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(54) **NON-THERMAL DISINFECTION OF BIOLOGICAL FLUIDS USING NON-THERMAL PLASMA**

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(21) Appl. No.: **11/345,582**

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Related U.S. Application Data

(63) Continuation of application No. 10/426,605, filed on Apr. 30, 2003, now Pat. No. 7,011,790, and which is a continuation-in-part of application No. 10/364,599, filed on Feb. 11, 2003, now Pat. No. 6,911,225, which is a continuation-in-part of application No. 09/850, 284, filed on May 7, 2001, now Pat. No. 6,562,386.

(60) Provisional application No. 60/377,130, filed on Apr. 30, 2002.

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(51) **Int. Cl.**
A61L 2/00 (2006.01)

(52) **U.S. Cl.** **422/22**

(57) **ABSTRACT**

A method and apparatus are provided for at least partially disinfecting biological fluid of a mammal, which comprises pathogens. The method includes contacting a non-thermal plasma with the biological fluid to kill at least a portion of the pathogens within the biological fluid, and filtering toxins from at least some components of the biological fluid.

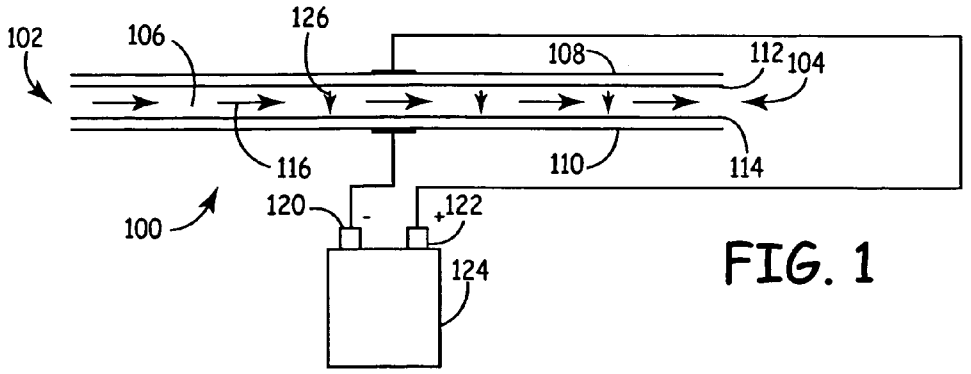


FIG. 1

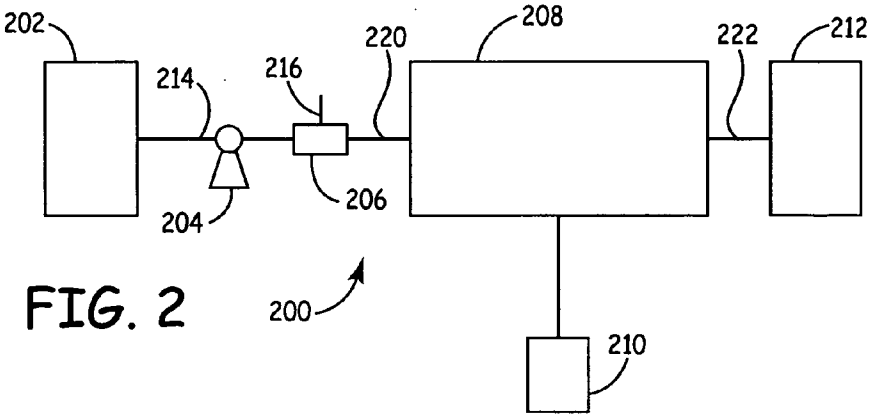


FIG. 2

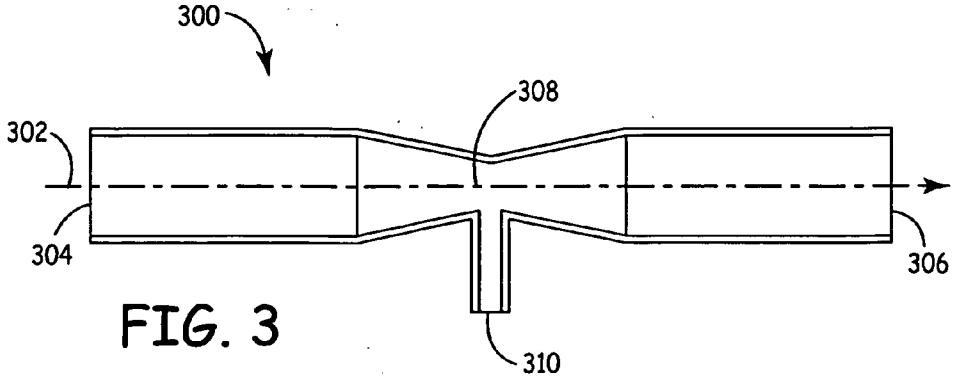


