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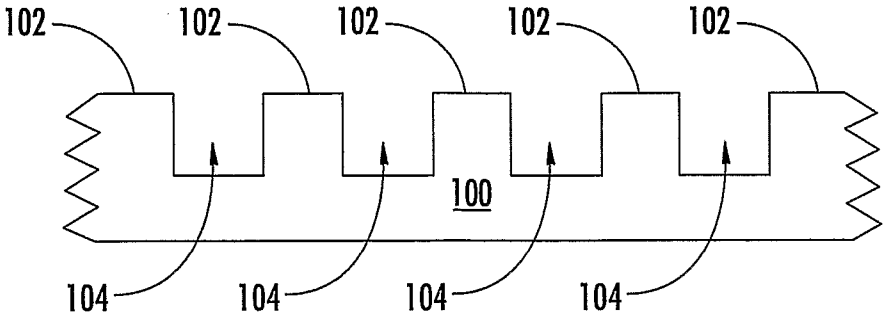
(54) Title: NANOPARTICLE FABRICATION METHODS, SYSTEMS, AND MATERIALS



(57) Abstract: Nano-particles are molded in nano-scale molds fabricated from non- wetting, low surface energy polymeric materials. The nano-particles can include pharmaceutical compositions, taggants, contrast agents, biologic drugs, drug compositions, organic materials, and the like. The molds can be virtually any shape and less than 10 micron in cross-sectional diameter.

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NANOPARTICLE FABRICATION METHODS, SYSTEMS, AND MATERIALS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is based on and claims priority to United States Provisional Patent Application Serial No. 60/691,607, filed June 17, 2005; United States Provisional Patent Application Serial No. 60/714,961, filed September 7, 2005; United States Provisional Patent Application Serial No. 60/734,228, filed November 7, 2005; United States Provisional Patent Application Serial No. 60/762,802, filed January 27, 2006; and United States Provisional Patent Application Serial No. 60/799,876 filed May 12, 2006; each of which is incorporated herein by reference in its entirety.

This application is also a continuation-in-part of PCT International Patent Application Serial NO. PCT/US04/42706, filed December 20, 2004, which is based on and claims priority to United States Provisional Patent Application Serial No. 60/531,531, filed December 19, 2003, United States Provisional Patent Application Serial No. 60/583,170, filed June 25, 2004, United States Provisional Patent Application Serial No. 60/604,970, filed August 27, 2004, each of which is incorporated herein by reference in its entirety.

GOVERNMENT INTEREST

A portion of the disclosure contained herein was made with U.S. Government support from the Office of Naval Research Grant No. N00014210185 and the Science and Technology Center program of the National Science Foundation under Agreement No. CHE-9876674. The U.S. Government has certain rights to that portion of the disclosure.

INCORPORATION BY REFERENCE

All documents referenced herein are hereby incorporated by reference as if set forth in their entirety herein, as well as all references cited therein.

TECHNICAL FIELD

Generally, this invention relates to micro and/or nano scale particle fabrication. More specifically, molds for casting micro and nano scale particles are disclosed, as well as, particles fabricated from the molds.

ABBREVIATIONS

°C	=	degrees Celsius
cm	=	centimeter
DBTDA	=	dibutyltin diacetate
DMA	=	dimethylacrylate
DMPA	=	2,2-dimethoxy-2-phenylacetophenone
EIM	=	2-isocyanatoethyl methacrylate
FEP	=	fluorinated ethylene propylene
Freon 113	=	1,1,2-trichlorotrifluoroethane
g	=	grams
h	=	hours
Hz	=	hertz
IL	=	imprint lithography
kg	=	kilograms
kHz	=	kilohertz
kPa	=	kilopascal
MCP	=	microcontact printing
MEMS	=	micro-electro-mechanical system
MHz	=	megahertz
MIMIC	=	micro-molding in capillaries
mL	=	milliliters
mm	=	millimeters
mmol	=	millimoles
mN	=	milli-Newton
m.p.	=	melting point
mW	=	milliwatts
NCM	=	nano-contact molding
NIL	=	nanoimprint lithography

nm	=	nanometers
PDMS	=	polydimethylsiloxane
PEG		poly(ethylene glycol)
PFPE	=	perfluoropolyether
PLA		poly(lactic acid)
PP	=	polypropylene
Ppy	=	poly(pyrrole)
psi	=	pounds per square inch
PVDF	=	poly(vinylidene fluoride)
PTFE	=	polytetrafluoroethylene
SAMIM	=	solvent-assisted micro-molding
SEM	=	scanning electron microscopy
S-FIL	=	"step and flash" imprint lithography
Si	=	silicon
Tg	=	glass transition temperature
Tm	=	crystalline melting temperature
TMPTA	=	trimethylolpropane triacrylate
μm	=	micrometers
UV	=	ultraviolet
W	=	watts
ZDOL	=	poly(tetrafluoroethylene oxide-*co*-difluoromethylene oxide)α,ω diol

BACKGROUND

The availability of viable nanofabrication processes is a key factor to realizing the potential of nanotechnologies. In particular, the availability of viable nanofabrication processes is important to the fields of photonics, electronics, and proteomics. Traditional imprint lithographic (IL) techniques are an alternative to photolithography for manufacturing integrated circuits, micro- and nano-fluidic devices, and other devices with micrometer and/or nanometer sized features. There is a need in the art, however, for new materials to advance IL techniques. See Xia, Y., et al., *Angew. Chem. Int. Ed.*, 1998, *37*, 550-575; Xia, Y., et al., *Chem. Rev.*, 1999, *99*, 1823-1848;

Resnick, D. J., et al., *Semiconductor International*, 2002, June, 71-78; Choi, K. M., et al., *J. Am. Chem. Soc.*, 2003, *125*, 4060-4061; McClelland, G. M., et al., *Appl. Phys. Lett.*, 2002, *81*, 1483; Chou, S. Y., et al., *J. Vac. Sci. Technol. B*, 1996, *14*, 4129; Otto, M., et al., *Microelectron. Eng.*, 2001, *57*, 361; and Bailey, T., et al., *J. Vac. Sci. Technol., B*, 2000, *18*, 3571.

Imprint lithography includes at least two areas: (1) soft lithographic techniques, see Xia, Y., et al., *Angew. Chem. Int. Ed.*, 1998, *37*, 550-575, such as solvent-assisted micro-molding (SAMIM); micro-molding in capillaries (MIMIC); and microcontact printing (MCP); and (2) rigid imprint lithographic techniques, such as nano-contact molding (NCM), see McClelland, G. M., et al., *Appl. Phys. Lett.*, 2002, *81*, 1483; Otto, M., et al., *Microelectron. Eng.*, 2001, *57*, 361; "step and flash" imprint lithographic (S-FIL), see Bailey, T., et al., *J. Vac. Sci. Technol., B*, 2000, *18*, 3571; and nanoimprint lithography (NIL), see Chou, S. Y., et al., *J. Vac. Sci. Technol. B*, 1996, *14*, 4129.

Polydimethylsiloxane (PDMS) based networks have been the material of choice for much of the work in soft lithography. See Quake, S. R., et al., *Science*, 2000, *290*, 1536; Y. N. Xia and G. M. Whitesides, *Angew. Chem. Int. Ed. Engl.* 1998, *37*, 551; and Y. N. Xia, et al., *Chem. Rev.* 1999, *99*, 1823.

The use of soft, elastomeric materials, such as PDMS, offers several advantages for lithographic techniques. For example, PDMS is highly transparent to ultraviolet (UV) radiation and has a very low Young's modulus (approximately 750 kPa), which gives it the flexibility required for conformal contact, even over surface irregularities, without the potential for cracking. In contrast, cracking can occur with molds made from brittle, high-modulus materials, such as etched silicon and glass. See Bietsch, A., et al., *J. Appl. Phys.*, 2000, *88*, 4310-4318. Further, flexibility in a mold facilitates the easy release of the mold from masters and replicates without cracking and allows the mold to endure multiple imprinting steps without damaging fragile features. Additionally, many soft, elastomeric materials are gas permeable, a property that can be used to advantage in soft lithography applications.

Although PDMS offers some advantages in soft lithography applications, several properties inherent to PDMS severely limit its capabilities in soft lithography. First, PDMS-based elastomers swell when exposed to most organic soluble compounds. See Lee, J. N., et al., *Anal. Chem.*, 2003, *75*, 6544-6554. Although this property is beneficial in microcontact printing (MCP) applications because it allows the mold to adsorb organic inks, see Xia, Y., et al., *Angew. Chem. Int. Ed.*, 1998, *37*, 550-575, swelling resistance is critically important in the majority of other soft lithographic techniques, especially for SAMIM and MIMIC, and for IL techniques in which a mold is brought into contact with a small amount of curable organic monomer or resin. Otherwise, the fidelity of the features on the mold is lost and an unsolvable adhesion problem ensues due to infiltration of the curable liquid into the mold. Such problems commonly occur with PDMS-based molds because most organic liquids swell PDMS. Organic materials, however, are the materials most desirable to mold. Additionally, acidic or basic aqueous solutions react with PDMS, causing breakage of the polymer chain.

Secondly, the surface energy of PDMS (approximately 25 mN/m) is not low enough for soft lithography procedures that require high fidelity. For this reason, the patterned surface of PDMS-based molds is often fluorinated using a plasma treatment followed by vapor deposition of a fluoroalkyl trichlorosilane. See Xia, Y., et al., *Angew. Chem. Int. Ed.*, 1998, *37*, 550-575. These fluorine-treated silicones swell, however, when exposed to organic solvents.

Third, the most commonly-used commercially available form of the material used in PDMS molds, e.g., Sylgard 184® (Dow Corning Corporation, Midland, Michigan, United States of America) has a modulus that is too low (approximately 1.5 MPa) for many applications. The low modulus of these commonly used PDMS materials results in sagging and bending of features and, as such, is not well suited for processes that require precise pattern placement and alignment. Although researchers have attempted to address this last problem, see Odom, T. W., et al., *J. Am. Chem. Soc.*, 2002, *124*, 12112-12113; Odom, T. W. et al., *Langmuir*, 2002,

18, 5314-5320; Schmid, H., et al., *Macromolecules*, 2000, *33*, 3042-3049; Csucs, G., et al., *Langmuir*, 2003, *19*, 6104-6109; Trimbach, D., et al., *Langmuir*, 2003, *19*, 10957-10961, the materials chosen still exhibit poor solvent resistance and require fluorination steps to allow for the release of the mold.

Rigid materials, such as quartz glass and silicon, also have been used in imprint lithography. See Xia, Y., et al., *Angew. Chem. Int. Ed.*, 1998, *37*, 550-575; Resnick, D. J., et al., *Semiconductor International*, 2002, June, 71-78; McClelland, G. M., et al., *Appl. Phys. Lett.*, 2002, *81*, 1483; Chou, S. Y., et al., *J. Vac. Sci. Technol. B*, 1996, *14*, 4129; Otto, M., et al., *Microelectron. Eng.*, 2001, *57*, 361; and Bailey, T., et al., *J. Vac. Sci. Technol., B*, 2000, *18*, 3571; Chou, S. Y., et al., *Science*, 1996, *272*, 85-87; Von Werne, T. A., et al., *J. Am. Chem. Soc.*, 2003, *125*, 3831-3838; Resnick, D. J., et al., *J. Vac. Sci. Technol. B*, 2003, *21*, 2624-2631. These materials are superior to PDMS in modulus and swelling resistance, but lack flexibility. Such lack of flexibility inhibits conformal contact with the substrate and causes defects in the mask and/or replicate during separation.

Another drawback of rigid materials is the necessity to use a costly and difficult to fabricate hard mold, which is typically made by using conventional photolithography or electron beam (e-beam) lithography. See Chou, S. Y., et al., *J. Vac. Sci. Technol. B*, 1996, *14*, 4129. More recently, the need to repeatedly use expensive quartz glass or silicon molds in NCM processes has been eliminated by using an acrylate-based mold generated from casting a photopolymerizable monomer mixture against a silicon master. See McClelland, G. M., et al., *Appl. Phys. Lett.*, 2002, *81*, 1483, and Jung, G. Y., et al., *Nanoletters*, 2004, ASAP. This approach also can be limited by swelling of the mold in organic solvents.

Despite such advances, other disadvantages of fabricating molds from rigid materials include the necessity to use fluorination steps to lower the surface energy of the mold, see Resnick, D. J., et al., *Semiconductor International*, 2002, June, 71-78, and the inherent problem of releasing a rigid mold from a rigid substrate without breaking or damaging the mold or the substrate. See Resnick, D. J., et al., *Semiconductor International*, 2002,

June, 71-78; Bietsch, A., *J. Appl. Phys.*, 2000, *88*, 4310-4318. Khang, D. Y., et al., *Langmuir*, 2004, *20*, 2445-2448, have reported the use of rigid molds composed of thermoformed Teflon AF® (DuPont, Wilmington, Delaware, United States of America) to address the surface energy problem. Fabrication of these molds, however, requires high temperatures and pressures in a melt press, a process that could be damaging to the delicate features on a silicon wafer master. Additionally, these molds still exhibit the intrinsic drawbacks of other rigid materials as outlined hereinabove.

Further, a clear and important limitation of fabricating structures on semiconductor devices using molds or templates made from hard materials is the usual formation of a residual or "scum" layer that forms when a rigid template is brought into contact with a substrate. Even with elevated applied forces, it is very difficult to completely displace liquids during this process due to the wetting behavior of the liquid being molded, which results in the formation of a scum layer. Thus, there is a need in the art for a method of fabricating a pattern or a structure on a substrate, such as a semiconductor device, which does not result in the formation of a scum layer.

The fabrication of solvent resistant, microfluidic devices with features on the order of hundreds of microns from photocurable perfluoropolyether (PFPE) has been reported. See Rolland, J. P., et al., *J. Am. Chem. Soc.*, 2004, *126*, 2322-2323. PFPE-based materials are liquids at room temperature and can be photochemically cross-linked to yield tough, durable elastomers. Further, PFPE-based materials are highly fluorinated and resist swelling by organic solvents, such as methylene chloride, tetrahydrofuran, toluene, hexanes, and acetonitrile among others, which are desirable for use in microchemistry platforms based on elastomeric microfluidic devices. There is a need in the art, however, to apply PFPE-based materials to the fabrication of nanoscale devices for related reasons.

Further, there is a need in the art for improved methods for forming a pattern on a substrate, such as method employing a patterned mask. See U.S. Patent No. 4,735,890 to Nakane et al.; U. S. Patent No. 5,147,763 to Kamitakahara et al.; U.S. Patent No. 5,259,926 to Kuwabara et al.; and

International PCT Publication No. WO 99/54786 to Jackson et al., each of which is incorporated herein by reference in their entirety.

There also is a need in the art for an improved method for forming isolated structures that can be considered "engineered" structures, including but not limited to particles, shapes, and parts. Using traditional IL methods, the scum layer that almost always forms between structures acts to connect or link structures together, thereby making it difficult, if not impossible to fabricate and/or harvest isolated structures.

There also is a need in the art for an improved method for forming micro- and nanoscale charged particles, in particular polymer electrets. The term "polymer electrets" refers to dielectrics with stored charge, either on the surface or in the bulk, and dielectrics with oriented dipoles, frozen-in, ferrielectric, or ferroelectric. On the macro scale, such materials are used, for example, for electronic packaging and charge electret devices, such as microphones and the like. See Kressman, R., et al., *Space-Charge Electrets*, Vol. 2, Laplacian Press, 1999; and Harrison, J. S., et al., *Piezoelectic Polymers*, NASA/CR-2001-211422, ICASE Report No. 2001-43. Poly(vinylidene fluoride) (PVDF) is one example of a polymer electret material. In addition to PVDF, charge electret materials, such as polypropylene (PP), Teflon-fluorinated ethylene propylene (FEP), and polytetrafluoroethylene (PTFE), also are considered polymer electrets.

Further, there is a need in the art for improved methods for delivering therapeutic agents, such as drugs, non-viral gene vectors, DNA, RNA, RNAi, and viral particles, to a target. See *Biomedical Polymers*, Shalaby, S. W., ed., Harner/Gardner Publications, Inc., Cincinnati, Ohio, 1994; *Polymeric Biomaterials*, Dumitrin, S., ed., Marcel Dekkar, Inc., New York, New York, 1994; Park, K., et al., *Biodegradable Hydrogels for Drug Delivery*, Technomic Publishing Company, Inc., Lancaster, Pennsylvania, 1993; Gumargalieva, et al., *Biodegradation and Biodeterioration of Polymers: Kinetic Aspects*, Nova Science Publishers, Inc., Commack, New York, 1998; *Controlled Drug Delivery*, American Chemical Society Symposium Series 752, Park, K., and Mrsny, R. J., eds., Washington, D.C., 2000; *Cellular Drug Delivery: Principles and Practices*, Lu, D. R., and Oie, S., eds., Humana

Press, Totowa, New Jersey, 2004; and *Bioreversible Carriers in Drug Design: Theory and Applications*, Roche, E. B., ed., Pergamon Press, New York, New York, 1987. For a description of representative therapeutic agents for use in such delivery methods, see U.S. Patent No. 6,159,443 to Hallahan, which is incorporated herein by reference in its entirety.

There is also a need in the art for an improved method for forming super absorbent particles. These particles can be used for specialty packaging, wire waterblocking, filtration, medical markets, spill control, therapy packs, composites and laminates, water retention.

There is also a need in the art for improved methods to create polymorphs. Polymorphs exist when there is more than one way for the particles of a particular substance to arrange themselves into a crystalline array. Different polymorphs of the same substance can have vastly different physical and chemical properties. Invariably, one of the crystal forms may be more stable or easier to handle than another although the conditions under which the various crystal forms appears may be so close as to be very difficult to control on the large scale. This effect can create differences in the bioavailability of the drug which leads to inconsistencies in efficacy. See "Drug polymorphism and dosage form design: a practical perspective" *Adv. Drug Deliv. Rev.*, Singhal D, Curatolo W. 2004 Feb 23;56(3):335-47; *Generic Drug Product Development: Solid Oral Dosage Forms*, Shargel, L., ed., Marcel Dekker, New York, 2005.

In sum, there exists a need in the art to identify new materials for use in imprint lithographic techniques. More particularly, there is a need in the art for methods for the fabrication of structures at the hundreds of micron level down to sub-100 nm feature sizes. Additionally, there is a need in the art for improved methods for polymorph creation.

Moreover, authentication and identification of articles is of particular concern in all industries, and particularly of financial documents, high-profile consumer and retail brands, pharmaceutics, and bulk materials. Billions of dollars are lost every year through counterfeiting and liability lawsuits that could be prevented with effective taggant technology.

What has been needed has been an authentication system with additional protections against counterfeiting that includes tagging materials and a system for detecting those materials. The system and method can be useful to the manufacturer to verify the authenticity of the article through processing, the first time it is sold, and throughout the lifetime of the product. The system and method should also be useful for purchasers in the secondary market to verify the identification or authenticity of articles for purchase.

It is also often desirable to monitor for, identify, report, and evaluate a presence of a solid, liquid, gaseous, or other substance of interest. It will be appreciated, for example, that it has become highly desirable or even necessary, particularly in light of recent terrorist activities, to monitor for, identify, report, and evaluate any presence of threatening chemical, biological, or radioactive substances. Many less sinister substances, however, are also often the subject of monitoring, including, for example, pollutants; illegal or otherwise regulated substances; substances of interest to science; and substances of interest to agriculture or industry.

In the case of threatening substances, for example, detection devices are well-known in the prior art, ranging from the extremely simple to the exceedingly complex. Simple detection devices are typically narrowly capable of detecting and identifying a single substance or group of closely related substances. These devices typically combine detection and identification into a single function by using a very specific test that can only detect the presence or non-presence of the specific substance and none other. More complex detection systems can be used to increase the level of security, with multiple, coupled detection methods.

An example of a detection system is disclosed in U.S. Pat. No. 3,897,284. This system discloses microparticles for tagging of explosives, which particles incorporate a substantial proportion of magnetite that enables the particles to be located by means of magnetic pickup. Ferrite has also been used. More recently, modified tagging particles with strips of color coding material having a layer of magnetite affixed to one side and layers of fluorescent material affixed to both exterior sides, has been developed. In

this system, the taggant can be located by visual detection of the luminescent response, or magnetic pickup, or both. Both the ferrite and the magnetite materials are, however, dark colored and absorptive of the radiation which excites the luminescent material, thereby making the particles somewhat difficult to locate after an explosion. Further developments produced similar particles that take advantage of the magnetic properties without diminishing the luminescent response of the materials, such as those described in U.S. Pat. No. 4,131,064.

Yet, another approach is the development of particles coded with ordered sequences of distinguishable colored segments, such as described in U.S. Pat. No. 4,053,433. Still further, other patents employ radioactive isotopes or other hazardous materials as taggants and many patents utilize inorganic materials as taggants, such as U.S. Pat. No 6,899,827.

However, some drawbacks of many current systems is that they are expensive; require sophisticated technology to produce, employ, and detect; inappropriate for many environments such as harsh chemical or thermal environments; time consuming to produce and incorporate into products to be protected; and the like.

SUMMARY

In some embodiments, the presently disclosed subject matter describes a nanoparticle composition that includes a particle having a shape that corresponds to a mold where the particle is less than about 100 μm in a broadest dimension. In some embodiments, the nanoparticle composition can include a plurality of particles, were the particles have a substantially constant mass. In some embodiments, the plurality of particles has a poly dispersion index of between about 0.80 and about 1.20. In alternative embodiments, the particles have a poly dispersion index of between about 0.90 and about 1.10, between about 0.95 and about 1.05, between about 0.99 and about 1.01, or between about 0.999 and about 1.001. In yet other embodiments, the nanoparticle composition includes a plurality of particles with a mono-dispersity.

According to some embodiments, the nanoparticle composition includes a therapeutic or diagnostic agent associated with the particle. The therapeutic or diagnostic agent can be physically coupled or chemically coupled with the particle, encompassed within the particle, at least partially encompassed within the particle, coupled to the exterior of the particle, or the like. In some embodiments, the composition includes a therapeutic agent selected from the group of a drug, a biologic, a ligand, an oligopeptide, a cancer treatment, a viral treatment, a bacterial treatment, an auto-immune treatment, a fungal treatment, a psychotherapeutic agent, a cardiovascular drug, a blood modifier, a gastrointestinal drug, a respiratory drug, an antiarthritic drug, a diabetes drug, an anticonvulsant, a bone metabolism regulator, a multiple sclerosis drug, a hormone, a urinary tract agent, an immunosuppressant, an ophthalmic product, a vaccine, a sedative, a sexual dysfunction therapy, an anesthetic, a migraine drug, an infertility agent, a weight control product, cell treatment, and combinations thereof. In some embodiments, the composition includes a diagnostic selected from the group of an imaging agent, a x-ray agent, a MRI agent, an ultrasound agent, a nuclear agent, a radiotracer, a radiopharmaceutical, an isotope, a contrast agent, a fluorescent tag, a radiolabeled tag, and combinations thereof. According to some embodiments, the nanoparticle includes an organic composition, a polymer, an inorganic composition, or the like.

In one embodiment, there is a nanoparticle that includes an organic composition having a substantially predetermined shape substantially corresponding to a mold, wherein the shape is less than about 100 microns in a broadest dimension.

In some embodiments, the nanoparticle includes a super absorbent polymer. The super absorbent polymer can be selected from the group of polyacrylates, polyacrylic acid, polyacrylamide, cellulose ethers, poly (ethylene oxide), poly (vinyl alcohol), polysuccinimides, polyacrylonitrile polymers, combinations of the above polymers blended or crosslinked together, combinations of the above polymers having monomers co-polymerized with monomers of another polymer, combinations of the above polymers with starch, and the like.

In some embodiments, the nanoparticle is less than about 50 μm in a dimension. In other embodiments, the nanoparticle can be between about 1 nm or about 10 micron in a dimension, between about 5 nm and about 1micron in a dimension. The dimension can be, in some embodiments, a cross-sectional dimension, a circumferential dimension, a surface area, a length, a height, a width, a linear dimension, or the like. According to alternative embodiments, the nanoparticle can be shaped as a substantially non-spherical object, substantially viral shaped, substantially bacteria shaped, substantially cell shaped, substantially rod shaped, substantially rod shaped, where the rod can be less than about 200 nm in diameter or less than about 2 nm in diameter. According to yet other embodiments, the nanoparticle can be shaped as a substantially chiral shaped particle, configured substantially as a right triangle, substantially flat having a thickness of about 2 nm, a substantially flat disc having a thickness between about 2 nm and about 200 nm, substantially boomerang shaped, and the like.

In some embodiments, the nanoparticle can be substantially coated, such as with a sugar based coating of, for example, glucose, sucrose, maltose, derivatives thereof, and combinations thereof.

According to some embodiments, the presently disclosed subject matter discloses a nanoparticle that is less than about 100 micron in a largest dimension and is fabricated from a mold, where the mold is composed of a fluoropolymer. In some embodiments, the nanoparticle includes ^{18}F. In other embodiments, the nanoparticle includes a charged particle, polymer electret, therapeutic agent, non-viral gene vector, viral particle, polymorph, or super absorbent polymer.

The presently disclosed subject matter describes methods for fabricating a nanoparticle. In some embodiments, the methods include providing a template, where the template defines a recess between about 1 nanometers and about 100 micron in average diameter, dispensing a substance to be molded onto the template such that the substance fills the recess, and hardening the substance in the recess such that a particle is

molded within the recess. In some embodiments the methods also include removing excess substance from the template such that remaining substance resides substantially within the recess. In some embodiments, the methods include the step of removing the particle from the recess. In some embodiments, the methods include the step of evaporation of a solvent of the substance. In one embodiment, the subtance includes a solution with a drug dissolved therein. In some embodiments, the method includes, including a therapeutic agent with the substance. In some embodiments, the method includes, including a diagnostic agent with the substance. In one embodiment, the method includes trerating a cell with the particle.

According to some embodiments, the template for fabricating nanoparticles can be composed of materials selected from the group of a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction. In some embodiments, the template is composed of a fluoropolymer that is selected from the group of a perfluoropolyether, a photocurable perfluoropolyether, a thermally curable perfluoropolyether, or a combination of photocurable and thermally curable perfluoropolyether. In one embodiment, the template is confligured from a low surface energy polymeric material.

According to other embodiments, the methods for fabricating nanoparticles can include placing a material that includes a liquid into a recess in a fluoropolymer mold, where the recess is less than about 100 μm in a broadest dimension, curing the material to make a particle, and removing the particle from the recess. In some embodiments, the nanoparticle can include a therapeutic agent selected from the group consisting of: a drug, a biologic, a cancer treatment, a viral treatment, a bacterial treatment, an auto-immune treatment, a fungal treatment, an enzyme, a protein, a nucleotide sequence, an antigen, an antibody, and a

diagnostic. In one embodiment, the particle has a smaller volume than a volume of the material placed into the recess.

In some embodiments, the recess for fabricating a nanoparticle can be less than about 10 μm in the broadest dimension, between about 1 nm and 1 micron in the broadest dimension, between about 1 nm and 500 nm in the broadest dimension, or between about 1 nm and about 150 nm in the broadest dimension.

In some embodiments, the nanoparticle can have a shape corresponding to a mold that is substantially non-spherical, substantially viral shaped, substantially bacteria shaped, substantially cell shaped, substantially rod shaped, substantially rod shaped wherein the rod is less than about 200 nm in diameter, substantially chiral shaped, substantially a right triangle, substantially flat disc shaped with a thickness of about 2 nm, substantially flat disc shaped with a thickness of between about 200 nm and about 2 nm, substantially boomerang shaped, and combinations thereof.

In some embodiments, methods for fabricating nanoparticles include placing a material into a recess defined in a fluoropolymer mold, treating the material in the recess to form a particle, and removing the particle from the recess. In some embodiments, the fluoropolymer includes a low-surface energy. According to some embodiments, the methods of fabricating a nanoparticle includes providing a template, where the template defines a recess less than about 100 micron in average diameter and where the template is a low-surface energy polymeric material, dispensing a substance to be molded onto the template such that the substance at least partially fills the recess, and hardening the substance in the recess such that a particle is molded within the recess. In some embodiments, a force is applied to the template to remove substance not contained within the recess and the force can be applied with a substrate having a surface configured to engage the template. In some embodiments, the force applied to the template is a manual pressure. According to some embodiments, the methods include removing the substrate from the template after removing excess substance from the template and before hardening the substance in the recess. Some

embodiments include passing a blade across the template to remove substance not contained within the recess, where the blade can be selected from the group of a metal blade, a rubber blade, a silicon based blade, a polymer based blade, and combinations thereof. According to some embodiments, the template can be selected from the group of a substantially rotatable cylinder, a conveyor belt, a roll-to-roll process, a batch process, or a continuous process.

According to some embodiments of the methods, the substance in the recess can be hardened by evaporation, a chemical process, treating the substance with UV light, a temperature change, treating the substance with thermal energy, or the like. In some embodiments, the methods include leaving the substrate in position on the template to reduce evaporation of the substance from the recess. Some embodiments of the methods include harvesting the particle from the recess after hardening the substance. According to alternative embodiments, the harvesting of nanoparticles includes applying an article that has affinity for the particles that is greater than an affinity between the particles and the template. In some embodiments, the harvesting can further include contacting the particle with an adhesive substance, where adhesion between the particle and the adhesive substance is greater than adhesive force between the particle and the template. In other embodiments, the harvesting substance can be selected from one or more of water, organic solvents, carbohydrates, epoxies, waxes, polyvinyl alcohol, polyvinyl pyrrolidone, polybutyl acrylate, polycyano acrylates, and polymethyl methacrylate.

According to other embodiments, the methods can further include purifying the particle after harvesting the particle. In some embodiments, the purifying of the particle can include purifying the particle from a harvesting substance, centrifugation, separation, vibration, gravity, dialysis, filtering, sieving, electrophoresis, gas stream, magnetism, electrostatic separation, dissolution, ultrasonics, megasonics, flexure of the template, suction, electrostatic attraction, electrostatic repulsion, magnetism, physical template manipulation, combinations thereof, and the like.

In some embodiments of the presently disclosed subject matter, the substance to be molded is selected from the group of a polymer, a solution, a monomer, a plurality of monomers, a polymerization initiator, a polymerization catalyst, an inorganic precursor, a metal precursor, a pharmaceutical agent, a tag, a magnetic material, a paramagnetic material, a ligand, a cell penetrating peptide, a porogen, a surfactant, a plurality of immiscible liquids, a solvent, and a charged species. According to some embodiments, the particle includes organic polymers, super absorbent polymers, charged particles, polymer electrets (poly(vinylidene fluoride), Teflon-fluorinated ethylene propylene, polytetrafluoroethylene), therapeutic agents, drugs, non-viral gene vectors, DNA, RNA, RNAi, viral particles, polymorphs, combinations thereof, and the like.

According to some embodiments, the presently disclosed subject matter includes methods for making nanoparticles that include providing a patterned template defining a nano-scale recess, submerging the nano-scale recess into a substance to be molded in the nano-scale recess, allowing the substance to enter the recess, and removing the patterned template from the substance. In other embodiments, the methods include providing a template, where the template defines a nano-scale recess, disposing a substance to be molded in the nano-scale recess onto the template, and allowing the substance to enter the nano-scale recess.

In some embodiments, the methods include configuring a contact angle between a liquid to be molded and a template mold to be a predetermined angel such that the liquid passively fills a nano-scale recess defined in the template mold. In some embodiments, the contact angle can be modified or altered by applying a voltage to the liquid.

In some embodiments, the methods include introducing a first substance to be molded into a nano-scale recess of a template, allowing a solvent component of the first substance to evaporate from the nano-scale recess, and curing the first substance in the nano-scale recess to form a particle. According to other embodiments, the methods include adding a

second substance to the nano-scale recess following evaporation and curing of the first substance such that a particle having two compositions is formed.

According to some embodiments, the methods include providing a template, where the template defines a nano-scale recess, disposing a substance to be molded onto the template, and applying a voltage across the substance to assist the substance to enter the nano-scale recess. In some embodiments, the methods include configuring a template with a predetermined permeability, where the template defines a nano-scale recess, subjecting the template with a substance having a predetermined permeability, allowing the substance to enter the nano-scale recess, and curing the substance in the nano-scale recess.

In yet other embodiments, the methods include a particle including a functional molecular imprint, where the particle has a shape corresponding to a mold, and wherein the particle is less than about 100 μm in a dimension. In some embodiments the dimension is one of less than about 1 μm, between about 1nm and and 500nm, between about 50nm and about 200nm, and between about 80nm and about 120nm. According to some embodiments, the functional molecular imprint comprises functional monomers arranged as a negative image of a template. In one embodiment the particle is an analytical material. In some embodiments, the functional molecular imprint substantially includes steric and chemical properties of a template.

In one embodiment, analytical material includes a particle having a shape selected from the group consisting of substantially spherical, substantially non-spherical, substantially viral shaped, substantially bacteria shaped, substantially protein shaped, substantially cell shaped, substantially rod shaped, substantially rod shaped wherein the rod is less than about 200 nm in diameter, substantially chiral shaped, substantially a right triangle, substantially flat disc shaped with a thickness of about 2 nm, substantially flat disc shaped with a thickness of greater than about 2 nm, substantially boomerang shaped, and combinations thereof. In some embodiments, the particle is a plurality of particles having a poly dispersion index of between

about 0.80 and about 1.20. In another embodiment, the particle is a plurality of particles having a poly dispersion index of between about 0.90 and about 1.10. In yet another embodiment, the particle is a plurality of particles having a poly dispersion index of between about 0.95 and about 1.05. In a still further embodiemnt, the particle is a plurality of particles having a poly dispersion index of between about 0.99 and about 1.01. In another embodiment, the the analytical material includes a particle that is a plurality of particles having a poly dispersion index of between about 0.999 and about 1.001. In another embodiment, the particle is a plurality of particles and the plurality of particles has a mono-dispersity.

In some embodiments, the methods include providing a substrate of perfluoropolyether and a functional template, wherein the substrate defines a recess and the recess include the functional template at least partially exposed therein, applying a material to the substrate, curing the material to form a particle, and removing the particle from the recess, where the particle includes a molecular imprint of the functional template. In some embodiments, the material includes a functional monomer and the functional template is selected from the group of an enzyme, a protein, an antibiotic, an antigen, a nucleotide sequence, an amino acid, a drug, a biologic, nucleic acid, and combinations thereof. In some embodiments, the perfluoropolyether is selected from the group of photocurable perfluoropolyether, thermally curable perfluoropolyether, and a combination of photocurable and thermally curable perfluoropolyether.

In other embodiments, the methods include a functionalized particle molded from a molecular imprint. In some embodiments, the functionalized particle further includes a functionalized monomer. In some embodiments, the functionalized particle includes substantially similar steric and chemical properties of a molecular imprint template. According to some embodiments, the functional monomers of the functionalized particle are arranged substantially as a negative image of functional groups of the molecular imprint. In other embodiments, the molecular imprint is a molecular imprint of a template selected from the group of an enzyme, a

protein, an antibiotic, an antigen, a nucleotide sequence, an amino acid, a drug, a biologic, nucleic acid, and combinations thereof.

According to some embodiments, the methods include providing a template defining a molecular imprint, where the template includes a low-surface energy polymeric material, applying a mixture of a material and a functional monomer to the molecular imprint, curing the mixture to form a polymerized artificial functional molecule, and removing the polymerized artificial functional molecule from the molecular imprint. The methods also can include allowing the functional monomers in the mixture to arrange with opposing entities to the functional molecular imprint. In one embodiment, the method includes treating a patient with a polymerized artifidical functional molecule.

In other embodiments, the methods include providing a patterned template defining a molecular imprint, where the patterned template includes a low-surface energy polymeric material, applying a mixture of a material and a functional monomer to the molecular imprint, curing the mixture to form a polymerized artificial functional molecule, removing the polymerized artificial functional molecule from the molecular imprint, and administering a therapeutically effective amount of the polymerized artificial functional molecule to a patient. According to some embodiments, the polymerized artificial functional molecule treats a patient by interacting with a cellular membrane, treats a patient by undergoing intracellular uptake, treats a patient by inducing an immune response, interacts with a cellular receptor, or is less than about 100 μm in a dimension.

In some embodiments, the methods include administering a therapeutically effective amount of a particle having a predetermined shape and a dimension of less than about 100 μm to a patient. In some embodiments, the particle undergoes intracellular uptake. In some embodiments, the particle includes a therapeutic or diagnostic at least partially encompassed within the particle or coupled to the exterior of the particle. In other embodiments, the methods include selecting the therapeutic from the group of a drug, a biologic, an anti-cancer treatment, an

anti-viral treatment, an anti-bacterial treatment, an auto-immune treatment, a fungal treatment, a psychotherapeutic agent, cardiovascular drug , a blood modifier, a gastrointestinal drug, a respiratory drug, an antiarthritic drug, a diabetes drug, an anticonvulsant, a bone metabolism regulator, a multiple sclerosis drug, a hormone, a urinary tract agent, an immunosuppressant, an ophthalmic product, a vaccine, a sedative, a sexual dysfunction therapy, an anesthetic, a migraine drug, an infertility agent, a weight control product, and combinations thereof. In some embodiments, the diagnostic is selected from the group of an imaging agent, a x-ray agent, a MRI agent, an ultrasound agent, a nuclear agent, a radiotracer, a radiopharmaceutical, an isotope, a contrast agent, a fluorescent tag, a radiolabeled tag, and combinations thereof. In one embodiment of the method, the particle has a dimension that is take from the group of that is less than about 10 μm, between 1nm and about 1 micron in diameter, and between about 1 nm and about 200nm in diameter. In one embodiment, the particle is substantially non-spherical, substantially viral shaped, substantially bacteria shaped, substantially protein shaped, substantially cell shaped, substantially rod shaped, substantially chiral shaped, substantially a right triangle, substantially a flat disc with a thickness of about 2 nm, substantially a flat disc with a thickness between about 2 nm and about 1 μm, and substantially boomerang shaped. In another embodiment, the particle is substantially rod-shaped and the rod is less than about 200 nm in diameter. In another embodiment, the particle is substantially coated. In a further embodiment, the particle is coated with a carbohydrate based coating. In a still further embodiment the particle includes an organic material. In one embodiment, the particle is molded from a patterned template that includes a low surface energy polymeric material.

In some embodiments, methods of delivering a treatment include forming a particle of a treatment compound, the particle having a predetermined shape and being less than about 100 μm in a dimension and administering the particle to a location of maxillofacial or orthopedic inquiry. In other embodiments, the methods include harvesting a nanoparticle from an article including, providing an article defining a recess, where the recess

is less than 100 micron in a greatest dimension, forming a particle in the recess, applying, to the article, a material having an affinity for the particle that is greater than an affinity between the article and the particle, and separating the material from the article wherein the material remains attached to the particle. In some embodiments, the methods include treating the material to increase the affinity of the material to the particle. In other embodiments, the methods include applying a force to at least one of the article, the material and combinations thereof. In some embodiments, the treating includes cooling the material, including one of the group of hardening the material, chemically modifying a surface of the particle to increase the affinity between the material and the particle, chemically modifying a surface of the material to increase the affinity between the particle and the material, a UV treatment, a thermal treatment, and combinations thereof. In some embodiments, the treating includes promoting a chemical interaction between the material and the particles or promoting a physical interaction between the material and the particles. In some embodiments, the physical interaction is a physical entrapment. In one embodiment, the article includes a low surface energy material. In one embodiment, the low surface energy material includes a material selected from the group consisting of a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction. In one embodiment, the method material is selected from the group consisting of carbohydrates, epoxies, waxes, polyvinyl alcohol, polyvinyl pyrrolidone, polybutyl acrylate, polycyano acrylates, polymethyl methacrylate and combinations thereof.

According to some embodiments of the presently disclosed subject matter, the methods include modifying a surface of a nanoparticle, such as providing an article defining a recess and having a particle formed therein, applying to the particle a solution containing modifying groups of molecules, and promoting a reaction between a first portion of the modifying groups of

molecules and at least a portion of a surface of the particle. In some embodiments, a second portion of the modifying groups of molecules are left unreacted. In other embodiments, the methods include removing the unreacted modifying groups of molecules. In some embodiments, the modifying group of molecules chemically attach to the particle through a linking group and the linking group can be selected from a group of sulfides, amines, carboxylic acids, acid chlorides, alcohols, alkenes, alkyl halides, isocyanates, imidazoles, halides, azides, and acetylenes. In some embodiments, the modifying group is selected from a group of dyes, fluorescence tags, radiolabeled tags, contrast agents, ligands, peptides, aptamers, antibodies, pharmaceutical agents, proteins, DNA, RNA, siRNA, and fragments thereof.

According to some embodiments, a system for harvesting a plurality of nanoparticles from an article includes an article defining a plurality of recesses wherein the recesses are less than about 100 micron in a dimension and wherein particles are formed within the recesses, a material having an affinity for the particles that is greater than an affinity between the particles and the article, and an applicator configured to separate the particles from the article. In some embodiments, the article includes a low-surface energy polymeric material. In some embodiments, a system for modifying at least a portion of a nanoparticle includes an article defining a recess, where the recess is less than about 100 micron in a dimension and wherein the recess has a particle formed therein, and a solution having modifying groups of molecules, the solution being in contact with at least a portion of the particle and being configured to promote a reaction between the molecules and the particle.

In other embodiments, the methods of the presently disclosed subject matter include methods for coating particles. In some embodiments, the method includes coating a particle with a sugar-based coating. In one embodiment the sugar-based coating is selected from the group consisting of clucose, sucrose, maltose, derivatives thereof, and combinations thereof. In some embodiments, the methods include seed coating, including suspending a seed in a liquid solution, depositing the liquid solution

containing the seed onto a template, where the template defines a recess that is less than about 100 micron in a dimension and where the template comprises a low-surface energy polymeric material, and hardening the liquid solution in the recesses such that the seed is coated with the hardened liquid solution. In some embodiments, the coating methods include engaging a surface with the template to sandwich the solution containing the seed into the recess. In some embodiments, the recess has a predetermined shape or size, the liquid solution is a polymer, or the liquid solution is a water soluble polymer. In one embodiment, the recess has a larger volume than an amount of liquid solution deposited into the recess. In some embodiments, the methods further include harvesting the hardened liquid solution containing the seed. According to some embodiments, the hardened liquid solution containing the seed is harvested by physical manipulation of the template, hardening includes evaporation of solvent from the substance, the substance in the recess is hardened by treating the substance with UV light, the substance in the recess is hardened by a chemical process, the substance in the recess is hardened by a temperature change, the substance in the recess is hardened by two or more of the group consisting of a thermal process, an evaporative process, a chemical process, and a optical process. In some embodiments, the method includes harvesting the hardened liquid solution containing the seed from the recess after curing the substance. In some embodiments, the hardened liquid solution containing the seed is harvested by an article that has affinity for the hardened liquid solution containing the seed that is greater than the affinity between the hardened liquid solution containing the seed and the template. In other embodiments, the methods include purifying the particle after it has been harvested.

According to some embodiments, a coated seed is prepared by the process including suspending a seed in a liquid solution, depositing the liquid solution containing the seed onto a template, where the template includes a recess, and hardening the liquid solution in the recesses such that the seed is coated with the hardened liquid solution.

In some embodiments, the presently disclosed subject matter describes taggants, including a particle having a shape corresponding to a mold, wherein the particle is less than about 100 micron is a dimension, and where the particle includes an identifying characteristic. In other embodiments, the presently disclosed subject matter describes methods of making taggants, including placing material into a mold formed from a low surface energy, non-wettable material, where the mold is less than about 100 micron in a dimension, and where the mold includes an identifying characteristic, curing the material to make a particle, and removing the particle from the mold.

In some embodiments, the presently disclosed subject matter includes a secure item including, an item coupled with a taggant including a particle having a shape corresponding to a mold, where the particle is less than about 100 micron in a dimension, and where the particle includes an identifying characteristic. In some embodiments, the presently disclosed subject matter includes methods of making a secure item, including placing material into a mold formed from a low surface energy, non-wettable material, where the mold is less than about 100 micron in a dimension, and where the mold includes an identifying characteristic, curing the material to make a particle, removing the particle from the mold, and coupling the particle with an item. In yet other embodiments, the presently disclosed subject matter includes a system for securing an item, including producing a taggant including a particle having a shape corresponding to a mold, where the particle is less than about 100 micron in a dimension, and where the particle includes an identifying characteristic, incorporating the taggant with an item to be secured, analyzing the item to detect and read the identifying characteristic, and comparing the identifying characteristic with an expected characteristic.

According to other embodiments, the presently disclosed subject matter describes an identification particle, including a taggant fabricated from a photoresist, where the taggant is configured and dimensioned using photolithography. In some embodiments, an identification particle, includes a taggant cast from a mold, where the mold includes low-surface energy

polymeric material, and where the taggant includes a substantially flat surface. According to alternative embodiments, the identification particle includes bosch etch lines on a surface of the taggant, chemical functionality, an active sensor, combinations thereof, and the like. According to some embodiments of the presently disclosed subject matter, methods of identifying a nanoparticle include providing a taggant configured and dimensioned in a predetermined shape, and recognizing the taggant according to the shape of the taggant.

In some embodiments, the presently disclosed subject matter describes a nanoparticle formed by the process of providing a template of a low surface energy polymeric material, where the template defines a nano-scale recess, disposing a liquid to be molded onto the template, where the liquid has a predetermined contact angle with a surface of the template such that the liquid passively enters the nano-scale recess, and forming a particle from the liquid in the nano-scale recess. In other embodiments, the presently disclosed subject matter includes a nanoparticle prepared by the process of providing a template having a first surface, where the first surface defines a recess between about 2 nanometers and about 1 millimeter in average diameter, dispensing a substance to be molded onto the first surface such that the substance fills the recess, removing substance from the first surface such that remaining substance resides substantially within the recess, and hardening the substance in the recess such that a particle is molded within the recess. In one embodiment, the nanoparticle includes at least one of an organic polymer, a super absorbent particle, a charged particle, a polymer electret, a therapeutic agent, a drug, a non-viral gene vector, DNA, RNA, RNAi, a viral particle, a polymorph, combinations thereof, and the like. In another embodiment, the process of producing the nanoparticle includes applying a press to the first surface to remove substance not contained within the recess. In one embodiment, the press is has substantially flat surface for engaging the first surface of the template. In another embodiment, the process further includes removing the press from the first surface after removing excess substance from the first surface and before hardening the substance in the recess. In a further embodiment,

the template is selected from the group consisting of a rotatable cylinder, a press, a conveyor belt, combinations thereof, and the like. In a still further embodiment of the method, the hardening comprises evaporation of solvent from the substance.

In one embodiment, the substance in the recess is hardened by treating the substance with UV light. In another embodiment, the substance in the recess is hardened by a chemical process. In a further embodiment, the substance in the recess is hardened by a temperature change. In a still further embodiment, the substance in the recess is hardened by treating the substance with thermal energy. In another embodiment, the substance in the recess is hardened by two or more of the group consisting of a thermal process, an evaporative process, a chemical process, and a optical process.

In yet another embodiment, the method includes harvesting the particle from the recess after curing the substance. In still another embodiment, the method includes purifying the particle after it has been harvested. In one embodiment, the purifying is selected from the group consisting of centrifugation, separation, vibration, gravity, dialysis, filtering, sieving, electrophoresis, gas stream, magnetism, electrostatic separation, combinations thereof, and the like.

In one embodiment, the particle is harvested by an article that has affinity for the particles that is greater than the affinity between the particles and the template. In another embodiment, the particle is harvested by contacting the particle with an adhesive substance. In still another embodiment, the method includes purifying the particle after it has been harvested.

In one embodiment, the material for the template comprises a polymeric material. In another embodiment, the material for the template comprises a solvent resistant, low surface energy polymeric material. In still another embodiment, the material for the template comprises a solvent resistant, elastomeric material. In a further embodiment, the template is selected from the group consisting of a material selected from the group consisting of a perfluoropolyether material, a silicone material, a fluoroolefin

material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction.

According to some embodiments, the particle includes a biocompatible material. The biocompatible material can be selected from the group of a poly(ethylene glycol), a poly(lactic acid), a poly(lactic acid-co-glycolic acid), a lactose, a phosphatidylcholine, a polylactide, a polyglycolide, a hydroxypropylcellulose, a wax, a polyester, a polyanhydride, a polyamide, a phosphorous-based polymer, a poly(cyanoacrylate), a polyurethane, a polyorthoester, a polydihydropyran, a polyacetal, a biodegradable polymer, a polypeptide, a hydrogel, a carbohydrate, and combinations thereof. The particle can also include, in some a therapeutic agent, a diagnostic agent, or a linker. In some embodiments, the therapeutic agent is combined with a crosslinked biocompatible component in the particle.

According to some embodiments, the crosslinked biocompatible component is configured to bioresorb over a predetermined time. In other embodiments, the bioresorbable crosslinker includes polymers functionalized with a disulfide group. In some embodiments, the biocompatible component has a crosslink density of less than about 0.50, and in other embodiments, the biocompatible component has a crosslink density of more than about 0.50. According to some embodiments, the biocompatible component is functionalized with a non-biodegradable group and in some embodiments the biocompatible component is functionalized with a biodegradable group. The biodegradable group can be a disulfide group in some embodiments. In one embodiment, the particle is configured to at least partially degrade from reacting with the stimuli. In some embodiments, the stimulus includes a reducing environment, a predetermined pH, a cellular byproduct, or cell component.

In some embodiments, the particle or a component of the particle includes a predetermined charge. In other embodiments, the particle can

include a predetermined zeta potential. In some embodiments, the particle is configured to react to a stimulus. The stimuli can be selected from the group of pH, radiation, oxidation, reduction, ionic strength, temperature, alternating magnetic or electric fields, acoustic forces, ultrasonic forces, time, and combinations thereof. In alternative embodiments, the particle includes a magnetic material. In some alternative embodiments, the composition of the particle further includes a carbon-carbon bond.

In some embodiments, the composition includes a charged particle, a polymer electret, a therapeutic agent, a non-viral gene vector, a viral particle, a polymorph, or a super absorbent polymer. The therapeutic agent can be selected from the group of a drug, an agent, a modifier, a regulator, a therapy, a treatment, and combinations thereof. The composition can also include a therapeutic agent selected from the group of a biologic, a ligand, an oligopeptide, an enzyme, DNA, an oligonucleotide, RNA, siRNA, a cancer treatment, a viral treatment, a bacterial treatment, an auto-immune treatment, a fungal treatment, a psychotherapeutic agent, a cardiovascular drug, a blood modifier, a gastrointestinal drug, a respiratory drug, an antiarthritic drug, a diabetes drug, an anticonvulsant, a bone metabolism regulator, a multiple sclerosis drug, a hormone, a urinary tract agent, an immunosuppressant, an ophthalmic product, a vaccine, a sedative, a sexual dysfunction therapy, an anesthetic, a migraine drug, an infertility agent, a weight control product, and combinations thereof.

In some embodiments, the composition can include a diagnostic selected from the group of an imaging agent, an x-ray agent, an MRI agent, an ultrasound agent, a nuclear agent, a radiotracer, a radiopharmaceutical, an isotope, a contrast agent, a fluorescent tag, a radiolabeled tag, and combinations thereof. In other embodiments, the particle further includes ^{18}F.

In other embodiments, the composition can include a shape selected from the group of substantially non-spherical, substantially viral, substantially bacterial, substantially cellular, substantially a rod, substantially chiral, and combinations thereof. The shape of the particle can be selected from the

group of substantially rod shaped wherein the rod is less than about 200 nm in diameter. In other embodiments, the shape of the particle can be selected from the group of substantially rod shaped wherein the rod is less than about 2 nm in diameter.

According to some embodiments, the composition includes a therapeutic agent or diagnostic agent or linker that is associated with the particle, physically coupled with the particle, chemically coupled with the particle, substantially encompassed within the particle, at least partially encompassed within the particle, or coupled with the exterior of the particle. In some embodiments, the particle can be functionalized with a targeting ligand.

In some embodiments of the composition, the linker is selected from the group of sulfides, amines, carboxylic acids, acid chlorides, alcohols, alkenes, alkyl halides, isocyanates, imidazoles, halides, azides, N-hydroxysuccimidyl (NHS) ester groups, acetylenes, diethylenetriaminepentaacetic acid (DPTA) and combinations thereof. In alternative embodiments, the composition further includes a modifying molecule chemically coupled with the linker. The modifying molecule can be selected from the group of dyes, fluorescence tags, radiolabeled tags, contrast agents, ligands, targeting ligands, peptides, aptamers, antibodies, pharmaceutical agents, proteins, DNA, RNA, siRNA, and fragments thereof.

According to some embodiments, the composition can further include a plurality of particles, where the particles have a substantially uniform mass, are substantially monodisperse, are substantially monodisperse in size or shape, or are substantially monodisperse in surface area. In some embodiments, the plurality of particles have a normalized size distribution of between about 0.80 and about 1.20, between about 0.90 and about 1.10, between about 0.95 and about 1.05, between about 0.99 and about 1.01, between about 0.999 and about 1.001. According to some embodiments, the normalized size distribution is selected from the group of a linear size, a volume, a three dimensional shape, surface area, mass, and shape. In yet other embodiments, the plurality of particles includes particles that are

monodisperse in surface area, volume, mass, three-dimensional shape, or a broadest linear dimension.

In some embodiments, the particle can have a broadest dimension of less than about 50 μm, between about 1 nm and about 10 micron, or between about 5 nm and about 1micron. In some embodiments, the particle has a ratio of surface area to volume greater than that of a sphere.

According to some embodiments, the composition can include a super absorbent polymer selected from the group of polyacrylates, polyacrylic acid, HEMA, neutralized acrylates, sodium acrylate, ammonium acrylate, methacrylates, polyacrylamide, cellulose ethers, poly (ethylene oxide), poly (vinyl alcohol), polysuccinimides, polyacrylonitrile polymers, combinations of the above polymers blended or crosslinked together, combinations of the above polymers having monomers co-polymerized with monomers of another polymer, combinations of the above polymers with starch, and combinations thereof.

According to some embodiments, the present invention includes methods for the fabrication of nanoparticles. According to such methods, a nanoparticle can be fabricated from a liquid material in a recess of a mold, where a contact angle between the liquid material and the mold is configured such that the liquid substantially passively fills the recess, and where the particle has a broadest dimension of less than about 250 micron. In some embodiments, the liquid material forms a meniscus with an edge of the recess and a portion of the resulting particle is configured as a lens defined by the meniscus. In some embodiments, the particle reflects a shape of the recess of the mold from which the particle was fabricated within. According to some embodiments, the method also includes hardening of the material that becomes the particle. In some embodiments, the hardening can be an evaporation or an evaporation of a carrier substance. An evaporation can be evaporation of one or more of the group of water soluble adhesives, acetone soluble adhesives, and organic solvent soluble adhesives.

According to other embodiments, the molds from which particles of the present disclosure are fabricated include low-surface energy polymeric

materials having a surface energy less than about 23 dynes/cm, less than about 19 dynes/cm, less than about 15 dynes/cm, less than about 12 dynes/cm, or less than about 8 dynes/cm.

According to some embodiments, methods of the present invention include attaching a linking group to the particle, wherein the linking group can be selected from a group of sulfides, amines, carboxylic acids, acid chlorides, alcohols, alkenes, alkyl halides, isocyanates, imidazoles, halides, diethylenetriaminepentaacetic acid (DPTA), azides, acetylenes, N-hydroxysuccimidyl (NHS) ester group, and combinations thereof.

In alternative embodiments, a system of particles can be utilized for diagnosis, testing, sampling, administration, packaging, transportation, handling, and the like. In some embodiments, the system includes attaching particles to a substrate, such as a flat smooth surface. In some embodiments, the system further includes a plurality of particles arranged in a two dimensional array on the substrate. In some embodiments, the particle includes an active selected from the group of a drug, an agent, a reactant, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Reference is made to the accompanying drawings in which are shown illustrative embodiments of the presently disclosed subject matter, from which its novel features and advantages will be apparent.

Figures 1A-1D are a schematic representation of an embodiment of the presently disclosed method for preparing a patterned template.

Figures 2A-2F are a schematic representation of the presently disclosed method for forming one or more micro- and/or nanoscale particles.

Figures 3A-3F are a schematic representation of the presently disclosed method for preparing one or more spherical particles.

Figures 4A-4D are a schematic representation of the presently disclosed method for fabricating charged polymeric particles. Fig. 4A represents the electrostatic charging of the molded particle during polymerization or crystallization; Fig. 4B represents a charged nano-disc;

Fig. 4C represents typical random juxtapositioning of uncharged nano-discs; and Fig. 4D represents the spontaneous aggregation of charged nano-discs into chain-like structures.

Figures 5A-5C are a schematic illustration of multilayer particles that can be formed using the presently disclosed soft lithography method.

Figures 6A-6C are a schematic representation of the presently disclosed method for making three-dimensional nanostructures using a soft lithography technique.

Figures 7A-7F are a schematic representation of an embodiment of the presently disclosed method for preparing a multi-dimensional complex structure.

Figures 8A-8E are a schematic representation of the presently disclosed imprint lithography process resulting in a "scum layer".

Figures 9A-9E are a schematic representation of the presently disclosed imprint lithography method, which eliminates the "scum layer" by using a functionalized, non-wetting patterned template and a non-wetting substrate.

Figures 10A-10E are a schematic representation of the presently disclosed solvent-assisted micro-molding (SAMIM) method for forming a pattern on a substrate.

Figure 11 is a scanning electron micrograph of a silicon master including 3-μm arrow-shaped patterns.

Figure 12 is a scanning electron micrograph of a silicon master including 500 nm conical patterns that are <50 nm at the tip.

Figure 13 is a scanning electron micrograph of a silicon master including 200 nm trapezoidal patterns.

Figure 14 is a scanning electron micrograph of 200-nm isolated trapezoidal particles of poly(ethylene glycol) (PEG) diacrylate.

Figure 15 is a scanning electron micrograph of 500-nm isolated conical particles of PEG diacrylate.

Figure 16 is a scanning electron micrograph of 3-μm isolated arrow-shaped particles of PEG diacrylate.

Figure 17 is a scanning electron micrograph of 200-nm x 750-nm x 250-nm rectangular shaped particles of PEG diacrylate.

Figure 18 is a scanning electron micrograph of 200-nm isolated trapezoidal particles of trimethylolpropane triacrylate (TMPTA).

Figure 19 is a scanning electron micrograph of 500-nm isolated conical particles of TMPTA.

Figure 20 is a scanning electron micrograph of 500-nm isolated conical particles of TMPTA, which have been printed using an embodiment of the presently described non-wetting imprint lithography method and harvested mechanically using a doctor blade.

Figure 21 is a scanning electron micrograph of 200-nm isolated trapezoidal particles of poly(lactic acid) (PLA).

Figure 22 is a scanning electron micrograph of 200-nm isolated trapezoidal particles of poly(lactic acid) (PLA), which have been printed using an embodiment of the presently described non-wetting imprint lithography method and harvested mechanically using a doctor blade.

Figure 23 is a scanning electron micrograph of 3-μm isolated arrow-shaped particles of PLA.

Figure 24 is a scanning electron micrograph of 500-nm isolated conical-shaped particles of PLA.

Figure 25 is a scanning electron micrograph of 200-nm isolated trapezoidal particles of poly(pyrrole) (Ppy).

Figure 26 is a scanning electron micrograph of 3-μm arrow-shaped Ppy particles.

Figure 27 is a scanning electron micrograph of 500-nm conical shaped Ppy particles.

Figures 28A-28C are fluorescence confocal micrographs of 200-nm isolated trapezoidal particles of PEG diacrylate that contain fluorescently tagged DNA. Fig. 28A is a fluorescent confocal micrograph of 200 nm trapezoidal PEG nanoparticles which contain 24-mer DNA strands that are tagged with CY-3. Fig. 28B is optical micrograph of the 200-nm isolated trapezoidal particles of PEG diacrylate that contain fluorescently tagged

DNA. Fig. 28C is the overlay of the images provided in Figures 28A and 28B, showing that every particle contains DNA.

Figure 29 is a scanning electron micrograph of fabrication of 200-nm PEG-diacrylate nanoparticles using "double stamping".

Figure 30 is an atomic force micrograph image of 140-nm lines of TMPTA separated by distance of 70 nm that were fabricated using a PFPE mold.

Figures 31A and 31B are a scanning electron micrograph of mold fabrication from electron-beam lithographically generated masters. Fig. 31A is a scanning electron micrograph of silicon/silicon oxide masters of 3 micron arrows. Fig. 31B is a scanning electron micrograph of silicon/silicon oxide masters of 200-nm x 800-nm bars.

Figures 32A and 32B are an optical micrographic image of mold fabrication from photoresist masters. Fig. 32A is a SU-8 master. Fig. 32B is a PFPE-DMA mold templated from a photolithographic master.

Figures 33A and 33B are an atomic force micrograph of mold fabrication from Tobacco Mosaic Virus templates. Fig. 33A is a master. Fig. 33B is a PFPE-DMA mold templated from a virus master.

Figures 34A and 34B are an atomic force micrograph of mold fabrication from block copolymer micelle masters. Fig. 34A is a polystyrene-polyisoprene block copolymer micelle. Fig. 34B is a PFPE-DMA mold templated from a micelle master.

Figures 35A and 35B are an atomic force micrograph of mold fabrication from brush polymer masters. Fig. 35A is a brush polymer master. Fig 35B is a PFPE-DMA mold templated from a brush polymer master.

Figures 36A–36D are schematic representations of one embodiment of a method for functionalizing particles of the presently disclosed subject matter.

Figures 37A–37F are schematic representations of one embodiment of a method of the presently disclosed subject matter for harvesting particles from an article.

Figures 38A–38G are schematic representations of one embodiment of a method of the presently disclosed subject matter for harvesting particles from an article.

Figures 39A–39F are schematic representations of one embodiment of one process of the presently disclosed subject matter for imprint lithography wherein 3-dimensional features are patterned.

Figures 40A–40D schematic representations of one embodiment of one process of the presently disclosed subject matter for harvesting particles from an article.

Figures 41A-41E show a sequence of forming small particles through evaporation according to an embodiment of the presently disclosed subject matter.

Figure 42 shows doxorubicin containing particles after removal from a template according to an embodiment of the presently disclosed subject matter.

Figure 43 shows a structure patterned with nano-cylindrical shapes according to an embodiment of the presently disclosed subject matter.

Figure 44 shows a sequence of molecular imprinting according to an embodiment of the presently disclosed subject matter.

Figure 45 shows a labeled particle associated with a cell according to an embodiment of the presently disclosed subject matter.

Figure 46 shows a labeled particle associated with a cell according to an embodiment of the presently disclosed subject matter.

Figure 47 shows particles fabricated through an open molding technique according to some embodiments of the present invention.

Figure 48 shows a process for coating a seed and seeds coated from the process according to some embodiments of the present invention.

Figure 49 shows a taggant having identifying characteristics according to an embodiment of the present invention.

Figure 50 shows a method of passively introducing a substance to a patterned template according to an embodiment of the present invention.

Figure 51 shows a method of dipping a patterned template to introduce a substance into recesses of the patterned template according to an embodiment of the present invention.

Figure 52 shows a method of flowing a substance across a patterned template surface to introduce the substance into recesses of the patterned template according to an embodiment of the present invention.

Figure 53 shows voltage assisted recess filling according to an embodiment of the present invention.

Figure 54 shows particles formed from methods described herein and released from a mold according to an embodiment of the present invention.

Figure 55 shows further particles formed from methods described herein and released from a mold according to an embodiment of the present invention.

Figure 56 shows introducing a substance to be molded to a patterned template by droplet rolling according to an embodiment of the present invention.

Figure 57 shows wetting angles and mold filling according to an embodiment of the present invention.

Figure 58 shows harvesting of particles according to an embodiment of the present invention.

Figure 59 shows permeability balancing between a mold and substance according to an embodiment of the present invention.

Figure 60 shows a method for harvesting particles with a sacrificial layer according to an embodiment of the present invention.

Figures 61A and 61B show cube-shaped PEG particles fabricated by a dipping method according to an embodiment of the present invention.

Figure 62 shows an SEM micrograph of 2 x 2 x 1 μm positively charged DEDSMA particles according to an embodiment of the present invention.

Figure 63 shows fluorescent micrograph of 2 x 2 x 1 μm positively charged DEDSMA particles according to an embodiment of the present invention.

Figure 64 shows fluorescence micrograph of calcein cargo incorporated into 2 μm DEDSMA particles according to an embodiment of the present invention.

Figure 65 shows 2 x 2 x 1 μm pDNA containing positively charged DEDSMA particles: Top Left: SEM, Top Right: DIC, Bottom Left: Particle-bound Polyflour 570 flourescence, Bottom Right: Fluorescein-labelled control plasmid fluorescence according to an embodiment of the present invention.

Figure 66 shows 2 x 2 x 1 μm pDNA containing positively charged PEG particles: Top Left: SEM, Top Right: DIC, Bottom Left: Particle-bound Polyflour 570 flourescence, Bottom Right: Fluorescein-labelled control plasmid fluorescence according to an embodiment of the present invention.

Figure 67 shows master templates containing 200 nm cylindrical shapes with varying aspect ratios according to an embodiment of the present invention.

Figure 68 shows scanning electron micrograph (at a 45° angle) of harvested neutral PEG-composite 200 nm (aspect ratio = 1:1) particles on the poly(cyanoacrylate) harvesting layer according to an embodiment of the present invention.

Figure 69 shows confocal micrographs of cellular uptake of purified PRINT PEG-composite particles into NIH 3T3 cells – trends in amount of cationic charge according to an embodiment of the present invention.

Figure 70 shows toxicity results obtained from an MTT assay on varying both the amount of cationic charge incorporated into a particle matrix, as well as an effect of particle concentration on cellular uptake according to an embodiment of the present invention.

Figure 71 shows confocal micrographs of cellular uptake of PRINT PEG particles into NIH 3T3 cells while the inserts show harvested particles on medical adhesive layers prior to cellular treatment according to an embodiment of the present invention.

Figure 72 shows a reaction scheme for conjugation of a radioactively labeled moiety to PRINT particles according to an embodiment of the present invention.

Figure 73 shows fabrication of pendant gadolinium PEG particles according to an embodiment of the present invention.

Figure 74 shows formation of a particle containing CDI linker according to an embodiment of the present invention.

Figure 75 shows tethering avidin to a CDI linker according to an embodiment of the present invention.

Figure 76 shows fabrication of PEG particles that target an HER2 receptor according to an embodiment of the present invention.

Figure 77 shows fabrication of PEG particles that target non-Hodgkin's lymphoma according to an embodiment of the present invention.

Figure 78 shows a controlled-release phantom study of 100% and 70% dPEG DOX loaded particles after 36 hour dialysis according to an embodiment of the present invention.

Figure 79A-79C shows particles fabricated by an evaporation process, according to an embodiment of the present invention.

DETAILED DESCRIPTION

The presently disclosed subject matter will now be described more fully hereinafter with reference to the accompanying Examples, in which representative embodiments are shown. The presently disclosed subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the embodiments to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Throughout the specification and claims, a given chemical formula or name shall encompass all optical and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

I. Materials

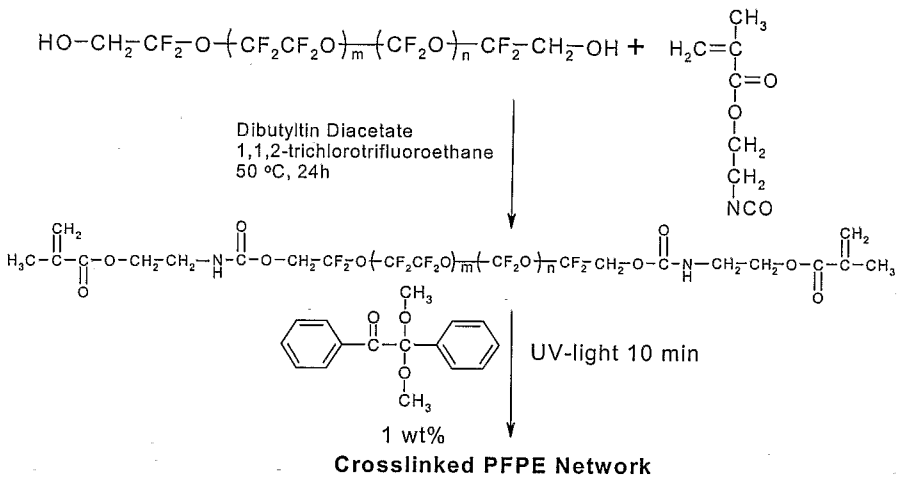
The presently disclosed subject matter broadly describes solvent resistant, low surface energy polymeric materials, derived from casting low viscosity liquid materials onto a master template and then curing the low viscosity liquid materials to generate a patterned template for use in high-resolution soft or imprint lithographic applications, such as micro- and nanoscale replica molding. In some embodiments, the patterned template or mold includes a solvent resistant elastomer-based material, such as but not limited to a fluoropolymer, such as for example, fluorinated elastomer-based materials.

Further, the presently disclosed subject matter describes nano-contact molding of organic materials to generate high fidelity features using an elastomeric mold. Accordingly, the presently disclosed subject matter describes a method for producing free-standing, isolated micro- and nanostructures of virtually any shape using soft or imprint lithography techniques. Representative micro- and nanostructures include but are not limited to micro- and nanoparticles, and micro- and nano-patterned substrates.

The nanostructures described by the presently disclosed subject matter can be used in several applications, including, but not limited to, semiconductor manufacturing, such as molding etch barriers without scum layers for the fabrication of semiconductor devices; crystals; materials for displays; photovoltaics; a solar cell device; optoelectronic devices; routers; gratings; radio frequency identification (RFID) devices; catalysts; fillers and additives; detoxifying agents; etch barriers; atomic force microscope (AFM) tips; parts for nano-machines; the delivery of a therapeutic agent, such as a drug or genetic material; cosmetics; chemical mechanical planarization

(CMP) particles; and porous particles and shapes of virtually any kind that will enable the nanotechnology industry.

Representative solvent resistant elastomer-based materials include but are not limited to fluorinated elastomer-based materials. As used herein, the term "solvent resistant" refers to a material, such as an elastomeric material that neither swells nor dissolves in common hydrocarbon-based organic solvents or acidic or basic aqueous solutions. Representative fluorinated elastomer-based materials include but are not limited to perfluoropolyether (PFPE)-based materials. A photocurable liquid PFPE exhibits desirable properties for soft lithography. A representative scheme for the synthesis and photocuring of functional PFPEs is provided in Scheme 1.



Scheme 1. Synthesis and Photocuring of Functional Perfluoropolyethers.

According to another embodiment, a material according to the presently disclosed subject matter includes one or more of a photo-curable constituent, a thermal-curable constituent, and mixtures thereof. In one embodiment, the photo-curable constituent is independent from the thermal-curable constituent such that the material can undergo multiple cures. A material having the ability to undergo multiple cures is useful, for example, in forming layered devices. For example, a liquid material having photo-

curable and thermal-curable constituents can undergo a first cure to form a first device through, for example, a photocuring process or a thermal curing process. Then the photocured or thermal cured first device can be adhered to a second device of the same material or virtually any material similar thereto that will thermally cure or photocure and bind to the material of the first device. By positioning the first device and second device adjacent one another and subjecting the first and second devices to a thermalcuring or photocuring process, whichever component that was not activated on the first curing can be cured by a subsequent curing step. Thereafter, either the thermalcure constituents of the first device that was left un-activated by the photocuring process or the photocure constituents of the first device that were left un-activated by the first thermal curing, will be activated and bind the second device. Thereby, the first and second devices become adhered together. It will be appreciated by one of ordinary skill in the art that the order of curing processes is independent and a thermal-curing could occur first followed by a photocuring or a photocuring could occur first followed by a thermal curing.

According to yet another embodiment, multiple thermo-curable constituents can be included in the material such that the material can be subjected to multiple independent thermal-cures. For example, the multiple thermo-curable constituents can have different activation temperature ranges such that the material can undergo a first thermal-cure at a first temperature range and a second thermal-cure at a second temperature range.

According to yet another embodiment, multiple independent photo-curable constituents can be included in the material such that the material can be subjected to multiple independent photo-cures. For example, the multiple photo-curable constituents can have different activation wavelength ranges such that the material can undergo a first photo-cure at a first wavelength range and a second photo-cure at a second wavelength range.

According to some embodiments, curing of a polymer or other material, solution, dispersion, or the like includes hardening, such as for example by chemical reaction like a polymerization, phase change, a melting

transition (e.g. mold above the melting point and cool after molding to harden), evaporation, combinations thereof, and the like.

Additional schemes for the synthesis of functional perfluoropolyethers are provided in Examples 7.1 through 7.6.

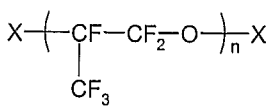
According to one embodiment this PFPE material has a surface energy below about 30 mN/m. According to another embodiment the surface energy of the PFPE is between about 10 mN/m and about 20 mN/m. According to a another embodiment, the PFPE has a low surface energy of between about 12 mN/m and about 15 mN/m. The PFPE is non-toxic, UV transparent, and highly gas permeable; and cures into a tough, durable, highly fluorinated elastomer with excellent release properties and resistance to swelling. The properties of these materials can be tuned over a wide range through the judicious choice of additives, fillers, reactive co-monomers, and functionalization agents. Such properties that are desirable to modify, include, but are not limited to, modulus, tear strength, surface energy, permeability, functionality, mode of cure, solubility and swelling characteristics, and the like. The non-swelling nature and easy release properties of the presently disclosed PFPE materials allows for nanostructures to be fabricated from virtually any material. Further, the presently disclosed subject matter can be expanded to large scale rollers or conveyor belt technology or rapid stamping that allow for the fabrication of nanostructures on an industrial scale.

In some embodiments, the patterned template includes a solvent resistant, low surface energy polymeric material derived from casting low viscosity liquid materials onto a master template and then curing the low viscosity liquid materials to generate a patterned template. In some embodiments, the patterned template includes a solvent resistant elastomeric material.

In some embodiments, at least one of the patterned template and substrate includes a material selected from the group including a perfluoropolyether material, a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated

epoxy resin, and a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction.

In some embodiments, the perfluoropolyether material includes a backbone structure selected from the group including: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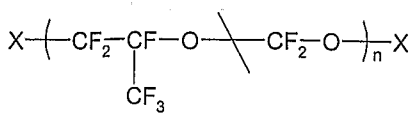
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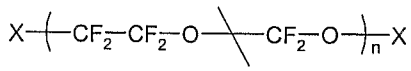
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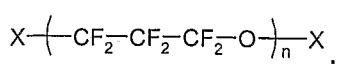
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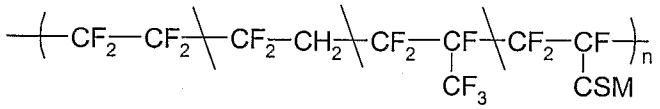
wherein X is present or absent, and when present includes an endcapping group.

In some embodiments, the fluoroolefin material is selected from the group including: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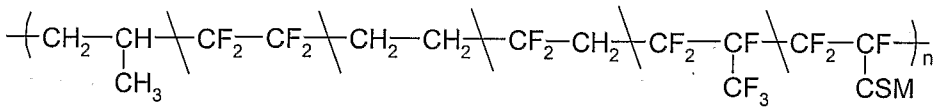
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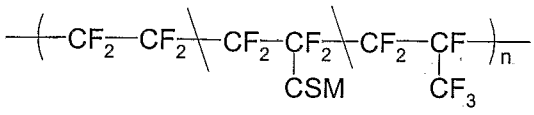
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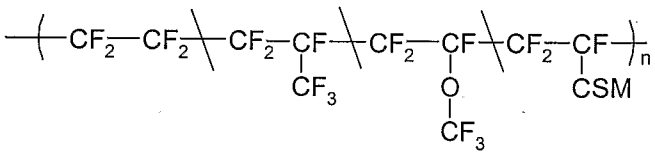
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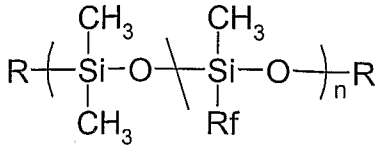
wherein CSM includes a cure site monomer.

In some embodiments, the fluoroolefin material is made from monomers which include tetrafluoroethylene, vinylidene fluoride, hexafluoropropylene, 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole, a functional fluoroolefin, functional acrylic monomer, and a functional methacrylic monomer.

In some embodiments, the silicone material includes a fluoroalkyl functionalized polydimethylsiloxane (PDMS) having the following structure:

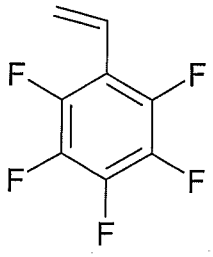


wherein:

R is selected from the group including an acrylate, a methacrylate, and a vinyl group; and

Rf includes a fluoroalkyl chain.

In some embodiments, the styrenic material includes a fluorinated styrene monomer selected from the group including:



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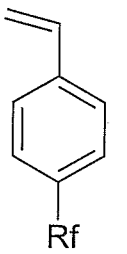

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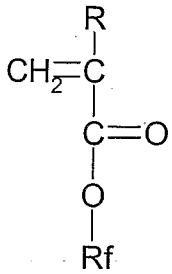
wherein Rf includes a fluoroalkyl chain.

In some embodiments, the acrylate material includes a fluorinated acrylate or a fluorinated methacrylate having the following structure:



wherein:

R is selected from the group including H, alkyl, substituted alkyl, aryl, and substituted aryl; and

Rf includes a fluoroalkyl chain.

In some embodiments, the triazine fluoropolymer includes a fluorinated monomer. In some embodiments, the fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction includes a functionalized olefin. In some embodiments, the functionalized olefin includes a functionalized cyclic olefin.

