(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 July 2017 (06.07.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/114398 A1

(51) International Patent Classification:
G01N 33/68 (2006.01) *A61B 5/00* (2006.01)

(21) International Application Number:
PCT/CN2016/112475

(22) International Filing Date:
27 December 2016 (27.12.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/271,256 27 December 2015 (27.12.2015) US

(71) Applicant: **TSINGHUA-BERKELEY SHENZHEN INSTITUTE** [CN/CN]; Nanshan Intelligent Park, No.1001 Xueyuan Avenue, Nanshan District, Shenzhen, Guangdong 518055 (CN).

(72) Inventors: **MARRIOTT, Myfanwy Emma**; 3772 Redwood Circle, Palo Alto, California 94306 (US). **MARRIOTT, Gerard Joseph David**; Nanshan Intelligent Park, No.1001 Xueyuan Avenue, Nanshan District, Shenzhen, Guangdong 518055 (CN).

(74) Agent: **BEYOND ATTORNEYS AT LAW**; F6, Xijin Centre, 39 Lianhuachi East Rd., Haidian District, Beijing 100036 (CN).

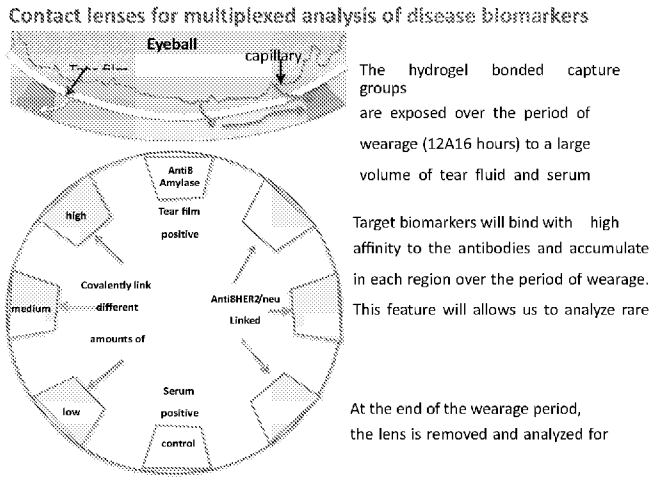
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) **Title**: COMPOSITIONS OF DIAGNOSTIC CONTACT LENSES AND USES THEREOF



(57) **Abstract**: A diagnostic composition that include a diagnostically effective capture group that is chemically bonded to defined regions of a contact lens, where each diagnostically effective capture group on the contact lens binds tightly and in long-lived complexes to specific and rare biomarkers associated with a diseased state during a period when the contact lens is on the eyeball of the subject. Also provided are methods and kits useful for detecting the bound biomarker in the contact lens by using an enzyme coupled antibody that also binds to the biomarker on the contact lens and turns over a chromogenic or fluorogenic substrate whose insoluble product is recorded at the site of biomarker capture on the contact lens using a cell phone camera or related imaging device and calibrated and quantified using an associated software application to determine the level of the disease biomarker in the patient.

Figure 3

WO 2017/114398 A1

COMPOSITIONS OF DIAGNOSTIC CONTACT LENSES AND USES THEREOF

BACKGROUND

Human diseases including cancer, diabetes, Alzheimer's, cardiac failure, HIV and viral associated conditions are usually accompanied with increases in the serum levels of disease-associated proteins, metabolites and oligonucleotides. Early detection of these biomarkers can drive early stage treatment, which can help to improve the outcome of a treatment. Early detection of cancer in part through the application of biomarker-detection strategies has resulted in a dramatic increase in cancer survival rate over the past 30 years. Early stage detection of cancer requires one to quantify very low levels of a specific disease biomarker – biomarker-specific probes including antibody conjugates of luminescence, colorimetric or fluorescence probes are commonly used to report on the amount of biomarker in a serum or biopsy sample. For example, HER-2/neu, a protein released from breast cancer cells is an FDA-approved biomarker for early detection of breast cancer - HER-2/neu is present in the blood of breast cancer patients at a fairly high concentration of 30 ng/mL, and is usually detected using a specific antibody conjugated with a fluorescent probes or radioactive metal ion chelate or else indirectly using ELISA. A typical HER-2/neu assay use ~1 ml of serum containing 30 ng of biomarker, which is readily detected by primary antibody conjugates and fluorescence detection, or colorimetrically, using an ELISA format. There can be no doubt that HER-2/neu and other cancer biomarkers also exist in tear-film, sweat and saliva although they are present at much lower quantities compared to serum. Moreover, biomarker levels in these fluids show much larger variation compared to serum. In principle, this problem can be overcome by collecting a larger sample volume or by conducting multiple measurements of smaller volumes to average out variations in the levels of biomarkers. Clearly, this is an impractical solution to analyze biomarkers in tear-films and sweat samples. Consequently, the FDA has not approved any protein biomarkers for cancer diagnosis that are present in tear-drops, sweat or saliva. The same problem is faced in detecting -amyloid derived biomarkers associated with Alzheimer's from non-serum fluids. Thus, although many biomarker detection systems have sufficient sensitivity to detect ultra-low levels of a protein biomarker, their application to cancer biomarker detection in non-serum samples is limited owing to large variations in the amount of biomarker within individual samples taken from the same patient, eg individual tear drops or sweat droplets may vary in their biomarker content at different times of the day time of day, depending on the method of collection and as a consequence of an unrelated health condition in the patient. Moreover, since POC devices are designed to analyze very small volumes of serum or tear-drops (~ L), it becomes difficult to analyze biomarkers that are present at a very low concentration.