FIG. 3

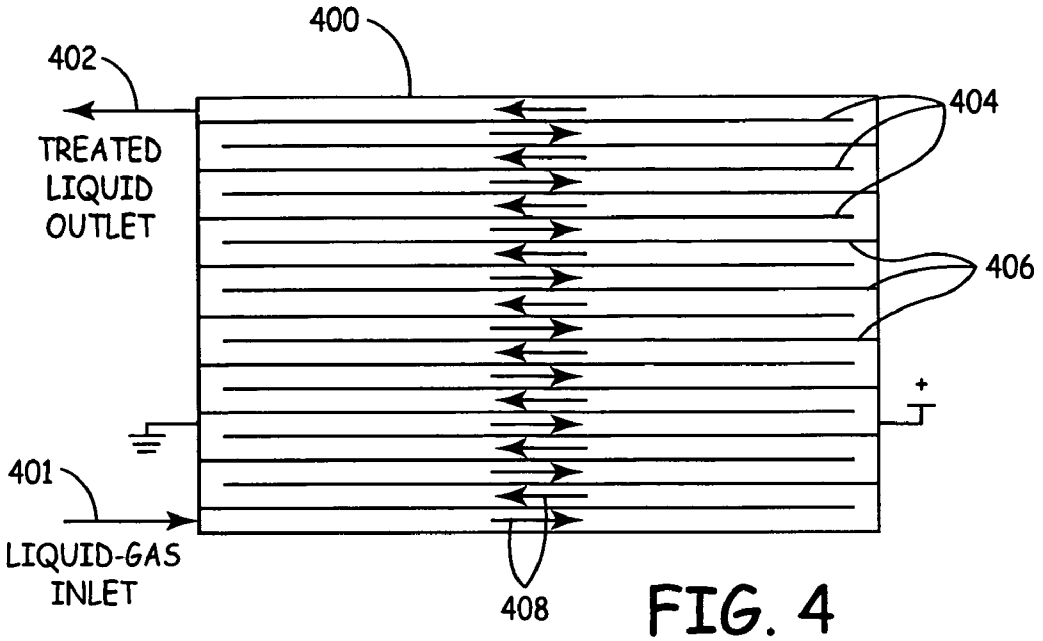


FIG. 4

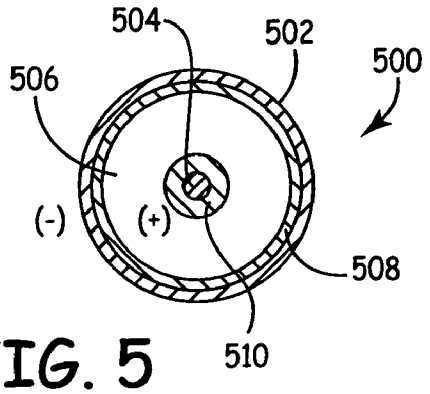


FIG. 5

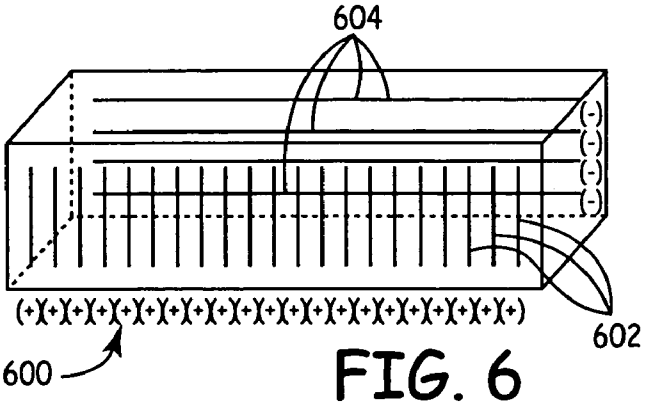


FIG. 6

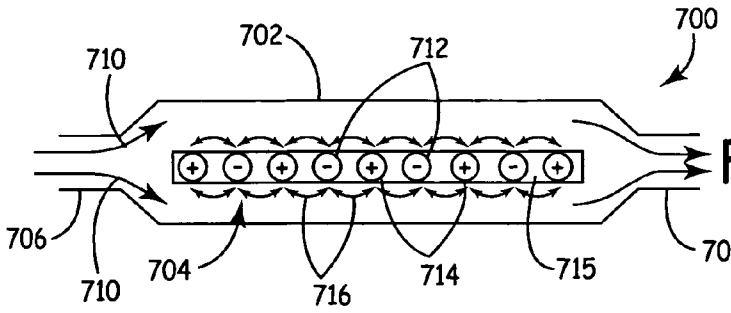


FIG. 7A

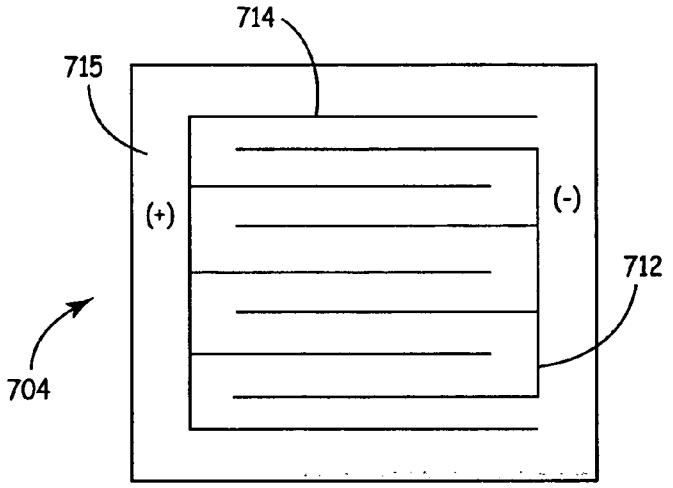


FIG. 7B

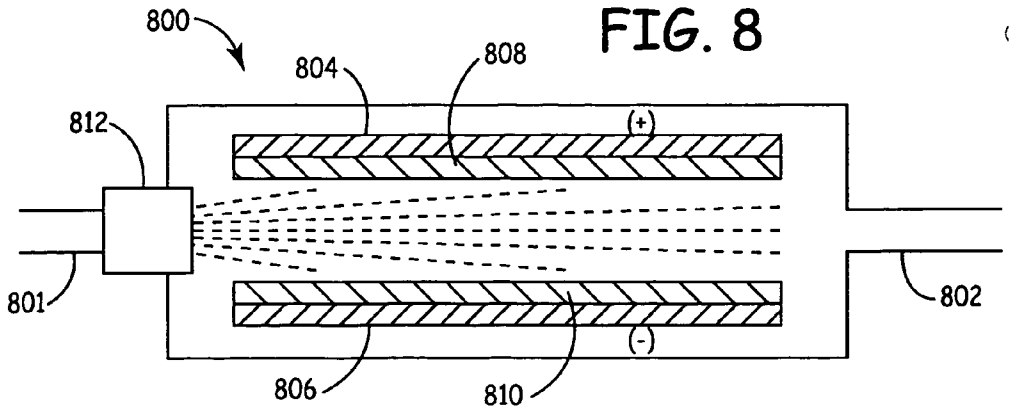


FIG. 8

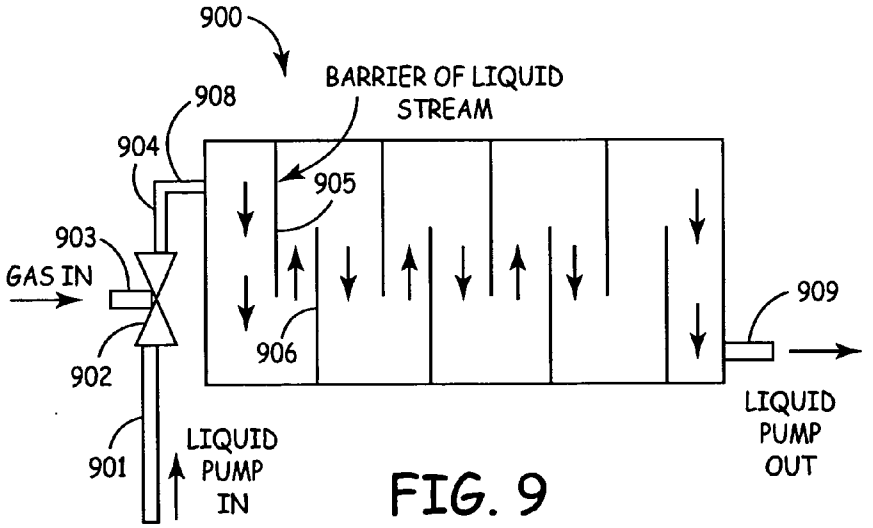

900
BARRIER OF LIQUID STREAM
908
904
905
903
GAS IN
909
906
902
LIQUID PUMP OUT
901
LIQUID PUMP IN

FIG. 9

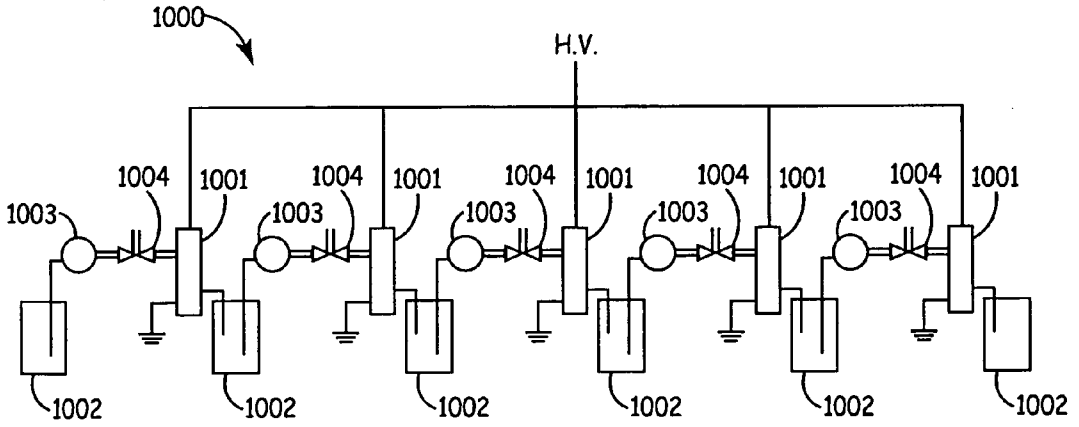

1000
H.V.
1004
1001
1003
1002

FIG. 11

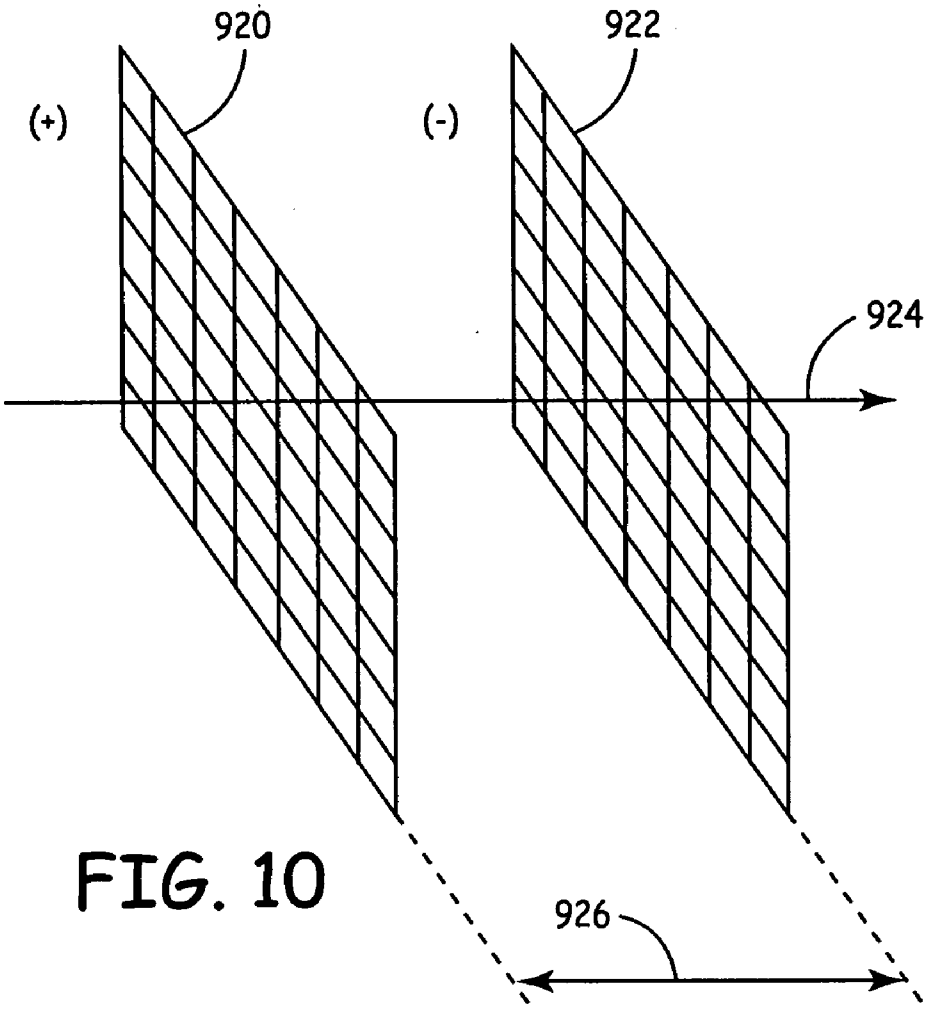


FIG. 10

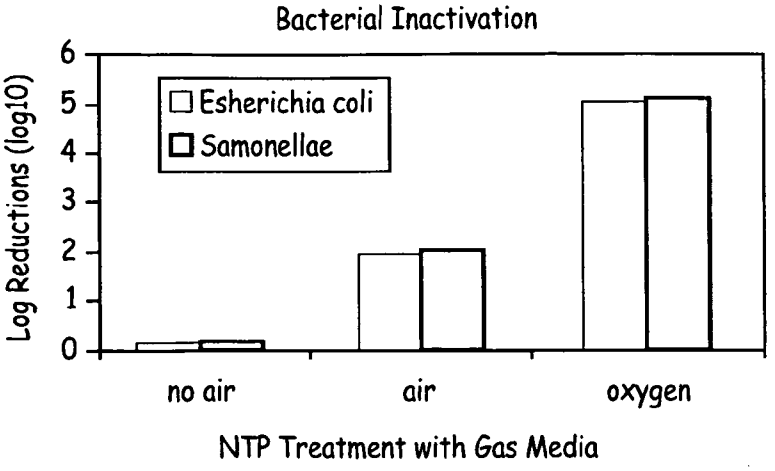


FIG. 12

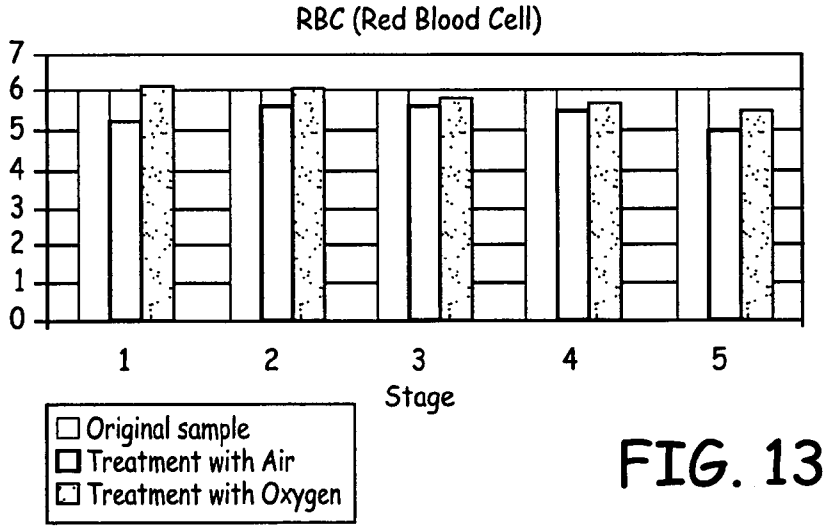


FIG. 13

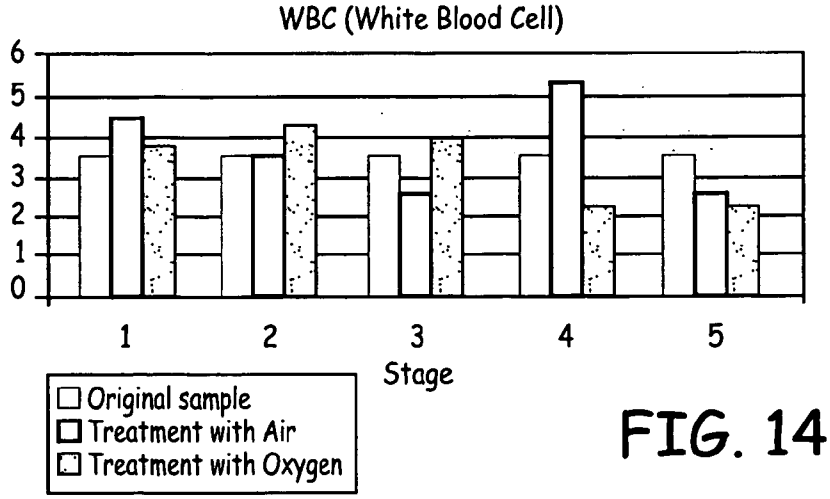


FIG. 14

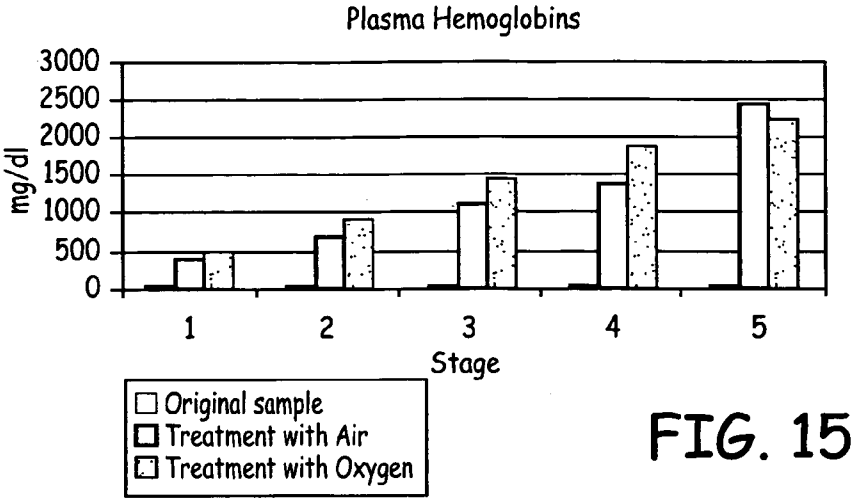


FIG. 15

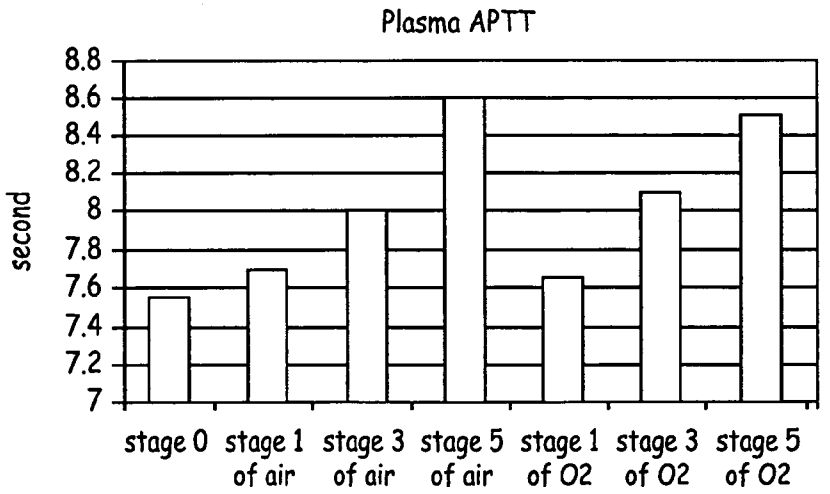


FIG. 16

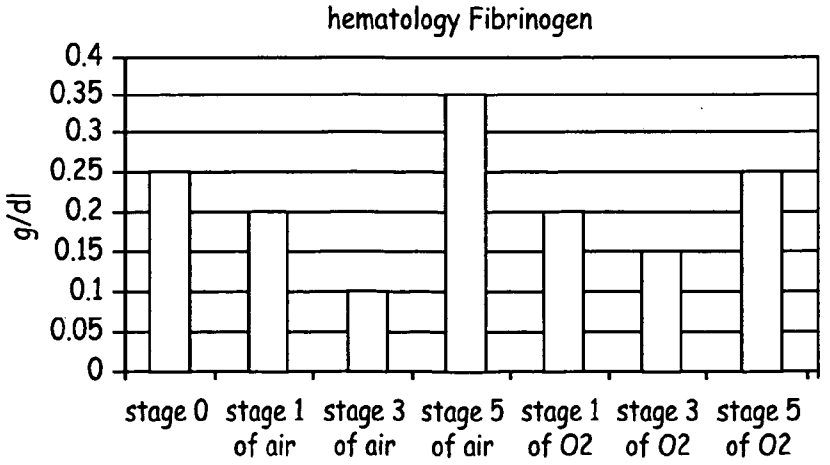


FIG. 17

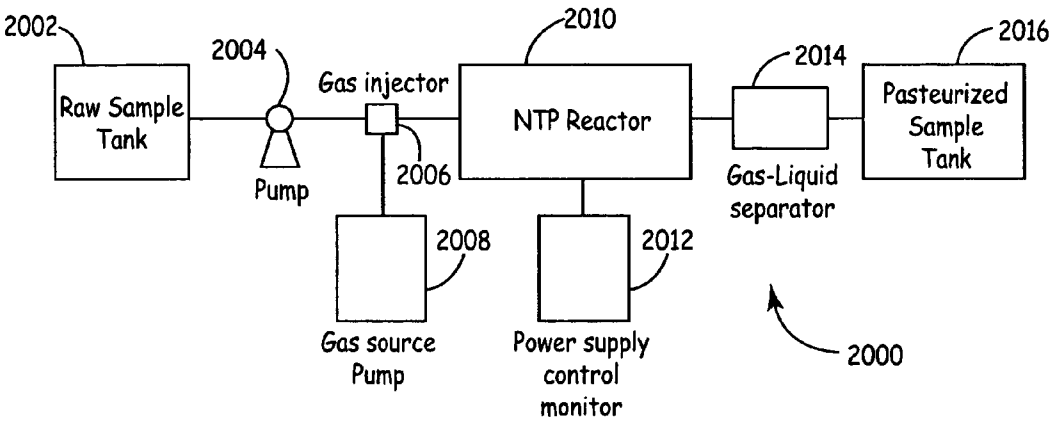

2002
Raw Sample Tank
2004
Pump
Gas injector
2006
2010
NTP Reactor
2008
Gas source Pump
2012
Power supply control monitor
2014
Gas-Liquid separator
2016
Pasteurized Sample Tank
2000

FIG. 18

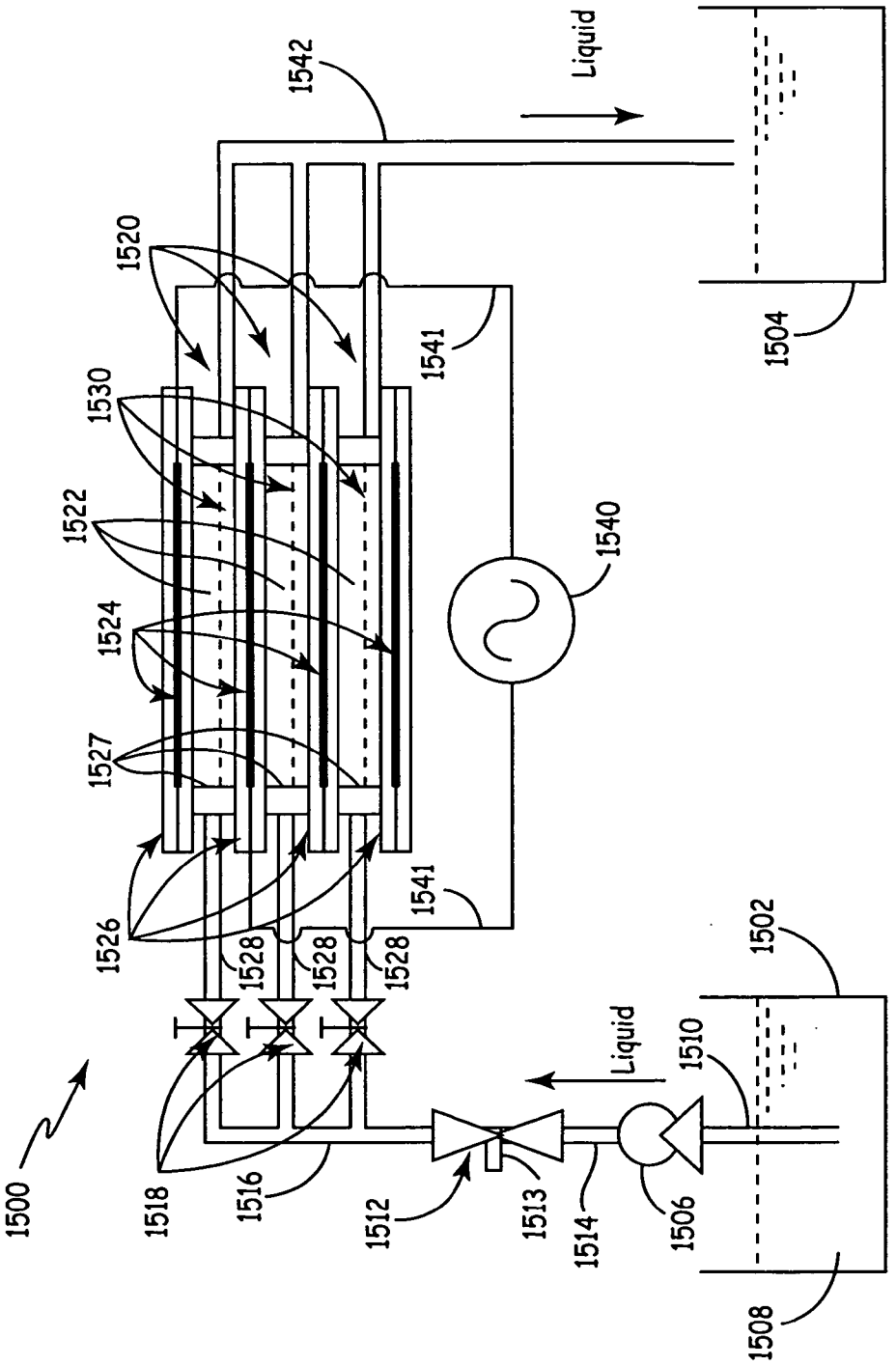

1542
Liquid
1520
1504
1541
1530
1522
1540
1524
1527
1526
1541
1528
1528
1528
1502
Liquid
1510
1500
1518
1516
1512
1513
1514
1506
1508