In some embodiments, the fluoropolymer is further subjected to a fluorine treatment after curing. In some embodiments, the fluoropolymer is subjected to elemental fluorine after curing.

In some embodiments, at least one of the patterned template and the substrate has a surface energy lower than about 18 mN/m. In some embodiments, at least one of the patterned template and the substrate has a surface energy lower than about 15 mN/m. According to a further embodiment the patterned template and/or the substrate has a surface energy between about 10 mN/m and about 20 mN/m. According to another embodiment, the patterned template and/or the substrate has a low surface energy of between about 12 mN/m and about 15 mN/m.

From a property point of view, the exact properties of these molding materials can be adjusted by adjusting the composition of the ingredients used to make the materials. In particular the modulus can be adjusted from low (approximately 1 MPa) to multiple GPa.

II. Formation of Isolated Micro- and/or Nanoparticles

In some embodiments, the presently disclosed subject matter provides a method for making isolated micro- and/or nanoparticles. In some embodiments, the process includes initially forming a patterned substrate. Turning now to Figure 1A, a patterned master **100** is provided. Patterned master **100** includes a plurality of non-recessed surface areas **102** and a plurality of recesses **104**. In some embodiments, patterned master **100** includes an etched substrate, such as a silicon wafer, which is etched in the desired pattern to form patterned master **100**.

Referring now to Figure 1B, a liquid material **106**, for example, a liquid fluoropolymer composition, such as a PFPE-based precursor, is then poured onto patterned master **100**. Liquid material **106** is treated by treating process $\mathbf{T_r}$, for example exposure to UV light, actinic radiation, or the like, thereby forming a treated liquid material **108** in the desired pattern.

Referring now to Figures 1C and 1D, a force $\mathbf{F_r}$ is applied to treated liquid material **108** to remove it from patterned master **100**. As shown in

Figures 1C and 1D, treated liquid material **108** includes a plurality of recesses **110**, which are mirror images of the plurality of non-recessed surface areas **102** of patterned master **100**. Continuing with Figures 1C and 1D, treated liquid material **108** includes a plurality of first patterned surface areas **112**, which are mirror images of the plurality of recesses **104** of patterned master **100**. Treated liquid material **108** can now be used as a patterned template for soft lithography and imprint lithography applications. Accordingly, treated liquid material **108** can be used as a patterned template for the formation of isolated micro- and nanoparticles. For the purposes of Figures 1A-1D, 2A-2E, and 3A-3F, the numbering scheme for like structures is retained throughout, where possible.

Referring now to Figure 2A, in some embodiments, a substrate **200**, for example, a silicon wafer, is treated or is coated with a non-wetting material **202**. In some embodiments, non-wetting material **202** includes an elastomer (such a solvent resistant elastomer, including but not limited to a PFPE elastomer) that can be further exposed to UV light and cured to form a thin, non-wetting layer on the surface of substrate **200**. Substrate **200** also can be made non-wetting by treating substrate **200** with non-wetting agent **202**, for example a small molecule, such as an alkyl- or fluoroalkyl-silane, or other surface treatment. Continuing with Figure 2A, a droplet **204** of a curable resin, a monomer, or a solution from which the desired particles will be formed is then placed on the coated substrate **200**.

Referring now to Figure 2A and Figure 2B, patterned template **108** (as shown in Figure 1D) is then contacted with droplet **204** of a particle precursor material so that droplet **204** fills the plurality of recessed areas **110** of patterned template **108**.

Referring now to Figures 2C and 2D, a force $\mathbf{F_a}$ is applied to patterned template **108**. While not wishing to be bound by any particular theory, once force $\mathbf{F_a}$ is applied, the affinity of patterned template **108** for non-wetting coating or surface treatment **202** on substrate **200** in combination with the non-wetting behavior of patterned template **108** and surface treated or coated substrate **200** causes droplet **204** to be excluded from all areas

except for recessed areas **110**. Further, in embodiments essentially free of non-wetting or low wetting material **202** with which to sandwich droplet **204**, a "scum" layer forms that interconnects the objects being stamped.

Continuing with Figures 2C and 2D, the particle precursor material filling recessed areas **110**, *e.g.*, a resin, monomer, solvent, combinations thereof, or the like, is then treated by a treating process $\mathbf{T_r}$, *e.g.*, photocured, UV-light treated, or actinic radiation treated, through patterned template **108** or thermally cured while under pressure, to form a plurality of micro- and/or nanoparticles **206**. In some embodiments, a material, including but not limited to a polymer, an organic compound, or an inorganic compound, can be dissolved in a solvent, patterned using patterned template **108**, and the solvent can be released.

Continuing with Figures 2C and 2D, once the material filling recessed areas **110** is treated, patterned template **108** is removed from substrate **200**. Micro- and/or nanoparticles **206** are confined to recessed areas **110** of patterned template **108**. In some embodiments, micro- and/or nanoparticles **206** can be retained on substrate **200** in defined regions once patterned template **108** is removed. This embodiment can be used in the manufacture of semiconductor devices where essentially scum-layer free features could be used as etch barriers or as conductive, semiconductive, or dielectric layers directly, mitigating or reducing the need to use traditional and expensive photolithographic processes.

Referring now to Figures 2D and 2E, micro- and/or nanoparticles **206** can be removed from patterned template **108** to provide freestanding particles by a variety of methods, which include but are not limited to: (1) applying patterned template **108** to a surface that has an affinity for the particles **206**; (2) deforming patterned template **108**, or using other mechanical methods, including sonication, in such a manner that the particles **206** are naturally released from patterned template **108**; (3) swelling patterned template **108** reversibly with supercritical carbon dioxide or another solvent that will extrude the particles **206**; (4) washing patterned template **108** with a solvent that has an affinity for the particles **206** and will

wash them out of patterned template **108**; (5) applying patterned template **108** to a liquid that when hardened physically entraps particles **206**; (6) applying patterned template **108** to a material that when hardened has a chemical and/or physical interaction with particles **206**.

In some embodiments, the method of producing and harvesting particles includes a batch process. In some embodiments, the batch process is selected from one of a semi-batch process and a continuous batch process. Referring now to Figure 2F, an embodiment of the presently disclosed subject matter wherein particles **206** are produced in a continuous process is schematically presented. An apparatus **199** is provided for carrying out the process. Indeed, while Figure 2F schematically presents a continuous process for particles, apparatus **199** can be adapted for batch processes, and for providing a pattern on a substrate continuously or in batch, in accordance with the presently disclosed subject matter and based on a review of the presently disclosed subject matter by one of ordinary skill in the art.

Continuing, then, with Figure 2F, droplet **204** of liquid material is applied to substrate **200'** via reservoir **203**. Substrate **200'** can be coated or not coated with a non-wetting agent. Substrate **200'** and pattern template **108'** are placed in a spaced relationship with respect to each other and are also operably disposed with respect to each other to provide for the conveyance of droplet **204** between patterned template **108'** and substrate **200'**. Conveyance is facilitated through the provision of pulleys **208**, which are in operative communication with controller **201**. By way of representative non-limiting examples, controller **201** can include a computing system, appropriate software, a power source, a radiation source, and/or other suitable devices for controlling the functions of apparatus **199**. Thus, controller **201** provides for power for and other control of the operation of pulleys **208** to provide for the conveyance of droplet **204** between patterned template **108'** and substrate **200'**. Particles **206** are formed and treated between substrate **200'** and patterned template **108'** by a treating process T_R, which is also controlled by controller **201**. Particles **206** are collected in an inspecting device **210**, which is also controlled by controller **201**.

Inspecting device **210** provides for one of inspecting, measuring, and both inspecting and measuring one or more characteristics of particles **206**. Representative examples of inspecting devices **210** are disclosed elsewhere herein.

By way of further exemplifying embodiments of particle harvesting methods described herein, reference is made to Figures 37A–37F and Figures 38A–38G. In Figures 37A–37C and Figures 38A–38C particles which are produced in accordance with embodiments described herein remain in contact with an article **3700**, **3800**. The article **3700, 3800** can have an affinity for particles **3705** and **3805**, respectively, or the particles can simple remain in the mold recesses following fabrication of the particles therein. In one embodiment, article **3700** is a patterned template or mold as described herein and article **3800** is a substrate as described herein.

Referring now to Figures 37D–37 F and Figures 38D–38G, material **3720**, **3820** having an affinity for particles **3705**, **3805** is put into contact with particles **3705**, **3805** while particles **3705**, **3805** remain in communication with articles **3700**, **3800**. In the embodiment of Fig. 37D, material **3720** is disposed on surface **3710**. In the embodiment of Fig. 38D, material **3820** is applied directly to article **3800** having particles **3820**. As illustrated in Figures 37E, 38D in some embodiments, article **3700**, **3800** is put in engaging contact with material **3720**, **3820**. In one embodiment material **3720**, **3820** is thereby dispersed to coat at least a portion of substantially all of particles **3705**, **3805** while particles **3705**, **3805** are in communication with article **3700**, **3800** (*e.g.*, a patterned template). In one embodiment, illustrated in Figures 37F and 38F, articles **3700**, **3800** are substantially disassociated with material **3720**, **3820**. In one embodiment, material **3720**, **3820** has a higher affinity for particles **3705**, **3805** than any affinity between article **3700**, **3800** and particles **3705**, **3805**. In Figures 37F and 38F, the disassociation of article **3700**, **3800** from material **3720**, **3820** thereby releases particles **3705**, **3805** from article **3700**, **3800** leaving particles **3705**, **3805** associated with material **3720**, **3820**.

In one embodiment material **3720**, **3820** has an affinity for particles **3705** and **3805**. For example, material **3720**, **3820** can include an adhesive or sticky surface such that when it is applied to particles **3705** and **3805** the particles remain associated with material **3720, 3820** rather than with article **3700**, **3800**. In other embodiments, material **3720**, **3820** undergoes a transformation after it is brought into contact with article 3700, 3800. In some embodiments that transformation is an inherent characteristic of material **3705**, **3805**. In other embodiments, material **3705**, **3805** is treated to induce the transformation. For example, in one embodiment material **3720**, **3820** is an epoxy that hardens after it is brought into contact with article **3700**, **3800**. Thus, when article **3700**, **3800** is pealed away from the hardened epoxy, particles **3705**, **3805** remain engaged with the epoxy and not article **3700**, **3800**. In other embodiments, material **3720**, **3820** is water that is cooled to form ice. Thus, when article **3700**, **3800** is stripped from the ice, particles **3705**, **3805** remain in communication with the ice and not article **3700**, **3800**. In one embodiment, the particle in connection with ice can be melted to create a liquid with a concentration of particles **3705**, **3805**. In some embodiments, material **3705**, **3805** include, without limitation, one or more of a carbohydrate, an epoxy, a wax, polyvinyl alcohol, polyvinyl pyrrolidone, polybutyl acrylate, a polycyano acrylate and polymethyl methacrylate. In some embodiments, material **3720**, **3820** includes, without limitation, one or more of liquids, solutions, powders, granulated materials, semi-solid materials, suspensions, combinations thereof, or the like.

Thus, in some embodiments, the method for forming and harvesting one or more particles includes:

(a) providing a patterned template and a substrate, wherein the patterned template includes a first patterned template surface having a plurality of recessed areas formed therein;

(b) disposing a volume of liquid material in or on at least one of:

(i) the first patterned template surface;

(ii) the plurality of recessed areas; and/or

(iii) a substrate; and

(c) forming one or more particles by one of:

(i) contacting the patterned template surface with the substrate and treating the liquid material; and

(ii) treating the liquid material.

In some embodiments, the plurality of recessed areas includes a plurality of cavities. In some embodiments, the plurality of cavities includes a plurality of structural features. In some embodiments, the plurality of structural features have a dimension ranging from about 10 microns to about 1 nanometer in size. In some embodiments, the plurality of structural features have a dimension ranging from about 1 micron to about 100 nm in size. In some embodiments, the plurality of structural features have a dimension ranging from about 100 nm to about 1 nm in size. In some embodiments, the plurality of structural features have a dimension in both the horizontal and vertical plane.

In some embodiments, the method includes positioning the patterned template and the substrate in a spaced relationship to each other such that the patterned template surface and the substrate face each other in a predetermined alignment.

In some embodiments, the disposing of the volume of liquid material on one of the patterned template or the substrate is regulated by a spreading process. In some embodiments, the spreading process includes:

(a) disposing a first volume of liquid material on one of the patterned template and the substrate to form a layer of liquid material thereon; and

(b) drawing an implement across the layer of liquid material to:

(i) remove a second volume of liquid material from the layer of liquid material on the one of the patterned template and the substrate; and

(ii) leave a third volume of liquid material on the one of the patterned template and the substrate.

In some embodiments, an article is contacted with the layer of liquid material and a force is applied to the article to thereby remove the liquid material from the one of the patterned material and the substrate. In some

embodiments, the article is selected from the group including a roller, a "squeegee" blade type device, a nonplanar polymeric pad, combinations thereof, or the like. In some embodiments, the liquid material is removed by some other mechanical apparatus.

In some embodiments, the contacting of the patterned template surface with the substrate forces essentially all of the disposed liquid material from between the patterned template surface and the substrate.

In some embodiments, the treating of the liquid material includes a process selected from the group including a thermal process, a phase change, an evaporative process, a photochemical process, and a chemical process.

In some embodiments as described in detail herein below, the method further includes:

(a) reducing the volume of the liquid material disposed in the plurality of recessed areas by one of:

(i) applying a contact pressure to the patterned template surface; and

(ii) allowing a second volume of the liquid to evaporate or permeate through the template;

(b) removing the contact pressure applied to the patterned template surface;

(c) introducing gas within the recessed areas of the patterned template surface;

(d) treating the liquid material to form one or more particles within the recessed areas of the patterned template surface; and

(e) releasing the one or more particles.

In some embodiments, the releasing of the one or more particles is performed by at least one of:

(a) applying the patterned template to a substrate, wherein the substrate has an affinity for the one or more particles;

(b) deforming the patterned template such that the one or more particles is released from the patterned template;

(c) swelling the patterned template with a first solvent to extrude the one or more particles;

(d) washing the patterned template with a second solvent, wherein the second solvent has an affinity for the one or more particles;

(e) applying a mechanical force to the one or more particles;

(f) applying the patterned template to a liquid that when hardened physically entraps particles; and

(g) applying the patterned template to a material that when hardened has a chemical and/or physical interaction with particles.

In some embodiments, the mechanical force is applied by contacting one of a doctor blade and a brush with the one or more particles. In some embodiments, the mechanical force is applied by ultrasonics, megasonics, electrostatics, or magnetics means.

In some embodiments, the method includes harvesting or collecting the particles. In some embodiments, the harvesting or collecting of the particles includes a process selected from the group including scraping with a doctor blade, a brushing process, a dissolution process, an ultrasound process, a megasonics process, an electrostatic process, and a magnetic process. In some embodiments, the harvesting or collecting of the particles includes applying a material to at least a portion of a surface of the particle wherein the material has an affinity for the particles. In some embodiments, the material includes an adhesive or sticky surface. In some embodiments, the material includes, without limitation, one or more of a carbohydrate, an epoxy, a wax, polyvinyl alcohol, polyvinyl pyrrolidone, polybutyl acrylate, a polycyano acrylate, a polyhydroxyethyl methacrylate, a polyacrylic acid and polymethyl methacrylate. In some embodiments, the harvesting or collecting of the particles includes cooling water to form ice (e.g., in contact with the particles). In some embodiments, the presently disclosed subject matter

describes a particle or plurality of particles formed by the methods described herein. In some embodiments, the plurality of particles includes a plurality of monodisperse particles. According to some embodiments, monodisperse particles are particles that have a physical characteristic that falls within a normalized size distribution tolerance limit. According to some embodiments, the size characteristic, or paramater, that is analyzed is the surface area, circumference, a linear dimension, mass, volume, three dimensional shape, shape, or the like.

According to some embodiments, the particles have a normalized size distribution of between about 0.80 and about 1.20, between about 0.90 and about 1.10, between about 0.95 and about 1.05, between about 0.99 and about 1.01, between about 0.999 and about 1.001, combinations thereof, and the like. Furthermore, in other embodiments the particles have a monodispersity. According to some embodiments, dispersity is calculated by averaging a dimension of the particles. In some embodiments, the dispersity is based on, for example, surface area, length, width, height, mass, volume, porosity, combinations thereof, and the like.

In some embodiments, the particle or plurality of particles is selected from the group including a semiconductor device, a crystal, a drug delivery vector, a gene delivery vector, a disease detecting device, a disease locating device, a photovoltaic device, a porogen, a cosmetic, an electret, an additive, a catalyst, a sensor, a detoxifying agent, an abrasive, such as a CMP, a micro-electro-mechanical system (MEMS), a cellular scaffold, a taggant, a pharmaceutical agent, and a biomarker. In some embodiments, the particle or plurality of particles include a freestanding structure.

According to some embodiments, a material can be incorporated into a particle composition or a particle according to the present invention, to treat or diagnose diseases including, but not limited to, Allergies; Anemia; Anxiety Disorders; Autoimmune Diseases; Back and Neck Injuries; Birth Defects; Blood Disorders; Bone Diseases; Cancers; Circulation Diseases; Dental Conditions; Depressive Disorders; Digestion and Nutrition Disorders; Dissociative Disorders; Ear Conditions; Eating Disorders; Eye Conditions; Foodborne Illnesses; Gastrointestinal Diseases; Genetic Disorders; Heart

Diseases; Heat and Sun Related Conditions; Hormonal Disorders; Impulse Control Disorders; Infectious Diseases; Insect Bites and Stings; Institutes; Kidney Diseases; Leukodystrophies; Liver Diseases; Mental Health Disorders; Metabolic Diseases; Mood Disorders; Neurological Disorders; Organizations; Personality Disorders; Phobias; Pregnancy Complications; Prion Diseases; Prostate Diseases; Registries; Respiratory Diseases; Sexual Disorders; Sexually Transmitted Diseases; Skin Conditions; Sleep Disorders; Speech-Language Disorders; Sports Injuries; Thyroid Diseases; Tropical Diseases; Vestibular Disorders; Waterborne Illnesses; and other diseases such as found at: http://www.mic.ki.se/Diseases/Alphalist.html, which is incorporated herein by reference in its entirety including each reference cited therein.

Further, in some embodiments, the presently disclosed subject matter describes a method of fabricating isolated liquid objects, the method including (a) contacting a liquid material with the surface of a first low surface energy material; (b) contacting the surface of a second low surface energy material with the liquid, wherein at least one of the surfaces of either the first or second low surface energy material is patterned; (c) sealing the surfaces of the first and the second low surface energy materials together; and (d) separating the two low surface energy materials to produce a replica pattern including liquid droplets.

In some embodiments, the liquid material includes poly(ethylene glycol)-diacrylate. In some embodiments, the low surface energy material includes perfluoropolyether-diacrylate. In some embodiments, a chemical process is used to seal the surfaces of the first and the second low surface energy materials. In some embodiments, a physical process is used to seal the surfaces of the first and the second low surface energy materials. In some embodiments, one of the surfaces of the low surface energy material is patterned. In some embodiments, one of the surfaces of the low surface energy material is not patterned.

In some embodiments, the method further includes using the replica pattern composed of liquid droplets to fabricate other objects. In some embodiments, the replica pattern of liquid droplets is formed on the surface

of the low surface energy material that is not patterned. In some embodiments, the liquid droplets undergo direct or partial solidification. In some embodiments, the liquid droplets undergo a chemical transformation. In some embodiments, the solidification of the liquid droplets or the chemical transformation of the liquid droplets produces freestanding objects. In some embodiments, the freestanding objects are harvested. In some embodiments, the freestanding objects are bonded in place. In some embodiments, the freestanding objects are directly solidified, partially solidified, or chemically transformed.

In some embodiments, the liquid droplets are directly solidified, partially solidified, or chemically transformed on or in the patterned template to produce objects embedded in the recesses of the patterned template. In some embodiments, the embedded objects are harvested. In some embodiments, the embedded objects are bonded in place. In some embodiments, the embedded objects are used in other fabrication processes.

In some embodiments, the replica pattern of liquid droplets is transferred to other surfaces. In some embodiments, the transfer takes place before the solidification or chemical transformation process. In some embodiments, the transfer takes place after the solidification or chemical transformation process. In some embodiments, the surface to which the replica pattern of liquid droplets is transferred is selected from the group including a non-low surface energy surface, a low surface energy surface, a functionalized surface, and a sacrificial surface. In some embodiments, the method produces a pattern on a surface that is essentially free of one or more scum layers. In some embodiments, the method is used to fabricate semiconductors and other electronic and photonic devices or arrays. In some embodiments, the method is used to create freestanding objects. In some embodiments, the method is used to create three-dimensional objects using multiple patterning steps. In some embodiments, the isolated or patterned object includes materials selected from the group including organic, inorganic, polymeric, and biological materials. In some

embodiments, a surface adhesive agent is used to anchor the isolated structures on a surface.

In some embodiments, the liquid droplet arrays or solid arrays on patterned or non-patterned surfaces are used as regiospecific delivery devices or reaction vessels for additional chemical processing steps. In some embodiments, the additional chemical processing steps are selected from the group including printing of organic, inorganic, polymeric, biological, and catalytic systems onto surfaces; synthesis of organic, inorganic, polymeric, biological materials; and other applications in which localized delivery of materials to surfaces is desired. Applications of the presently disclosed subject matter include, but are not limited to, micro and nanoscale patterning or printing of materials. In some embodiments, the materials to be patterned or printed are selected from the group including surface-binding molecules, inorganic compounds, organic compounds, polymers, biological molecules, nanoparticles, viruses, biological arrays, and the like.

In some embodiments, the applications of the presently disclosed subject matter include, but are not limited to, the synthesis of polymer brushes, catalyst patterning for CVD carbon nanotube growth, cell scaffold fabrication, the application of patterned sacrificial layers, such as etch resists, and the combinatorial fabrication of organic, inorganic, polymeric, and biological arrays.

In some embodiments, non-wetting imprint lithography, and related techniques, are combined with methods to control the location and orientation of chemical components within an individual object. In some embodiments, such methods improve the performance of an object by rationally structuring the object so that it is optimized for a particular application. In some embodiments, the method includes incorporating biological targeting agents into particles for drug delivery, vaccination, and other applications. In some embodiments, the method includes designing the particles to include a specific biological recognition motif. In some embodiments, the biological recognition motif includes biotin/avidin and/or other proteins.

In some embodiments, the method includes tailoring the chemical composition of these materials and controlling the reaction conditions, whereby it is then possible to organize the biorecognition motifs so that the efficacy of the particle is optimized. In some embodiments, the particles are designed and synthesized so that recognition elements are located on the surface of the particle in such a way to be accessible to cellular binding sites, wherein the core of the particle is preserved to contain bioactive agents, such as therapeutic molecules. In some embodiments, a non-wetting imprint lithography method is used to fabricate the objects, wherein the objects are optimized for a particular application by incorporating functional motifs, such as biorecognition agents, into the object composition. In some embodiments, the method further includes controlling the microscale and nanoscale structure of the object by using methods selected from the group including self-assembly, stepwise fabrication procedures, reaction conditions, chemical composition, crosslinking, branching, hydrogen bonding, ionic interactions, covalent interactions, and the like. In some embodiments, the method further includes controlling the microscale and nanoscale structure of the object by incorporating chemically organized precursors into the object. In some embodiments, the chemically organized precursors are selected from the group including block copolymers and core-shell structures.

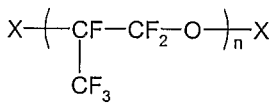
In some embodiments, a non-wetting imprint lithography technique is scalable and offers a simple, direct route to particle fabrication without the use of self-assembled, difficult to fabricate block copolymers and other systems.

II.A. Materials of the Patterned Template and Substrate

In some embodiments of the method for forming one or more particles, the patterned template includes a solvent resistant, low surface energy polymeric material derived from casting low viscosity liquid materials onto a master template and then curing the low viscosity liquid materials to generate a patterned template. In some embodiments, the patterned template includes a solvent resistant elastomeric material.

In some embodiments, at least one of the patterned template and substrate includes a material selected from the group including a perfluoropolyether material, a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction.

In some embodiments, the perfluoropolyether material includes a backbone structure selected from the group including: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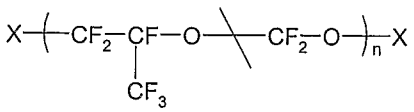
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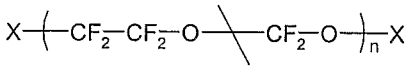
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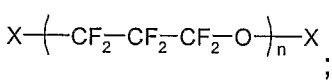
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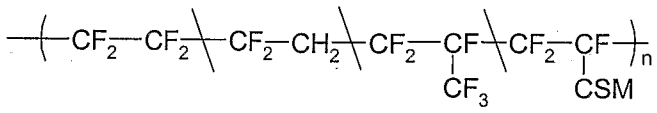
wherein X is present or absent, and when present includes an endcapping group.

In some embodiments, the fluoroolefin material is selected from the group including: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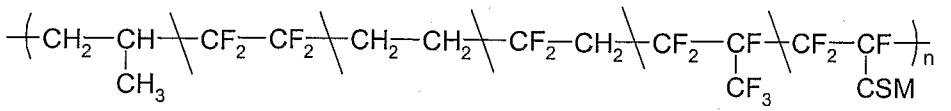
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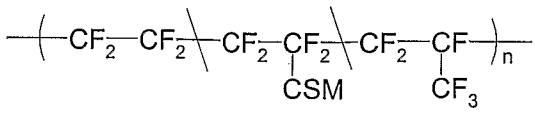
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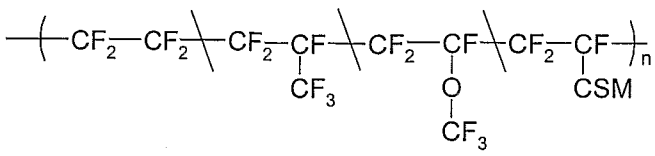
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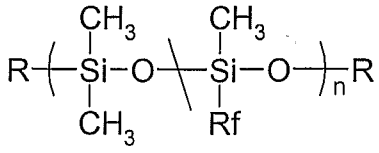
wherein CSM includes a cure site monomer.

In some embodiments, the fluoroolefin material is made from monomers which include tetrafluoroethylene, vinylidene fluoride, hexafluoropropylene, 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole, a

functional fluoroolefin, functional acrylic monomer, and a functional methacrylic monomer.

In some embodiments, the silicone material includes a fluoroalkyl functionalized polydimethylsiloxane (PDMS) having the following structure:

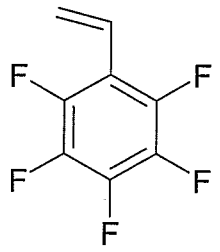


wherein:

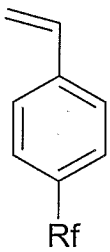
R is selected from the group including an acrylate, a methacrylate, and a vinyl group; and

Rf includes a fluoroalkyl chain.

In some embodiments, the styrenic material includes a fluorinated styrene monomer selected from the group including:



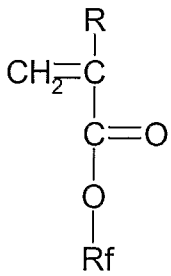
and



,

wherein Rf includes a fluoroalkyl chain.

In some embodiments, the acrylate material includes a fluorinated acrylate or a fluorinated methacrylate having the following structure:



wherein:

R is selected from the group including H, alkyl, substituted alkyl, aryl, and substituted aryl; and

Rf includes a fluoroalkyl chain.

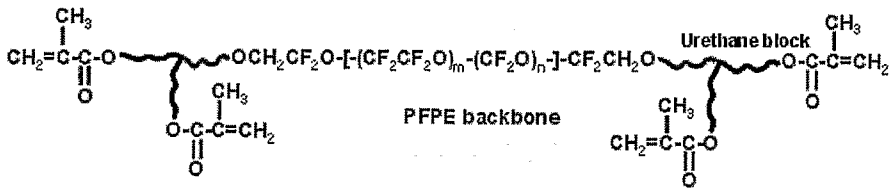
In some embodiments, the triazine fluoropolymer includes a fluorinated monomer. In some embodiments, the fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction includes a functionalized olefin. In some embodiments, the functionalized olefin includes a functionalized cyclic olefin.

In some embodiments, at least one of the patterned template and the substrate has a surface energy lower than 18 mN/m. In some embodiments, at least one of the patterned template and the substrate has a surface energy lower than 15 mN/m. According to a further embodiment the patterned template and/or the substrate has a surface energy between about 10 mN/m and about 20 mN/m. According to another, the patterned template and/or the substrate has a low surface energy of between about 12 mN/m and about 15 mN/m.

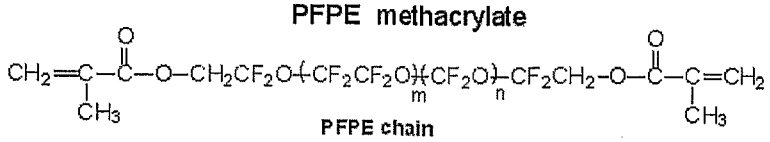
In some embodiments, the substrate is selected from the group including a polymer material, an inorganic material, a silicon material, a quartz material, a glass material, and surface treated variants thereof. In some embodiments, the substrate includes a patterned area.

According to an alternative embodiment, the PFPE material includes a urethane block as described and shown in the following structures:

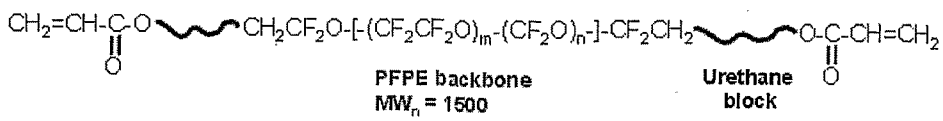
PFPE urethane tetrafunctional methacrylate



PFPE methacrylate



PFPE urethane acrylate



According to an embodiment of the presently disclosed subject matter, PFPE urethane tetrafunctional methacrylate materials, such as the above described material, can be used as the materials and methods of the presently disclosed subject matter or can be used in combination with other materials and methods described herein.

In some embodiments, the patterned template includes a patterned template formed by a replica molding process. In some embodiments, the replica molding process includes: providing a master template; contacting a liquid material with the master template; and curing the liquid material to form a patterned template.

In some embodiments, the master template includes, without limitation, one or more of a template formed from a lithography process, a naturally occurring template, combinations thereof, or the like. In some embodiments, the natural template is selected from one of a biological structure and a self-assembled structure. In some embodiments, the one of a biological structure and a self-assembled structure is selected from the group including a naturally occurring crystal, an enzyme, a virus, a protein, a micelle, and a tissue surface.

In some embodiments, the method includes modifying the patterned template surface by a surface modification step. In some embodiments, the surface modification step is selected from the group including a plasma treatment, a chemical treatment, and an adsorption process. In some embodiments, the adsorption process includes adsorbing molecules selected from the group including a polyelectrolyte, a poly(vinylalcohol), an alkylhalosilane, and a ligand.

II.B. Micro and Nano Particles

According to some embodiments of the presently disclosed subject matter, a particle is formed that has a shape corresponding to a mold (e.g., the particle has a shape reflecting the shape of the mold within which the particle was formed) having a desired shape and is less than about 100 μm in a given dimension (*e.g.* minimum, intermediate, or maximum dimension). In some embodiments, the particle is a nano-scale particle. According to some embodiments, the nano-scale particle has a dimension, such as a

diameter or linear measurement that is less than 500 micron. The dimension can be measured across the largest portion of the particle that corresponds to the parameter being measured. In other embodiments, the dimension is less than 250 micron. In other embodiments, the dimension is less than 100 micron. In other embodiments, the dimension is less than 50 micron. In other embodiments, the dimension is less than 10 micron. In other embodiments, the dimension is between 1 nm and 1,000 nm. In some embodiments, the dimension is less than 1,000 nm. In other embodiments, the dimension is between 1 nm and 500 nm. In yet other embodiments, the dimension is between 1 nm and 100 nm. The particle can be of an organic material or an inorganic material and can be one uniform compound or component or a mixture of compounds or components. In some embodiments, an organic material molded with the materials and methods of the present invention includes a material that includes a carbon molecule. According to some embodiments, the particle can be of a high molecular weight material. According to some embodiments, a particle is composed of a matrix that has a predetermined surface energy. In some embodiments, the material that forms the particle includes more than about 50 percent liquid. In some embodiments, the material that forms the particle includes less than about 50 percent liquid. In some embodiments, the material that forms the particle includes less than about 10 percent liquid.

In some embodiments, the particle includes a therapeutic or diagnostic agent coupled with the particle. The therapeutic or diagnostic agent can be physically coupled or chemically coupled with the particle, encompassed within the particle, at least partially encompassed within the particle, coupled to the exterior of the particle, combinations thereof, and the like. The therapeutic agent can be a drug, a biologic, a ligand, an oligopeptide, a cancer treating agent, a viral treating agent, a bacterial treating agent, a fungal treating agent, combinations thereof, or the like.

According to some embodiments, the particle is hydrophilic such that the particle avoids clearance by biological organism, such as a human.

According to other embodiments, the particle can be substantially coated. The coating, for example, can be a sugar based coating where the

sugar is preferably a glucose, sucrose, maltose, derivatives thereof, combinations thereof, or the like.

In yet other embodiments, the particle can include a functional location such that the particle can be used as an analytical material. According to such embodiments, a particle includes a functional molecular imprint. The functional molecular imprint can include functional monomers arranged as a negative image of a functional template. The functional template, for example, can be but is not limited to, chemically functional and size and shape equivalents of an enzyme, a protein, an antibiotic, an antigen, a nucleotide sequence, an amino acid, a drug, a biologic, nucleic acid, combinations thereof, or the like. In other embodiments, the particle itself, for example, can be, but is not limited to, an artificial functional molecule. In one embodiment, the artificial functional molecule is a functionalized particle that has been molded from a molecular imprint. As such, a molecular imprint is generated in accordance with methods and materials of the presently disclosed subject matter and then a particle is formed from the molecular imprint, in accordance with further methods and materials of the presently disclosed subject matter. Such an artificial functional molecule includes substantially similar steric and chemical properties of a molecular imprint template. In one embodiment, the functional monomers of the functionalized particle are arranged substantially as a negative image of functional groups of the molecular imprint.

According to some embodiments, particles formed in the patterned templates described herein are less than about 10 μm in a dimension. In other embodiments, the particle is between about 10 μm and about 1μm in dimension. In yet further embodiments, the particle is less than about 1μm in dimension. According to some embodiments the particle is between about 1 nm and about 500 nm in a dimension. According to other embodiments, the particle is between about 10 nm and about 200 nm in a dimension. In still further embodiments, the particle is between about 80 nm and 120 nm in a dimension. According to still more embodiments the particle is between about 20 nm and about 120 nm in dimension. The dimension of the particle

can be a predetermined dimension, a cross-sectional diameter, a circumferential dimension, or the like.

According to further embodiments, the particles include patterned features that are about 2 nm in a dimension. In still further embodiments, the patterned features are between about 2 nm and about 200 nm. In other embodiments, the particle is less than about 80 nm in a widest dimension.

According to other embodiments, the particles produced by the methods and materials of the presently disclosed subject matter have a poly dispersion index (i.e., normalized size distribution) of between about 0.80 and about 1.20, between about 0.90 and about 1.10, between about 0.95 and about 1.05, between about 0.99 and about 1.01, between about 0.999 and about 1.001, combinations thereof, and the like. Furthermore, in other embodiments the particle has a mono-dispersity. According to some embodiments, dispersity is calculated by averaging a dimension of the particles. In some embodiments, the dispersity is based on, for example, surface area, length, width, height, mass, volume, porosity, combinations thereof, and the like.

According to other embodiments, particles of many predetermined regular and irregular shape and size configurations can be made with the materials and methods of the presently disclosed subject matter. Examples of representative particle shapes that can be made using the materials and methods of the presently disclosed subject matter include, but are not limited to, non-spherical, spherical, viral shaped, bacteria shaped, cell shaped, rod shaped (*e.g.*, where the rod is less than about 200 nm in diameter), chiral shaped, right triangle shaped, flat shaped (*e.g.*, with a thickness of about 2 nm, disc shaped with a thickness of greater than about 2 nm, or the like), boomerang shaped, combinations thereof, and the like.

In some embodiments, the material from which the particles are formed includes, without limitation, one or more of a polymer, a liquid polymer, a solution, a monomer, a plurality of monomers, a polymerization initiator, a polymerization catalyst, an inorganic precursor, an organic material, a natural product, a metal precursor, a pharmaceutical agent, a tag, a magnetic material, a paramagnetic material, a ligand, a cell penetrating

peptide, a porogen, a surfactant, a plurality of immiscible liquids, a solvent, a charged species, combinations thereof, or the like.

In some embodiments, the monomer includes butadienes, styrenes, propene, acrylates, methacrylates, vinyl ketones, vinyl esters, vinyl acetates, vinyl chlorides, vinyl fluorides, vinyl ethers, acrylonitrile, methacrylnitrile, acrylamide, methacrylamide allyl acetates, fumarates, maleates, ethylenes, propylenes, tetrafluoroethylene, ethers, isobutylene, fumaronitrile, vinyl alcohols, acrylic acids, amides, carbohydrates, esters, urethanes, siloxanes, formaldehyde, phenol, urea, melamine, isoprene, isocyanates, epoxides, bisphenol A, alcohols, chlorosilanes, dihalides, dienes, alkyl olefins, ketones, aldehydes, vinylidene chloride, anhydrides, saccharide, acetylenes, naphthalenes, pyridines, lactams, lactones, acetals, thiiranes, episulfide, peptides, derivatives thereof, and combinations thereof.

In yet other embodiments, the polymer includes polyamides, proteins, polyesters, polystyrene, polyethers, polyketones, polysulfones, polyurethanes, polysiloxanes, polysilanes, cellulose, amylose, polyacetals, polyethylene, glycols, poly(acrylate)s, poly(methacrylate)s, poly(vinyl alcohol), poly(vinylidene chloride), poly(vinyl acetate), poly(ethylene glycol), polystyrene, polyisoprene, polyisobutylenes, poly(vinyl chloride), poly(propylene), poly(lactic acid), polyisocyanates, polycarbonates, alkyds, phenolics, epoxy resins, polysulfides, polyimides, liquid crystal polymers, heterocyclic polymers, polypeptides, conducting polymers including polyacetylene, polyquinoline, polyaniline, polypyrrole, polythiophene, and poly(p-phenylene), dendimers, fluoropolymers, derivatives thereof, combinations thereof,

In still further embodiments, the material from which the particles are formed includes a non-wetting agent. According to another embodiment, the material is a liquid material in a single phase. In other embodiments, the liquid material includes a plurality of phases. In some embodiments, the liquid material includes, without limitation, one or more of multiple liquids, multiple immiscible liquids, surfactants, dispersions, emulsions, micro-emulsions, micelles, particulates, colloids, porogens, active ingredients, combinations thereof, or the like.

In some embodiments, additional components are included with the material of the particle to functionalize the particle. According to these embodiments the additional components can be encased within the isolated structures, partially encased within the isolated structures, on the exterior surface of the isolated structures, combinations thereof, or the like. Additional components can include, but are not limited to, drugs, biologics, more than one drug, more than one biologic, combinations thereof, and the like.

In some embodiments, the drug is a psychotherapeutic agent. In other embodiments, the psychotherapeutic agent is used to treat depression and can include, for example, sertraline, venlafaxine hydrochloride, paroxetine, bupropion, citalopram, fluoxetine, mirtazapine, escitalopram, and the like. In some embodiments, the psychotherapeutic agent is used to treat schizophrenia and can include, for example, olanazapine, risperidone, quetiapine, aripiprazole, ziprasidone, and the like. According to other embodiments, the psychotherapeutic agent is used to treat attention deficit disorder (ADD) or attention deficit hyperactivity disorder (ADHD), and can include, for example, methylphenidate, atomoxetine, amphetamine, dextroamphetamine, and the like. In some other embodiments, the drug is a cholesterol drug and can include, for example, atorvastatin, simvastatin, pravastatin, ezetimibe, rosuvastatin, fenofibrate fluvastatin, and the like. In yet some other embodiments, the drug is a cardiovascular drug and can include, for example, amlodipine, valsartan, losartan, hydrochlorothiazide, metoprolol, candesartan, ramipril, irbesartan, amlodipine, benazepril, nifedipine, carvedilol, enalapril, telemisartan, quinapril, doxazosin mesylate, felodipine, lisinopril, and the like. In some embodiments, the drug is a blood modifier and can include, for example, epoetin alfa, darbepoetin alfa, epoetin beta, clopidogrel, pegfilgrastim, filgrastim, enoxaparin, Factor VIIA, antihemophilic factor, immune globulin, and the like. According to a further embodiment, the drug can include a combination of the above listed drugs.

In some embodiments, the material of the particles or the additional components included with the particles of the presently disclosed subject matter can include, but are not limited, to anti-infective agents. In some

embodiments, the anti-infective agent is used to treat bacterial infections and can include, for example, azithromycin, amoxicillin, clavulanic acid, levofloxacin, clarithromycin, ceftriaxone, ciprofloxacin, piperacillin, tazobactam sodium, imipenem, cilastatin, linezolid, meropenem, cefuroxime, moxifloxacin, and the like. In some embodiments the anti-infective agent is used to treat viral infections and can include, for example, lamivudine, zidovudine, valacyclovir, peginterferon, lopinavir, ritonavir, tenofovir, efavirenz, abacavir, lamivudine, zidovudine, atazanavir, and the like. In other embodiments, the anti-infective agent is used to treat fungal infections and can include, for example, terbinafine, fluconazole, itraconazole, caspofungin acetate, and the like. In some embodiments, the drug is a gastrointestinal drug and can include, for example, esomeprazole, lansoprazole, omeprazole, pantoprazole, rabeprazole, ranitidine, ondansetron, and the like. According to yet other embodiments, the drug is a respiratory drug and can include, for example, fluticasone, salmeterol, montelukast, budesonide, formoterol, fexofenadine, cetirizine, desloratadine, mometasone furoate, tiotropium, albuterol, ipratropium, palivizumab, and the like. In yet other embodiments, the drug is an antiarthritic drug and can include, for example, celecoxib, infliximab, etanercept, rofecoxib, valdecoxib, adalimumab, meloxicam, diclofenac, fentanyl, and the like. According to a further embodiment, the drug can include a combination of the above listed drugs.

According to alternative embodiments, the material of the particles or the additional components included with the particles of the presently disclosed subject matter can include, but are not limited to an anticancer agent and can include, for example, nitrogen mustard, cisplatin, doxorubicin, docetaxel, anastrozole, trastuzumab, capecitabine, letrozole, leuprolide, bicalutamide, goserelin, rituximab, oxaliplatin, bevacizumab, irinotecan, paclitaxel, carboplatin, imatinib, gemcitabine, temozolomide, gefitinib, and the like. In some embodiments, the drug is a diabetes drug and can include, for example, rosiglitazone, pioglitazone, insulin, glimepiride, voglibose, and the like. In other embodiments, the drug is an anticonvulsant and can include, for example, gabapentin, topiramate, oxcarbazepine,

carbamazepine, lamotrigine, divalproex, levetiracetam, and the like. In some embodiments, the drug is a bone metabolism regulator and can include, for example, alendronate, raloxifene, risedronate, zoledronic, and the like. In some embodiments, the drug is a multiple sclerosis drug and can include, for example, interferon, glatiramer, copolymer-1, and the like. In other embodiments, the drug is a hormone and can include, for example, somatropin, norelgestromin, norethindrone, desogestrel, progestin, estrogen, octreotide, levothyroxine, and the like. In yet other embodiments, the drug is a urinary tract agent, and can include, for example, tamsulosin, finasteride, tolterodine, and the like. In some embodiments, the drug is an immunosuppressant and can include, for example, mycophenolate mofetil, cyclosporine, tacrolimus, and the like. In some embodiments, the drug is an ophthalmic product and can include, for example, latanoprost, dorzolamide, botulinum, verteporfin, and the like. In some embodiments, the drug is a vaccine and can include, for example, pneumococcal, hepatitis, influenza, diphtheria, and the like. In other embodiments, the drug is a sedative and can include, for example, zolpidem, zaleplon, eszopiclone, and the like. In some embodiments, the drug is an Alzheimer disease therapy and can include, for example, donepexil, rivastigmine, tacrine, and the like. In some embodiments, the drug is a sexual dysfunction therapy and can include, for example, sildenafil, tadalafil, alprostadil, levothyroxine, and the like. In an alternative embodiment, the drug is an anesthetic and can include, for example, sevoflurane, propofol, mepivacaine, bupivacaine, ropivacaine, lidocaine, nesacaine, etidocaine, and the like. In some embodiments, the drug is a migraine drug and can include, for example, sumatriptan, almotriptan, rizatriptan, naratriptan, and the like. In some embodiments, the drug is an infertility agent and can include, for example, follitropin, choriogonadotropin, menotropin, follicle stimulating hormone (FSH), and the like. In some embodiments, the drug is a weight control product and can include, for example, orlistat, dexfenfluramine, sibutramine, and the like. According to a further embodiment, the drug can include a combination of the above listed drugs.

In some embodiments, one or more additional components are included with the particles. The additional components can include: targeting ligands such as cell-targeting peptides, cell-penetrating peptides, integrin receptor peptide (GRGDSP), melanocyte stimulating hormone, vasoactive intestional peptide, anti-Her2 mouse antibodies and antibody fragments, and the like; vitamins; viruses; polysaccharides; cyclodextrins; liposomes; proteins; oligonucleotides; aptamers; optical nanoparticles such as CdSe for optical applications; borate nanoparticles to aid in boron neutron capture therapy (BNCT) targets; combinations thereof; and the like.

According to some embodiments, the particles can be controlled or time-release drug delivery vehicles. A co-constituent of the particle, such as a polymer for example, can be cross-linked to varying degrees. Depending upon the amount of cross-linking of the polymer, another co-constituent of the particle, such as an active agent, can be configured to be released from the particle as desired. The active can be released with no restraint, controlled release, or can be completely restrained within the particle. In some embodiments, the particle can be functionalized, according to methods and materials disclosed herein, to target a specific biological site, cell, tissue, agent, combinations thereof, or the like. Upon interaction with the targeted biological stimulus, a co-constituent of the particle can be broken down to begin releasing the active co-constituent of the particle. In one example, the polymer can be poly(ethylene glycol) (PEG), which can be cross-linked between about 5% and about 100%. The active co-constituent that can be doxorubicin that is included in the cross-linked PEG particle. In one embodiment, when the PEG co-constituent is cross-linked about 100%, no doxorubicin leaches out of the particle.

In certain embodiments, the particle includes a composition of material that imparts controlled, delayed, immediate, or sustained release of cargo of the particle or composition, such as for example, sustained drug release. According to some embodiments, materials and methods used to form controlled, delayed, immediate, or sustained release characteristics of the particles of the present invention include the materials, methods, and formulations disclosed in U.S. Patent Application nos. 2006/0099262;

2006/0104909; 2006/0110462; 2006/0127484; 2004/0175428; 2004/0166157; and U.S. Patent no. 6,964,780, each of which are incorporated herein by reference in their entirety.

In some embodiments, imaging agents are the material of the particle or can be included with the particles. In some embodiments, the imaging agent is an x-ray agent and can include, for example, barium sulfate, ioxaglate meglumine, ioxaglate sodium, diatrizoate meglumine, diatrizoate sodium, ioversol, iothalamate meglumine, iothalamate sodium, iodixanol, iohexol, iopentol, iomeprol, iopamidol, iotroxate meglumine, iopromide, iotrolan, sodium amidotrizoate, meglumine amidotrizoate, and the like. In some embodiments, the imaging agent is a MRI agent and can include, for example, gadopentetate dimeglumine, ferucarbotran, gadoxetic acid disodium, gadobutrol, gadoteridol, gadobenate dimeglumine, ferumoxsil, gadoversetamide, gadolinium complexes, gadodiamide, mangafodipir, and the like. In some embodiments, the imaging agent is an ultrasound agent and can include, for example, galactose, palmitic acid, SF_6, and the like. In some embodiments, the imaging agent is a nuclear agent and can include, for example, technetium (Tc99m) tetrofosmin, ioflupane, technetium (Tc99m) depreotide, technetium (Tc99m) exametazime, fluorodeoxyglucose (FDG), samarium (Sm153) lexidronam, technetium (Tc99m) mebrofenin, sodium iodide (I125 and I131), technetium (Tc99m) medronate, technetium (Tc99m) tetrofosmin, technetium (Tc99m) fanolesomab, technetium (Tc99m) mertiatide, technetium (Tc99m) oxidronate, technetium (Tc99m) pentetate, technetium (Tc99m) gluceptate, technetium (Tc99m) albumin, technetium (Tc99m) pyrophosphate, thallous (Tl201) chloride, sodium chromate (Cr51), gallium (Ga67) citrate, indium (In111) pentetreotide, iodinated (I125) albumin, chromic phosphate (P32), sodium phosphate (P32), and the like. According to a further embodiment, the agent can include a combination of the above listed agents, drugs, biologics, and the like.

According to other embodiments, one or more other drugs can be included with the particles of the presently disclosed subject matter and can be found in Physician's Desk Reference, Thomson Healthcare, 59th Bk&Cr edition (2004), which is incorporated herein by reference in its entirety.

In some embodiments, the particles are coated with a patient appealing substance to facilitate and encourage consumption of the particles as oral drug delivery vehicles. The particles can be coated or substantially coated with a substance (*e.g.*, a food substance) that can mask a taste of the particle and/or drug combinations. According to some embodiments, the particle is coated with a sugar-based substance to impart to the particle an appealing sweet taste. According to other embodiments, the particles can be coated with materials described in relation to the fast-dissolve embodiments described herein above.

According to some embodiments, radiotracers and/or radiopharmaceuticals are the material of the particle or can be included with the particles. Examples of radiotracers and/or radiopharmaceuticals that can be combined with the isolated structures of the presently disclosed subject matter include, but are not limited to, [^{15}O]oxygen, [^{15}O]carbon monoxide, [^{15}O]carbon dioxide, [^{15}O]water, [^{13}N]ammonia, [^{18}F]FDG, [^{18}F]FMISO, [^{18}F]MPPF, [^{18}F]A85380, [^{18}F]FLT, [^{11}C]SCH23390, [^{11}C]flumazenil, [^{11}C]PK11195, [^{11}C]PIB, [^{11}C]AG1478, [^{11}C]choline, [^{11}C]AG957, [^{18}F]nitroisatin, [^{18}F]mustard, combinations thereof, and the like. In some embodiments elemental isotopes are included with the particles. In some embodiments, the isotopes include ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{32}P, ^{51}Cr, ^{57}Co, ^{67}Ga, ^{81}Kr, ^{82}Rb, ^{89}Sr, ^{99}Tc, ^{111}In, ^{123}I, ^{125}I, ^{131}I, ^{133}Xe, ^{153}Sm, ^{201}Tl, or the like. According to a further embodiment, the isotope can include a combination of the above listed isotopes, and the like. Likewise, the particles can include a fluorescent label such that the particle can be identified. Examples of fluorescent labeled particles are shown in Figures 45 and 46. Figure 45 shows a particle that has been fluorescently labeled and is associated with a cell membrane and the particle shown in Figure 46 is within the cell.

According to still further embodiments, contrast agents can be included with the material from which the particles are formed or can make up the entire particle or can be tethered to the particle's exterior. Adding contrast agents enhances diagnostic imaging of physiologic structures for clinical evaluations and other testing. For example, ultrasound imaging techniques often involve the use of contrast agents, as contrast agents can

serve to improve the quality and usefulness of images which are obtained with ultrasound. The viability of currently available ultrasound contrast agents and methods involving their use is highly dependent on a variety of factors, including the particular region being imaged. For example, difficulty is encountered in obtaining useful diagnostic images of heart tissue and the surrounding vasculature due, at least in part, to the large volume of blood that flows through the chambers of the heart relative to the volume of blood that flows in the blood vessels of the heart tissue itself. The high volume of blood flowing through the chambers of the heart can result in insufficient contrast in ultrasound images of the heart region, especially the heart tissue. The high volume of blood flowing through the chambers of the heart also can produce diagnostic artifacts including, for example, shadowing or darkening, in ultrasound images of the heart. Diagnostic artifacts can be highly undesirable since they can hamper or even prevent visualization of a region of interest. Thus, in certain circumstances, diagnostic artifacts can render a diagnostic image substantially unusable.

In addition to ultrasound, computed tomography (CT) is a valuable diagnostic imaging technique for studying various areas of the body. Like ultrasound, CT imaging is greatly enhanced with the aid of contrast agents. In CT, the radiodensity (electron density) of matter is measured. Because of the similarity in the measured densities of various tissues in the body, it has been necessary to use contrast agents that can change the relative densities of different tissues. This characteristic has resulted in an overall improvement in the diagnostic efficacy of CT. Barium and iodine compounds, for example, have been developed for this purpose and can be included with the particles of the presently disclosed subject matter in some embodiments. Accordingly, in other embodiments, contrast agents that can be used with the materials of the presently disclosed subject matter, include for example, but are not limited to, barium sulfate, Iodinated water-soluble contrast media, combinations thereof, and the like.

Magnetic resonance imaging (MRI) is another diagnostic imaging technique that is used for producing cross-sectional images of a tissue in a variety of scanning planes. Like ultrasound and CT, MRI also benefits from

the use of contrast agents. In some embodiments of the presently disclosed subject matter, contrast agents for MRI are used with the materials of the presently disclosed subject matter to enhance MRI imaging. Contrast agents for MRI imaging that can be useful with the materials of the presently disclosed subject matter include, but are not limited to, paramagnetic contrast agents, metal ions, transition metal ions, metal ions that are chelated with ligands, metal oxides, iron oxides, nitroxides, stable free radicals, stable nitroxides, lanthanide and actinide elements, lipophilic derivatives, proteinaceous macromolecules, alkylated, nitroxides 2,2,5,5-tetramethyl-1-pyrrolidinyloxy, free radical, 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical, combinations thereof, and the like.

According to yet other embodiments contrast agents that can be used as the materials or with the materials of the presently disclosed subject matter include, but are not limited to, superparamagnetic contrast agents, ferro- or ferrimagnetic compounds such as pure iron, magnetic iron oxide, such as magnetite, γ-Fe_2O_3, Fe_3O_4, manganese ferrite, cobalt ferrite, nickel ferrite; paramagnetic gases such as oxygen 17 gas, hyperpolarized xenon, neon, helium gas, combinations thereof, and the like. If desired, the paramagnetic or superparamagnetic contrast agents used with the materials of the presently disclosed include, but are not limited to, paramagnetic or superparamagetic agents that are delivered as alkylated or having other derivatives incorporated into the compositions, combinations thereof, and the like.

In yet another embodiment, contrast agents for X-ray techniques useful for combination with the particles of the presently disclosed subject matter include, but are not limited to, carboxylic acid and non-ionic amide contrast agents typically containing at least one 2,4,6-triiodophenyl group having substituents such as carboxyl, carbamoyl, N-alkylcarbamoyl, N-hydroxyalkylcarbamoyl, acylamino, N-alkylacylamino or acylaminomethyl at the 3- and/or 5-positions, as in metrizoic acid, diatrizoic acid, iothalamic acid, ioxaglic acid, iohexol, iopentol, iopamidol, iodixanol, iopromide, metrizamide, iodipamide, meglumine iodipamide, meglumine acetrizoate, meglumine diatrizoate, combinations thereof, and the like.

Still other contrast agents that can be included with the particle materials of the presently disclosed subject matter include, but are not limited to, barium sulfate, a barium sulfate suspension, sodium bicarbonate and tartaric acid mixtures, lothalamate meglumine, lothalamate sodium, hydroxypropyl methylcellulose, ferumoxsil, ioxaglate meglumine, ioxaglate sodium, diatrizoate meglumine, diatrizoate sodium, gadoversetamide, ioversol, organically bound iodine, methiodal sodium, ioxitalamate meglumine, iocarmate meglumine, metrizamide, iohexal, iopamidol, combinations thereof, and the like.

U.S. patent nos. 6,884,407 and 6,331,289, along with the references cited therein, disclose contrasts that are useful with the particles of the presently disclosed subject matter, these references are incorporated by reference herein along with the references cited therein.

According to further embodiments the particle can include or can be formed into and used as a tag or a taggant. A taggant that can be included in the particle or can be the particle includes, but is not limited to, a fluorescent, radiolabeled, magnetic, biologic, shape specific, size specific, combinations thereof, or the like.

In some embodiments, a therapeutic agent for combination with the particles of the presently disclosed subject matter is selected from one of a drug and genetic material. In some embodiments, the genetic material includes, without limitation, one or more of a non-viral gene vector, DNA, RNA, RNAi, a viral particle, agents described elsewhere herein, combinations thereof, or the like.

In some embodiments, the particle includes a biodegradable polymer. In other embodiments, the polymer is modified to be a biodegradable polymer (*e.g.*, a poly(ethylene glycol) that is functionalized with a disulfide group). In some embodiments, the biodegradable polymer includes, without limitation, one or more of a polyester, a polyanhydride, a polyamide, a phosphorous-based polymer, a poly(cyanoacrylate), a polyurethane, a polyorthoester, a polydihydropyran, a polyacetal, combinations thereof, or the like.

In some embodiments, the polyester includes, without limitation, one or more of polylactic acid, polyglycolic acid, poly(hydroxybutyrate), poly(ϵ-caprolactone), poly(β-malic acid), poly(dioxanones), combinations thereof, or the like. In some embodiments, the polyanhydride includes, without limitation, one or more of poly(sebacic acid), poly(adipic acid), poly(terpthalic acid), combinations thereof, or the like. In yet other embodiments, the polyamide includes, without limitation, one or more of poly(imino carbonates), polyaminoacids, combinations thereof, or the like.

According to some embodiments, the phosphorous-based polymer includes, without limitation, one or more of a polyphosphate, a polyphosphonate, a polyphosphazene, combinations thereof, or the like. Further, in some embodiments, the biodegradable polymer further includes a polymer that is responsive to a stimulus. In some embodiments, the stimulus includes, without limitation, one or more of pH, radiation, ionic strength, oxidation, reduction, temperature, an alternating magnetic field, an alternating electric field, combinations thereof, or the like. In some embodiments, the stimulus includes an alternating magnetic field.

In some embodiments, a pharmaceutical agent can be combined with the particle material. The pharmaceutical agent can be, but is not limited to, a drug, a peptide, RNAi, DNA, combinations thereof, or the like. In other embodiments, the tag is selected from the group including a fluorescence tag, a radiolabeled tag, a contrast agent, combinations thereof, or the like. In some embodiments, the ligand includes a cell targeting peptide, or the like.

In use, the particles of the presently disclosed subject matter can be used as treatment devices. In such uses, the particle is administered in a therapeutically effective amount to a patient. According to yet other uses, the particle can be utilized as a physical tag. In such uses, a particle of a predetermined shape having a diameter of less than about 1 μm in a dimension is used as a taggant to identify products or the origin of a product. The particle as a taggant can be either identifiable to a particular shape or a particular chemical composition.

Further uses of the micro and/or nano particles include medical treatments such as orthopedic, oral, maxillofacial, and the like. For example, the particles described above that are or include pharmaceutical agents can be used in combination with traditional hygiene and/or surgical procedures. According to such an application, the particles can be used to directly and locally deliver pharmaceutical agents, or the like to an area of surgical interest. In some embodiments, medications used in oral medicine can fight oral diseases, prevent or treat infections, control pain, relieve anxiety, assist in the regeneration of damaged tissue, combinations thereof, and the like. For example, during oral or maxillofacial treatments, bleeding often occurs. As a result, bacteria from the mouth can directly enter the bloodstream and easily reach the heart. This occurrence presents a risk for some persons with cardiac abnormalities because the bacteria can cause bacterial endocarditis, a serious inflammation of the heart valves or tissues. Antibiotics reduce this risk. Traditional antibiotic delivery techniques, however, can be slow to reach the bloodstream, thus giving the bacterial a head start. To the contrary, applying particles of the presently disclosed subject matter, made from or including appropriate antibiotics, directly to the site of oral or maxillofacial treatment can greatly reduce the probability of a serious bacterial infection. Such procedures aided by the particles can include professional teeth cleaning, incision and drainage of infected oral tissue, oral injections, extractions, surgeries that involve the maxillary sinus, combinations thereof, and the like.

According to further embodiments, compositions can be formulated and made into particles according to materials and methods of the presently disclosed subject matter that are designed to be applied to defective teeth and gums for preventing diseases, such as carious tooth, pyorrhea alveolaris, or the like.

Further embodiments include particles having a composition for the repair and healing of tissue, bone defects and bone voids, resins for artificial teeth, resins for tooth bed, and other tooth fillers. For example, particles can be constructed from calcium based component, such as, but not limited to, calcium phosphates, calcium sulfates, calcium carbonates, calcium bone

cements, amorphous calcium phosphate, crystalline calcium phosphate, combinations thereof, and the like. In use, such particles can be locally applied to a site of orthopedic treatment to facilitate recovery of the natural bone material. Furthermore, because of the small size of the particles and the ability to form the particles in practically any shape and configuration desirable, the particles can be administered to a site of orthopedic interest and interact with the site on a scale of the particle size. That is, the particles can integrate into very small spaces, cracks, gaps, and the like within the bone, such as a bone fracture, or between the bone and an implant. Thus, the particles can deliver pharmaceutical, regenerative, or the like materials to the orthopedic treatment site and integrate these materials where they were not previously applyable. Still further, the particles can increase the mechanical strength and integrity of fixation of a bone implant, such as an artificial joint fixation, because, due to control over the size and shape of the particles, they can neatly and orderly fill small voids between the implant and the natural bone tissue.

In other embodiments, medications to control pain and anxiety that are commonly used in oral, maxillofacial, orthopedic, and other procedures can be included in the particles. Such agents that can be incorporated with the particle include, but are not limited to, anti-inflammatory medications that are used to relieve the discomfort of mouth and gum problems, and can include corticosteroids, opioids, carprofen, meloxicam, etodolac, diclofenac, flurbiprofen, ibuprofen, ketorolac, nabumetone, naproxen, naproxen sodium, and oxaprozin. Oral anesthetics are used to relieve pain or irritation caused by many conditions, including toothaches, teething, sores, or dental appliances, and can include articaine, epinephrine, ravocaine, novocain, levophed, propoxycaine, procaine, norepinephrine bitartrate, marcaine, lidocaine, carbocaine, neocobefrin, mepivacaine, levonordefrin, etidocaine, dyclonine, and the like. Antibiotics are commonly used to control plaque and gingivitis in the mouth, treat periodontal disease, as well as reduce the risk of bacteria from the mouth entering the bloodstream. Oral antibiotics can include chlorhexidine, doxycycline, demeclocycline, minocycline, oxytetracycline, tetracycline, triclosan, clindamycin, orfloxacin,

metronidazole, tinidazole, and ketoconazole. Fluoride also can be or be included in the particles of the presently disclosed subject matter and is used to prevent tooth decay. Fluoride is absorbed by teeth and helps strengthen teeth to resist acid and block the cavity-forming action of bacteria. As a varnish or a mouth rinse, fluoride helps reduce tooth sensitivity. Other useful agents for dental applications are substances such as flavonoids, benzenecarboxylic acids, benzopyrones, steroids, pilocarpine, terpenes, and the like. Still further agents used within the particles include anethole, anisaldehyde, anisic acid, cinnamic acid, asarone, furfuryl alcohol, furfural, cholic acid, oleanolic acid, ursolic acid, sitosterol, cineol, curcumine, alanine, arginine, homocerine, mannitol, berterine, bergapten, santonin, caryophyllene, caryophyllene oxide, terpinene, chymol, terpinol, carvacrol, carvone, sabinene, inulin, lawsone, hesperedin, naringenin, flavone, flavonol, quercetin, apigenin, formonoretin, coumarin, acetyl coumarin, magnolol, honokiol, cappilarin, aloetin, and the like. Still further oral and maxillofacial treatment compounds include sustained release biodegradable compounds, such as, for example (meth)acrylate type monomers and/or polymers. Other compounds useful for the particles of the presently disclosed subject matter can be found in U.S. Patent no. 5,006,340, which is incorporated herein by reference in its entirety.

In some embodiments, the particle fabrication process provides control of particle matrix composition, the ability for the particle to carry a wide variety of cargos, the ability to functionalize the particle for targeting and enhanced circulation, and/or the versatility to configure the particle into different dosage forms, such as inhalation, dermatological, injectable, and oral, to name a few.

According to some embodiments, the matrix composition is tailored to provide control over biocompatibility. In some embodiments, the matrix composition is tailored to provide control over cargo release. The matrix composition, in some embodiments, contains biocompatible materials with solubility and/or philicity, controlled mesh density and charge, stimulated degradation, and/or shape and size specificity while maintaining relative monodispersity.

According to further embodiments, the method for making particles containing cargo does not require the cargo to be chemically modified. In one embodiment, the method for producing particles is a gentle processing technique that allows for high cargo loading without the need for covalent bonding. In one embodiment, cargo is physically entrapped within the particle due to interactions such as Van der Waals forces, electrostatic, hydrogen bonding, other other intra- and inter-molecular forces, combinations thereof, and the like.

In some embodiments, the particles are functionalized for targeting and enhanced circulation. In some embodiments, these features allow for tailored bioavailability. In one embodiment, the tailored bioavailability increases delivery effectiveness. In one embodiment, the tailored bioavailability reduces side effects.

In some embodiments, a non-sperical particle has a surface area that is greater than the surface area of spherical particle of the same volume. In some embodiments, the number of surface ligands on the particle is greater than the number of surface ligands on a spherical particle of the same volume.

In some embodiments, one or more particles contain chemical moiety handles for the attachment of protein. In some embodiments, the protein is avidin. In some embodiments biotinylated reagents are subsequently bound to the avidin. In some embodiments the protein is a cell penetrating protein. In some embodiments, the protein is an antibody fragment. In one embodiment, the particles are used for specific targeting (e.g., breast tumors in female subjects). In some embodiments, the particles contain chemotherapeutics. In some embodiments, the particles are composed of a cross link density or mesh density designed to allow slow release of the chemotherapeutic. The term crosslink density means the mole fraction of prepolymer units that are crosslink points. Prepolymer units include monomers, macromonomers and the like.

In some embodiments, the physical properties of the particle are varied to enhance cellular uptake. In some embodiments, the size (e.g.,

mass, volume, length or other geometric dimension) of the particle is varied to enhance cellular uptake. In some embodiments, the charge of the particle is varied to enhance cellular uptake. In some embodiments, the charge of the particle ligand is varied to enhance cellular uptake. In some embodiments, the shape of the particle is varied to enhance cellular uptake.

In some embodiments, the physical properties of the particle are varied to enhance biodistribution. In some embodiments, the size (e.g., mass, volume, length or other geometric dimension) of the particle is varied to enhance biodistribution. In some embodiments, the charge of the particle matrix is varied to enhance biodistribution. In some embodiments, the charge of the particle ligand is varied to enhance biodistribution. In some embodiments, the shape of the particle is varied to enhance biodistribution. In some embodiments, the aspect ratio of the particles is varied to enhance biodistribution.

In some embodiments, the physical properties of the particle are varied to enhance cellular adhesion. In some embodiments, the size (e.g., mass, volume, length or other geometric dimension) of the particle is varied to enhance cellular adhesion. In some embodiments, the charge of the particle matrix is varied to enhance cellular adhesion. In some embodiments, the charge of the particle ligand is varied to enhance cellular adhesion. In some embodiments, the shape of the particle is varied to enhance cellular adhesion.

In some embodiments, the particles are configured to degrade in the presence of an intercellular stimulus. In some embodiments, the particles are configured to degrade in a reducing environment. In some embodiments, the particles contain crosslinking agents that are configured to degrade in the presence of an external stimulus. In some embodiments, the crosslinking agents are configured to degrade in the presence of a pH condition, a radiation condition, an ionic strength condition, an oxidation condition, a reduction condition, a temperature condition, an alternating magnetic field condition, an alternating electric field condition, combinations thereof, or the like. In some embodiments, the particles contain crosslinking

agents that are configured to degrade in the presence of an external stimulus and/or a therapeutic agent.

In some embodiments, the particles contain crosslinking agents that are configured to degrade in the presence of an external stimulus, a targeting ligand, and a therapeutic agent. In some embodiments, the therapeutic agent is a drug or a biologic. In some embodiments the therapeutic agent is DNA, RNA, or siRNA.

In some embodiments, particles are configured to degrade in the cytoplasm of a cell. In some embodiments, particles are configured to degrade in the cytoplasm of a cell and release a therapeutic agent. In some embodiments, the therapeutic agent is a drug or a biologic. In some embodiments the therapeutic agent is DNA, RNA, or siRNA. In some embodiments, the particles contain poly(ethylene glycol) and crosslinking agents that degrade in the presence of an external stimulus.

In some embodiments, the particles are used for ultrasound imaging. In some embodiments, the particles used for ultrasound imaging are composed of bioabsorbable polymers. In some embodiments, particles used for ultrasound imaging are porous. In some embodiments, particles used for ultrasound imaging are composed of poly(lactic acid), poly(D,L-lactic acid-co-glycolic acid), and combinations thereof.

In some embodiments, the particles contain magnetite and are used as contrast agents. In some embodiments, the particles contain magnetite and are functionalized with linker groups and are used as contrast agents. In some embodiments, the particles are functionalized with a protein. In some embodiments, the particles are functionalized with N-hydroxysuccinimidyl ester groups. In some embodiments, avidin is bound to the particles. In some embodiments, particles containing magnetite are covalently bound to avidin and exposed to a biotinylated reagent.

In some embodiments, the particles are shaped to mimic natural structures. In some embodiments, the particles are substantially cell-shaped. In some embodiments, the particles are substantially red blood cell-shaped. In some embodiments, the particles are substantially red blood cell-

shaped and composed of a matrix with a modulus less than 1 MPa. In some embodiments, the particles are shaped to mimic natural structures and contain a therapeutic agent, a contrast agent, a targeting ligand, combination thereof, and the like.

In some embodiments, the particles are configured to elicit an immune response. In some embodiments, the particles are configured to stimulate B-cells. In some embodiments, the B-cells are stimulated by targeting ligands covalently bound to the particles. In some embodiments, the B-cells are stimulated by haptens bound to the particles. In some embodiments, the B-cells are stimulated by antigens bound to the particles.

In some embodiments, the particles are functionalized with targeting ligands. In some embodiments, the particles are functionalized to target tumors. In some embodiments, the particles are functionalized to target breast tumors. In some embodiments, the particles are functionalized to target the HER2 receptor. In some embodiments, the particles are functionalized to target breast tumors and contain a chemotherapeutic. In some embodiments, the particles are functionalized to target dendritic cells.

According to some embodiments, the particles have a predetermined zeta-potential.

II.C. Introduction of Particle Precursor to Patterned Templates

According to some embodiments, the recesses of the patterned templates can be configured to receive a substance to be molded. According to such embodiments, variables such as, for example, the surface energy of the patterned template, the volume of the recess, the permeability of the patterned template, the viscosity of the substance to be molded as well as other physical and chemical properties of the substance to be molded interact and affect the willingness of the recess to receive the substance to be molded.

II.C.i. Passive Mold Filling

According to some embodiments, a substance **5000** to be molded is introduced to a patterned template **5002**, as shown in FIG. 50. Substance

5000 can be introduced to patterned template **5002** as a droplet, by spin coating, a liquid stream, a doctor blade, jet droplet, or the like. Patterned template **5002** includes recesses **5012** and can be fabricated, according to methods disclosed herein, from materials disclosed herein such as, for example, low surface energy polymeric materials. Because patterned template **5002** is fabricated from low surface energy polymeric materials, substance **5000** does not wet the surface of patterned template **5002**, however, substance **5000** fills recesses **5012**. Next, a treatment **5008**, such as treatments disclosed herein, is applied to substance **5000** to cure substance **5000**. According to some embodiments, treatment **5008** can be, for example, photo-curing, thermal curing, oxidative curing, evaporation, reductive curing, combinations thereof, evaporation, and the like. Following treating substance **5000**, substance **5000** is formed into particles **5010** that can be harvested according to methods disclosed herein.

According to some embodiments, the method for forming particles includes providing a patterned template and a liquid material, wherein the patterned template includes a first patterned template surface having a plurality of recessed areas formed therein. Next, a volume of liquid material is deposited onto the first patterned template surface. A subvolume of the liquid material than fills a recessed area of the patterned template. The subvolumes of the liquid material is then solidified into a solid or semi-solid and harvested from the recesses.

In some embodiments, the plurality of recessed areas includes a plurality of cavities. In some embodiments, the plurality of cavities includes a plurality of structural features. In some embodiments, the plurality of structural features have a dimension ranging from about 10 microns to about 1 nanometer in size. In some embodiments, the plurality of structural features have a dimension ranging from about 1 micron to about 100 nm in size. In some embodiments, the plurality of structural features have a dimension ranging from about 100 nm to about 1 nm in size. In some embodiments, the plurality of structural features have a dimension in both the horizontal and vertical plane.

II.C.ii. Dipping Mold Filling

According to some embodiments, the patterned template is dipped into the substance to be molded, as shown in FIG. 51. Referring to FIG. 51, patterned template **5104** is submerged into a volume of substance **5102**. Substance **5102** enters recesses **5106** and following removal of patterned template **5104** from substance **5102**, substance **5108** remains in recesses **5106** of patterned template **5104**.

II.C.iii. Moving Droplet Mold Filling

According to some embodiments, the patterned template can be positioned on an angle, as shown in FIG. 52. A volume of particle precursor **5204** is introduced onto the surface of patterned template **5200** that includes recesses **5206**. The volume of particle precursor **5204** travels down the sloped surface of patterned template **5200**. As the volume of particle precursor **5204** travels over recesses **5206**, subvolumes of particle precursor **5208** enter and fill recesses **5206**. According to some embodiments, patterned template **5200** can be positioned at about a 20 degree angle from the horizontal. According to some embodiments, the liquid can be moved by a doctor blade.

II.C.iv. Voltage Assist Filling

According to some embodiments, a voltage can assist in introducing a particle precursor into recesses in a patterned template. Referring to FIG. 53, a patterned template **5300** having recesses **5302** on a surface thereof can be positioned on an electrode surface **5308**. A volume of particle precursor **5304** can be introduced onto the recess surface of patterned template **5300**. Particle precursor **5304** can also be in communication with an opposite electrode **5306** to electrode **5308** that is in communication with patterned template **5300**. The voltage difference between electrodes **5306** and **5308** travels through particle precursor **5304** and patterned template **5300**. The voltage difference alters the wetting angle of particle precursor **5304** with respect to patterned template **5300** and, thereby, facilitating entry of particle precursor **5304** into recesses **5302**. In some embodiments, electrode **5306**, in communication with particle precursor **5304**, is moved

across the surface of patterned template **5300** thereby facilitating filling of recesses **5304** across the surface of patterned template **5300**.

According to some embodiments, patterned template **5300** and particle precursor **5304** are subjected to about 3000 DC volts, however, the voltage applied to a combination of patterned template and particle precursor can be tailored to the specific requirements of the combinations. In some embodiments, the voltage is altered to arrive at a preferred contact angle between particle precursor and patterned template to facilitate entry of particle precursor into the recesses of the patterned template.

II.D. Thermodynamics of Recess Filling

Recesses in a patterned template, such as recesses **5012** in patterned template **5002** of FIG. 50 can be configured to receive a substance to be molded. The physical and chemical characteristics of both the recess and the particular substance to be molded can be configured to increase how readily the substance is received by the recess. Factors that can influence the filling of a recess include, but are not limited to, recess volume, diameter, surface area, surface energy, contact angle between a substance to be molded and the material of the recess, voltage applied across a substance to be molded, temperature, environmental conditions surrounding the patterned template such as for example the removal of oxygen or impurities from the atmosphere, combinations thereof, and the like. In some embodiments, a recess that is about 2 micron in diameter has a capillary pressure of about 1 atmosphere. In some embodiments, a recess with a diameter of about 200 nm has a capillary pressure of about 10 atmospheres.

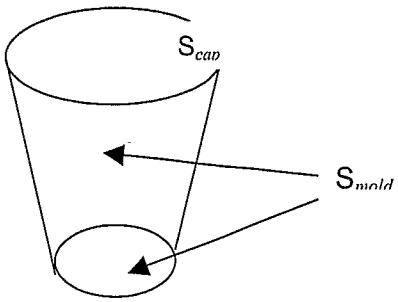
A surface ratio of a recess can be defined according to the following equation:

$$\varepsilon = \frac{S_{cap}}{S_{mold}}$$

where;

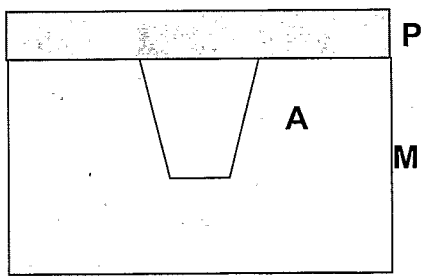
S_{cap} - surface area of air or substrate (if used) contact and

S_{mold} - surface area of the cavity.

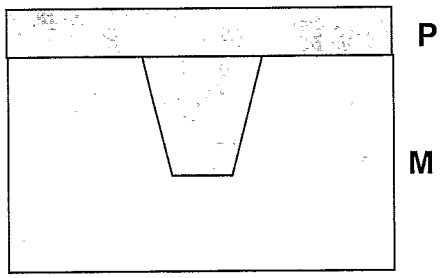


For example, a cube will have a surface ratio of $\varepsilon = \frac{1}{5}$ and a cylinder that has an aspect ratio a=height/diameter will have a surface ratio of $\varepsilon = \frac{1}{1+4a}$.

The thermodynamics of recess filling can be explained by the following equations.