BRIEF SUMMARY OF THE INVENTION

This invention introduces a novel approach to overcome the serious limitations posed by time-dependent variations in the sampling of non-serum samples *ie* the need to measure either large volumes of tear drops, or to measure a target biomarker frequently in multiple tear drops over an extended period of time. The new approach is based on a sensor platform where rare biomarker molecules are trapped by an antibody on a contact lens where they accumulate over a period of 12-16 hours. The entrapment of soluble biomarkers present in tear films is realized by coupling antibodies to the polymer backbone of a hydrophilic contact lens that bind with high affinity to the biomarker. The accumulated biomarker is detected and quantified after removing the lens using sensitive and rapid immuno-based image detection systems. The immuno-detection generated readout of the target biomarker is recorded at different regions of the diagnostic contact lens using a cell phone camera or dedicated imaging device, and analyzed using a software application that quantifies the amount of known amounts of specific biomarkers. Furthermore, a single contact lens can be fabricated to analyze a panel of reference and disease-associated biomarkers to improve the diagnosis. The concentration of the targeted biomarker can be determined on the same contact lens using a novel calibration system. By sampling a large volume of tear film over a long period of wearage, the lens allows one to accumulate ay number of rare biomarkers in the patient that would not necessarily be detected by sampling blood or single tear-droplets. The invention thereby affords a less costly means of detecting disease-associated biomarkers in a patient, reduces the effect of time-dependent concentration-fluctuations in biomarkers in tear films, and quantitative analysis of reference and/or multiple biomarkers on the same lens.

Aspects of the present disclosure includes multiple regions on the skirt of the contact lens, each of which is chemically bonded to a specific capture group that forms a tight, long-lived complex with a biomarker whose level correlates with the presence of a diseased state. The capture groups may take form of an antibody directed against such a biomarker, or a DNA or RNA oligonucleotide whose sequence is complementary to serum localized, disease-associated DNA or RNA. Other regions on the rim of the contact lens are covalently linked with capture groups that serve as positive or negative controls to evaluate specific and non-specific binding of proteins to the contact lens. Furthermore, the contact lens integrates a means to quantify the amount of bound biomarker to the capture groups; in particular, by including controls for each capture group biomarker pair in which the capture group covalently binds exact amounts of the biomarker. These controls are used to calibrate the color generated by the detection of the

patient's biomarker to its actual concentration. In addition, we provide are methods and kits to quantify the amount of captured biomarker(s) on the contact lens after a given period of usage by the subject: the ELISA system may be integrated with an enzyme conjugated second antibody against the captured biomarker that uses a specific chromogenic substrate for the enzyme to generate a colored insoluble product in the hydrogel. This product may then be recorded by a cell phone camera (or other such device) and analyzed using a software application.

In some embodiments, the capture group is composed of an antibody, engineered protein, DNA or RNA that binds to a target biomarker.

In some embodiments, the same capture group is bonded to different regions on the contact lens to improve the accuracy of the detection.

In some embodiments, capture groups that bind to a panel of biomarkers are chemically-bonded to different regions of the contact lens.

In some embodiments, different amounts of the authentic biomarker are chemically bonded to different regions on the contact lens where they are used to calibrate the color developed in the contact lens from the ELISA reaction with the actual concentration of the biomarker in the hydrogel.

In some embodiments, the bonding between the capture group and the target biomarker is detected during actual usage of the contact lens.

Aspects of the present disclosure include a method of preparing multiple regions within a contact lens that harbor specific functional groups that are used to covalently link the capture group. The method involves the chemical bonding of antibodies against tumor marker and control and calibration proteins to functional groups at specific regions of the contact lens. The site specific coupling is brought about in one instance by chemical coupling of maleimide conjugated antibodies to free thiol groups in these regions that are generated by the reaction of a slight excess of 4-ARM thiol PEG with 4-ARM maleimido-PEG. Alternatively, the antibodies can be bonded to specific sites on the contact lens by molecular imprinting, by addition methacrylate conjugates of the antibody to a polymerizing mixture of HEPA or other standard methods for spatial defined coupling of proteins to surfaces.

In some embodiments, regions of the contact lens may include functional groups that include but are not limited to amino, carboxyl, azide, alkyne, acrylate, hydroxyl, aldehyde or epoxide.

In some embodiments, the reactive group on the capture groups includes a NHS-ester, azide, alkyne, epoxide, bromoacetyl or acrylate.

Aspects of the present disclosure include a method of creating multiple regions of the contact lens, each with a specific capture group. The method includes the chemical bonding of specific capture groups to functional groups at defined regions on the contact lens where the composition of the capture group includes a reactive group that is used to form a covalent bond with the functional group within specific regions of the contact lens.

Aspects of the present disclosure include a contact lens that allows for sensitive detection of multiple bound biomarkers.