FIG. 19

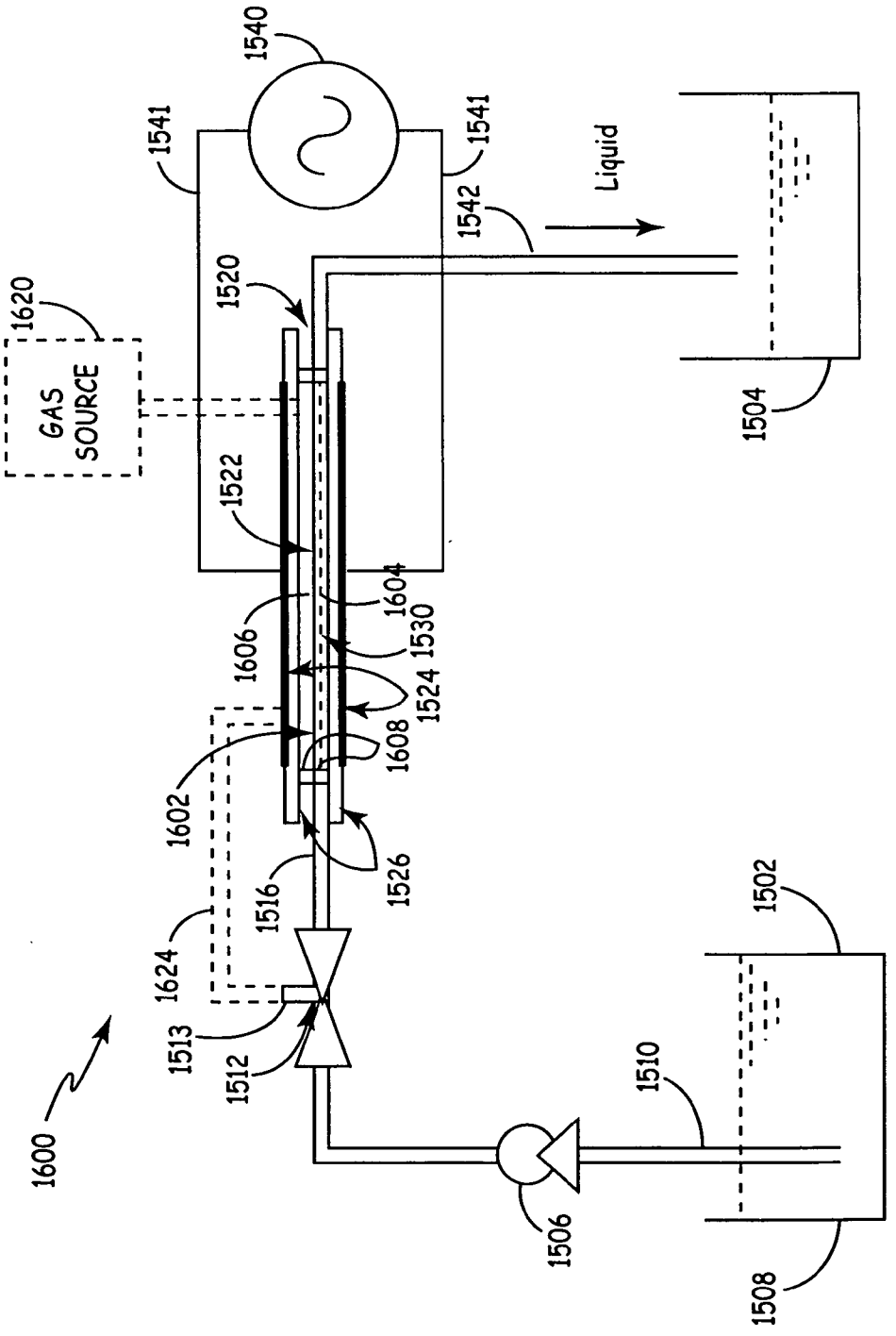

1540
1541
1541
1542
Liquid
1620
GAS SOURCE
1520
1522
1504
1604
1606
1530
1524
1608
1602
1526
1516
1624
1513
1512
1600
1502
1510
1506
1508

FIG. 20

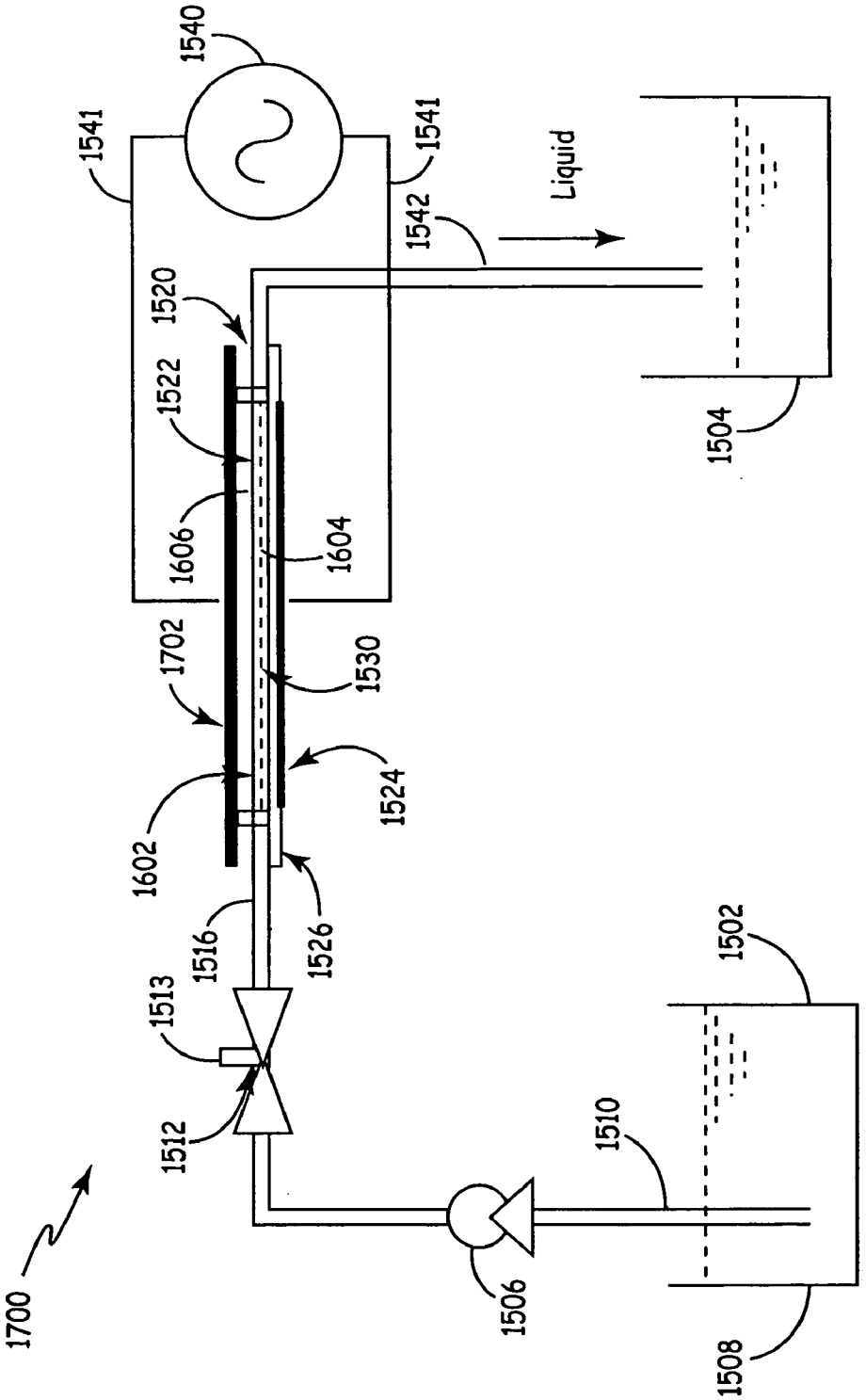

1700
1502
1504
1506
1508
1510
1512
1513
1516
1520
1522
1524
1526
1530
1540
1541
1542
1602
1604
1606
1702
Liquid

FIG. 21

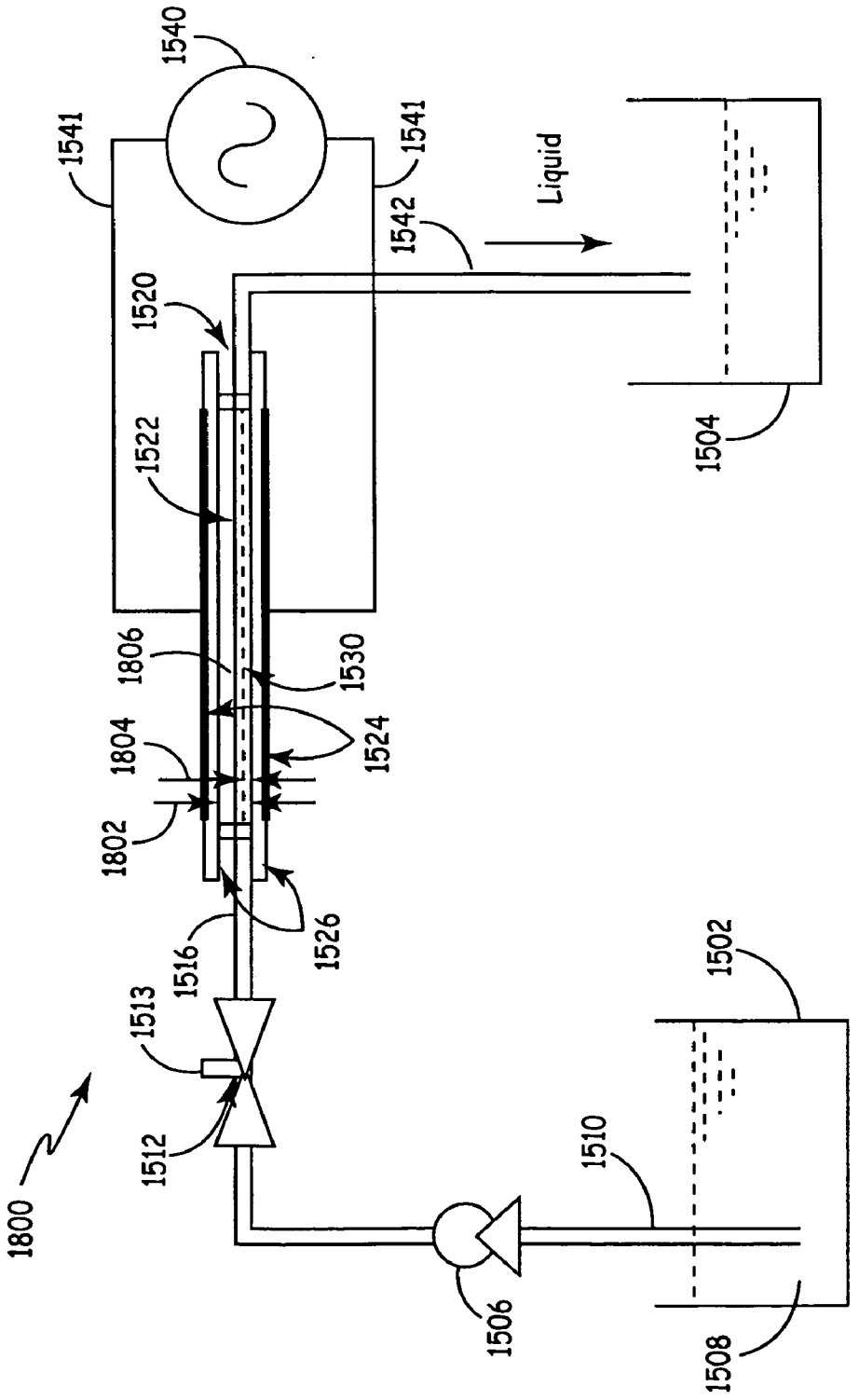

1540
1541
1541
Liquid
1542
1520
1522
1504
1806
1530
1804
1524
1802
1526
1516
1513
1512
1800
1502
1510
1506
1508