I Non-wetting recess



II Wetting recess

M – mold: **P** – polymer: **A** - air

γ_{ij} - interfacial tension between **i** and **j**

The surface energy for the non-wetting recess (I) is determined by the equation:

$E_I = S_{cap}\gamma_{PA} + S_{mold}\gamma_{MA}$; and

the surface energy for the wetting recess (II) is determined by the equation:

$E_{II} = S_{mold}\gamma_{PM}$.

According to some embodiments, a condition for recess wetting is $E_I > E_{II}$, which can be written as the following equation:

$$\varepsilon\gamma_{PA} + \gamma_{MA} > \gamma_{PM}$$

Taking into account that a contact angle θ_{PM} formed by the patterned template polymer on a plain surface of the mold is given as the following equation:

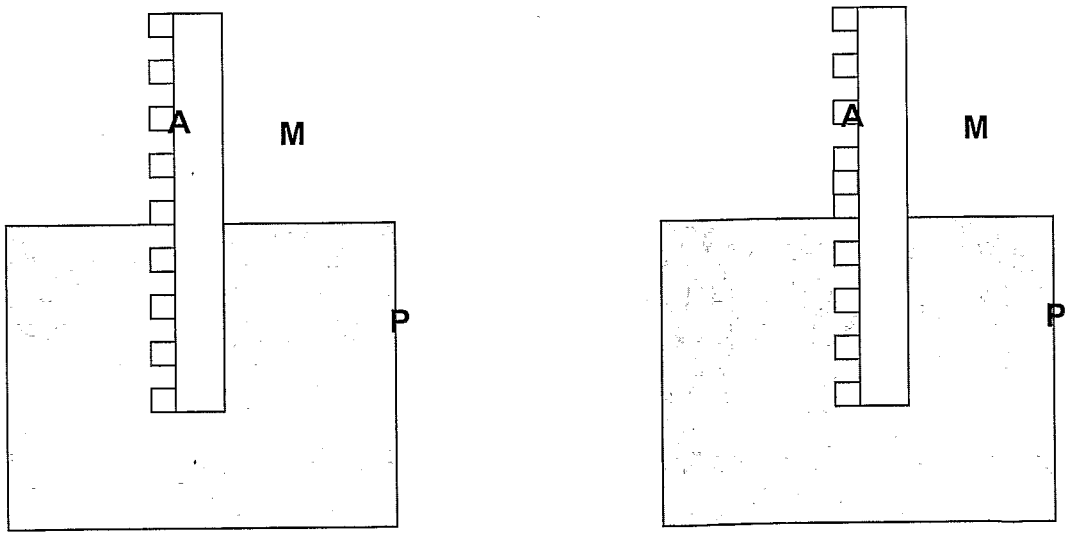
$$\cos\theta_{PM} = \frac{\gamma_{MA} - \gamma_{PM}}{\gamma_{PA}}$$

Recess wetting criteria is determined as:

$$\cos\theta_{PM} > -\varepsilon$$

As a result, a recess can be filled even for wetting angles (θ_{PM}) greater than 90 degrees.

According to some embodiments, the thermodynamics of filling a recess is determined based on the method of filling the recess. According to some embodiments, as further described herein, a patterned template can be dipped into a substance to be molded and the recesses of the patterned template become filled. The thermodynamics of dipping a patterned template are explained by the following equations.



$E_I = S_{mold}\gamma_{MA}$ $E_{II} = S_{mold}\gamma_{PM} + S_{cap}\gamma_{PA}$

According to an embodiment, a dip coating criteria is given by: $E_I > E_{II}$, which can be written as the following equation:

$$\gamma_{MA} > \gamma_{PM} + \varepsilon\gamma_{PA}$$

Taking into account that a contact angle θ_{PM} formed by the patterned template polymer on a plain surface of the mold is given as the following equation:

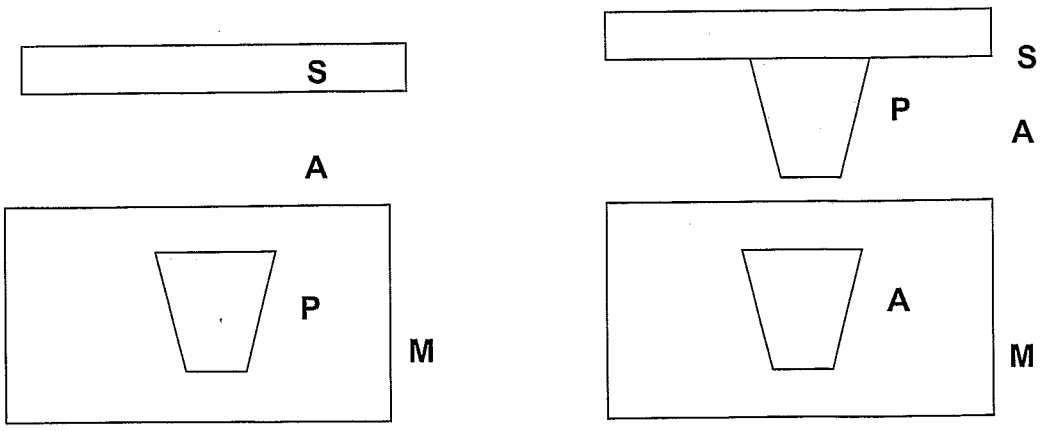
$$\cos\theta_{PM} = \frac{\gamma_{MA} - \gamma_{PM}}{\gamma_{PA}}$$

Dip coating criteria is determined as:

$$\cos\theta_{PM} > \varepsilon$$

II.E. Thermodynamics of Mold Release

In some embodiments, particles formed in recesses of a patterned template are removed by application of a force or energy. According to other embodiments, characteristics of the mold and substance molded facilitate release of particles from the recesses. Mold release characteristics can be related to, for example, the materials molded, recess filing characteristics, permeability of materials of the mold, surface energy of the materials of the mold, combinations thereof, and the like.



$$E_I = S_{cap}(\gamma_{SA} + \sigma_{PA}) + S_{mold}\sigma_{PM} \qquad E_{II} = S_{cap}\sigma_{PS} + S_{mold}(\sigma_{PA} + \gamma_{MA})$$

S – substrate: **P** – particle: **M** – mold: **A** – atmosphere/air

Where polymer-air and polymer-mold interfacial tensions are σ_{PA} and σ_{PM}, respectively, and polymer-substrate interfacial tension is σ_{PS}. Two different notations are used for polymer-air interface and polymer-mold interface because after curing the polymer has different interfacial properties than it has in a liquid state.

According to some embodiments, mold release criteria can be $E_I > E_{II}$; which is represented by the following equations:

$$\varepsilon(\gamma_{SA} + \sigma_{PA}) + \sigma_{PM} > \varepsilon\sigma_{PS} + \sigma_{PA} + \gamma_{MA}$$

$$\varepsilon\left(1 + \frac{\gamma_{SA} - \sigma_{PS}}{\sigma_{PA}}\right) > 1 + \frac{\gamma_{MA} - \sigma_{PM}}{\sigma_{PA}}$$

Next, the effective contact angles of can be represented by:

$$\cos\theta_{PM}^{erff} = \frac{\gamma_{MA} - \sigma_{PM}}{\sigma_{PA}}$$

$$\cos\theta_{PS}^{erff} = \frac{\gamma_{SA} - \sigma_{PS}}{\sigma_{PA}}$$

Which are the angles that the polymer would form on a plain surfaces of the mold and substrate respectively if it was a liquid with interfacial tensions σ_{PM}, σ_{PA}, and σ_{PS}.

Finally, mold release criteria can be written as

$$\frac{1 + \cos\theta_{PM}^{eff}}{1 + \cos\theta_{PS}^{eff}} < \varepsilon$$

III. Formation of Rounded Particles Through "Liquid Reduction"

Referring now to Figures 3A through 3F, the presently disclosed subject matter provides a "liquid reduction" process for forming particles that have shapes that do not conform to the shape of the template, including but not limited to spherical and non-spherical, regular and non-regular micro-

and nanoparticles. For example, a "cube-shaped" template can allow for sphereical particles to be made, whereas a "Block arrow-shaped" template can allow for "lolli-pop" shaped particles or objects to be made wherein the introduction of a gas allows surface tension forces to reshape the resident liquid prior to treating it. While not wishing to be bound by any particular theory, the non-wetting characteristics that can be provided in some embodiments of the presently disclosed patterned template and/or treated or coated substrate allows for the generation of rounded, e.g., spherical, particles.

Referring now to Figure 3A, droplet **302** of a liquid material is disposed on substrate **300**, which in some embodiments is coated or treated with a non-wetting material **304**. A patterned template **108**, which includes a plurality of recessed areas **110** and patterned surface areas **112**, also is provided.

Referring now to Figure 3B, patterned template **108** is contacted with droplet **302**. The liquid material including droplet **302** then enters recessed areas **110** of patterned template **108**. In some embodiments, a residual, or "scum," layer **RL** of the liquid material including droplet **302** remains between the patterned template **108** and substrate **300**.

Referring now to Figure 3C, a first force F_{a1} is applied to patterned template **108**. A contact point **CP** is formed between the patterned template **108** and the substrate and displacing residual layer **RL**. Particles **306** are formed in the recessed areas **110** of patterned template **108**.

Referring now to Figure 3D, a second force F_{a2}, wherein the force applied by F_{a2} is greater than the force applied by F_{a1}, is then applied to patterned template **108**, thereby forming smaller liquid particles **308** inside recessed areas **112** and forcing a portion of the liquid material including droplet **302** out of recessed areas **112**.

Referring now to Figure 3E, the second force F_{a2} is released, thereby returning the contact pressure to the original contact pressure applied by first force F_{a1}. In some embodiments, patterned template **108** includes a gas permeable material, which allows a portion of space with recessed areas

112 to be filled with a gas, such as nitrogen, thereby forming a plurality of liquid spherical droplets **310**. Once this liquid reduction is achieved, the plurality of liquid spherical droplets **310** are treated by a treating process T_r.

Referring now to Figure 3F, treated liquid spherical droplets **310** are released from patterned template **108** to provide a plurality of freestanding spherical particles **312**.

IIIA. Formation of Small Particles through Evaporation

Referring now to Figures 41A through 41E, an embodiment of the presently disclosed subject matter includes a process for forming particles through evaporation. In one embodiment, the process produces a particle having a shape that does not necessarily conform to the shape of the template. The shape can include, but is not limited to, a three dimensional shape. According to some embodiments, the particle forms a spherical or non-spherical and regular or non-regular shaped micro- and nanoparticle. While not wishing to be bound by a particular theory, an example of producing a spherical or substantially spherical particle includes using a patterned template and/or substrate of a non-wetting material or treating the surfaces of the patterned template and substrate particle forming recesses with a non-wetting agent such that the material from which the particle will be formed does not wet the surfaces of the recess. Because the material from which the particle will be formed cannot wet the surfaces of the patterned template and/or substrate the particle material has a greater affinity for itself than the surfaces of the recesses and thereby forms a rounded, curved, or substantially spherical shape.

A non-wetting substance can be defined through the concept of the contact angle (Θ), which can be used quantitatively to measure interaction between virtually any liquid and solid surface. When the contact angle between a drop of liquid on the surface is $90 < \Theta < 180$, the surface is considered non-wetting. In general, fluorinated surfaces are non-wetting to aqueous and organic liquids. Fluorinated surfaces can include a fluoropolyether material, a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer

(TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and/or a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction, surfaces created by treating a silicon or glass surface with a fluorinated silane, or coating a surface with a fluorinated polymer. Further, surfaces of materials that are typically wettable materials can be made non-wettable by surface treatments. Materials that can be made substantially non-wetting by surface treatments include, but are not limited to, a typical wettable polymer material, an inorganic material, a silicon material, a quartz material, a glass material, combinations thereof, and the like. Surface treatments to make these types of materials non-wetting include, for example, layering the wettable material with a surface layer of the above described non-wetting materials, and techniques of the like that will be appreciated by one of ordinary skill in the art.

Referring now to Figure 41A, droplet **4102** of a liquid material of the presently disclosed subject matter that is to become the particle is disposed on non-wetting substrate **4100**, which in some embodiments is a material or a surface coated or treated with a non-wetting material, as described herein above. A patterned template **4108**, which includes a plurality of recessed areas **4110** and patterned surface areas **4112**, also is provided.

Referring now to Figure 41B, patterned template **4108** is contacted with droplet **4102**. The material of droplet **4102** then enters recessed areas **4110** of patterned template **4108**. According to some embodiments, mechanical or physical manipulation of droplet **4102** and patterned template **4108** is provided to facilitate the droplet **4102** in substantially filling and conforming to recessed areas **4110**. Such mechanical and/or physical manipulation can include, but is not limited to, vibration, rotation, centrifugation, pressure differences, a vacuum environment, combinations thereof, or the like. A contact point **CP** is formed between the patterned surface areas **4112** and the substrate **4100**. In other embodiments, liquid material of the droplet 4102 enters the recess 4110 upon dipping the patterned template 4108 into liquid material, upon applying a voltage across the template and the liquid material, by capillary action forces, combinations

thereof, and the like as described herein. Particles **4106** are then formed in the recessed areas **4110** of patterned template **4108**, from the liquid material that entered the recess.

Referring now to Figure 41C, an evaporative process, **E**, is performed, thereby reducing the volume of liquid particles **4106** inside recessed areas **4110**. Examples of an evaporative process **E** that can be used with the present embodiments include forming patterned template **4108** from a gas permeable material, which allows volatile components of the particle precursor material to pass through the template, thereby reducing the volume of the particles precursor material in the recesses. According to another embodiment, an evaporative process **E**, suitable for use with the presently disclosed subject matter includes providing a portion of the recessed areas **4110** filled with a gas, such as nitrogen, which thereby increases the evaporation rate of the material to become the particles. According to futher embodiments, after the recesses are filled with material to become the particles, a space can be left between the patterned template and substrate such that evaporation is enhanced. In yet another embodiment, the combination of the patterned template, substrate, and material to become the particle can be heated or otherwise treated to enhance evaporation of the material to become the particle. Combinations of the above described evaporation processes are encompassed by the presently disclosed subject matter.

Referring now to Figure 41D, once liquid reduction is achieved, the plurality of liquid droplets **4114** are treated by a treating process $\mathbf{T_r}$. Treating process $\mathbf{T_r}$ can be photo curing, thermal curing, phase change, solvent evaporation, crystallization, oxidative/reductive processes, evaporation, combinations thereof, or the like to solidify the material of droplet **4102**.

Referring now to Figure 41E, patterned template **4108** is separated from substrate **4100** according to methods and techniques described herein. After separation of patterned template **4108** from substrate **4100**, treated liquid spherical droplets **4114** are released from patterned template **4108** to provide a plurality of freestanding spherical particles **4116**. In some

embodiments release of the particles **4116** is facilitated by a solvent, applying a substance to the particles with an affinity for the particles, subjecting the particles to gravitational forces, combinations thereof, and the like.

Figures 79A-79C show representative particles fabricated from evaporation techniques of some embodiments of the present invention. According to some embodiments, a dimension of the particles is shown with length bar L, as shown in Figure 79C. According to some embodiments the particles are less than about 200 nm in diameter. According to some embodiments the particles are between about 80 nm and 200 nm in diameter. According to some embodiments the particles are between about 100 nm and about 200 nm in diameter.

IV. Formation of Polymeric Nano- to Micro-Electrets

Referring now to Figures 4A and 4B, in some embodiments, the presently disclosed subject matter describes a method for preparing polymeric nano- to micro-electrets by applying an electric field during the polymerization and/or crystallization step during molding (Figure 4A) to yield a charged polymeric particle (Figure 4B). In one embodiment, the particles are configured to have a predetermined zeta potential. In some embodiments, the charged polymeric particles spontaneously aggregate into chain-like structures (Figure 4D) instead of the random configurations shown in Figure 4C.

In some embodiments, the charged polymeric particle includes a polymeric electret. In some embodiments, the polymeric electret includes a polymeric nano-electret. In some embodiments, the charged polymeric particles aggregate into chain-like structures. In some embodiments, the charged polymeric particles include an additive for an electro-rheological device. In some embodiments, the electro-rheological device is selected from the group including clutches and active dampening devices. In some embodiments, the charged polymeric particles include nano-piezoelectric

devices. In some embodiments, the nano-piezoelectric devices are selected from the group including actuators, switches, and mechanical sensors.

V. Formation of Multilayer Structures

In some embodiments, the presently disclosed subject matter provides a method for forming multilayer structures, including multilayer particles. In some embodiments, the multilayer structures, including multilayer particles, include nanoscale multilayer structures. In some embodiments, multilayer structures are formed by depositing multiple thin layers of immisible liquids and/or solutions onto a substrate and forming particles as described by methods hereinabove. The immiscibility of the liquid can be based on virtually any physical characteristic, including but not limited to density, polarity, and volatility. Examples of possible morphologies of the presently disclosed subject matter are illustrated in Figures 5A-5C and include, but are not limited to, multi-phase sandwich stuctures, core-shell particles, and internal emulsions, microemulsions and/or nano-sized emulsions.

Referring now to Figure 5A, a multi-phase sandwich structure **500** of the presently disclosed subject matter is shown, which by way of example, includes a first liquid material **502** and a second liquid material **504**.

Referring now to Figure 5B, a core-shell particle **506** of the presently disclosed subject matter is shown, which by way of example, includes a first liquid material **502** and a second liquid material **504**.

Referring now to Figure 5C, an internal emulsion particle **508** of the presently disclosed subject matter is shown, which by way of example, includes a first liquid material **502** and a second liquid material **504**.

More particularly, in some embodiments, the method includes disposing a plurality of immiscible liquids between the patterned template and substrate to form a multilayer structure, e.g., a multilayer nanostructure. In some embodiments, the multilayer structure includes a multilayer particle. In some embodiments, the multilayer structure includes a structure selected from the group including multi-phase sandwich structures, core-shell particles, internal emulsions, microemulsions, and nanosized emulsions.

VI. Fabrication of Complex Multi-Dimensional Structures

In some embodiments, the currently disclosed subject matter provides a process for fabricating complex, multi-dimensional structures. In some embodiments, complex multi-dimensional structures can be formed by performing the steps illustrated in Figures 2A-2E. In some embodiments, the method includes imprinting onto a patterned template that is aligned with a second patterned template (instead of imprinting onto a smooth substrate) to generate isolated multi-dimensional structures that are cured and released as described herein. A schematic illustration of an embodiment of a process for forming complex multi-dimensional structures and examples of such structures are provided in Figures 6A-6C.

Referring now to Figure 6A, a first patterned template **600** is provided. First patterned template **600** includes a plurality of recessed areas **602** and a plurality of non-recessed surfaces **604**. Also provided is a second patterned template **606**. Second patterned template **606** includes a plurality of recessed areas **608** and a plurality of non-recessed surfaces **610**. As shown in Figure 6A, first patterned template **600** and second patterned template **606** are aligned in a predetermined spaced relationship. A droplet of liquid material **612** is disposed between first patterned template **600** and second patterned template **606**.

Referring now to Figure 6B, patterned template **600** is contacted with patterned template **606**. A force $\mathbf{F_a}$ is applied to patterned template **600** causing the liquid material including droplet **612** to migrate to the plurality of recessed areas **602** and **608**. The liquid material including droplet **612** is then treated by treating process $\mathbf{T_r}$ to form a patterned, treated liquid material **614**.

Referring now to Figure 6C, the patterned, treated liquid material **614** of Figure 6B is released by the releasing methods described herein to provide a plurality of multi-dimensional patterned structures **616**.

In some embodiments, patterned structure **616** includes a nanoscale-patterned structure. In some embodiments, patterned structure **616** includes a multi-dimensional structure. In some embodiments, the multi-dimensional

structure includes a nanoscale multi-dimensional structure. In some embodiments, the multi-dimensional structure includes a plurality of structural features. In some embodiments, the structural features include a plurality of heights.

In some embodiments, a microelectronic device including patterned structure **616** is provided. Indeed, patterned structure **616** can be virtually any structure, including "dual damscene" structures for microelectronics. In some embodiments, the microelectronic device is selected from the group including integrated circuits, semiconductor particles, quantum dots, and dual damascene structures. In some embodiments, the microelectronic device exhibits certain physical properties selected from the group including etch resistance, low dielectric constant, high dielectric constant, conducting, semiconducting, insulating, porosity, and non-porosity.

In some embodiments, the presently disclosed subject matter discloses a method of preparing a multidimensional, complex structure. Referring now to Figures 7A-7F, in some embodiments, a first patterned template **700** is provided. First patterned template **700** includes a plurality of non-recessed surface areas **702** and a plurality of recessed surface areas **704**. Continuing particularly with Figure 7A, also provided is a substrate **706**. In some embodiments, substrate **706** is coated with a non-wetting agent **708**. A droplet of a first liquid material **710** is disposed on substrate **706**.

Referring now to Figures 7B and 7C, first patterned template **700** is contacted with substrate **706**. A force $\mathbf{F_a}$ is applied to first patterned template **700** such that the droplet of the first liquid material **710** is forced into recesses **704**. The liquid material including the droplet of first liquid material **710** is treated by a first treating process $\mathbf{T_{r1}}$ to form a treated first liquid material within the plurality of recesses **704**. In some embodiments, first treating process $\mathbf{T_{r1}}$ includes a partial curing process causing the treated first liquid material to adhere to substrate **706**. Referring particularly to Figure 7C, first patterned template **700** is removed to provide a plurality of structural features **712** on substrate **706**.

Referring now to Figures 7D-7F, a second patterned template **714** is provided. Second patterned substrate **714** includes a plurality of recesses **716**, which are filled with a second liquid material **718**. The filling of recesses **716** can be accomplished in a manner similar to that described in Figures 7A and 7B with respect to recesses **704**. Referring particularly to Figure 7E, second patterned template **714** is contacted with structural features **712**. Second liquid material **718** is treated with a second treating process T_{r2} such that the second liquid material **718** adheres to the plurality of structural feature **712**, thereby forming a multidimensional structure **720**. Referring particularly to Figure 7F, second patterned template **714** and substrate **706** are removed, providing a plurality of free-standing multidimensional structures **722**. In some embodiments, the process schematically presented in Figures 7A-7F can be carried out multiple times as desired to form intricate nanostructures.

Accordingly, in some embodiments, a method for forming multidimensional structures is provided, the method including:

(a) providing a particle prepared by the process described in the figures;

(b) providing a second patterned template;

(c) disposing a second liquid material in the second patterned template;

(d) contacting the second patterned template with the particle of step (a); and

(e) treating the second liquid material to form a multidimensional structure.

VII. Functionalization of Particles

In some embodiments, the presently disclosed subject matter provides a method for functionalizing isolated micro- and/or nanoparticles. In one embodiment, the functionalization includes introducing chemical functional groups to a surface either physically or chemically. In some

embodiments, the method of functionalization includes introducing at least one chemical functional group to at least a portion of microparticles and/or nanoparticles. In some embodiments, particles **3605** are at least partially functionalized while particles **3605** are in contact with an article **3600**. In one embodiment, the particles **3605** to be functionalized are located within a mold or patterned template **108** (Figs. 35A – 36D). In some embodiments, particles **3605** to be functionalized are attached to a substrate (e.g., substrate **4010** of Figs. 40A – 40D). In some embodiments, at least a portion of the exterior of the particles **3605** can be chemically modified by performing the steps illustrated in Figures 36A – 36D. In one embodiment, the particles **3605** to be functionalized are located within article **3600** as illustrated in Fig. 36A and 40A. As illustrated in Figures 36A-36D and 40A - 40D, some embodiments include contacting an article **3600** containing particles **3605** with a solution **3602** containing a modifying agent **3604**.

In one embodiment, illustrated in Figures 36C and 40C, modifying agent **3604** attaches (e.g., chemically) to exposed particle surface **3606** by chemically reacting with or physically adsorbing to a linker group on particle surface **3606**. In one embodiment, the linker group on particle **3606** is a chemical functional group that can attach to other species via chemical bond formation or physical affinity. In some embodiments, modifying agents **3611** are contained within or partially within particles **3605**. In some embodiments, the linker group includes a functional group that includes, without limitation, sulfides, amines, carboxylic acids, acid chlorides, alcohols, alkenes, alkyl halides, isocyanates, compounds disclosed elsewhere herein, combinations thereof, or the like.

In one embodiment, illustrated in Fig. 36D and 40D, excess solution is removed from article **3600** while particle **3605** remains in communication with article **3600**. In some embodiments, excess solution is removed from the surface containing the particles. In some embodiments, excess solution is removed by rinsing with or soaking in a liquid, by applying an air stream, or by physically shaking or scraping the surface. In some embodiments, the modifying agent includes an agent selected from the group including dyes, fluorescent tags, radiolabeled tags, contrast agents, ligands, peptides,

pharmaceutical agents, proteins, DNA, RNA, siRNA, compounds and materials disclosed elsewhere herein, combinations thereof, and the like.

In one embodiment, functionalized particles **3608**, **4008** are harvested from article **3600** using, for example, methods described herein. In some embodiments, functionalizing and subsequently harvesting particles that reside on an article (*e.g.*, a substrate, a mold or patterned template) have advantages over other methods (*e.g.*, methods in which the particles must be functionalized while in solution). In one embodiment of the presently disclosed subject matter, fewer particles are lost in the process, giving a high product yield. In one embodiment of the presently disclosed subject matter, a more concentrated solution of the modifying agent can be applied in lower volumes. In one embodiment of the presently disclosed subject matter, where particles are functionalized while they remain associated with article **3600**, functionalization does not need to occur in a dilute solution. In one embodiment, the use of more concentrated solution facilitates, for example, the use of lower volumes of modifying agent and/or lower times to functionalize. According to another embodiment, the functionalized particles are uniformly functionalized and each has substantially an identical physical load. In some embodiments, particles in a tight, 2-dimensional array, but not touching, are susceptible to application of thin, concentrated solutions for faster functionalization. In some embodiments, lower volume/higher concentration modifying agent solutions are useful, for example, in connection with modifying agents that are difficult and expensive to make and handle (*e.g.*, biological agents such as peptides, DNA, or RNA). In some embodiments, functionalizing particles that remain connected to article **3600** eliminates difficult and/or time-consuming steps to remove excess unreacted material (*e.g.*, dialysis, extraction, filtration and column separation). In one embodiment of the presently disclosed subject matter, highly pure functionalized product can be produced at a reduced effort and cost. Because the particles are molded in a substantially inert polymer mold, the contents of the particle can be controlled, thereby yielding a highly pure (*e.g.*, greater than 95%) functionalized product.

VIII. Imprint Lithography

Referring now to Figures 8A-8D, a method for forming a pattern on a substrate is illustrated. In the embodiment illustrated in Figure 8, an imprint lithography technique is used to form a pattern on a substrate.

Referring now to Figure 8A, a patterned template **810** is provided. In some embodiments, patterned template **810** includes a solvent resistant, low surface energy polymeric material, derived from casting low viscosity liquid materials onto a master template and then curing the low viscosity liquid materials to generate a patterned template as defined hereinabove. In some embodiments, patterned template **810** can further include a first patterned template surface **812** and a second template surface **814**. First patterned template surface **812** further includes a plurality of recesses **816**. The patterned template derived from a solvent resistant, low surface energy polymeric material can then be mounted on another material to facilitate alignment of the patterned template or to facilitate continuous processing such as a conveyor belt, which can be particularly useful in some embodiments, such as for example in the fabrication of precisely placed structures on a surface, such as in the fabrication of a complex devices, a semiconductor, electronic devices, photonic devices, combinations thereof, and the like.

Referring again to Figure 8A, a substrate **820** is provided. Substrate **820** includes a substrate surface **822**. In some embodiments, substrate **820** is selected from the group including a polymer material, an inorganic material, a silicon material, a quartz material, a glass material, and surface treated variants thereof. In some embodiments, at least one of patterned template **810** and substrate **820** has a surface energy lower than 18 mN/m. In some embodiments, at least one of patterned template **810** and substrate **820** has a surface energy lower than 15 mN/m. According to a further embodiment the patterned template **810** and/or the substrate **820** has a surface energy between about 10 mN/m and about 20 mN/m. According to some embodiments, the patterned template **810** and/or the substrate **820** has a low surface energy of between about 12 mN/m and about 15 mN/m. In some embodiments, the material is PFPE.

In some embodiments, as illustrated in Figure 8A, patterned template **810** and substrate **820** are positioned in a spaced relationship to each other such that first patterned template surface **812** faces substrate surface **822** and a gap **830** is created between first patterned template surface **812** and substrate surface **822**. This is an example of a predetermined relationship.

Referring now to Figure 8B, a volume of liquid material **840** is disposed in gap **830** between first patterned template surface **812** and substrate surface **822**. In some embodiments, the volume of liquid material **840** is disposed directed on a non-wetting agent, which is disposed on first patterned template surface **812**.

Referring now to Figure 8C, in some embodiments, first patterned template **812** is contacted with the volume of liquid material **840**. In some embodiments, a force $\mathbf{F_a}$ is applied to second template surface **814** thereby forcing the volume of liquid material **840** into the plurality of recesses **816**. In some embodiments, as illustrated in Figure 8C, a portion of the volume of liquid material **840** remains between first patterned template surface **812** and substrate surface **820** after force $\mathbf{F_a}$ is applied.

Referring again to Figure 8C, in some embodiments, the volume of liquid material **840** is treated by a treating process $\mathbf{T_r}$ while force $\mathbf{F_a}$ is being applied to form a treated liquid material **842**. In some embodiments, treating process $\mathbf{T_r}$ includes a process selected from the group including a thermal process, a photochemical process, and a chemical process.

Referring now to Figure 8D, a force $\mathbf{F_r}$ is applied to patterned template **810** to remove patterned template **810** from treated liquid material **842** to reveal a pattern **850** on substrate **820** as shown in Figure 8E. In some embodiments, a residual, or "scum," layer **852** of treated liquid material **842** remains on substrate **820**.

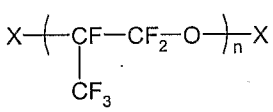
More particularly, a method for forming a pattern on a substrate can include (a) providing patterned template and a substrate, where the patterned template includes a patterned template surface having a plurality of recessed areas formed therein. Next, a volume of liquid material is disposed in or on at least one of: (i) the patterned template surface; (ii) the plurality of recessed areas; and (iii) the substrate. Next, the patterned

template surface is contacted with the substrate, and the liquid material is treated to form a pattern on the substrate.

In some embodiments, the patterned template includes a solvent resistant, low surface energy polymeric material derived from casting low viscosity liquid materials onto a master template and then curing the low viscosity liquid materials to generate a patterned template. In some embodiments, the patterned template includes a solvent resistant elastomeric material.

In some embodiments, at least one of the patterned template and substrate includes a material selected from the group including a perfluoropolyether material, a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction.

In some embodiments, the perfluoropolyether material includes a backbone structure selected from the group including: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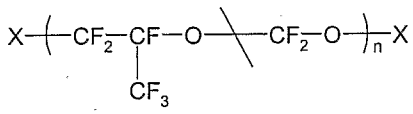
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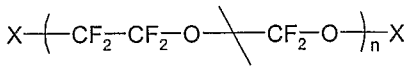
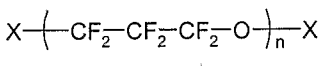
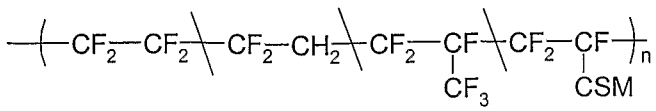
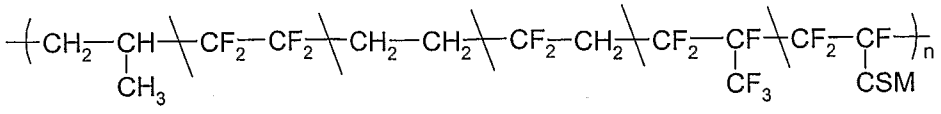

, and

;

wherein X is present or absent, and when present includes an endcapping group.

In some embodiments, the fluoroolefin material is selected from the group including:


,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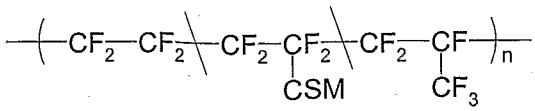
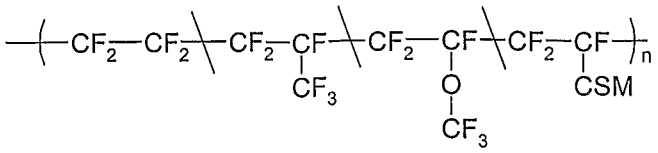
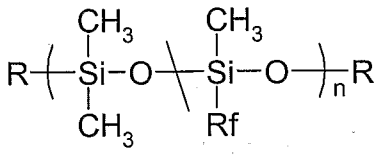

, and



wherein CSM includes a cure site monomer.

In some embodiments, the fluoroolefin material is made from monomers which include tetrafluoroethylene, vinylidene fluoride, hexafluoropropylene, 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole, a functional fluoroolefin, functional acrylic monomer, and a functional methacrylic monomer.

In some embodiments, the silicone material includes a fluoroalkyl functionalized polydimethylsiloxane (PDMS) having the following structure:

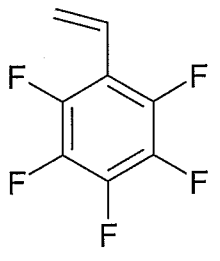


wherein:

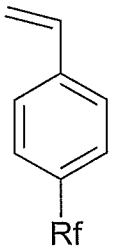
R is selected from the group including an acrylate, a methacrylate, and a vinyl group; and

Rf includes a fluoroalkyl chain.

In some embodiments, the styrenic material includes a fluorinated styrene monomer selected from the group including:

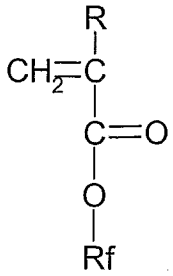


and



wherein Rf includes a fluoroalkyl chain.

In some embodiments, the acrylate material includes a fluorinated acrylate or a fluorinated methacrylate having the following structure:



wherein:

R is selected from the group including H, alkyl, substituted alkyl, aryl, and substituted aryl; and

Rf includes a fluoroalkyl chain.

In some embodiments, the triazine fluoropolymer includes a fluorinated monomer.

In some embodiments, the fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction includes a functionalized olefin. In some embodiments, the functionalized olefin includes a functionalized cyclic olefin.

In some embodiments, at least one of the patterned template and the substrate has a surface energy lower than 18 mN/m. In some embodiments, at least one of the patterned template and the substrate has a surface energy lower than 15 mN/m. According to a further embodiment the patterned template and/or the substrate has a surface energy between about 10 mN/m and about 20 mN/m. According to some embodiments, the patterned template and/or the substrate has a low surface energy of between about 12 mN/m and about 15 mN/m. In some embodiments the material is PFPE, a PFPE derivative, or partially composed of PFPE.

In some embodiments, the substrate is selected from the group including a polymer material, an inorganic material, a silicon material, a quartz material, a glass material, and surface treated variants thereof. In some embodiments, the substrate is selected from one of an electronic device in the process of being manufactured and a photonic device in the process of being manufactured. In some embodiments, the substrate includes a patterned area.

In some embodiments, the plurality of recessed areas can include a plurality of cavities. In some embodiments, the plurality of cavities includes a plurality of structural features. In some embodiments, the plurality of structural features has a dimension ranging from about 10 microns to about 1 nanometer in size. In some embodiments, the plurality of structural features has a dimension ranging from about 10 microns to about 1 micron in size. In some embodiments, the plurality of structural features has a dimension ranging from about 1 micron to about 100 nm in size. In some embodiments, the plurality of structural features has a dimension ranging from about 100 nm to about 1 nm in size. In some embodiments, the plurality of structural features has a dimension in both the horizontal and vertical plane.

Referring now to Figures 39A-39F, one embodiment of a method for forming a complex pattern on a substrate is illustrated. In the embodiment illustrated in Figure 39, an imprint lithography technique is used to form a pattern on a substrate.

Referring now to Figure 39A, a patterned master **3900** is provided. Patterned master **3900** includes a plurality of non-recessed surface **3920** areas and a plurality of recesses **3930**. In some embodiments, recesses **3930** include one or more sub-recesses **3932**. In some embodiments, recesses **3930** include a multiplicity of sub-recesses **3932**. In some embodiments, patterned master **3900** includes an etched substrate, such as a silicon wafer, which is etched in the desired pattern to form patterned master **3900**.

Referring now to Figure 39B, a flowable material **3901**, for example, a liquid fluoropolymer composition, such as a PFPE-based precursor, is poured onto patterned master **3900**. In some embodiments, flowable material **3901** is treated by a treating process, for example exposure to UV light, thereby forming a treated material mold **3910** in the desired pattern.

In one embodiment, illustrated in Figure 39C, mold **3910** is removed from patterned master **3900**. In one embodiment, treated material mold **3910** is a cross-linked polymer. In one embodiment, treated material mold **3910** is an elastomer. In one embodiment, a force is applied to one or more

of mold **3910** or patterned master **3900** to separate mold **3910** from patterned master **3900**. Figure 39C illustrates one embodiment of mold **3910** and patterned master **3900** wherein mold **3910** includes a plurality of recesses and sub-recesses that are mirror images of the plurality of non-recessed surface areas of patterned master **3900**. In one embodiment of mold **3910** the plurality of non-recessed areas elastically deform to facilitate removal of mold **3910** from master **3900**. Mold **3910**, in one embodiment, is a useful patterned template for soft lithography and imprint lithography applications.

Referring now to Figure 39D, a mold **3910** is provided. In some embodiments, mold **3910** includes a solvent resistant, low surface energy polymeric material, derived from casting low viscosity liquid materials onto a master template and then curing the low viscosity liquid materials to generate a patterned template as defined hereinabove. Mold **3910** further includes a first patterned template surface **812** and a second template surface **814**. The first patterned template surface **812** further includes a plurality of recesses **816** and subrecesses **3942**. In one embodiment, multiple layers of subrecesses **3942** form sub-sub-recesses and so on. In some embodiments, mold **3910** is derived from a solvent resistant, low surface energy polymeric material and is mounted on another material to facilitate alignment of the mold or to facilitate continuous processing, such as a continuous process using a roll-to-roll or conveyor belt type mechanism. In one emboidment, such continuous processing is useful in the fabrication of precisely placed structures on a surface, such as in the fabrication of a complex device or a semiconductor, electronic or photonic device.

Referring again to Figure 39D, a substrate **3903** is provided. In some embodiments, substrate **3903** includes, without limitation, one or more of a polymer material, an inorganic material, a silicon material, a quartz material, a glass material, and surface treated variants thereof. In some embodiments, at least one of mold **3910** and substrate **3903** has a surface energy lower than 18 mN/m. In some embodiments, at least one of mold **3910** and substrate **3903** has a surface energy lower than 15 mN/m. According to a further embodiment the mold **3910** and/or the substrate **3903**

has a surface energy between about 10 mN/m and about 20 mN/m. According to some embodiments, the mold **3910** and/or the substrate **3903** has a low surface energy of between about 12 mN/m and about 15 mN/m.

In some embodiments, as illustrated in Figure 39D, mold **3910** and substrate **3903** are positioned in a spaced relationship to each other such that first patterned template surface **812** faces substrate surface **822** and a gap **830** is created between first patterned template surface **812** and the substrate surface **822**. This is merely one example of a predetermined relationship.

Referring again to Figure 39D, a volume of liquid material **3902** is disposed in the gap between first patterned template surface **812** and substrate surface **822**. In some embodiments, the volume of liquid material **3902** is disposed directly on a non-wetting agent, which is disposed on first patterned template surface **812**.

Referring now to Figure 39E, in some embodiments, mold **3910** is contacted with the volume of liquid material **3902** (not shown in Fig. 39E) . A force **F** is applied to the mold **3910** thereby forcing the volume of liquid material **3902** into the plurality of recesses **816** and sub-recesses . In some embodiments, such as was illustrated in Figure 8C, a portion of the volume of liquid material **3902** remains between mold **3910** and substrate **3903** surface after force **F** is applied.

Referring again to Figure 39E, in some embodiments, the volume of liquid material **3902** is treated by a treating process while force **F** is being applied to form a product **3904**. In some embodiments, the treating process includes, without limitation, one or more of a photochemical process, a chemical process, a thermal process, combinations thereof, or the like.

Referring now to Figure 39F, mold **3910** is removed from product **3904** to reveal a patterned product on substrate **3903** as shown in Figure 39F. In some embodiments, a residual, or "scum," layer of treated liquid material remains on substrate **3903**.

In some embodiments, the liquid material from which the particles will be formed, or particle precursor, is selected from the group including a polymer, a solution, a monomer, a plurality of monomers, a polymerization

initiator, a polymerization catalyst, an inorganic precursor, an organic material, a natural product, a metal precursor, a pharmaceutical agent, a tag, a magnetic material, a paramagnetic material, a superparamagnetic material, a ligand, a cell penetrating peptide, a porogen, a surfactant, a plurality of immiscible liquids, a solvent, a pharmaceutical agent with a binder, a charged species, combinations thereof, and the like. In some embodiments, the pharmaceutical agent is selected from the group including a drug, a peptide, RNAi, DNA, combinations thereof, and the like. In some embodiments, the tag is selected from the group including a fluorescence tag, a radiolabeled tag, a contrast agent, combinations thereof, and the like. In some embodiments, the ligand includes a cell targeting peptide.

Representative superparamagnetic or paramagnetic materials include but are not limited to Fe_2O_3, Fe_3O_4, FePt, Co, $MnFe_2O_4$, $CoFe_2O_4$, $CuFe_2O_4$, $NiFe_2O_4$ and ZnS doped with Mn for magneto-optical applications, CdSe for optical applications, borates for boron neutron capture treatment, combinations thereof, and the like.

In some embodiments, the liquid material is selected from one of a resist polymer and a low-k dielectric. In some embodiments, the liquid material includes a non-wetting agent.

In some embodiments, the disposing of the volume of liquid material is regulated by a spreading process. In some embodiments, the spreading process includes disposing a first volume of liquid material on the patterned template to form a layer of liquid material on the patterned template, and drawing an implement across the layer of liquid material to remove a second volume of liquid material from the layer of liquid material on the patterned template and leave a third volume of liquid material on the patterned template.

In some embodiments, the contacting of the first template surface with the substrate eliminates essentially all of the disposed volume of liquid material. In some embodiments, the treating of the liquid includes, without limitation, one or more of a thermal process, a photochemical process, a chemical process, an evaporative process, a phase change, an oxidative process, a reductive process, combinations thereof, or the like. In some

embodiments, the method includes a batch process. In some embodiments, the batch process is selected from one of a semi-batch process and a continuous batch process. In some embodiments, the presently disclosed subject matter describes a patterned substrate formed by the presently disclosed methods.

VIII.A. Methods for Fabrication by Imprint Lithography

According to other embodiments, the liquid material can be introduced to the patterned template and the recesses formed therein by one of or a combination of the following techniques. In some embodiments, the recesses of the patterned templates can be configured to receive a predetermined substance to be molded. According to such embodiments, variables such as, for example, the surface energy of the patterned template, the volume of the recess, the permeability of the patterned template, the viscosity of the substance to be molded, the relative energies between the template surface and the substance to be molded, as well as other physical and chemical properties of the substance to be molded interact and affect the readiness of reception of the substance to be molded into the recess.

VIII.A.i. Passive Mold Filling

Referring now to FIG. 50, in some embodiments a substance **5000** to be molded is introduced to a patterned template **5002**. Substance **5000** can be introduced to patterned template **5002** as a droplet, by spin coating, a liquid stream, a doctor blade, or the like. Patterned template **5002** includes recesses **5012** and can be fabricated, according to methods disclosed herein, from materials disclosed herein such as, for example, low surface energy polymeric materials. Because patterned template **5002** is fabricated from low surface energy polymeric materials, substance **5000** does not wet the surface of patterned template **5002**, however, substance **5000** fills recesses **5012**. Next, a treatment **5008**, such as treatments disclosed herein, is applied to substance **5000** to cure substance **5000**. According to some embodiments, treatment **5008** can be, for example, photo-curing, thermal curing, oxidative curing, reductive curing, combinations thereof, evaporation, and the like.

In some embodiments, the plurality of recessed areas includes a plurality of cavities. In some embodiments, the plurality of cavities includes a plurality of structural features. In some embodiments, the plurality of structural features have a dimension ranging from about 10 microns to about 1 nanometer in size. In some embodiments, the plurality of structural features have a dimension ranging from about 1 micron to about 100 nm in size. In some embodiments, the plurality of structural features have a dimension ranging from about 100 nm to about 1 nm in size. In some embodiments, the plurality of structural features have a dimension in both the horizontal and vertical plane.

VIII.A.ii. Dipping Mold Filling

According to some embodiments, the patterned template is dipped into the substance to be molded, as shown in FIG. 51. Referring to FIG. 51, patterned template **5104** is submerged into a volume of substance **5102**. Substance **5102** enters recesses **5106** and following removal of patterned template **5104** from substance **5102**, substance **5108** remains in recesses **5106** of patterned template **5104**.

VIII.A.iii. Moving Droplet Mold Filling

According to some embodiments, the patterned template can be positioned on an angle, as shown in FIG. 52. A volume of material to be fabricated **5204** is introduced onto the surface of patterned template **5200** that includes recesses **5206**. The volume of material to be fabricated 5204 travels down the sloped surface of patterned template **5200**. As the volume of material to be fabricated **5204** travels over recesses **5206**, subvolumes of material to be fabricated **5208** enter and fill recesses **5206**. According to some embodiments, patterned template **5200** can be positioned at about a 20 degree angle from the horizontal. According to some embodiments, the liquid can be moved by a doctor blade.

VIII.A.iv. Voltage Assist Filling

According to some embodiments, a voltage can assist in introducing a material to be fabricated into recesses in a patterned template. Referring to FIG. 53, a patterned template **5300** having recesses **5302** on a surface

thereof can be positioned on an electrode surface **5308**. A volume of material to be fabricated **5304** can be introduced onto the recess surface of patterned template **5300**. Material to be fabricated **5304** can also be in communication with an opposite electrode **5306** to electrode **5308** that is in communication with patterned template **5300**. The voltage difference between electrodes **5306** and **5308** travels through material to be fabricated **5304** and patterned template **5300**. The voltage difference alters the wetting angle of material to be fabricated **5304** with respect to patterned template **5300** and, thereby, facilitating entry of material to be fabricated **5304** into recesses **5302**. In some embodiments, electrode **5306**, in communication with material to be fabricated **5304**, is moved across the surface of patterned template **5300** thereby facilitating filling of recesses **5302** across the surface of patterned template **5300**.

According to some embodiments, patterned template **5300** and material to be fabricated **5304** are subjected to about 3000 DC volts, however, the voltage applied to a combination of patterned template and material to be fabricated can be tailored to the specific requirements of the combinations. In some embodiments, the voltage is altered to arrive at a preferred contact angle between material to be fabricated and patterned template to facilitate entry of material to be fabricated into the recesses of the patterned template.

VIII.B. Thermodynamics of Recess Filling

Recesses in a patterned template, such as recesses **5012** in patterned template **5002** of FIG. 50 can be configured to receive a substance for imprint lithography. The physical and chemical characteristics of both the recess and the particular substance to be molded can be configured to increase how readily the substance is received by the recess. Factors that can influence the filling of a recess include, but are not limited to, recess volume, diameter, surface area, surface energy, contact angle between a substance to be molded and the material of the recess, voltage applied across a substance to be molded, temperature, environmental conditions surrounding the patterned template such as for example the

removal of oxygen or impurities from the atmosphere, combinations thereof, and the like. In some embodiments, a recess that is about 2 micron in diameter has a capillary pressure of about 1 atmosphere. In some embodiments, a recess with a diameter of about 200 nm has a capillary pressure of about 10 atmospheres.

IX. Imprint Lithography Free of a Residual "Scum Layer"

A characteristic of imprint lithography that has restrained its full potential is the formation of a "scum layer" once the liquid material, e.g., a resin, is patterned. The "scum layer" includes residual liquid material that remains between the stamp and the substrate. In some embodiments, the presently disclosed subject matter provides a process for generating patterns essentially free of a scum layer.

Referring now to Figures 9A-9E, in some embodiments, a method for forming a pattern on a substrate is provided, wherein the pattern is essentially free of a scum layer. Referring now to Figure 9A, a patterned template **910** is provided. Patterned template **910** further includes a first patterned template surface **912** and a second template surface **914**. The first patterned template surface **912** further includes a plurality of recesses **916**. In some embodiments, a non-wetting agent **960** is disposed on the first patterned template surface **912**.

Referring again to Figure 9A, a substrate **920** is provided. Substrate **920** includes a substrate surface **922**. In some embodiments, a non-wetting agent **960** is disposed on substrate surface **920**.

In some embodiments, as illustrated in Figure 9A, patterned template **910** and substrate **920** are positioned in a spaced relationship to each other such that first patterned template surface **912** faces substrate surface **922** and a gap **930** is created between first patterned template surface **912** and substrate surface **922**.

Referring now to Figure 9B, a volume of liquid material **940** is disposed in the gap **930** between first patterned template surface **912** and substrate surface **922**. In some embodiments, the volume of liquid material **940** is disposed directly on first patterned template surface **912**. In some

embodiments, the volume of liquid material **940** is disposed directly on non-wetting agent **960**, which is disposed on first patterned template surface **912**. In some embodiments, the volume of liquid material **940** is disposed directly on substrate surface **920**. In some embodiments, the volume of liquid material **940** is disposed directly on non-wetting agent **960**, which is disposed on substrate surface **920**.

Referring now to Figure 9C, in some embodiments, first patterned template surface **912** is contacted with the volume of liquid material **940**. A force $\mathbf{F_a}$ is applied to second template surface **914** thereby forcing the volume of liquid material **940** into the plurality of recesses **916**. In contrast with the embodiment illustrated in Figure 8, a portion of the volume of liquid material **940** is forced out of gap **930** by force $\mathbf{F_o}$ when force $\mathbf{F_a}$ is applied.

Referring again to Figure 9C, in some embodiments, the volume of liquid material **940** is treated by a treating process $\mathbf{T_r}$ while force $\mathbf{F_a}$ is being applied to form a treated liquid material **942**.

Referring now to Figure 9D, a force $\mathbf{F_r}$ is applied to patterned template **910** to remove patterned template **910** from treated liquid material **942** to reveal a pattern **950** on substrate **920** as shown in Figure 9E. In this embodiment, substrate **920** is essentially free of a residual, or "scum," layer of treated liquid material **942**.

In some embodiments, at least one of the template surface and substrate includes a functionalized surface element. In some embodiments, the functionalized surface element is functionalized with a non-wetting material. In some embodiments, the non-wetting material includes functional groups that bind to the liquid material. In some embodiments, the non-wetting material is a trichloro silane, a trialkoxy silane, a trichloro silane including non-wetting and reactive functional groups, a trialkoxy silane including non-wetting and reactive functional groups, and/or mixtures thereof.

In some embodiments, the point of contact between the two surface elements is free of liquid material. In some embodiments, the point of contact between the two surface elements includes residual liquid material. In some embodiments, the height of the residual liquid material is less than

30% of the height of the structure. In some embodiments, the height of the residual liquid material is less than 20% of the height of the structure. In some embodiments, the height of the residual liquid material is less than 10% of the height of the structure. In some embodiments, the height of the residual liquid material is less than 5% of the height of the structure. In some embodiments, the volume of liquid material is less than the volume of the patterned template. In some embodiments, substantially all of the volume of liquid material is confined to the patterned template of at least one of the surface elements. In some embodiments, having the point of contact between the two surface elements free of liquid material retards slippage between the two surface elements.

X. Solvent-Assisted Micro-molding (SAMIM)

In some embodiments, the presently disclosed subject matter describes a solvent-assisted micro-molding (SAMIM) method for forming a pattern on a substrate.

Referring now to Figure 10A, a patterned template **1010** is provided. Patterned template **1010** further includes a first patterned template surface **1012** and a second template surface **1014**. The first patterned template surface **1012** further includes a plurality of recesses **1016**.

Referring again to Figure 10A, a substrate **1020** is provided. Substrate **1020** includes a substrate surface **1022**. In some embodiments, a polymeric material **1070** is disposed on substrate surface **1022**. In some embodiments, polymeric material **1070** includes a resist polymer.

Referring again to Figure 10A, patterned template **1010** and substrate **1020** are positioned in a spaced relationship to each other such that first patterned template surface **1012** faces substrate surface **1022** and a gap **1030** is created between first patterned template surface **1012** and substrate surface **1022**. As shown in Figure 10A, a solvent **S** is disposed within gap **1030**, such that solvent **S** contacts polymeric material **1070** forming a swollen polymeric material **1072**.

Referring now to Figures 10B and 10C, first patterned template surface **1012** is contacted with swollen polymeric material **1072**. A force $\mathbf{F_a}$ is applied to second template surface **1014** thereby forcing a portion of swollen polymeric material **1072** into the plurality of recesses **1016** and leaving a portion of swollen polymeric material **1072** between first patterned template surface **1012** and substrate surface **1020**. The swollen polymeric material **1072** is then treated by a treating process $\mathbf{T_r}$ while under pressure.

Referring now to Figure 10D, a force $\mathbf{F_r}$ is applied to patterned template **1010** to remove patterned template **1010** from treated swollen polymeric material **1072** to reveal a polymeric pattern **1074** on substrate **1020** as shown in Figure 10E.

XI. Removing/Harvesting the Patterned Structures from the Patterned Template and/or Substrate

In some embodiments, the patterned structure (e.g., a patterned micro- or nanostructure) is removed from at least one of the patterned template and/or the substrate. This can be accomplished by a number of approaches, including but not limited to applying the surface element containing the patterned structure to a surface that has an affinity for the patterned structure; applying the surface element containing the patterned structure to a material that when hardened has a chemical and/or physical interaction with the patterned structure; deforming the surface element containing the patterned structure such that the patterned structure is released from the surface element; swelling the surface element containing the patterned structure with a first solvent to extrude the patterned structure; and washing the surface element containing the patterned structure with a second solvent that has an affinity for the patterned structure.

In some embodiments, a surface has an affinity for the particles. The affinity of the surface can be a result of, in some embodiments, an adhesive or sticky surface, such as for example but not limitation, carbohydrates, epoxies, waxes, polyvinyl alcohol, polyvinyl pyrrolidone, polybutyl acrylate, polycyano acrylates, polyhydroxyethyl methacrylate, polymethyl methacrylate, combinations thereof, and the like. In some embodiments, the

liquid is water that is cooled to form ice. In some embodiments, the water is cooled to a temperature below the Tm of water but above the Tg of the particle. In some embodiments the water is cooled to a temperature below the Tg of the particles but above the Tg of the mold or substrate. In some embodiments, the water is cooled to a temperature below the Tg of the mold or substrate.

In some embodiments, the first solvent includes supercritical fluid carbon dioxide. In some embodiments, the first solvent includes water. In some embodiments, the first solvent includes an aqueous solution including water and a detergent. In embodiments, the deforming the surface element is performed by applying a mechanical force to the surface element. In some embodiments, the method of removing the patterned structure further includes a sonication method.

According to yet another embodiment the particles are harvested on a fast dissolving substrate, sheet, or films. The film-forming agents can include, but are not limited to pullulan, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, polyvinyl pyrrolidone, carboxymethyl cellulose, polyvinyl alcohol, sodium alginate, polyethylene glycol, xanthan gum, tragacanth gum, guar gum, acacia gum, arabic gum, polyacrylic acid, methylmethacrylate copolymer, carboxyvinyl polymer, amylose, high amylose starch, hydroxypropylated high amylose starch, dextrin, pectin, chitin, chitosan, levan, elsinan, collagen, gelatin, zein, gluten, soy protein isolate, whey protein isolate, casein, combinations thereof, and the like. In some embodiments, pullulan is used as the primary filler. In still other embodiments, pullulan is included in amounts ranging from about 0.01 to about 99 wt %, preferably about 30 to about 80 wt %, more preferably from about 45 to about 70 wt %, and even more preferably from about 60 to about 65 wt % of the film.

The film can further include water, plasticizing agents, natural and/or artificial flavoring agents, sulfur precipitating agents, saliva stimulating agents, cooling agents, surfactants, stabilizing agents, emulsifying agents, thickening agents, binding agents, coloring agents, sweeteners, fragrances, combinations thereof, and the like.

Suitable sweeteners include both natural and artificial sweeteners. Examples of some sweeteners that can be used with the sheets of the presently disclosed subject matter include, but are not limited to: (a) water-soluble sweetening agents, such as monosaccharides, disaccharides and polysaccharides such as xylose, ribose, glucose (dextrose), mannose, galactose, fructose (levulose), sucrose (sugar), maltose, invert sugar (a mixture of fructose and glucose derived from sucrose), partially hydrolyzed starch, corn syrup solids, dihydrochalcones, monellin, steviosides, and glycyrrhizin; (b) water-soluble artificial sweeteners, such as the soluble saccharin salts, sodium or calcium saccharin salts, cyclamate salts, the sodium, ammonium or calcium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2, 2-dioxide, the potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide (acesulfame-K), the free acid form of saccharin, and the like; (c) dipeptide based sweeteners, such as L-aspartic acid derived sweeteners, L-aspartyl-L-phenylalanine methyl ester (aspartame) and materials described in U.S. Pat. No. 3,492,131, which is incorporated herein by reference in its entirety, L-alpha-aspartyl-N-(2,2,4,4-tetramethyl-3-thietanyl)-D-alaninamide hydrate, methyl esters of L-aspartyl-L-phenylglycerin and L-aspartyl-L-2,5,dihydrophenyl-glycine, L-aspartyl-2,5-dihydro-L-phenylalanine, L-aspartyl-L-(1-cyclohexyen)-alanine, and the like; (d) water-soluble sweeteners derived from naturally occurring water-soluble sweeteners, such as a chlorinated derivative of ordinary sugar (sucrose); and (e) protein based sweeteners, such as thaumatoccous danielli (Thaumatin I and II) and the like.

In general, an effective amount of auxiliary sweetener is utilized to provide the level of sweetness desired for a particular composition, and this amount will vary with the sweetener selected. The amount will normally be between about 0.01% to about 10% by weight of the composition when using an easily extractable sweetener. The water-soluble sweeteners described in category (a) above, are usually used in amounts of between about 0.01 to about 10 wt %, and preferably in amounts of between about 2 to about 5 wt %. The sweeteners described in categories (b)-(e) are generally used in amounts of between about 0.01 to about 10 wt %, with

between about 2 to about 8 wt % being preferred and between about 3 to about 6 wt % being most preferred. These amounts can be used to achieve a desired level of sweetness independent from the flavor level achieved from optional flavor oils used. Of course, sweeteners need not be added to films intended for non-oral administration.

The flavorings that can be used in the films include natural and artificial flavors. These flavorings can be chosen from synthetic flavor oils and flavoring aromatics, and/or oils, oleo resins and extracts derived from plants, leaves, flowers, fruits, combinations thereof, and the like. Representative flavor oils include: spearmint oil, cinnamon oil, peppermint oil, clove oil, bay oil, thyme oil, cedar leaf oil, oil of nutmeg, oil of sage, and oil of bitter almonds. Also useful are artificial, natural or synthetic fruit flavors, such as vanilla, chocolate, coffee, cocoa and citrus oil, including lemon, orange, grape, lime and grapefruit, and fruit essences including apple, pear, peach, strawberry, raspberry, cherry, plum, pineapple, apricot and so forth. These flavorings can be used individually or in admixture. Flavorings such as aldehydes and esters including cinnamyl acetate, cinnamaldehyde, citral, diethylacetal, dihydrocarvyl acetate, eugenyl formate, p-methylanisole, and so forth also can be used. Generally, any flavoring or food additive can be used, such as those described in Chemicals Used in Food Processing, publication 1274 by the National Academy of Sciences, pages 63-258, which is incorporated herein by reference in its entirety. Further examples of aldehyde flavorings include, but are not limited to, acetaldehyde (apple); benzaldehyde (cherry, almond); cinnamic aldehyde (cinnamon); citral, *i.e.*, alpha citral (lemon, lime); neral, *i.e.* beta citral (lemon, lime); decanal (orange, lemon); ethyl vanillin (vanilla, cream); heliotropine, *i.e.*, piperonal (vanilla, cream); vanillin (vanilla, cream); alpha-amyl cinnamaldehyde (spicy fruity flavors); butyraldehyde (butter, cheese); valeraldehyde (butter, cheese); citronellal; decanal (citrus fruits); aldehyde C-8 (citrus fruits); aldehyde C-9 (citrus fruits); aldehyde C-12 (citrus fruits); 2-ethyl butyraldehyde (berry fruits); hexenal, *i.e.* trans-2 (berry fruits); tolyl aldehyde (cherry, almond); veratraldehyde (vanilla); 2,6-dimethyl-5-heptenal,

i.e. melonal (melon); 2-6-dimethyloctanal (green fruit); 2-dodecenal (citrus, mandarin); cherry; grape; mixtures thereof; and the like.

The amount of flavoring employed is normally a matter of preference subject to such factors as flavor type, individual flavor, strength desired, strength necessary to mask other less desirable flavors, and the like. Thus, the amount can be varied to obtain the result desired in the final product. In general, amounts of between about 0.1 to about 30 wt % are useable with amounts of about 2 to about 25 wt % being preferred and amounts from about 8 to about 10 wt % are more preferred.

The films also can contain coloring agents or colorants. The coloring agents are used in amounts effective to produce a desired color. The coloring agents useful in the presently disclosed subject matter, include pigments, such as titanium dioxide, which can be incorporated in amounts of up to about 5 wt %, and preferably less than about 1 wt %. Colorants can also include natural food colors and dyes suitable for food, drug and cosmetic applications. These colorants are known as FD&C dyes and lakes. The materials acceptable for the foregoing spectrum of use are preferably water-soluble, and include FD&C Blue No. 2, which is the disodium salt of 5,5-indigotindisulfonic acid. Similarly, the dye known as Green No. 3 comprises a triphenylmethane dye and is the monosodium salt of 4-[4-N-ethyl-p-sulfobenzylamino) diphenyl-methylene]-[1-N-ethyl-N-p-sulfonium benzyl)-2,5-cyclo-hexadienimine]. A full recitation of all FD&C and D&C dyes and their corresponding chemical structures can be found in the Kirk-Othmer Encyclopedia of Chemical Technology, Volume 5, Pages 857-884, which is incorporated herein by reference in its entirety. Furthermore, the materials and methods described in U.S. Patent 6,923,981 and the references cited therein, all of which are incorporated herein by reference, disclose appropriate fast-dissolve films for use with the particles of the presently disclosed subject matter.

After the particles are harvested on such sugar sheets, for example, the fast dissolving sheet can act as the delivery device. According to such embodiments, the fast dissolve films can be placed on biological tissues and

as the film is dissolved and/or absorbed, the particles contained therein are also dissolved or absorbed. The films can be configured for transdermal delivery, trans mucosal delivery, nasal delivery, anal delivery, vaginal delivery, combinations thereof, and the like.

According to some embodiments, a method for harvesting particles from a patterned template includes the use of a sacrificial layer. Referring to FIG. 60, a template **6002** having cured particles **6004** contained within the recesses is prepared by techniques described herein. Next, a droplet or thin film of a monomer **6008** is deposited onto a substrate **6006**. In some embodiments, the monomer **6008** can be polymerized thermally or by UV irradiation such that an adhesive bond forms between monomer layer **6008** and particles **6004** in template **6002**. Template **6002** is then released from polymerized monomer **6008** leaving particles **6004** in an array (C). Next, a solvent can be introduced to monomer **6008** that can dissolve the sacrificial monomer layer **6008**, thereby releasing particles **6004** (D).

In alternative embodiments, the method can be adapted such that template **6002** contains uncured liquid droplets **6004**. Template **6002** containing droplets **6004** can then be pressed into an unpolymerized liquid monomeric adhesive **6008**. Next, particles **6004** and adhesive **6008** are cured in the same step such that they both become solidified and bonded together. Template **6002** is then released leaving particles **6004** in an array (C). When a solvent in introduced to the particle **6004** monomeric adhesive layer **6008**, the sacrificial adhesive layer **6008** is washed away, leaving particles **6004** (D). According to other embodiments, particle droplets **6004** contain a predetermined amount of a crosslinking agent while adhesive layer **6008** contains no crosslinker. Prior to curing, when the liquids of particles **6004** are in contact with the liquid of monomeric adhesive layer **6008**, laminar flow prevents diffusion of particle **6004** into monomeric adhesive layer **6008**.

In some embodiments, the monomer adhesive grafts to the particle during polymerization. In some embodiments, the particles contain a crosslinker. In further embodiments, the adhesive monomer is formed of the

same composition as the particles minus a crosslinking agent, making the adhesive soluble when exposed to a solvent while leaving the particles intact. In some embodiments, the monomer contains a predetermined amount of free radical photoinitiator or thermal initiator. In some embodiments, the monomer is polymerized to generate a polymer with a glass transition temperature above the working temperature. In some embodiments the adhesive layer contains a monomer which, through grafting, adds a desired functionality to one face of the particle such as: reactive chemical species, magnetic components, targeting ligands, fluorescent tags, imaging agents, catalysts, biomolecules, combinations thereof, and the like.

In some embodiments, suitable monomers to be used in the adhesive layer include but are not limited to: methacrylate and acrylate containing compounds, acrylic acid, nitrocellulose, cellulose acetate, 2-hydroxyethyl methacrylate, cyanoacrylates, styrenics, monomers containing vinylic groups, vinyl pyrrolidinone, poly(ethylene glycol) acrylate, poly(ethylene glycol) methacrylate, hydroxyl ethyl acrylate, hydroxyl ethyl methacrylate, epoxy containing monomers, combinations thereof, and the like.

XII. Method of Fabricating Molecules and for Delivering a Therapeutic Agent to a Target

In some embodiments, the presently disclosed subject matter describes methods, processes, and products by processes, for fabricating delivery molecules, for use in drug discovery and drug therapies. In some embodiments, the method or process for fabricating a delivery molecule includes a combinatorial method or process. In some embodiments, the method for fabricating molecules includes a non-wetting imprint lithography method.

XII.A. Method of Fabricating Molecules

In some embodiments, the non-wetting imprint lithography method of the presently disclosed subject matter is used to generate a surface derived from or including a solvent resistant, low surface energy polymeric material.

The surface is derived from casting low viscosity liquid materials onto a master template and then curing the low viscosity liquid materials to generate a patterned template, as described herein. In some embodiments, the surface includes a solvent resistant elastomeric material.

In some embodiments, the non-wetting imprint lithography method is used to generate isolated structures. In some embodiments, the isolated structures include isolated micro-structures. In some embodiments, the isolated structures include isolated nano-structures. In some embodiments, the isolated structures include a biodegradable material. In some embodiments, the isolated structures include a hydrophilic material. In some embodiments, the isolated structures include a hydrophobic material. In some embodiments, the isolated structures include a particular shape. In another embodiment, the isolated structures include or are configured to hold "cargo." According to one embodiment, the cargo held by the isolated structure can include an element, a molecule, a chemical substance, an agent, a drug, a biologic, a protein, DNA, RNA, a diagnostic, a therapeutic, a cancer treatment, a viral treatment, a bacterial treatment, a fungal treatment, an auto-immune treatment, combinations thereof, or the like. According to an alternative embodiment, the cargo protrudes from the surface of the isolated structure, thereby functionalizing the isolated structure. According to yet another embodiment, the cargo is completely contained within the isolated particle such that the cargo is stealthed or sheltered from an environment to which the isolated structure can be subjected. According to yet another embodiment, the cargo is contained substantially on the surface of the isolated structure. In a further embodiment, the cargo is associated with the isolated structure in a combination of one of the above techniques, or the like.

According to another embodiment, the cargo is attached to the isolated structure by chemical binding or physical constraint. In some embodiments, the chemical binding includes, but is not limited to, covalent binding, ionic bonding, other intra- and inter-molecular forces, hydrogen bonding, van der Waals forces, combinations thereof, and the like.

In some embodiments, the non-wetting imprint lithography method further includes adding molecular modules, fragments, or domains to the solution to be molded. In some embodiments, the molecular modules, fragments, or domains impart functionality to the isolated structures. In some embodiments, the functionality imparted to the isolated structure includes a therapeutic functionality.

In some embodiments, a therapeutic agent, such as a drug, a biologic, combinations thereof, and the like, is incorporated into the isolated structure. In some embodiments, the physiologically active drug is tethered to a linker to facilitate its incorporation into the isolated structure. In some embodiments, the domain of an enzyme or a catalyst is added to the isolated structure. In some embodiments, a ligand or an oligopeptide is added to the isolated structure. In some embodiments, the oligopeptide is functional. In some embodiments, the functional oligopeptide includes a cell targeting peptide. In some embodiments, the functional oligopeptide includes a cell penetrating peptide. In some embodiments an antibody or functional fragment thereof is added to the isolated structure.

In some embodiments, a binder is added to the isolated structure. In some embodiments, the isolated structure including the binder is used to fabricate identical structures. In some embodiments, the isolated structure including the binder is used to fabricate structures of a varying structure. In some embodiments, the structures of a varying structure are used to explore the efficacy of a molecule as a therapeutic agent. In some embodiments, the shape of the isolated structure mimics a biological agent. In some embodiments, the method further includes a method for drug discovery.

XII.B. Method of Delivering a Therapeutic Agent to a Target

In some embodiments, a method of delivering a therapeutic agent to a target is disclosed, the method including: providing a particle produced as described herein; admixing the therapeutic agent with the particle; and delivering the particle including the therapeutic agent to the target.

In some embodiments, the therapeutic agent includes a drug. In some embodiments, the therapeutic agent includes genetic material. In

some embodiments, the genetic material includes, without limitation, one or more of a non-viral gene vector, DNA, RNA, RNAi, a viral particle, combinations thereof, or the like.

In some embodiments, the particle has a diameter of less than 100 microns. In some embodiments, the particle has a diameter of less than 10 microns. In some embodiments, the particle has a diameter of less than 1 micron. In some embodiments, the particle has a diameter of less than 100 nm. In some embodiments, the particle has a diameter of less than 10 nm.

In some embodiments, the particle includes a biodegradable polymer. In some embodiments, a biodegradable polymer can be a polymer that undergoes a reduction in molecular weight upon either a change in biological condition or exposure to a biological agent. In some embodiments, the biodegradable polymer includes, without limitation, one or more of a polyester, a polyanhydride, a polyamide, a phosphorous-based polymer, a poly(cyanoacrylate), a polyurethane, a polyorthoester, a polydihydropyran, a polyacetal, combinations thereof, or the like. In some embodiments, the polymer is modified to be a biodegradable polymer (e.g. a poly(ethylene glycol) that is functionalized with a disulfide group). In some embodiments, the polyester includes, without limitation, one or more of polylactic acid, polyglycolic acid, poly(hydroxybutyrate), poly(ϵ-caprolactone), poly(β-malic acid), poly(dioxanones), combinations thereof, or the like. In some embodiments, the polyanhydride includes, without limitation, one or more of poly(sebacic acid), poly(adipic acid), poly(terpthalic acid), combinations thereof, or the like. In some embodiments, the polyamide includes, without limitation, one or more of a poly(imino carbonate), a polyaminoacid, combinations thereof, or the like. In some embodiments, the phosphorous-based polymer includes, without limitation, one or more of polyphosphates, polyphosphonates, polyphosphazenes, combinations thereof, or the like. In some embodiments, the polymer is responsive to stimuli, such as pH, radiation, oxidation, reduction, ionic strength, temperature, alternating magnetic or electric fields, acoustic forces, ultrasonic forces, time, combinations thereof, and the like.

Responses to such stimuli can include swelling, bond cleavage, heating, combinations thereof, or the like, which can facilitate release of the isolated structures cargo, degradation of the isolated structure itself, combinations thereof, and the like.

In some embodiments, the presently disclosed subject matter describes magneto containing particles for applications in hyperthermia therapy, cancer and gene therapy, drug delivery, magnetic resonance imaging contrast agents, vaccine adjuvants, memory devices, spintronics, combinations thereof, and the like.

Without being bound to any one particular theory, the magneto containing particles, e.g., a magnetic nanoparticle, produce heat by the process of hyperthermia (between 41 and 46°C) or thermo ablation (greater than 46°C), *i.e.*, the controlled heating of the nanoparticles upon exposure to an AC-magnetic field. The heat is used to *(i)* induce a phase change in the polymer component (for example melt and release an encapsulated material) and/or *(ii)* hyperthermia treatment of specific cells and/or *(iii)* increase the effectiveness of the encapsulated material. The triggering mechanism of the magnetic nanoparticles via electromagnetic heating enhance the *(iv)* degradation rate of the particulate; *(v)* can induce swelling; and/or (*vi)* induce dissolution/phase change that can lead to a greater surface area, which can be beneficial when treating a variety of diseases.

In some embodiments, the presently disclosed subject matter describes an alternative therapeutic agent delivery method, which utilizes "non-wetting" imprint lithography to fabricate monodisperse magnetic nanoparticles for use in a drug delivery system. Such particles can be used for: (1) hyperthermia treatment of cancer cells; (2) MRI contrast agents; (3) guided delivery of the particle; and (4) triggered degradation of the drug delivery vector.

In some embodiments, the therapeutic agent delivery system includes a biocompatible material and a magnetic nanoparticle. In some embodiments, the biocompatible material has a melting point below 100°C. In some embodiments, the biocompatible material includes, without

limitation, one or more of a polylactide, a polyglycolide, a hydroxypropylcellulose, a wax, combinations thereof, or the like.

In some embodiments, once the magnetic nanoparticle is delivered to the target or is in close proximity to the target, the magnetic nanoparticle is exposed to an AC-magnetic field. The exposure to the AC-magnetic field causes the magnetic nanoparticle to undergo a controlled heating. Without being bound to any one particular theory, the controlled heating is a result of a thermo ablation process. In some embodiments, the heat is used to induce a phase change in the polymer component of the nanoparticle. In some embodiments, the phase change includes a melting process. In some embodiments, the phase change results in the release of an encapsulated material. In some embodiments, the release of an encapsulated material includes a controlled release. In some embodiments, the controlled release of the encapsulated material results in a concentrated dosing of the therapeutic agent. In some embodiments, the heating results in the hyperthermic treatment of the target, *e.g.*, specific cells. In some embodiments, the heating results in an increase in the effectiveness of the encapsulated material. In some embodiments, the triggering mechanism of the magnetic nanoparticles induced by the electromagnetic heating enhances the degradation rate of the particle and can induce swelling and/or a dissolution/phase change that can lead to a greater surface area which can be beneficial when treating a variety of diseases.

The presently described magnetic containing materials also lend themselves to other applications. The magneto-particles can be assembled into well-defined arrays driven by their shape, functionalization of the surface and/or exposure to a magnetic field for investigations of and not limited to magnetic assay devices, memory devices, spintronic applications, and separations of solutions.

Thus, the presently disclosed subject matter provides a method for delivering a therapeutic agent to a target, the method including:

(a) providing a particle prepared by the presently disclosed methods;

(b) admixing the therapeutic agent with the particle; and

(c) delivering the particle including the therapeutic agent to the target.

In some embodiments, the method includes exposing the particle to an alternating magnetic field once the particle is delivered to the target. In some embodiments, the exposing of the particle to an alternating magnetic field causes the particle to produce heat through one of a hypothermia process, a thermo ablation process, combinations thereof, or the like.

In some embodiments, the heat produced by the particle induces one of a phase change in the polymer component of the particle and a hyperthermic treatment of the target. In some embodiments, the phase change in the polymer component of the particle includes a change from a solid phase to a liquid phase. In some embodiments, the phase change from a solid phase to a liquid phase causes the therapeutic agent to be released from the particle. In some embodiments, a constituent of the particle, such as a polymer (*e.g.*, PEG), can be cross-linked in varying degrees to provide for varying degrees of release of another constituent, such as an active agent, of the particle. In some embodiments, the release of the therapeutic agent from the particle includes a controlled release.

In some embodiments, the target includes, without limitation, one or more of a cell-targeting peptide, a cell-penetrating peptide, an integrin receptor peptide (GRGDSP), a melanocyte stimulating hormone, a vasoactive intestional peptide, an anti-Her2 mouse antibody, a vitamin, combinations thereof, or the like.

In one embodiment, the presently disclosed subject matter provides a method for modifying a particle surface. In one embodiment the method of modifying a particle surface includes: (a) providing particles in or on at least one of: (i) a patterned template; or (ii) a substrate; (b) disposing a solution containing a modifying group in or on at least one of: (i) the patterned template; or (ii) the substrate; and (c) removing excess unreacted modifying groups.

In one embodiment of the method for modifying a particle, the modifying group chemically attaches to the particle through a linking group.

In another embodiment of the method for modifying a particle, the linker group includes, without limitation, one or more of sulfides, amines, carboxylic acids, acid chlorides, alcohols, alkenes, alkyl halides, isocyanates, combinations thereof, or the like. In another embodiment, the method of modifying the particles includes a modifying agent that includes, without limitation, one or more of dyes, fluorescence tags, radiolabeled tags, contrast agents, ligands, peptides, antibodies or fragments thereof, pharmaceutical agents, proteins, DNA, RNA, siRNA, combinations thereof, or the like.

With respect to the methods of the presently disclosed subject matter, an animal subject can be treated. The term "subject" as used herein refers to a vertebrate species. The methods of the presently claimed subject matter are particularly useful in the diagnosis of warm-blooded vertebrates. Thus, the presently claimed subject matter concerns mammals. In some embodiments provided is the diagnosis and/or treatment of mammals such as humans, as well as those mammals of importance due to being endangered (such as Siberian tigers), of economical importance (animals raised on farms for consumption by humans) and/or social importance (animals kept as pets or in zoos) to humans, for instance, carnivores other than humans (such as cats and dogs), swine (pigs, hogs, and wild boars), ruminants (such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels), and horses. Also provided is the diagnosis and/or treatment of livestock, including, but not limited to domesticated swine (pigs and hogs), ruminants, horses, poultry, and the like.