In some embodiments, the binding of a target biomarker to the immobilized capture antibody on the contact lens is detected by visual means using a detection antibody that binds to a different site on the biomarker and that is chemically coupled with a bright chromophore, fluorophore or metallic nanoparticle immuno-detection.

In some embodiments, the detection of biomarker in the contact lens is based on a colorimetric, chemi- or bioluminescence or fluorescence signal that is generated by the action of an enzyme-coupled antibody on a substrate to produce an insoluble colored, bioluminescent or fluorescent product at the biomarker capture site

In some embodiments, the detection of the bound biomarker at sites on the contact lens is based on a change in the refractive index, SPR, fluorescence emission or light scattering associated with the binding of a detection antibody that is coupled to a metallic nanoparticle probe.

In some embodiments, the detection of the bound biomarker at sites on the contact lens over the period of wearage is based on a mass spectrophotometric measurement.

In some embodiments, the contact lens is coupled at specific sites with a ligand that can bind to any protein that accumulate on the contact lens during the period of wearage to generate a contact

lens proteome that is analyzed after given time periods by mass spectrophotometry.

In some embodiments, the detection of colorimetric indicator generated by the ELISA assay is recorded on a cell phone or other image capture device and analyzed by a software comparing the transmission of light through the regions of the contact lens that contain known amounts of chemically bonded pure biomarker with those of the test regions.

Aspects of the present disclosure include a kit that includes a diagnostic contact lens composition and a detection chamber configured to generate a photometric signal to quantify the amounts of specific biomarkers that accumulate in each region of the lens during the usage period. The diagnostic composition includes: 1) A contact lens vehicle where diagnostically effective capture groups are bonded to specific regions on the contact lens skirt and are used to accumulate target biomarkers during the usage period; 2) An immuno-detection system for visual detection of the target biomarker on the lens comprising either a detection antibody directed against the biomarker or to a second antibody bound to the biomarker on the lens that is chemically coupled with either a bright colored probe or fluorescent probe or a gold or silver nanoparticle; 3) an ELISA-based detection system where the detection antibody is chemically coupled with an enzyme that generates an insoluble choromophore or fluorophore from a specific substrate that is deposited at the site of biomarker capture; 4) the spectroscopic signals associated with the detection antibody and positive control and reference biomarkers are recorded from one or more lens using a cell phone camera or a dedicated image capture device and analyzed to provide quantitative measures of specific serum biomarkers.

In some embodiments, the ELISA employs an antibody directed against the biomarker that is chemically coupled to a phosphatase, glycosidase, luciferase, catalase, peroxidase or protease that catalyzes the formation of an insoluble colored product or fluorescent product at the site of the biomarker on the lens.

Before the present invention is further described, it is to be understood that this invention is not limited to the particular embodiments. It is also to be understood that the terminology used in describing particular embodiments is not intended to be limiting, as the scope of the invention will only be limited by the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the patterning of a contact lens with hydrogel regions that harbor functional groups (amino, carboxy, thiol, azide or alkyne) that are used for the coupling of specific capture groups within these regions

Figure 2 illustrates the patterning of a contact lens with specific capture groups within defined hydrogel regions, where a reactive form of the capture group (maleimide, NHS-ester, epoxide) is used to form a covalent bond with the complementary functional group on the hydrogel polymer.

Figure 3 illustrates a diagnostic contact lens to show regions of the lens that harbor specific capture groups each of which can bind tightly and in long lived complex to a target biomarker that is present in the tear film or from leakage from capillaries in the eyelid or eyeball.

Figure 4 illustrates the process for detecting and quantifying specific biomarkers on a single contact lens, showing that after a period of wearage the lens is first washed and then incubated with an antibody directed against a different epitope on the biomarker, that is chemically coupled to either a bright chromophore, fluorophore or metallic nanoparticle that allows for visual detection of the biomarker on the lens, or else the detection antibody is chemically coupled with an enzyme such as a phosphatase that on treatment with a solution of a chromogenic or fluorogenic substrate generates an insoluble colored or fluorescent product at the site of the captured biomarker on the lens that is recorded on a cell phone camera or a modified imaging device and the image analyzed using a software application that quantifies the transmission or fluorescence signal from the lens and using a calibration function calculates the concentration of the biomarker(s) and compares the value to that associated with a diseased state.

Figure 5 shows a cell phone image showing site specific covalent linking of an MBS-conjugated antibody (Cy3-Goat anti rabbit IgG) to a specific region of a hydrogel composed of 4-ARM Thiol-PEG.

DETAILED DESCRIPTION AND BEST MODE OF IMPLEMENTATION

Aspects of the present disclosure include a contact lens composition that includes a contact lens, which acts as a vehicle in providing defined regions on its rim (skirt) that harbor a functional group that serves to covalently link a capture group that binds tightly to a specific biomarker

present in the tear fluid. Also provided are methods and kits useful for sensitive and specific detection of bound biomarkers in each region of the contact lens during or after a period of wearage by the subject. Also provided are methods and software applications that allow the user to record using a cell phone, and to quantify the amount of a biomarker that accumulates in each region of the contact lens and to relate that level to those associated with the diseased state including cancer, stroke, Alzheimer's, diabetes.