FIG. 22

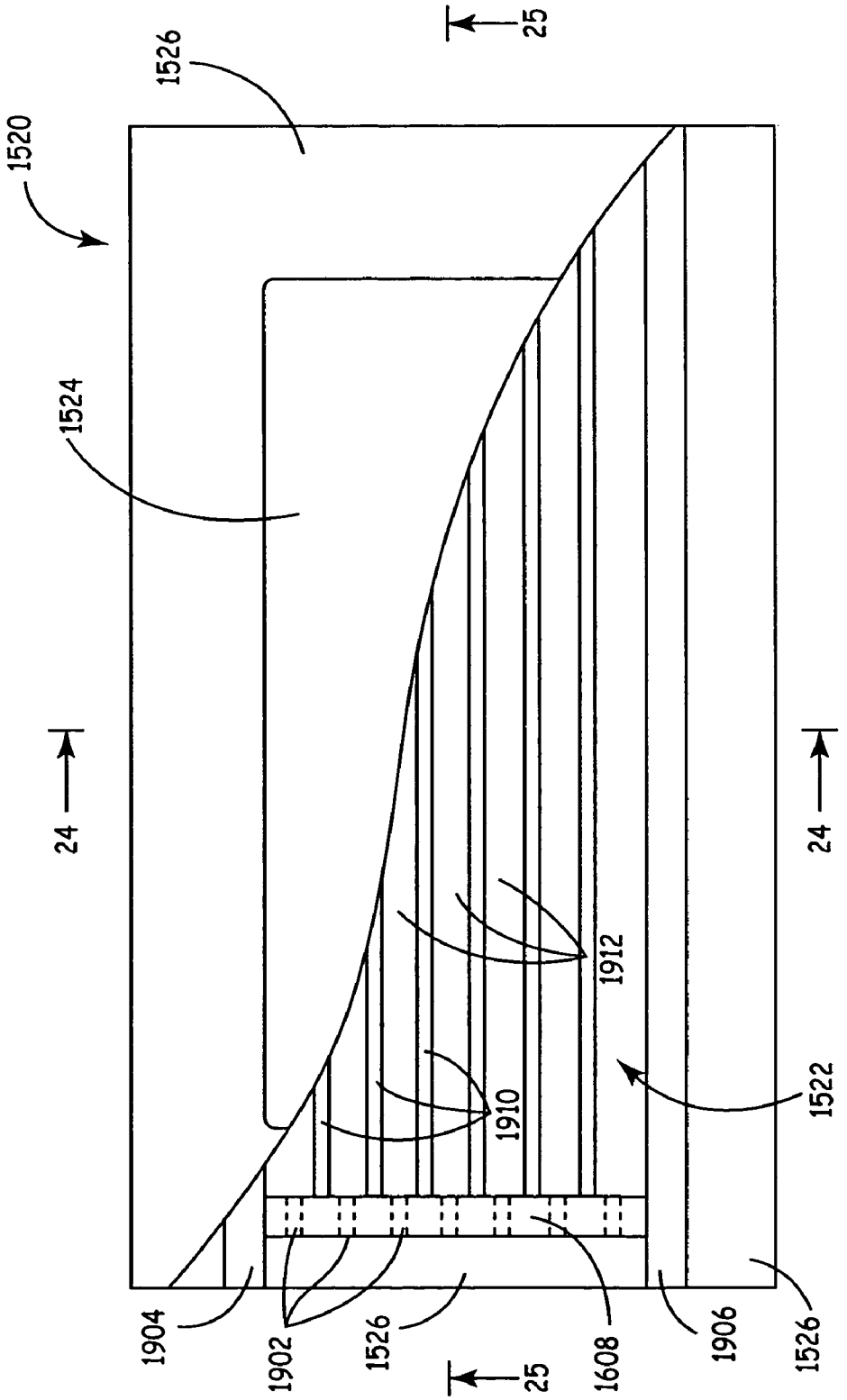

25
1526
1520
1524
24
24
1912
1910
1522
1904
1902
1526
25
1608
1906
1526

FIG. 23

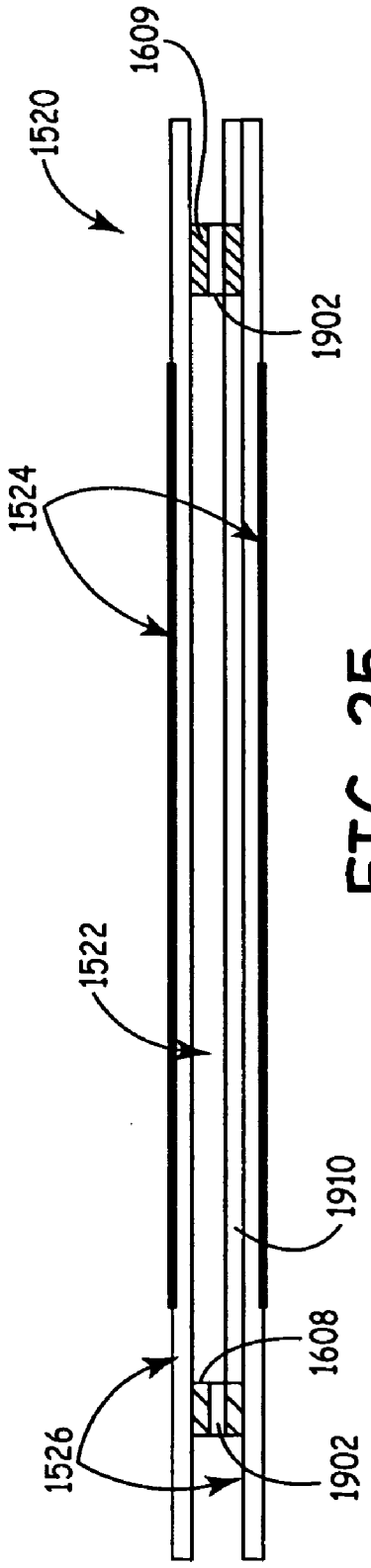

1609
1520
1902
1524
1522
1910
1608
1526
1902

FIG. 25

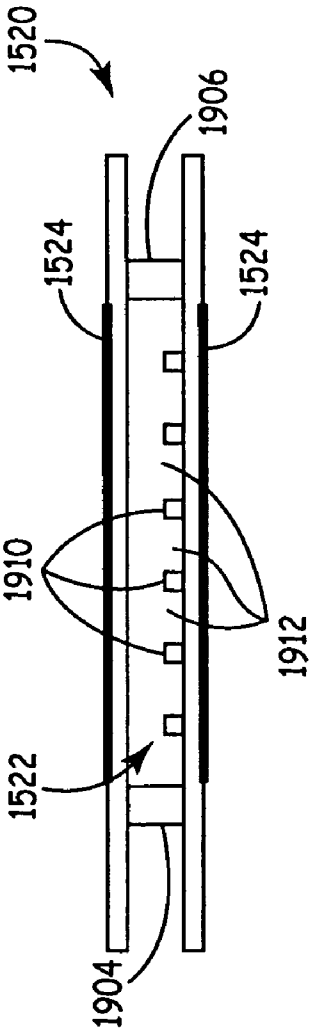

1520
1906
1524
1524
1910
1912
1522
1904

FIG. 24

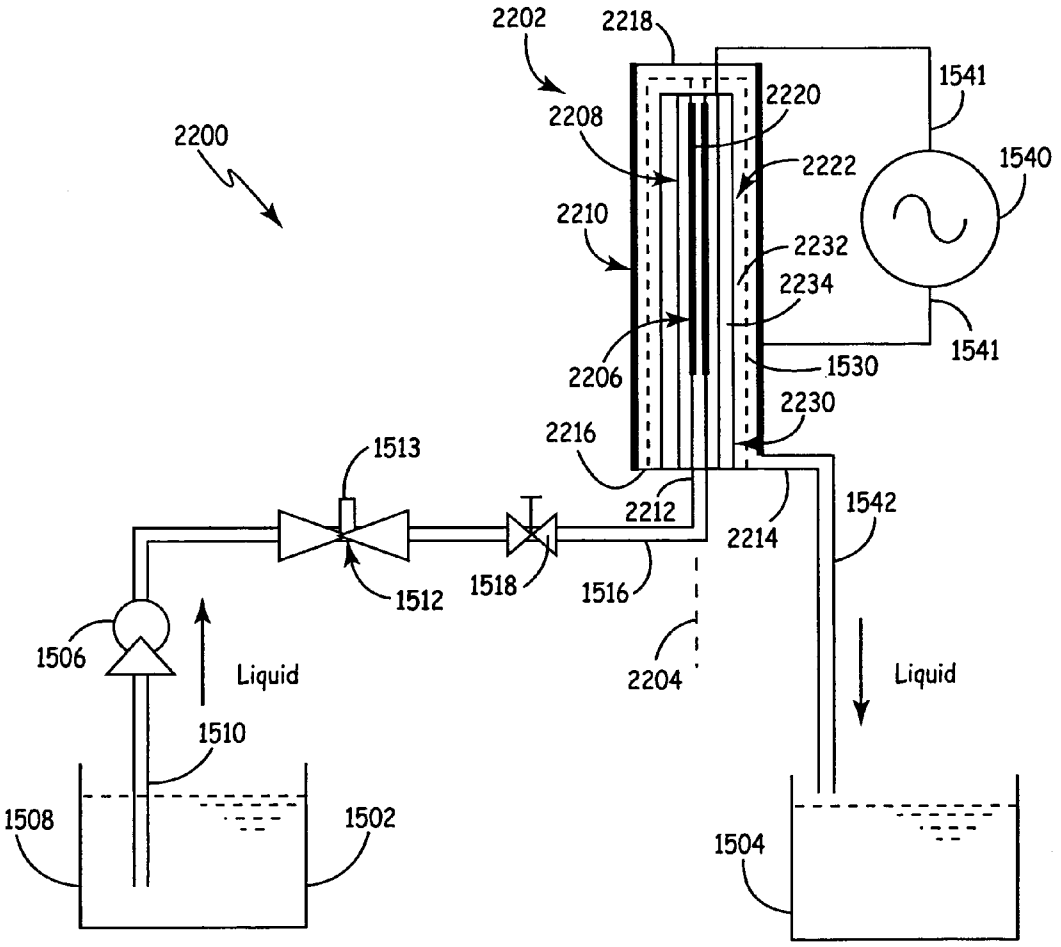

2202
2218
2200
2208
2220
1541
1540
2222
2210
2232
2234
1541
2206
1530
2230
2216
1513
2212
2214
1542
1512
1518
1516
1506
Liquid
2204
Liquid
1510
1508
1502
1504

FIG. 26

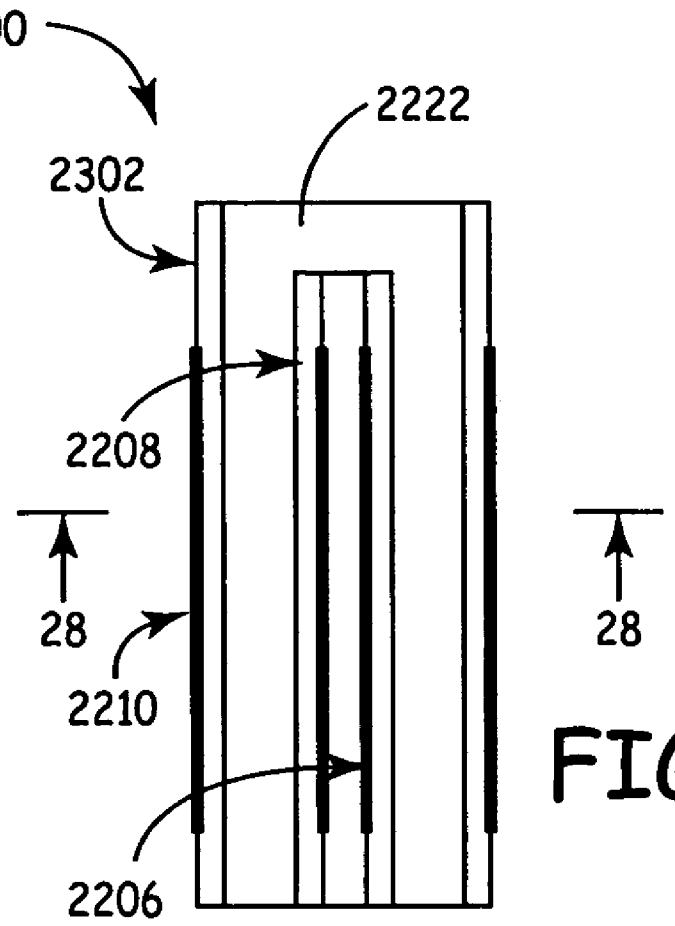

2300
2222
2302
2208
28
28
2210
2206

FIG. 27

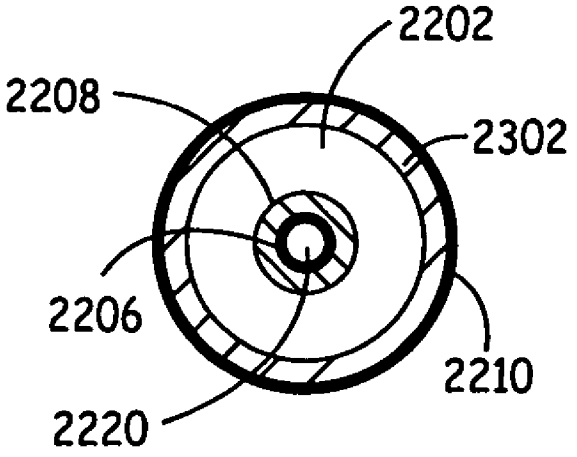

2202
2208
2302
2206
2210
2220

FIG. 28

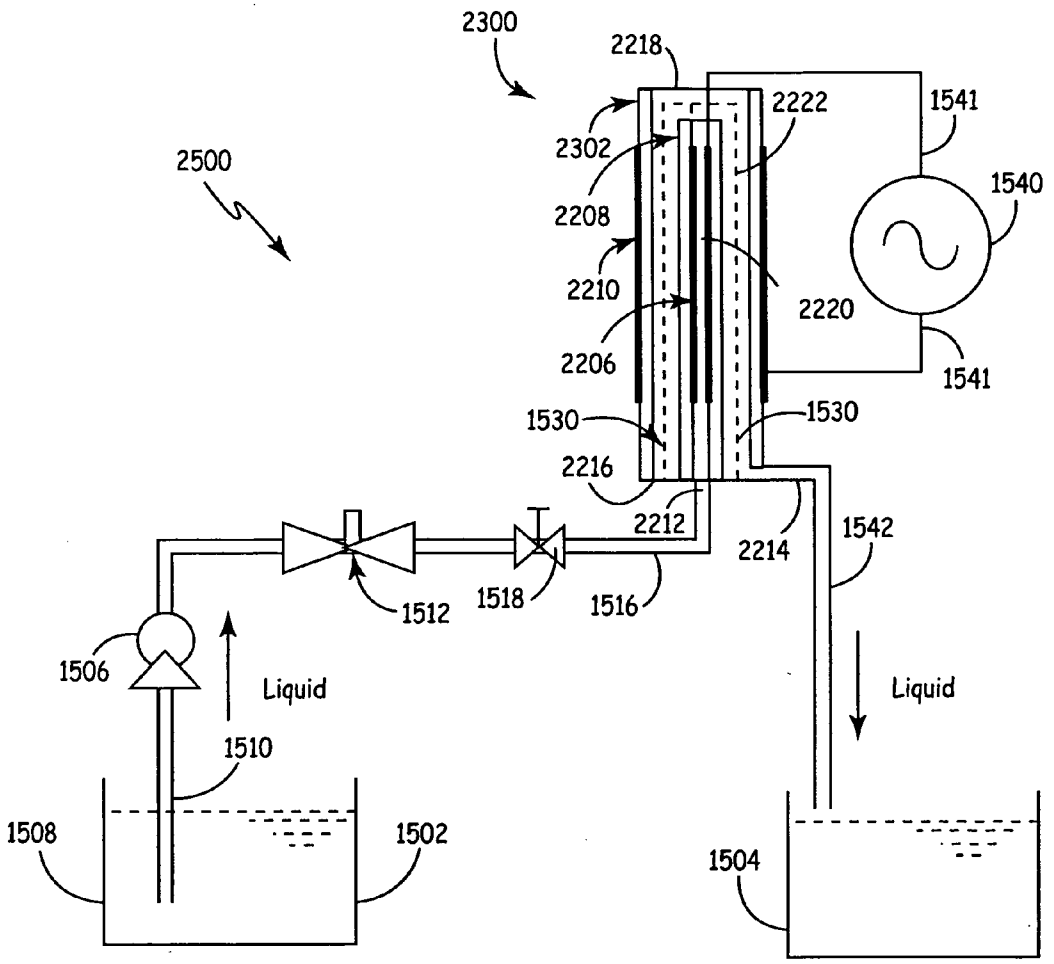

2300
2218
2222
1541
2302
2500
1540
2208
2210
2220
2206
1541
1530
1530
2216
2212
2214
1542
1512
1518
1516
1506
Liquid
Liquid
1510
1508
1502
1504