The following references are incorporated herein by reference in their entirety. Published International PCT Application No. WO2004081666 to DeSimone et al., U.S. Patent No. 6,528,080 to Dunn et al.; U.S. Patent No. 6,592,579 to Arndt et al., Published International PCT Application No. WO0066192 to Jordan; Hilger, I. et al., *Radiology* 570-575 (2001); Mornet, S. et al., *J. Mat. Chem.*, 2161-2175 (2004); Berry, C.C. et al., *J. Phys. D: Applied Physics* 36, R198-R206 (2003); Babincova, M. et al., *Bioelectrochemistry* 55, 17-19 (2002); Wolf, S.A. et al., *Science* 16, 1488-1495 (2001); and Sun, S. et al., *Science* 287, 1989-1992 (2000); United

States Patent No. 6,159,443 to Hallahan; and Published PCT Application No. WO 03/066066 to Hallahan et al.

XIII. Method of Patterning Natural and Synthetic Structures

In some embodiments, the presently disclosed subject matter describes methods and processes, and products by processes, for generating surfaces and molds from natural structures, single molecules, or self-assembled structures. Accordingly, in some embodiments, the presently disclosed subject matter describes a method of patterning a natural structure, single molecule, and/or a self-assembled structure. In some embodiments, the method further includes replicating the natural structure, single molecule, and/or a self-assembled structure. In some embodiments, the method further includes replicating the functionality of the natural structure, single molecule, and/or a self-assembled structure.

More particularly, in some embodiments, the method further includes taking the impression or mold of a natural structure, single molecule, and/or a self-assembled structure. In some embodiments, the impression or mold is taken with a low surface energy polymeric precursor. In some embodiments, the low surface energy polymeric precursor includes a perfluoropolyether (PFPE) functionally terminated diacrylate. In some embodiments, the natural structure, single molecule, and/or self-assembled structure includes, without limitation, one or more of enzymes, viruses, antibodies, micelles, tissue surfaces, combinations thereof, or the like.

In some embodiments, the impression or mold is used to replicate the features of the natural structure, single molecule, and/or a self-assembled structure into an isolated object or a surface. In some embodiments, a non-wetting imprint lithography method is used to impart the features into a molded part or surface. In some embodiments, the molded part or surface produced by this process can be used in many applications, including, but not limited to, drug delivery, medical devices, coatings, catalysts, or mimics of the natural structures from which they are derived. In some embodiments, the natural structure includes biological tissue. In some embodiments, the biological tissue includes tissue from a bodily organ, such as a heart. In

some embodiments, the biological tissue includes vessels and bone. In some embodiments, the biological tissue includes tendon or cartilage. For example, in some embodiments, the presently disclosed subject matter can be used to pattern surfaces for tendon and cartilage repair. Such repair typically requires the use of collagen tissue, which comes from cadavers and must be machined for use as replacements. Most of these replacements fail because one cannot lay down the primary pattern that is required for replacement. The soft lithographic methods described herein alleviate this problem.

In some embodiments, the presently disclosed subject matter can be applied to tissue regeneration using stem cells. Almost all stem cell approaches known in the art require molecular patterns for the cells to seed and then grow, thereby taking the shape of an organ, such as a liver, a kidney, or the like. In some embodiments, the molecular scaffold is cast and used as crystals to seed an organ in a form of transplant therapy. In some embodiments, the stem cell and nano-substrate is seeded into a dying tissue, e.g., liver tissue, to promote growth and tissue regeneration. In some embodiments, the material to be replicated in the mold includes a material that is similar to or the same as the material that was originally molded. In some embodiments, the material to be replicated in the mold includes a material that is different from and/or has different properties than the material that was originally molded. This approach could play an important role in addressing the organ transplant shortage.

In some embodiments, the presently disclosed subject matter is used to take the impression of one of an enzyme, a bacterium, and a virus. In some embodiments, the enzyme, bacterium, or virus is then replicated into a discrete object or onto a surface that has the shape reminiscent of that particular enzyme, bacterium, or virus replicated into it. In some embodiments, the mold itself is replicated on a surface, wherein the surface-attached replicated mold acts as a receptor site for an enzyme, bacterium, or virus particle. In some embodiments, the replicated mold is useful as a catalyst, a diagnostic sensor, a therapeutic agent, a vaccine, combinations

thereof, and the like. In some embodiments, the surface-attached replicated mold is used to facilitate the discovery of new therapeutic agents.

In some embodiments, the macromolecular, *e.g.*, enzyme, bacterial, or viral, molded "mimics" serve as non-self-replicating entities that have the same surface topography as the original macromolecule, bacterium, or virus. In some embodiments, the molded mimics are used to create biological responses, *e.g.*, an allergic response, to their presence, thereby creating antibodies or activating receptors. In some embodiments, the molded mimics function as a vaccine. In some embodiments, the efficacy of the biologically-active shape of the molded mimics is enhanced by a surface modification technique.

XIII.A. Molecular Imprinting

According to some embodiments, the materials and methods of the presently disclosed subject matter can be used with molecular imprinting techniques to form particles with recognition cites. For recognition to be viable the size, shape, and/or chemical functionality of the particle must simulate a portion of a biological system, such as an enzyme-substrate system, antibody-antigen system, hormone-receptor system, combinations thereof, or the like. Drug research and development often requires the analysis of highly specific and sensitive chemical and/or biologic agents collectively called "recognition agents." Natural recognition agents, such as for example, enzymes, proteins, drug candidates, biomolecules, herbicides, amino acids, derivatives of amino acids, peptides, nucleotides, nucleotide bases, combinations thereof, and the like, tend to be very specific and sensitive as well as being labile and have a low density of binding sites. Because of the delicacy of natural recognition agents, artificial recognition agents are more stable and have become popular research tools. Molecular imprinting has emerged in recent years as a highly accepted tool for the development of artificial recognition agents.

Imprinting of molecules occurs by the polymerization of functional and cross-linking monomers in the presence of a template molecule. First, a template molecule, such as, for example but not limitation, an enzyme, a protein, a drug candidate, a biomolecule, a herbicide, an amino acid, a

derivative of an amino acid, a peptide, nucleotides, nucleotide bases, a virus, combinations thereof, and the like is introduced to a liquid polymer solution. In some embodiments, the liquid polymer solution is a liquid polymer of the presently disclosed subject matter and includes functional and cross-linked monomers. The functional and cross-linked monomers are allowed to establish bond formations and other chemical and physical associations and orientations with the template in the polymer. In some embodiments, a functional monomer includes two functional groups. At one end of the monomer, the monomer is configured to interact with the template, for example through noncovalent interactions (*i.e.*, hydrogen bonding, van der Waals forces, or hydrophobic interactions). The other end of the monomer, *i.e.*, the end that is not interacting with the template, includes a group that is capable of binding with the polymer. During polymerization, the monomers are locked in position around the template, for example with covalent binding, thereby forming an imprint of the template in size, shape, and/or chemical functionality which remains in such a position after the template is removed.

After polymerization or curing the template is removed from the polymer. The template can be removed by dissolving the template in a solvent in some embodiments. The resultant imprint of the template has a steric (size and shape) and chemical (spatial arrangements or complementary functionality) memory of the template. After polymerization and removal of the template, the functional groups of the polymer molecular imprint can then bind a target provided that the binding sites of the imprint and the target molecule complement each other in size, shape, and chemical functionality. This process provides a material with a high stability against physicochemical perturbations that has specificity toward a target molecule and, as such, the material can be used in high throughput assays and in conjunction with physical and chemical parameters that a natural recognition agent may not be capable of withstanding.

According to some embodiments, applications of molecular imprinting include, but are not limited to, purification, separation, screening of bioactive molecules, sensors, catalysis, chromatographic separation, drug screening,

chemosensors, catalysis, biodefense, immunoassays, combinations thereof, and the like.

Useful applications and experimentations of molecular imprinting that can be used in combination with the materials and methods of the presently disclosed subject matter can be found in: Vivek Babu Kandimalla, Hunagxian Ju, *Molecular Imprinting: A Dynamic Technique for Diverse Applications in Analytical Chemistry*, Anal. Bioanal. Chem. (2004) 380: 587-605, and the references cited therein, which are all hereby incorporated by reference in their entirety herein.

XIII.B. Artificial Functional Molecules

According to some embodiments of the presently disclosed subject matter, following the formation of a molecular imprint of a template molecule, as described herein, the molecular imprint can then be used as a mold and receive the materials and methods of the presently disclosed subject matter to form, for example, an artificial functional molecule. After forming the functionalized molecular imprint mold in the polymer material, a polymer precursor solution including, but not limited to, functional and cross-linked monomers, can be applied to the functionalized imprint mold in accord with the materials and methods disclosed herein to form an artificial functional molecule. During molding of the artificial functional molecule, the functionalized monomers in the polymer precursor will align with the functionalized parts of the imprint mold such that the artificial functional molecule will posses a steric (size and shape) and chemical (spatial arrangements or complementary functionality) memory of the imprint mold. The artificial functional molecule, which is the steric and chemical memory of the imprint mold, has similar chemical and physical properties to the original template molecule and can trigger membrane channels; bind to receptors; enter cells; interact with proteins and enzymes; trigger immune responses; trigger physiological responses; trigger release of bioregulatory agents such as, for example, hormones, "feel good" molecules, neurotransmitters, and the like; inhibit responses; trigger regulatory functions; combinations thereof; and the like.

According to other embodiments, molecular imprints and artificial functional molecules of the presently disclosed subject matter can be used in conjunction with particles of the presently disclosed subject matter, as disclosed herein, that have drugs, biologics, or other agents for analysis associated with the particle. Accordingly, the particles with drugs, biologics, or other agents can be analyzed for interaction and/or binding with the artificial functional molecule particles and/or molecular imprint, thereby, making a complete analysis system having high stability against physicochemical perturbations and, as such, the materials can be used in high throughput assays and in conjunction with physical and chemical parameters that natural recognition agents can not withstand. Further, the presently disclosed analysis systems made of the materials and methods of the presently disclosed subject matter are economical to manufacture, increase throughput of drug and biomolecule research and development, and the like.

Referring now to FIG. 44, an embodiment of forming an artificial functional molecule includes creating a molecular imprinting such as shown in FIG. 44A. A substrate material **4410**, such as liquid perfluoropolyether, contains functional monomers **4412** and **4414**. Substrate material **4410** is imprinted with template molecules **4420** having specific steric and chemical groupings **4418** associated therewith. Template molecules **4420** form imprint wells **4416** in substrate material **4410**. Substrate material **4410** is then cured, for example by photocuring, thermal curing, combinations thereof, or the like as described herein.

Next, in FIG. 44B, template molecules **4420** are removed, dissociated, or dissolved from association with substrate material **4410**. Before curing of substrate material **4410**, however, functional monomers **4412** and **4414** of substrate material **4410** associate with their negative or mirror image in template molecules **4420** and during polymerization the functional monomers become locked in position. Thereby, a molecular imprint **4430**, that is the steric and chemical mirror image of the template molecule **4420** is formed in the substrate material.

Next, an artificial functional molecule **4440** is formed in molecular imprint **4430**. According to an embodiment, the materials and methods of the presently disclosed subject matter are utilized, as described elsewhere herein, to make particles that mimic, both stericly and chemically template molecule **4420** that made imprint **4430**. According to one embodiment, a polymer, such as for example liquid PFPE, is prepared and mixed with functional monomers **4444** and the mixture is introduced into molecular imprint cavity **4442** in substrate **4410**. Functional monomers **4444** in the polymer associate with their mirror image functional monomer **4412** and **4414**, which become locked into place in substrate material **4410**. The polymer mixture is then cured such that artificial functional molecules **4440** are formed in imprint cavity **4442** and mimic template molecule **4420** both stericly and chemically. Artificial functional molecules **4444** are then removed from the substrate **4410** as described herein.

XIV. Method of Modifying the Surface of an Imprint Lithography Mold to Impart Surface Characteristics to Molded Products

In some embodiments, the presently disclosed subject matter describes a method of modifying the surface of an imprint lithography mold. In some embodiments, the method further includes imparting surface characteristics to a molded product. In some embodiments, the molded product includes an isolated molded product. In some embodiments, the isolate molded product is formed using a non-wetting imprint lithography technique. In some embodiments, the molded product includes a contact lens, a medical device, and the like.

More particularly, the surface of a solvent resistant, low surface energy polymeric material, or more particularly a PFPE mold is modified by a surface modification step, wherein the surface modification step includes, without limitation, one or more of plasma treatment, chemical treatment, the adsorption of molecules, combinations thereof, or the like. In some embodiments, the molecules adsorbed during the surface modification step include, without limitation, one or more of polyelectrolytes, poly(vinylalcohol), alkylhalosilanes, ligands, combinations thereof, or the like. In some

embodiments, the structures, particles, or objects obtained from the surface-treated molds can be modified by the surface treatments in the mold. In some embodiments, the modification includes the pre-orientation of molecules or moieties with the molecules including the molded products. In some embodiments, the pre-orientation of the molecules or moieties imparts certain properties to the molded products, including catalytic, wettable, adhesive, non-stick, interactive, or not interactive, when the molded product is placed in another environment. In some embodiments, such properties are used to facilitate interactions with biological tissue or to prevent interaction with biological tissues. Applications of the presently disclosed subject matter include sensors, arrays, medical implants, medical diagnostics, disease detection, and separation media.

XV. Methods for Selectively Exposing the Surface of an Article to an Agent

Also disclosed herein is a method for selectively exposing the surface of an article to an agent. In some embodiments the method includes:

(a) shielding a first portion of the surface of the article with a masking system, wherein the masking system includes a elastomeric mask in conformal contact with the surface of the article; and

(b) applying an agent to be patterned within the masking system to a second portion of the surface of the article, while preventing application of the agent to the first portion shielded by the masking system.

In some embodiments, the elastomeric mask includes a plurality of channels. In some embodiments, each of the channels has a cross-sectional dimension of less than about 1 millimeter. In some embodiments, each of the channels has a cross-sectional dimension of less than about 1 micron. In some embodiments, each of the channels has a cross-sectional dimension of less than about 100 nm. In some embodiments, each of the channels has a cross-sectional dimension of about 1 nm. In some embodiments, the agent swells the elastomeric mask less than 25%.

In some embodiments, the agent includes an organic electroluminescent material or a precursor thereof. In some embodiments, the method further including allowing the organic electroluminescent material to form from the agent at the second portion of the surface, and establishing electrical communication between the organic electroluminescent material and an electrical circuit.

In some embodiments, the agent includes a liquid or is carried in a liquid. In some embodiments, the agent includes the product of chemical vapor deposition. In some embodiments, the agent includes a product of deposition from a gas phase. In some embodiments, the agent includes a product of e-beam deposition, evaporation, or sputtering. In some embodiments, the agent includes a product of electrochemical deposition. In some embodiments, the agent includes a product of electroless deposition. In some embodiments, the agent is applied from a fluid precursor. In some embodiments, includes a solution or suspension of an inorganic compound. In some embodiments, the inorganic compound hardens on the second portion of the article surface.

In some embodiments, the fluid precursor includes a suspension of particles in a fluid carrier. In some embodiments, the method further includes allowing the fluid carrier to dissipate thereby depositing the particles at the first region of the article surface. In some embodiments, the fluid precursor includes a chemically active agent in a fluid carrier. In some embodiments, the method further includes allowing the fluid carrier to dissipate thereby depositing the chemically active agent at the first region of the article surface.

In some embodiments, the chemically active agent includes a polymer precursor. In some embodiments, the method further includes forming a polymeric article from the polymer precursor. In some embodiments, the chemically active agent includes an agent capable of promoting deposition of a material. In some embodiments, the chemically active agent includes an etchant. In some embodiments, the method further includes allowing the second portion of the surface of the article to be etched. In some embodiments, the method further includes removing the elastomeric mask of

the masking system from the first portion of the article surface while leaving the agent adhered to the second portion of the article surface.

XVI. Methods for Forming Engineered Membranes

The presently disclosed subject matter also describes a method for forming an engineered membrane. In some embodiments, a patterned non-wetting template is formed by contacting a first liquid material, such as a PFPE material, with a patterned substrate and treating the first liquid material, for example, by curing through exposure to UV light to form a patterned non-wetting template. The patterned substrate includes a plurality of recesses or cavities configured in a specific shape such that the patterned non-wetting template includes a plurality of extruding features. The patterned non-wetting template is contacted with a second liquid material, for example, a photocurable resin. A force is then applied to the patterned non-wetting template to displace an excess amount of second liquid material or "scum layer." The second liquid material is then treated, for example, by curing through exposure to UV light to form an interconnected structure including a plurality of shape and size specific holes. The interconnected structure is then removed from the non-wetting template. In some embodiments, the interconnected structure is used as a membrane for separations.

XVII. Methods for Inspecting Processes and Products by Processes

It will be important to inspect the objects/structures/particles described herein for accuracy of shape, placement and utility. Such inspection can allow for corrective actions to be taken or for defects to be removed or mitigated. The range of approaches and monitoring devices useful for such inspections include: air gages, which use pneumatic pressure and flow to measure or sort dimensional attributes; balancing machines and systems, which dynamically measure and/or correct machine or component balance; biological microscopes, which typically are used to study organisms and their vital processes; bore and ID gages, which are designed for internal diameter dimensional measurement or assessment; boroscopes, which are inspection

tools with rigid or flexible optical tubes for interior inspection of holes, bores, cavities, and the like; calipers, which typically use a precise slide movement for inside, outside, depth or step measurements, some of which are used for comparing or transferring dimensions; CMM probes, which are transducers that convert physical measurements into electrical signals, using various measuring systems within the probe structure; color and appearance instruments, which, for example, typically are used to measure the properties of paints and coatings including color, gloss, haze and transparency; color sensors, which register items by contrast, true color, or translucent index, and are based on one of the color models, most commonly the RGB model (red, green, blue); coordinate measuring machines, which are mechanical systems designed to move a measuring probe to determine the coordinates of points on a work piece surface; depth gages, which are used to measure of the depth of holes, cavities or other component features; digital/video microscopes, which use digital technology to display the magnified image; digital readouts, which are specialized displays for position and dimension readings from inspection gages and linear scales, or rotary encoders on machine tools; dimensional gages and instruments, which provide quantitative measurements of a product's or component's dimensional and form attributes such as wall thickness, depth, height, length, I.D., O.D., taper or bore; dimensional and profile scanners, which gather two-dimensional or three-dimensional information about an object and are available in a wide variety of configurations and technologies; electron microscopes, which use a focused beam of electrons instead of light to "image" the specimen and gain information as to its structure and composition; fiberscopes, which are inspection tools with flexible optical tubes for interior inspection of holes, bores, and cavities; fixed gages, which are designed to access a specific attribute based on comparative gaging, and include Angle Gages, Ball Gages, Center Gages, Drill Size Gages, Feeler Gages, Fillet Gages, Gear Tooth Gages, Gage or Shim Stock, Pipe Gages, Radius Gages, Screw or Thread Pitch Gages, Taper Gages, Tube Gages, U.S. Standard Gages (Sheet / Plate), Weld Gages and Wire Gages; specialty/form gages, which are used to inspect parameters such as roundness, angularity, squareness,

straightness, flatness, runout, taper and concentricity; gage blocks, which are manufactured to precise gagemaker tolerance grades for calibrating, checking, and setting fixed and comparative gages; height gages, which are used for measuring the height of components or product features; indicators and comparators, which measure where the linear movement of a precision spindle or probe is amplified; inspection and gaging accessories, such as layout and marking tolls, including hand tools, supplies and accessories for dimensional measurement, marking, layout or other machine shop applications such as scribes, transfer punches, dividers, and layout fluid; interferometers, which are used to measure distance in terms of wavelength and to determine wavelengths of particular light sources; laser micrometers, which measure extremely small distances using laser technology; levels, which are mechanical or electronic tools that measure the inclination of a surface relative to the earth's surface; machine alignment equipment, which is used to align rotating or moving parts and machine components; magnifiers, which are inspection instruments that are used to magnify a product or part detail via a lens system; master and setting gages, which provide dimensional standards for calibrating other gages; measuring microscopes, which are used by toolmakers for measuring the properties of tools, and often are used for dimensional measurement with lower magnifying powers to allow for brighter, sharper images combined with a wide field of view; metallurgical microscopes, which are used for metallurgical inspection; micrometers, which are instruments for precision dimensional gaging including a ground spindle and anvil mounted in a C-shaped steel frame. Noncontact laser micrometers are also available; microscopes (all types), which are instruments that are capable of producing a magnified image of a small object; optical/light microscopes, which use the visible or near-visible portion of the electromagnetic spectrum; optical comparators, which are instruments that project a magnified image or profile of a part onto a screen for comparison to a standard overlay profile or scale; plug/pin gages, which are used for a "go/no-go" assessment of hole and slot dimensions or locations compared to specified tolerances; protractors and angle gages, which measure the angle between two surfaces of a part or

assembly; ring gages, which are used for "go/no-go" assessment compared to the specified dimensional tolerances or attributes of pins, shafts, or threaded studs; rules and scales, which are flat, graduated scales used for length measurement, and which for OEM applications, digital or electronic linear scales are often used; snap gages, which are used in production settings where specific diametrical or thickness measurements must be repeated frequently with precision and accuracy; specialty microscopes, which are used for specialized applications including metallurgy, gemology, or use specialized techniques like acoustics or microwaves to perform their function; squares, which are used to indicate if two surfaces of a part or assembly are perpendicular; styli, probes, and cantilevers, which are slender rod-shaped stems and contact tips or points used to probe surfaces in conjunction with profilometers, SPMs, CMMs, gages and dimensional scanners; surface profilometers, which measure surface profiles, roughness, waviness and other finish parameters by scanning a mechanical stylus across the sample or through noncontact methods; thread gages, which are dimensional instruments for measuring thread size, pitch or other parameters; and videoscopes, which are inspection tools that capture images from inside holes, bores or cavities.

XVIII. Open Molding Techniques

According to some embodiments, the particles described herein are formed in an open mold. Open molding can reduce the number of steps and sequences of events required during molding of particles and can improve the evaporation rate of solvent from the particle precursor material, thereby, increasing the efficiency and rate of particle production.

Referring to Figure 47, surface or template **4700** includes cavities or recesses **4702** formed therein. A substance **4704**, which can be, but is not limited to a liquid, a powder, a paste, a gel, a liquified solid, combinations thereof, and the like, is then deposited on surface **4700**. The substance **4704** is introduced into recesses **4702** of surface **4700** and excess substance remaining on surface **4700** is removed **4706**. Excess substance **4704** can be removed from the surface by, but is not limited to, doctor

blading, applying pressure with a substrate, electrostatics, magnetics, gravitational forces, air pressure, combinations thereof, and the like. Next, substance **4704** remaining in recesses **4702** is hardened into particles **4708** by, but is not limited to, photocuring, thermal curing, solvent evaporation, oxidation or reductive polymerization, change of temperature, combinations thereof, and the like. After substance **4704** is hardened, the particles **4708** are harvested from recesses **4702**.

According to some embodiments, surface **4700** is configured such that particle fabrication is accomplished in high throughput. In some embodiments, the surface is configured, for example, planer, cylindrical, spherical, curved, linear, a convery belt type arrangement, a gravure printing type arrangement (such as described in U.S. Patent no's. 4,557,195 and 4,905,594, all of which are incorporated herein by reference in their entirity), in large sheet arrangements, in multi-layered sheet arrangements, combinations thereof, and the like. According to such embodiments some recesses in the surface can be in a stage of being filled with substance while at another station of the surface excess substance is being removed. Meanwhile, yet another station of the surface can be hardening the substance and still another station being responsible for harvesting the particles from the recesses. In such embodiments, particles are fabricated effeciently and effectively in high throughput. In some embodiments the method and system are continuous, in other embodiments the method and system are batch, and in some embodiments the method and system are a combination of continuous and batch.

The composition of surface **4700** itself can be fabricated from virtually any material that is chemically, physically, and commercially viable for a particular process to be carried out. According to some embodiments, the material for fabrication of surface **4700** is a material described herein. More particularly, the material of surface **4700** is a material that has a low surface energy, is non-wettable, highly chemically inert, a solvent resistant low surface energy polymeric material, a solvent resistant elastomeric material, combinations thereof, and the like. Even more particularly, the material from which surface **4700** is fabricated is a perfluoropolyether material, a silicone

material, a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction, combinations thereof, and the like.

According to some embodiments, recesses **4702** in surface **4700** are recesses of particular shapes and sizes. Recesses **4702** can be, but are not limited to, regular shaped, irregular shaped, variable shaped, and the like. In some embodiments, recesses **4702** are, but are not limited to, arched recesses, recesses with right angles, tapered recesses, diamond shaped, spherical, rectangle, triangle, polymorphic, molecular shaped, protein shaped, combinations thereof, and the like. In some embodiments, recesses **4702** can be electrically and/or chemically charged such that functional monomers within substance **4704** are attracted and/or repelled, thereby resulting in a functional particle as described elsewhere herein. According to some embodiments, recess **4702** is less than about 1 mm in a dimension. According to some embodiments, the recess is less than about 1 mm in its largest cross-sectional dimension. In other embodiments the recess includes a dimension that is between about 20 nm and about 1 mm. In other embodiments, the recess is between about 20 nm and about 500 micron in a dimension and/or in a largest dimension. More particularly, the recess is between about 50 nm and about 250 micron in a dimension and/or in a largest dimension.

According to embodiments of the present invention, a substance disclosed herein, for example, a drug, DNA, RNA, a biological molecule, a super absorptive material, combinations thereof, and the like can be substance **4704** that is deposited into recesses **4702** and molded into a particle. According to still further embodiments, substance **4704** to be molded is, but is not limited to, a polymer, a solution, a monomer, a plurality of monomers, a polymerization initiator, a polymerization catalyst, an inorganic precursor, a metal precursor, a pharmaceutical agent, a tag, a magnetic material, a paramagnetic material, a ligand, a cell penetrating

peptide, a porogen, a surfactant, a plurality of immiscible liquids, a solvent, a charged species, combinations thereof, and the like. In still further embodiments, particle **4708** is, but is not limited to, organic polymers, charged particles, polymer electrets (poly(vinylidene fluoride), Teflon-fluorinated ethylene propylene, polytetrafluoroethylene), therapeutic agents, drugs, non-viral gene vectors, RNAi, viral particles, polymorphs, combinations thereof, and the like.

According to embodiments of the invention, substance **4704** to be molded into particles **4708** is deposited onto template surface **4700**. In some embodiments substance **4704** is in a liquid form and therefore flows into recesses **4702** of surface **4700** according to techniques disclosed herein. According to other embodiments, substance **4704** takes on another physical form, such as for example, a powder, a gel, a paste, or the like, such that a force or other manipulation, such as heating or the like, may be required to ensure substance **4704** becomes introduced into recesses **4702**. Such a force that can be useful in introducing substance **4704** into recesses **4702** can be, but is not limited to, vibration, centrifugal, electrostatic, magnetic, heating, electromagnetic, gravity, compression, combinations thereof, and the like. The force can also be utilized in embodiments where substance **4704** is a liquid to further ensure substance **4704** enters into recesses **4702**.

Following introduction of substance **4704** onto template surface **4700** and recesses **4702** thereof, excess substance is removed from surface **4700** in some embodiments. Removal of excess substance **4704** can be accomplished by engaging surface **4700** with a second surface **4712** such that the excess substance is squeezed out. Second surface **4712** can be, but is not limited to, a flat surface, an arched surface, and the like. In some embodiments second surface **4712** is brought into contact with template surface **4700**. According to other embodiments second surface **4712** is brought within a predetermine distance of template surface **4700**. According to some embodiments, second surface **4712** is positioned with respect to template surface **4700** normal to the plane of template surface **4700**. According to other embodiments second surface **4712** engages template

surface **4700** with a predetermined contact angle. According to still further embodiments, second surface **4712** can be an arched surface, such as a cylinder, and can be rolled with respect to template surface **4700** to remove excess substance. According to yet further embodiments, second surface **4712** is composed of a composition that repells or attracts the excess substance, such as for example, a non-wetting substance, a hydrophobic surface repelling a hydrophilic substance, and the like.

According to other embodments, excess substance **4704** can be removed from template surface **4700** by doctor blading, or otherwise passing a blade across template surface **4700**. According to some embodiments, blade **4714** is composed of a metal, rubber, polymer, silicon based material, glass, hydrophobic substance, hydrophilic substance, combinations thereof, and the like. In some embodiments blade **4714** is positioned to contact surface **4700** and wipe away excess substance. In other embodiments, blade **4714** is positioned a predetermined distance from surface **4700** and drawn across surface **4700** to remove excess substance from template surface **4700**. The distance blade **4714** is positioned from surface **4700** and the rate at which blade **4714** is drawn across surface **4700** are variable and determined by the material properties of blade **4714**, template surface **4700**, substance **4704** to be molded, combinations thereof, and the like. Doctor blading and similar techniques are disclosed in Lee *et al.*, Two-Polymer Microtransfer Molding for Highly Layered Microstructures, Adv. Mater., 17, 2481-2485, 2005, which is incorporated herein by reference in its entirity.

Substance **4704** in recesses **4702** is then hardened to form particles **4708**. The hardening of substance **4704** can be achieved by a method and by utilizing a material described herein. According to some embodiments the hardening is accomplished by, but is not limited to, solvent evaporation, photo curing, thermal curing, cooling, combinations thereof, and the like.

After substance **4704** has been hardened, particles **4708** are harvested from recesses **4702**. According to some embodiments particle **4708** is harvested by contacting particle **4708** with an article that has affinity for particles **4708** that is greater than the affinity between particle **4708** and

recess **4702**. By way of example, but not limitation, particle **4708** is harvested by contacting particle **4708** with an adhesive substance that adheres to particle **4708** with greater affinity than affinity between particle **4708** and template recess **4702**. According to some embodiments, the harvesting substance is, but is not limited to, water, organic solvents, carbohydrates, epoxies, waxes, polyvinyl alcohol, polyvinyl pyrrolidone, polybutyl acrylate, polycyano acrylates, polymethyl methacrylate, combinations thereof, and the like. According to still further embodiments substance **4704** in recesses **4702** forms a porous particle by solvent casting.

According to other embodiments, particles **4708** are harvested by subjecting the particle/recess combination and/or template surface to a physical force or energy such that particles **4708** are released from the recess **4702**. In some embodiments the force is, but is not limited to, centrifugation, dissolution, vibration, ultrasonics, megasonics, gravity, flexure of the template, suction, electrostatic attraction, electrostatic repulsion, magnetism, physical template manipulation, combinations thereof, and the like.

According to some embodiments, particles **4708** are purified after being harvested. In some embodiments particles **4708** are purified from the harvesting substance. The harvesing can be, but is not limited to, centrifugation, separation, vibration, gravity, dialysis, filtering, sieving, electrophoresis, gas stream, magnetism, electrostatic separation, combinations thereof, and the like.

XVIII.A. Particles Formed From Open Molding

According to some embodiments, recesses **4702** are sized and shaped such that particles formed therefrom will make polymorphs of drugs. Forming a drug from particles **4708** of specific sizes and shapes can increase the efficacy, efficiency, potency, and the like, of a drug substance. For more on polymorphs, see Lee *et al.*, Crystalliztion on Confined Engineered Surfaces: A Method to Control Crystal Size and Generate Different Polymorphs, J. Am. Chem. Soc., 127 (43), 14982 -14983, 2005, which is incorporated herein by reference in its entirity.

According to some embodiments, particles **4708** form super absorbent polymer particles. Examples of super absorbent polymer materials that can be made into particles **4708** according to the present invention, include, but are not limited to, polyacrylates, polyacrylic acid, polyacrylamide, cellulose ethers, poly (ethylene oxide), poly (vinyl alcohol), polysuccinimides, polyacrylonitrile polymers, combinations thereof, and the like. According to further embodiments, these super absorbent polymers can be blended or crosslinked with other polymers, or their monomers can be co-polymerized with other monomers, or the like. According to still further embodiments, a starch is grafted onto these polymers.

According to further embodiments, particle **4708** formed from the methods and materials of the present invention include, but are not limited to, particles between 20 nm and 10 microns of a drug, a charged particle, a polymer electret, a therapeutic agent, a viral particle, a polymorph, a super absorbent particle, combinations thereof, and the like.

According to some embodiments, liquid material to be molded is dispersed into a mold with no substrate associated with the mold, such that the mold has open pores. Because the mold is open, evaporation occurs in the pores. Next, the first substance entered into the mold can be solidified or cured by the methods described herein. Because the first substance was allowed to evaporate in the open mold, there is empty volume in the recess of the mold to receive a second substance. After the second substance is introduced into the empty volume of the mold recesses, the combination can be treated to solidify or cure the second substance. Curing can be done by any of the methods disclosed herein and the first and second substances can be adhered to each other by utilizing methods and materials disclosed herein. Therefore, a micro or nano-scale particle can be formed from more than one layer of material.

XVIV. Seed Coating

According to some embodiments of the present invention, the materials and methods disclosed herein are used to coat seeds. Referring

now to Figure 48, to coat seeds, the seeds are suspended in a liquid solution **4808**. The liquid solution containing the seeds **4808** is deposited onto a template **4802**, where the template includes a recess **4812**. The liquid solution containing the seed **4808** is brought into the recesses **4812** and the liquid is hardened such that the seed becomes coated. The coated seeds are then harvested from the recesses **4810**. Harvesting of the coated seeds can be accomplished by a harvesting method described herein.

According to some embodiments, template **4802** is generated by introducing a liquid template precursor to scaffolding **4800** which contains a pattern that template **4802** will mask. The liquid template precursor is then hardened to form template **4802**. The liquid template precursor can be a material disclosed herein and can be hardened by a method and material disclosed herein. For example, the liquid template precursor can be a liquid PFPE precursor and contain a curable component (*e.g.*, UV, photo, thermal, combinations thereof, and the like). According to this example, the liquird PFPE precursor is introduced to scaffolding **4800** and treated with UV radiation to cure the liquid PFPE into solid form.

According to further embodiments, liquid solution containing the seed **4808** is desposited onto a platform **4804** that is configured to sandwich liquid solution **4808** with template **4802**. When liquid solution **4808** has been sandwiched into recesses **4812** of template **4802**, liquid solution containing the seed **4808** is hardened such that the seed is coated in a solidified material **4810**. Hardening can be by a method and system described herein, including, but not limited to, photo curing, thermal curing, evaporation, and the like. Following hardening of liquid solution **4808**, platform **4804** and template **4802** are removed from each other and solidified coated seeds **4810** are harvested from template **4802** and/or the surface of platform **4804**. Harvesting can be any of the harvesting methods described herein.

The coating of seeds with the materials and methods disclosed herein can, but is not limited to, preparing the seed for packaging, prepairing coated seeds of a uniform size, prepairing seeds with a uniform coating, preparing seeds with a uniform coated shape, eliminating surfactants, preserving seed

viability, combinations thereof, and the like. Seed coating techniques compatible with the present invention are disclosed in U.S. Patent no. 4,245,432, which is incorporated herein by reference in its entirity.

XX. Taggants

In some embodiments the invention relates to formulations comprising a taggant, articles marked with a taggant, and methods for detecting a taggant. Generally, taggants incorporate a unique "mark", or group of "marks" in or on the article that is invisible to an end user of the article, virtually incapable of being counterfeited, cannot be removed from the article without destroying or altering it, and harmless to the article or its end-user. In some embodiments, the taggant comprises a plurality of micro- or nanoparticles, fabricated in accord with the materials and methods disclosed herein, and have a defined shape, size, composition, material, or the like. In other embodiments, micro- or nanoparticles disclosed herein can include substances that act as a taggant. In still other embodiments, the taggant can include a bar code or similar code with up to millions of letter, number, shape, or the like, combinations that make identification of the taggant unique and non-replicable.

In some embodiments, Particle Replication in Nonwetting Templates (PRINT) particles are used as taggants. PRINT particles, fabricated according to particle fabrication embodiments described herein, can contain one or more unique characteristic. The unique characteristic of the particle imparts specific identification information to the particle while rendering the particle non-replicable. In some embodiments the particle can be detected and identified by: inorganic materials, polymeric materials, organic molecules, fluorescent moieties, phosphorescent moieties, dye molecules, more dense segments, less dense segments, magnetic materials, ions, chemiluminescent materials, molecules that respond to a stimulus, volatile segments, photochromic materials, thermochromic materials, radio frequency identification, infrared detection, bar-code detection, surface enhanced raman spectroscopy (SERS), and combinations thereof. In other embodiments, the inorganic materials are one or more of the following: iron

oxide, rare earths and transitional metals, nuclear materials, semiconducting materials, inorganic nanoparticles, metal nanoparticles, alumina, titania, zirconia, yttria, zirconium phosphate, or yttrium aluminum garnet.

In some embodiments, PRINT particles are made in one or more unique shapes and/or sizes and used as a taggant. In another preferred embodiment, PRINT particles are made in one or more unique shapes and/or sizes and composed of one or more of the following for use in detection: inorganic materials, polymeric materials, organic molecules, fluorescent moieties, phosphorescent moieties, dye molecules, more dense segments, less dense segments, magnetic materials, ions, chemiluminescent materials, molecules that respond to a stimulus, volatile segments, photochromic materials, thermochromic materials, and combinations thereof. In yet other embodiment, the PRINT particles are made with a desired porosity.

In some embodiments, the mark or taggant can be a shape, a chemical signature, a spectroscopic signature, a material, a size, a density, and combinations thereof. It is desirable to configure the taggant to supply more information than merely its presence. In some embodiments it is preferred to have the taggant also encode information such as a product date, expiration date, product origin, product destination, identify the source, type, production conditions, composition of the material, or the like. Furthermore, the additional ability to contain randomness or uniqueness is a feature of a preferred taggant. Randomness and/or uniqueness of a taggant based on shape specificity can impart a level of uniqueness not found with other taggant technology. According to other embodiments, the taggant is configured from materials that can survive harsh manufacturing and/or use processes. In other embodiment, the taggant can be coated with a substance that can withstand harsh manufacturing and/or use processes or conditions. In other embodiments, the PRINT particles are distinctly coded with attributes such as shape, size, cargo, and/or chemical functionality that are assigned to a particular meaning, such as the source or identity of goods marked with the particles.

In some embodiments, the particle taggant is configured with a predetermined shape and is between about 20 nm and about 100 micron in a widest dimension. In other embodiments, the particle taggant is molded into a predetermined configuration and is between about 50 nm and about 50 micron in a widest dimension. In some embodiments, the particle taggant is between about 500 nm and about 50 micron in a widest dimension. In some embodiments, the particle taggant is less than 1000 nm in diameter. In other embodiments, the particle taggant is less than 500 nm in its widest diameter. In some embodiments, the particle taggant is between about 250 nm and about 500 nm in a widest dimension. In some embodiments, the particle taggant is between about 100 nm and about 250 nm in a widest dimension. In yet other embodiments, the particle taggant is between about 20 nm and about 100 nm in its widest diameter. U.S. published application no. 2005/0218540, incorporated herein by reference in its entirety, discloses inorganic size and shape specific particles that can be used in combination with the present disclosure.

In some embodiments, the particle taggant can be incorporated into paper pulp or woven fibers, printing inks, copier and printer toners, varnishes, sprays, powders, paints, glass, building materials, molded or extruded plastics, molten metals, fuels, fertilizers, explosives, ceramics, raw materials, finished consumer goods, historic artifacts, pharmaceuticals, biological specimens, biological organisms, laboratory equipment, and the like.

According to some embodiments, a combination of molecules is incorporated into the PRINT particles to yield a unique spectral signature upon detection. In other embodiments, a master, mold, or particle fabrication methodology, such as the particle fabrication methodology disclosed herein, can be rationally designed to produce features or patterns on individual elements of the master, mold, or particles, and these features or patterns can then be incorporated into some or all of the particles either through master and mold replication or by direct structuring of the particle. Methods to produce these additional features or patterns can include chemical or physical etching, photolithography, electron beam lithography,

scanning probe lithography, ion beam lithography, indentation, mechanical deformation, dissolution, deposition of material, chemical modification, chemical transformation, or other methods to control addition, removal, processing, modification, or structuring of material. These features can be used to assign a particular meaning, such as, for example, the source or identity of goods marked with the particle taggants.

Particle taggants, such as described herein, enable a variety of methods of "interrogating" the particles to confirm the authenticity of an article or item. Some of the embodiments include labels that can be viewed and compared with the naked eye. Other embodiments include features that can be viewed with optical microscopy, electron microscopy, or scanning probe microscopy. Other embodiments require exposure of the mark to an energy stimulus, such as temperature changes, radiation of a particular frequency, x-ray, IR, radio, UV, infrared, visible, Raman spectroscopy, or the like. Other embodiments involve accessing a database and comparing information. Still further embodiments can be viewed using fluorescence or phosphorescence methods. Other embodiments include features that can be detected using particle counting instruments, such as flow cytometry. Other embodiments include features that can be detected with atomic spectroscopy, including atomic absorption, atomic emission, mass spectrometry, and x-ray spectrometry. Still further embodiments include features that can be detected by Raman spectroscopy, and nuclear magnetic resonance spectroscopy. Other embodiments require electroanalytical methods for detection. Still further embodiments require chromatographic separation. Other embodiments include features that can be detected with thermal or radiochemical methods such as therogravimetry, differential thermal analysis, differential scanning calorimetry, scintillation counters, and isotope dilution methods.

According to some embodiments, the particle taggant is configured in the form of a radio frequency identification (RFID) tag. The object of an RFID system is to carry data and make the data accessible as machine-readable. RFID systems are typically categorized as either "active" or "passive". In an active RFID system, tags are powered by an internal

battery, and data written into active tags may be rewritten and modified. In a passive RFID system, tags operate without an internal power source and are usually programmed, encoded, or imprinted with a unique set of data that cannot be modified, is invisible to the human senses, is virtually indestructible, virtually not reproducible, and machine readable. A typical passive RFID system comprises two components: a reader and a passive tag. The main component of every passive RFID system is information carried on the tags that respond to a coded RF signals that are typically sent from the reader. Active RFID systems typically include a memory that stores data, an RF transceiver that supports long range RF communications with a long range reader, and an interface that supports short range communications with a short range reader over a secure link.

In some embodiments, the micro- or nanoparticle taggant can be encoded or imprinted with RFID information. According to such embodiments, a RFID reader can be used to read the encoded data. In other embodiments of the present invention, the methods and materials disclosed here can be utilized to imprint RFID data and signals into an RFID tag.

According to other embodiments, authentication and identification of articles is enabled. Some of the embodiments can be used in the fields of regulated materials such as narcotics, pollutants, and explosives. Other embodiments can be used for security in papers and inks. Still further embodiments can be utilized as anti-counterfeiting measures. Other embodiments can be used in pharmaceutical products, including formulations and packaging. Further embodiments can be used in bulk materials, including plastic resins, films, petroleum materials, paint, textiles, adhesives, coatings, and sealants, to name a few. Other embodiments can be used in consumer goods. Still further embodiments can be used in labels and holograms. Other embodiments can be used to prevent counterfeit in collectables and sporting goods. Still further embodiments can be used in tracking and point of source measurements.

According to an example, a particle taggant of the present invention can be used to detect biological specimens. According to such an example, a magnetoelectronic sensor can detect magnetically tagged biological specimens. For example, magnetic particles can be used for biological tagging by coating the particles with a suitable antibody that will only bind to specific analyte (virus, bacteria, etc.). One can then test for the presence of that analyte, by mixing the test solution with the taggant. The prepared solution can then be applied over an integrated circuit chip containing an array of giant magneto-resistance (GMR) sensor elements. The sensor elements are individually coated with the specific antibody of interest. An analyte in the solution will bind to the sensor and carry with it the magnetic tag whose magnetic fringing field will act upon the GMR sensor and alter its resistance. By electrically monitoring an array of these chemically coated GMR sensors, a statistical assay of the concentration of the analyte in the test solution is generated.

According to another example, as shown in FIG. 49, a structural identity of a particle **4900** can be a "Bar-code" type identification **4910**. According to this example, "Bar-code" identification elements **4910** are fabricated on particles **4900** by producing structural features on a master or template that are transferred to the mold and the particles **4900** during PRINT fabrication. In FIG. 49, for example, a Bosch-type etch is used to process a master which introduces a recognizable pattern ("Bosch etch lines") on the sidewalls of individual particles **4900**. The number, morphology and/or pattern of features on the particle sidewalls can be defined by controlling the specific Bosch etching conditions, time, or number of Bosch etch iterations used to process the master from which the particles are derived. Figure 49A shows two distinct particles derived from the same master that show a similar sidewall pattern resulting from the specific Bosch-type etch process used on the master. In this case, this pattern can be recognized using SEM imaging and identifies these particles as originating from the same master.

In some embodiments, the taggants fabricated according to the methods and materials described herein can be fabricated with a controlled

size, shape, and chemical functionality. According to some embodiments, the taggants are fabricated from a photoresist using photolithography to control the size and/or shape of the taggants. In some embodiments, the taggants are particles that have one substantially flat side, or shapes that are not geometric solids. According to some embodiments, the taggants fabricated by the materials and methods of the present invention can be recognized based on the shape, or plurality of shapes, or ratio of known shapes of the taggants. In further embodiments, the taggants can be made of particles in an addressable array, janus particles in which a polymer or monomer is dissolved in a solvent, molded, and let the solvent evaporate, then filling the rest of the mold with a different material, tag, fluorescence, or the like. In other embodiments, taggants are formed with Bosch etch lines on their sides like "bar codes."

In some embodiments, the taggants are fabricated to be included in pharmaceutical formulations. According to such embodiments, the materials of the taggants are FDA approved materials or useful in the formulation of the pharmaceutical. According to other embodiments, taggants are fabricated by the materials and methods of the present invention that form "smart" taggants. A smart taggant can contain sensors or transmitters that let manufacturers, raw material suppliers, or end customers know, for example, if a material has been processed out of specification or mis-treated, stressed, or the like.

According to other embodiments, the taggant particles fabricated from the materials and methods of the present invention can be configured such as the bar-code particles described in Nicewarner-Pena, S.R., et. al., *Science*, 294, 137-141 (2001), which is incorporated herein by reference in their entirety.

Further disclosure and use of taggants and associated systems useful with the present invention can be found in U.S. Patent No's. 6,946,671; 6,893,489; 6,936,828; and U.S. Published Application No's. 2005/0205846; 2005/0171701; 2004/0120857; 2004/0046644; 2004/0046642; 2003/0194578; 2005/0258240; 2004/0101469; 2004/0142106;

2005/0009206; 2005/0272885; 2006/0014001, each of which is incorporated herein by reference in their entirety.

The following references are incorporated herein by reference in their entirety, including each reference cited therein: Jackman, et.al., *Anal. Chem.*, 70, 280-2287 (1998); Moran et al., *Appl. Phys. Lett.*, 78, 3741-3743 (2001); Lee et al., *Adv. Mater.*, 17, 2481-2485 (2005); Yin et al., *Adv. Mater.*, 13, 267-271 (2001); Barton and Odom, *Nano. Lett.*, 4, 1525-1528 (2004); U.S. Patents 6,355,198; 6,752,942; and Published U.S. Application 2002/0006978.

EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

Example 1

Representative Procedure for Synthesis and Curing Photocurable Perfluoropolyethers

In some embodiments, the synthesis and curing of PFPE materials of the presently disclosed subject matter is performed by using the method described by Rolland, J. P., et al., *J. Am. Chem. Soc.*, 2004, *126*, 2322-2323. Briefly, this method involves the methacrylate-functionalization of a commercially available PFPE diol (M_n = 3800 g/mol) with isocyanatoethyl methacrylate. Subsequent photocuring of the material is accomplished through blending with 1 wt% of 2,2-dimethoxy-2-phenylacetophenone and exposure to UV radiation (λ = 365 nm).

More particularly, in a typical preparation of perfluoropolyether dimethacrylate (PFPE DMA), poly(tetrafluoroethylene oxide-*co*-difluoromethylene oxide)α,ω diol (ZDOL, average M_n *ca.* 3,800 g/mol, 95%, Aldrich Chemical Company, Milwaukee, Wisconsin, United States of America) (5.7227g, 1.5 mmol) was added to a dry 50 mL round bottom flask and purged with argon for 15 minutes. 2-isocyanatoethyl methacrylate (EIM, 99%, Aldrich) (0.43 mL, 3.0 mmol) was then added via syringe along with 1,1,2-trichlorotrifluoroethane (Freon 113 99%, Aldrich) (2 mL), and dibutyltin diacetate (DBTDA, 99%, Aldrich) (50 µL). The solution was immersed in an oil bath and allowed to stir at 50 °C for 24 h. The solution was then passed through a chromatographic column (alumina, Freon 113, 2 x 5 cm). Evaporation of the solvent yielded a clear, colorless, viscous oil, which was further purified by passage through a 0.22-µm polyethersulfone filter.

In a representative curing procedure for PFPE DMA, 1 wt% of 2,2-dimethoxy-2-phenyl acetophenone (DMPA, 99% Aldrich), (0.05g, 2.0 mmol) was added to PFPE DMA (5g, 1.2 mmol) along with 2 mL Freon 113 until a clear solution was formed. After removal of the solvent, the cloudy viscous oil was passed through a 0.22-µm polyethersulfone filter to remove any DMPA that did not disperse into the PFPE DMA. The filtered PFPE DMA was then irradiated with a UV source (Electro-Lite Corporation, Danbury, Connecticut, United States of America, UV curing chamber model no. 81432-ELC-500, λ = 365 nm) while under a nitrogen purge for 10 min. This resulted in a clear, slightly yellow, rubbery material.

Example 2

Representative Fabrication of a PFPE DMA Device

In some embodiments, a PFPE DMA device, such as a stamp, was fabricated according to the method described by Rolland, J. P., et al., *J. Am. Chem. Soc.*, 2004, *126*, 2322-2323. Briefly, the PFPE DMA containing a photoinitiator, such as DMPA, was spin coated (800 rpm) to a thickness of 20 μm onto a Si wafer containing the desired photoresist pattern. This coated wafer was then placed into the UV curing chamber and irradiated for 6 seconds. Separately, a thick layer (about 5 mm) of the material was

produced by pouring the PFPE DMA containing photoinitiator into a mold surrounding the Si wafer containing the desired photoresist pattern. This wafer was irradiated with UV light for one minute. Following this, the thick layer was removed. The thick layer was then placed on top of the thin layer such that the patterns in the two layers were precisely aligned, and then the entire device was irradiated for 10 minutes. Once complete, the entire device was peeled from the Si wafer with both layers adhered together.

Example 3

Fabrication of Isolated Particles using Non-Wetting Imprint Lithography

3.1 Fabrication of 200-nm trapezoidal PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (See Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus was then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold was then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of PEG diacrylate is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate. The pressure used was at least about 100 N/cm^2. The entire apparatus was then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (*see* Figure 14).

3.2 Fabrication of 500-nm conical PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 500-nm conical shapes (*see* Figure 12). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of PEG diacrylate is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (*see* Figure 15).

3.3 Fabrication of 3-μm arrow-shaped PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 3-μm arrow shapes (*see* Figure 11). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha"

solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of PEG diacrylate is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (*see* Figure 16).

3.4 Fabrication of 200-nm x 750-nm x 250-nm rectangular PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm x 750-nm x 250-nm rectangular shapes. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of PEG diacrylate is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (*see* Figure 17).

3.5 Fabrication of 200-nm trapezoidal trimethylopropane triacrylate (TMPTA) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (*see* Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, TMPTA is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of TMPTA is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess TMPTA. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (see Figure 18).

3.6 Fabrication of 500-nm conical trimethylopropane triacrylate (TMPTA) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 500-nm conical shapes (see Figure 12). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, TMPTA is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a

silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of TMPTA is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess TMPTA. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (see Figure 19). Further, Figure 20 shows a scanning electron micrograph of 500-nm isolated conical particles of TMPTA, which have been printed using an embodiment of the presently described non-wetting imprint lithography method and harvested mechanically using a doctor blade. The ability to harvest particles in such a way offers conclusive evidence for the absence of a "scum layer."

3.7 Fabrication of 3-μm arrow-shaped TMPTA particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 3-μm arrow shapes (see Figure 11). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, TMPTA is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of TMPTA is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess TMPTA. The entire apparatus is then subjected to UV light (λ = 365

nm) for ten minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM).

3.8 Fabrication of 200-nm trapezoidal poly(lactic acid) (PLA) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ= 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, one gram of (3S)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione (LA) is heated above its melting temperature (92 °C) to 110 °C and approximately 20 μL of stannous octoate catalyst/initiator is added to the liquid monomer. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of molten LA containing catalyst is then placed on the treated silicon wafer preheated to 110°C and the patterned PFPE mold is placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess monomer. The entire apparatus is then placed in an oven at 110°C for 15 hours. Particles are observed after cooling to room temperature and separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (see Figure 21). Further, Figure 22 is a scanning electron micrograph of 200-nm isolated trapezoidal particles of poly(lactic acid) (PLA), which have been printed using an embodiment of the presently described non-wetting imprint lithography method and harvested mechanically using a doctor blade. The ability to harvest particles in such a way offers conclusive evidence for the absence of a "scum layer."

3.9 Fabrication of 3-μm arrow-shaped (PLA) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 3-μm arrow shapes (see Figure 11). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, one gram of (3S)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione (LA) is heated above its melting temperature (92 °C) to 110 °C and approximately 20 μL of stannous octoate catalyst/initiator is added to the liquid monomer. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of molten LA containing catalyst is then placed on the treated silicon wafer preheated to 110°C and the patterned PFPE mold is placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess monomer. The entire apparatus is then placed in an oven at 110°C for 15 hours. Particles are observed after cooling to room temperature and separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (see Figure 23).

3.10 Fabrication of 500-nm conical shaped (PLA) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 500-nm conical shapes (see Figure 12). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, one gram of (3S)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione (LA) is heated above its melting temperature (92°C) to 110°C and approximately 20 μL of stannous

octoate catalyst/initiator is added to the liquid monomer. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of molten LA containing catalyst is then placed on the treated silicon wafer preheated to 110°C and the patterned PFPE mold is placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess monomer. The entire apparatus is then placed in an oven at 110°C for 15 hours. Particles are observed after cooling to room temperature and separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) (see Figure 24).

3.11 Fabrication of 200-nm trapezoidal poly(pyrrole) (Ppy) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, 50 μL of a 1:1 v:v solution of tetrahydrofuran:pyrrole is added to 50 μL of 70% perchloric acid (aq). A clear, homogenous, brown solution quickly forms and develops into black, solid, polypyrrole in 15 minutes. A drop of this clear, brown solution (prior to complete polymerization) is placed onto a treated silicon wafer and into a stamping apparatus and a pressure is applied to remove excess solution. The apparatus is then placed into a vacuum oven for 15 h to remove the THF and water. Particles are observed using scanning electron

microscopy (SEM) (see Figure 25) after release of the vacuum and separation of the PFPE mold and the treated silicon wafer.

3.12 Fabrication of 3-μm arrow-shaped (Ppy) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 3-μm arrow shapes (*see* Figure 11). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, 50 μL of a 1:1 v:v solution of tetrahydrofuran:pyrrole is added to 50 μL of 70% perchloric acid (aq). A clear, homogenous, brown solution quickly forms and develops into black, solid, polypyrrole in 15 minutes. A drop of this clear, brown solution (prior to complete polymerization) is placed onto a treated silicon wafer and into a stamping apparatus and a pressure is applied to remove excess solution. The apparatus is then placed into a vacuum oven for 15 h to remove the THF and water. Particles are observed using scanning electron microscopy (SEM) (*see* Figure 26) after release of the vacuum and separation of the PFPE mold and the treated silicon wafer.

3.13 Fabrication of 500-nm conical (Ppy) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 500-nm conical shapes (*see* Figure 12). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform,

non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, 50 μL of a 1:1 v:v solution of tetrahydrofuran:pyrrole is added to 50 μL of 70% perchloric acid (aq). A clear, homogenous, brown solution quickly forms and develops into black, solid, polypyrrole in 15 minutes. A drop of this clear, brown solution (prior to complete polymerization) is placed onto a treated silicon wafer and into a stamping apparatus and a pressure is applied to remove excess solution. The apparatus is then placed into a vacuum oven for 15 h to remove the THF and water. Particles are observed using scanning electron microscopy (SEM) (see Figure 27) after release of the vacuum and separation of the PFPE mold and the treated silicon wafer.

3.14 Encapsulation of fluorescently tagged DNA inside 200-nm trapezoidal PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. 20 μL of water and 20 μL of PEG diacrylate monomer are added to 8 nanomoles of 24 bp DNA oligonucleotide that has been tagged with a fluorescent dye, CY-3. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of the PEG diacrylate solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The

substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold and the treated silicon wafer using confocal fluorescence microscopy (see Figure 28). Further, Figure 28A shows a fluorescent confocal micrograph of 200-nm trapezoidal PEG nanoparticles, which contain 24-mer DNA strands that are tagged with CY-3. Figure 28B is optical micrograph of the 200-nm isolated trapezoidal particles of PEG diacrylate that contain fluorescently tagged DNA. Figure 28C is the overlay of the images provided in Figures 28A and 28B, showing that every particle contains DNA.

3.15 Encapsulation of magnetite nanoparticles inside 500-nm conical PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 500-nm conical shapes (see Figure 12). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, citrate capped magnetite nanoparticles were synthesized by reaction of ferric chloride (40 mL of a 1 M aqueous solution) and ferrous chloride (10 mL of a 2 M aqueous hydrochloric acid solution) which is added to ammonia (500 mL of a 0.7 M aqueous solution). The resulting precipitate is collected by centrifugation and then stirred in 2 M perchloric acid. The final solids are collected by centrifugation. 0.290 g of these perchlorate-stabilized nanoparticles are suspended in 50 mL of water and heated to 90°C while stirring. Next, 0.106 g of sodium citrate is added. The solution is stirred at 90°C for 30 min to

yield an aqueous solution of citrate-stabilized iron oxide nanoparticles. 50 μL of this solution is added to 50 μL of a PEG diacrylate solution in a microtube. This microtube is vortexed for ten seconds. Following this, 50 μL of this PEG diacrylate/particle solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate/particle solution. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Nanoparticle-containing PEG-diacrylate particles are observed after separation of the PFPE mold and the treated silicon wafer using optical microscopy.

3.16 Fabrication of isolated particles on glass surfaces using "double stamping"

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. A flat, non-wetting surface is generated by photocuring a film of PFPE-DMA onto a glass slide, according to the procedure outlined for generating a patterned PFPE-DMA mold. 5 μL of the PEG-diacrylate/photoinitiator solution is pressed between the PFPE-DMA mold and the flat PFPE-DMA surface, and pressure is applied to squeeze out excess PEG-diacrylate monomer. The PFPE-DMA mold is then removed from the flat PFPE-DMA surface and pressed against a clean glass microscope slide and photocured using UV radiation (λ = 365 nm) for 10 minutes while under a nitrogen purge. Particles are observed after cooling to room temperature and separation of the PFPE mold and the

glass microscope slide, using scanning electron microscopy (SEM) (see Figure 29).

3.17. Encapsulation of viruses in PEG-diacrylate nanoparticles.

A patterned perfluoropolyether (PFPE) mold is generated by pouring PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Fluorescently-labeled or unlabeled Adenovirus or Adeno-Associated Virus suspensions are added to this PEG-diacrylate monomer solution and mixed thoroughly. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of the PEG diacrylate/virus solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Virus-containing particles are observed after separation of the PFPE mold and the treated silicon wafer using transmission electron microscopy or, in the case of fluorescently-labeled viruses, confocal fluorescence microscopy.

3.18 Encapsulation of proteins in PEG-diacrylate nanoparticles.

A patterned perfluoropolyether (PFPE) mold is generated by pouring PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid

PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Fluorescently-labeled or unlabeled protein solutions are added to this PEG-diacrylate monomer solution and mixed thoroughly. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of the PEG diacrylate/virus solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Protein-containing particles are observed after separation of the PFPE mold and the treated silicon wafer using traditional assay methods or, in the case of fluorescently-labeled proteins, confocal fluorescence microscopy.

3.19 Fabrication of 200-nm titania particles

A patterned perfluoropolyether (PFPE) mold can be generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes, such as shown in Figure 13. A poly(dimethylsiloxane) mold can be used to confine the liquid PFPE-DMA to the desired area. The apparatus can then be subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, 1 g of Pluronic P123 is dissolved in 12 g of absolute ethanol. This solution was added to a solution of 2.7 mL of concentrated hydrochloric acid and 3.88 mL titanium (IV) ethoxide. Flat, uniform, non-wetting surfaces can be generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated

sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of the sol-gel solution can then be placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess sol-gel precursor. The entire apparatus is then set aside until the sol-gel precursor has solidified. After solidification of the sol-gel precursor, the silicon wafer can be removed from the patterned PFPE and particles will be present.

3.20 Fabrication of 200-nm silica particles

A patterned perfluoropolyether (PFPE) mold can be generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes, such as shown in Figure 13. A poly(dimethylsiloxane) mold can then be used to confine the liquid PFPE-DMA to the desired area. The apparatus can then be subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, 2 g of Pluronic P123 is dissolved in 30 g of water and 120 g of 2 M HCl is added while stirring at 35°C. To this solution, add 8.50 g of TEOS with stirring at 35°C for 20 h. Flat, uniform, non-wetting surfaces can then be generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of the sol-gel solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess sol-gel precursor. The entire apparatus is then set aside until the sol-gel precursor has solidified. Particles should be observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM).

3.21 Fabrication of 200-nm europium-doped titania particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, 1 g of Pluronic P123 and 0.51 g of $EuCl_3 \bullet 6\ H_2O$ are dissolved in 12 g of absolute ethanol. This solution is added to a solution of 2.7 mL of concentrated hydrochloric acid and 3.88 mL titanium (IV) ethoxide. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of the sol-gel solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess sol-gel precursor. The entire apparatus is then set aside until the sol-gel precursor has solidified. Next, after the sol-gel precursor has solidified, the PFPE mold and the treated silicon wafer are separated and particles should be observed using scanning electron microscopy (SEM).

3.22 Encapsulation of CdSe nanoparticles inside 200-nm PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide

(aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, 0.5 g of sodium citrate and 2 mL of 0.04 M cadmium perchlorate are dissolved in 45 mL of water, and the pH is adjusted to of the solution to 9 with 0.1 M NaOH. The solution is bubbled with nitrogen for 15 minutes. 2 mL of 1 M *N,N*-dimethylselenourea is added to the solution and heated in a microwave oven for 60 seconds. 50 μL of this solution is added to 50 μL of a PEG diacrylate solution in a microtube. This microtube is vortexed for ten seconds. 50 μL of this PEG diacrylate/CdSe particle solution is placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. PEG-diacrylate particles with encapsulated CdSe nanoparticles will be observed after separation of the PFPE mold and the treated silicon wafer using TEM or fluorescence microscopy.

3.23 Synthetic replication of adenovirus particles using Non-Wetting Imprint Lithography

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated by dispersing adenovirus particles on a silicon wafer. This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master. Separately, TMPTA is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of TMPTA is then placed on

the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess TMPTA. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Synthetic virus replicates are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

3.24 Synthetic replication of earthworm hemoglobin protein using Non-Wetting Imprint Lithography

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated by dispersing earthworm hemoglobin protein on a silicon wafer. This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master. Separately, TMPTA is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of TMPTA is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess TMPTA. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Synthetic protein replicates are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

3.25. Combinatorial engineering of 100-nm nanoparticle therapeutics

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 100-nm cubic shapes. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Other therapeutic agents (i.e., small molecule drugs, proteins, polysaccharides, DNA, etc.), tissue targeting agents (cell penetrating peptides and ligands, hormones, antibodies, etc.), therapeutic release/transfection agents (other controlled-release monomer formulations, cationic lipids, etc.), and miscibility enhancing agents (cosolvents, charged monomers, etc.) are added to the polymer precursor solution in a combinatorial manner. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid:30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of the combinatorially-generated particle precursor solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess solution. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. The PFPE-DMA mold is then separated from the treated wafer, particles can be harvested, and the therapeutic efficacy of each combinatorially generated nanoparticle is established. By repeating this methodology with different particle formulations, many combinations of therapeutic agents, tissue targeting agents, release agents, and other important compounds can be rapidly screened to determine the optimal combination for a desired therapeutic application.

3.26 Fabrication of a shape-specific PEG membrane

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 3-μm cylindrical holes that are 5 μm deep. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid:30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 50 μL of PEG diacrylate is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. An interconnected membrane will be observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM). The membrane will release from the surface by soaking in water and allowing it to lift off the surface.

3.27 Harvesting of PEG particles by ice formation

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 5-μm cylinder shapes. The substrate is then subjected to a nitrogen purge for 10 minutes, then UV light (λ = 365 nm) is applied for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform,

non-wetting surfaces are generated by coating a glass slide with PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone. The slide is then subjected to a nitrogen purge for 10 minutes, then UV light (λ = 365 nm) is applied for 10 minutes while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of PEG diacrylate is then placed on the flat PFPE-DMA substrate and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate. The entire apparatus is then purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. PEG particles are observed after separation of the PFPE-DMA mold and substrate using optical microscopy. Water is applied to the surface of the substrate and mold containing particles. A gasket is used to confine the water to the desired location. The apparatus is then placed in the freezer at a temperature of –10° C for 30 minutes. The ice containing PEG particles is peeled off the PFPE-DMA mold and substrate and allowed to melt, yielding an aqueous solution containing PEG particles.

3.28 Harvesting of PEG particles with vinyl pyrrolidone

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 5-μm cylinder shapes. The substrate is then subjected to a nitrogen purge for 10 minutes, and then UV light (λ = 365 nm) is applied for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by coating a glass slide with PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone. The slide is then subjected to a nitrogen purge for 10 minutes, then UV light (λ = 365 nm) is applied for 10 minutes while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of PEG diacrylate is then placed on the flat PFPE-DMA substrate and the patterned

PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate. The entire apparatus is then purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. PEG particles are observed after separation of the PFPE-DMA mold and substrate using optical microscopy. In some embodiments, the material includes an adhesive or sticky surface. In some embodiments, the material includes carbohydrates, epoxies, waxes, polyvinyl alcohol, polyvinyl pyrrolidone, polybutyl acrylate, polycyano acrylates, polymethyl methacrylate. In some embodiments, the harvesting or collecting of the particles includes cooling water to form ice (e.g., in contact with the particles) drop of n-vinyl-2-pyrrolidone containing 5% photoinitiator, 1-hydroxycyclohexyl phenyl ketone, is placed on a clean glass slide. The PFPE-DMA mold containing particles is placed patterned side down on the n-vinyl-2-pyrrolidone drop. The slide is subjected to a nitrogen purge for 5 minutes, then UV light (λ = 365 nm) is applied for 5 minutes while under a nitrogen purge. The slide is removed, and the mold is peeled away from the polyvinyl pyrrolidone and particles. Particles on the polyvinyl pyrrolidone were observed with optical microscopy. The polyvinyl pyrrolidone film containing particles was dissolved in water. Dialysis was used to remove the polyvinyl pyrrolidone, leaving an aqueous solution containing 5 μm PEG particles.

3.29 Harvesting of PEG particles with polyvinyl alcohol

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 5-μm cylinder shapes. The substrate is then subjected to a nitrogen purge for 10 minutes, then UV light (λ = 365 nm) is applied for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces are generated by coating a glass slide with PFPE-DMA

containing 1-hydroxycyclohexyl phenyl ketone. The slide is then subjected to a nitrogen purge for 10 minutes, then UV light (λ = 365 nm) is applied for 10 minutes while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of PEG diacrylate is then placed on the flat PFPE-DMA substrate and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate. The entire apparatus is then purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. PEG particles are observed after separation of the PFPE-DMA mold and substrate using optical microscopy. Separately, a solution of 5 weight percent polyvinyl alcohol (PVOH) in ethanol (EtOH) is prepared. The solution is spin coated on a glass slide and allowed to dry. The PFPE-DMA mold containing particles is placed patterned side down on the glass slide and pressure is applied. The mold is then peeled away from the PVOH and particles. Particles on the PVOH were observed with optical microscopy. The PVOH film containing particles was dissolved in water. Dialysis was used to remove the PVOH, leaving an aqueous solution containing 5 μm PEG particles.

3.30 Fabrication of 200 nm phosphatidylcholine particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200-nm trapezoidal shapes (see Figure 13). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to a nitrogen purge for 10 minutes followed by UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Following this, 20 mg of the

phosphatidylcholine was placed on the treated silicon wafer and heated to 60 degrees C. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess phosphatidylcholine. The entire apparatus is then set aside until the phosphatidylcholine has solidified. Particles are observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM).

3.31 Functionalizing PEG particles with FITC

Poly(ethylene glycol) (PEG) particles with 5 weight percent aminoethyl methacrylate were created. Particles are observed in the PFPE mold after separation of the PFPE mold and the PFPE substrate using optical microscopy. Separately, a solution containing 10 weight percent fluorescein isothiocyanate (FITC) in dimethylsulfoxide (DMSO) was created. Following this, the mold containing the particles was exposed to the FITC solution for one hour. Excess FITC was rinsed off the mold surface with DMSO followed by deionized (DI) water. The tagged particles were observed with fluorescence microscopy, with an excitation wavelength of 492 nm and an emission wavelength of 529 nm.

3.32 Encapsulation of doxorubicin inside 500 nm conical PEG particles

A patterned perfluoropolyether (PFPE) mold was generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 500-nm conical shapes (see Figure 12). A poly(dimethylsiloxane) mold was used to confine the liquid PFPE-DMA to the desired area. The apparatus was then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold was then released from the silicon master. Flat, uniform, non-wetting surfaces were generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, a solution of 1 wt% doxorubicin in PEG diacrylate was formulated with 1 wt% photoinitiator. Following this, 50 μL of this PEG

diacrylate/doxorubicin solution was then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate was then placed in a molding apparatus and a small pressure was applied to push out excess PEG-diacrylate/doxorubicin solution. The small pressure in this example was at least about 100 N/cm^2. The entire apparatus was then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Doxorubicin-containing PEG-diacrylate particles were observed after separation of the PFPE mold and the treated silicon wafer using fluorescent microscopy (see Figure 42).

3.33 Encapsulation of avidin (66 kDa) in 160 nm PEG particles

A patterned perfluoropolyether (PFPE) mold was generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 160-nm cylindrical shapes (see Figure 43). A poly(dimethylsiloxane) mold was used to confine the liquid PFPE-DMA to the desired area. The apparatus was then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold was then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, a solution of 1 wt% avidin in 30:70 PEG monomethacrylate:PEG diacrylate was formulated with 1 wt% photoinitiator. Following this, 50 μL of this PEG/avidin solution was then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate was then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate/avidin solution. The small pressure in this example was at least about 100 N/cm^2. The entire apparatus was then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Avidin-containing PEG particles were observed after separation of the PFPE mold and the treated silicon wafer using fluorescent microscopy.

3.34 Encapsulation of 2-fluoro-2-deoxy-d-glucose in 80 nm PEG Particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a 6 inch silicon substrate patterned with 80-nm cylindrical shapes. The substrate is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, a solution of 0.5 wt% 2-fluoro-2-deoxy-d-glucose (FDG) in 30:70 PEG monomethacrylate:PEG diacrylate is formulated with 1 wt% photoinitiator. Following this, 200 μL of this PEG/FDG solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG/FDG solution. The small pressure should be at least about 100 N/cm^2. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. FDG-containing PEG particles will be observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy.

3.35 Encapsulated DNA in 200 nm x 200 nm x 1 µm bar-shaped poly(lactic acid) particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 200 nm x 200 nm x 1 µm bar shapes. The substrate is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with

trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, a solution of 0.01 wt% 24 base pair DNA and 5 wt% poly(lactic acid) in ethanol is formulated. 200 μL of this ethanol solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG/FDG solution. The small pressure should be at least about 100 N/cm^2. The entire apparatus is then placed under vacuum for 2 hours. DNA-containing poly(lactic acid) particles will be observed after separation of the PFPE mold and the treated silicon wafer using optical microscopy.

3.36 100 nm paclitaxel particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 500-nm conical shapes (see Figure 12). A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, a solution of 5 wt% paclitaxel in ethanol was formulated. Following this, 100 μL of this paclitaxel solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess solution. The pressure applied was at least about 100 N/cm^2. The entire apparatus is then placed under vacuum for 2 hours. Separation of the mold and surface yielded approximately 100 nm spherical paclitaxel particles, which were observed with scanning electron microscopy.

3.37 Triangular particles functionalized on one side

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a 6 inch silicon substrate patterned with 0.6 μm x 0.8 μm x 1 μm right triangles. The substrate is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, a solution of 5 wt% aminoethyl methacrylate in 30:70 PEG monomethacrylate:PEG diacrylate is formulated with 1 wt% photoinitiator. Following this, 200 μL of this monomer solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess solution. The small pressure should be at least about 100 N/cm^2. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Aminoethyl methacrylate-containing PEG particles are observed in the mold after separation of the PFPE mold and the treated silicon wafer using optical microscopy. Separately, a solution containing 10 weight percent fluorescein isothiocyanate (FITC) in dimethylsulfoxide (DMSO) is created. Following this, the mold containing the particles is exposed to the FITC solution for one hour. Excess FITC is rinsed off the mold surface with DMSO followed by deionized (DI) water. Particles, tagged only on one face, will be observed with fluorescence microscopy, with an excitation wavelength of 492 nm and an emission wavelength of 529 nm.

3.38 Formation of an imprinted protein binding cavity and an artificial protein.

The desired protein molecules are adsorbed onto a mica substrate to create a master template. A mixture of PFPE-dimethacrylate (PFPE-DMA)

containing a monomer with a covalently attached disaccharide, and1-hydroxycyclohexyl phenyl ketone as a photoinitiator was poured over the substrate. The substrate is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the mica master, creating polysaccharide-like cavities that exhibit selective recognition for the protein molecule that was imprinted. The polymeric mold was soaked in NaOH/NaClO solution to remove the template proteins.

Flat, uniform, non-wetting surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid: 30% hydrogen peroxide (aq) solution) with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane via vapor deposition in a desiccator for 20 minutes. Separately, a solution of 25% (w/w) methacrylic acid (MAA), 25% diethyl aminoethylmethacrylate (DEAEM), and 48% PEG diacrylate was formulated with 2 wt% photoinitiator. Following this, 200 μL of this monomer solution is then placed on the treated silicon wafer and the patterned PFPE/disaccharide mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess solution. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Removal of the mold yields artificial protein molecules which have similar size, shape, and chemical functionality as the original template protein molecule.

3.39 Template Filling with "Moving Drop"

A mold (6 inch in diameter) with 5x5x10 micron pattern was placed on an inclined surface that has an angle of 20 degrees to horizon. Then a set of 100 μL drops of 98 % PEG-diacrylate and 2% photo initiator solution was placed on the surface of the mold at a higher end. Each drop then would slide down leaving the trace with filled cavities.

After all the drops reached the lower end the mold was put in UV oven, purged with nitrogen for 15 minutes and then cured for 15 minutes. The particles were harvested on glass slide using cyanoacrylate adhesive.

No scum was detected and monodispersity of the particles was confirmed first using optical microscope and then scanning electron microscope.

3.40 Template Filling through Dipping

A mold of size 0.5x3 cm with 3x3x8 micron pattern was dipped into the vial with 98 % PEG-diacrylate and 2% photo initiator solution. After 30 seconds the mold was withdrawn at a rate of approximately 1 mm per second.

Then the mold was put into an UV oven, purged with nitrogen for 15 minutes, and then cured for 15 minutes. The particles were harvested on the glass slide using cyanoacrylate adhesive. No scum was detected and monodispersity of the particles was confirmed using optical microscope.

3.41 Template Filling by Voltage Assist

A voltage of about 3000 volts DC can be applied across a substance to be molded, such as PEG. The voltage makes the filling process easier as it changes the contact angle of substance on the patterned template.

3.42 Fabrication of 2 μm Cube-shaped PEG Particles by Dipping

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 2-μm x 2-µm x 1-µm cubes. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Fluorescently-labeled methacrylate is added to this PEG-diacrylate monomer solution and mixed thoroughly. The mold is dipped into this solution and withdrawn slowly. The mold is subjected to UV light for 10 minutes under nitrogen purge. The particles are harvested by placing cyanoacrylate onto a glass slide, placing the mold in

contact with the cyanoacylate, and allowing the cyanoacrylate to cure. The mold is removed from the cured film, leaving the particles entrapped in the film. The cyanoacrylate is dissolved away using acetone, and the particles are collected in an acetone solution, and purified with centrifugation. Particles are observed using scanning electron microscopy (SEM) after drying (see Figures 61A and 61B).

Example 4

Molding of Features for Semiconductor Applications

4.1 Fabrication of 140-nm lines separated by 70 nm in TMPTA

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 140-nm lines separated by 70 nm. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, TMPTA is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid:30% hydrogen peroxide (aq) solution) and treating the wafer with an adhesion promoter, (trimethoxysilyl propyl methacryalte). Following this, 50 μL of TMPTA is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to ensure a conformal contact. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Features are observed after separation of the PFPE mold and the treated silicon wafer using atomic force microscopy (AFM) (see Figure 30).

4.2 Molding of a polystyrene solution

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl

ketone over a silicon substrate patterned with 140-nm lines separated by 70 nm. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, polystyrene is dissolved in 1 to 99 wt% of toluene. Flat, uniform, surfaces are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid:30% hydrogen peroxide (aq) solution) and treating the wafer with an adhesion promoter. Following this, 50 μL of polystyrene solution is then placed on the treated silicon wafer and the patterned PFPE mold is placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to ensure a conformal contact. The entire apparatus is then subjected to vacuum for a period of time to remove the solvent. Features are observed after separation of the PFPE mold and the treated silicon wafer using atomic force microscopy (AFM) and scanning electron microscopy (SEM).