The contact lens vehicle harbors a number of defined sites each of which is chemically bound to an antibody, protein or non-protein capture group whose function is to bind tightly in long lived complexes to their target biomarker that are present in the tear fluid during an extended period of usage and to quantify the amount of each biomarker using a sensitive immune-detection approach including the ELISA method that generates a colorimetric, fluorescence, SPR or bioluminescence signal at the site of the captured biomarker that is recorded and quantified by the user using a cell phone camera or related imaging device.

We have addressed this challenge through the design of a hydrogel based contact lens that is patterned along its skirt with regions that harbor functional groups used to link covalently a specific capture group, each of which binds specifically and in a long lived complex to a target biomarker. Each region of the contact lens will bind to specific biomarkers that are present at a low concentration in the tear film via their immobilized capture groups allowing for an accumulation of the biomarker over a defined period of usage. The contact lens also harbors sites that function as a positive and negative control and to calibrate the color generated in test regions of the contact lens with the concentration of the target biomarker. The contact lens once removed from the subject is placed in a container that performs wash steps, incubation with a second capture group that bears either a detection probe, comprising chemically-coupled chromophores or fluorophores or metallic nanoparticles including gold and silver, or else coupled to a covalently bound enzyme followed by a subsequent wash step and incubation with a chromogenic or fluorogenic substrate for the enzyme that generates a colored, insoluble fluorescent or non-fluorescent product that precipitates at the site of enzyme action and is detected from either its absorption of light at a specific wavelength or from fluorescence emission using a cell phone camera or a purpose built detection device.

Aspects of the present disclosure include the design, synthesis and quantification of contact lens harboring capture groups at specific sites on the skirt of the lens with demonstrations of proof of

principle for the covalent linkage of capture groups at defined sites on the lens and their application in detecting a validated cancer biomarker. The approach can be applied to a range of diagnostic tests for biomarkers associated with human disease, such as but not limited to cancer, Alzheimer's, diabetes, stroke, and inflammation.

Aspects of the present disclosure include the design, synthesis and quantification of diagnostic contact lens, with an example for the detection of a specific biomarker. The approach can be applied to a range of biomarkers that are currently used to diagnose human diseases, such as but not limited to cancer, diabetes stroke and inflammation. We also show that the diagnostic contact lens can be used for quantitative, multiplexed analysis of disease biomarkers. For example, capture groups for multiple biomarkers associated with a diseased state can be integrated within a single lens that also includes regions that serve to calibrate the signal generated from the immuno-detection system including ELISA with the actual amount of each biomarker. We have demonstrated that biomarkers are bound tightly and in long lived complexes to capture groups in each region of the contact lens and that they react further with a second capture group that harbors an enzyme that acts on a soluble fluorogenic substrate to form an insoluble product at the site of catalysis that is recorded by either the change in absorption of specific wavelengths of light at the site of precipitation, or else by recording the fluorescence emission of the precipitate colorimetric signal by turning over a that is recorded and quantified using a cell phone camera and associated software. Under this condition the sensitivity of the contact lens for a target biomarker is on the order of 1 ng/ml. The contact lens can be adapted for multiple types of capture group including antibodies, DNA and RNA oligonucleotides and capture groups that integrate an enzyme whose activity is activated upon binding to the target biomarker.

Diagnostic compositions. Aspects of the present disclosure include an example of a diagnostic composition that includes a mock contact lens that harbors diagnostically effective agents, where the diagnostically effective agent is chemically bonded to defined sites on the contact lens that, over an extended period of usage (1-16 hours), bind tightly to low concentrations of target biomarkers in the tear film. Also provided are methods and kits useful for the detection and quantification of bound biomarkers on the contact lens.

In certain embodiments, the disclosed diagnostic compositions are useful for the detection or monitoring of a disease or disorder, such as cancer, diabetes, stroke or Alzheimer's. For example, the present disclosure provides diagnostic compositions that include a diagnostically effective

agent and a vehicle (e.g., a contact lens patterned with multiple capture groups for specific biomarkers) of the present disclosure. "Diagnostically effective amount" refer to an amount of a biomarker linked to a diseased state that is detected on the contact lens.

"Patient" refers to human and non-human subjects. The term "diagnosis" as used herein means the quantification of a validated biomarker or biomarkers of a disease or medical condition in a patient that includes: (a) detecting a diseased condition or monitoring the progression of that condition; (b) monitoring the progress of a treatment for the disease or medical condition in a patient.

In certain embodiments, the disclosed diagnostic compositions are useful for the evaluation of a disease or disorder, such as cancer, diabetes, stroke and Alzheimer's. For example, the present disclosure provides diagnostic compositions that include a contact lens capable of detecting validated biomarkers of the described diseased states.

Methods of diagnosis. The subject diagnostic compositions are useful in the detection or evaluation of a therapy of a disease or disorder, such as cancer, diabetes, stroke or Alzheimer's disease, in a subject. Accordingly, the present disclosure provides methods of diagnosis biomarkers associated with the disease in a subject by administering the subject diagnostic contact lens composition. For example, the present disclosure provides a method of quantifying low levels of biomarkers associated with a disease in a subject.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

1. A diagnostic composition comprising: immobilized capture groups; and a contact lens vehicle, wherein the capture groups or the vehicle comprises an antibody or oligonucleotide sequence harboring a reactive group that is used to form a chemical bond to a region on the contact lens that harbors a complementary functional group.