FIG. 29

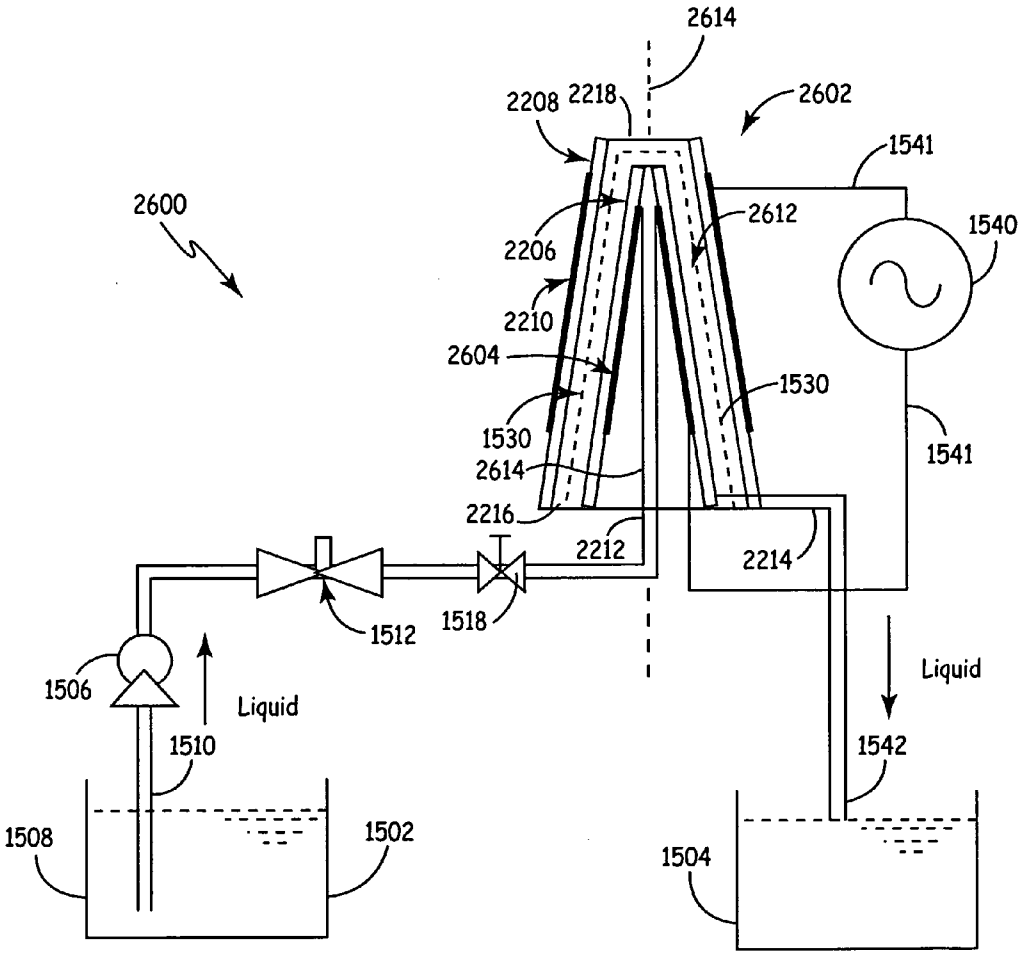

2614
2208
2218
2602
1541
2600
2612
1540
2206
2210
2604
1530
1530
1541
2614
2216
2212
2214
1512
1518
1506
Liquid
Liquid
1510
1542
1508
1502
1504

FIG. 30

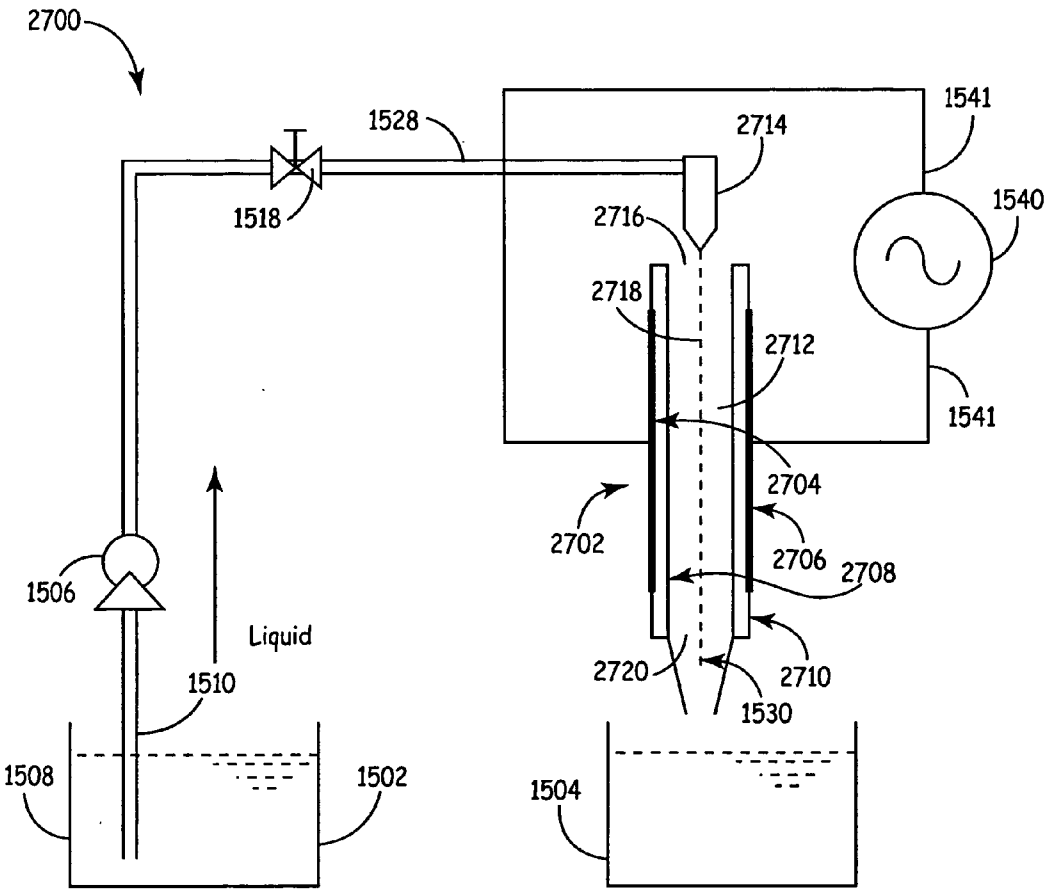

2700
1528
1518
1541
2714
1540
2716
2718
2712
1541
2704
2702
2706
2708
1506
Liquid
1510
2720
2710
1530
1508
1502
1504

FIG. 31

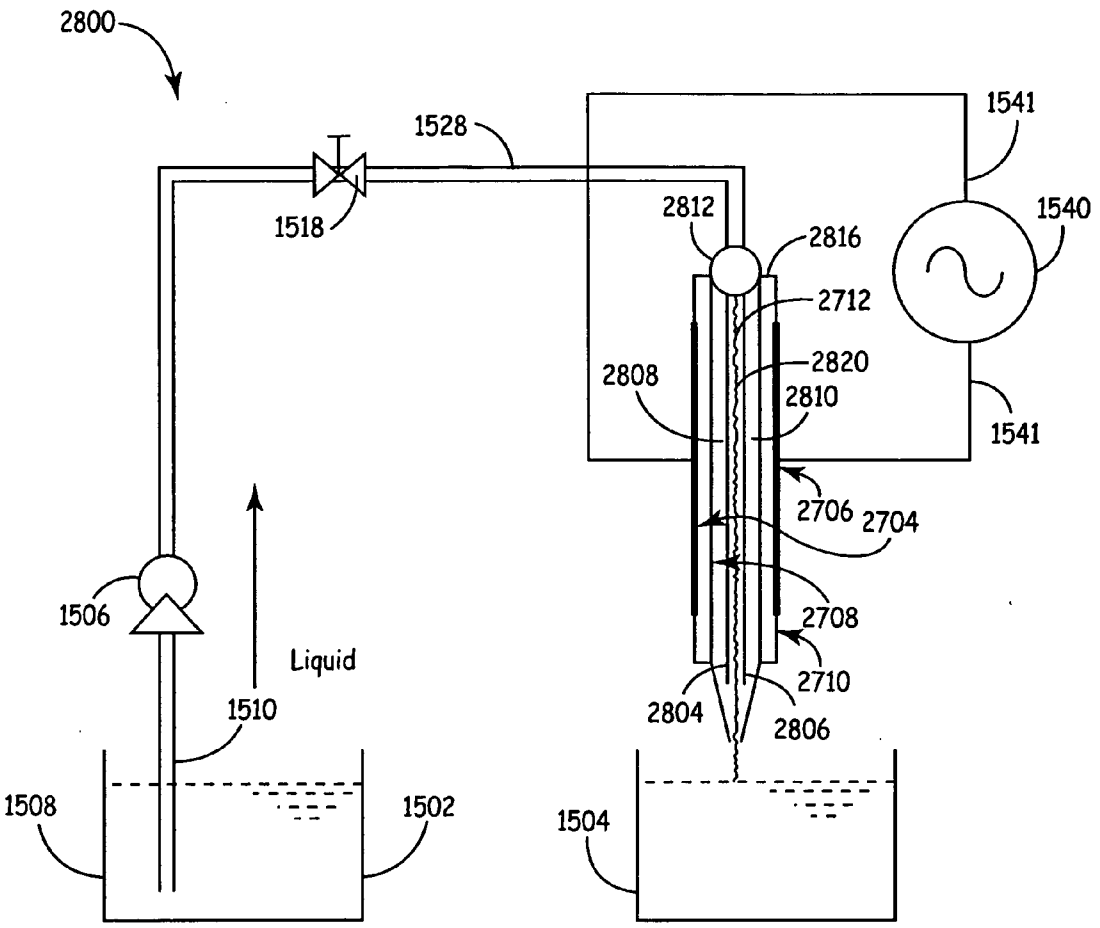

2800
1528
1541
1518
2812
2816
1540
2712
2820
2808
2810
1541
2706
2704
1506
2708
Liquid
2710
1510
2804
2806
1508
1502
1504

FIG. 32

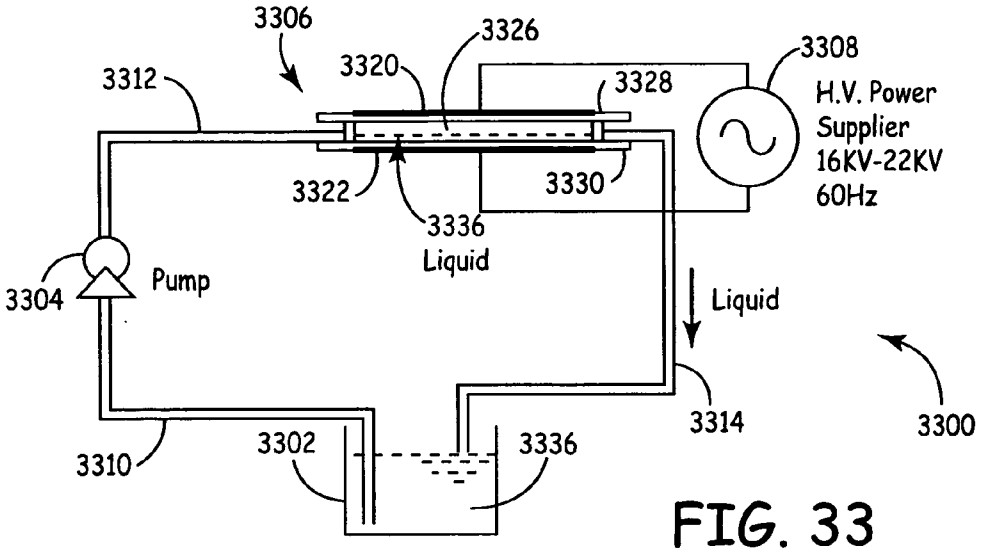

3306
3326
3320
3308
3312
3328
H.V. Power
Supplier
16KV-22KV
60Hz
3322
3330
3336
Liquid
Pump
3304
Liquid
3314
3300
3310
3302
3336

FIG. 33

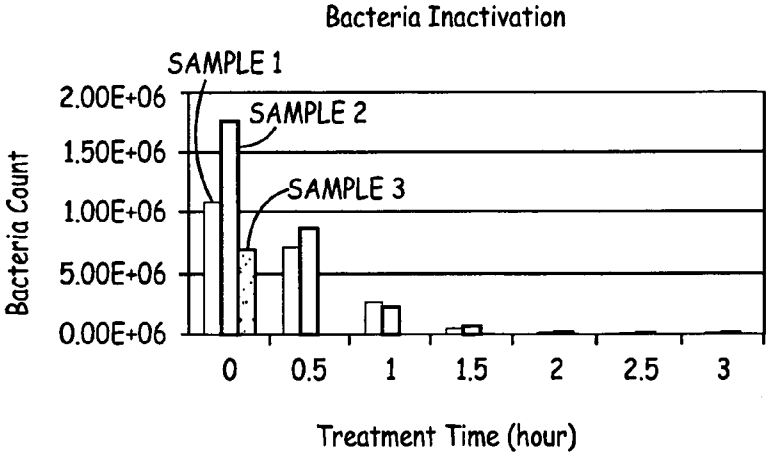
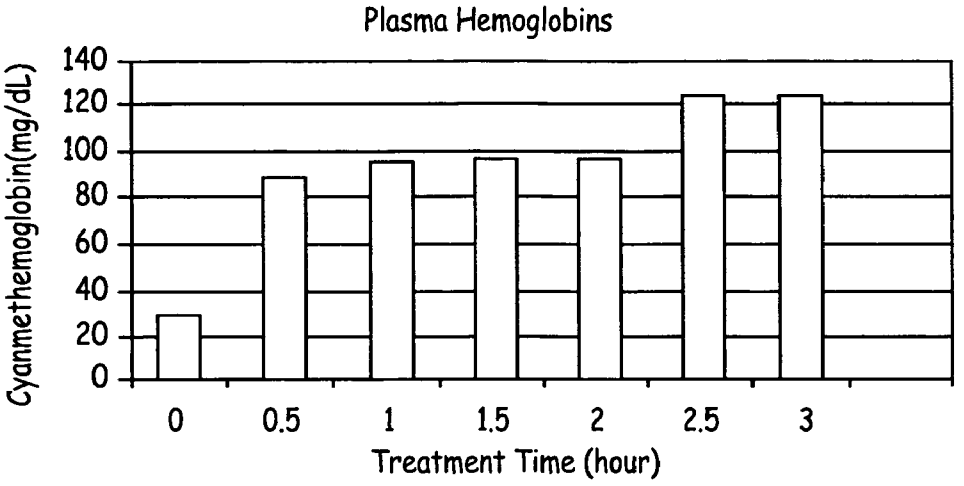
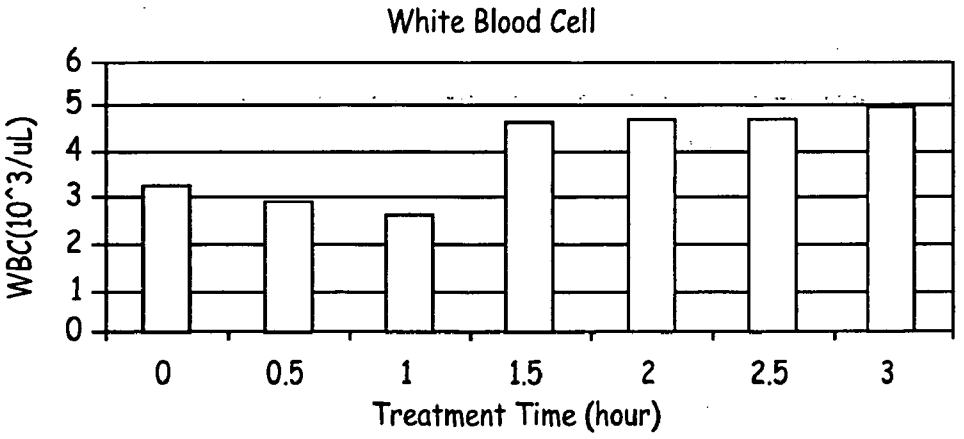

Bacteria Inactivation
SAMPLE 1
SAMPLE 2
SAMPLE 3
2.00E+06
1.50E+06
1.00E+06
5.00E+06
0.00E+06
Bacteria Count
0
0.5
1
1.5
2
2.5
3
Treatment Time (hour)

FIG. 34



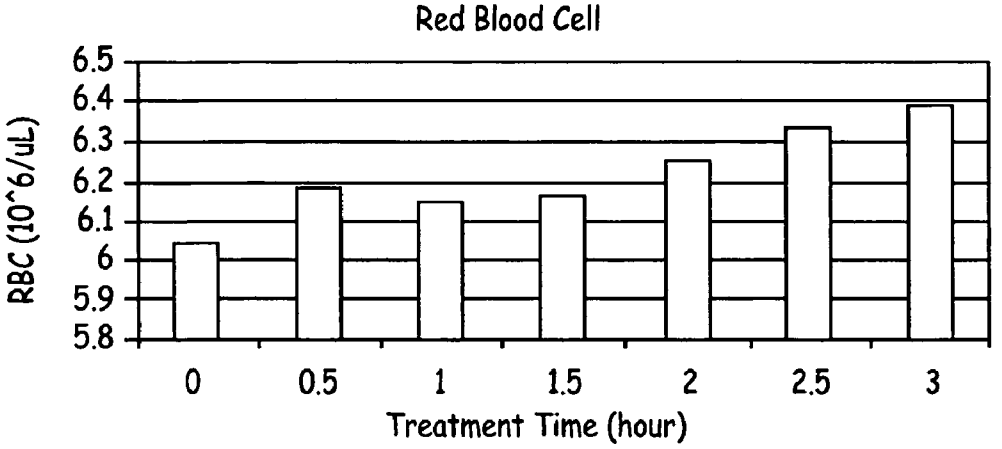
Plasma hemoglobin as a function of treatment time