4.3 Molding of isolated features on microelectronics-compatible surfaces using "double stamping"

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 140-nm lines separated by 70 nm. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, TMPTA is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. A flat, non-wetting surface is generated by photocuring a film of PFPE-DMA onto a glass slide, according to the procedure outlined for generating a patterned PFPE-DMA mold. 50 μL of the TMPTA/photoinitiator solution is pressed between the PFPE-DMA mold and the flat PFPE-DMA surface, and pressure is applied to squeeze out excess TMPTA monomer. The PFPE-DMA mold is then removed from the flat PFPE-DMA surface and pressed

against a clean, flat silicon/silicon oxide wafer and photocured using UV radiation (λ = 365 nm) for 10 minutes while under a nitrogen purge. Isolated, poly(TMPTA) features are observed after separation of the PFPE mold and the silicon/silicon oxide wafer, using scanning electron microscopy (SEM).

4.4 Fabrication of 200-nm titania structures for microelectronics

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 140-nm lines separated by 70 nm. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, 1 g of Pluronic P123 is dissolved in 12 g of absolute ethanol. This solution was added to a solution of 2.7 mL of concentrated hydrochloric acid and 3.88 mL titanium (IV) ethoxide. Flat, uniform, surfaces are generated by treating a silicon/silicon oxide wafer with "piranha" solution (1:1 concentrated sulfuric acid:30% hydrogen peroxide (aq) solution) and drying. Following this, 50 μL of the sol-gel solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess sol-gel precursor. The entire apparatus is then set aside until the sol-gel precursor has solidified. Oxide structures will be observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM).

4.5 Fabrication of 200-nm silica structures for microelectronics

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 140-nm lines separated by 70 nm. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-

DMA mold is then released from the silicon master. Separately, 2 g of Pluronic P123 is dissolved in 30 g of water and 120 g of 2 M HCl is added while stirring at 35°C. To this solution, add 8.50g of TEOS with stirring at 35°C for 20h. Flat, uniform, surfaces are generated by treating a silicon/silicon oxide wafer with "piranha" solution (1:1 concentrated sulfuric acid:30% hydrogen peroxide (aq) solution) and drying. Following this, 50 μL of the sol-gel solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess sol-gel precursor. The entire apparatus is then set aside until the sol gel precursor has solidified. Oxide structures will be observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM).

4.6 Fabrication of 200-nm europium-doped titania structures for microelectronics

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 140-nm lines separated by 70 nm. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, 1 g of Pluronic P123 and 0.51g of $EuCl_3 \bullet 6\ H_2O$ are dissolved in 12g of absolute ethanol. This solution was added to a solution of 2.7 mL of concentrated hydrochloric acid and 3.88 mL titanium (IV) ethoxide. Flat, uniform, surfaces are generated by treating a silicon/silicon oxide wafer with "piranha" solution (1:1 concentrated sulfuric acid:30% hydrogen peroxide (aq) solution) and drying. Following this, 50 μL of the sol-gel solution is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess sol-gel precursor. The entire apparatus is then set aside until the sol-gel precursor has solidified. Oxide structures will be

observed after separation of the PFPE mold and the treated silicon wafer using scanning electron microscopy (SEM).

4.7 Fabrication of isolated "scum free" features for microelectronics

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 140-nm lines separated by 70 nm. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, TMPTA is blended with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. Flat, uniform, non-wetting surfaces capable of adhering to the resist material are generated by treating a silicon wafer cleaned with "piranha" solution (1:1 concentrated sulfuric acid:30% hydrogen peroxide (aq) solution) and treating the wafer with a mixture of an adhesion promoter, (trimethoxysilyl propyl methacrylate) and a non-wetting silane agent (1H, 1H, 2H, 2H-perfluorooctyl trimethoxysilane). The mixture can range from 100% of the adhesion promoter to 100% of the non-wetting silane. Following this, 50 μL of TMPTA is then placed on the treated silicon wafer and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to ensure a conformal contact and to push out excess TMPTA. The entire apparatus is then subjected to UV light (λ = 365 nm) for ten minutes while under a nitrogen purge. Features are observed after separation of the PFPE mold and the treated silicon wafer using atomic force microscopy (AFM) and scanning electron microscopy (SEM).

Example 5

Molding of Natural and Engineered Templates

5.1. Fabrication of a perfluoropolyether-dimethacrylate (PFPE-DMA) mold from a template generated using Electron-Beam Lithography

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated using electron beam lithography by spin coating a bilayer resist of 200,000 MW PMMA and 900,000 MW PMMA onto a silicon wafer with 500-nm thermal oxide, and exposing this resist layer to an electron beam that is translating in a pre-programmed pattern. The resist is developed in 3:1 isopropanol:methyl isobutyl ketone solution to remove exposed regions of the resist. A corresponding metal pattern is formed on the silicon oxide surface by evaporating 5 nm Cr and 15 nm Au onto the resist covered surface and lifting off the residual PMMA/Cr/Au film in refluxing acetone. This pattern is transferred to the underlying silicon oxide surface by reactive ion etching with CF_4/O_2 plasma and removal of the Cr/Au film in aqua regia (see Figure 31). This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master. This mold can be used for the fabrication of particles using non-wetting imprint lithography as specified in Particle Fabrication Examples 3.3 and 3.4.

5.2 Fabrication of a perfluoropolyether-dimethacrylate (PFPE-DMA) mold from a template generated using photolithography.

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated using photolithography by spin coating a film of SU-8 photoresist onto a silicon wafer. This resist is baked on a hotplate at 95°C and exposed through a pre-patterned photomask. The wafer is baked again at 95°C and developed using a commercial developer solution to remove unexposed SU-8 resist. The resulting patterned surface

is fully cured at 175°C. This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master, and can be imaged by optical microscopy to reveal the patterned PFPE-DMA mold (see Figure 32).

5.3 Fabrication of a perfluoropolyether-dimethacrylate (PFPE-DMA) mold from a template generated from dispersed Tobacco Mosaic Virus Particles

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated by dispersing tobacco mosaic virus (TMV) particles on a silicon wafer (Figure 33a). This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master. The morphology of the mold can then be confirmed using Atomic Force Microscopy (Figure 33b).

5.4 Fabrication of a perfluoropolyether-dimethacrylate (PFPE-DMA) mold from a template generated from block-copolymer micelles

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated by dispersing polystyrene-polyisoprene block copolymer micelles on a freshly-cleaved mica surface. This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold

is then released from the master. The morphology of the mold can then be confirmed using Atomic Force Microscopy (see Figure 34).

5.5 Fabrication of a perfluoropolyether-dimethacrylate (PFPE-DMA) mold from a template generated from brush polymers.

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated by dispersing poly(butyl acrylate) brush polymers on a freshly-cleaved mica surface. This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master. The morphology of the mold can then be confirmed using Atomic Force Microscopy (Figure 35).

5.6 Fabrication of a perfluoropolyether-dimethacrylate (PFPE-DMA) mold from a template generated from earthworm hemoglobin protein.

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated by dispersing earthworm hemoglobin proteins on a freshly-cleaved mica surface. This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master. The morphology of the mold can then be confirmed using Atomic Force Microscopy.

5.7 Fabrication of a perfluoropolyether-dimethacrylate (PFPE-DMA) mold from a template generated from patterned DNA nanostructures.

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated by dispersing DNA nanostructures on a

freshly-cleaved mica surface. This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master. The morphology of the mold can then be confirmed using Atomic Force Microscopy.

5.8 Fabrication of a perfluoropolyether-dimethacrylate (PFPE-DMA) mold from a template generated from carbon nanotubes

A template, or "master," for perfluoropolyether-dimethacrylate (PFPE-DMA) mold fabrication is generated by dispersing or growing carbon nanotubes on a silicon oxide wafer. This master can be used to template a patterned mold by pouring PFPE-DMA containing 1-hydroxycyclohexyl phenyl ketone over the patterned area of the master. A poly(dimethylsiloxane) mold is used to confine the liquid PFPE-DMA to the desired area. The apparatus is then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the master. The morphology of the mold can then be confirmed using Atomic Force Microscopy.

Example 6

Method of Making Monodisperse Nanostructures Havinga Plurality of Shapes and Sizes

In some embodiments, the presently disclosed subject matter describes a novel "top down" soft lithographic technique; non-wetting imprint lithography (NoWIL) which allows completely isolated nanostructures to be generated by taking advantage of the inherent low surface energy and swelling resistance of cured PFPE-based materials.

The presently described subject matter provides a novel "top down" soft lithographic technique; non-wetting imprint lithography (NoWIL) which

allows completely isolated nanostructures to be generated by taking advantage of the inherent low surface energy and swelling resistance of cured PFPE-based materials. Without being bound to any one particular theory, a key aspect of NoWIL is that both the elastomeric mold and the surface underneath the drop of monomer or resin are non-wetting to this droplet. If the droplet wets this surface, a thin scum layer will inevitably be present even if high pressures are exerted upon the mold. When both the elastomeric mold and the surface are non-wetting (i.e. a PFPE mold and fluorinated surface) the liquid is confined only to the features of the mold and the scum layer is eliminated as a seal forms between the elastomeric mold and the surface under a slight pressure. Thus, the presently disclosed subject matter provides for the first time a simple, general, soft lithographic method to produce nanoparticles of nearly any material, size, and shape that are limited only by the original master used to generate the mold.

Using NoWIL, nanoparticles composed of 3 different polymers were generated from a variety of engineered silicon masters. Representative patterns include, but are not limited to, 3-μm arrows (see Figure 11), conical shapes that are 500 nm at the base and converge to <50 nm at the tip (see Figure 12), and 200-nm trapezoidal structures (see Figure 13). Definitive proof that all particles were indeed "scum-free" was demonstrated by the ability to mechanically harvest these particles by simply pushing a doctor's blade across the surface. See Figures 20 and 22.

Polyethylene glycol (PEG) is a material of interest for drug delivery applications because it is readily available, non-toxic, and biocompatible. The use of PEG nanoparticles generated by inverse microemulsions to be used as gene delivery vectors has previously been reported. K. McAllister *et al.*, *Journal of the American Chemical Society* 124, 15198-15207 (Dec 25, 2002). In the presently disclosed subject matter, NoWIL was performed using a commercially available PEG-diacrylate and blending it with 1 wt% of a photoinitiator, 1-hydroxycyclohexyl phenyl ketone. PFPE molds were generated from a variety of patterned silicon substrates using a dimethacrylate functionalized PFPE oligomer (PFPE DMA) as described previously. See J. P. Rolland, E. C. Hagberg, G. M. Denison, K. R. Carter,

J. M. DeSimone, *Angewandte Chemie-International Edition* 43, 5796-5799 (2004). In one embodiment, flat, uniform, non-wetting surfaces were generated by using a silicon wafer treated with a fluoroalkyl trichlorosilane or by casting a film of PFPE-DMA on a flat surface and photocuring. A small drop of PEG diacrylate was then placed on the non-wetting surface and the patterned PFPE mold placed on top of it. The substrate was then placed in a molding apparatus and a small pressure was applied to push out the excess PEG-diacrylate. The entire apparatus was then subjected to UV light (λ= 365 nm) for ten minutes while under a nitrogen purge. Particles were observed after separation of the PFPE mold and flat, non-wetting substrate using optical microscopy, scanning electron microscopy (SEM), and atomic force microscopy (AFM).

Poly(lactic acid) (PLA) and derivatives thereof, such as poly(lactide-*co*-glycolide) (PLGA), have had a considerable impact on the drug delivery and medical device communities because it is biodegradable. See K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, K. M. Shakesheff, *Chemical Reviews* 99, 3181-3198 (Nov, 1999); A. C. Albertsson, I. K. Varma, *Biomacromolecules* 4, 1466-1486 (Nov-Dec, 2003). As with PEG-based systems, progress has been made toward the fabrication of PLGA particles through various dispersion techniques that result in size distributions and are strictly limited to spherical shapes. See C. Cui, S. P. Schwendeman, *Langmuir* 34, 8426 (2001).

The presently disclosed subject matter demonstrates the use of NoWIL to generate discrete PLA particles with total control over shape and size distribution. For example, in one embodiment, one gram of (3S)-*cis*-3,6-dimethyl-1,4-dioxane-2,5-dione was heated above its melting temperature to 110°C and ~20 μL of stannous octoate catalyst/initiator was added to the liquid monomer. A drop of the PLA monomer solution was then placed into a preheated molding apparatus which contained a non-wetting flat substrate and mold. A small pressure was applied as previously described to push out excess PLA monomer. The apparatus was allowed to heat at 110°C for 15h until the polymerization was complete. The PFPE-DMA mold and the flat, non-wetting substrate were then separated to reveal the PLA particles.

To further demonstrate the versatility of NoWIL, particles composed of a conducting polymer polypyrrole (PPy) were generated. PPy particles have been formed using dispersion methods, see M. R. Simmons, P. A. Chaloner, S. P. Armes, *Langmuir* 11, 4222 (1995), as well as "lost-wax" techniques, see P. Jiang, J. F. Bertone, V. L. Colvin, *Science* 291, 453 (2001).

The presently disclosed subject matter demonstrates for the first time, complete control over shape and size distribution of PPy particles. Pyrrole is known to polymerize instantaneously when in contact with oxidants such as perchloric acid. Dravid et al. has shown that this polymerization can be retarded by the addition of tetrahydrofuran (THF) to the pyrrole. See M. Su, M. Aslam, L. Fu, N. Q. Wu, V. P. Dravid, *Applied Physics Letters* 84, 4200-4202 (May 24, 2004).

The presently disclosed subject matter takes advantage of this property in the formation of PPy particles by NoWIL. For example, 50 μL of a 1:1 v/v solution of THF:pyrrole was added to 50 μL of 70% perchloric acid. A drop of this clear, brown solution (prior to complete polymerization) into the molding apparatus and applied pressure to remove excess solution. The apparatus was then placed into the vacuum oven overnight to remove the THF and water. PPy particles were fabricated with good fidelity using the same masters as previously described.

Importantly, the materials properties and polymerization mechanisms of PLA, PEG, and PPy are completely different. For example, while PLA is a high-modulus, semicrystalline polymer formed using a metal-catalyzed ring opening polymerization at high temperature, PEG is a malleable, waxy solid that is photocured free radically, and PPy is a conducting polymer polymerized using harsh oxidants. The fact that NoWIL can be used to fabricate particles from these diverse classes of polymeric materials that require very different reaction conditions underscores its generality and importance.

In addition to its ability to precisely control the size and shape of particles, NoWIL offers tremendous opportunities for the facile encapsulation of agents into nanoparticles. As described in Example 3-14, NoWIL can be used to encapsulate a 24-mer DNA strand fluorescently tagged with CY-3

inside the previously described 200 nm trapezoidal PEG particles. This was accomplished by simply adding the DNA to the monomer/water solution and molding them as described. We were able to confirm the encapsulation by observing the particles using confocal fluorescence microscopy (see Figure 28). The presently described approach offers a distinct advantage over other encapsulation methods in that no surfactants, condensation agents, and the like are required. Furthermore, the fabrication of monodisperse, 200 nm particles containing DNA represents a breakthrough step towards artificial viruses. Accordingly, a host of biologically important agents, such as gene fragments, pharmaceuticals, oligonucleotides, and viruses, can be encapsulated by this method.

The method also is amenable to non-biologically oriented agents, such as metal nanoparticles, crystals, or catalysts. Further, the simplicity of this system allows for straightforward adjustment of particle properties, such as crosslink density, charge, and composition by the addition of other comonomers, and combinatorial generation of particle formulations that can be tailored for specific applications.

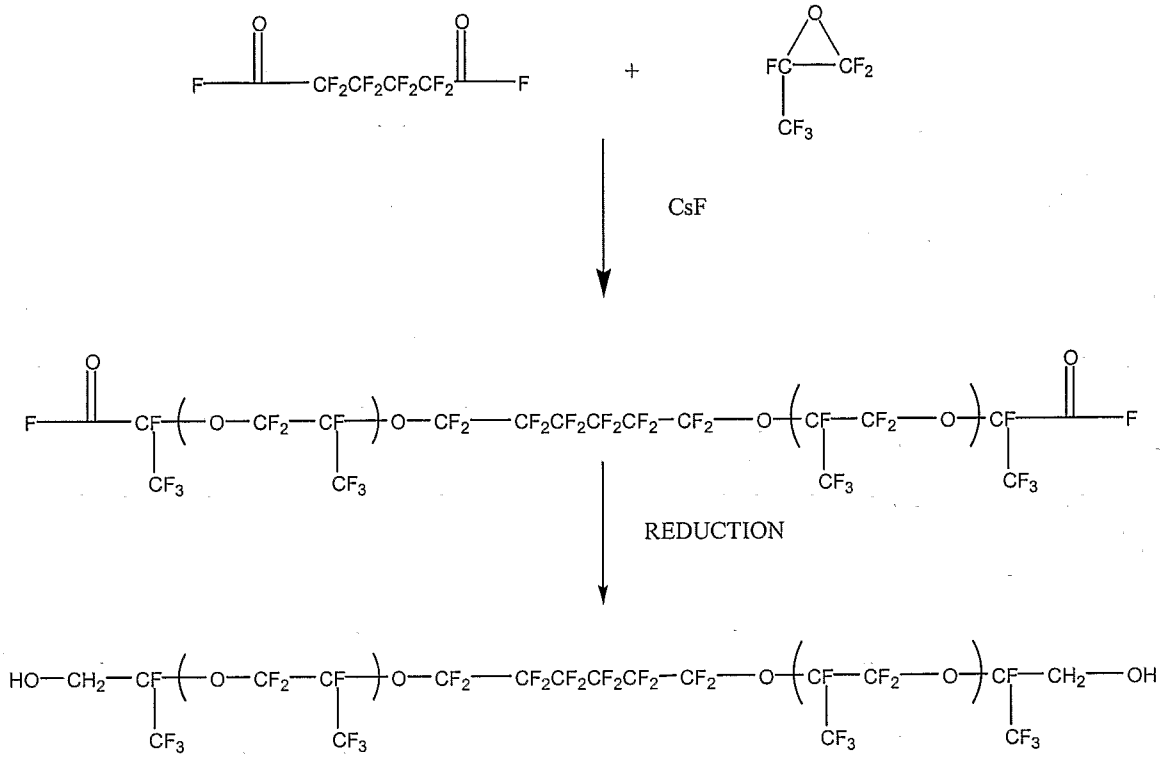
Accordingly, NoWIL is a highly versatile method for the production of isolated, discrete nanostructures of nearly any size and shape. The shapes presented herein were engineered non-arbitrary shapes. NoWIL can easily be used to mold and replicate non-engineered shapes found in nature, such as viruses, crystals, proteins, and the like. Furthermore, the technique can generate particles from a wide variety of organic and inorganic materials containing nearly any cargo. The method is simplistically elegant in that it does not involve complex surfactants or reaction conditions to generate nanoparticles. Finally, the process can be amplified to an industrial scale by using existing soft lithography roller technology, see Y. N. Xia, D. Qin, G. M. Whitesides, *Advanced Materials* 8, 1015-1017 (Dec, 1996), or silk screen printing methods.

Example 7

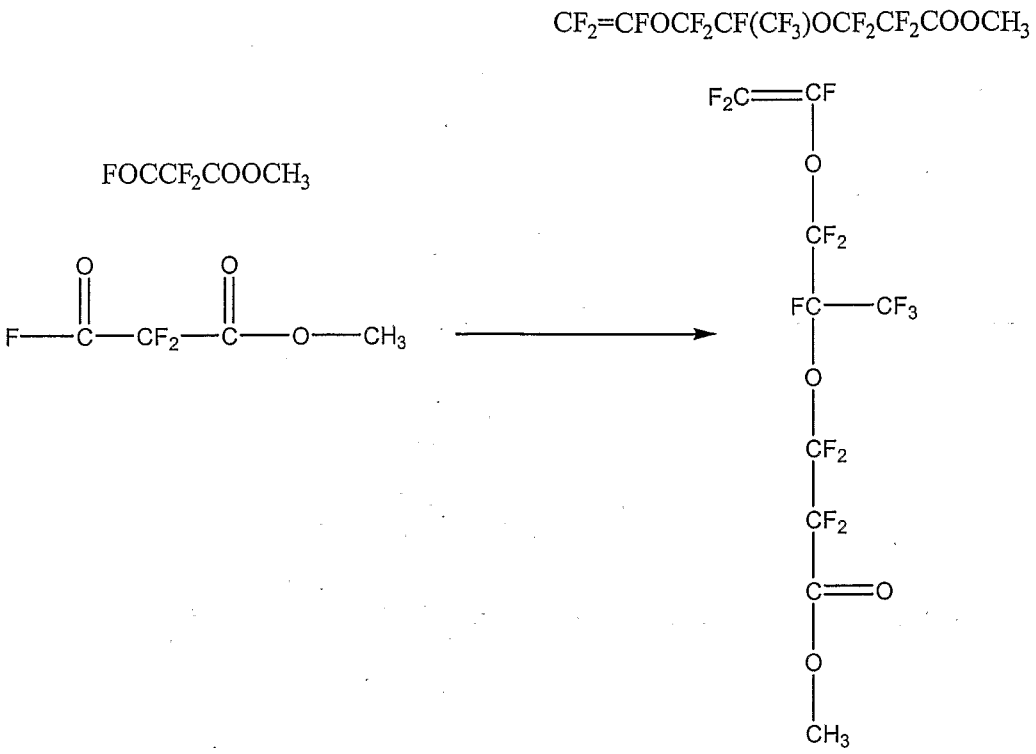
Synthesis of Functional Perfluoropolyethers

7.1 Synthesis of Krytox® (DuPont, Wilmington, Delaware, United States of America) Diol to be Used as a Functional PFPE

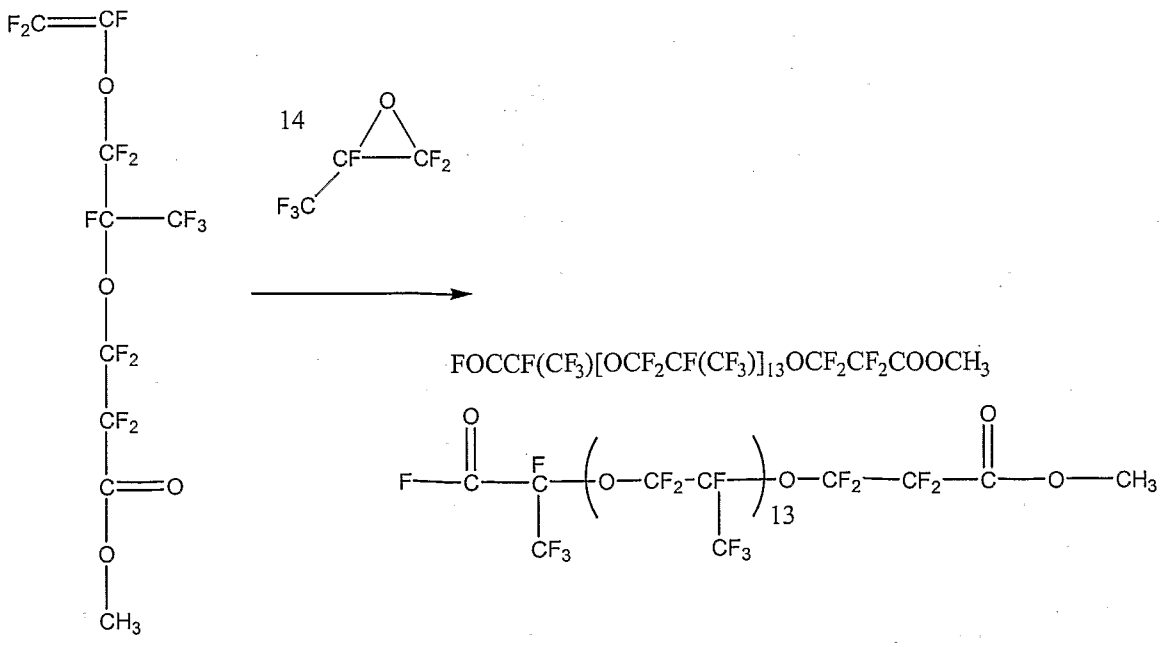
5



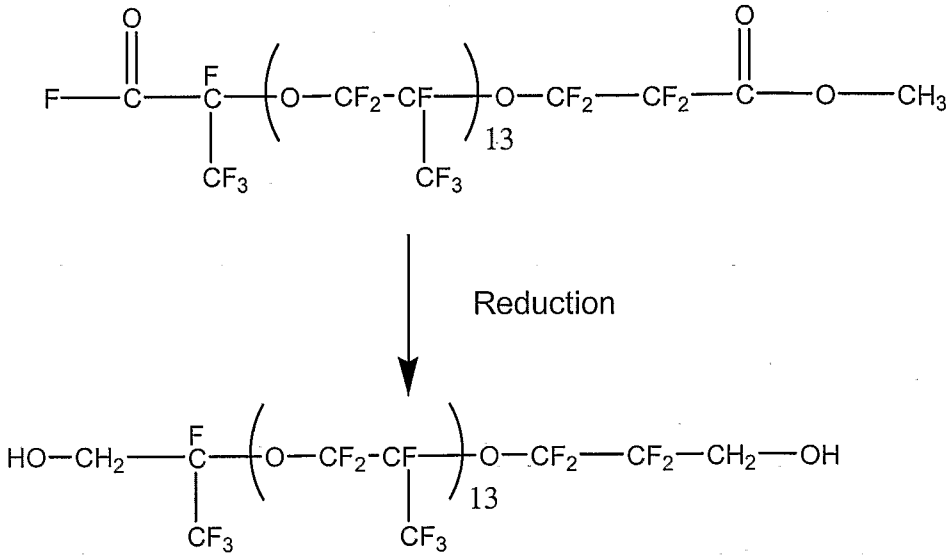
7.2 Synthesis of Krytox® (DuPont, Wilmington, Delaware, United States of America) Diol to be Used as a Functional PFPE



7.3 Synthesis of Krytox® (DuPont, Wilmington, Delaware, United States of America)Diol to be Used as a Functional PFPE

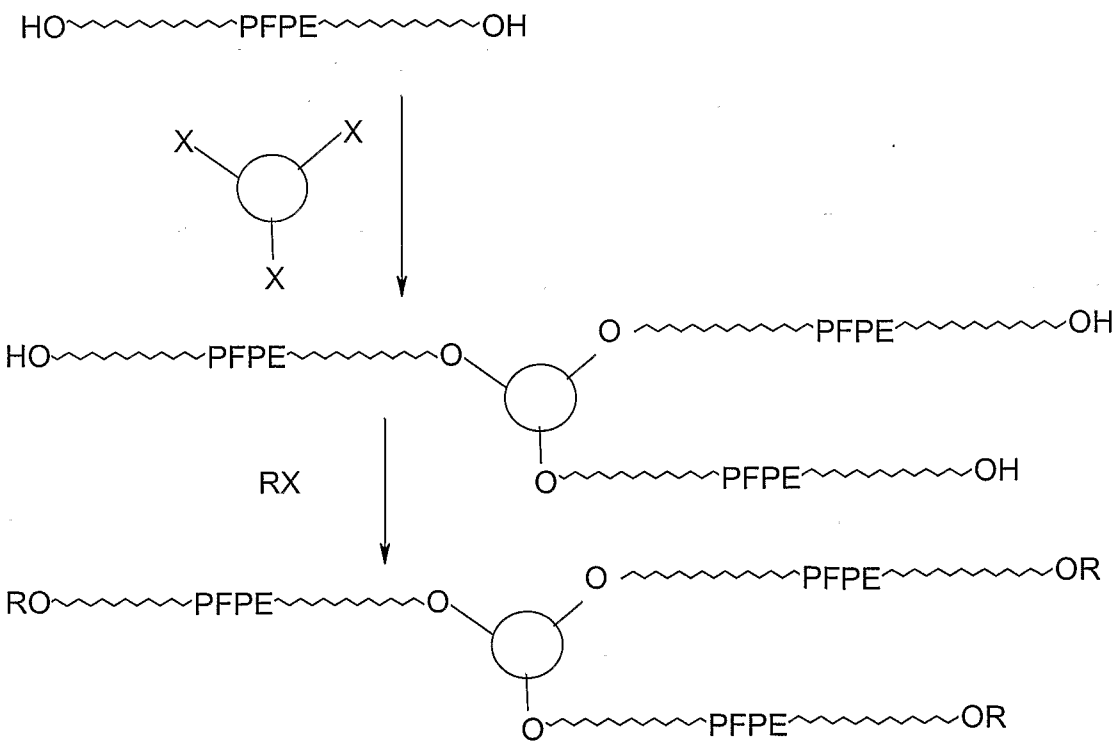


7.4 Example of Krytox® (DuPont, Wilmington, Delaware, United States of America) Diol to be Used as a Functional PFPE

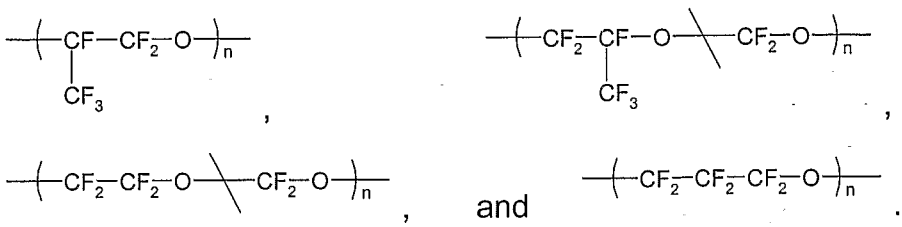


mw = 2436

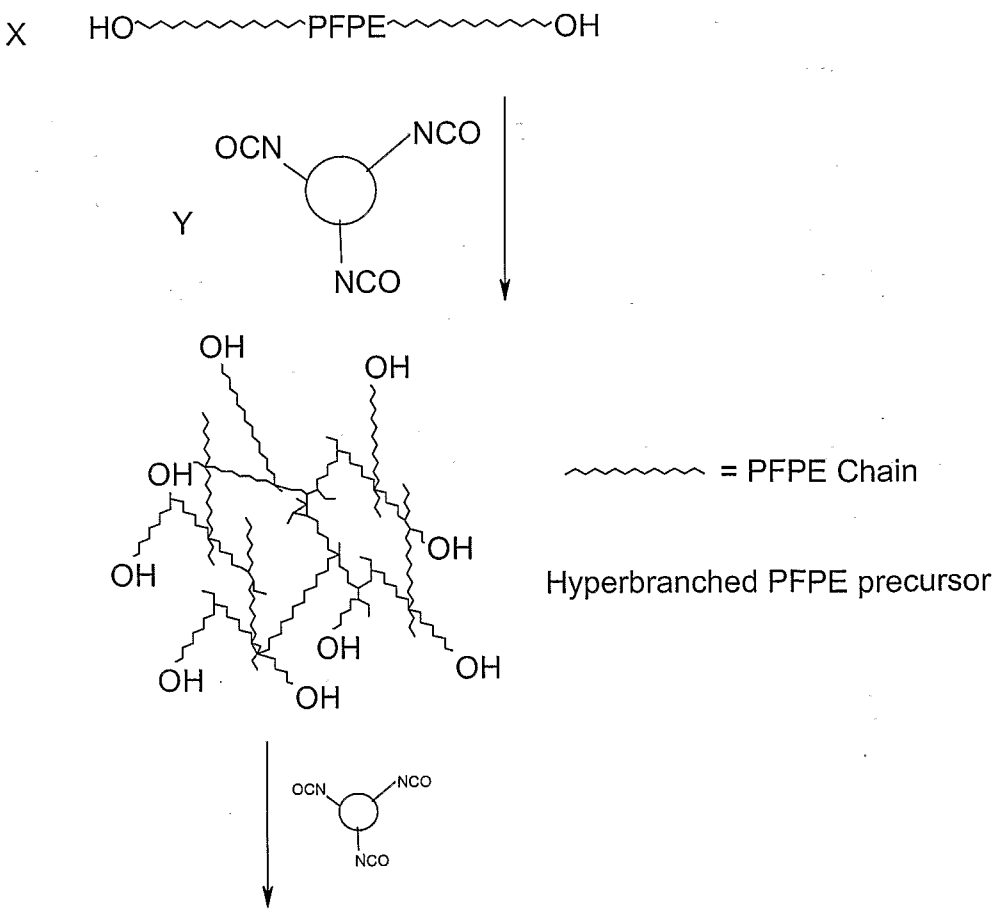
7.5 Synthesis of a Multi-arm PFPE Precursor



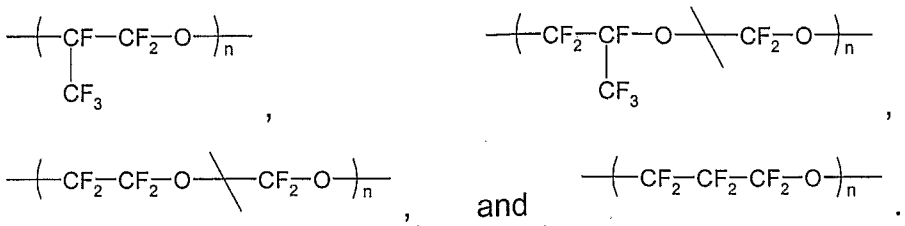
wherein, X includes, but is not limited to an isocyanate, an acid chloride, an epoxy, and a halogen; R includes, but is not limited to an acrylate, a methacrylate, a styrene, an epoxy, and an amine; and the circle represents any multifunctional molecule, such a cyclic compound. PFPE can be any perfluoropolyether material as described herein, including, but not limited to a perfluoropolyether material including a backbone structure as follows:



7.6 Synthesis of a Hyperbranched PFPE Precursor



wherein, PFPE can be any perfluoropolyether material as described herein, including, but not limited to a perfluoropolyether material including a backbone structure as follows:

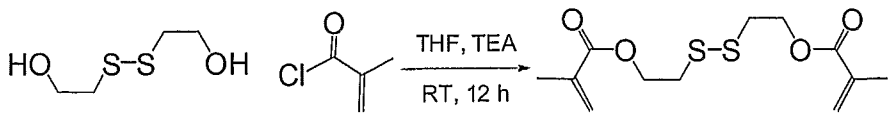


Example 8

Synthesis of Degradable Crosslinkers for Hydrolysable PRINT Particles

Bis(ethylene methacrylate) disulfide (DEDSMA) was synthesized using methods described in Li et al. *Macromolecules* 2005, *38*, 8155-8162 from 2-hyrdoxyethane disulfide and methacroyl chloride (Scheme 8). Analogously, bis(8-hydroxy-3,6-dioxaoctyl methacrylate) disulfide (TEDSMA) was synthesized from bis(8-hydroxy-3,6-dioxaoctyl) disulfide (Lang et al. *Langmuir* 1994, *10*, 197-210). Methacroyl chloride (0.834 g, 8 mmole) was slowly added to a stirred solution of bis(8-hydroxy-3,6-dioxaoctyl) disulfide (0.662 g, 2 mmole) and triethylamine (2 mL) in acetonitrile (30 mL) chilled in an ice bath. The reaction was allowed to warm to room temperature and stirred for 16 hours. The mixture was diluted with 5 % NaOH solution (50 mL) and stirred for an additional hour. The mixture was extracted with 2 x 60 mL of methylene chloride, the organic layer was washed 3 x 100 mL of 1 M NaOH, dried with anhydrous K_2CO_2, and filtered. Removal of the solvent yielded 0.860 g of the TEDSMA as a pale yellow oil. ^1H NMR ($CDCl_3$) δ = 6.11 (2H, s), 5.55 (2H, s), 4.29 (4H, t), 3.51 – 3.8 (16H, m), 2.85 (4H, t), 1.93 (6H, s).

Scheme 8.



DEDSMA

8.1 Fabrication of 2 μm Postively Charged DEDSMA particles

A patterned perfluoropolyether (PFPE) mold was generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 2 μm rectangles. A poly(dimethylsiloxane) mold was used to confine the liquid PFPE-DMA to the desired area. The apparatus was then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold was then released from the silicon master. Separately, a mixture composed of acryloxyethyltrimethylammonium chloride (24.4 mg), DEDSMA (213.0 mg), Polyflour 570 (2.5 mg), diethoxyacetophenone (5.0 mg), methanol (39.0 mg), acetonitrile (39.0 mg), water (8.0 mg), and N,N-dimethylformamide (6.6 mg) was prepared. This mixture was spotted directly onto the patterned PFPE-DMA surface and covered with a separated unpatterned PFPE-DMA surface. The mold and surface were placed in molding apparatus, purge with N_2 for ten minutes, and placed under at least 500 N/cm^2 pressure for 2 hours. The entire apparatus was then subjected to UV light (λ = 365 nm) for 40 minutes while maintaining nitrogen purge. DEDSMA particles were harvested on glass slide using cyanoacrylate adhesive. The particles were purified by dissolving the adhesive layer with acetone followed by centrifugation of the suspended particles (see Figure 62 and 63).

8.2 Encapsulation of Calcein inside 2 μm Postively Charged DEDSMA particles

A patterned perfluoropolyether (PFPE) mold was generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 2 μm rectangles. A poly(dimethylsiloxane) mold was used to confine the liquid

PFPE-DMA to the desired area. The apparatus was then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold was then released from the silicon master. Separately, a mixture composed of acryloxyethyltrimethylammonium chloride (3.4 mg), DEDSMA (29.7 mg), calcein (0.7 mg), Polyflour 570 (0.35 mg), diethoxyacetophenone (0.7 mg), methanol (5.45 mg), acetonitrile (5.45 mg), water (1.11 mg), and N,N-dimethylformamide (6.6 mg) was prepared. This mixture was spotted directly onto the patterned PFPE-DMA surface and covered with a separated unpatterned PFPE-DMA surface. The mold and surface were placed in molding apparatus, purge with N_2 for ten minutes, and placed under at least 500 N/cm^2 pressure for 2 hours. The entire apparatus was then subjected to UV light (λ = 365 nm) for 40 minutes while maintaining nitrogen purge. Calcein containing DEDSMA particles were harvested on glass slide using cyanoacrylate adhesive. The particles were purified by dissolving the adhesive layer with acetone followed by centrifugation of the suspended particles (see Figure 64).

8.3 Encapsulation of Plasmid DNA into Charged DEDSMA particles

A patterned perfluoropolyether (PFPE) mold was generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 2 μm rectangles. A poly(dimethylsiloxane) mold was used to confine the liquid PFPE-DMA to the desired area. The apparatus was then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold was then released from the silicon master. Separately, 0.5 μg of flourescein-labelled plasmid DNA (Mirus Biotech) as a 0.25 μg/μL solution in TE buffer and a 2.0 μg of pSV β-galactosidase control vector (Promega) as a 1.0 μg/μL solution in TE buffer were sequentially added to a mixture composed of acryloxyethyltrimethylammonium chloride (1.44 mg), DEDSMA (12.7 mg), , Polyflour 570 (Polysciences, 0.08 mg), 1-hydroxycyclohexyl phenyl ketone (0.28 mg), methanol (5.96 mg), acetonitrile (5.96 mg), water (0.64 mg), and N,N-dimethylformamide (14.16 mg). This mixture was spotted directly onto the patterned PFPE-DMA surface and

covered with a separated unpatterned PFPE-DMA surface. The mold and surface were placed in molding apparatus, purge with N_2 for ten minutes, and placed under at least 500 N/cm^2 pressure for 2 hours. The entire apparatus was then subjected to UV light (λ = 365 nm) for 40 minutes while maintaining nitrogen purge. These particles were harvested on glass slide using cyanoacrylate adhesive. The particles were purified by dissolving the adhesive layer with acetone followed by centrifugation of the suspended particles (see Figure 65).

8.4 Encapsulation of Plasmid DNA into PEG Particles

A patterned perfluoropolyether (PFPE) mold was generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 1-hydroxycyclohexyl phenyl ketone over a silicon substrate patterned with 2 μm rectangles. A poly(dimethylsiloxane) mold was used to confine the liquid PFPE-DMA to the desired area. The apparatus was then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold was then released from the silicon master. Separately, 0.5 μg of flourescein-labelled plasmid DNA (Mirus Biotech) as a 0.25 μg/μL solution in TE buffer and a 2.0 μg of pSV β-galactosidase control vector (Promega) as a 1.0 μg/μL solution in TE buffer were sequentially added to a mixture composed of acryloxyethyltrimethylammonium chloride (1.2 mg), polyethylene glycol diacrylate (n=9) (10.56 mg), Polyflour 570 (Polysciences, 0.12 mg), diethoxyacetophenone (0.12 mg), methanol (1.5 mg), water (0.31 mg), and N,N-dimethylformamide (7.2 mg). This mixture was spotted directly onto the patterned PFPE-DMA surface and covered with a separated unpatterned PFPE-DMA surface. The mold and surface were placed in molding apparatus, purge with N_2 for ten minutes, and placed under at least 500 N/cm^2 pressure for 2 hours. The entire apparatus was then subjected to UV light (λ = 365 nm) for 40 minutes while maintaining nitrogen purge. These particles were harvested on glass slide using cyanoacrylate adhesive. The particles were purified by dissolving the

adhesive layer with acetone followed by centrifugation of the suspended particles (see Figure 66).

The following references may provide information and techniques to supplement some of the techniques and parameters of the present examples, therefore, the references are incorporated by reference herein in their entirety including any and all references cited therein. Li, Y., and Armes, S. P. Synthesis and Chemical Degradation of Branched Vinyl Polymers Prepared via ATRP: Use of a Cleavable Disulfide-Based Branching Agent. Macromolecules 2005; 38: 8155-8162; and Lang, H., Duschl, C., and Vogel, H. (1994), A new class of thiolipids for the attachment of lipid bilayers on gold surfaces. Langmuir *10*, 197-210.

Example 9

Cellular Uptake of PRINT Particles - Effect of Charge

9.1 Fabrication of 200 nm cylindrical fluorescently-tagged neutral PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone over a silicon substrate patterned with 200 nm cylindrical shapes (see Figure 67). The apparatus is then subjected to a nitrogen purge for 10 minutes before the application of UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 28 wt% PEG methacrylate (n=9), 2 wt% azobisisobutyronitrile (AIBN), and 0.25 wt% rhodamine methacrylate. Flat, uniform, non-wetting surfaces are generated by coating a glass slide with PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone. The slide is then subjected to a nitrogen purge for 10 minutes, then UV light is applied (λ = 365 nm) while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of the monomer blend is evenly spotted onto the flat PFPE-DMA surface and then the patterned PFPE-DMA mold placed on top of it. The surface and mold are then placed in a molding apparatus and a small amount of pressure is

applied to remove any excess monomer solution. The entire apparatus is purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. Neutral PEG nanoparticles are observed after separation of the PFPE-DMA mold and substrate using scanning electron microscopy (SEM). The harvesting process begins by spraying a thin layer of cyanoacrylate monomer onto the PFPE-DMA mold filled with particles. The PFPE-DMA mold is immediately placed onto a glass slide and the cyanoacrylate is allowed to polymerize in an anionic fashion for one minute. The mold is removed and the particles are embedded in the soluble adhesive layer (see Figure 68), which provides isolated, harvested colloidal particle dispersions upon dissolution of the soluble adhesive polymer layer in acetone. Particles embedded in the harvesting layer, or dispersed in acetone can be visualized by SEM. The dissolved poly(cyanoacrylate) can remain with the particles in solution, or can be removed via centrifugation.

9.2 Fabrication of 200 nm cylindrical fluorescently-tagged 14 wt% cationically charged PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone over a silicon substrate patterned with 200 nm cylindrical shapes (see Figure 67). The apparatus is then subjected to a nitrogen purge for 10 minutes before the application of UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 14 wt% PEG methacrylate (n=9), 14 wt% 2-acryloxyethyltrimethylammonium chloride (AETMAC), 2 wt% azobisisobutyronitrile (AIBN), and 0.25 wt% rhodamine methacrylate. Flat, uniform, non-wetting surfaces are generated by coating a glass slide with PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone. The slide is then subjected to a nitrogen purge for 10 minutes, then UV light is applied (λ = 365 nm) while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of

the monomer blend is evenly spotted onto the flat PFPE-DMA surface and then the patterned PFPE-DMA mold placed on top of it. The surface and mold are then placed in a molding apparatus and a small amount of pressure is applied to remove any excess monomer solution. The entire apparatus is purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. Cationically charged PEG nanoparticles are observed after separation of the PFPE-DMA mold and substrate using scanning electron microscopy (SEM). The harvesting process begins by spraying a thin layer of cyanoacrylate monomer onto the PFPE-DMA mold filled with particles. The PFPE-DMA mold is immediately placed onto a glass slide and the cyanoacrylate is allowed to polymerize in an anionic fashion for one minute. The mold is removed and the particles are embedded in the soluble adhesive layer (see Figure 68), which provides isolated, harvested colloidal particle dispersions upon dissolution of the soluble adhesive polymer layer in acetone. Particles embedded in the harvesting layer or dispersed in acetone can be visualized by SEM. The dissolved poly(cyanoacrylate) can remain with the particles in solution, or can be removed via centrifugation.

9.3 Fabrication of 200 nm cylindrical fluorescently-tagged 28 wt% cationically charged PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone over a silicon substrate patterned with 200 nm cylindrical shapes (see Figure 67). The apparatus is then subjected to a nitrogen purge for 10 minutes before the application of UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 28 wt% 2-acryloxyethyltrimethylammonium chloride (AETMAC), 2 wt% azobisisobutyronitrile (AIBN), and 0.25 wt% rhodamine methacrylate. Flat, uniform, non-wetting surfaces are generated by coating a glass slide with PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone. The slide is then subjected to a nitrogen purge for

10 minutes, then UV light is applied (λ = 365 nm) while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of the monomer blend is evenly spotted onto the flat PFPE-DMA surface and then the patterned PFPE-DMA mold placed on top of it. The surface and mold are then placed in a molding apparatus and a small amount of pressure is applied to remove any excess monomer solution. The entire apparatus is purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. Cationically charged PEG nanoparticles are observed after separation of the PFPE-DMA mold and substrate using scanning electron microscopy (SEM). The harvesting process begins by spraying a thin layer of cyanoacrylate monomer onto the PFPE-DMA mold filled with particles. The PFPE-DMA mold is immediately placed onto a glass slide and the cyanoacrylate is allowed to polymerize in an anionic fashion for one minute. The mold is removed and the particles are embedded in the soluble adhesive layer (see Figure 68), which provides isolated, harvested colloidal particle dispersions upon dissolution of the soluble adhesive polymer layer in acetone. Particles embedded in the harvesting layer or dispersed in acetone can be visualized by SEM. The dissolved poly(cyanoacrylate) can remain with the particles in solution, or can be removed via centrifugation.

9.4 Cellular uptake of 200 nm cylindrically shaped neutral PEG PRINT particles

The neutral 200 nm cylindrical PEG particles (aspect ratio = 1:1, 200 nm x 200 nm particles) fabricated using PRINT were dispersed in 250 µL of water to be used in cellular uptake experiments. These particles were exposed to NIH 3T3 (mouse embryonic) cells at a final concentration of particles of 60 µg/mL. The particles and cells were incubated for 4 hrs at 5 % CO_2 at 37°C. The cells were then characterized via confocal microscopy (see Figure 69) and cell toxicities were assessed using an MTT assay (see Figure 70).

9.5 Cellular uptake of 200 nm cylindrically shaped 14 wt% cationically charged PEG PRINT particles

The 14 wt% cationically charged 200 nm cylindrical PEG particles (aspect ratio = 1:1, 200 nm x 200 nm particles) fabricated using PRINT were dispersed in 250 µL of water to be used in cellular uptake experiments. These particles were exposed to NIH 3T3 (mouse embryonic) cells at a final concentration of particles of 60 µg/mL. The particles and cells were incubated for 4 hrs at 5 % CO_2 at 37°C. The cells were then characterized via confocal microscopy (see Figure 69) and cell toxicities were assessed using an MTT assay (see Figure 70).

9.6 Cellular uptake of 200 nm cylindrically shaped 28 wt% cationically charged PEG PRINT particles

The 28 wt% cationically charged 200 nm cylindrical PEG particles (aspect ratio = 1:1, 200 nm x 200 nm particles) fabricated using PRINT were dispersed in 250 µL of water to be used in cellular uptake experiments. These particles were exposed to NIH 3T3 (mouse embryonic) cells at a final concentration of particles of 60 µg/mL. The particles and cells were incubated for 4 hrs at 5 % CO_2 at 37°C. The cells were then characterized via confocal microscopy (see Figure 69) and cell toxicities were assessed using an MTT assay (see Figure 70).

Example 10

Cellular Uptake of PRINT Particles – Effect of Size

10.1 Fabrication of 200 nm cylindrical fluorescently-tagged 14 wt% cationically charged PEG particles – *repeat*

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone over a silicon substrate patterned with 200 nm cylindrical shapes (see Figure 67). The apparatus is then subjected to a nitrogen purge for 10 minutes before the application of UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate

(n=9) is blended with 14 wt% PEG methacrylate (n=9), 14 wt% 2-acryloxyethyltrimethylammonium chloride (AETMAC), 2 wt% azobisisobutyronitrile (AIBN), and 0.25 wt% rhodamine methacrylate. Flat, uniform, non-wetting surfaces are generated by coating a glass slide with PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone. The slide is then subjected to a nitrogen purge for 10 minutes, then UV light is applied (λ = 365 nm) while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of the monomer blend is evenly spotted onto the flat PFPE-DMA surface and then the patterned PFPE-DMA mold placed on top of it. The surface and mold are then placed in a molding apparatus and a small amount of pressure is applied to remove any excess monomer solution. The entire apparatus is purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. Cationically charged PEG nanoparticles are observed after separation of the PFPE-DMA mold and substrate using scanning electron microscopy (SEM). The harvesting process begins by spraying a thin layer of cyanoacrylate monomer onto the PFPE-DMA mold filled with particles. The PFPE-DMA mold is immediately placed onto a glass slide and the cyanoacrylate is allowed to polymerize in an anionic fashion for one minute. The mold is removed and the particles are embedded in the soluble adhesive layer (see Figure 68), which provides isolated, harvested colloidal particle dispersions upon dissolution of the soluble adhesive polymer layer in acetone. Particles embedded in the harvesting layer or dispersed in acetone can be visualized by SEM. The dissolved poly(cyanoacrylate) can remain with the particles in solution, or can be removed via centrifugation.

10.2 Fabrication of 2 μm x 2 μm x 1 μm cubic fluorescently-tagged 14 wt% cationically charged PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone over a silicon substrate patterned with 2 μm x 2 μm x 1 μm cubic shapes. The apparatus is then subjected to a nitrogen purge for 10 minutes before

the application of UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 14 wt% PEG methacrylate (n=9), 14 wt% 2-acryloxyethyltrimethylammonium chloride (AETMAC), 2 wt% azobisisobutyronitrile (AIBN), and 0.25 wt% rhodamine methacrylate. Flat, uniform, non-wetting surfaces are generated by coating a glass slide with PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone. The slide is then subjected to a nitrogen purge for 10 minutes, then UV light is applied (λ = 365 nm) while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of the monomer blend is evenly spotted onto the flat PFPE-DMA surface and then the patterned PFPE-DMA mold placed on top of it. The surface and mold are then placed in a molding apparatus and a small amount of pressure is applied to remove any excess monomer solution. The entire apparatus is purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. Cationically charged PEG nanoparticles are observed after separation of the PFPE-DMA mold and substrate using scanning electron microscopy (SEM), optical and fluorescence microscopy (excitation λ= 526nm, emission λ=555 nm). The harvesting process begins by spraying a thin layer of cyanoacrylate monomer onto the PFPE-DMA mold filled with particles. The PFPE-DMA mold is immediately placed onto a glass slide and the cyanoacrylate is allowed to polymerize in an anionic fashion for one minute. The mold is removed and the particles are embedded in the soluble adhesive layer, which provides isolated, harvested colloidal particle dispersions upon dissolution of the soluble adhesive polymer layer in acetone. Particles embedded in the harvesting layer or dispersed in acetone can be visualized by SEM. The dissolved poly(cyanoacrylate) can remain with the particles in solution, or can be removed via centrifugation.

10.3 Fabrication of 5 μm x 5 μm x 5 μm cubic fluorescently-tagged 14 wt% cationically charged PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone over a silicon substrate patterned with 5 μm x 5 μm x 5 μm cubic shapes. The apparatus is then subjected to a nitrogen purge for 10 minutes before the application of UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 14 wt% PEG methacrylate (n=9), 14 wt% 2-acryloxyethyltrimethylammonium chloride (AETMAC), 2 wt% azobisisobutyronitrile (AIBN), and 0.25 wt% rhodamine methacrylate. Flat, uniform, non-wetting surfaces are generated by coating a glass slide with PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone. The slide is then subjected to a nitrogen purge for 10 minutes, then UV light is applied (λ = 365 nm) while under a nitrogen purge. The flat, fully cured PFPE-DMA substrate is released from the slide. Following this, 0.1 mL of the monomer blend is evenly spotted onto the flat PFPE-DMA surface and then the patterned PFPE-DMA mold placed on top of it. The surface and mold are then placed in a molding apparatus and a small amount of pressure is applied to remove any excess monomer solution. The entire apparatus is purged with nitrogen for 10 minutes, then subjected to UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. Cationically charged PEG nanoparticles are observed after separation of the PFPE-DMA mold and substrate using scanning electron microscopy (SEM), optical and fluorescence microscopy (excitation λ= 526nm, emission λ=555 nm). The harvesting process begins by spraying a thin layer of cyanoacrylate monomer onto the PFPE-DMA mold filled with particles. The PFPE-DMA mold is immediately placed onto a glass slide and the cyanoacrylate is allowed to polymerize in an anionic fashion for one minute. The mold is removed and the particles are embedded in the soluble adhesive layer, which provides isolated, harvested colloidal particle dispersions upon dissolution of the soluble adhesive polymer layer in acetone. Particles

embedded in the harvesting layer, or dispersed in acetone can be visualized by SEM. The dissolved poly(cyanoacrylate) can remain with the particles in solution, or can be removed via centrifugation.

10.4 Cellular uptake of 200 nm cylindrically shaped 14 wt% cationically charged PEG PRINT particles – *Repeat*

The 14 wt% cationically charged 200 nm cylindrical PEG particles (aspect ratio = 1:1, 200 nm x 200 nm particles) fabricated using PRINT were dispersed in 250 µL of water to be used in cellular uptake experiments. These particles were exposed to NIH 3T3 (mouse embryonic) cells at a final concentration of particles of 60 µg/mL. The particles and cells were incubated for 4 hrs at 5 % CO_2 at 37 °C. The cells were then characterized via confocal microscopy (see Figure 71).

10.5 Cellular uptake of 2 µm x 2 µm x 1 µm cubic shaped 14 wt% cationically charged PEG PRINT particles

The 14 wt% cationically charged 2 µm x 2 µm x 1 µm cubic PEG particles fabricated using PRINT were dispersed in 250 µL of water to be used in cellular uptake experiments. These particles were exposed to NIH 3T3 (mouse embryonic) cells at a final concentration of particles of 60 µg/mL. The particles and cells were incubated for 4 hrs at 5 % CO_2 at 37°C. The cells were then characterized via confocal microscopy (see Figure 71).

10.6 Cellular uptake of 5 µm x 5 µm x 5 µm cubic shaped 14 wt% cationically charged PEG PRINT particles

The 14 wt% cationically charged 5 µm x 5 µm x 5 µm cubic PEG particles fabricated using PRINT were dispersed in 250 µL of water to be used in cellular uptake experiments. These particles were exposed to NIH 3T3 (mouse embryonic) cells at a final concentration of particles of 60 µg/mL. The particles and cells were incubated for 4 hrs at 5 % CO_2 at 37°C. The cells were then characterized via confocal microscopy (see Figure 71).

Example 11

Cellular Uptake of DEDSMA PRINT Particles

11.1 Cellular uptake of DEDSMA PRINT particles

The DEDSMA particles fabricated using PRINT were dispersed in 250 µL of water to be used in cellular uptake experiments. These particles were exposed to NIH 3T3 (mouse embryonic) cells at a final concentration of particles of 60 µg/mL. The particles and cells were incubated for 4 hrs at 5 % CO_2 at 37°C. The cells were then characterized via confocal microscopy.

Example 12

Radiolabeling PRINT particles

12.1 Synthesis of ^{14}C radiolabeled 2 µm x 2 µm x 1 µm cubic PRINT particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone over a silicon substrate patterned with 2 µm x 2 µm x 1 µm cubic shapes. The apparatus is then subjected to a nitrogen purge for 10 minutes before the application of UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 30 wt% 2-aminoethylmethacrylate hydrochloride (AEM), and 1 wt% 2,2-diethoxyacetophenone. The monomer solution is applied to the mold by spraying a diluted (10X) blend of the monomers with isopropyl alcohol. A polyethylene sheet is placed onto the mold, and any residual air bubbles are pushed out with a roller. The sheet is slowly pulled back from the mold at a rate of 1 inch/minute. The mold is then subjected to a nitrogen purge for 10 minutes, then UV light is applied (λ = 365 nm) while under a nitrogen purge. The harvesting process begins by spraying a thin layer of cyanoacrylate monomer onto the PFPE-DMA mold filled with particles. The PFPE-DMA mold is immediately placed onto a glass slide and the cyanoacrylate is allowed to polymerize in an anionic fashion for one minute. The mold is removed and the particles are embedded in the soluble adhesive layer, which provides isolated, harvested colloidal particle

dispersions upon dissolution of the soluble adhesive polymer layer in acetone. Particles embedded in the harvesting layer, or dispersed in acetone can be visualized by SEM, and optical microscopy. The dissolved poly(cyanoacrylate) can remain with the particles in solution, or can be removed via centrifugation. The dry, purified particles are then exposed to ^{14}C-acetic anhydride in dry dichloromethane in the presence of triethylamine, and 4-dimethylaminopyridine for 24 hours (see Figure 72). Unreacted reagents are removed via centrifugation. Efficiency of the reaction is monitored by measured the emitted radioactivity in a scintillation vial.

12.2 Synthesis of ^{14}C radiolabeled 200 nm cylindrical PRINT particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2-diethoxyacetophenone over a silicon substrate patterned with 200 nm cylindrical shapes. The apparatus is then subjected to a nitrogen purge for 10 minutes before the application of UV light (λ = 365 nm) for 10 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 30 wt% 2-aminoethylmethacrylate hydrochloride (AEM), and 1 wt% 2,2-diethoxyacetophenone. The monomer solution is applied to the mold by spraying a diluted (10X) blend of the monomers with isopropyl alcohol. A polyethylene sheet is placed onto the mold, and any residual air bubbles are pushed out with a roller. The sheet is slowly pulled back from the mold at a rate of 1 inch/minute. The mold is then subjected to a nitrogen purge for 10 minutes, then UV light is applied (λ = 365 nm) while under a nitrogen purge. The harvesting process begins by spraying a thin layer of cyanoacrylate monomer onto the PFPE-DMA mold filled with particles. The PFPE-DMA mold is immediately placed onto a glass slide and the cyanoacrylate is allowed to polymerize in an anionic fashion for one minute. The mold is removed and the particles are embedded in the soluble adhesive layer, which provides isolated, harvested colloidal particle dispersions upon dissolution of the soluble adhesive polymer layer in acetone. Particles embedded in the harvesting layer, or dispersed in

acetone can be visualized by SEM. The dissolved poly(cyanoacrylate) can remain with the particles in solution, or can be removed via centrifugation. The dry, purified particles are then exposed to ^{14}C-acetic anhydride in dry dichloromethane in the presence of triethylamine, and 4-dimethylaminopyridine for 24 hours (see Figure 72). Unreacted reagents are removed via centrifugation. Efficiency of the reaction is monitored by measured the emitted radioactivity in a scintillation vial.

12.3 Fabrication of pendant gadolinium PEG particles

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon substrate patterned with 3 x 3 x 11 um pillar shapes. The apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 2,2'-diethoxy-acetophenone. 20 μL of chloroform, 70 μL of PEG diacrylate monomer and 30 uL of DPTA-PEG-acrylate are mixed. Flat, uniform, non-wetting surfaces are generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon wafer and then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Following this, 50 μL of the PEG diacrylate solution is then placed on the non-wetting surface and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold. The particles were harvested utilizing a sacrificial adhesive layer and verified via DIC microscopy. These particles were subsequently treated with an aqueous solution of $Gd(NO_3)_3$. These particles were then dispersed in a agrose gel and T1 weighted imaging profiles were examined utilizing a Siemens Allegra 3T head magnetic resonance instrument (see Figure 73).

12.4 Forming a particle containing CDI linker

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon substrate patterned with 200 nm shapes. The apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 2,2'-diethoxy-acetophenone. 70 μL of PEG diacrylate monomer and 30 uL of CDI-PEG monomer were mixed. Specifically, the CDI-PEG monomer was synthesized by adding 1,1'-carbonyl diimidazole (CDI) to a solution of PEG (n=400) monomethylacrylate in chloroform. This solution was allowed to stir overnight. This solution was then further purified by an extraction with cold water. The resulting CDI-PEG monomethacrylate was then isolated via vacuum. Flat, uniform, non-wetting surfaces are generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon wafer and then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Following this, 50 μL of the PEG diacrylate solution is then placed on the non wetting surface and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold. The particles were harvested utilizing a sacrificial adhesive layer and verified via DIC microscopy. This linker can be utilized to attach an amine containing target onto the particle (see Figure 74).

12.5 Tethering avidin to the CDI linker

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon substrate patterned with 200 nm shapes. The apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon

master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 2,2'-diethoxy-acetophenone. 70 μL of PEG diacrylate monomer and 30 uL of CDI-PEG monomer were mixed. Specifically, the CDI-PEG monomer was synthesized by adding 1,1'-carbonyl diimidazole (CDI) to a solution of PEG (n=400) monomethylacrylate in chloroform. This solution was allowed to stir overnight. This solution was then further purified by an extraction with cold water. The resulting CDI-PEG monomethacrylate was then isolated via vacuum. Flat, uniform, non-wetting surfaces are generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon wafer and then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Following this, 50 μL of the PEG diacrylate solution is then placed on the non wetting surface and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold. The particles were harvested utilizing a sacrificial adhesive layer and verified via DIC microscopy. These particles containing the CDI linker group were subsequently treated with and aqueous solution of fluorescently tagged avidin. These particles were allowed to stir at room temperature for four hours. These particles were then isolated via centrifugation and rinsed with deionized water. Attachment was confirmed via confocal microscopy (see Figure 75).

12.6 Fabrication of PEG particles that target the HER2 receptor

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon substrate patterned with 200 nm shapes. The apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 2,2'-diethoxy-acetophenone. 70 μL of

PEG diacrylate monomer and 30 uL of CDI-PEG monomer were mixed. Specifically, the CDI-PEG monomer was synthesized by adding 1,1'-carbonyl diimidazole (CDI) to a solution of PEG (n=400) monomethylacrylate in chloroform. This solution was allowed to stir overnight. This solution was then further purified by an extraction with cold water. The resulting CDI-PEG monomethacrylate was then isolated via vacuum. Flat, uniform, non-wetting surfaces are generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon wafer and then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Following this, 50 μL of the PEG diacrylate solution is then placed on the non wetting surface and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold. The particles were harvested utilizing a sacrificial adhesive layer and verified via DIC microscopy. These particles containing the CDI linker group were subsequently treated with and aqueous solution of fluorescently tagged avidin. These particles were allowed to stir at room temperature for four hours. These particles were then isolated via centrifugation and rinsed with deionized water. These avidin labeled particles were then treated with biotinylated FAB fragments. Attachment was confirmed via confocal microscopy (see Figure 76).

12.7 Fabrication of PEG particles that target non-Hodgkin's lymphoma

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon substrate patterned with 200 nm shapes. The apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 2,2'-diethoxy-acetophenone. 70 μL of PEG diacrylate monomer and 30 uL of CDI-PEG monomer were mixed.

Specifically, the CDI-PEG monomer was synthesized by adding 1,1'-carbonyl diimidazole (CDI) to a solution of PEG (n=400) monomethylacrylate in chloroform. This solution was allowed to stir overnight. This solution was then further purified by an extraction with cold water. The resulting CDI-PEG monomethacrylate was then isolated via vacuum. Flat, uniform, non-wetting surfaces are generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon wafer and then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Following this, 50 μL of the PEG diacrylate solution is then placed on the non wetting surface and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold. The particles were harvested utilizing a sacrificial adhesive layer and verified via DIC microscopy. These particles containing the CDI linker group were subsequently treated with and aqueous solution of fluorescently tagged avidin. These particles were allowed to stir at room temperature for four hours. These particles were then isolated via centrifugation and rinsed with deionized water. These avidin labeled particles were then treated with biotinylated-SUP-B8 (peptide specific to the specific surface immunoglobulin (sIg) known as the idiotype, which is distinct from the sIg of all of the patient's non-neoplastic cells) (see Figure 77).

12.8 Controlled mesh density: phantom study and cellular uptake/MTT assay

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon substrate patterned with 3 x 3 x 11 um pillar shapes. The apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 2,2'-diethoxy-acetophenone.

56 μL of PEG diacrylate monomer, 19 uL of PEG monomethacrylate, 10 ug 2-acryloxyethyltrimethylammonium chloride (AETMAC), and 23 uL of a doxorubicin (26 mg/mL) are mixed. Flat, uniform, non-wetting surfaces are generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon wafer and then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Following this, 50 μL of the PEG diacrylate solution is then placed on the non-wetting surface and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold. The particles were harvested utilizing a sacrificial adhesive layer and verified via DIC microscopy. These particles were then dispersed in an aqueous solution and exposed to NIH 3T3 mouse embryo fibroblasts cell lines at a concentration of nanoparticles of 50 ug/mL. The particles and cells were incubated for 48 hrs at 5 % CO_2 at 37 °C. The cells were then characterized via confocal and MTT assay.

12.9 Fabrication of particles by dipping methods

A mold (**5104**) of size 0.5x3 cm with 3x3x8 micron patterned recesses (**5106**) was dipped into the vial (**5102**) with 98 % PEG-diacrylate and 2% photo initiator solution. After 30 seconds the mold was withdrawn at a rate of approximately 1 mm per second. The process is schematically shown in Figure 51. Next, the mold was put into a UV oven, purged with nitrogen for 15 minutes and then cured for 15 minutes. The particles were then harvested on a glass slide using cyanoacrylate adhesive. No scum was detected and monodispersity of the particles was confirmed using optical microscope, as shown in the image of Figure 54. Furthermore, as evident in Figure 54, the material contained in the recesses formed a meniscus with the sides of the recesses, as shown by reference number **5402**. This meniscus, when cured formed a lens on a portion of the particle.

12.10 Fabrication of particles by droplet moving

A mold (**5200**), 6 inch in diameter with 5x5x10 micron pattern recesses (**5206**) was placed on an incline surface having an angle of 20 degrees (**5210**) to the horizon. Next, a set of 100 micro liter drops (**5204**) were placed on the surface of the mold at a higher end. Each drop slid down the mold leaving a trace of filled recesses (**5208**). The process is schematically shown in Figure 52.

After all the drops reached the lower end of the mold, the mold was put in a UV oven, purged with nitrogen for 15 minutes and then cured for 15 minutes. The particles were harvested on a glass slide using cyanoacrylate adhesive. No scum was detected and monodispersity of the particles was confirmed first using optical microscope (Figure 55) and then by scanning electron microscope (Figure 55). Furthermore, as evident in Figure 55, the material contained in the recesses formed a meniscus with the sides of the recesses, as shown by reference number **5502**. This meniscus, when cured formed a lens on a portion of the particle.

Example 13

Control Mouse Studies

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon substrate patterned with 200 nm shapes. The apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master. Separately, a poly(ethylene glycol) (PEG) diacrylate (n=9) is blended with 1 wt% of a photoinitiator, 2,2'-diethoxy-acetophenone. 70 μL of PEG diacrylate monomer and 30 uL of CDI-PEG monomer were mixed. Specifically, the CDI-PEG monomer was synthesized by adding 1,1'-carbonyl diimidazole (CDI) to a solution of PEG (n=400) monomethylacrylate in chloroform. This solution was allowed to stir overnight. This solution was then further purified by an extraction with cold water. The resulting CDI-PEG monomethacrylate was then isolated via vacuum. Flat, uniform, non-wetting

surfaces are generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon wafer and then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Following this, 50 μL of the PEG diacrylate solution is then placed on the non wetting surface and the patterned PFPE mold placed on top of it. The substrate is then placed in a molding apparatus and a small pressure is applied to push out excess PEG-diacrylate solution. The entire apparatus is then subjected to UV light (λ= 365 nm) for 15 minutes while under a nitrogen purge. Particles are observed after separation of the PFPE mold. The particles were harvested utilizing a sacrificial adhesive layer and verified via DIC microscopy. These particles containing the CDI linker group were subsequently treated with and aqueous solution of fluorescently tagged avidin. These particles were allowed to stir at room temperature for four hours. These particles were then isolated via centrifugation and rinsed with deionized water. These avidin labeled particles were then treated with biotin. A solution (2.5 mg avidin/biotin nanoparticles/200 uL saline) was administered to 4 Neu transgenic mice (2.5 mg avidin/biotin nanoparticles/200 uL saline) every 14 days for 2 cycles (total 28 days) versus a control group 4 Neu transgenic mice that was treated with 200 uL saline every 14 days for 2 cycles (total 28 days). Both sets of mice seemed to produce no adverse side effects from either treatment.

Example 14

Particle Fabrication

14.1 Synthesis of 200 nm cationic PEG particles for pharmacokinetics

A patterned perfluoropolyether (PFPE) mold is generated by pouring a PFPE-dimethacrylate (PFPE-DMA) containing 2,2'-diethoxy-acetophenone over a silicon substrate patterned with 200 nm shapes. The apparatus is purged with nitrogen for 10 minutes, and then subjected to UV light (λ= 365 nm) for 6 minutes while under a nitrogen purge. The fully cured PFPE-DMA mold is then released from the silicon master, and blown with air to remove dust. Separately, a solution containing 84 mol % PEG diacrylate, 5 mol % PEG monoacrylate, 10 mol% aminoethylmethacrylate hydrochloride, and 1

mol% photoinitiator was prepared. The mold was placed in a fume hood and the hydrogel-monomer solution was atomized onto mold. A polyethylene sheet was then placed over the mold and bubbles were removed by manual pressure with a roller. The polyethylene cover was slowly removed to fill the particle chambers. The mold/solution combination was placed into a UV curing chamber, purged for 10 minutes with nitrogen, and UV cured for 8 minutes. The particle/mold combination was placed in the spin coater and the spin coater started at approx 1000rpm. Approx 20 mls of nitro-cellulose was put into the center of the spinning mold and left to cure for 1 minute while rotating. The nitro-cellulose is then carefully lifted off the mold with particles attached and placed in a vial. Acetone is then added to dissolve the cellulose and leave the particles. The particles were purified via centrifugation, and then strained through a 100 mesh screen. The remaining acetone is carefully aspirated and the particles dried under nitrogen.

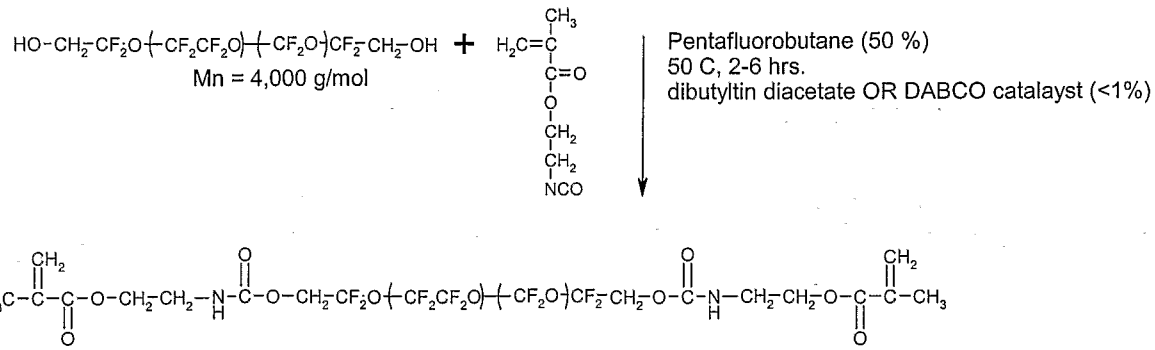
14.2 Synthesis of 200 nm triacrylate particles

Molds suitable for PRINT fabrication of 200x200x200 nm particles were prepared by pooling end-functionalized PFPE dimethacrylate precursor containing 0.1% diethoxyacetophenone (DEAP) photoinitiator onto a master template containing 200x200x200 nm posts. The telechelic PFPE precursor was UV polymerized under a blanket of nitrogen into a cross-linked rubber (the "mold"). The mold was then peeled away from the master, revealing 200x200x200 nm patterned cavities in the mold. 1 part trimethylolpropane triacrylate containing 10% DEAP ("triacrylate resin") was then dissolved in 10 parts methanol and spray-coated onto the patterned side of the mold until full coverage was achieved. A thin polyethylene sheet was placed over the patterned side of the mold and sealed to the mold by manually applying a small amount of pressure. The polyethylene sheet was then slowly peeled away from the mold (~1 mm/sec), allowing capillary filling of the cavities in the mold. Excess triacrylate resin was gathered at the PFPE/polyethylene interface and removed from the mold as the polyethylene sheet was peeled away. Once the polyethylene sheet was fully peeled away from the mold, any residual macroscopic droplets of triacrylate resin were removed from the

mold. The triacrylate resin filling the patterned cavities in the mold was then UV polymerized under a blanket of nitrogen for about 5 minutes. Collodion solution (Fisher Scientific) was then spin-cast onto the patterned side of the mold to produce a robust nitrocellulose-based film. This film was then peeled away from the mold to remove particles by adhesive transfer to the nitrocellulose film. The nitrocellulose film was then dissolved in acetone. The particles were purified from the dissolved nitrocellulose by a repetitive process of sedimenting the particles, decanting nitrocellulose/acetone solution, and resuspension of the particles in clean acetone. This process was repeated until all the nitrocellulose was separated from the particles.

Example 15

Polymer Synthesis



15.1 Synthesis of PFPE Diurethane Dimethacrylate

Firstly, 50 mL (0.0125 moles) of ZDOL 4000 is measured and added to a three-neck, 250 mL round bottom flask which has been thoroughly dried in the oven. To this is added 50 mL of Solkane (1,1,1-3,3-pentafluorobutane). The flask is equipped with a condenser, rubber septa, a magnetic stir bar and outfitted with a nitrogen purge. Under a steady nitrogen purge, the flask is allowed to purge for 10 minutes. To the clear solution, 3.879g (0.025 moles) (3.54 mL) of 2- isocyanatoethyl methacrylate (EIM) is injected. Following this, 0.2 wt% (~0.1 mL) of dibutyltin diacetate catalyst is added to the solution. Alternatively, tertiary amine catalysts such as DABCO™ can be added in typical concentrations of 1 wt%. The solution

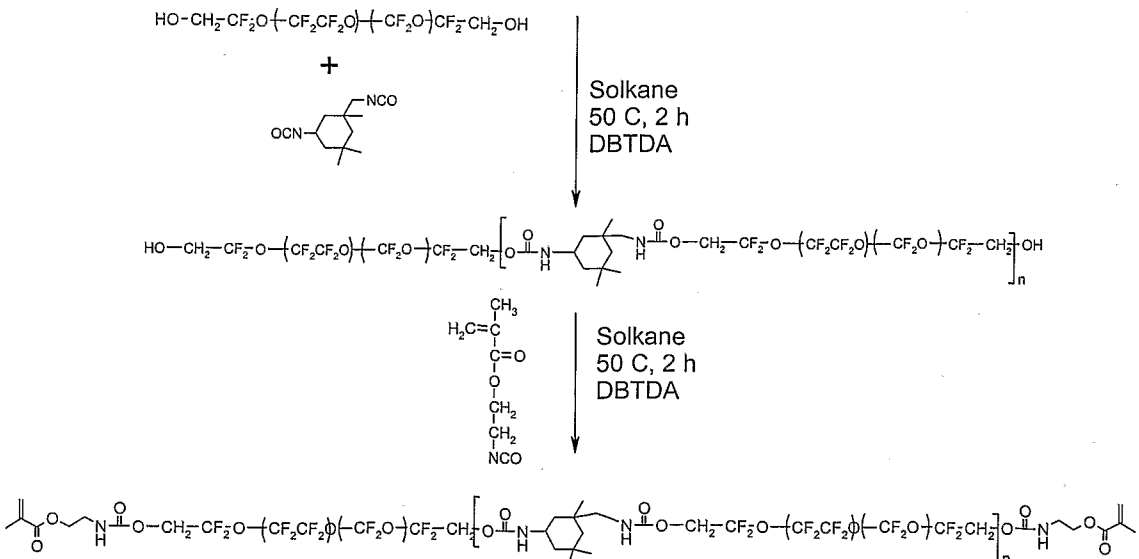
is heated to 50°C and allowed to reflux for 2-6 h under a slow, constant nitrogen purge. The flask is removed from heat and 25 mL of Solkane are added to the flask to further dilute the solution.

Next, A flash column is prepared using neutral alumina (the purpose of the flash column is to remove residual catalyst and any unreacted EIM). The column is typically 24 mm in diameter and filled with ~ 15 cm of alumina. The alumina is first wetted by running ~ 50 mL of Solkane until it begins to drip out of the column. The diluted reaction solution is then passed through the column under slight nitrogen pressure.