2. The diagnostic composition of Claim 1, wherein the immobilized capture group comprises diagnostically effective agent.

3. The diagnostic composition of Claims 1-2, wherein the vehicle comprises a region of the contact lens harboring a functional group.

4. The diagnostic composition of Claims 1-3, wherein the capture group is an antibody, oligonucleotide, aptamer, or non-protein moiety that binds to a specific biomarker.

5. The diagnostic composition of Claims 1-4, wherein the vehicle comprises a functionalized hydrogel or polymer within a contact lens.

6. The diagnostic composition of Claims 1-5, wherein the capture group comprising an antibody or aptamer or other biomolecule that recognizes the target biomarker is cross-linked to a specific region on the contact lens.

7. The diagnostic composition of Claims 1-6, wherein the binding of a biomarker to the capture antibody forms a tight and long-lived complex.

8. The diagnostic composition of Claim 1-7, wherein the biomarker bound to the capture antibody is treated with a second antibody directed against a different site on the biomarker that is chemically-coupled to an enzyme that generates a spectroscopic signal in the presence of a chromogenic substrate or other spectroscopic probe.

9. The diagnostic composition of Claim 1-8, wherein the biomarker bound to the capture antibody is treated with a second antibody that is directed against a different site on the biomarker and is chemically-coupled to a bright chromophore or fluorescent probe, or to a gold or silver nanoparticle that generates a spectroscopic signal at the biomarker site.

10. An ELISA-based diagnostic composition of Claim 1-9, wherein the biomarker bound to the capture antibody is treated in the device with an antibody directed against a different site on the biomarker and subsequently washed and treated with a second antibody directed against the first antibody and is chemically-coupled to an enzyme that generates a spectroscopic signal in the presence of a chromogenic substrate or other spectroscopic probe.

11. An immuno-label based diagnostic composition of Claim 1-7, wherein the biomarker bound to the capture antibody is treated in the device with a second antibody directed against a different site on the biomarker that after subsequent washing is treated with a detection antibody that is chemically-coupled to a gold or silver nanoparticle that generates a spectroscopic signal to indicate

the site and quantity of a specific biomarker.

12. The diagnostic composition of Claim 1-8 and 10, wherein enzyme linked to the second antibody is a peroxidase, catalase, phosphatase, glycosidase, protease, or other hydrolytic enzyme.

13. The diagnostic composition of Claim 1-8 and 10, wherein disease biomarkers that accumulate at specific sites on the diagnostic contact lens are identified by use of mass spectrometry.

14. The diagnostic composition of Claim 1-8 and 10, wherein all proteins within the tear film during a period of wearage are able to accumulate on the diagnostic contact lens by way of a protein capture group and are identified by use of mass spectrometry.

15. A compound of claims 1-8 and 10 comprising a substrate, an example of which is the ELF-87^{TM} substrate, that is acted upon by the enzyme attached to the detection antibody, which is a phosphatase in the case of the ELF-87 substrate, to generate an insoluble colored and fluorescent product at the site of the captured biomarker in the contact lens.

16. The compound of Claim 9 and 11, wherein the antibody that binds to the biomarker bound to specific sites on the device is detected by a biomarker-induced change in absorption, scattering or surface plasmon resonance of a metallic nanoparticle.

17. A method of administering a diagnostically effective agent to a subject, the method comprising: administering a diagnostic composition to a subject, the therapeutic composition comprising of a contact lens harboring chemically bonded capture groups at defined functionalized regions on the rim of the contact lens, wherein the capture group comprises an antibody, oligonucleotide or non-protein group that binds tightly and in a long-lived complex to a specific biomarker and that allows for the further binding of a second antibody to the captured biomarker that is chemically-coupled to a bright chromophore, fluorescent probe gold or silver nanoparticle, or else coupled to an enzyme that generates an insoluble product that is detected using a cell phone camera and quantified using dedicated software.

18. The method of Claims 1-15, wherein the diagnostically effective agent comprises a capture group.

19. The method of Claims 1-16, wherein the vehicle comprises a region on the contact lens that reacts with a specific capture group.

20. The compound of Claims 1-17, wherein an insoluble colored or fluorescent product is generated at the site of the captured biomarker.

21. The method of Claims 1-18, wherein the enzyme coupled to the detection antibody generates a product whose color, fluorescence, or other spectroscopic signal can be detected using a dedicated detection system or a cell phone camera and quantified using dedicated software.

22. The methods of Claim 1-19, wherein the colored or fluorescent product generated from regions of the contact lens with known amount of the biomarker is used to calibrate the signal recorded from the product to a concentration or presence of the biomarker.

23. A kit comprising:

a diagnostic composition comprising: a capture group; and

a contact lens vehicle, wherein the capture groups not limited to antibodies directed against a specific biomarker are covalently linked to defined regions of the contact lens and form tight and long lived-complexes with specific biomarkers during the period of usage; and a detection antibody that binds to a site on the captured biomarker and is chemically-coupled to either a bright chromophore, fluorescent probe or metallic nanoparticle, or to an enzyme that catalyzes the turnover of a chromogenic or fluorogenic substrate to an insoluble product that collects at the site of the capture biomarker; and a dedicated detection system or cellphone camera and software application that records the intensity of colors or fluorescence generated in different regions of the diagnostic contact lens and returns a quantitative measure of the amount of a biomarker captured within the contact lens during the period of usage.