FIG. 35



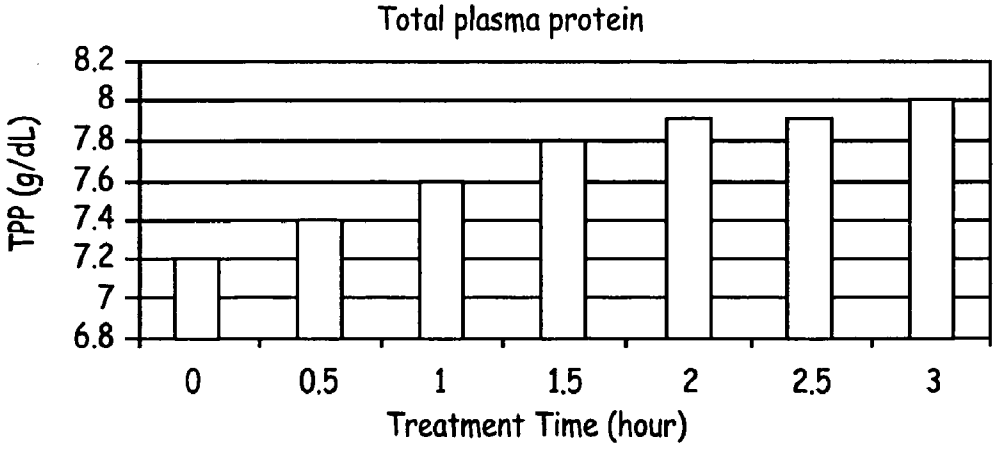
White blood cell as a function of treatment time
(Ref. Range: 4.1 - 11.3 x 10^3/uL)

FIG. 36



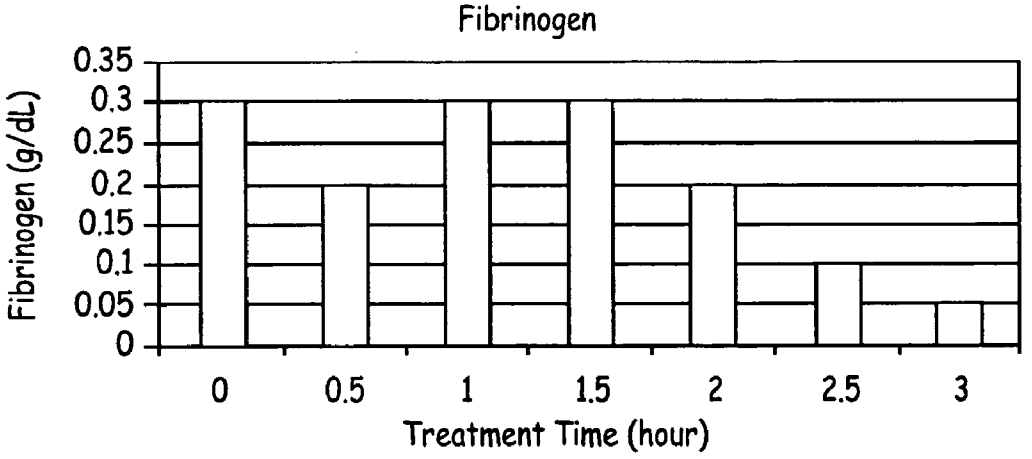
Red blood cell as a function of treatment time
(Ref. Range: 5.52 - 8.48 x 10^6/uL)

FIG. 37



Total Plasma Protein as a function of treatment time
(Ref. Range: 6.8 - 9.2 g/dL)

FIG. 38



Fibrinogen as a function of treatment time
(Ref. Range: 0.1 - 0.74 g/dL)

FIG. 39

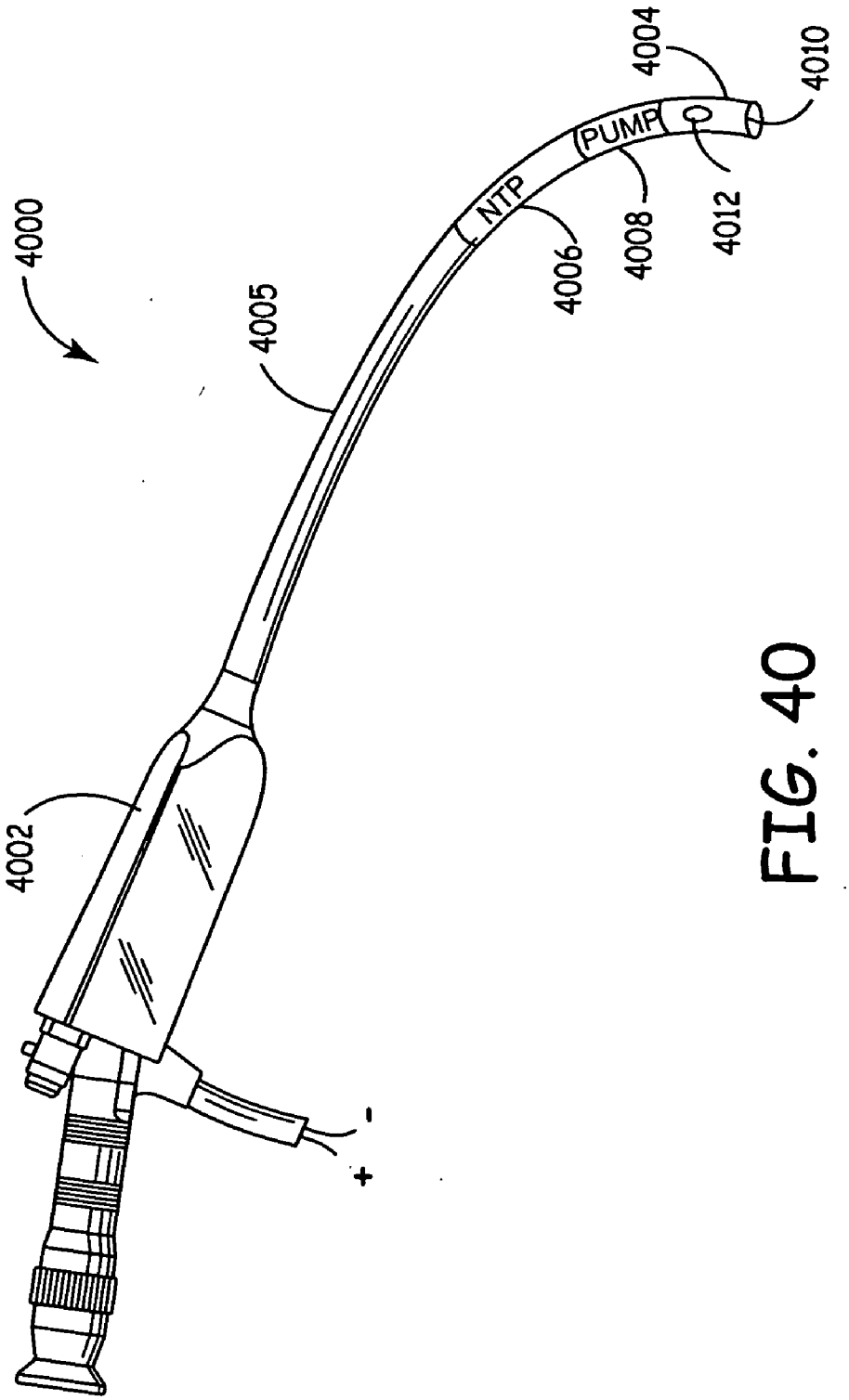


FIG. 40

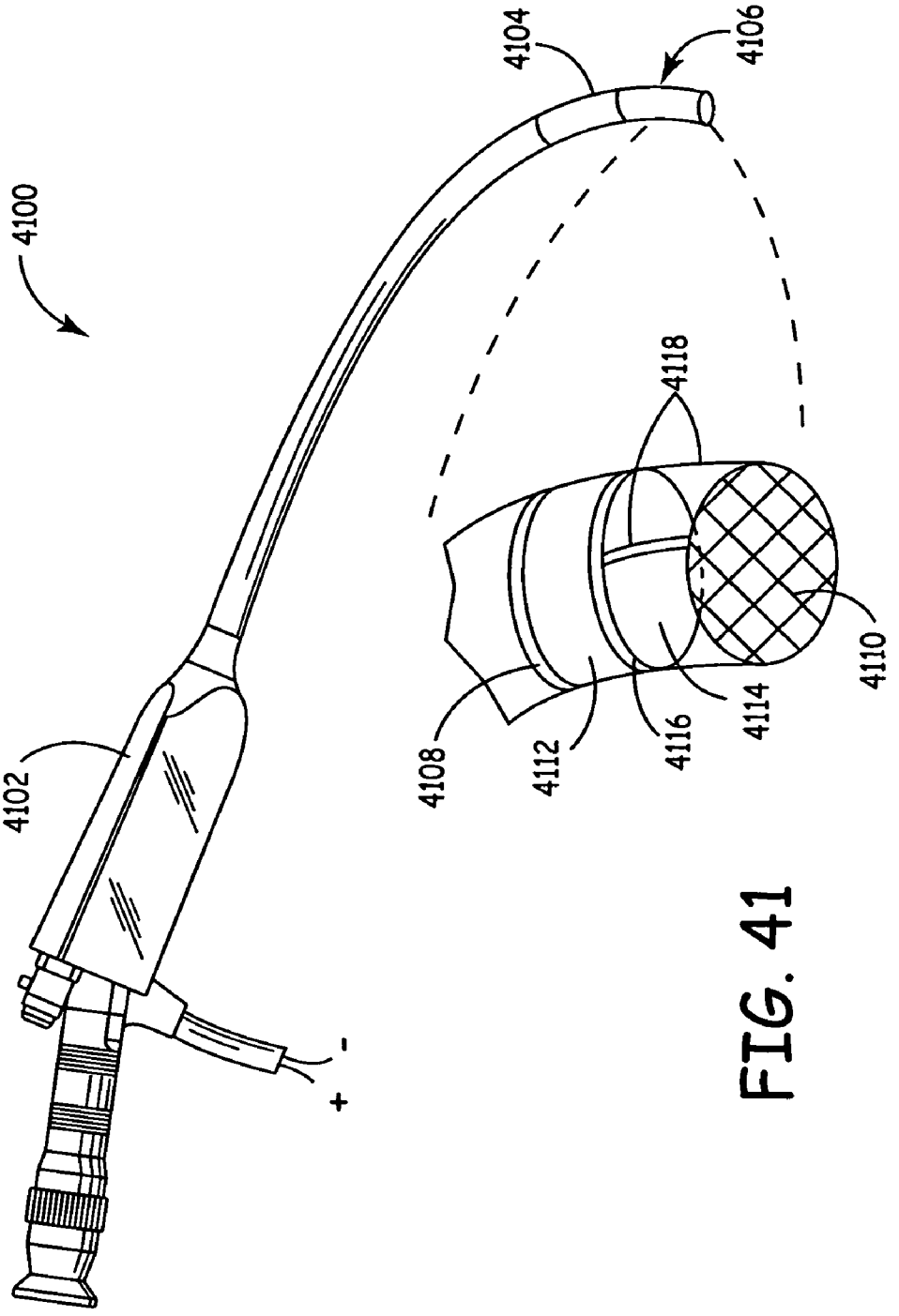


FIG. 41

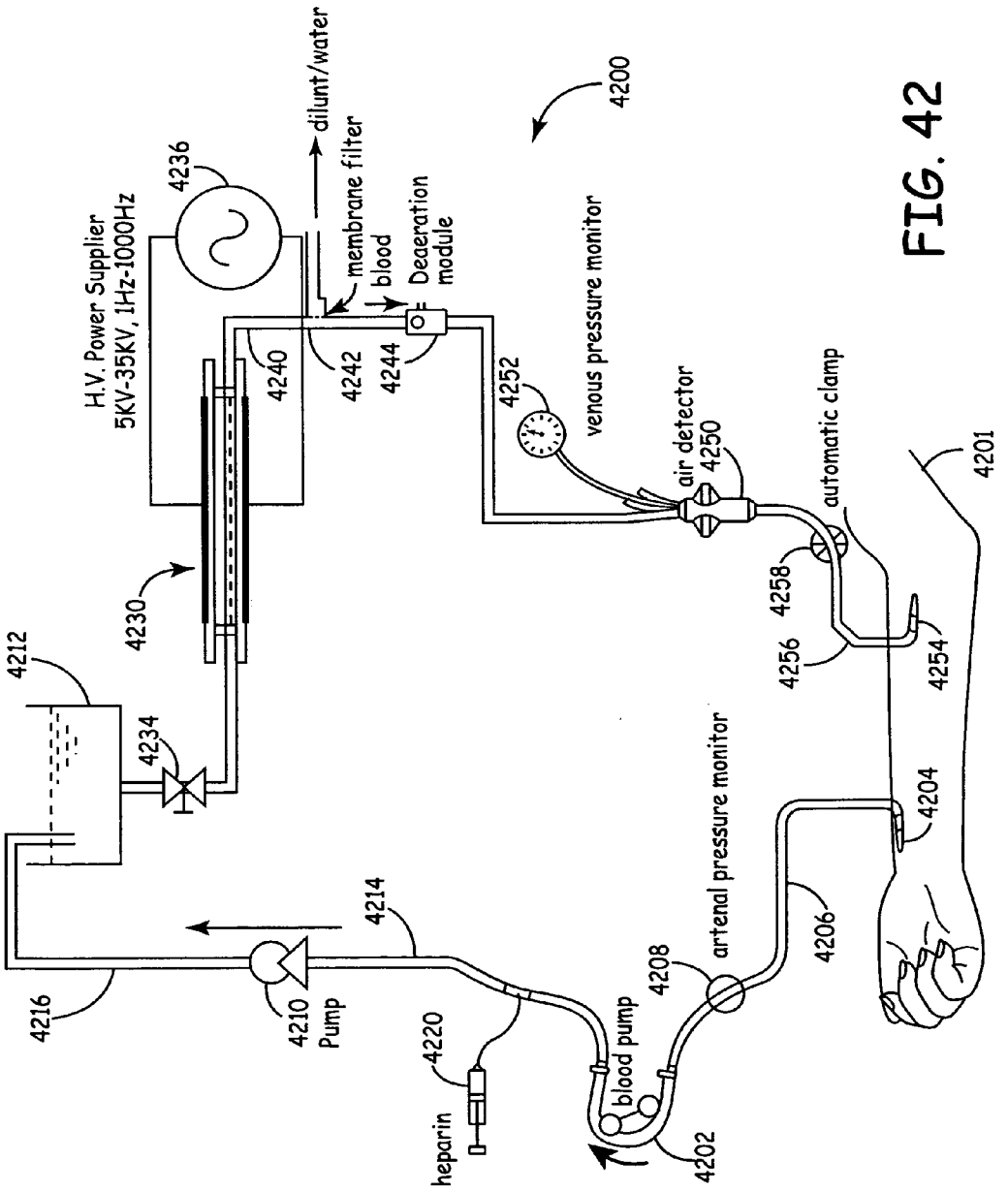

4200
4201
4202
4204
4206
4208
4210
Pump
4212
4214
4216
4220
heparin
4230
4234
4236
H.V. Power Supplier
5KV-35KV, 1Hz-1000Hz
4240
4242
4244
membrane filter
blood
Deaeration
module
diluntwater
4250
air detector
4252
venous pressure monitor
4254
4256
4258
automatic clamp
blood pump
artenal pressure monitor

FIG. 42

NON-THERMAL DISINFECTION OF BIOLOGICAL FLUIDS USING NON-THERMAL PLASMA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 10/426,605, Filed Apr. 30, 2003 and entitled "NON-THERMAL DISINFECTION OF BIOLOGICAL FLUIDS USING NON-THERMAL PLASMA;" which claims the benefit of U.S. Provisional Application No. 60/377,130, filed Apr. 30, 2002, and is a continuation-in-part of U.S. application Ser. No. 10/364,599, filed Feb. 11, 2003 and entitled "METHOD AND APPARATUS FOR NON-THERMAL PASTEURIZATION OF LIVING-MAMMAL INSTILLABLE LIQUIDS;" which is a continuation-in-part of U.S. application Ser. No. 09/850,284, filed May 7, 2001 and entitled "METHOD AND APPARATUS FOR NON-THERMAL PASTEURIZATION."