To the purified solution, 0.5g (0.1 – 1.0 wt% relative to ZDOL) of photoinitiator (particularly useful photoinitiators include: 1-hydroxycyclohexyl phenyl ketone, diethoxyacetophenone, and dimethoxy phenylacetophenone) is added and agitated until completely dissolved. Most of the Solkane is removed from the solution via rotovap. The remaining trace amounts are removed by placing the flask under vacuum for 3 hours while stirring. The clear solution will turn into a cloudy mixture as immiscible photoinitiator crashes out. This method ensures the maximum amount of photoinitiator is dissolved in the PFPE oil.

Finally, the cloudy oil is passed through a 0.22 µm Poly(ether sulfone) filter. A clear, water-white, viscous oil is collected at the bottom of the vacuum filtration vessel.

15.2 Synthesis of PFPE Chain-Extended Diurethane Dimethacrylate



Firstly, 50 g (0.0125 moles) of ZDOL 4000 is measured and added to a three-neck, 250 mL round bottom flask which has been thoroughly dried in the oven. 50 mL of Solkane is added to the flask. The flask is equipped with a condenser, rubber septa, a magnetic stir bar and outfitted with a nitrogen purge. Under a steady nitrogen purge, the flask is allowed to purge for 10 minutes. To the clear solution, 1.389 g (0.00625 moles) (1.31 mL) of IPDI is injected. Following this, 0.2 wt% (~0.1 mL) of dibutyltin diacetate catalyst is added to the solution. Alternatively, tertiary amine catalysts such as DABCO™ can be added in typical concentrations of 1 wt%. The solution is heated to 50°C and allowed to reflux for 2h under a slow, constant nitrogen purge (1 bubble every second on bubbler). To the clear solution, 1.9395 g (0.0125) (1.77 mL) of EIM is injected and the solution is allowed to reflux at 50 °C for an additional 2h under a slow, constant nitrogen purge.

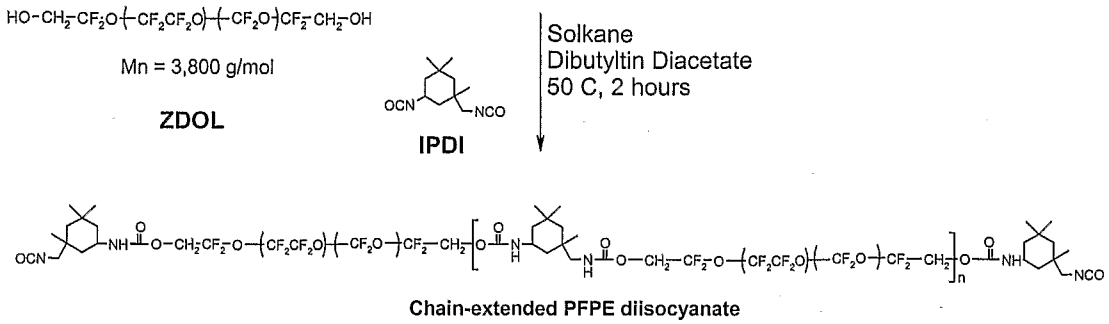
The reaction is taken off heat and 25 mL of solkane is added to further dilute the solution.

A flash column is prepared using neutral alumina (the purpose of the flash column is to remove residual catalyst and any unreacted EIM or IPDI). The column is typically 24 mm in diameter and filled with ~ 15 cm of alumina. The alumina is first wetted by running ~ 50 mL of Solkane until it begins to drip out of the column. The diluted reaction solution is then passed through the column under slight nitrogen pressure.

To the purified solution, 0.5g (0.1–1.0 wt% relative to ZDOL) of photoinitiator (particularly useful photoinitiators include: 1-hydroxycyclohexyl phenyl ketone, diethoxyacetophenone, and dimethoxy phenylacetophenone) is added and agitated until completely dissolved. Most of the Solkane is removed from the solution via rotovap. The remaining trace amounts are removed by placing the flask under vacuum for 3 hours while stirring. The clear solution will turn into a cloudy mixture as immiscible photoinitiator crashes out. This method ensures the maximum amount of photoinitiator is dissolved in the PFPE oil.

Finally, the cloudy oil is passed through a 0.22 μm Poly(ether sulfone) filter. A clear, water-white, viscous oil is collected at the bottom of the vacuum filtration vessel.

15.3 Synthesis of PFPE Diisocyanate



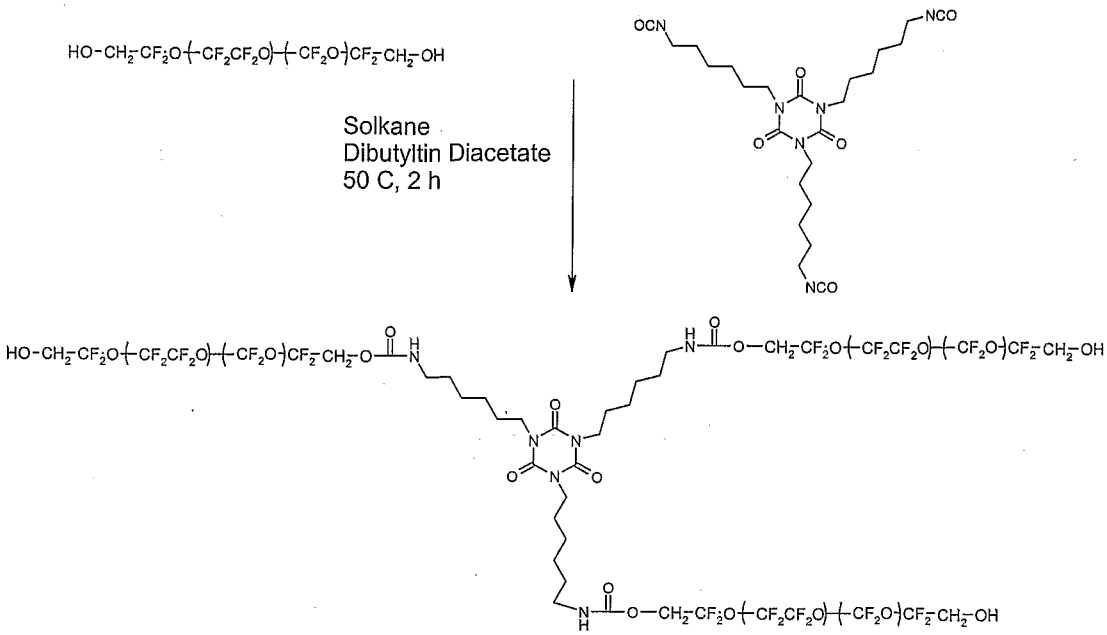
Firstly, 50 g (0.0125 moles) of ZDOL 4000 is measured and added to a three-neck, 250 mL round bottom flask which has been thoroughly dried in the oven.50 mL of Solkane is added to the flask. The flask is equipped with a condenser, rubber septa, a magnetic stir bar, and outfitted with a nitrogen purge. Under a steady nitrogen purge, the flask is allowed to purge for 10 minutes. To the clear solution, 4.167 g (0.01875 moles) (3.93 mL) of IPDI is injected. Following this, 0.2 wt% (~0.1 mL) of dibutyltin diacetate catalyst is added to the solution. Alternatively, tertiary amine catalysts such as DABCO™ can be added in typical concentrations of 1 wt%. The solution is heated to 50 °C and allowed to reflux for 2h under a slow, constant nitrogen purge. The reaction is taken off heat and 25 mL of solkane is injected to further dilute the solution.

A flash column is prepared using neutral alumina (the purpose of the flash column is to remove residual catalyst and any unreacted IPDI). The column is typically 24 mm in diameter and filled with ~ 15 cm of alumina. The alumina is first wetted by running ~ 50 mL of Solkane until it begins to drip out of the column. The diluted reaction solution is then passed through the column under slight nitrogen pressure. Once all of the solution has been run through, 50 mL of Solkane is passed through the column to pick up residual product. To prevent exposure to moisture the collection flask is sealed to the column using parafilm.

Most of the Solkane is removed from the solution via rotovap. The remaining trace amounts are removed by placing the flask under vacuum for

3 hours while stirring. The final product is a clear viscous oil and should be stored under vacuum in a dessicator.

15.4 Synthesis of PFPE Triol



Firstly, 50 g (0.033 moles) of Fluorolink-D (Solvay Solexis) is measured and added to a three-neck, 250 mL round bottom flask which has been thoroughly dried in the oven.50 mL of Solkane is added to the flask. The flask is equipped with a condenser, rubber septa, a magnetic stir bar, and outfitted with a nitrogen purge. Under a steady nitrogen purge, the flask is allowed to purge for 10 minutes. To the clear solution, 5.6 g (0.0112 moles) of Desmodur® N3600 (Bayer) dissolved in 10 mL of Solkane is injected. Following this, 0.2 wt% (~0.1 mL) of dibutyltin diacetate catalyst is added to the solution. Alternatively, tertiary amine catalysts such as DABCO™ can be added in typical concentrations of 1 wt%. The solution is heated to 50 °C and allowed to reflux for 2h under a slow, constant nitrogen purge. The reaction is taken off heat and 25 mL of solkane is injected to further dilute the solution.

A flash column is prepared using neutral alumina (the purpose of the flash column is to remove residual catalyst and any unreacted Desmodur). The column is typically 24 mm in diameter and filled with ~ 15 cm of alumina. The alumina is first wetted by running ~ 50 mL of Solkane until it begins to drip out of the column. The diluted reaction solution is then passed through the column under slight nitrogen pressure. Once all of the solution has been run through, 50 mL of Solkane is passed through the column to pick up residual product.

Most of the Solkane is removed from the solution via rotovap. The remaining trace amounts are removed by placing the flask under vacuum for 3 hours while stirring. The final product is a clear, water-white, viscous oil.

Example 16

Device Fabrication from Materials Synthesized in Examples 15.2, 15.3, and 15.4.

This Example describes the fabrication of microfluidic chips from the polymers synthesized herein:

To a 20 mL syringe were added the following: 20g of the material synthesized in Example 15.2 (Material 2), 2g of the material synthesized in Example 15.4 (Material 4), and 18.0 g of the material synthesized in Example 15.3 (Material 3). The materials were thoroughly mixed and degassed in a vacuum oven. The mixture was deposited onto a patterned master template to a thickness of 5 mm. Separately, a drop of the mixed liquids was spin coated at 1000 RPM. Both layers were cured in a UV chamber at 365 mW/cm^2 for 10 minutes under nitrogen. The 5 mm thick layer was peeled from the master template and inlet/outlet holes were punched into it. The layer was sealed to the cured flat layer and allowed to bake at 130°C for 2 hours, forming an adhesive bond between layers. Multilayer chips could be formed by spin coating fresh materials onto patterned wafers and UV curing as described above. Thick layers can be aligned on top of the new layers and heated to form an adhesive bond. The layers can then be peeled up together and realigned to the next layer. This process is repeated for each consecutive layer with very strong adhesion.

It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

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CLAIMS

What is claimed is:

1. A nanoparticle composition, comprising:

a particle having a shape corresponding to a mold, wherein the particle is less than about 100 μm in a broadest dimension.

2. The composition of claim 1, wherein the particle comprises a biocompatible material.

3. The composition of claim 2, wherein the biocompatible material is selected from the group consisting of a poly(ethylene glycol), a poly(lactic acid), a poly(lactic acid-co-glycolic acid), a lactose, a phosphatidylcholine, a polylactide, a polyglycolide, a hydroxypropylcellulose, a wax, a polyester, a polyanhydride, a polyamide, a phosphorous-based polymer, a poly(cyanoacrylate), a polyurethane, a polyorthoester, a polydihydropyran, a polyacetal, a biodegradable polymer, a polypeptide, a hydrogel, a carbohydrate, and combinations thereof.

4. The composition of claim 1, wherein the particle comprises a therapeutic agent, a diagnostic agent, or a linker.

5. The composition of claim 1, wherein the particle includes a therapeutic agent and a crosslinked biocompatible component.

6. The composition of claim 5, wherein the crosslinked biocompatible component is configured to bioresorb over a predetermined time.

7. The composition of claim 6, wherein the bioresorbable crosslinker comprises polymers functionalized with a disulfide group.

8. The composition of claim 5, wherein the biocompatible component has a crosslink density of less than about 0.50.

9. The composition of claim 5, wherein the biocompatible component has a crosslink density of more than about 0.50.

10. The composition of claim 5, wherein the biocompatible component is functionalized with a non-biodegradable group.

11. The composition of claim 5, wherein the biocompatible component is functionalized with a biodegradable group.

12. The composition of claim 11, wherein the biodegradable group is a disulfide group.

13. The composition of claim 1, wherein the particle comprises a predetermined charge.

14. The composition of claim 1, wherein the particle comprises a predetermined zeta potential.

15. The composition of claim 2, wherein the biocompatible material has a crosslink density of less than about 0.50.

16. The composition of claim 2, wherein the biocompatible material has a crosslink density of less than about 0.50.

17. The composition of claim 1, wherein the particle comprises a bioresorbable material.

18. The composition of claim 1, wherein the particle is configured to react to a stimuli.

19. The composition of claim 18, wherein the particle is configured to at least partially degrade from reacting with the stimuli.

20. The composition of claim 18, wherein the stimuli comprises a reducing environment, a predetermined pH, a cellular byproduct, or cell component.

21. The composition of claim 1, wherein the particle includes a magnetic material.

22. The composition of claim 1, wherein the particle comprises a charged particle, a polymer electret, a therapeutic agent, a non-viral gene vector, a viral particle, a polymorph, or a super absorbent polymer.

23. The composition of claim 4, wherein the therapeutic agent is selected from the group consisting of a drug, an agent, a modifier, a regulator, a therapy, a treatment, and combinations thereof.

24. The composition of claim 23, wherein the therapeutic agent is selected from the group consisting of a biologic, a ligand, an oligopeptide, an enzyme, DNA, an oligonucleotide, RNA, siRNA, a cancer treatment, a viral treatment, a bacterial treatment, an auto-immune treatment, a fungal treatment, a psychotherapeutic agent, a cardiovascular drug, a blood modifier, a gastrointestinal drug, a respiratory drug, an antiarthritic drug, a diabetes drug, an anticonvulsant, a bone metabolism regulator, a multiple sclerosis drug, a hormone, a urinary tract agent, an immunosuppressant, an ophthalmic product, a vaccine, a sedative, a sexual dysfunction therapy, an anesthetic, a migraine drug, an infertility agent, a weight control product, and combinations thereof.

25. The composition of claim 4, wherein the diagnostic is selected from the group consisting of an imaging agent, an x-ray agent, an MRI agent, an ultrasound agent, a nuclear agent, a radiotracer, a radiopharmaceutical, an isotope, a contrast agent, a fluorescent tag, a radiolabeled tag, and combinations thereof.

26. The composition of claim 1, wherein the shape of the particle is selected from the group consisting of substantially non-spherical, substantially viral, substantially bacterial, substantially cellular, substantially a rod, substantially chiral, and combinations thereof.

27. The composition of claim 1, wherein the shape of the particle is selected from the group consisting of substantially rod shaped wherein the rod is less than about 200 nm in diameter.

28. The composition of claim 1, wherein the shape of the particle is selected from the group consisting of substantially rod shaped wherein the rod is less than about 2 nm in diameter.

29. The composition of claim 1, wherein the particle further comprises a carbon-carbon bond.

30. The composition of claim 4, wherein the therapeutic agent or diagnostic agent or linker is associated with the particle.

31. The composition of claim 4, wherein the therapeutic agent or diagnostic agent or linker is physically coupled with the particle.

32. The composition of claim 4, wherein the therapeutic agent or diagnostic agent or linker is chemically coupled with the particle.

33. The composition of claim 4, wherein the therapeutic agent or diagnostic agent or linker is substantially encompassed within the particle.

34. The composition of claim 4, wherein the therapeutic agent or diagnostic agent or linker is at least partially encompassed within the particle.

35. The composition of claim 4, wherein the therapeutic or diagnostic agent is coupled with the exterior of the particle.

36. The composition of claim 4, wherein the linker is selected from the group consisting of sulfides, amines, carboxylic acids, acid chlorides, alcohols, alkenes, alkyl halides, isocyanates, imidazoles, halides, azides, *N*-hydroxysuccimidyl (NHS) ester groups, acetylenes, diethylenetriaminepentaacetic acid (DPTA) and combinations thereof.

37. The composition of claim 36, further comprising a modifying molecule chemically coupled with the linker.

38. The composition of claim 37, wherein the modifying molecule is selected from the group consisting of dyes, fluorescence tags, radiolabeled tags, contrast agents, ligands, targeting ligands, peptides, aptamers, antibodies, pharmaceutical agents, proteins, DNA, RNA, siRNA, and fragments thereof.

39. The composition of claim 18, wherein the stimuli is selected from the group consisting of pH, radiation, oxidation, reduction, ionic strength, temperature, alternating magnetic or electric fields, acoustic forces, ultrasonic forces, time, and combinations thereof.

40. The composition of claim 1, further comprising a plurality of particles, wherein the particles have a substantially uniform mass.

41. The composition of claim 1, further comprising a plurality of particles, wherein the particles are substantially monodisperse.

42. The composition of claim 41, wherein the particles are substantially monodisperse in size or shape.

43. The composition of claim 41, wherein the particles are substantially monodisperse in surface area.

44. The composition of claim 1, further comprising a plurality of particles having a normalized size distribution of between about 0.80 and about 1.20.

45. The composition of claim 1, further comprising a plurality of particles having a normalized size distribution of between about 0.90 and about 1.10.

46. The composition of claim 1, further comprising a plurality of particles having a normalized size distribution of between about 0.95 and about 1.05.

47. The composition of claim 1, further comprising a plurality of particles having a normalized size distribution of between about 0.99 and about 1.01.

48. The composition of claim 1, further comprising a plurality of particles having a normalized size distribution of between about 0.999 and about 1.001.

49. The composition of claims 44 to 48, wherein the normalized size distribution is selected from the group consisting of a linear size, a volume, a three dimensional shape, surface area, mass, and shape.

50. The composition of claim 1, further comprising a plurality of particles wherein the particles are monodisperse in surface area, volume, mass, three dimensional shape, or a broadest linear dimension.

51. The composition of claim 1, wherein the particle has a broadest dimension of less than about 50 μm.

52. The composition of claim 1, wherein the particle has a broadest dimension of between about 1 nm and about 10 micron.

53. The composition of claim 1, wherein the particle has a broadest dimension of between about 5 nm and about 1micron.

54. The composition of claim 1, wherein the dimension is a cross-sectional dimension.

55. The composition of claim 1, wherein the dimension is a circumferential dimension.

56. The composition of claim 1, wherein the particle comprises an organic composition.

57. The composition of claim 1, wherein the particle comprises a polymer.

58. The composition of claim 1, wherein the particle comprises an inorganic composition.

59. The composition of claim 1, wherein the particle is configured from the group consisting of substantially a triangle, substantially flat having a thickness of about 2 nm, substantially a flat disc having a thickness between about 2 nm and about 200 nm, and substantially boomerang-shaped.

60. The composition of claim 1, wherein the particle is substantially coated with a coating.

61. The composition of claim 60, wherein the coating includes a sugar.

62. The composition of claim 61, wherein the sugar is selected from the group consisting of glucose, sucrose, maltose, carbohydrate derivatives, and combinations thereof.

63. The composition of claim 1, wherein the particle further comprises ^{18}F.

64. The composition of claim 22, wherein the super absorbent polymer is selected from the group consisting of polyacrylates, polyacrylic

acid, HEMA, neutralized acrylates, sodium acrylate, ammonium acrylate, methacrylates, polyacrylamide, cellulose ethers, poly (ethylene oxide), poly (vinyl alcohol), polysuccinimides, polyacrylonitrile polymers, combinations of the above polymers blended or crosslinked together, combinations of the above polymers having monomers co-polymerized with monomers of another polymer, combinations of the above polymers with starch, and combinations thereof.

65. The composition of claim 1, wherein the particle has a ratio of surface area to volume greater than that of a sphere.

66. A particle, comprising:

an organic composition comprising a substantially predetermined shape substantially corresponding to a mold, wherein the shape is less than about 100 μm in a broadest dimension.

67. The particle of claim 66, wherein the organic composition further comprises a therapeutic agent, a diagnostic agent, or a linker.

68. The particle of claim 67, wherein the organic composition comprises a biocompatible material.

69. The particle of claim 67, wherein the therapeutic agent is selected from the group consisting of a drug, a biologic, a ligand, an oligopeptide, a cancer treatment, a viral treatment, a bacterial treatment, an auto-immune treatment, a fungal treatment, a psychotherapeutic agent, a cardiovascular drug, a blood modifier, a gastrointestinal drug, a respiratory drug, an antiarthritic drug, a diabetes drug, an anticonvulsant, a bone metabolism regulator, a multiple sclerosis drug, a hormone, a urinary tract agent, an immunosuppressant, an ophthalmic product, a vaccine, a sedative, a sexual dysfunction therapy, an anesthetic, a migraine drug, an infertility agent, a weight control product, and combinations thereof.

70. A nanoparticle, comprising:

a particle fabricated from a liquid material in a recess of a mold, wherein a contact angle between the liquid material and the mold is configured such that the liquid substantially passively fills the recess, and

wherein the particle has a broadest dimension of less than about 250 micron.

71. The nanoparticle of claim 70, wherein the liquid material forms a meniscus with an edge of the recess.

72. The nanoparticle of claim 71, wherein a portion of the particle is configured as a lens defined by the meniscus.

73. A nanoparticle, comprising:

a particle reflecting a shape of a recess of a mold, wherein the mold comprises a fluoropolymer and wherein the particle has a largest dimension that is less than about 100 micron.

74. A nanoparticle, comprising:

a particle prepared by a process comprising:

providing a template, wherein the template defines a recess between about 1 nanometers and about 100 micron in average dimension;

filling the recess; and

hardening the substance in the recess such that the particle is molded within the recess.

75. A nanoparticle, comprising:

a particle fabricated from a liquid material in a mold, wherein the liquid partially wets the mold, and wherein the particle is less than about 100 micron in a dimension.

76. A nanoparticle, comprising:

a particle fabricated from a liquid material in a mold, wherein the liquid does not wet the mold, and wherein the particle is less than about 100 micron in a dimension.

77. A method of making a nanoparticle, comprising:

placing a material comprising a liquid into a recess in a fluoropolymer mold, wherein the recess is less than about 100 μm in a broadest dimension;

hardening the material to make a particle; and

removing the particle from the recess.

78. The method of claim 77, wherein the particle includes a therapeutic agent selected from the group consisting of: a drug, a biologic, a cancer treatment, a viral treatment, a bacterial treatment, an auto-immune treatment, a fungal treatment, an enzyme, a protein, a nucleotide sequence, an antigen, an antibody, a diagnostic, and combinations thereof.

79. The method of claim 77, further comprising, before the placing step, adding a therapeutic agent, a diagnostic agent, or a linking group with the material.

80. The method of claim 77, further comprising, after the placing step, infusing a therapeutic agent, a diagnostic agent, or a linking group into the material.

81. The method of claim 77, further comprising, after the hardening step, infusing a therapeutic agent, a diagnostic agent, or a linking group into the material.

82. The method of claim 77, further comprising, after the removing step, infusing a therapeutic agent, a diagnostic agent, or a linking group into the material.

83. The method of claim 77, further comprising, after the hardening step, attaching a therapeutic agent, a diagnostic agent, or a linking group with a surface of the material.

84. The method of claim 77, further comprising loading a predetermined amount of a therapeutic agent, a diagnostic agent, a linking group, or a combination thereof into the particle.

85. The method of claim 79, wherein the therapeutic agent, diagnostic agent, or linking group is not modified before the mixing.

86. The method of claim 77, wherein the recess is less than about 10 μm in the broadest dimension.

87. The method of claim 77, wherein the recess is between about 1 nm and 1 micron in the broadest dimension.

88. The method of claim 77, wherein the recess is between about 1 nm and 500 nm in the broadest dimension.

89. The method of claim 77, wherein the recess is between about 1 nm and about 150 nm in the broadest dimension.

90. The method of claim 77, wherein the particle has a shape selected from the group consisting of substantially non-spherical, substantially viral shaped, substantially bacteria shaped, substantially cell shaped, substantially rod shaped, substantially chiral shaped, substantially a triangle, substantially flat disc shaped, substantially boomerang shaped, and combinations thereof.

91. The method of claim 77, wherein the rod is less than about 200 nm in diameter.

92. The method of claim 77, wherein the flat disc has a thickness of about 2 nm.

93. The method of claim 77, wherein the flat disc has a thickness of less than about 200 nm.

94. The method of claim 77, further comprising coating the particle.

95. The method of claim 77, wherein the fluoropolymer mold is formed from a material selected from the group consisting of perfluoropolyether, photocurable perfluoropolyether, thermally curable perfluoropolyether, and a combination of photocurable perfluoropolyether and thermally curable perfluoropolyether.

96. The method of claim 77, further comprising including a therapeutic agent with the material.

97. The method of claim 77, further comprising including a diagnostic agent with the material.

98. The method of claim 77, further comprising treating a cell with the particle.

99. The method of claim 77, further comprising before the hardening step, removing excess material from the mold such that substantially all remaining material resides substantially within the recess.

100. The method of claim 77, wherein the mold comprises a low-surface energy polymeric material.

101. The method of claim 77, wherein the mold is formed from a material selected from the group consisting of a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction.

102. The method of claim 77, wherein the mold comprises perfluoropolyether.

103. The method of claim 77, wherein the material comprises a solution containing a drug.

104. The method of claim 77, wherein the hardening is evaporation.

105. A method for making a nanoparticle, comprising:

placing a material into a recess defined in a fluoropolymer mold;

treating the material in the recess to form a particle; and

removing the particle from the recess.

106. The method of claim 105, wherein the fluoropolymer comprises a low-surface energy.

107. The method of claim 106, wherein the fluoropolymer comprises perfluoropolyether.

108. The method of claim 105, wherein treating is evaporation.

109. The method of claim 105, wherein the recess is less than 500 micron in a largest dimension.

110. A method of open molding, comprising:

providing a template, wherein the template includes a recess less than about 100 micron in average dimension and wherein the template comprises a low-surface energy polymeric material;

dispensing a substance comprising a liquid into the recess; and

hardening the substance in the recess such that a particle is molded within the recess.

111. The method of claim 110, further comprising after the dispensing step, applying a force to the template to remove substance not contained within the recess.

112. The method of claim 111, wherein the force is applied with a substrate having a surface configured to engage the template.

113. The method of claim 111, wherein the force applied to the template is a manual pressure.

114. The method of claim 110, further comprising passing a blade across the template to remove substance not contained within the recess.

115. The method of claim 114, wherein the blade is selected from the group consisting of a metal blade, a rubber blade, a silicon based blade, a polymer based blade, an air knife, and combinations thereof.

116. The method of claim 110, wherein the template is selected from the group consisting of a substantially rotatable cylinder, a conveyor belt, a roll-to-roll process, a batch process, and a continuous process.

117. The method of claim 110, wherein the substance in the recess is hardened by evaporation.

118. The method of claim 110, wherein the substance in the recess is hardened by a chemical process.

119. The method of claim 110, wherein the substance in the recess is hardened by treating the substance with UV light.

120. The method of claim 110, wherein the substance in the recess is hardened by a temperature change.

121. The method of claim 110, wherein the substance in the recess is hardened by treating the substance with thermal energy.

122. The method of claim 110, wherein the substance in the recess is hardened by evaporation of a carrier substance.

123. The method of claim 112, further comprising leaving the substrate in position on the template to reduce evaporation of the substance from the recess.

124. The method of claim 110, further comprising harvesting the particle from the recess after hardening the substance.

125. The method of claim 124, wherein harvesting comprises applying an article that has affinity for the particles that is greater than an affinity between the particles and the template.

126. The method of claim 125, wherein the harvesting step comprises contacting the particle with an adhesive substance.

127. The method of claim 126, wherein adhesion between the particle and the adhesive substance is greater than an adhesive force between the particle and the template.

128. The method of claim 125, wherein the harvesting article is selected from one or more of the group consisting of water soluble adhesives, acetone soluble adhesives, and organic solvent soluble adhesives.

129. The method of claim 125, wherein the harvesting article is selected from one or more of the group consisting of water, organic solvents, carbohydrates, epoxies, waxes, polyvinyl alcohols, poly(vinyl pyrrolidone)s, poly(acrylic acid), poly(butyl acrylate)s, polycyano acrylates, cellulosis, gelatins, poly(hydroxy ethyl methacrylate)s, and poly(methyl methacrylate).

130. The method of claim 124, further comprising purifying the particle after harvesting the particle.

131. The method of claim 130, wherein purifying the particle comprises purifying the particle from a harvesting substance.

132. The method of claim 130, wherein purifying is selected from the group consisting of centrifugation, separation, chromatography, vibration, gravity, dialysis, filtering, sieving, electrophoresis, gas stream, magnetism, electrostatic separation, combinations thereof, and the like.

133. The method of claim 124, wherein the particle is harvested by centrifugation, dissolution, vibration, ultrasonics, megasonics, gravity, flexure of the template, suction, electrostatic attraction, electrostatic repulsion, magnetism, physical template manipulation, combinations thereof, and the like.

134. The method of claim 110, wherein the low surface energy ploymeric material is substantially solvent resistant.

135. The method of claim 134, wherein the low-surface energy polymeric material has a surface energy less than about 23 dynes/cm.

136. The method of claim 134, wherein the low-surface energy polymeric material has a surface energy less than about 19 dynes/cm.

137. The method of claim 134, wherein the low-surface energy polymeric material has a surface energy less than about 15 dynes/cm.

138. The method of claim 134, wherein the low-surface energy polymeric material has a surface energy less than about 12 dynes/cm.

139. The method of claim 134, wherein the low-surface energy polymeric material has a surface energy less than about 8 dynes/cm.

140. The method of claim 110, wherein the low-surface energy polymeric material for the template comprises a solvent resistant, elastomeric material.

141. The method of claim 110, wherein the low-surface energy polymeric material for the template is selected from the group consisting of a perfluoropolyether material, a silicone material, a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and a fluorinated

monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction.

142. The method of claim 110, wherein the substance to be molded is selected from the group consisting of a polymer, a solution, a monomer, a plurality of monomers, a polymerization initiator, a polymerization catalyst, an inorganic precursor, a metal precursor, a pharmaceutical agent, a tag, a magnetic material, a paramagnetic material, a ligand, a cell penetrating peptide, a porogen, a surfactant, a plurality of immiscible liquids, a solvent, and a charged species.

143. The method of claim 110, wherein the particle comprises organic polymers, super absorbent polymers, charged particles, polymer electrets (poly(vinylidene fluoride), Teflon-fluorinated ethylene propylene, polytetrafluoroethylene), therapeutic agents, drugs, non-viral gene vectors, DNA, RNA, RNAi, viral particles, polymorphs, combinations thereof, and the like.

144. A method of loading a nano-scale recess, comprising:

providing a patterned template defining a nano-scale recess;

submerging the patterned template into a substance to be molded in the nano-scale recess;

allowing the substance to enter the recess; and

removing the patterned template from the substance.

145. The method of claim 144, wherein the template comprises a low surface energy polymeric material.

146. The method of claim 144, wherein the template comprises PFPE.

147. A method of filling a nano-scale recess, comprising:

providing a template, wherein the template defines a nano-scale recess;

disposing a substance to be molded in the nano-scale recess onto the template; and

allowing the substance to enter the nano-scale recess.

148. The method of claim 147, wherein the template comprises a low surface energy polymeric material.

149. The method of claim 147, wherein the template comprises PFPE.

150. A method for molding nano-scale structures, comprising:

configuring a contact angle between a liquid to be molded and a template mold to be a predetermined angel such that the liquid passively fills a nano-scale recess defined in the template mold.

151. The method of claim 150, wherein the contact angle is modified by applying a voltage to the liquid.

152. The method of claim 150, wherein the contact angle is modified by applying a voltage to the template.

153. The method of claim 150, wherein the liquid forms a meniscus with a portion of the nano-scale recess.

154. The method of claim 153, wherein a portion of a particle fabricated from the nano-scale recess forms a lens as a result of the meniscus.

155. A method of forming a nanoparticle, comprising:

introducing a first substance to be molded into a nano-scale recess of a template;

evaporating a solvent component of the first substance; and

curing the first substance in the nano-scale recess to form a particle.

156. The method of claim 155, further comprising:

adding a second substance to the nano-scale recess following the evaporation and curing of the first substance such that a particle having two compositions is formed.

157. The method of claim 156, wherein the template comprises a low surface energy polymeric material.

158. The method of claim 157, wherein the template comprises PFPE.

159. A method of filling a nano-scale recess, comprising:

providing a template, wherein the template defines a nano-scale recess;

disposing a substance to be molded onto the template; and

applying a voltage across the substance to assist the substance to enter the nano-scale recess.

160. A method of forming a nanoparticle, comprising:

configuring a template with a predetermined permeability, wherein the template defines a nano-scale recess;

subjecting the template with a substance having a predetermined permeability;

allowing the substance to enter the nano-scale recess; and

hardening the substance in the nano-scale recess.

161. A method of treating a patient, comprising:

providing a patterned template defining a recess, wherein the recess is less than about 100 micron in a broadest dimension, and wherein the patterned template comprises a low-surface energy polymeric material;

applying a material recess such that the material enters the recess;

hardening the material to form a nanoparticle;

removing the nanoparticle from the recess; and

administering a therapeutically effective amount of the nanoparticle to a patient.

162. The method of claim 161, wherein the nanoparticle treats a patient by interacting with a cellular membrane.

163. The method of claim 161, wherein the nanoparticle treats a patient by undergoing intracellular uptake.

164. The method of claim 161, wherein the nanoparticle induces an immune response.

165. The method of claim 161, wherein the nanoparticle interacts with a cellular receptor.

166. The method of claim 161, wherein the low-surface energy polymeric material comprises perfluoropolyether.

167. A method of treatment, comprising:

administering a therapeutically effective amount of a particle having a predetermined shape and a broadest dimension of less than about 100 μm to a patient.

168. The method of claim 167, wherein the particle undergoes intracellular uptake.

169. The method of claim 167, further comprising a therapeutic or diagnostic at least partially encompassed within the particle.

170. The method of claim 169, wherein the therapeutic or diagnostic is coupled to the exterior of the particle.

171. The method of claim 169, wherein the therapeutic is selected from the group consisting of a drug, a biologic, an anti-cancer treatment, an anti-viral treatment, an anti-bacterial treatment, an auto-immune treatment, a fungal treatment, and combinations thereof.

172. The method of claim 169, wherein the diagnostic is selected from the group consisting of an imaging agent, an x-ray agent, a MRI agent, an ultrasound agent, a nuclear agent, radiotracer, radiopharmaceutical, isotope, contrast agent, a fluorescent tag, a radiolabeled tag, and combinations thereof.

173. The method of claim 169, wherein the therapeutic is selected from the group consisting of a psychotherapeutic agent, a cardiovascular drug, a blood modifier, a gastrointestinal drug, a respiratory drug, an antiarthritic drug, a diabetes drug, an anticonvulsant, a bone metabolism regulator, a multiple sclerosis drug, a hormone, a urinary tract agent, an

immunosuppressant, an ophthalmic product, a vaccine, a sedative, a sexual dysfunction therapy, an anesthetic, a migraine drug, an infertility agent, a weight control product, and combinations thereof.

174. The method of claim 167, wherein the particle is less than about 10 μm in a dimension.

175. The method of claim 167, wherein the particle is between about 1 nm and about 1 micron in a dimension.

176. The method of claim 167, wherein the particle is between about 1 nm and about 200 nm in a dimension.

177. The method of claim 167, wherein the particle is substantially non-spherical, substantially viral shaped, substantially bacteria shaped, substantially protein shaped, substantially cell shaped, substantially rod shaped, substantially chiral shaped, substantially a triangle, substantially a flat disc with a thickness of about 2 nm, substantially a flat disc with a thickness between about 2 nm and about 1 μm, and substantially boomerang shaped.

178. The method of claim 148, wherein the particle is substantially rod shaped and wherein the rod is less than about 200 nm in diameter.

179. The method of claim 167, wherein the particle is substantially coated.

180. The method of claim 179, wherein the coating includes a carbohydrate based coating.

181. The method of claim 180, wherein the carbohydrate is selected from the group consisting of glucose, sucrose, maltose, derivatives thereof, and combinations thereof.

182. The method of claim 167, wherein the particle includes an organic material.

183. The method of claim 167, wherein the particle is molded from a patterned template comprising a low-surface energy polymeric material.

184. The method of Claim 167, wherein the particle is functionalized with a targeting ligand.

185. A method of delivering a treatment, comprising:

forming a particle of a treatment compound, the particle having a predetermined shape and being less than about 100 μm in a dimension; and

administering the particle to a location of inquiry.

186. A method of harvesting a nanoparticle from an article comprising:

providing an article defining a recess, wherein the recess is less than 100 micron in a greatest dimension;

forming a particle in the recess;

applying, to the article, a material having an affinity for the particle that is greater than an affinity between the article and the particle; and

separating the material from the article wherein the material remains attached to the particle.

187. The method of claim 186, wherein the applying step comprises treating the material to increase the affinity of the material to the particle.

188. The method of claim 186, wherein the separating step comprises applying a force to at least one of the article, the material and combinations thereof.

189. The method of claim 187, wherein the treating step comprises cooling the material.

190. The method of claim 187, wherein the treating step comprises one of the group consisting of hardening the material, chemically modifying a surface of the particle to increase the affinity between the material and the particle, chemically modifying a surface of the material to increase the affinity between the particle and the material, a UV treatment, a thermal treatment, and combinations thereof.

191. The method of claim 186, wherein the article comprises a low-surface energy material.

192. The method of claim 186, wherein the article comprises a perfluoropolyether material.

193. The method of claim 191, wherein the low surface energy material comprises a material selected from the group consisting of a fluoroolefin material, an acrylate material, a silicone material, a styrenic material, a fluorinated thermoplastic elastomer (TPE), a triazine fluoropolymer, a perfluorocyclobutyl material, a fluorinated epoxy resin, and a fluorinated monomer or fluorinated oligomer that can be polymerized or crosslinked by a metathesis polymerization reaction.

194. The method of claim 186, wherein the material is selected from the group consisting of carbohydrates, epoxies, waxes, polyvinyl alcohol, polyvinyl pyrrolidone, polybutyl acrylate, polycyano acrylates, polymethyl methacrylate, poly(acrylic acid), cellulose, gelatin, and combinations thereof.

195. The method of claim 187, wherein the treating step includes promoting a chemical interaction between the material and the particles.

196. The method of claim 187, wherein the treating step includes promoting a physical interaction between the material and the particles.

197. The method of claim 196, wherein physical interaction is a physical entrapment.

198. A method of modifying a surface of a nanoparticle comprising:

providing an article defining a recess and having a particle formed therein;

applying to the particle a solution containing modifying groups of molecules; and

promoting a reaction between a first portion of the modifying groups of molecules and at least a portion of a surface of the particle.

199. The method of claim 198, wherein a second portion of the modifying groups of molecules are left unreacted.

200. The method of claim 198, further comprising removing the unreacted modifying groups of molecules.

201. The method of claim 198, wherein the modifying group of molecules chemically attach to the particle through a linking group.

202. The method of claim 201, wherein the linking group is selected from a group consisting of sulfides, amines, carboxylic acids, acid chlorides, alcohols, alkenes, alkyl halides, isocyanates, imidazoles, halides, diethylenetriaminepentaacetic acid (DPTA), azides, acetylenes, N-hydroxysuccimidyl (NHS) ester group, and combinations thereof.

203. The method of claim 201, wherein the modifying group is selected from a group consisting of dyes, fluorescence tags, radiolabeled tags, contrast agents, ligands, peptides, aptamers, antibodies, pharmaceutical agents, proteins, DNA, RNA, siRNA, and fragments thereof.

204. A system for harvesting a plurality of nanoparticles from an article comprising:

an article defining a plurality of recesses wherein the recesses are less than about 100 micron in a dimension and wherein particles are formed within the recesses;

a material having an affinity for the particles that is greater than an affinity between the particles and the article; and

an applicator configured to separate the particles from the article.

205. The system of claim 204, wherein the article comprises a low-surface energy polymeric material.

206. A particle system, comprising:

a substrate; and

a particle having a shape corresponding to a mold, wherein the particle is less than about 100 μm in a broadest dimension;

wherein the particle is coupled with the substrate.

207. The system of claim 206, further comprising a plurality of particles arranged in a two dimensional array on the substrate.

208. The system of claim 206, wherein the particle further comprises an active.

209. The system of claim 208, wherein the active is selected from the group consisting of a drug, an agent, a reactant, and combinations thereof.

210. A system for modifying at least a portion of a nanoparticle comprising:

a substrate coupled with a particle having a major dimension of less than about 100 micron in a dimension and fabricated from a mold; and

a solution having a modifying group of molecules;

wherein the solution is configured to promote a reaction between the molecules and the particle upon contacting at least a portion of the particle with the solution.

211. A method for coating, comprising:

suspending a seed in a liquid solution;

depositing the liquid solution containing the seed onto a template, wherein the template comprises a low-surface energy polymeric material; and

hardening the liquid solution in the recesses such that the seed is coated with the hardened liquid solution.

212. A taggant, comprising:

a particle having a shape corresponding to a mold, wherein the particle is less than about 100 micron is a largest dimension; and

wherein the particle includes an identifying characteristic.

213. A method of making a taggant, comprising:

placing material into a mold formed from a low surface energy, non-wettable material, wherein the mold is less than about 100 micron in a

largest dimension, and wherein the mold includes an identifying characteristic;

curing the material to make a particle; and

removing the particle from the mold.

214. A secure item, comprising:

an item coupled with a taggant comprising a particle having a shape corresponding to a mold, wherein the particle is less than about 100 micron in a largest dimension, and wherein the particle includes an identifying characteristic.

215. A method of making a secure item, comprising:

placing material into a mold formed from a low surface energy, non-wettable material, wherein the mold is less than about 100 micron in a largest dimension, and wherein the mold includes an identifying characteristic;

curing the material to make a particle;

removing the particle from the mold; and

coupling the particle with an item.

216. A system for securing an item, comprising:

producing a taggant comprising a particle having a shape corresponding to a mold, wherein the particle is less than about 100 micron in a largest dimension, and wherein the particle includes an identifying characteristic;

incorporating the taggant with an item to be secured;

analyzing the item to detect and read the identifying characteristic; and

comparing the identifying characteristic with an expected characteristic.

217. An identification particle, comprising:

a taggant fabricated from a photoresist, wherein the taggant is configured and dimensioned using photolithography.

218. An identification particle, comprising:

a taggant cast from a mold, wherein the mold comprises low-surface energy polymeric material, and wherein the taggant includes a substantially flat surface.

219. The identification particle of claim 218, further comprising Bosch etch lines on a surface of the taggant.

220. The identification particle of claim 218, wherein the taggant further comprises chemical functionality.

221. The identification particle of claim 218, wherein the taggant further comprises an active sensor.

222. A nanoparticle identification method, comprising:

providing a taggant configured and dimensioned in a predetermined shape; and

recognizing the taggant according to the shape of the taggant.

223. A nanoparticle formed by the process comprising:

providing a template comprising a low surface energy polymeric material, wherein the template defines a nano-scale recess;

disposing a liquid to be molded onto the template, wherein the liquid has a predetermined contact angle with a surface of the template such that the liquid passively enters the nano-scale recess; and

forming a particle from the liquid in the nano-scale recess.

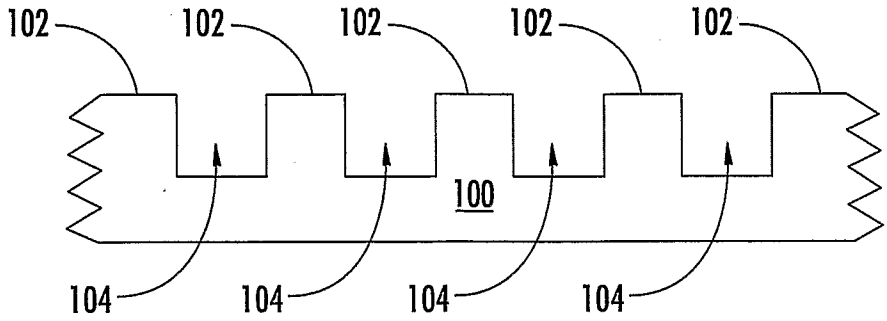
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FIG. 1A

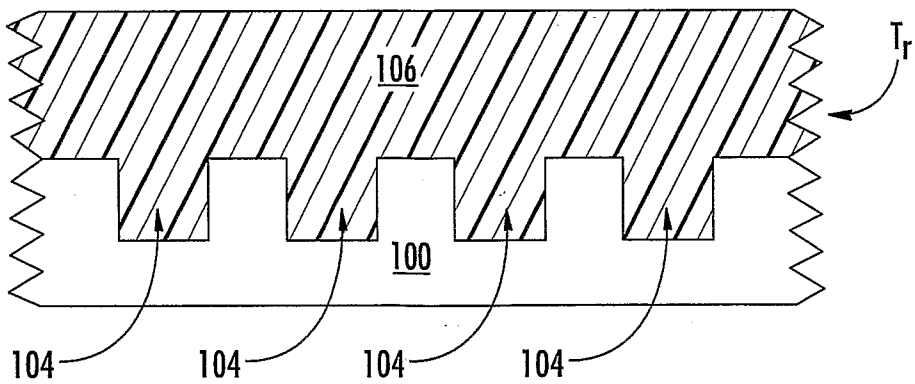
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FIG. 1B

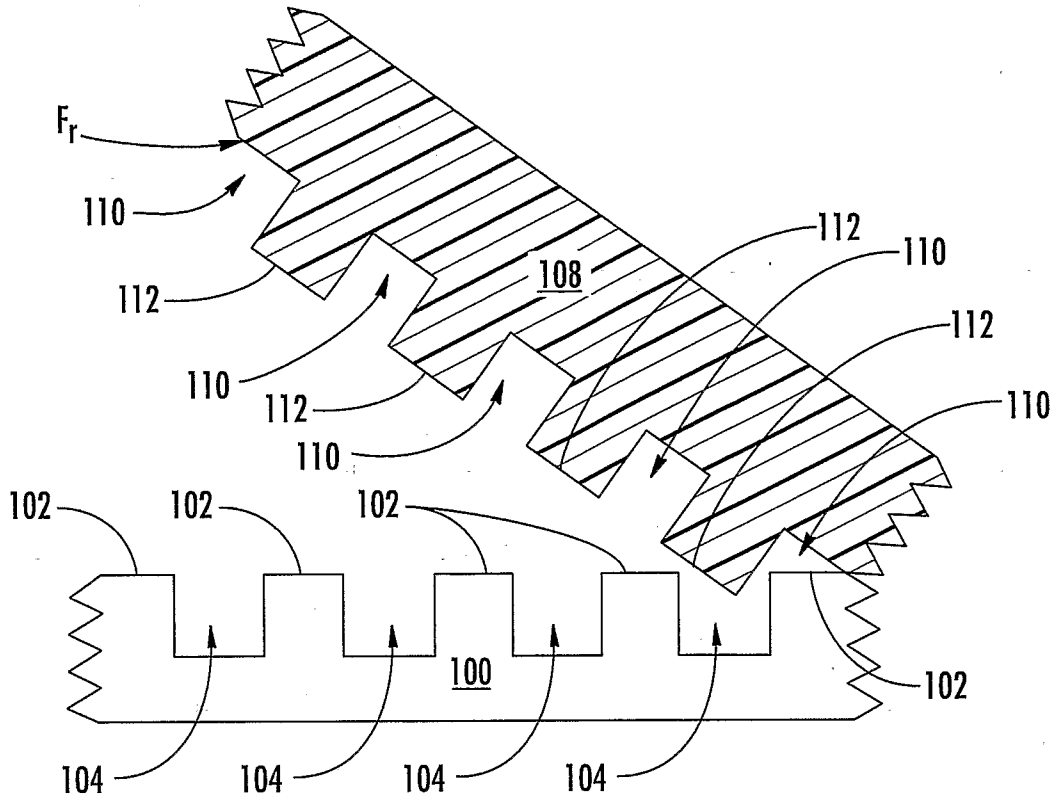
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FIG. 1C

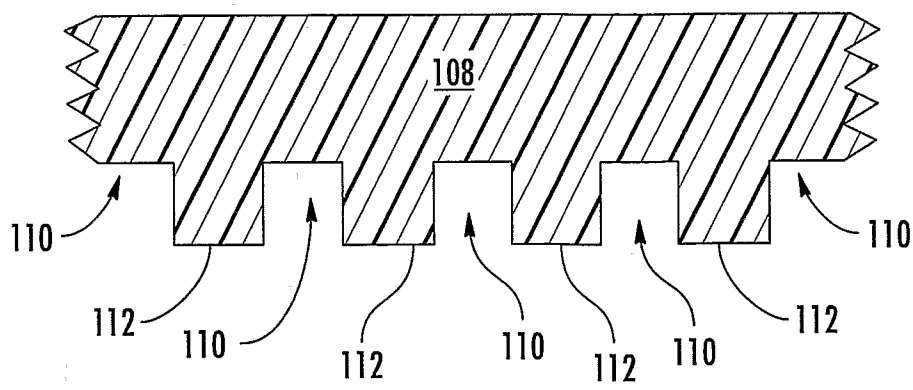
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FIG. 1D

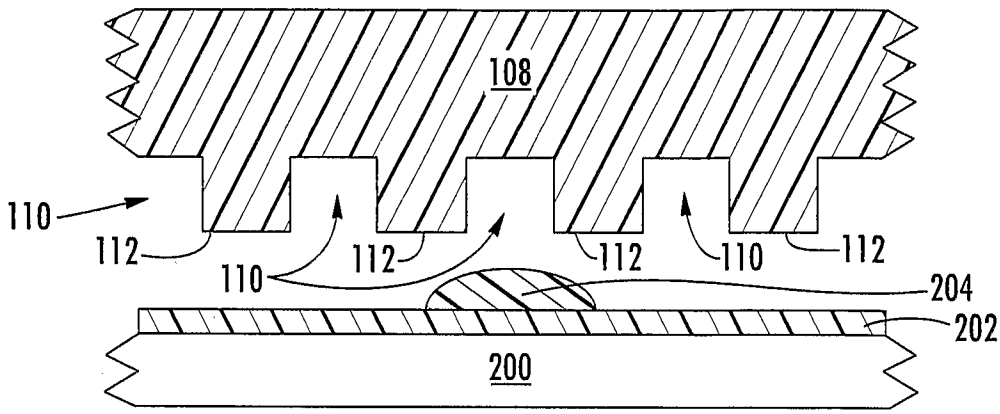
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FIG. 2A

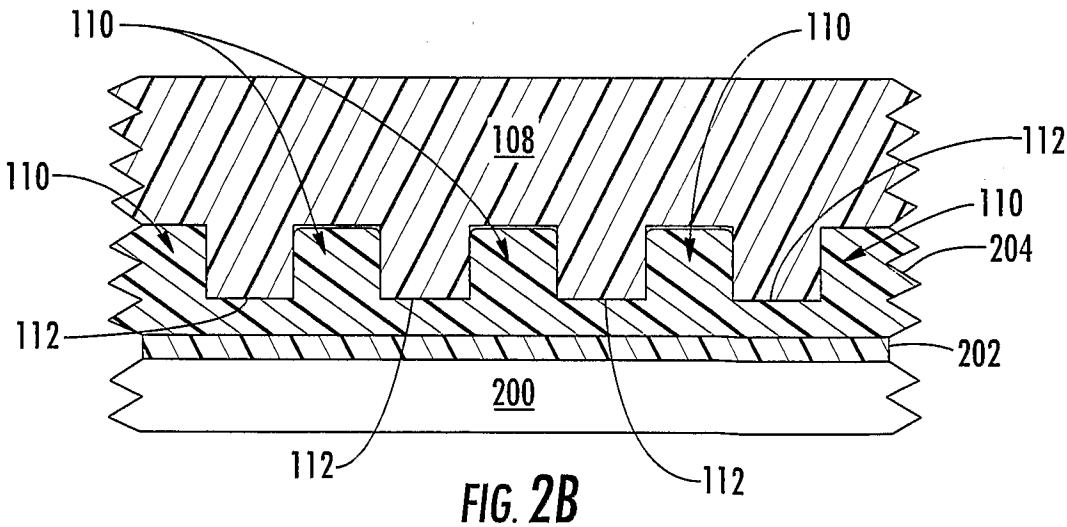
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FIG. 2B

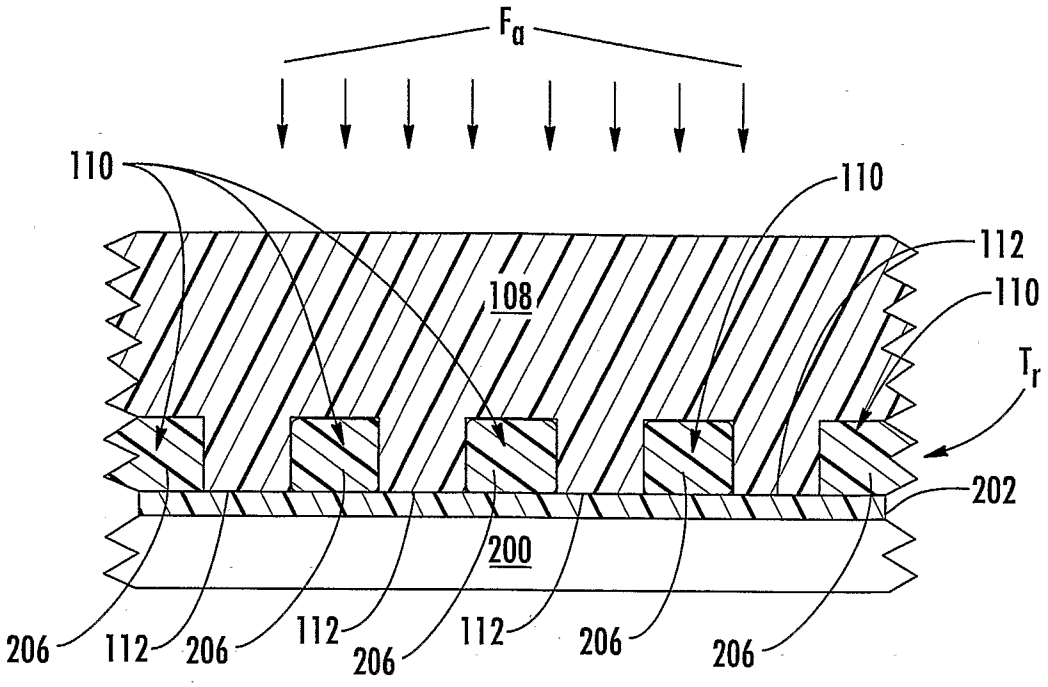

FIG. 2C

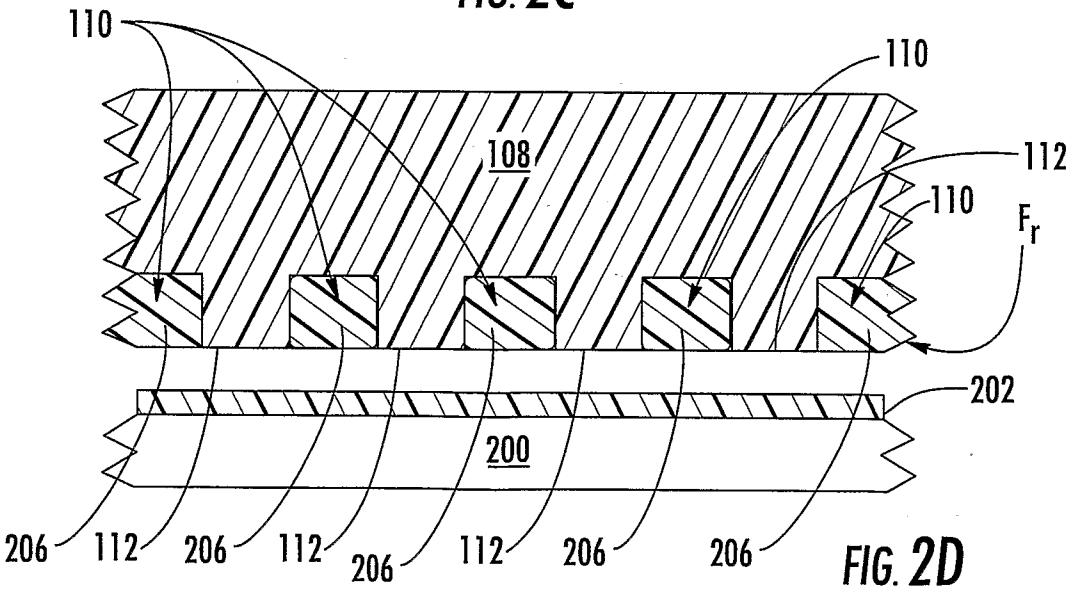

FIG. 2D

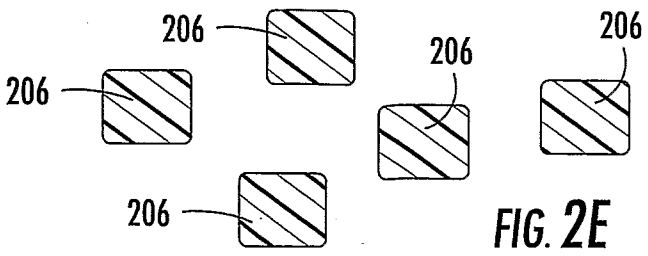

FIG. 2E

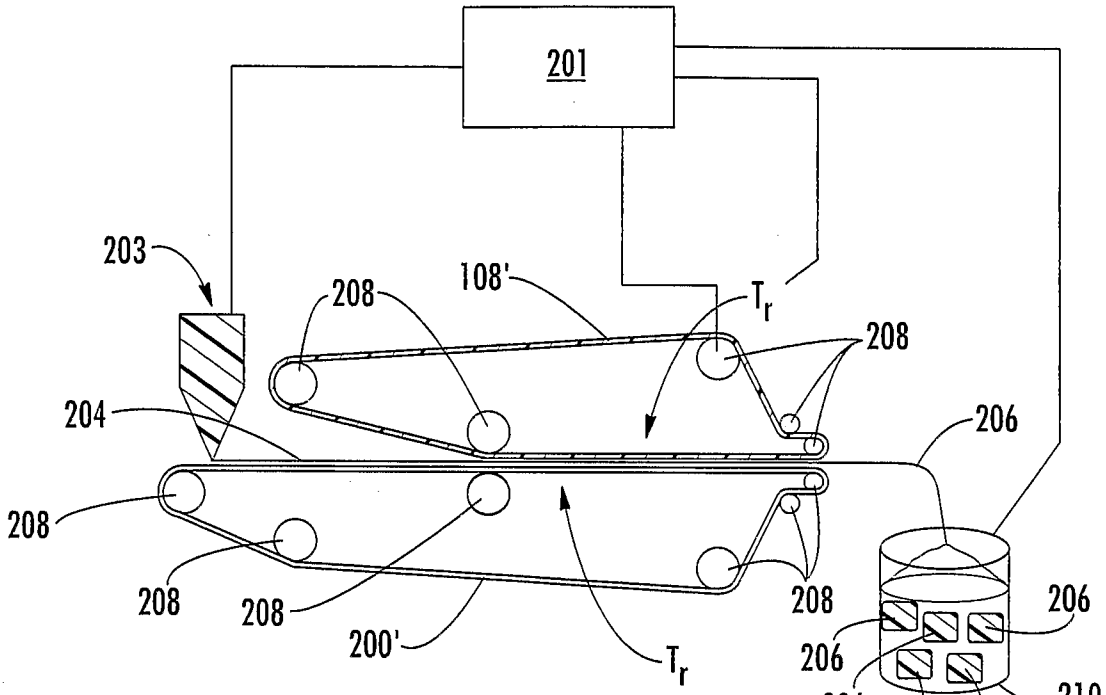
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FIG. 2F

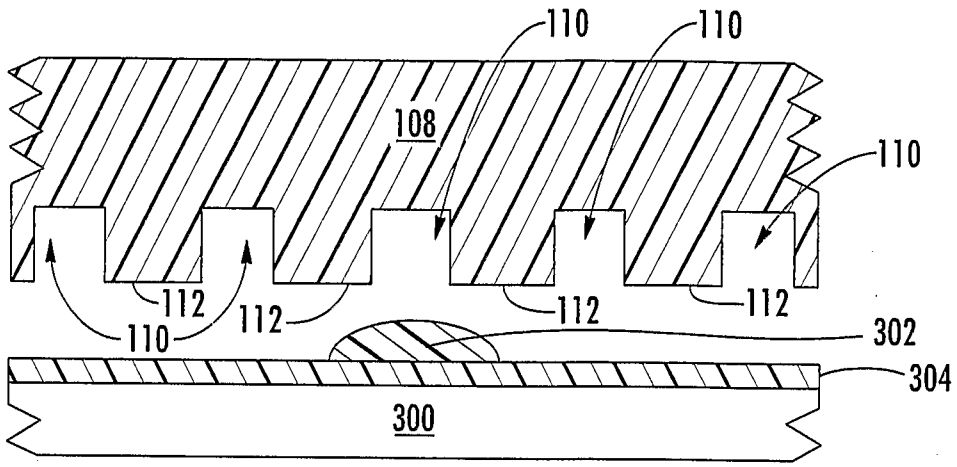
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FIG. 3A

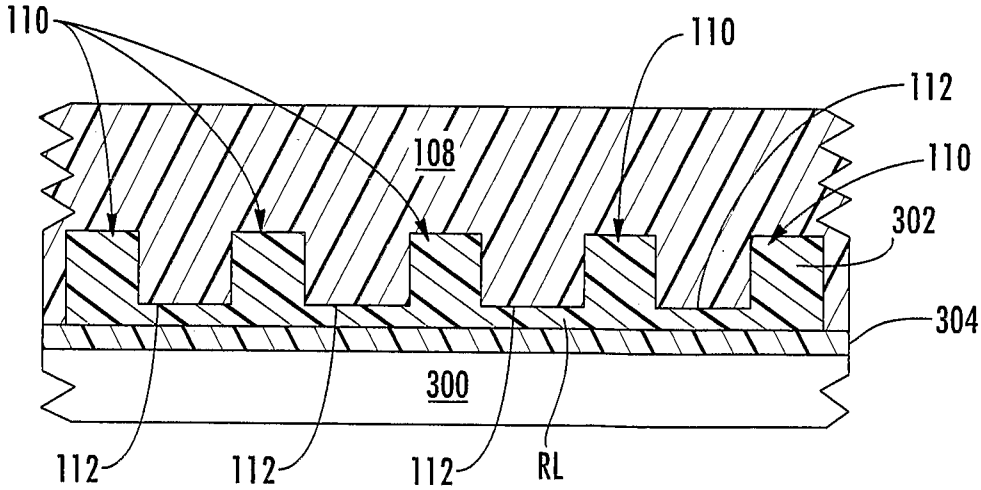
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FIG. 3B

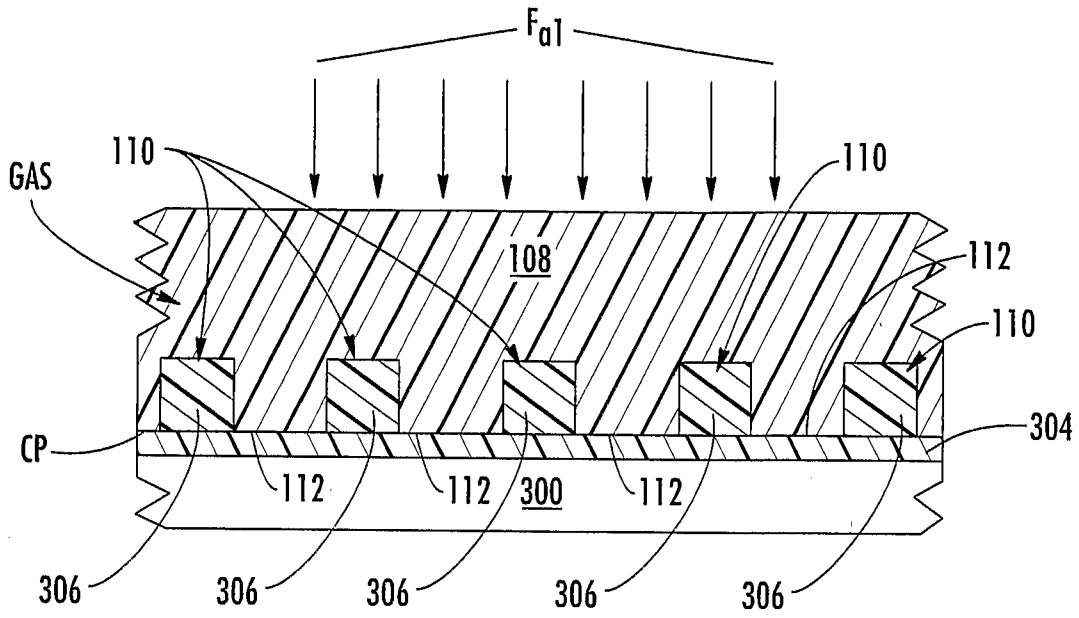
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FIG. 3C

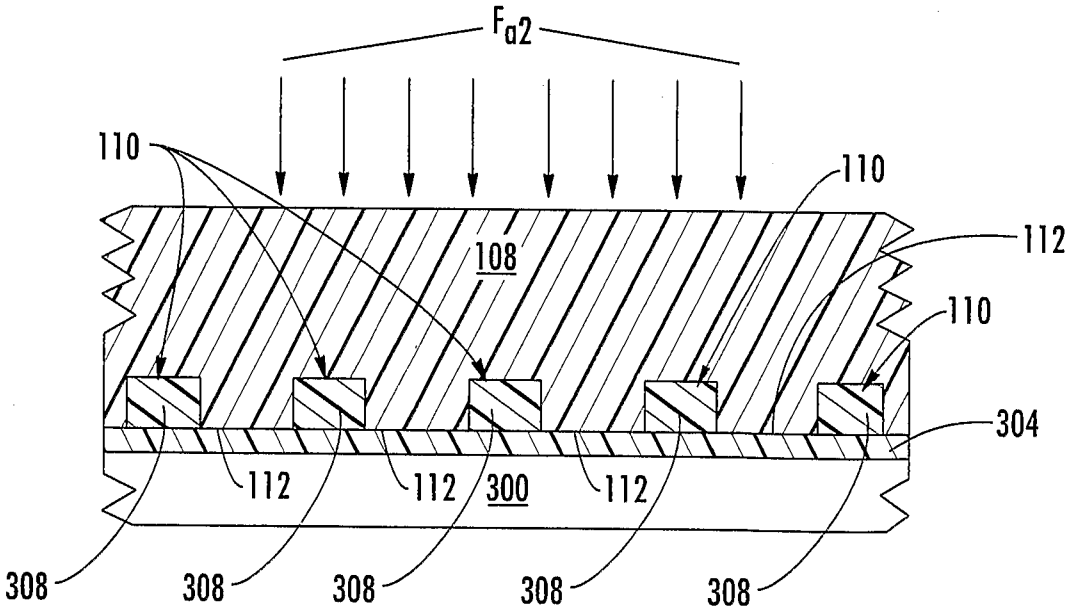
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FIG. 3D

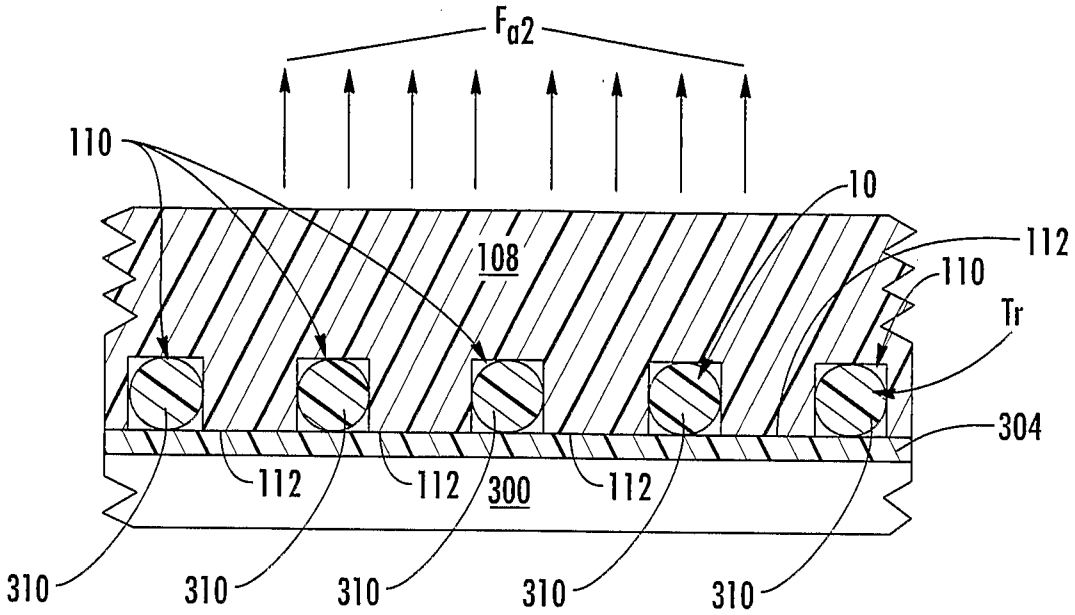
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FIG. 3E

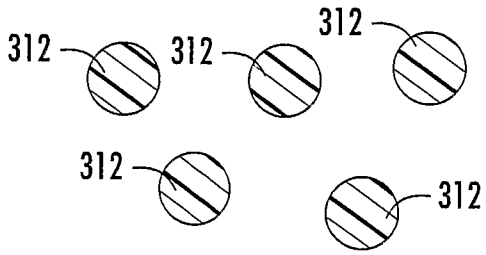
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FIG. 3F

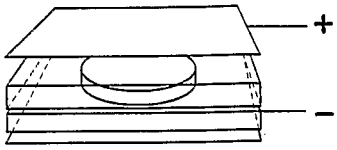


FIG. 4A

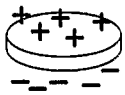


FIG. 4B

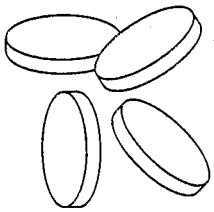


FIG. 4C

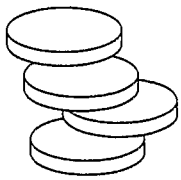


FIG. 4D

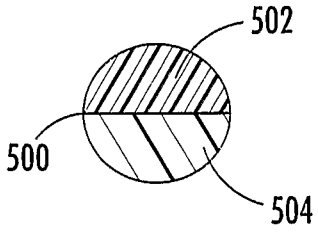


FIG. 5A

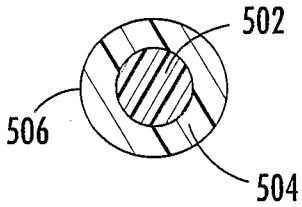


FIG. 5B

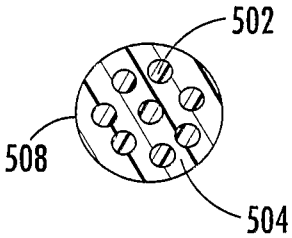


FIG. 5C

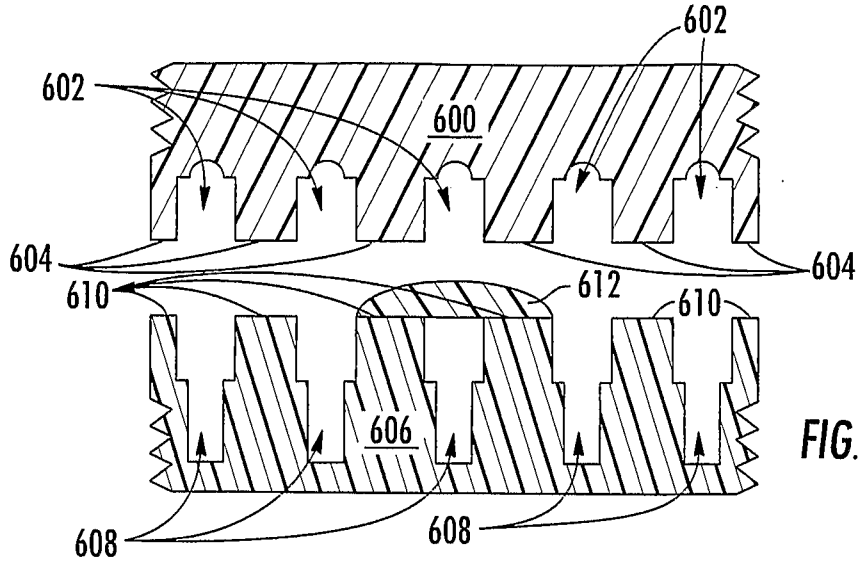


FIG. 6A

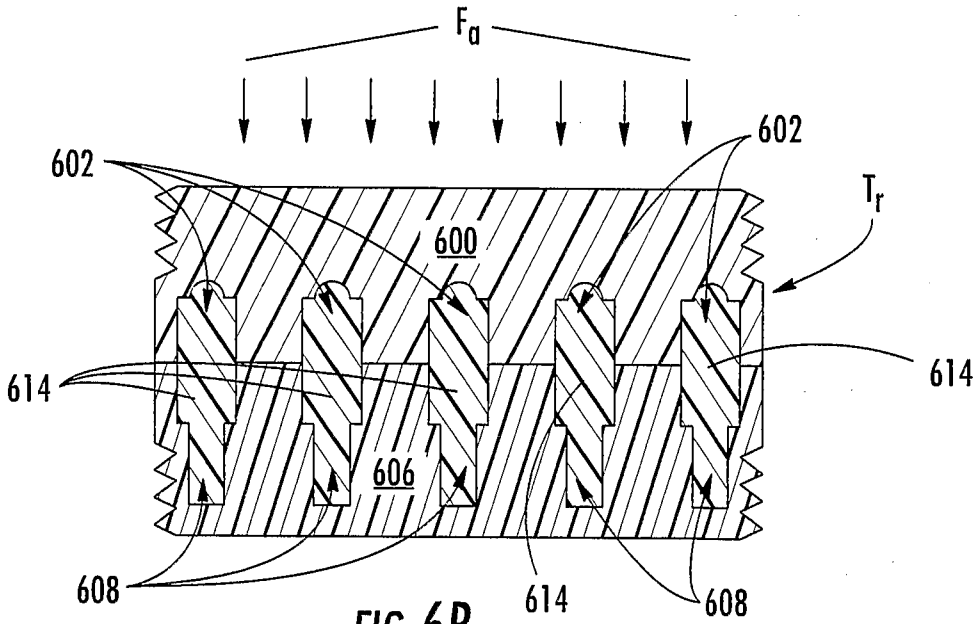


FIG. 6B

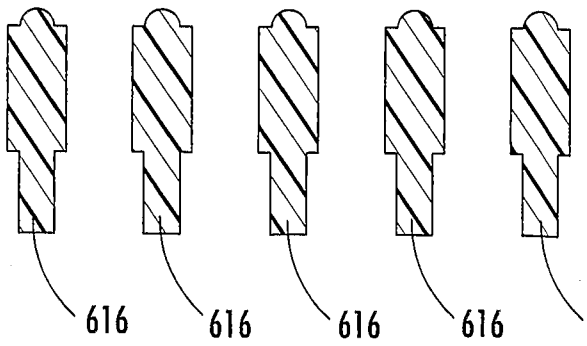


FIG. 6C

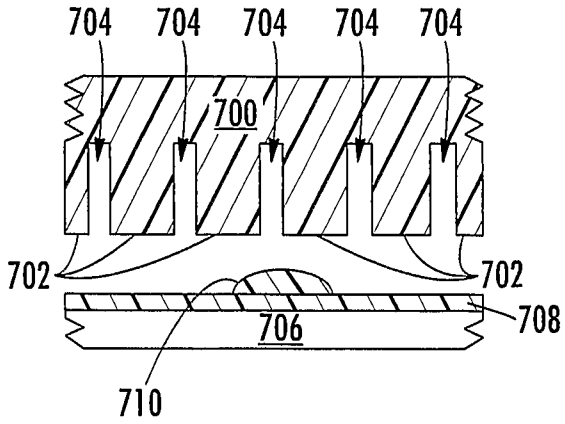


FIG. 7A

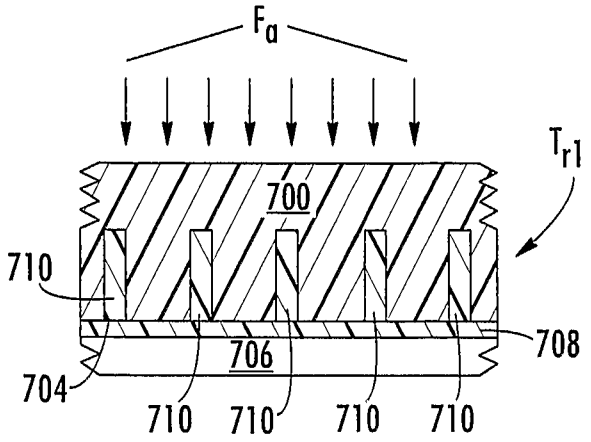


FIG. 7B

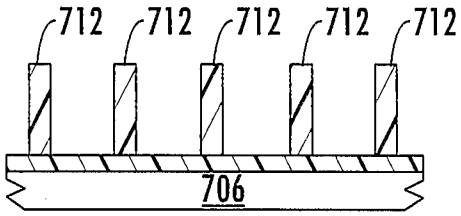


FIG. 7C

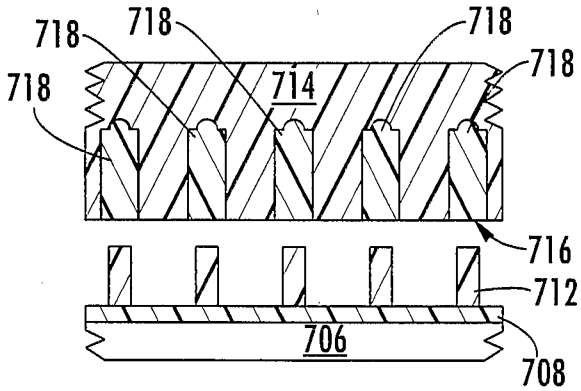


FIG. 7D

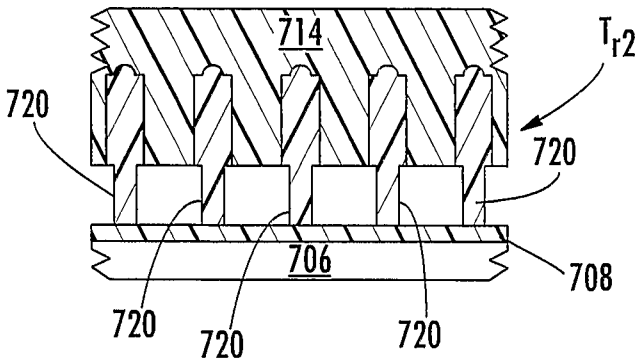


FIG. 7E

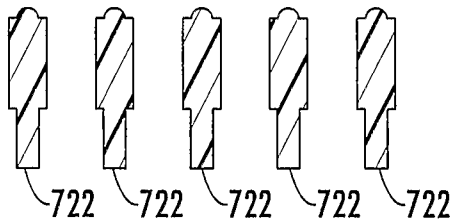


FIG. 7F

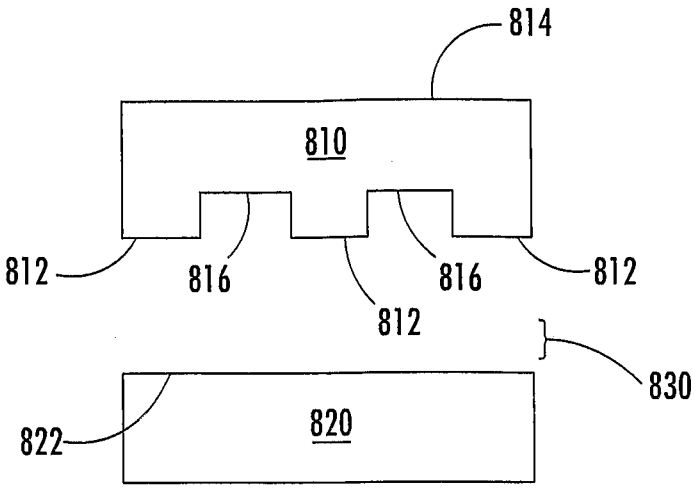


FIG. 8A

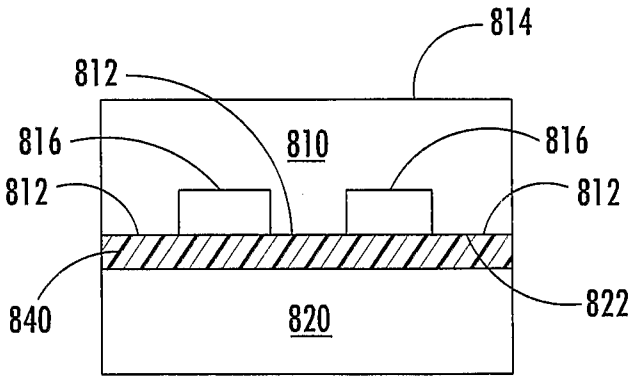


FIG. 8B

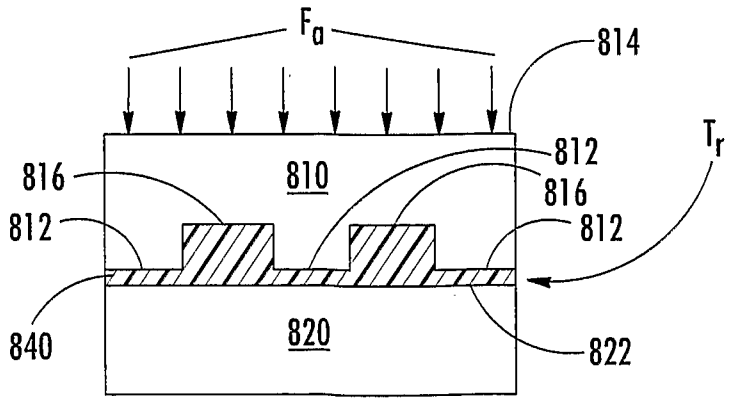
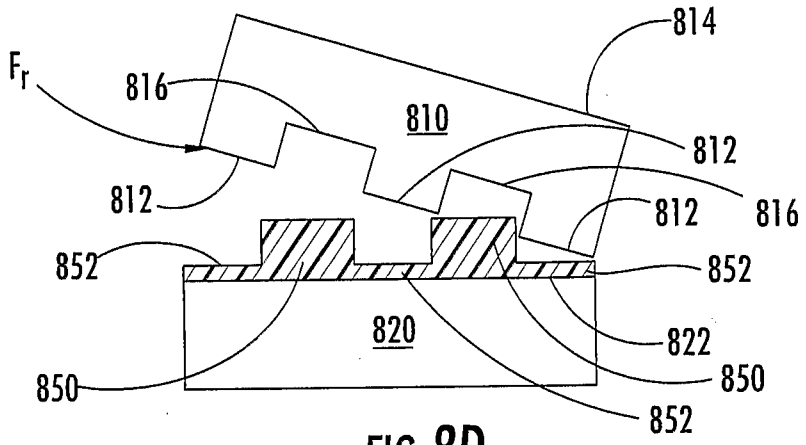