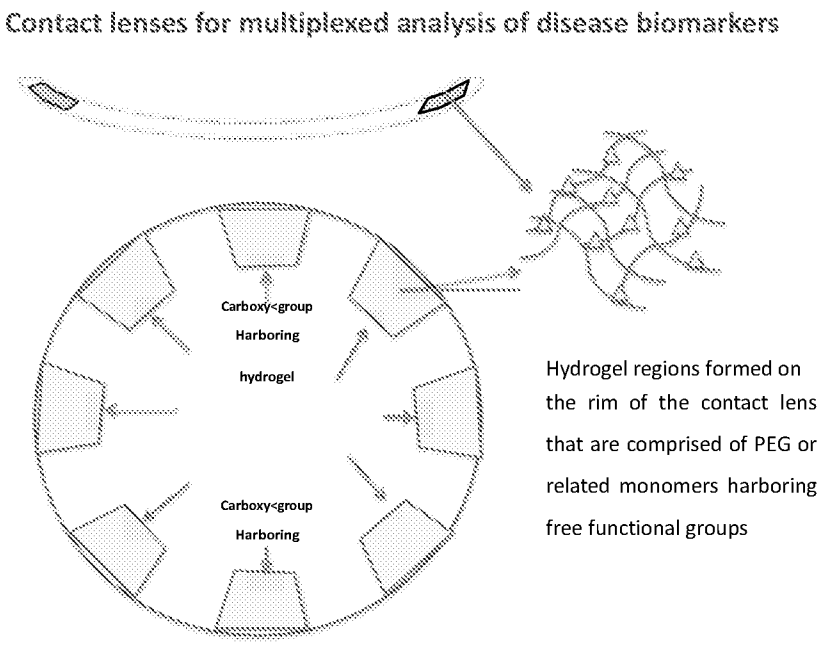

Contact lenses for multiplexed analysis of disease biomarkers
Carboxy<group
Harboring
hydrogel
Carboxy<group
Harboring
Hydrogel regions formed on the rim of the contact lens that are comprised of PEG or related monomers harboring free functional groups

Figure 1

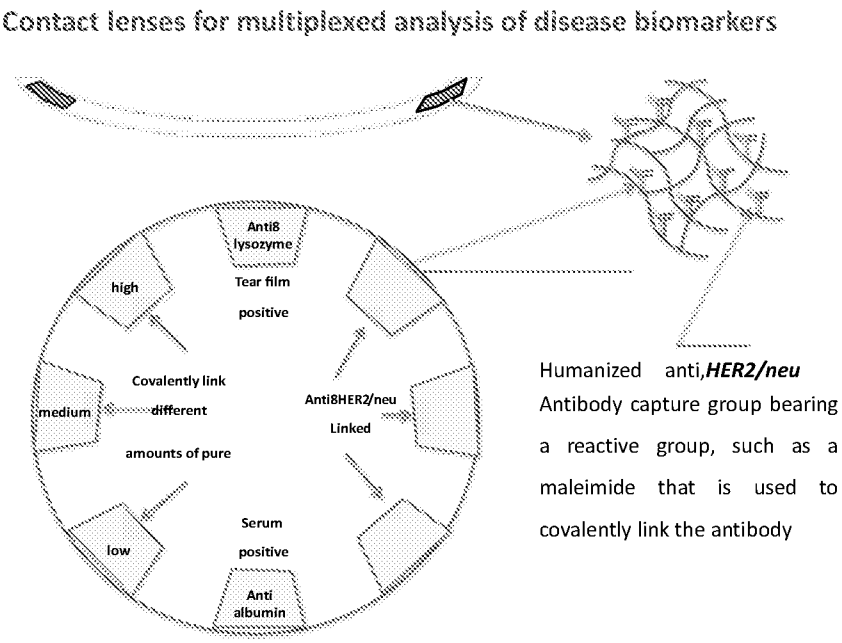

Contact lenses for multiplexed analysis of disease biomarkers
Anti8 lysozyme
Tear film
positive
high
Covalently link
different
medium
amounts of pure
Anti8HER2/neu
Linked
Serum
positive
low
Anti albumin
Humanized anti,HER2/neu
Antibody capture group bearing a reactive group, such as a maleimide that is used to covalently link the antibody