FIELD OF THE INVENTION

[0002] The present invention relates to a method and apparatus for disinfection of biological fluids to cleanse the fluids of pathogenic microorganisms.

BACKGROUND OF THE INVENTION

[0003] The phenomenon of bacterial resistance through micro-evolutionary change has been well documented. Substantial concerns over the degrading effectiveness of classic broad spectrum antibiotics, such as penicillin and its analogues, as the microbes have adapted new protection mechanisms, has led to medical protocols that attempt to minimize their usage. The goal is to delay the time when larger segments of the population may find the effectiveness of critically needed antibiotics compromised for their needs. To delay this problem, such powerful anti-microbial agents as Vancomycin are prescribed only under the most stringent use protocols. In addition, there are few pharmacological compounds that have been found to be effective for treatment of viruses, and the few that exist are very strain-specific.

[0004] The pharmaceutical industry is desperately seeking new ways to battle infection. The myriad attempts to develop improved types of antibotics and anti-viral agents include mechanisms ranging from various innovative chemical inactivation mechanisms, gene-based techniques of drug production, and tailored anti-infective medications for individual patients. What all of these approaches have in common is that they are chemical: drugs that are ingested and then act biochemically on the host. The disadvantage is that, while the pathogens may be destroyed, the patient may also be compromised or worse, due to drug toxicity.

[0005] A further problem is that, in general for a severe systemic infection, there still exist antibiotic drugs that can effectively destroy bacterial pathogens. However, what often kills the patient are the toxic poisons that are released by the bacteria as they are lysed (destroyed) due to either immune system action or antibiotic effects. There are two categories of such substances: endotoxins, lipopolysaccharides that are associated with the cellular walls of gram-negative bacteria, and exotoxins, soluble proteins which are diffusible and may act at different sites from the bacterial invasion as some of the most potent poisons known, comparable to strychnine and snake venom. It may help the patient very little to destroy a bacterium that leaves a parting gift of poison for the host as it dies. A treatment is needed that has the potential to both destroy bacteria in biological fluids such as blood, and also allow toxins to be filtered out of the blood before they can injure the patient. This phenomenon is applicable to both humans and animals.

[0006] Various methods of non-chemical purification of biological fluids, medicines, vaccines, etc., have been proposed to destroy or inactivate pathogens, including bacteria, viruses, and fungi, in the liquids. These methods typically generate heat during the purification process and may introduce impurities depending on the process. This heat can easily damage the active ingredients or critical live tissues that perform the essential functions of the fluids. In the case of biological fluids, if these tissues are thermally processed, they may become non-functional, as in the case of some clotting compounds, or vital living cells such as erythrocytes (red blood cells, RBC) or leukocytes (white blood cells, WBC) may be altered or damaged in ways that both mar instant function or compromise cellular stability, hardiness, or reproduction. When treating delicate biological fluids such as blood, these processes have had marginal success because blood functions properly in a very narrow temperature range around the normal 37 degrees C. (nominally 98.6 degrees F.).

[0007] Blood that is drawn from donors and banked for transfusion currently must go through multiple exhaustive steps to ensure safety for use, and there are large volumes of scarce blood and plasma that must be destroyed when they are found to be contaminated. A pasteurization technology that is able to cycle fluids such as blood through an apparatus outside the body to destroy the bacterial infection, and then filter out the toxins released in the process, and permit blood or plasma to be safely used rather than wasted will provide significant benefit to the health care system. Such a safe and efficacious technology should also permit cost-saving protocol changes in the collection and handling of biological fluids such as blood.

[0008] A number of minimal thermal processes have been developed for some of these applications, including ultrafiltration, ozonation, pulsed ultraviolet light, irradiation, high hydrostatic pressure (HHP) and pulsed electric field (PEF) discharge.

[0009] PEF discharge has been shown to be very effective for killing bacteria within medically useful liquids that are not subject to degradation, such as vaccines, medications, and other sterile substances. PEF discharge is considered to be one of the premier new technologies with a great potential of replacing thermal, chemical and other pasteurization and sterilization technologies. However, there are a number of drawbacks of the PEF discharge technology. For example, ohmic heating still occurs during the PEF discharge, which causes the temperature of the liquid being treated to rise. Hence, a cooling system must be used in order to maintain the liquid at a low temperature. A significant amount of energy is wasted with unwanted heating and cooling of the liquid. Also, the requirement of a cooling system adversely increases the time required to treat the liquid. In addition, the PEF electrodes are immersed directly in the liquid. Since the electrodes contact the liquid, they are regarded as a major contamination source to the liquid due to oxidation of the

electrodes during discharge. The electrodes must therefore be replaced regularly, which increases maintenance time and costs.

[0010] With respect to ozonation, numerous research reports have demonstrated the antiviral effect of ozone exposure. For example, K. H. Wells, J. Latino, J. Gavalchin and B. J. Poiesz, *Blood,* 78, 1882-90 (1991), reported more than 11 logs inactivation of HIV-1 virus in human blood that was exposed to ozone at a concentration of 1,200 ppm for two hours. J. M. Vaughn, Y. S. Chen, K. Lindburg and D. Morales, *Appl. Environ. Microbiol.,* 53, 2218-21 (1987), reported the use of ozone to inactivate simian rotavirus SA-11 and human rotavirus type 2 (Wa) at 4° C. by using single-particle virus stocks, and found that although the human strain was clearly more sensitive, both virus types were rapidly inactivated by ozone concentrations of 0.25 mg/liter or greater at all pH levels tested.

[0011] Use of ozone to kill viruses in blood and blood products has received increasing attention in the medical field, due to its high effectiveness, cost efficiency, and simplicity, with minimal collateral damage to blood cellular metabolism. Ozone is a strong oxidative, and can react with blood to form compounds which are identical to those produced by a human's own immune system to destroy viruses and bacteria. Some of these compounds include oxygen atoms, hydrogen peroxide, and lipid peroxide. Research in the Wells et al. article has indicated that the antiviral effects of ozone include viral particle disruption, reverse transcriptase inactivation, and/or a perturbation of the ability of the virus to bind to its receptor on target cells. Based on a study on the mechanism of enteroviral inactivation by ozone with poliovirus **1** as the model virus, D. Roy, P. K. Wong, R. S. Engelbrecht and E. S. Chian, *Appl. Environ. Microbiol.,* 41, 718-23 (1981) concluded that the damage to the viral nucleic acid is the major cause of poliovirus **1** inactivation by ozone.

[0012] In addition, use of ozone is entering commercial practice for purification of bottled water, but requires strict limitation of its concentration because of concerns over potential formation of the suspected carcinogen bromate when excess ozone concentration is permitted to interact with minerals in the water. This becomes an even greater concern when treating biological fluids, such as blood products since ozone can dissolve in the water of the blood plasma and therefore remain for an extended period of time. With biological fluids, the ozone must therefore be "inactivated" through the passage of time or by some other method. The lingering ozone may be an advantage for purifying bottled water, but in biological fluids, control of all biochemical process is critical.

[0013] Thus, while ozone and other minimally thermal methods are being researched for treatment of biological fluids, improved methods are desired for treating biological fluids such as blood without degrading their natural characteristics or generating toxic byproducts.

SUMMARY

[0014] One embodiment of the present invention is directed to a method of at least partially disinfecting biological fluid of a mammal, which comprises pathogens. The method includes: (a) drawing the biological fluid from the mammal; (b) contacting a non-thermal plasma with the biological fluid to kill at least a portion of the pathogens within the biological fluid; (c) filtering toxins from at least some components of the biological fluid after contacting in step (b); and (d) returning the biological fluid to the mammal after filtering the toxins in step (c).

[0015] Another embodiment of the present invention is directed to a method of at least partially disinfecting biological fluid of a mammal, which comprises pathogens. The method includes: (a) contacting a non-thermal plasma with the biological fluid to kill at least a portion of the pathogens within the biological fluid; and (b) filtering toxins from at least some components of the biological fluid after contacting in step (a).

[0016] Another embodiment of the present invention is directed to a liquid-gas mixture comprising a mammalian biological liquid, gas bubbles, and a non-thermal plasma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1** is a diagrammatic view of a "silent type", volume discharge non-thermal plasma reactor, which can be used for disinfecting biological fluids to destroy live pathogens living in the fluids.

[0018] **FIG. 2** is a diagram, which schematically illustrates a non-thermal plasma liquid disinfection system that introduces gas bubbles into the liquid being disinfected according to one embodiment of the present invention.

[0019] **FIG. 3** is a diagram illustrating a Venturi tube injector, which can be used for introducing gas bubbles within the system shown in **FIG. 2**.

[0020] **FIG. 4** is a diagram, which schematically illustrates a cross-sectional view of a non-thermal plasma reactor, which has a winding, serpentine flow path, according to one embodiment of the present invention.

[0021] **FIG. 5** is a cross-sectional view of a tubular non-thermal plasma reactor according to an alternative embodiment of the present invention.

[0022] **FIG. 6** is a perspective view of a non-thermal plasma reactor having narrow strip electrodes.

[0023] **FIG. 7A** is a side plan view of a surface discharge-type non-thermal plasma reactor according to another alternative embodiment of the present invention.

[0024] **FIG. 7B** is a plan view of a surface discharge electrode used in the reactor shown in **FIG. 7A**.

[0025] **FIG. 8** is a side view of a non-thermal plasma reactor in which the liquid is sprayed into the reaction volume, according to another alternative embodiment of the present invention.

[0026] **FIG. 9** illustrates a non-thermal plasma reactor having a set of barriers used to increase back pressure within the liquid being treated.

[0027] **FIG. 10** is a simplified, perspective view of two mesh-type non-thermal plasma electrodes that can be used for disinfecting liquids according to another alternative embodiment of the present invention.

[0028] **FIG. 1** is a diagram of a disinfection system having five NTP reactors connected together in series.

[0029] **FIG. 12** is a graph showing the level of inactivation of *Escherichia coli* and *Salmonella* in cow blood samples.

[0030] **FIG. 13** is a graph illustrating red blood cell count as a function of the number of NTP stages in and NTP system and the type of injected gas (air or oxygen).

[0031] **FIG. 14** is a graph illustrating white blood cell count as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen).

[0032] **FIG. 15** is a graph illustrating plasma hemoglobin in milligrams per deciliter (mg/dl) as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen).

[0033] **FIG. 16** is a graph illustrating plasma APTT in seconds as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen).

[0034] **FIG. 17** is a graph illustrating hematology fibrinogen in grams per deciliter (g/dl) as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen).

[0035] **FIG. 18** is a block diagram illustrating an NTP system having a gas-liquid separator according to one embodiment of the present invention.

[0036] **FIG. 19** is a diagram, which schematically illustrates a multiple-plate non-thermal plasma reactor according to another embodiment of the present invention.

[0037] **FIG. 20** is a diagram that schematically illustrates a two-dielectric barrier NTP reactor having a discharge initiation region according to another alternative embodiment of the present invention.

[0038] **FIG. 21** is a diagram, which illustrates an NTP reactor according to another alternative embodiment of the present invention.

[0039] **FIG. 22** is a diagram, which schematically illustrates an NTP reactor according to another embodiment of the present invention.

[0040] **FIG. 23** is a top plan view of one of the NTP cells shown in **FIGS. 19-22**, according to one embodiment of the present invention.

[0041] **FIG. 24** is a cross-sectional view of the NTP cell, taken along lines **24-24** of **FIG. 23**.

[0042] **FIG. 25** is a cross-sectional view of the NTP cell taken along lines **25-25** of **FIG. 23**.

[0043] **FIG. 26** is a diagram, which schematically illustrates an NTP reactor according to another alternative embodiment of the present invention.

[0044] **FIG. 27** is a cross-sectional view of a cylindrical NTP cell according to an alternative of the present invention.

[0045] **FIG. 28** is a cross-sectional view of the NTP cell taken along lines **28-28** of **FIG. 27**.

[0046] **FIG. 29** is a diagram, which schematically illustrates an NTP reactor in which the NTP cell shown in **FIGS. 27 and 28** can be used.

[0047] **FIG. 30** is a diagram, which illustrates a conical NTP reactor according to another alternative embodiment of the present invention.

[0048] **FIG. 31** illustrates a non-thermal plasma reactor in which the liquid is sprayed into the reaction volume, according to another alternative embodiment of the present invention.

[0049] **FIG. 32** is a diagram, which illustrates an NTP reactor that forms a liquid curtain according to another alternative embodiment of the present invention.

[0050] **FIG. 33** is a diagram illustrating an experimental test apparatus that was used for testing NTP treatments on fresh cow blood.

[0051] **FIG. 34** is a graph illustrating bacterial inactivation for each sample treated in the apparatus shown in **FIG. 33** as a function of treatment time.

[0052] **FIG. 35** is a graph illustrating concentration of plasma hemoglobin in the blood samples as a function of treatment time for the apparatus shown in **FIG. 33**.

[0053] **FIG. 36** is a graph illustrating concentration of white blood cells (WBCs) in the blood samples as a function of treatment time.

[0054] **FIG. 37** is a graph illustrating concentration of red blood cells (RBCs) in the blood samples as a function of treatment time.

[0055] **FIG. 38** is a graph illustrating concentration of Total Plasma Protein (TPP) in the blood samples as a function of treatment time.

[0056] **FIG. 39** is a graph illustrating concentration of fibronigen in the blood samples as a function of treatment time.

[0057] **FIG. 40** is a diagram, which schematically illustrates a catheter that can be used for in-vivo NTP treatment according to one embodiment of the present invention.

[0058] **FIG. 41** is a diagram, which schematically illustrates a further catheter that can be used for in-vivo NTP treatment according to an alternative embodiment of the present invention.

[0059] **FIG. 42** is a diagram illustrating an ex-vivo circulatory system and method for disinfecting the blood of a live mammal patient using non-thermal plasma according to another embodiment of the present invention.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0060] **FIG. 1** is a diagrammatic view of a "silent type", volume discharge non-thermal plasma reactor **100**, which can be used for pasteurizing and/or at least partially sterilizing or disinfecting living-mammal-instillable liquids, such as biological fluids, to kill live pathogens living in the liquids. Non-thermal plasma reactor **100** includes a liquid inlet **102**, a liquid outlet **104**, a reaction volume **106** between liquid inlet **102** and liquid outlet **104**, electrodes **108** and **110**, and dielectric barriers **112** and **114**. Flow path **116** indicates the liquid flow path from inlet **102** to outlet **104**, through reaction volume **106**. Each of the electrodes **108** and **110** is physically and electrically isolated from the liquid in flow path **112** by a respective one of the dielectric barriers **112** and **114**.