FIG. 8C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F_r
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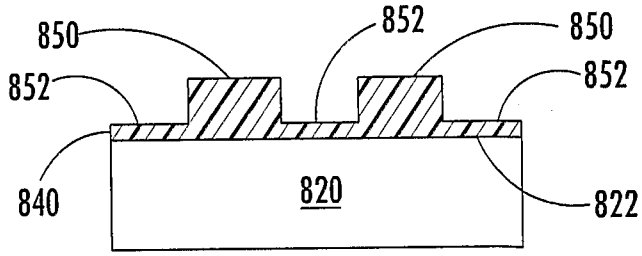
812
852
852
820
822
850
850
852

FIG. 8D


850
852
850
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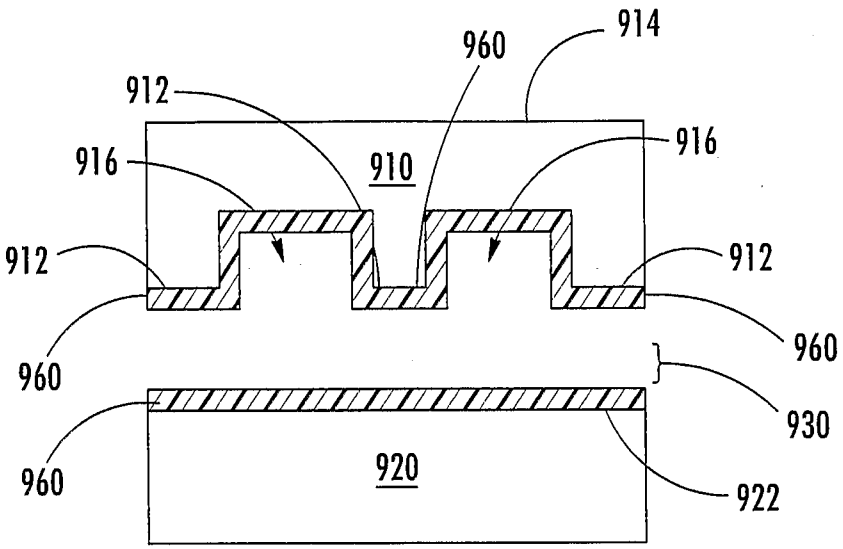
852
840
820
822

FIG. 8E


914
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960
960
930
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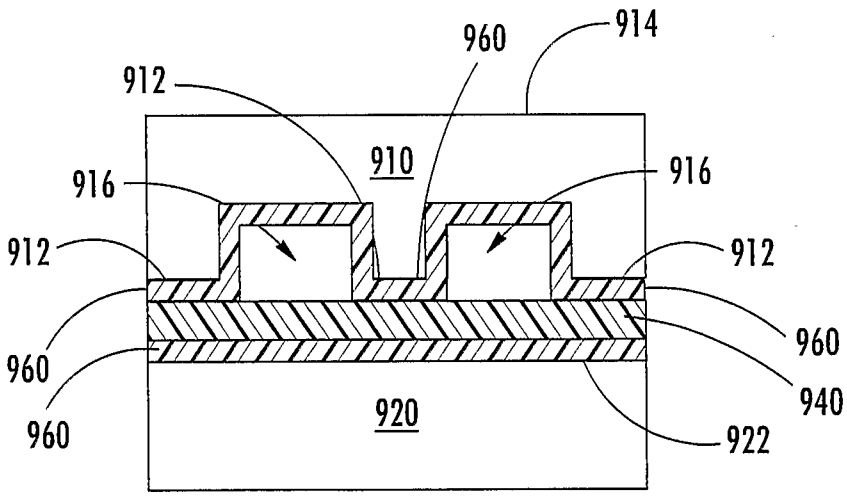
920
922

FIG. 9A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940
960
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922

FIG. 9B

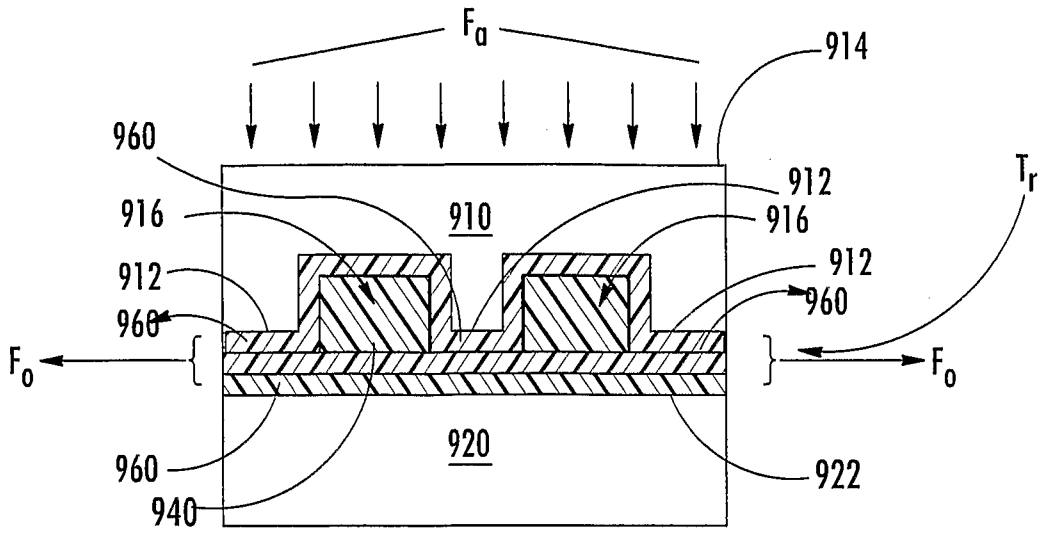


FIG. 9C

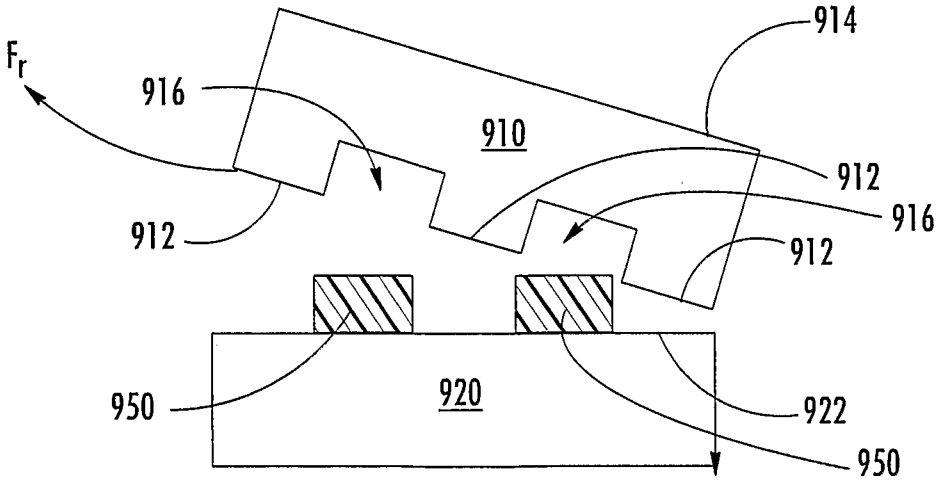


FIG. 9D

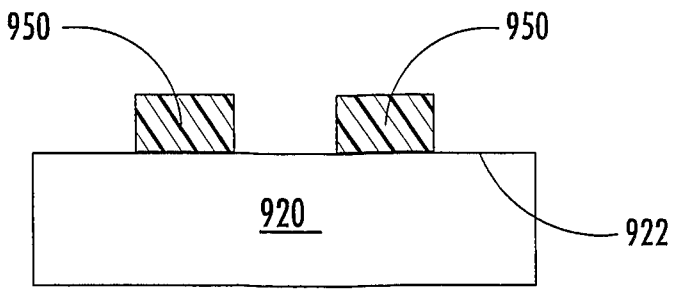


FIG. 9E

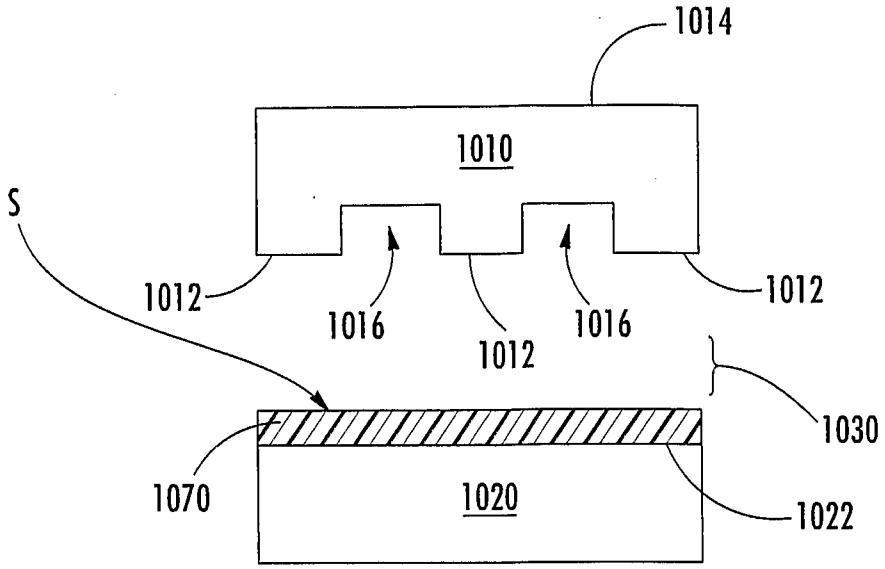


FIG. 10A

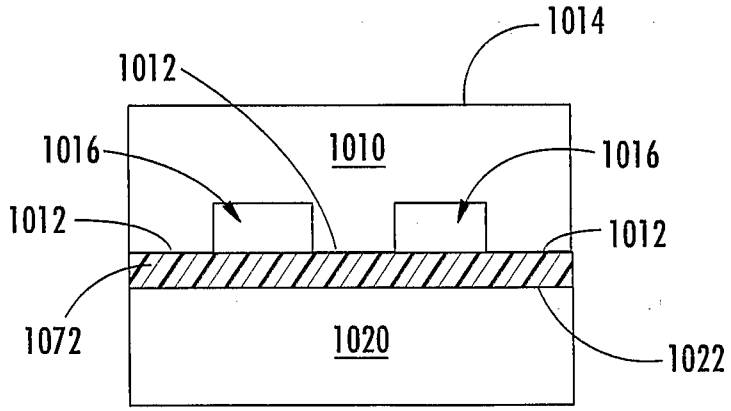


FIG. 10B

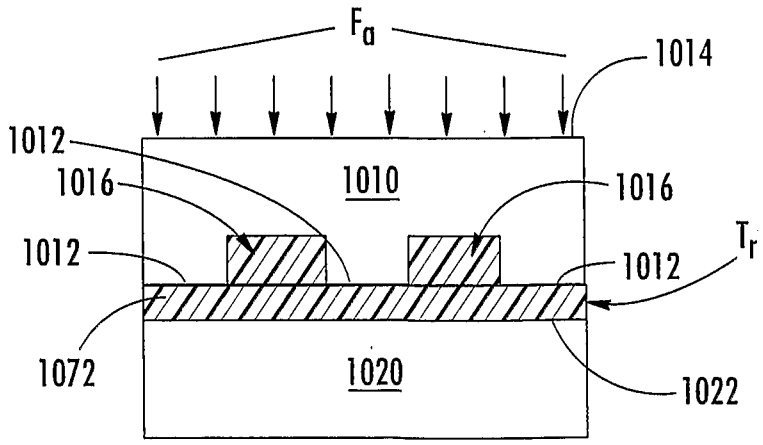


FIG. 10C

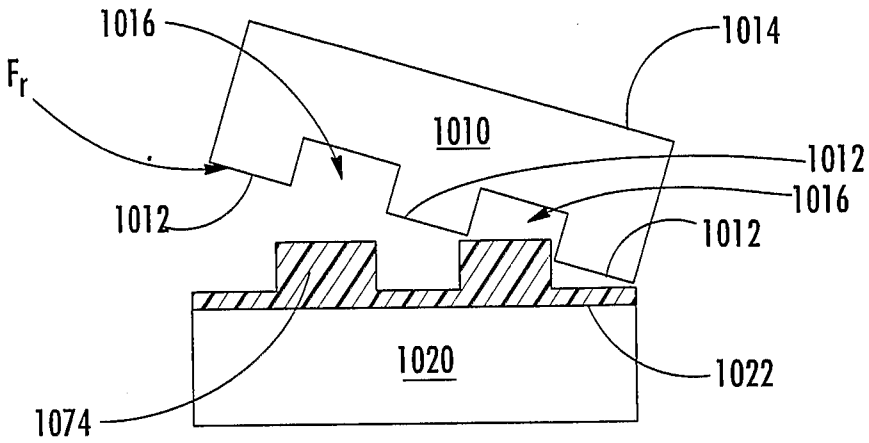


FIG. 10D

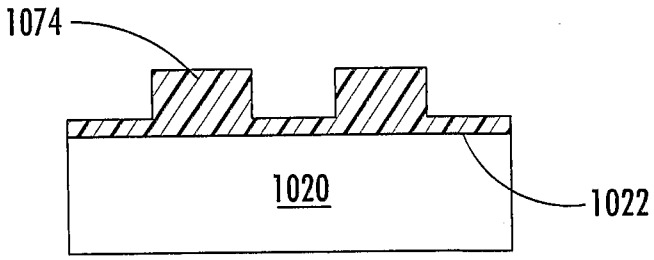


FIG. 10E

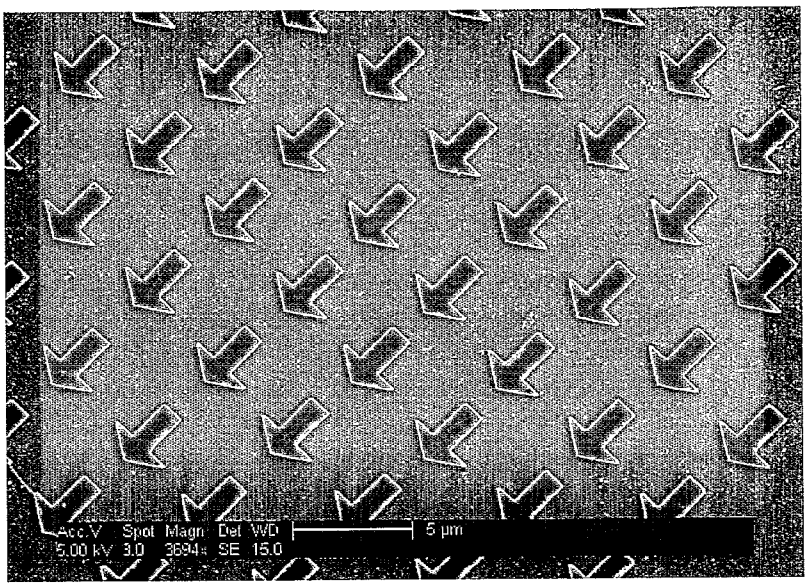


Fig. 11

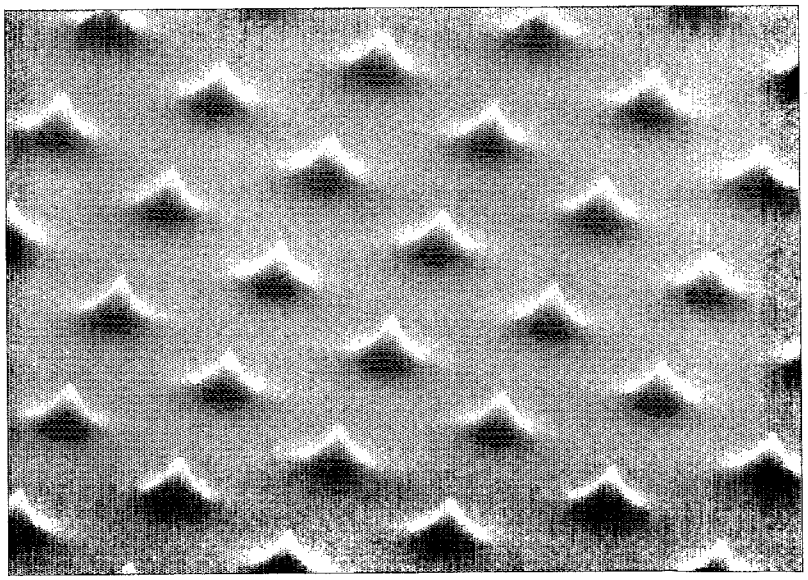


Fig. 12

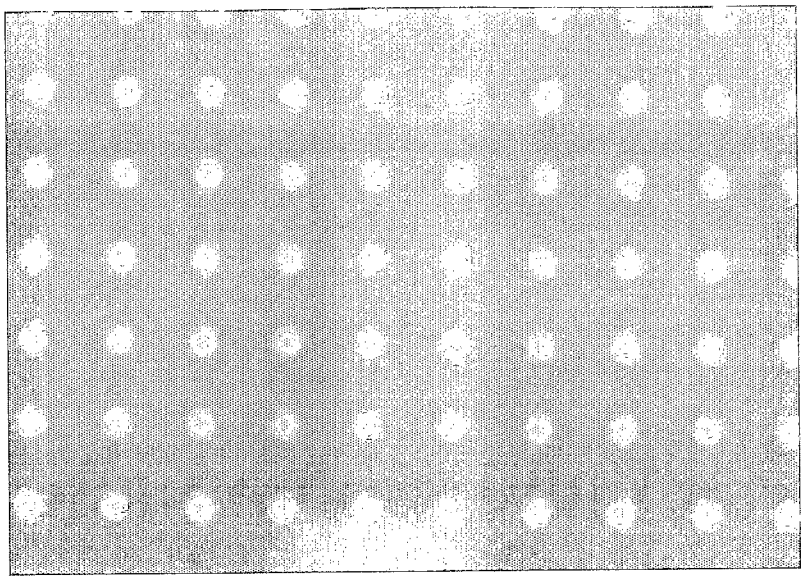


Fig. 13

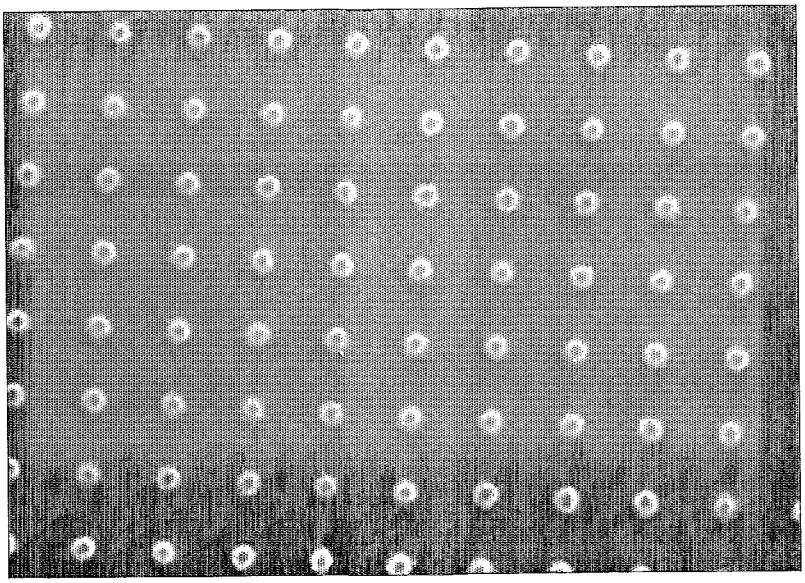


Fig. 14

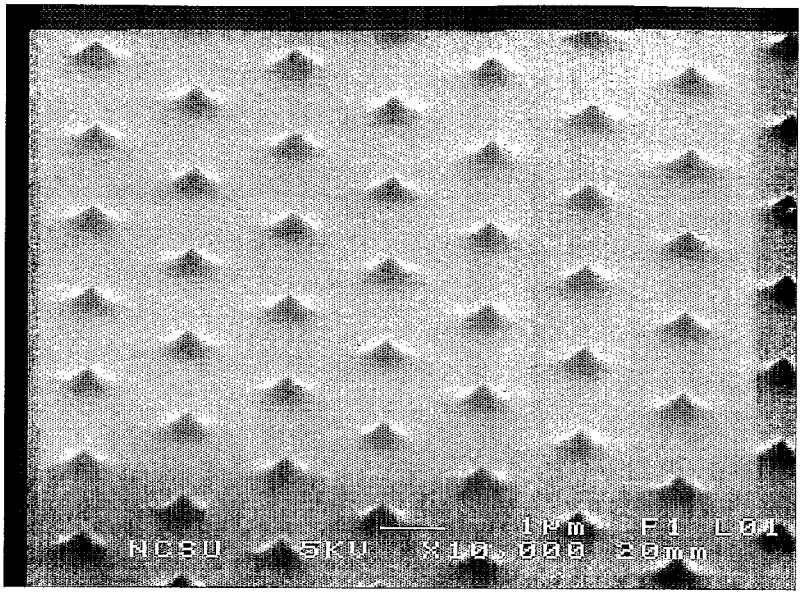

NCSU 5kV X10,000 2.9mm
1μm F1 L01

Fig. 15

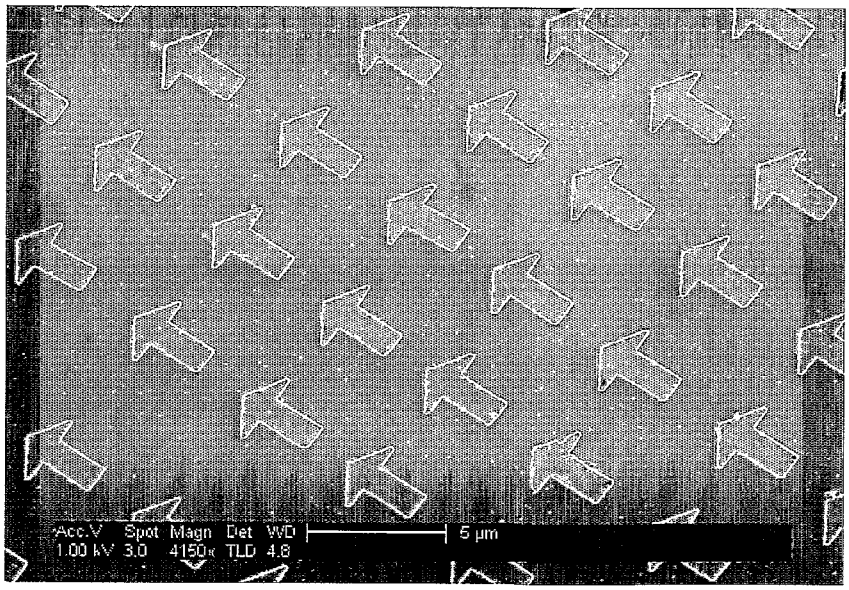

Acc.V Spot Magn Det WD
1.00 kV 3.0 4150x TLD 4.8
5 μm

Fig. 16

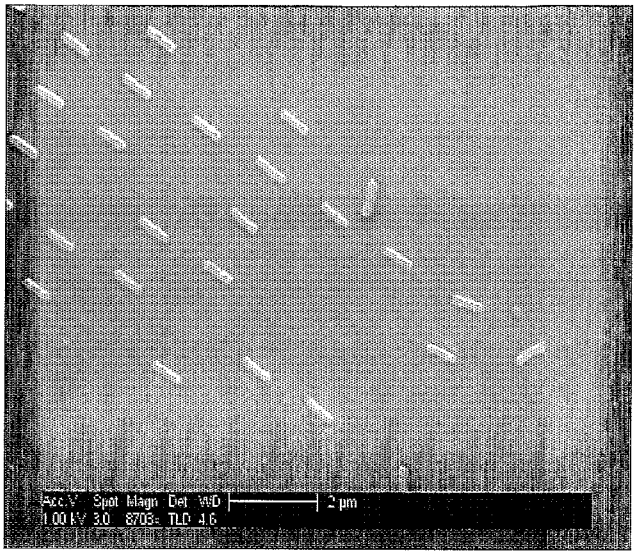

Acc.V Spot Magn Det WD 2 µm
1.00 kV 3.0 8703x TLD 4.6

Fig. 17

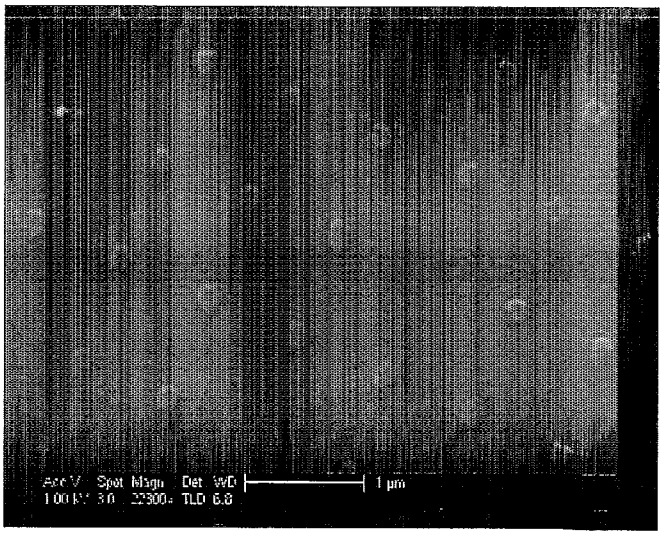

Acc.V Spot Magn Det WD 1 µm
1.00 kV 3.0 22300x TLD 6.8

Fig. 18

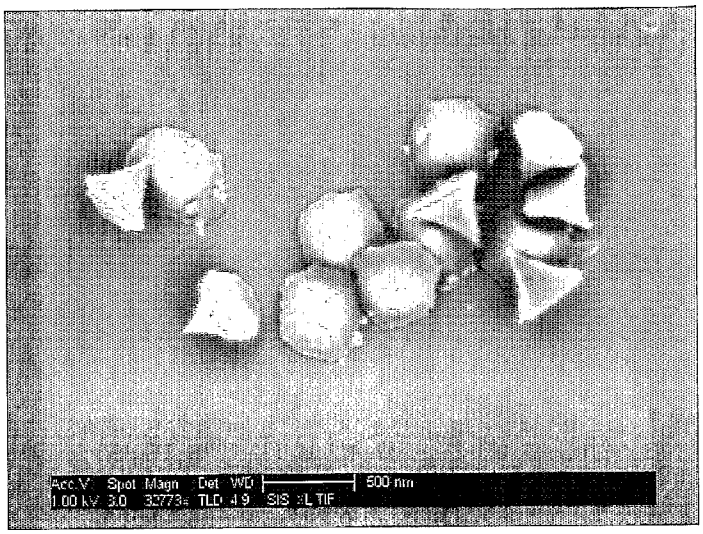

Acc.V Spot Magn Det WD 500 nm
1.00 kV 3.0 32773x TLD 4.9 SIS XL TIF

Fig. 19

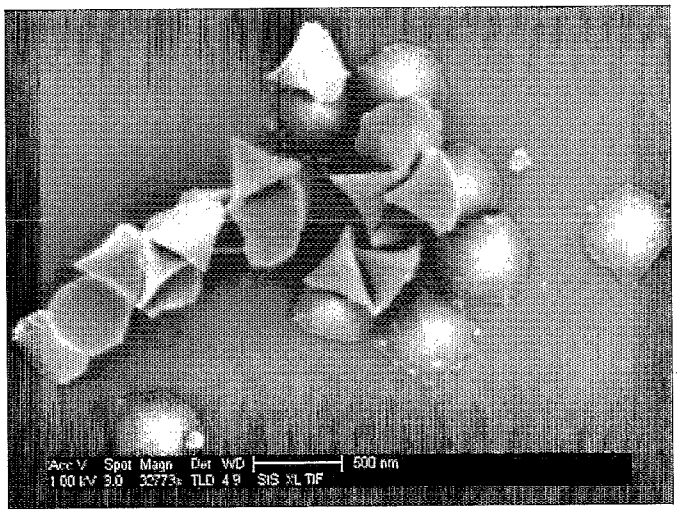

Acc.V Spot Magn Det WD 500 nm
1.00 kV 3.0 32773x TLD 4.9 SIS XL TIF

Fig. 20

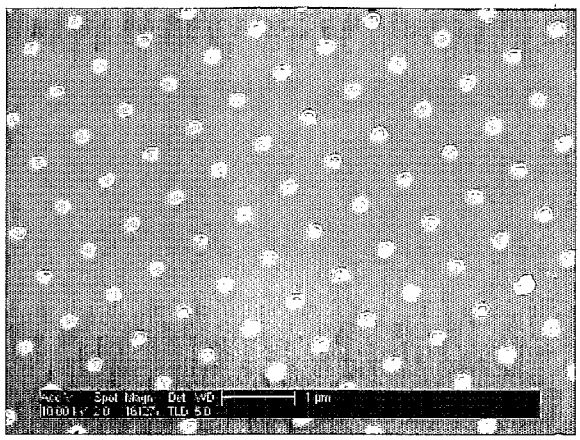


Fig. 21

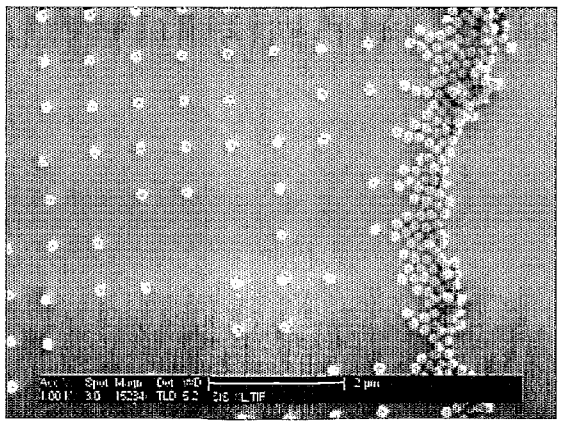


Fig. 22

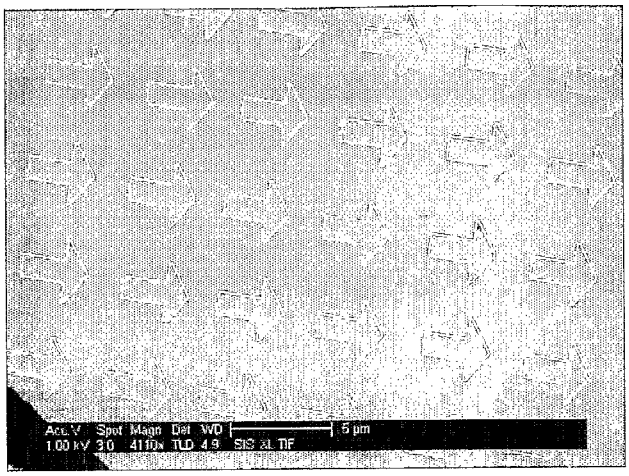

Acc.V Spot Magn Det WD 5 µm
1.00 kV 3.0 4110x TLD 4.9 SIS at TF

Fig. 23

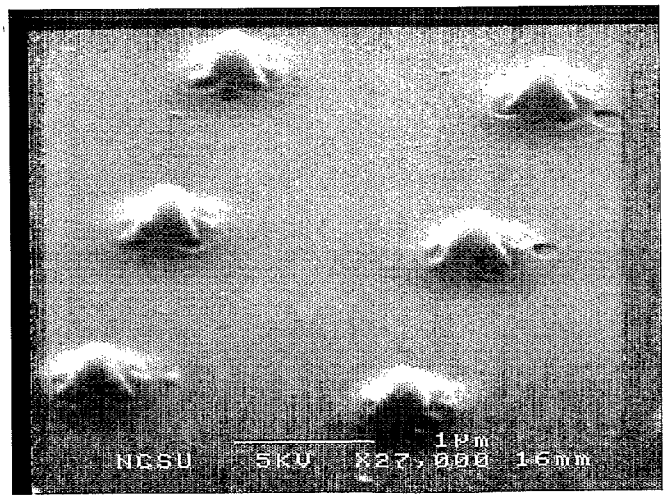

1µm
NCSU 5KV X27,000 1.6mm

Fig. 24

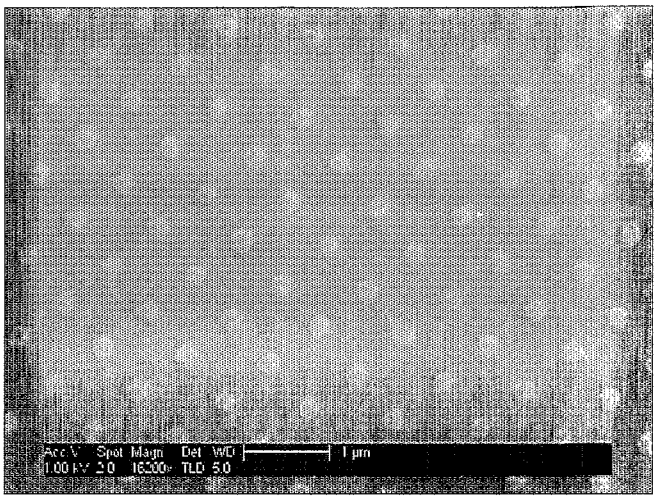

Acc.V Spot Magn Det WD 1 µm
1.00 kV 2.0 16200x TLD 5.0

Fig. 25

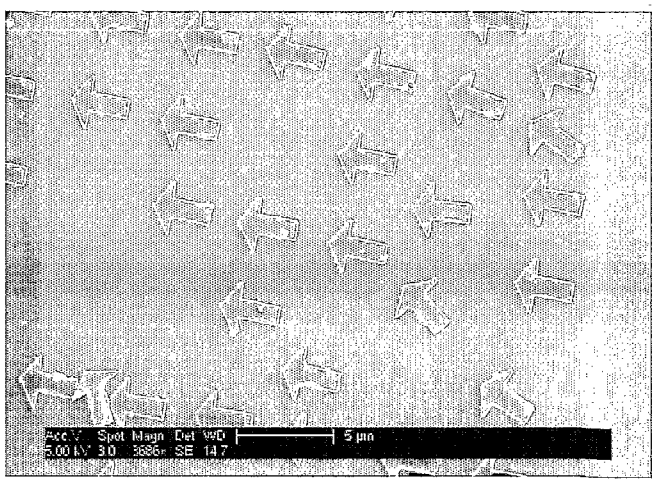

Acc.V Spot Magn Det WD 5 µm
5.00 kV 3.0 3686x SE 14.7

Fig. 26

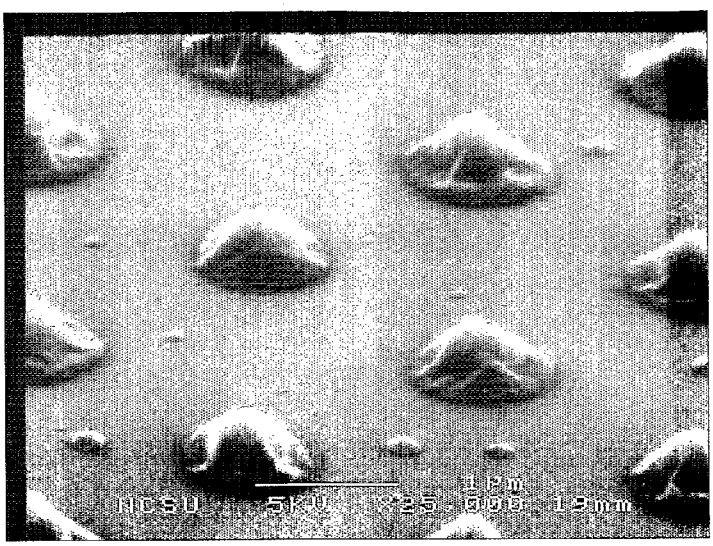


Fig. 27

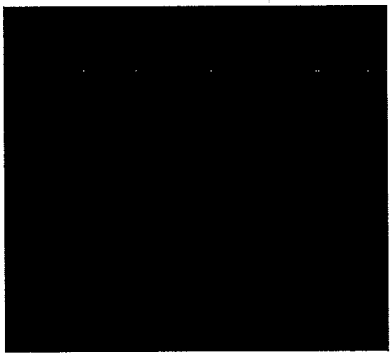


Fig. 28A

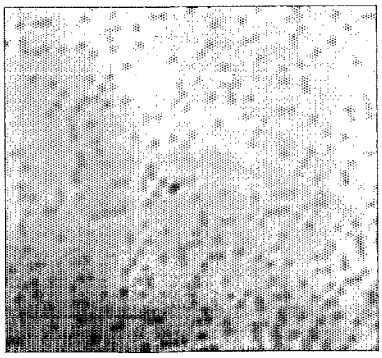


Fig. 28B

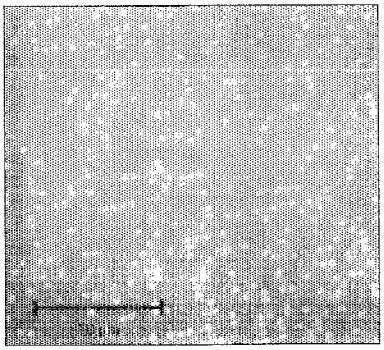


Fig. 28C

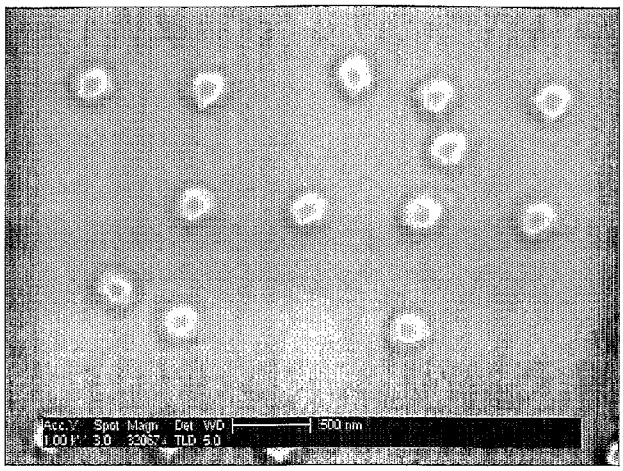


Fig. 29

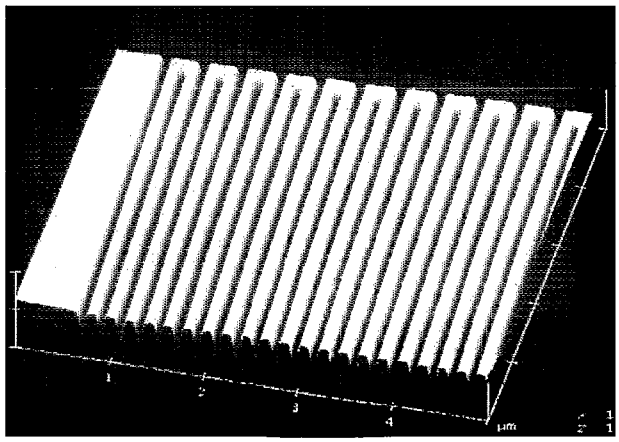


Fig. 30

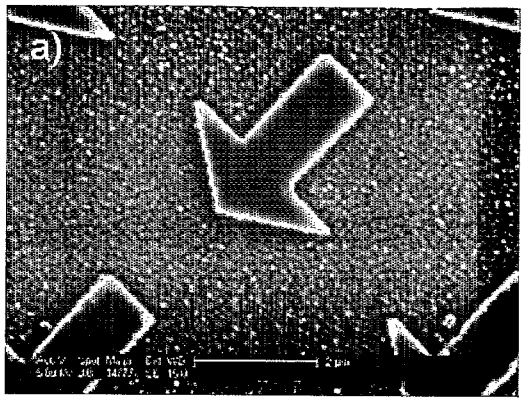


Fig. 31A

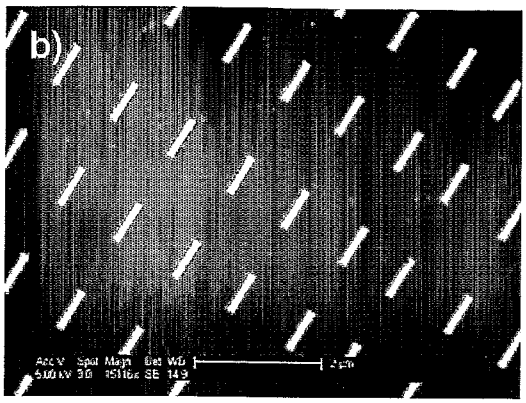


Fig. 31B

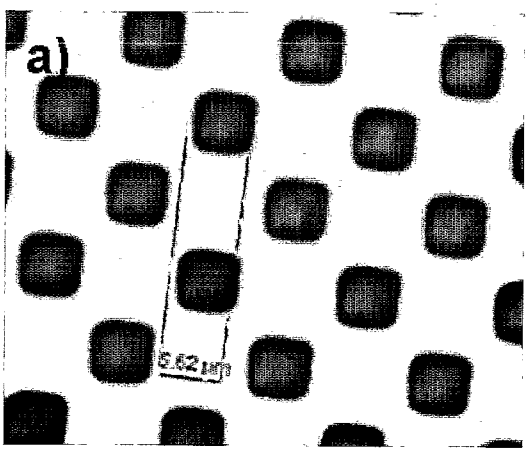


Fig. 32A

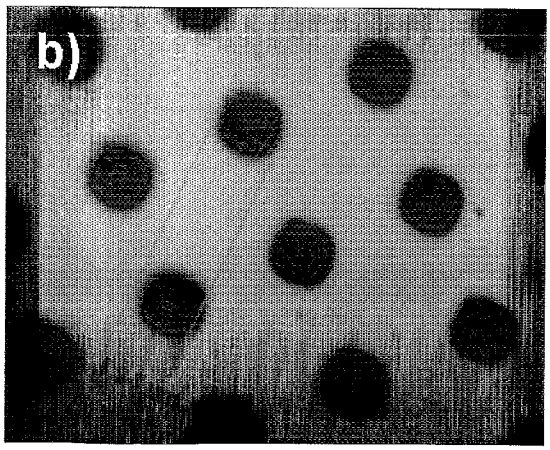


Fig. 32B

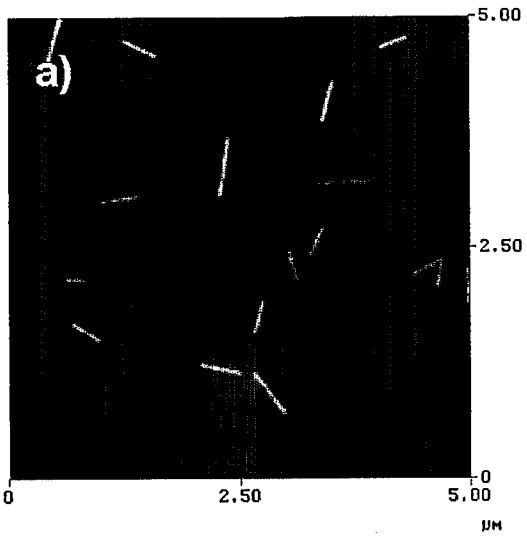


Fig. 33A

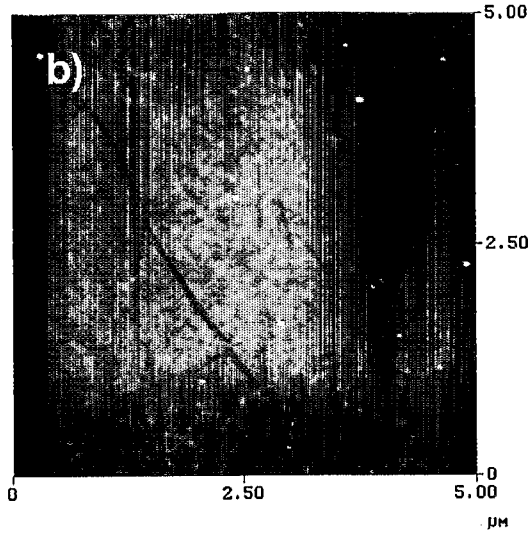


Fig. 33B

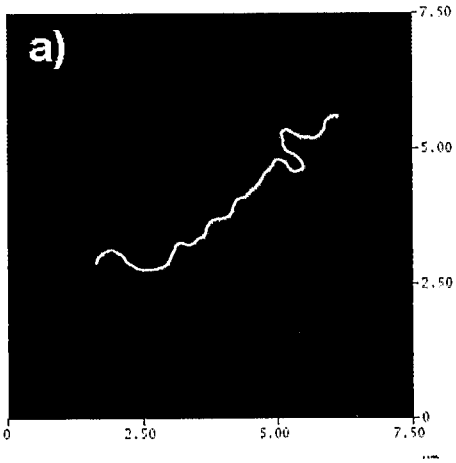


Fig. 34A

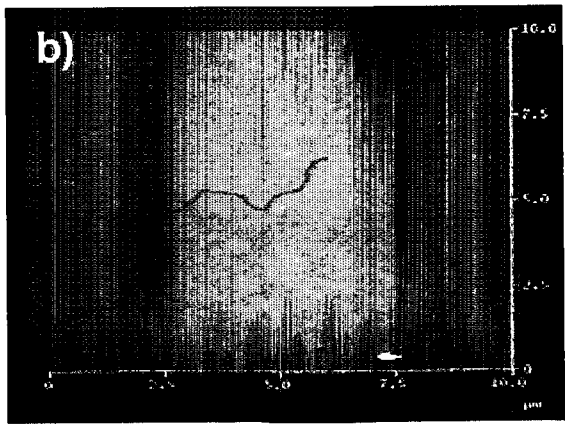


Fig. 34B

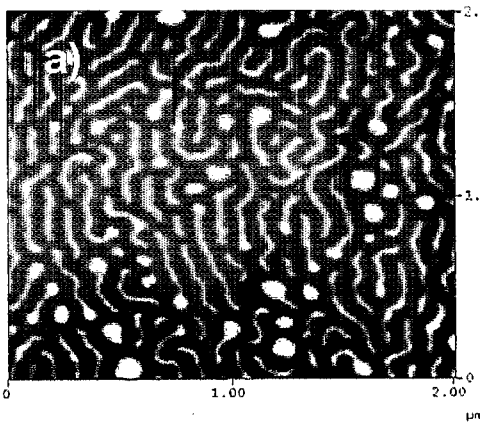


Fig. 35A

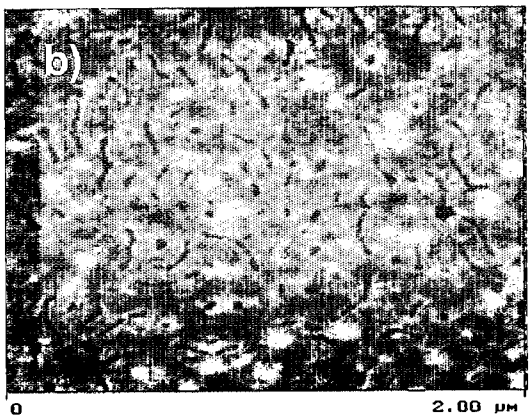


Fig. 35B

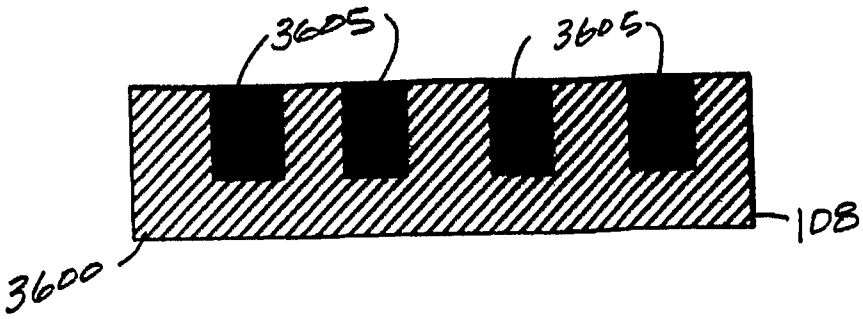


FIG. 36A

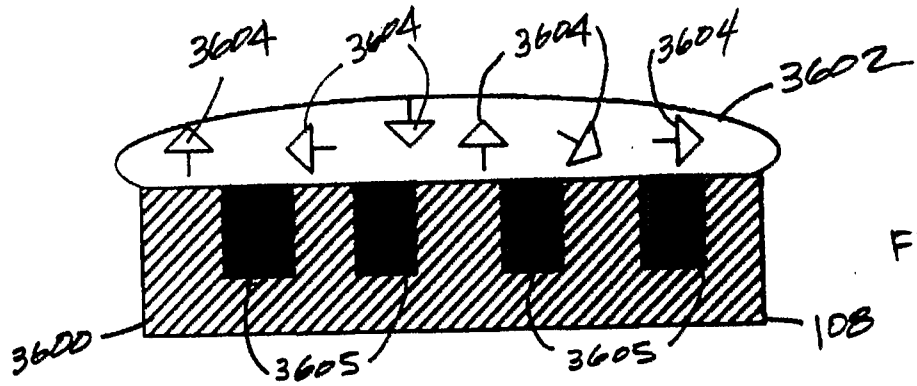


FIG. 36B

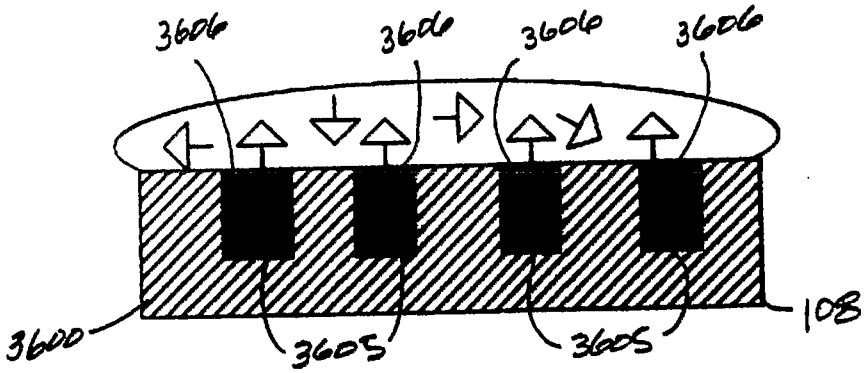


FIG. 36C

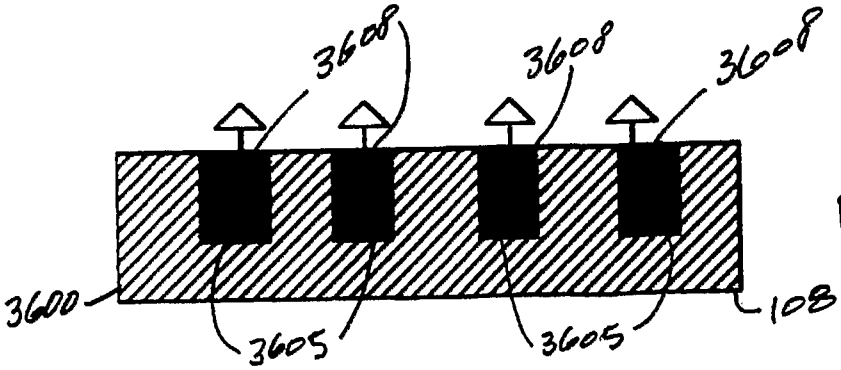


FIG. 36D

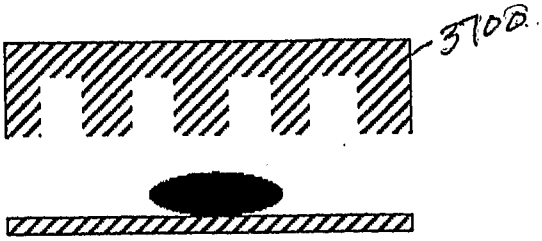


FIG. 37A

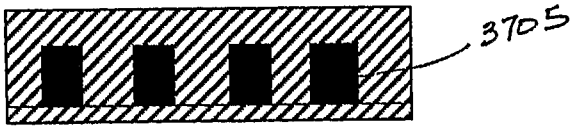


FIG. 37B

FIG. 37C

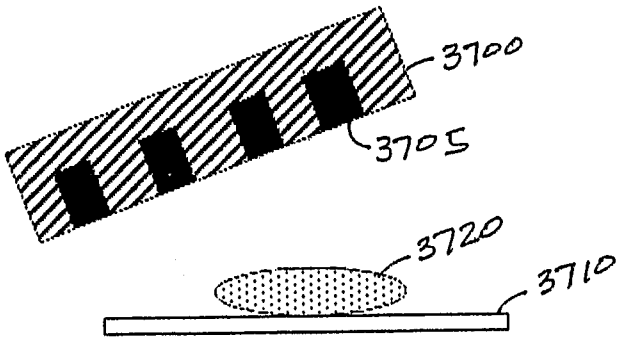


FIG. 37D

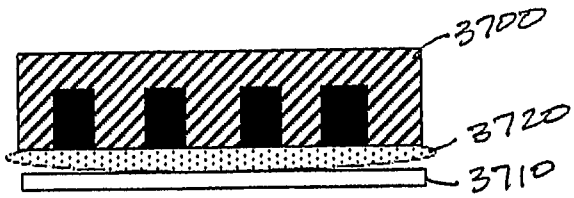


FIG 37E

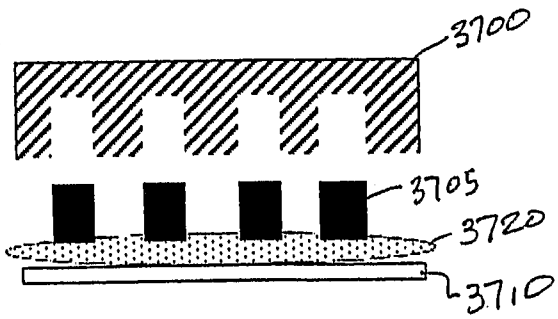


FIG. 37F

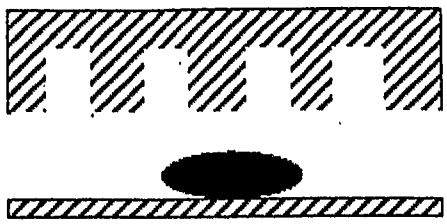


FIG. 38A

FIG. 38B

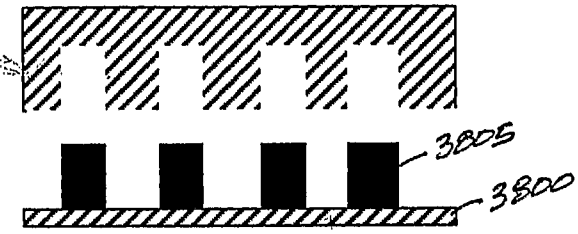


FIG. 38C

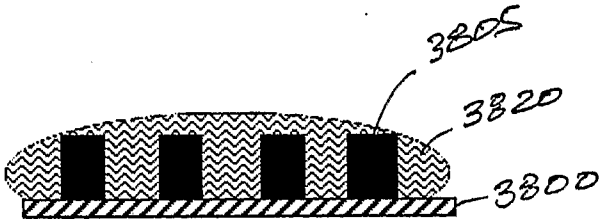


FIG. 38D

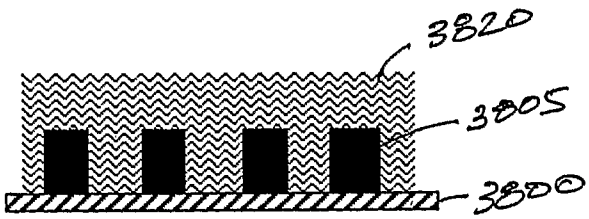


FIG. 38E

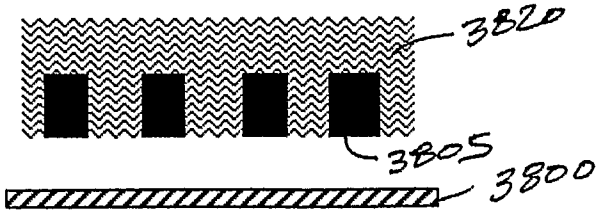


FIG. 38F

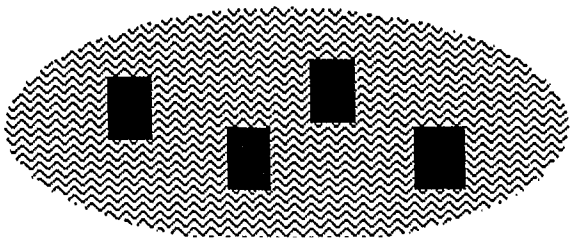
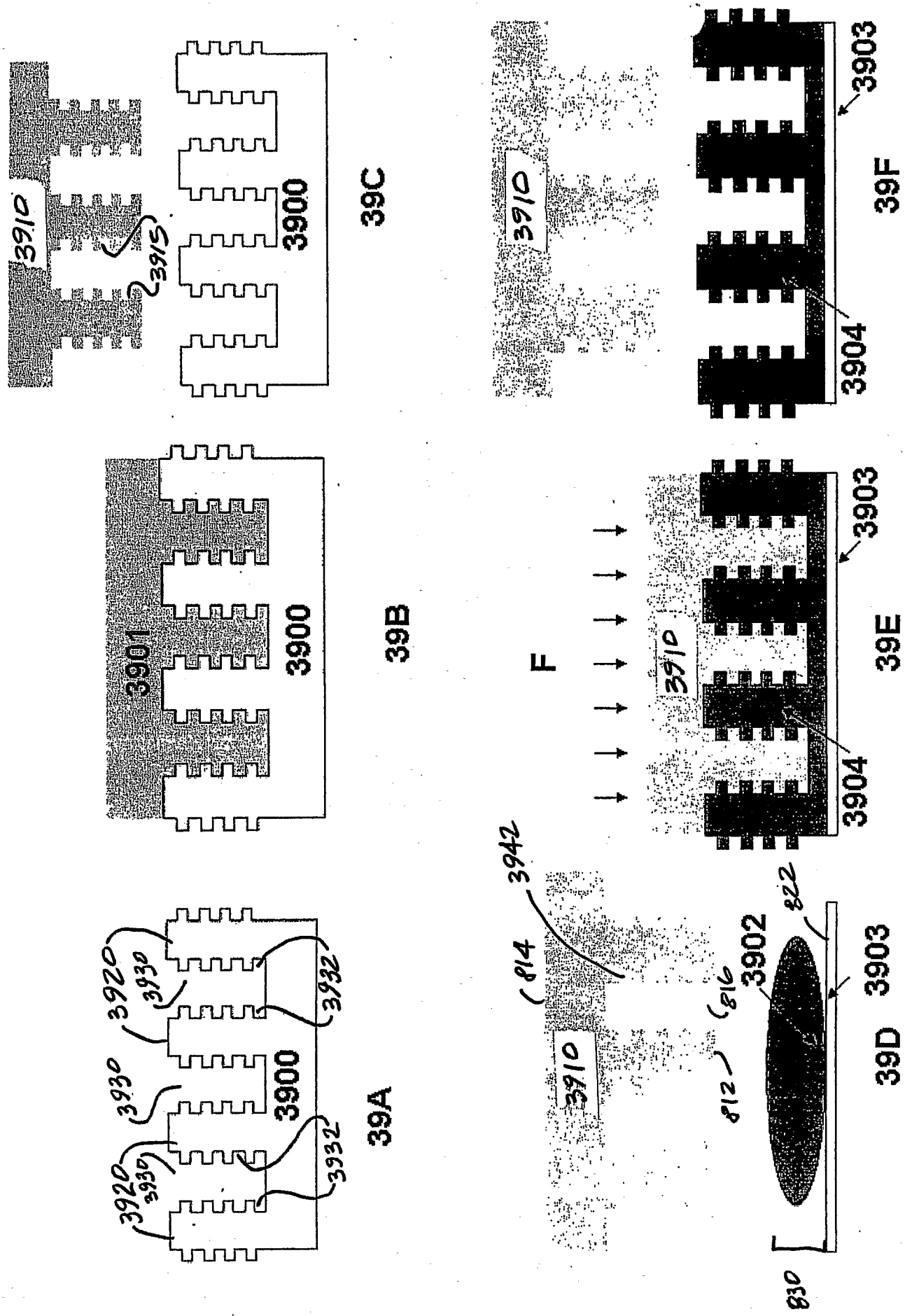


FIG. 38G


3910
3915
3900
39C
3901
3900
39B
3920
3930
3932
3930
3900
3920
3930
3932
39A
3910
3903
3904
39F
F
3910
3903
3904
39E
3942
814
3910
812
816
3902
822
3903
39D
830

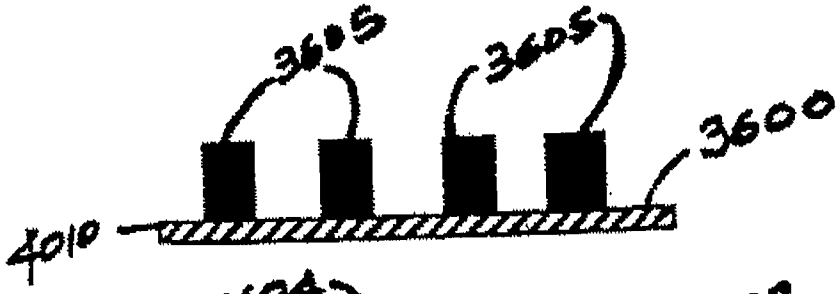


FIG. 40A

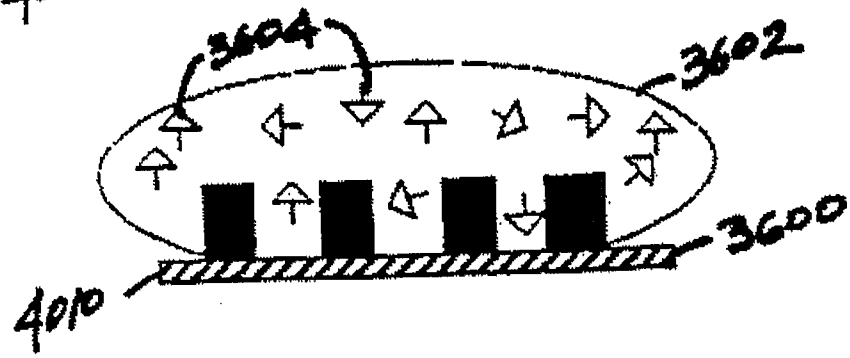


FIG. 40B

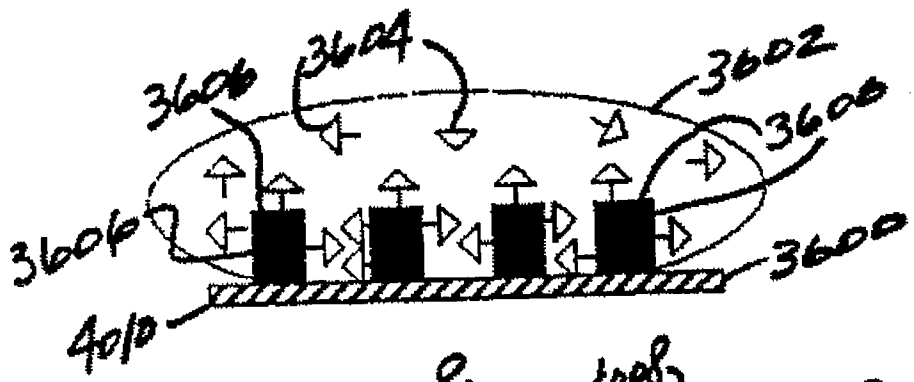


FIG. 40C

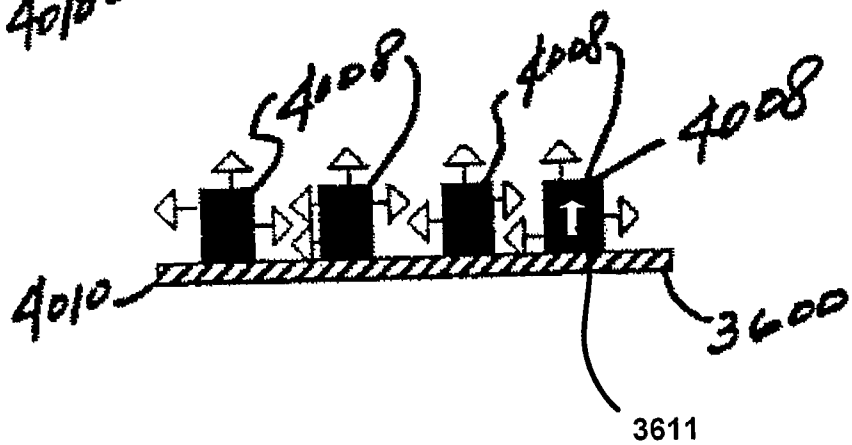
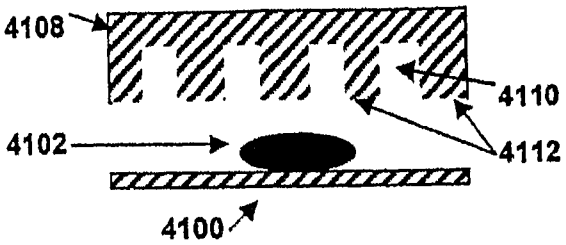
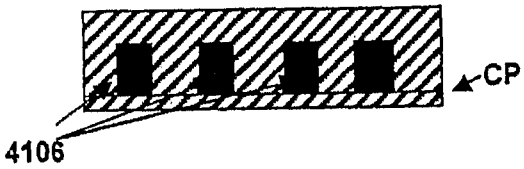


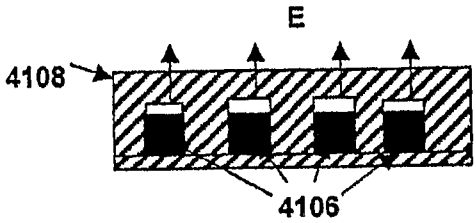
FIG. 40D



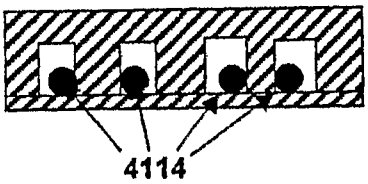
41A



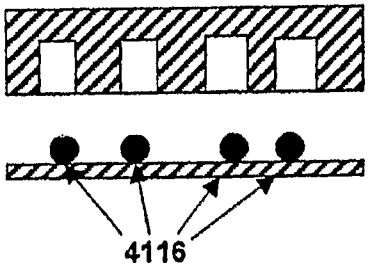
41B



41C



41D



41E

Figure 42

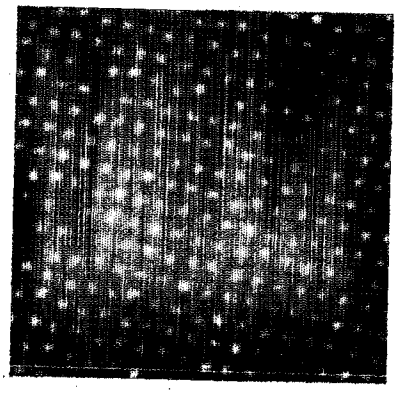
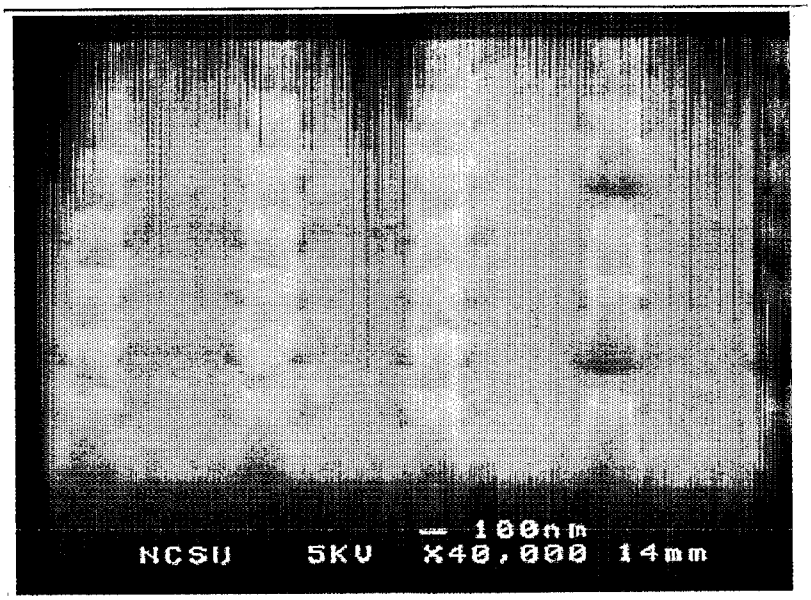