Figure 2

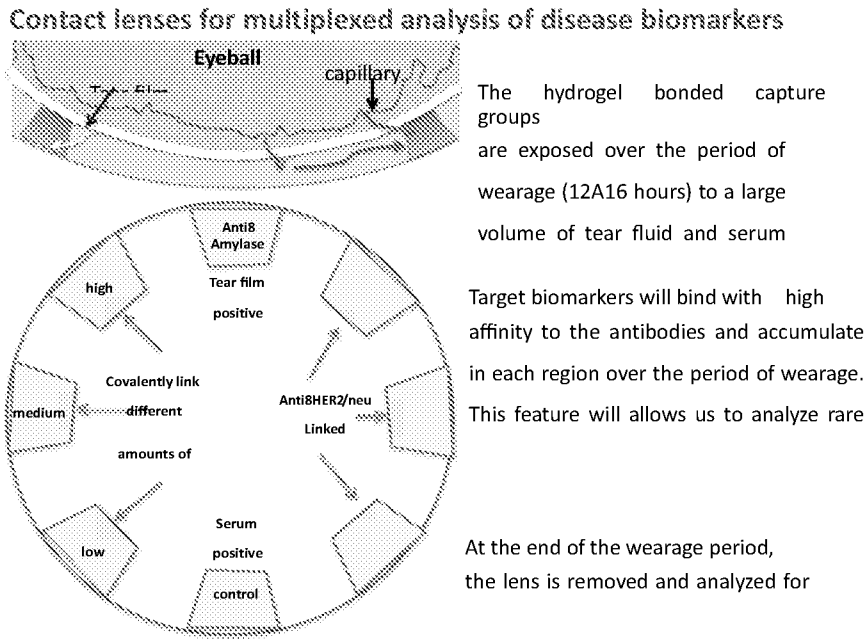

Contact lenses for multiplexed analysis of disease biomarkers
Eyeball
capillary
Anti8 Amylase
Tear film
positive
high
Covalently link
different
amounts of
medium
Anti8HER2/neu
Linked
Serum
positive
low
control
The hydrogel bonded capture groups
are exposed over the period of wearage (12A16 hours) to a large volume of tear fluid and serum
Target biomarkers will bind with high affinity to the antibodies and accumulate in each region over the period of wearage. This feature will allows us to analyze rare
At the end of the wearage period, the lens is removed and analyzed for

Figure 3

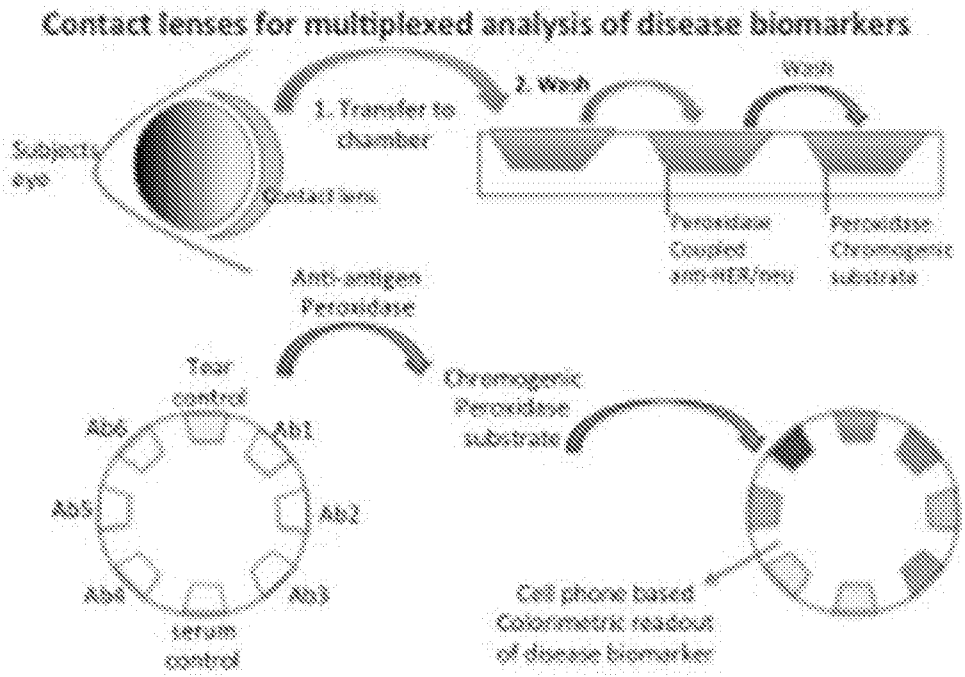

Contact lenses for multiplexed analysis of disease biomarkers
Subject's eye
1. Transfer to chamber
2. Wash
Wash
Peroxidase Coupled anti-HER2/neu
Peroxidase Chromogenic substrate
Anti-antigen Peroxidase
Tear control
Chromogenic Peroxidase substrate
Ab6
Ab1
Ab5
Ab2
Ab4
Ab3
serum control
Cell phone based Colorimetric readout of disease biomarker

Figure 4

Cell phone image showing the site specific covalent linking of an MBS-conjugated antibody (Cy3-Goat anti rabbit IgG) to a specific region of a hydrogel composed of 4-ARM Thiol-PEG.

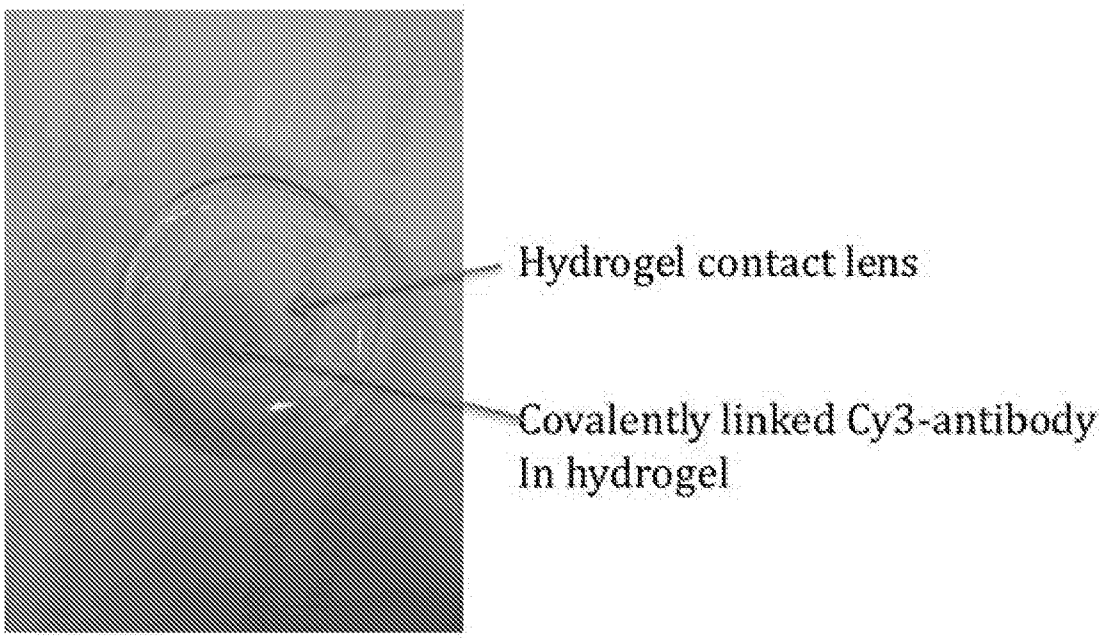


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/112475

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/68(2006.01)i; A61B 5/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N33; A61B5; A61B3; C12Q1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, CNTXT, CNKI, EPODOC, WPI, ISI, ELSEVIER, PUBMED: contact lens, ophthalmic lens, biomarker, hydrogel, antibody, oligonucleotide, aptamer, ELISA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	WO 2016029139 A1 (UNIV CENTRAL FLORIDA RES FOUND) 25 February 2016 (2016-02-25) Page 2 line 3 to page 6 line 8, examples 1-2, claims 1-32, figures 1-3	1-16, 18-23
X	US 2004181172 A1 (NOVARTIS AG ET AL.) 16 September 2004 (2004-09-16) Paragraphs 12-17, 21, 40, 42, 74-84, 90-98	1-7, 13-14, 18-19
Y	US 2004181172 A1 (NOVARTIS AG ET AL.) 16 September 2004 (2004-09-16) Paragraphs 12-17, 21, 40, 42, 74-84, 90-98	8-12, 15-16, 20-23
Y	US 2003198967 A1 (BECKMAN COULTER INC ET AL.) 23 October 2003 (2003-10-23) Paragraphs 6-9, 28, 31-32, 36, 43, examples 2-7, claims 1, 5, 8-9, 14-16, figure 3	8-12, 15-16, 20-23
A	WO 2014210526 A1 (GOOGLE INC) 31 December 2014 (2014-12-31) the whole document	1-16, 18-23
A	US 4014321 A (MARCH WAYNE F) 29 March 1977 (1977-03-29) the whole document	1-16, 18-23
A	US 3958560 A (MARCH WAYNE FRONT) 25 May 1976 (1976-05-25) the whole document	1-16, 18-23

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 March 2017	**24 March 2017**
Name and mailing address of the ISA/CN **STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **HU,Xiaojia** Telephone No. **(86-10)62085681**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/112475

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Wing Cheung Mak et al. "Surface-Engineered Contact Lens as an Advanced Theranostic Platform for Modulation and Detection of Viral Infection" *ACS Appl. Mater. Interfaces,* Vol. 7, 29 October 2015 (2015-10-29), Abstract, pages 25487-25488, 25492, figures 1, 6	1-14, 16, 18-19, 21
Y	Wing Cheung Mak et al. "Surface-Engineered Contact Lens as an Advanced Theranostic Platform for Modulation and Detection of Viral Infection" *ACS Appl. Mater. Interfaces,* Vol. 7, 29 October 2015 (2015-10-29), Abstract, pages 25487-25488, 25492, figures 1, 6	15, 20, 22-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/112475

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **17**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] According to PCT Rule 39.1(iv), the subject-matter of claim 17 refers to diagnostic method practiced on the human or animal body.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2016/112475

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2016029139	A1	25 February 2016	None			
US	2004181172	A1	16 September 2004	US	2008139963	A1	12 June 2008
				DE	602004022793	D1	08 October 2009
				AT	440537	T	15 September 2009
				EP	1617757	B1	26 August 2009
				WO	2004080297	A1	23 September 2004
				EP	1617757	A1	25 January 2006
US	2003198967	A1	23 October 2003	US	2005287590	A1	29 December 2005
				JP	2005524059	A	11 August 2005
				WO	03091446	A2	06 November 2003
				WO	03091446	A3	07 October 2004
				EP	1504258	A4	07 February 2007
				EP	1504258	A2	09 February 2005
				CN	1646908	A	27 July 2005
WO	2014210526	A1	31 December 2014	US	9028772	B2	12 May 2015
				US	2015004058	A1	01 January 2015
US	4014321	A	29 March 1977	None			
US	3958560	A	25 May 1976	GB	1521113	A	09 August 1978
				JP	S5175498	A	30 June 1976
				DE	2538985	A1	26 May 1976