Figure 43



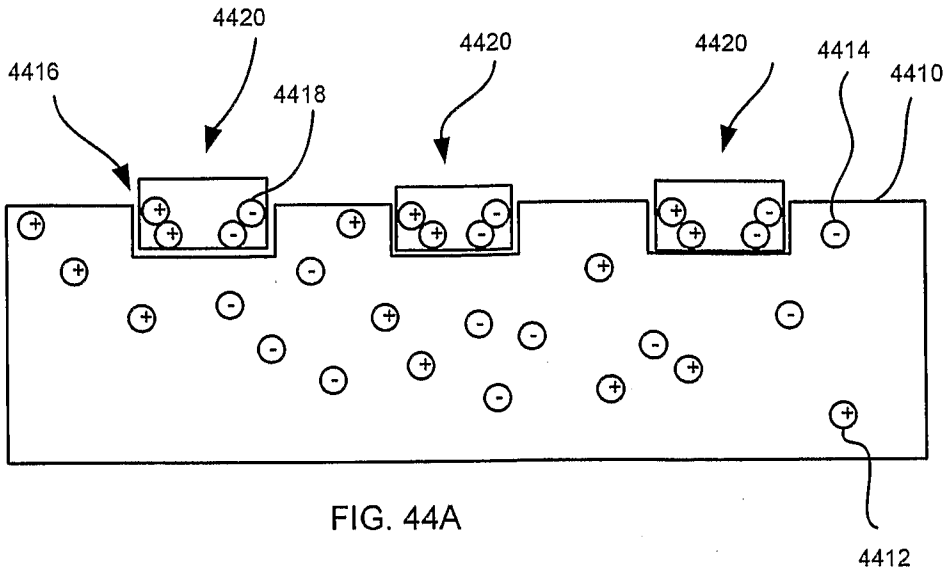


FIG. 44A

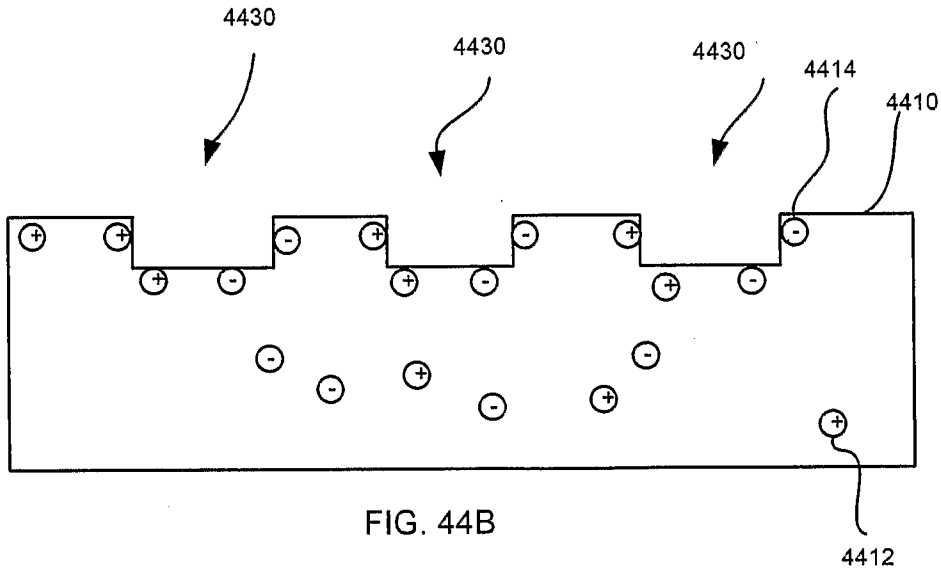


FIG. 44B

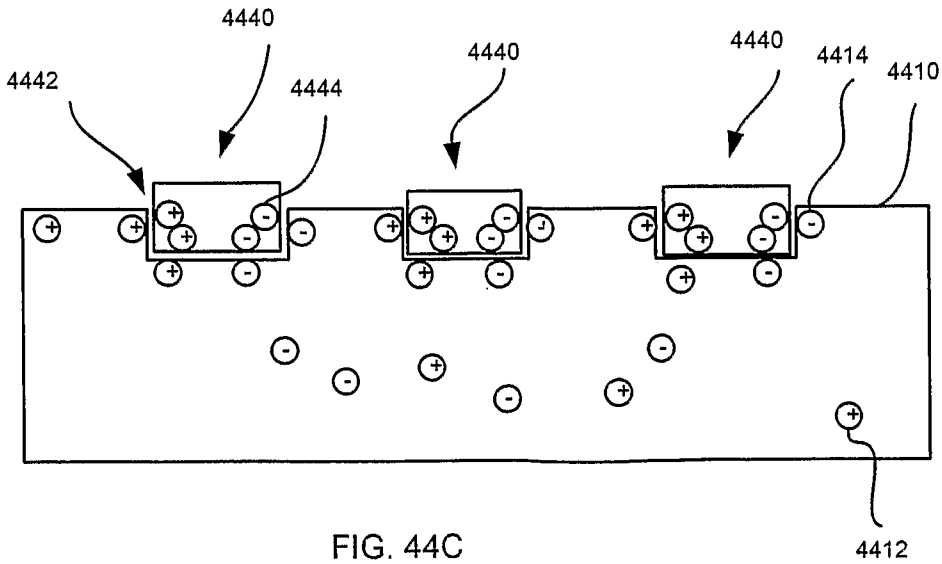


FIG. 44C

Fluorescence/DIC overlay image of a particle attached to a cell

FIG. 45

Fluorescence/DIC overlay image of a particle in a cell

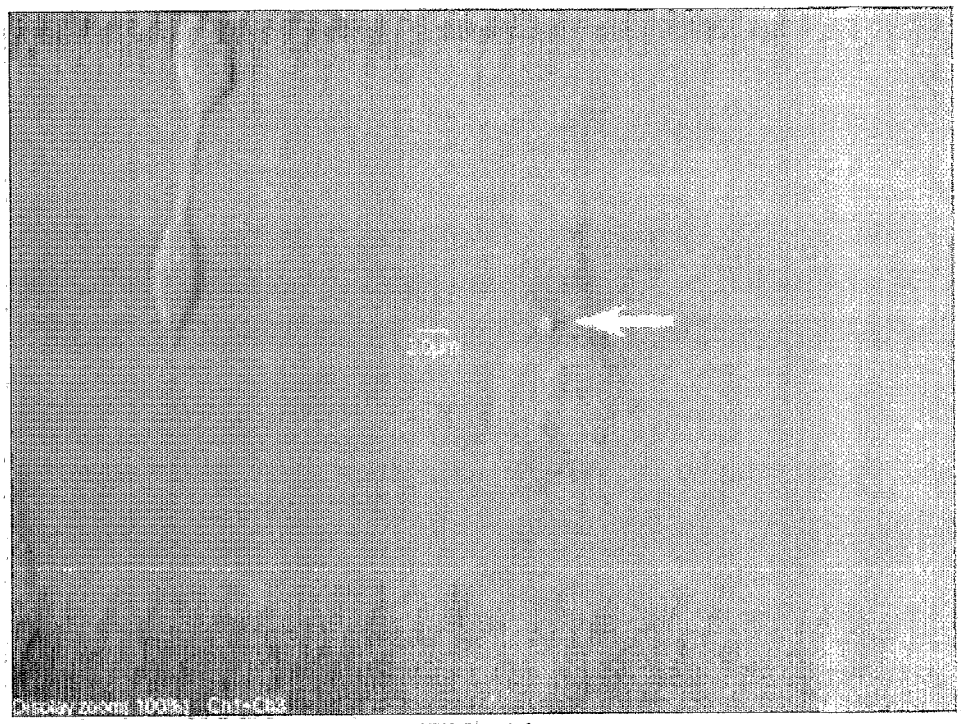


FIG. 46

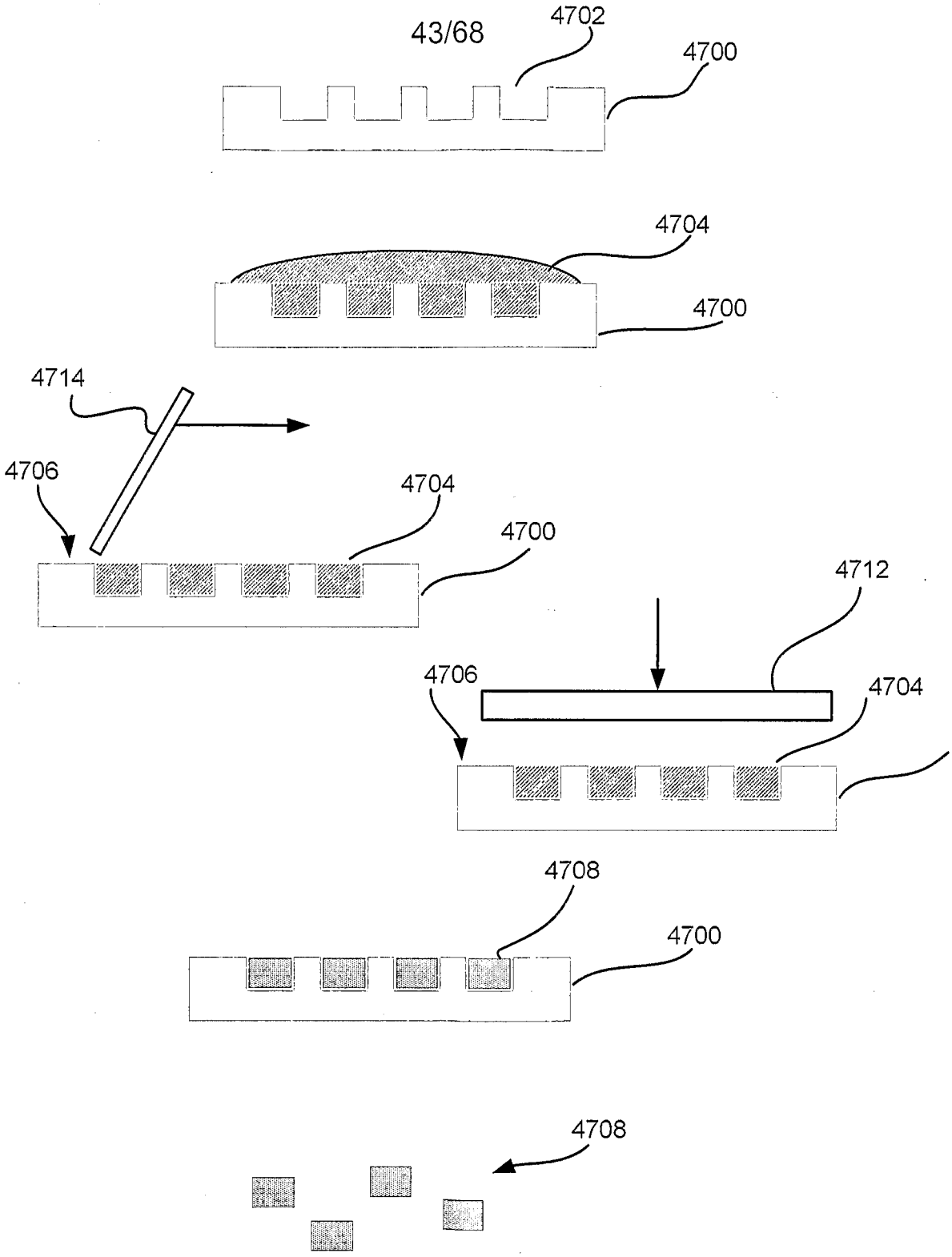

4702
4700
4704
4700
4714
4706
4704
4700
4712
4706
4704
4708
4700
4708

FIG. 47

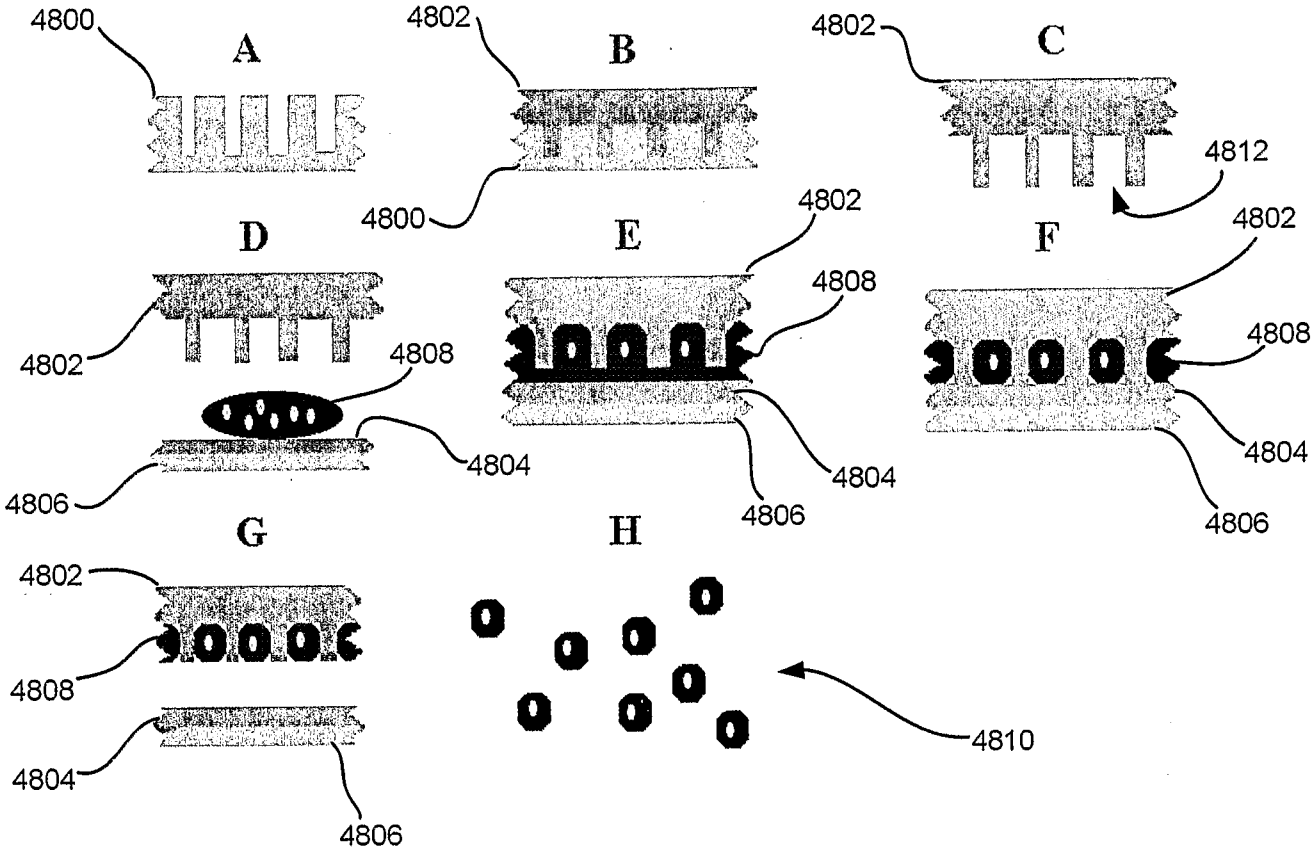

A
4800
B
4802
4800
C
4802
4812
D
4802
4808
4804
4806
E
4802
4808
4804
4806
F
4802
4808
4804
4806
G
4802
4808
4804
4806
H
4810

FIG. 48

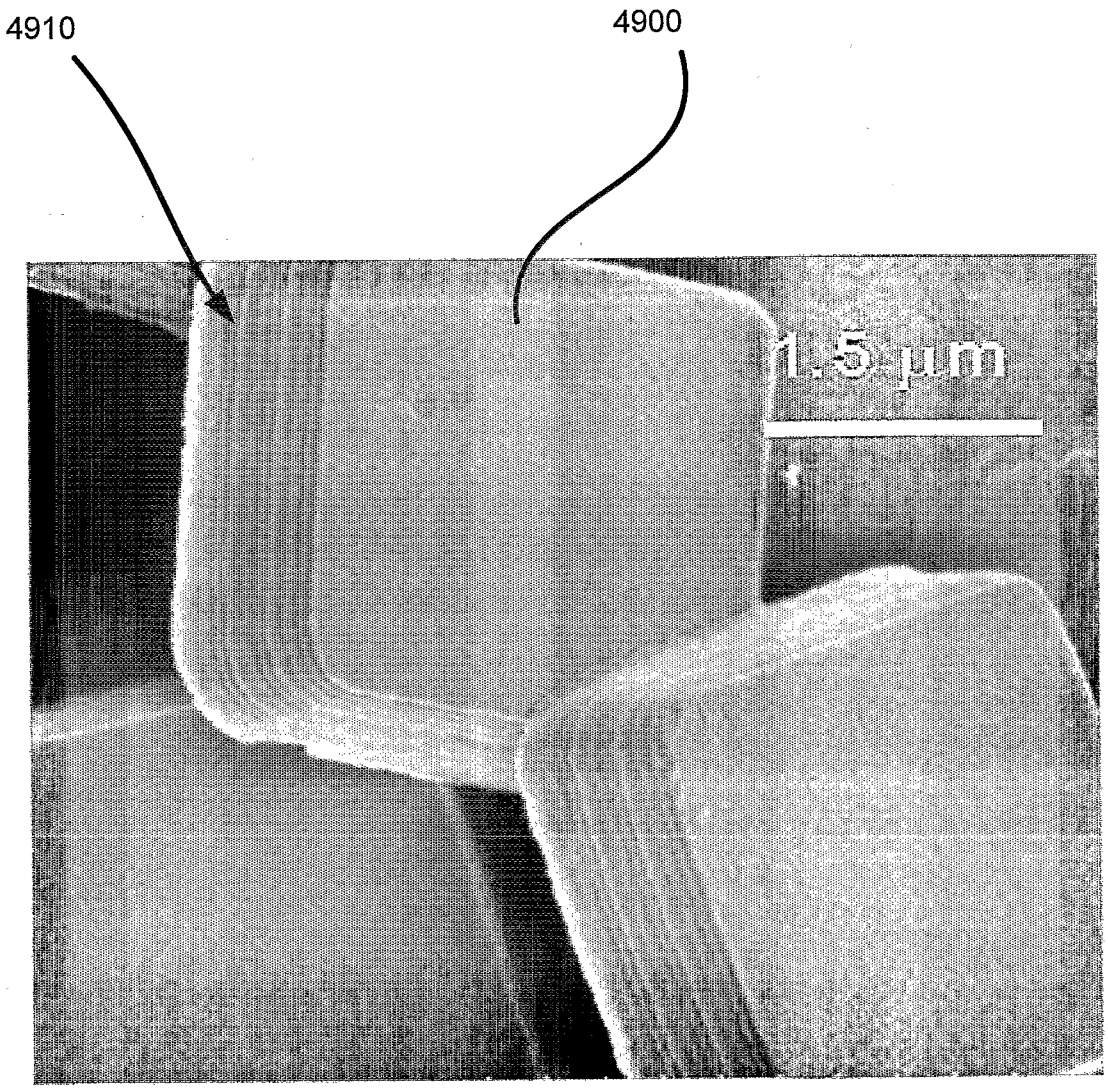


FIG. 49

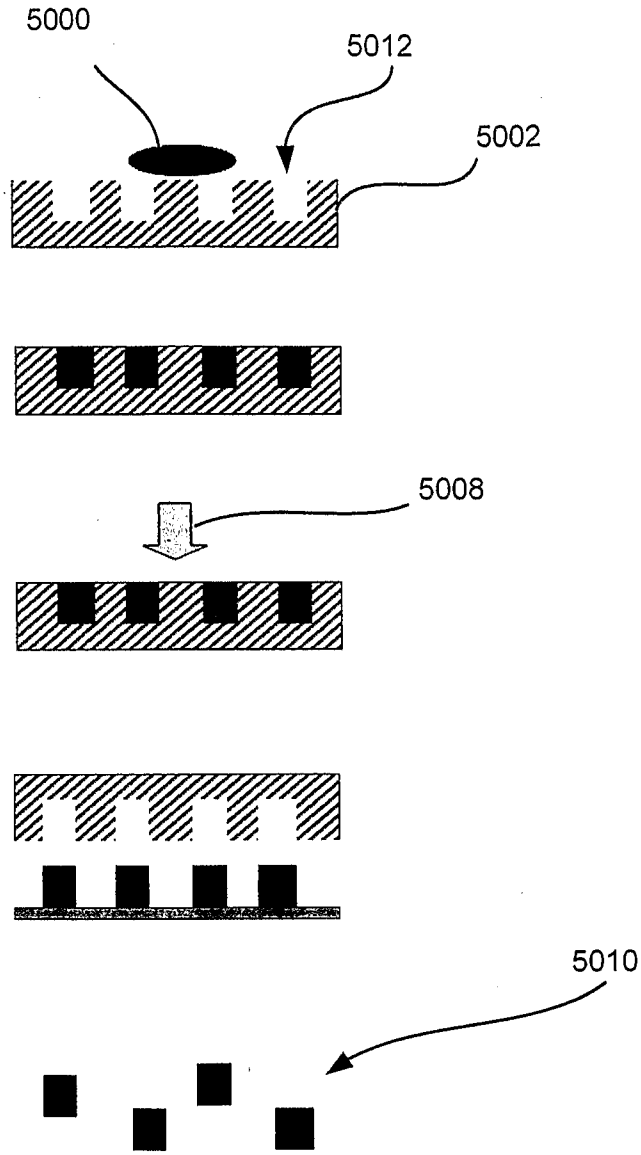


FIG. 50

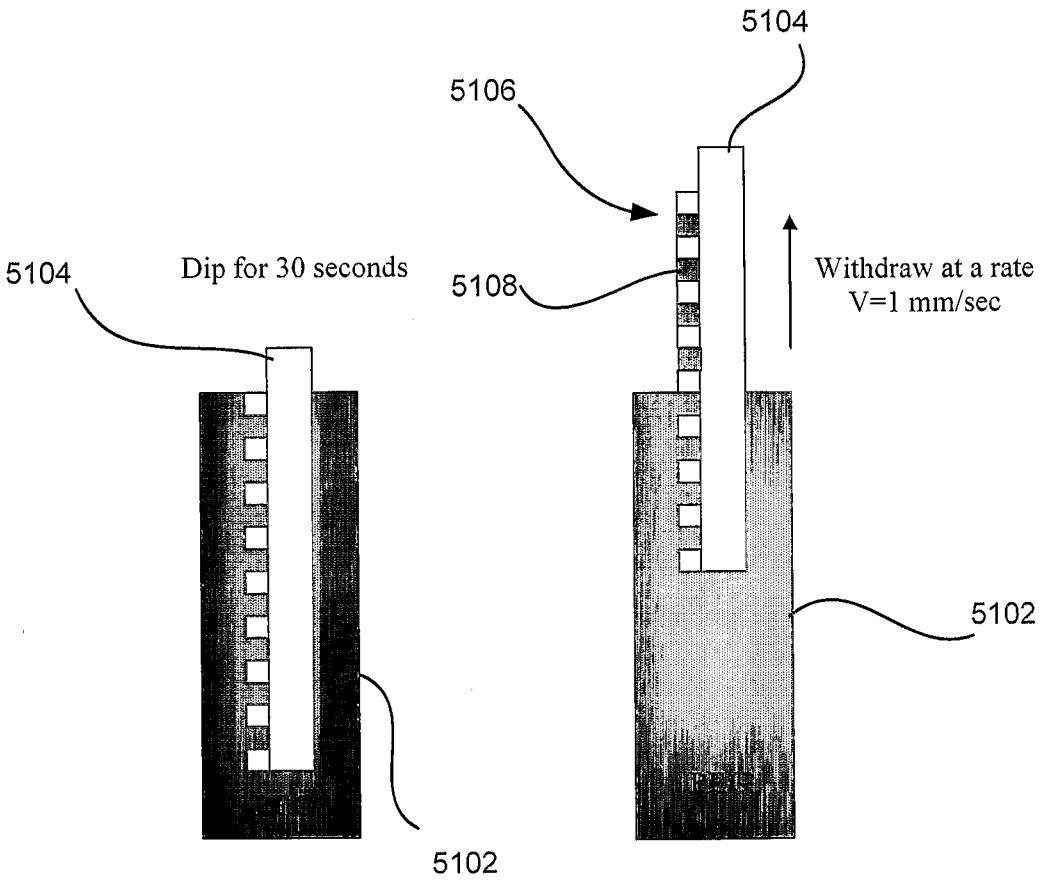

5104
5106
5104
Dip for 30 seconds
5108
Withdraw at a rate
V=1 mm/sec
5102
5102

FIG. 51

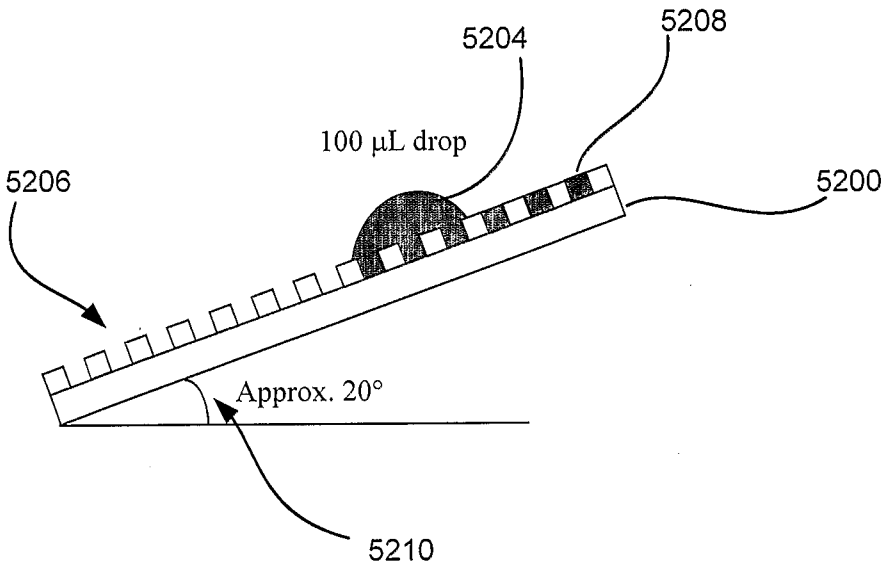


FIG. 52

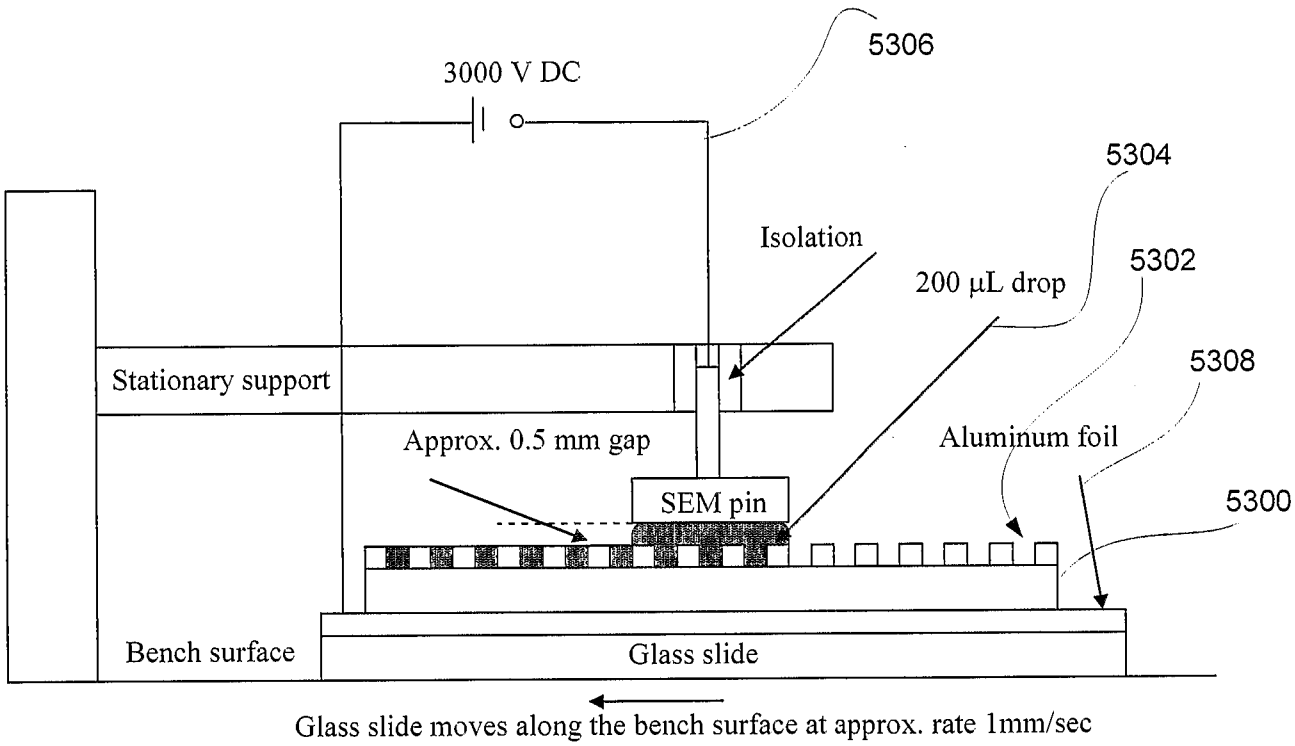

5306
3000 V DC
5304
Isolation
200 µL drop
5302
Stationary support
5308
Approx. 0.5 mm gap
Aluminum foil
SEM pin
5300
Bench surface
Glass slide
Glass slide moves along the bench surface at approx. rate 1mm/sec

FIG. 53

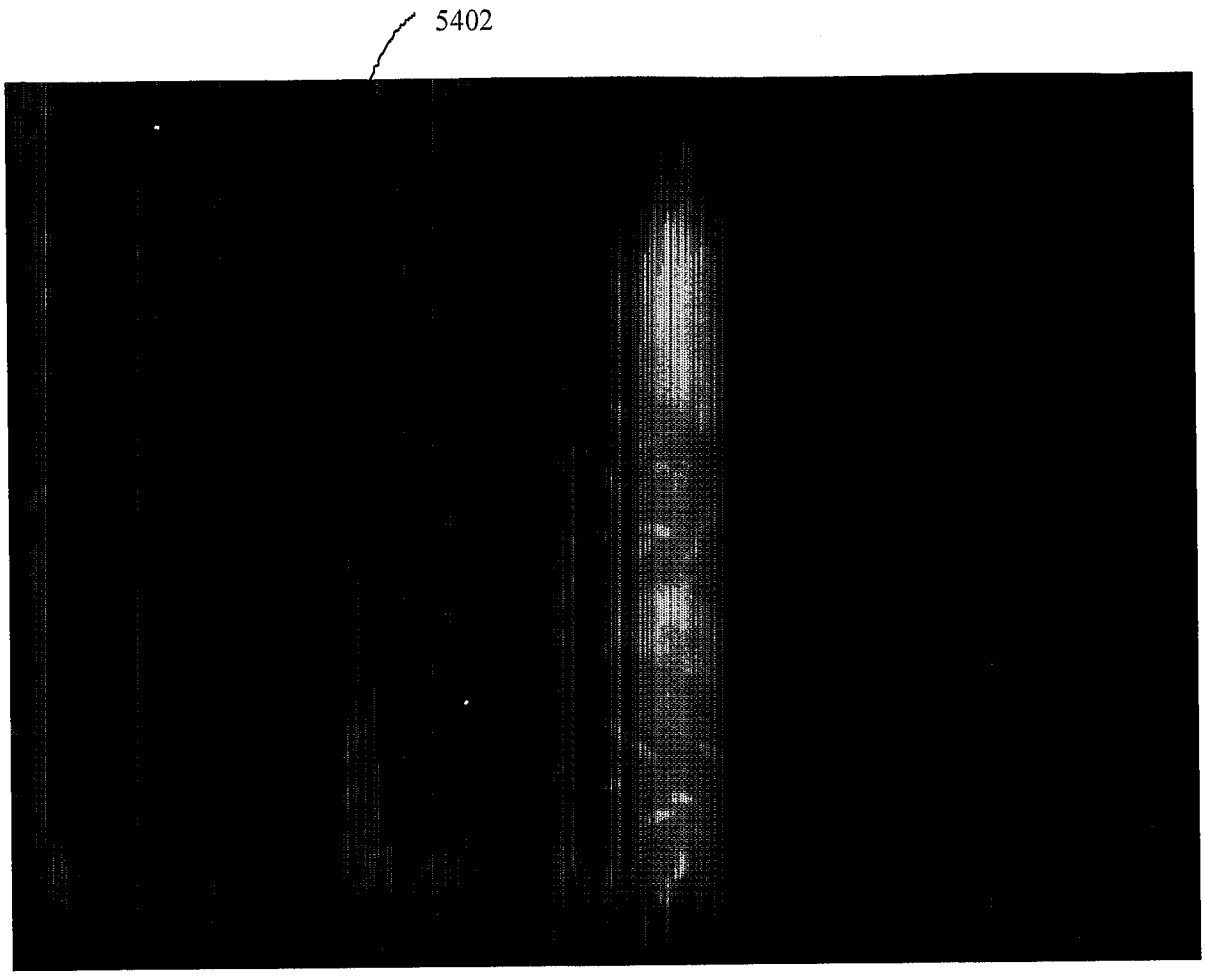


FIG. 54

20 μm

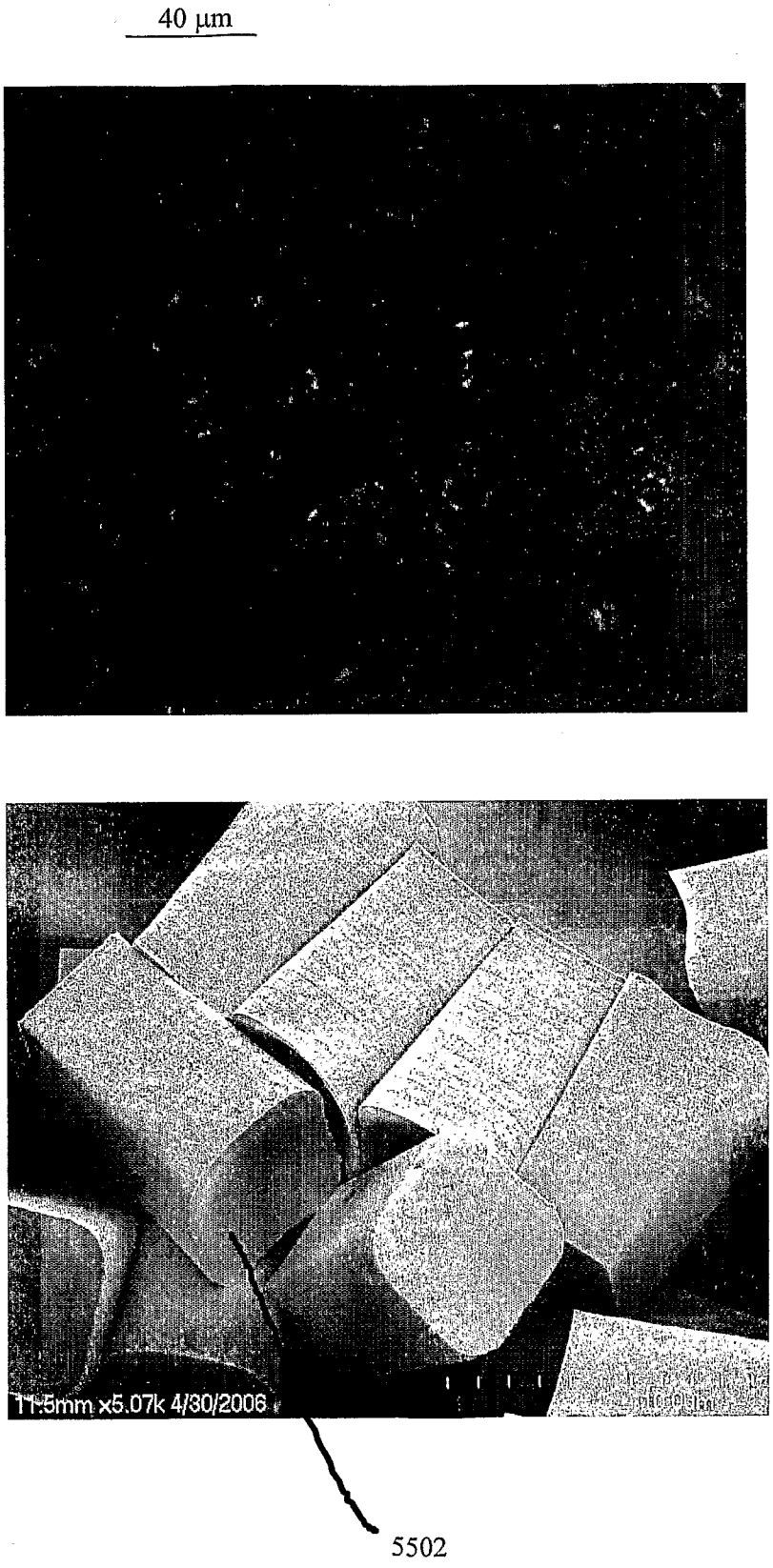
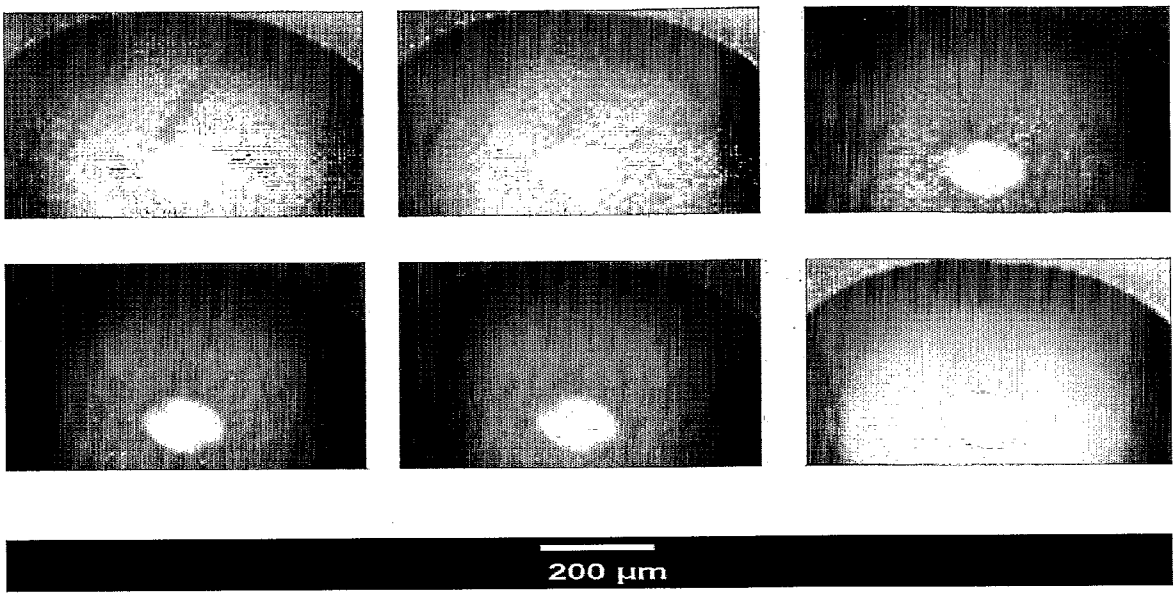


FIG. 55



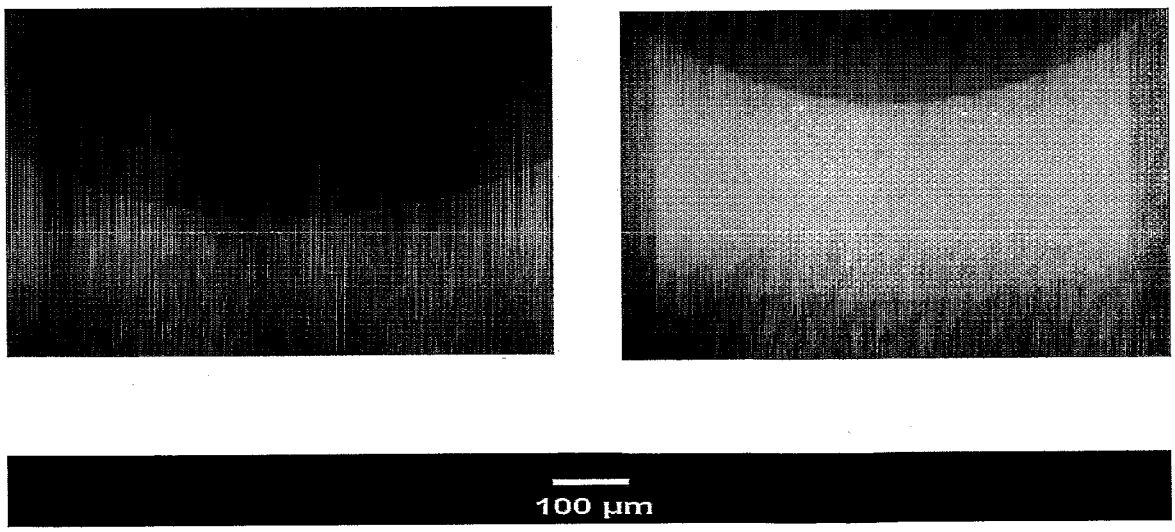


FIG. 56

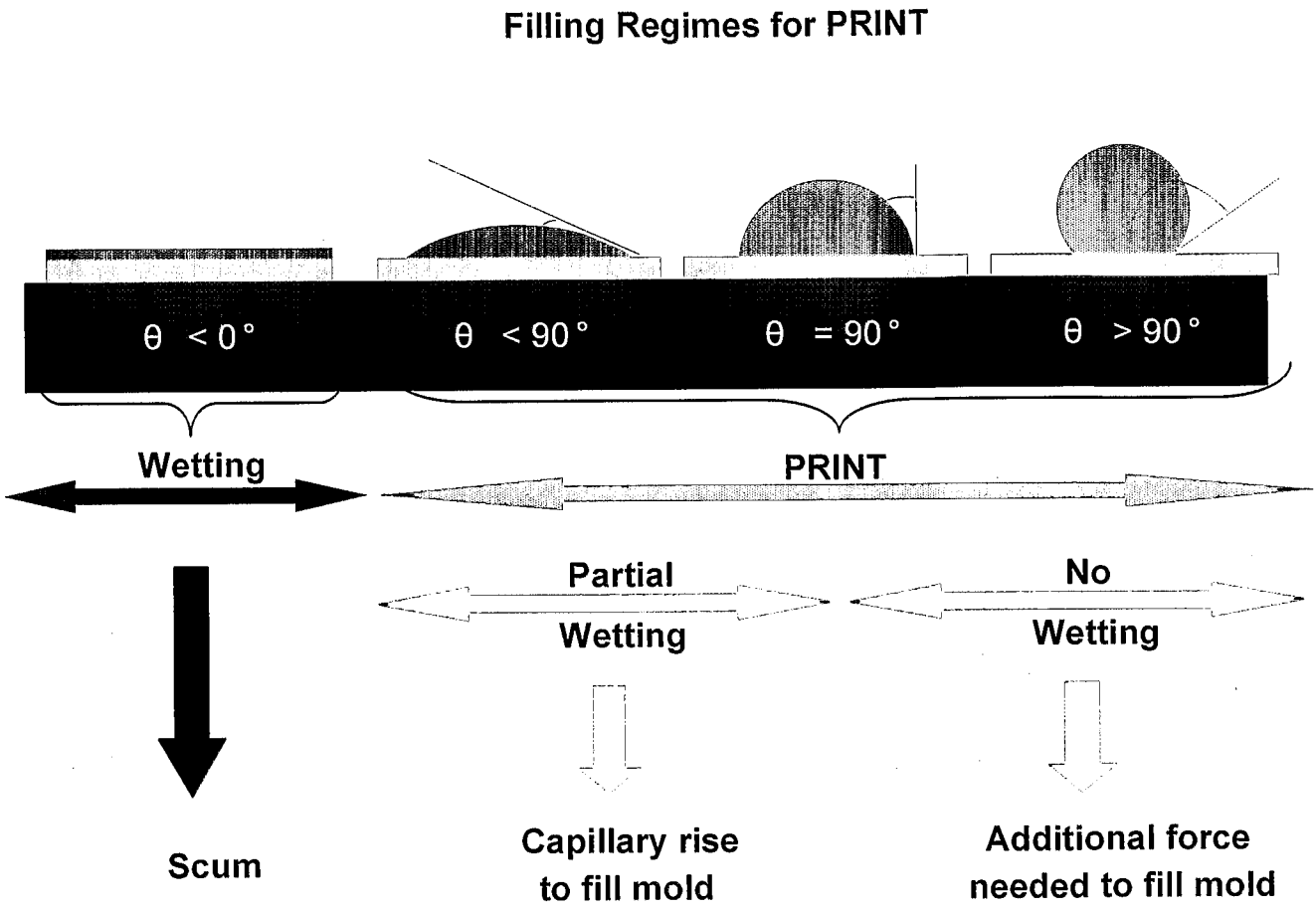
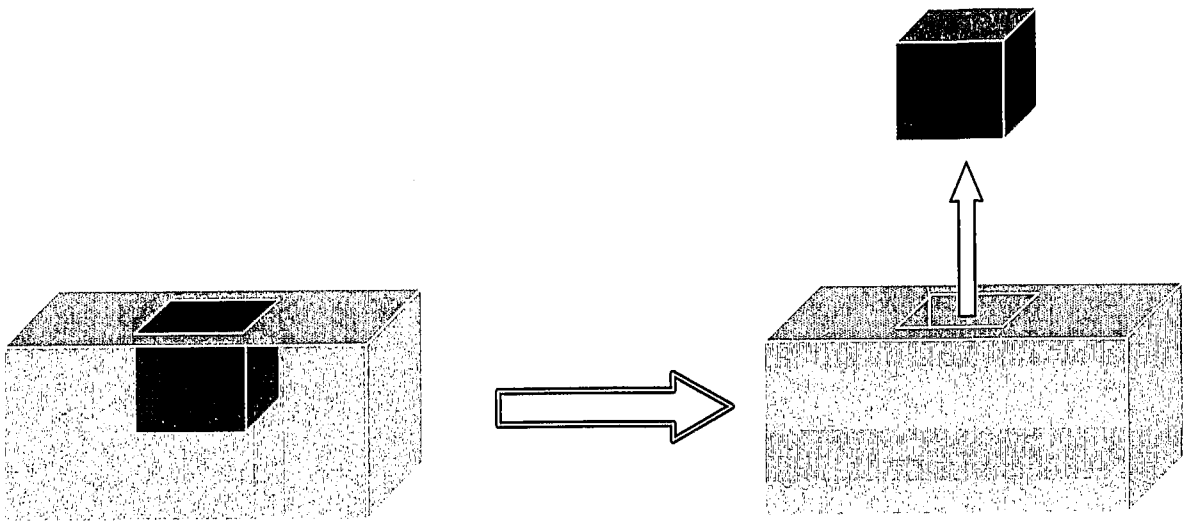

Filling Regimes for PRINT
θ < 0°
θ < 90°
θ = 90°
θ > 90°
Wetting
PRINT
Partial
Wetting
No
Wetting
Scum
Capillary rise
to fill mold
Additional force
needed to fill mold

FIG. 57

Harvesting Issues for PRINT



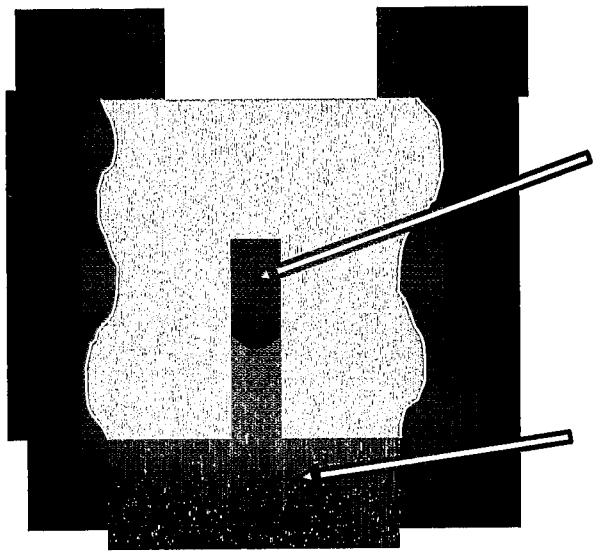
Need to overcome work of adhesion for harvesting

(Facilitated by having a mold with as low a surface energy as possible and no partitioning of liquid-to-be- molded into the mold)

FIG. 58

Permeability Balance

for PRINT



The air pocket will counter - act the capillary rise depending on the permeability of the mold *and* the liquid - to - be - molded to air.

For PRINT to be effective it is important that the mold is not highly permeable to the liquid - to - be - molded

FIG. 59

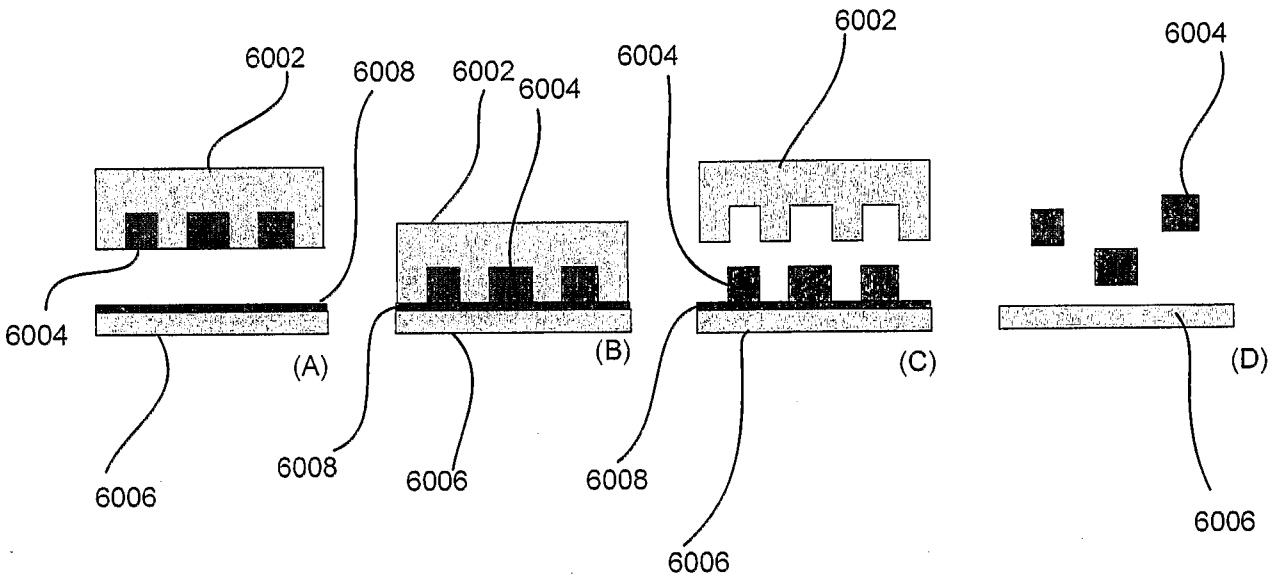

6002
6008
6004
6004
6006
(A)
6002
6004
6008
6006
(B)
6002
6004
6008
6006
(C)
6004
6006
(D)

FIG. 60

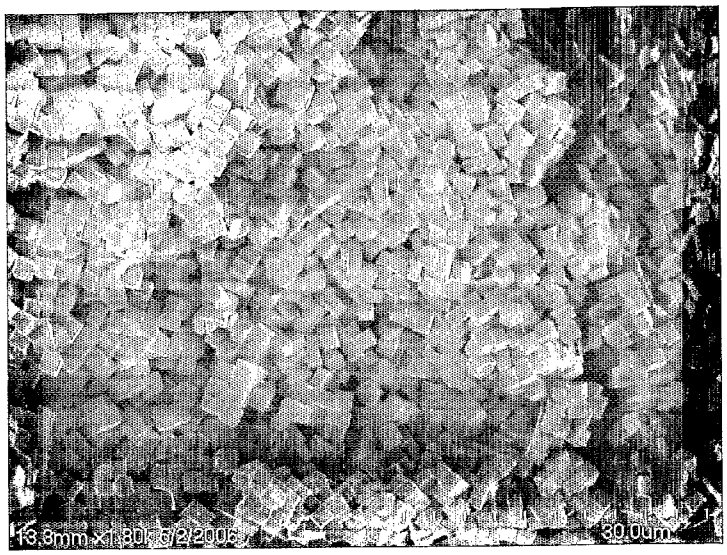


FIG. 61A

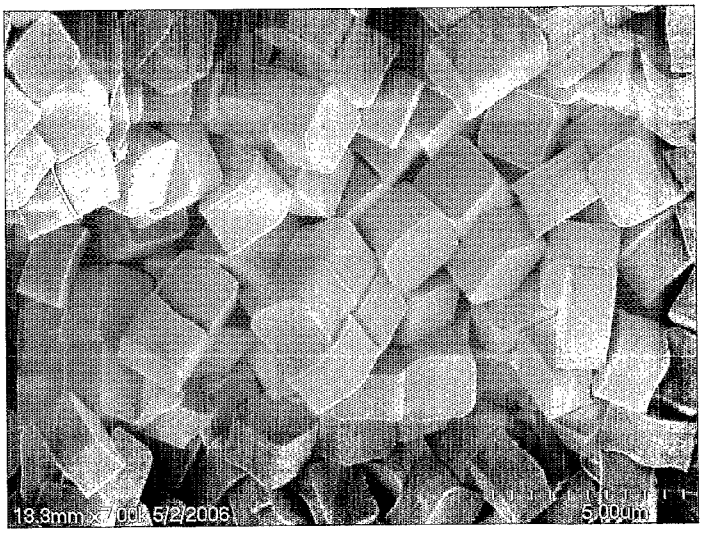


FIG. 61B

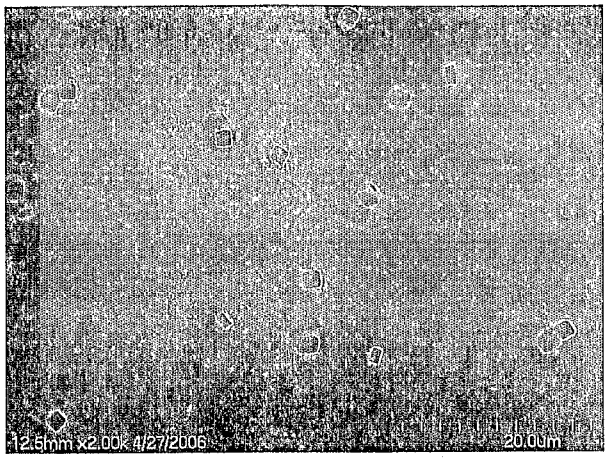


FIG. 62

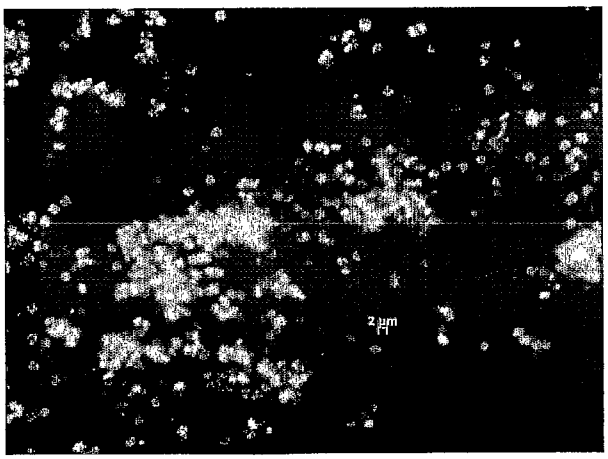


FIG. 63

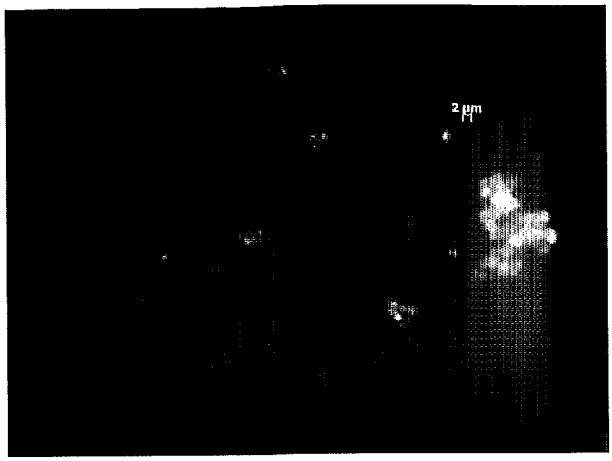


FIG. 64

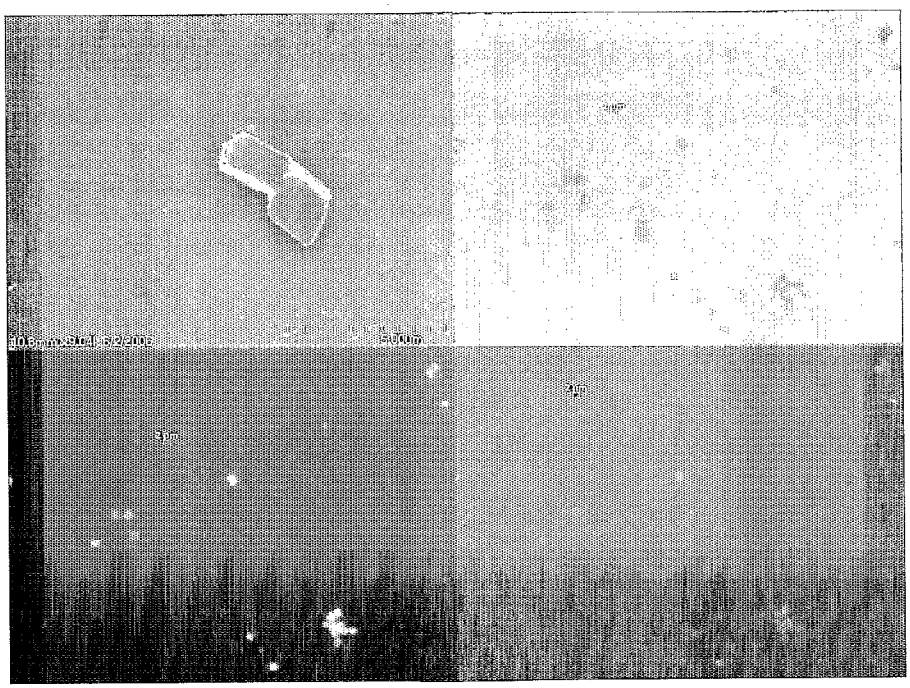


FIG. 65

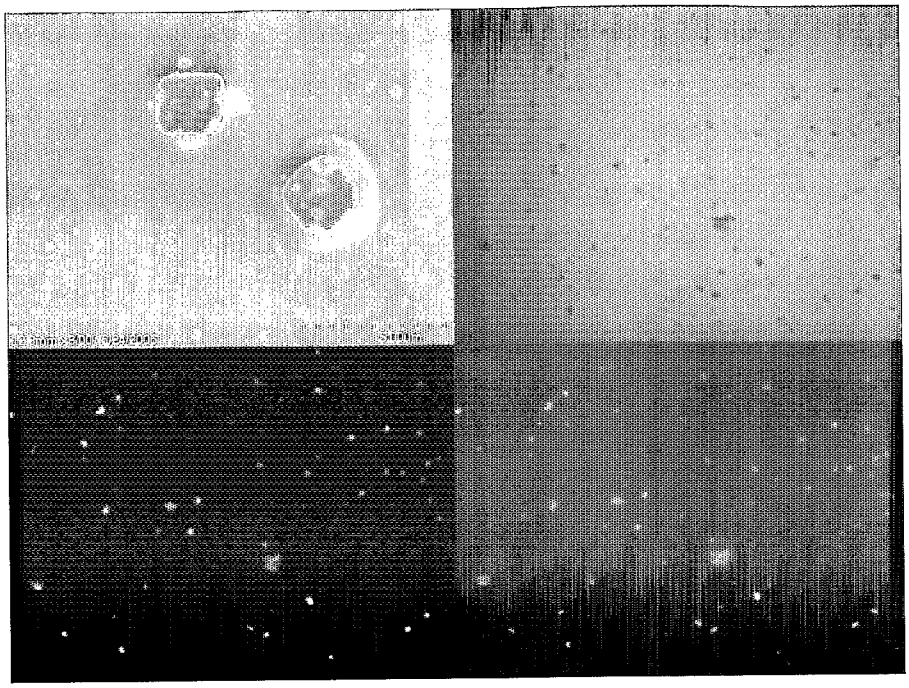


FIG. 66

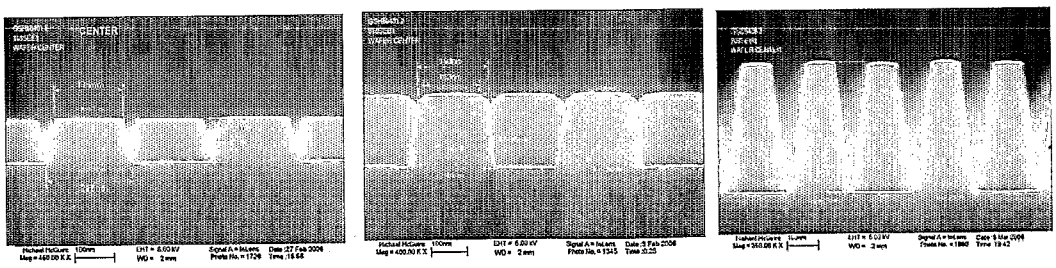


FIG. 67

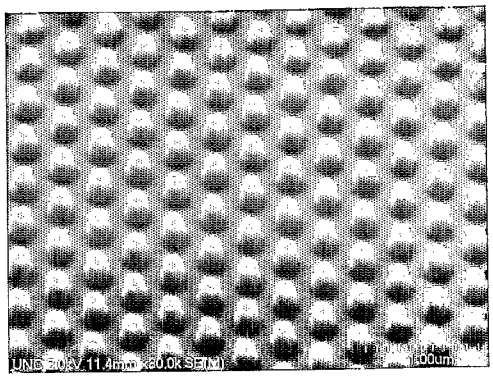


FIG. 68

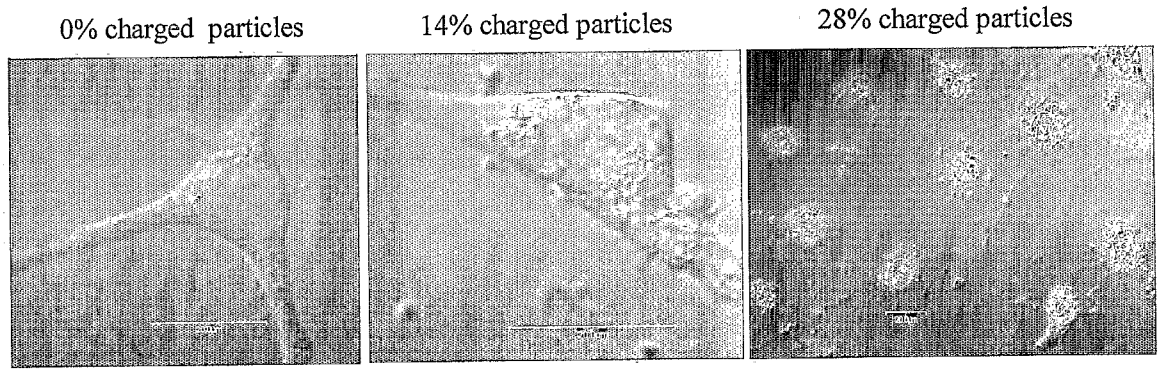

0% charged particles
14% charged particles
28% charged particles

FIG. 69

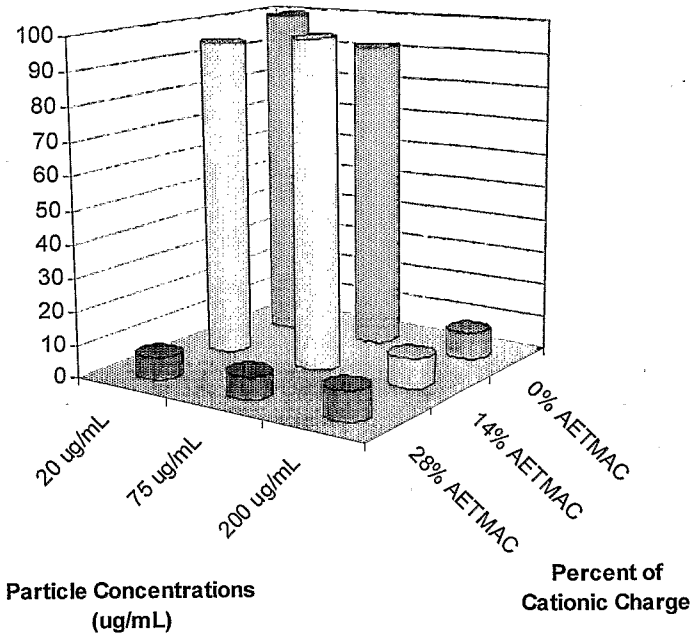

100
90
80
70
60
50
40
30
20
10
0
20 ug/mL
75 ug/mL
200 ug/mL
0% AETMAC
14% AETMAC
28% AETMAC
Particle Concentrations (ug/mL)
Percent of Cationic Charge

FIG. 70

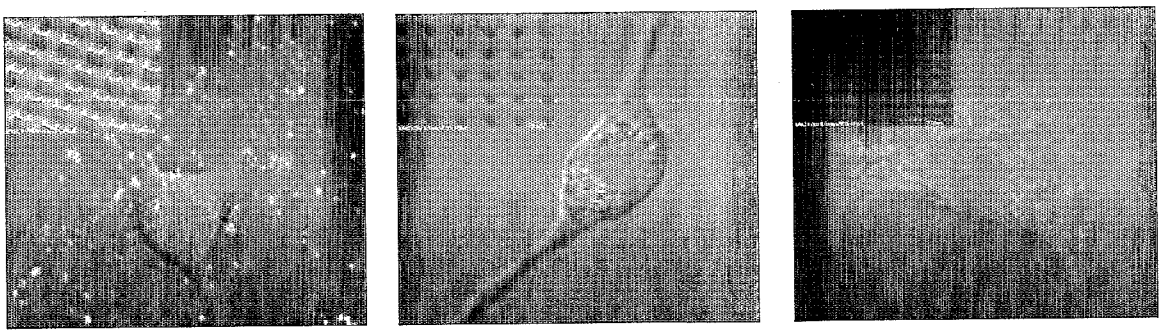


FIG. 71

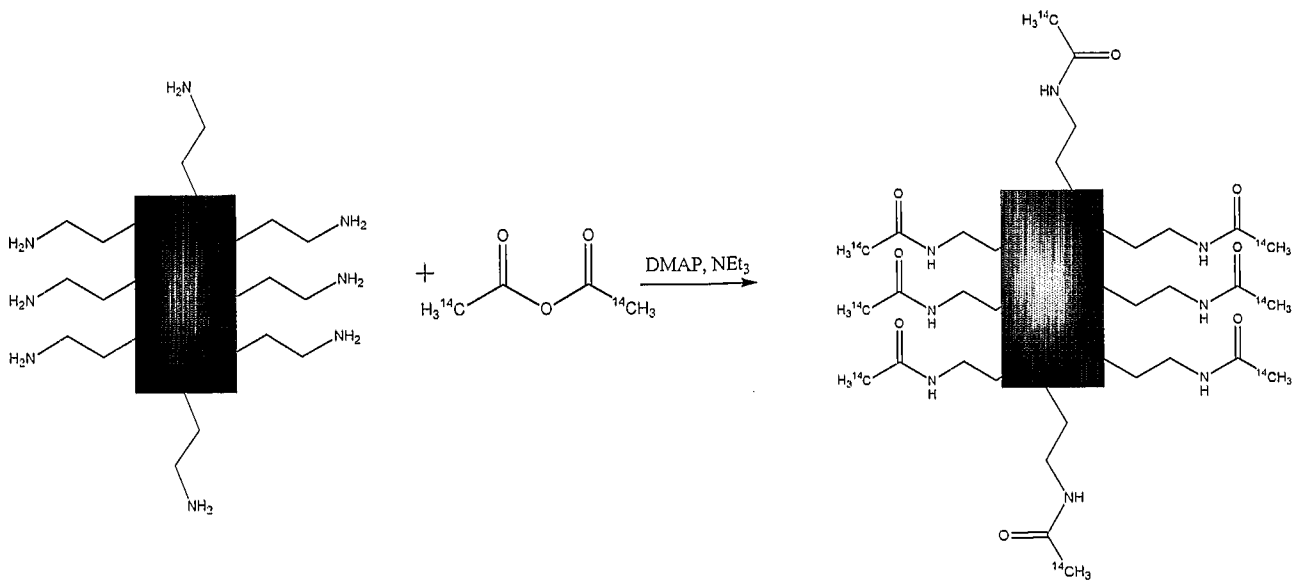

DMAP, NEt3

FIG. 72

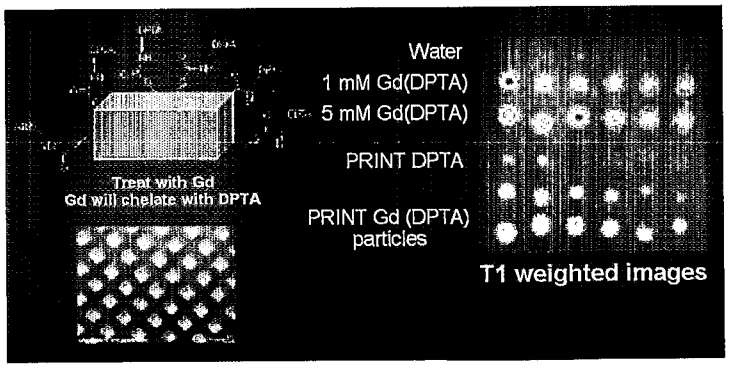

Water
1 mM Gd(DPTA)
5 mM Gd(DPTA)
PRINT DPTA
PRINT Gd (DPTA) particles
Treat with Gd
Gd will chelate with DPTA
T1 weighted images

FIG. 73

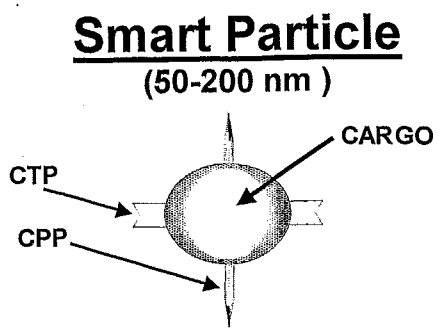

Smart Particle
(50-200 nm)
CARGO
CTP
CPP

FIG. 74

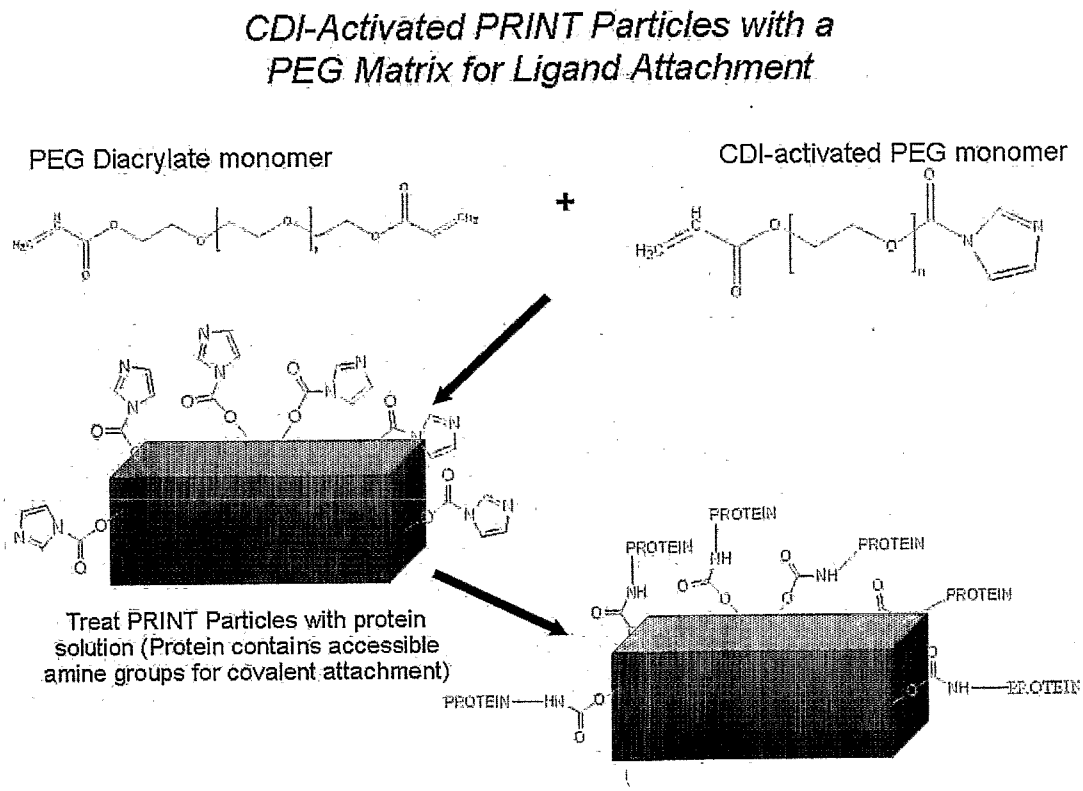
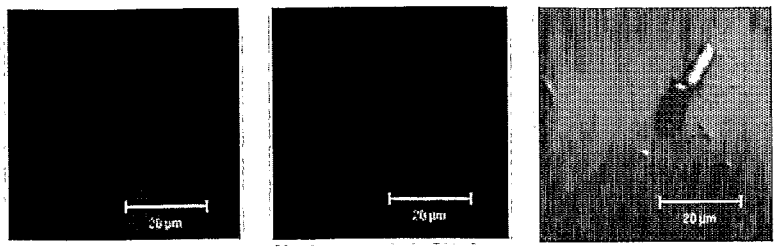

CDI-Activated PRINT Particles with a PEG Matrix for Ligand Attachment
PEG Diacrylate monomer
CDI-activated PEG monomer
+
Treat PRINT Particles with protein solution (Protein contains accessible amine groups for covalent attachment)
PROTEIN
PROTEIN
PROTEIN
PROTEIN
PROTEIN—HN
NH—PROTEIN

FIG. 75



CY-3 labeled Avidin attached to the periphery of PRINT particles

FITC-biotin FAB attached to PRINT particle through avidin

DIC Overlay

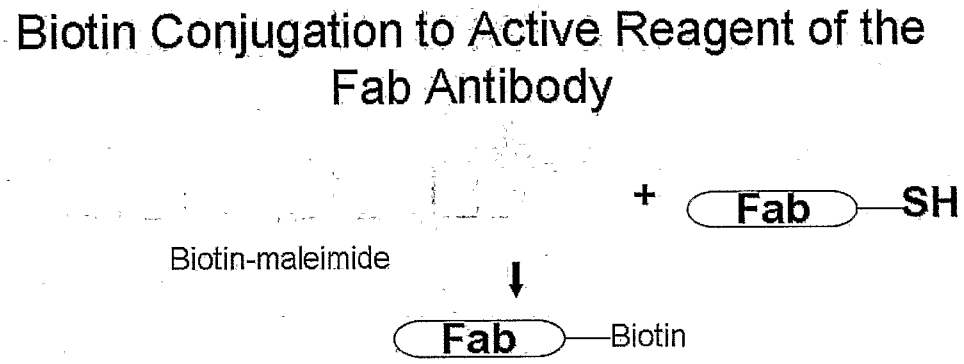


FIG. 76

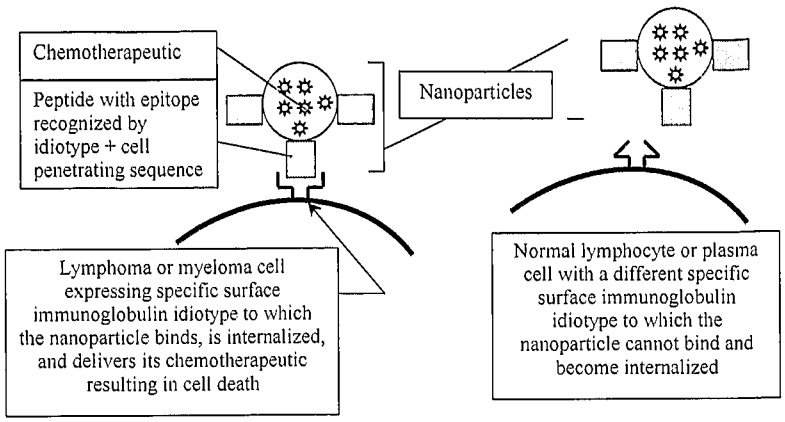

Chemotherapeutic
Peptide with epitope recognized by idiotype + cell penetrating sequence
Nanoparticles
Lymphoma or myeloma cell expressing specific surface immunoglobulin idiotype to which the nanoparticle binds, is internalized, and delivers its chemotherapeutic resulting in cell death
Normal lymphocyte or plasma cell with a different specific surface immunoglobulin idiotype to which the nanoparticle cannot bind and become internalized

FIG. 77

Controlled-Release Phantom Study

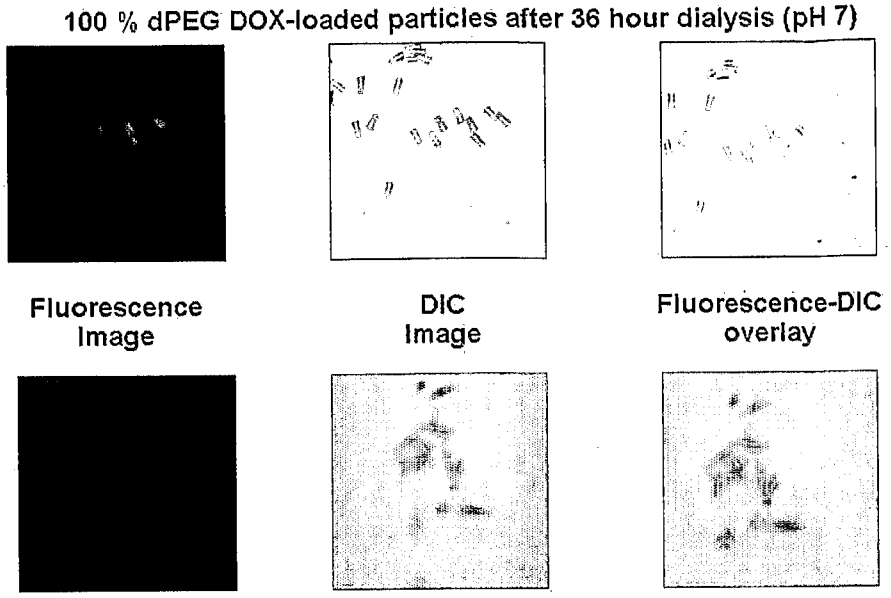


FIG. 78

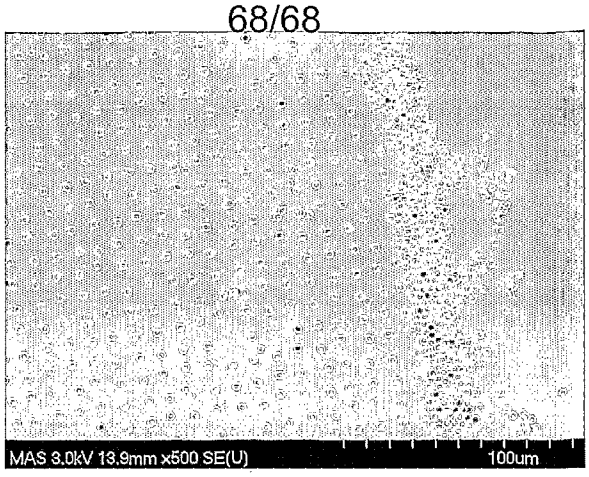


FIG. 79A

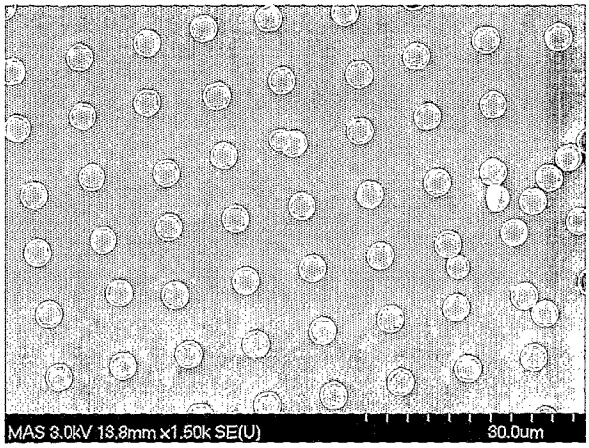


FIG. 79B

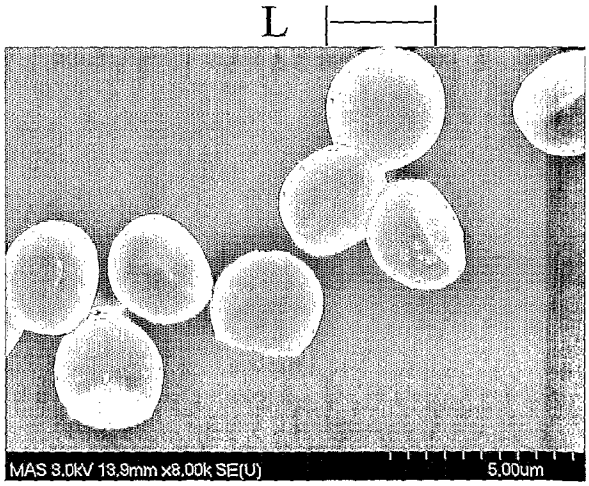


FIG. 79C