[0061] Dielectric barriers **112** and **114** are separated from one another by a gap, which defines the effective width of

reaction volume **106**. Dielectric barriers **112** and **114** can include Teflon, glass, ceramic or epoxy resin, for example. Other insulating materials can also be used. In one embodiment, each electrode **108** and **110** is embedded within an epoxy resin. The discharge gap between electrodes **108** and **110** can be sized to suit a particular application. For example, electrodes **108** and **110** can be separated by a distance of up to 30 centimeters. A larger gap can be used if voltage and insulation conditions permit. In one particular embodiment, electrodes **108** and **110** are separated by 10 millimeters, with an effective gap between dielectric layers **112** and **114** of about 7 millimeters.

[**0062**] Electrodes **108** and **100** can have a variety of configurations. For example in the embodiment shown in **FIG. 1**, electrodes **108** and **110** are each formed of a thin, planar sheet of conductive metal, such as a copper foil. Other conductive structures can also be used such as a conductive mesh, wire or strip. The combination of electrodes **108** and **110** can have a variety of different types, such as plate-to-plate, mesh-to-mesh, plate-to-wire, wire-to-wire, plate-to-mesh and wire-to-mesh, for example. The shapes of electrodes **108** and **110** can also be varied. For example, electrodes **108** and **110** can be arranged coaxially with one another, wherein the outer electrode is tubular and the inner electrode is either tubular or a wire. Other arrangements can also be used. However, in each arrangement, both electrodes **108** and **110** are physically and electrically isolated from the liquid in the reaction volume by a dielectric barrier in order to prevent an electrical conduction path through the liquid and contamination of the liquid due to contact with the electrodes.

[**0063**] High voltage power supply **124** supplies power to electrodes **108** and **110**. Electrode **108** is electrically coupled to a first terminal **120** of power supply **124**, and electrode **110** is electrically coupled to a second terminal **122** of power supply **124**. One of the electrodes **108** and **110** serves a ground electrode, such as electrode **110**, and the other, such as electrode **108**, serves as a high voltage electrode. Power supply **124** can include a continuous direct-current (DC) or an alternating-current (AC) power supply that is capable of producing a voltage across electrodes **108** and **110** so as to form an electric discharge path, shown by arrows **126**, across reaction volume **106**. In one embodiment, the voltage potential generated between electrodes **108** and **110** is in the range of 1 kV-35 kV, for example. Other voltage ranges can also be used, such as voltage ranges between 1 kV and 500 kV. Power supply **124** can be operated at either low or high frequencies and can produce pulses with a single polarity or can produce bipolar pulses.

[**0064**] With electrodes **108** and **110** having opposite polarity, electrodes **108** and **110** generate a strong electrical field across reaction volume **106**. The strong electrical field is applied to gas in the liquid, which generates non-thermal plasma species, including electrically neutral gas molecules, charged particles in the form of positive ions, negative ions, free radicals and electrons, and quanta of electromagnetic radiation (photons). Examples of the generation of these highly reactive species include, but are not limited to:

[**0065**] $e+O_2 \text{-----} > O_2^+ + 2e$

[**0066**] $O^+ + O + 2e$

[**0067**] $O^* + O + e$

[**0068**] $O^- + O$

[**0069**] $e+N_2 \text{-----} > N_2^+ + 2e$

[**0070**] $N^+ + N + 2e$

[**0071**] $N_2^* + e$

[**0072**] $N^* + N + e$

[**0073**] $O^* + H_2O \text{-----} > 2OH$

[**0074**] $O + O_2 + O_2 \text{-----} > O_3 + O_2$

[**0075**] $e + H_2O \text{-----} > H + OH$

[**0076**] These non-thermal plasma species are highly reactive and are effective in destroying live pathogens, such as bacteria, viruses and fingi, living in the liquid being treated. The non-thermal plasma species are extremely strong oxidants that break down chemicals and kill microorganisms instantly. A non-thermal plasma is one in which the mean electron energy is much higher than that of the bulk-gas (or liquid) molecules. Energy is added to the electrons instead of the ions and bulk gas (or liquid) molecules. The electrons can therefore attain high energies, while the background molecules and ions remain at ambient temperature. Because of the non-thermal nature of reactor **100**, reactor **100** preserves the quality and other heat-sensitive attributes of the liquids being disinfected.

[**0077**] Examples of liquids that can be treated include any liquid that is produced by or secreted by a living mammal, such as blood, tissue fluid, cerebrospinal fluid, or lymph; or a liquid that is biocompatible with the physiology of, and therefore beneficially instillable in a living mammal, such as a human, dog, horse, cat, etc. The term "instillable" includes all liquids that are non-toxic to a living mammal when introduced into the mammal by methods such as oral ingestion, inhaling, transdermal absorption, rectal (as with enema or other such solutions), direct insertion into arterial vessels, venal vessels (IV), lymphatic vessels, the spinal canal, and body cavities such as the abdomen, the lungs or the liver, intramuscular injection, and subcutaneous injection.

[**0078**] One example of such a liquid is a liquid that is capable of being consumed and assimilated by a living mammal as nourishment. Other treatable liquids may include fermentation broth, medications and vaccines of all types, total parenteral nutrition (TPN) liquids, including sugars and lipids, etc., intravenous (IV) fluids such as Lactated Ringers or D5, etc., renal dialyzing fluids (which are instilled and drawn back off), and biological fluids that must be returned to the body without damage to viable components such as platelets and leukocytes. These biological fluids can include heat-sensitive fluids such as human and animal bloods, blood products, such as plasma or lymph, extracellular tissue fluid, and cerebrospinal fluid (CSF).

[**0079**] Of these biologic fluids, the most important is blood, which brings the vital oxygen and nourishment to the organs of the body. When a mammal is infected with either a bacterial or viral pathogen, the blood is the carrier. Bacteria feed on the blood elements and reproduce uncontrollably; virus enters the blood cells and reproduces, but free virus is also carried throughout the body to contaminate other types of cells. The mammal's immune system combats and destroys the pathogens, but serious infections can overwhelm the body's immune system and compromise the innate ability to recover.

[0080] Blood is made up of plasma, the saline-and-water-based liquid that dissolves electrolytes such as sodium, calcium, and potassium, lipids, and vitamins, and carries the red blood cells (RBC, or erythrocytes), white blood cells (WBC, or leukocytes), and platelets (clot-supporting cells). Other minor fractions include hormones, antibodies, and albumin. But, when an infection occurs, the critical issue is cleansing the plasma, preventing it from carrying the infectious agents throughout the body, and choking off the major blood cell functions. Generally, lipids, electrolytes, vitamins, and other nutrients can be added intravenously as long as there is a minimal level of viable blood plasma and healthy cells to carry out basic functions. When blood volume is low, frequently fresh frozen plasma is added to increase blood volume.

[0081] Electrically-generated non-thermal plasma species can be used to inactivate pathogenic and spoilage microorganisms under "ambient" conditions, such as ambient temperature and atmospheric pressure or in non-ambient conditions. In one embodiment, "ambient" refers to the body's environment of approximately 37 degrees C. Non-thermal plasma is not the same substance as blood plasma. The term "non-thermal plasma" used in the specification and claims refers to a state of matter, such as a gas, atmosphere or liquid to which a specific amount of energy has been added to separate the component molecules into a collection of ions, electrons, free radicals, charge-neutral molecules, and other species in varying degrees of excitation. The term "blood plasma" refers to a saline and water carrier of the various blood fractions.

[0082] The non-thermal plasma used in this embodiment is a true non-thermal process, which disinfects or sterilizes biological fluids at the temperature of the host organism, but does not appear to pose a threat to the desirable properties of the fluids. For example, a five-log reduction in bacteria such as *Escherichia coli* and Salmonellae inoculated in biological fluids such as blood can be achieved with non-thermal plasma. As described in more detail below, hematology and morphology studies have shown that non-thermal plasma treatment does not cause significant changes in blood properties. The values for blood cell count, plasma hemoglobin, coagulation profile, and morphology of the treated blood samples were within the normal ranges following treatment.

[0083] In one embodiment of the present invention, it has been found that the reduction in pathogens living in the liquid being treated can be greatly enhanced if fine gas bubbles are introduced into the liquid being treated by the non-thermal plasma. The interaction of gas bubbles with the non-thermal plasma has been found to enhance the disinfection effectiveness by significantly enhancing the exposure of the host fluid to the highly reactive NTP species. The resulting liquid-gas mixture can include a gas dispersed in a liquid or a liquid dispersed in a gas. The gas can be mixed with the liquid in a variety of ways, such as by diffusion or injection. Various gas injection devices can be used, such as a Venturi tube gas injector made by Mazzei Injector Corporation. Alternatively, the liquid can be sprayed through the reaction chamber to form droplets of liquid separated by gas. In one embodiment, the liquid-gas mixture has a thickness along flow path **116** of 0.1 millimeters to 30 millimeters, for example. Other thicknesses can also be used. Reactor **100** can be constructed in various arrangements to expose the liquid-gas mixture to the plasma discharge for any treatment time, such as between 0.1 second to 10 minutes, for example. Other treatment times can also be used.

[0084] Introducing fine gas bubbles into the liquid greatly enhances the generation of plasma in reactor **100** for killing pathogens living in the liquid being treated. As the gas-liquid mixture is passed through NTP reactor **100**, the gas bubbles in the liquid become excited by the applied electric field, generating non-thermal plasma. The non-thermal plasma species then interact with and kill pathogens living in the liquid. Parameters associated with gas injection include composition of the gas, amount and distribution of the gas in the liquid, the size of the gas bubbles, velocity of the liquid relative to the physical motion of the gas, and the gas injector orifice size. Experiments have shown in liquid containing gas bubbles, especially with a gas containing 90% oxygen, bacteria kill is increased substantially as compared to the bacteria kill in liquid containing no gas bubbles.

[0085] Various factors that may affect the killing power of the reactive NTP species within reaction volume **106** include the ratio of gas to liquid (from very low to very high), size of gas bubbles, degree of mixing of gas and liquid, and compositions of the gas and liquid. Preferably, the system is adapted to obtain at least a one log, and more preferably at least a 5 log to 10 log reduction in pathogens living in the liquid. This is a highly controllable and scalable process. In general, the liquid flow rates as described in this embodiment are sufficient to permit the liquid to pass through consecutive NTP chambers, with a pathogen reduction of approximately one log per chamber, for example. Thus, liquid can cycle three, or five, or ten times, and the pathogen-inactivation effect is essentially proportional. A high gas-to-liquid ratio can be obtained by injecting the liquid into a gas phase. For example, it was observed that the killing power of the NTP species was greater with smaller gas bubbles than with larger gas bubbles. Also, it has been found that the more evenly the gas bubbles are distributed in the liquid, the more effective the non-thermal plasma generation and pathogen reduction. In one embodiment, the ratio of gas volume to liquid volume (Gas Volume/Liquid Volume) is preferably 0.1 to 20, more preferably 0.3 to 5, and most preferably 0.5 to 1. However, other ratios outside these ranges can also be used. A variety of gas compositions can be used, such as air, oxygen, ozone and nitrogen, or a mixture of these or other gases. One type of gas may be more effective than another in a particular application, depending on the type of liquid and the types of pathogens being killed. For example, the gas bubbles can consist of 100% by volume oxygen (e.g., O_2) or 100% by volume nitrogen.

[0086] **FIG. 2** is a diagram, which schematically illustrates a non-thermal plasma liquid pasteurization system **200** that introduces gas bubbles into the liquid according to one embodiment of the present invention. System **200** includes liquid source tank **202**, pump **204**, gas mixing device **206**, non-thermal plasma reactor **208**, high voltage power supply **210** and liquid receiving tank **212**. Source tank **202**, pump **204**, gas mixing device **206**, non-thermal plasma reactor **208** and receiving tank **212** are coupled in series with one another within a treatment flow path **214**, which can be formed of a series of tubes or other liquid channels for passing the liquid to be treated from one element in path **214** to the next.

[0087] Tank **202** contains the liquid to be treated. Pump **204** pumps liquid from tank **202** to tank **212**, through treatment flow path **214**. Additional pumps can be placed at various locations along treatment flow path **214** in alternative embodiments. Also, pump **204** can be eliminated in embodiments in which another mechanism, such as gravity, is used for moving the liquid along treatment flow path **214**. The output of pump **204** is coupled to the input of gas mixing device **206**. The flow rate of the pump is set based on factors such as the desired treatment time, the applied voltage, the dimensions/structures of reactor **208**, and the size of gas mixing device **206**. Gas mixing device **206** can include any device that is capable of introducing gas bubbles into the liquid flowing through treatment flow path **214**. Various mixing devices can be used, such as a gas diffuser or a gas injector. In one embodiment, gas mixing device **206** includes a Venturi tube injector. Other types of gas mixers can also be used. Gas mixing device **206** has a gas inlet **216** for receiving the gas to be mixed into the liquid.

[0088] The gas-liquid mixture is then provided to liquid inlet **220** of non-thermal plasma reactor **208**. Reactor **208** can include reactor **100** shown in **FIG. 1**, for example. High voltage power supply **210** is electrically coupled to the electrodes within reactor **208**. As the gas-liquid mixture passes through reactor **208**, from liquid inlet **220** to liquid outlet **222**, the non-thermal plasma generated in reactor **208** disinfects the liquid by destroying at least a portion of the live pathogens such as bacteria or viruses living in the liquid. The treated liquid then exits through liquid outlet **222** and is collected in receiving tank **212**.

[0089] In one embodiment, the liquid being treated within reactor **208** is kept under a pressure that is greater than an ambient pressure surrounding the reactor so as to maintain the gas bubbles substantially uniformly distributed in the liquid and of a small size. The pressure can be increased by providing liquid outlet **222** with a cross-sectional area that is less than the cross-sectional area of liquid inlet **222**. Also, the internal reactor flow path can be designed to provide a back pressure in the liquid and to provide turbulent flow.

[0090] **FIG. 3** is a diagram illustrating a Venturi tube injector **300**, which can be used for the gas mixing device **204** shown in **FIG. 2**. Injector **300** has a main flow path **302** between an inlet **304** and an outlet **306** and has a flow constriction **308**. A gas inlet **310** is coupled to the main flow path **302** at the flow constriction **308**. As liquid flows along main flow path **302** a pressure difference between inlet **304** and outlet **306** creates a vacuum inside the injector body, which draws gas into the injector through gas inlet **310** and results in a mixture of gas and liquid at outlet **306**. A Venturi tube injector is a high efficiency, differential pressure injector. It has been found that this type of injector mixes gases with liquids very well. As a result, bubbles in the gas-liquid mixture produced at the output of injector **300** are extremely fine and uniformly distributed.

[0091] **FIG. 4** is a diagram, which schematically illustrates a cross-sectional view of a non-thermal plasma reactor which has a winding, serpentine flow path and can be used for reactor **208** (shown in **FIG. 2**) according to one embodiment of the present invention. Reactor **400** includes a liquid-gas inlet **401**, a treated liquid-gas outlet **402** and a plurality of oppositely polarized non-thermal plasma electrodes **404** and **406** which are arranged to form a serpentine liquid flow path indicated by arrows **408**. As described above, each electrode **404** and **406** is physically and electrically isolated from the liquid flow path by a respective dielectric barrier. In one embodiment, electrodes **404** and **406** are each formed as a planar electrode panel that is parallel to and separated from the other electrode panels. Each electrode panel **404** and **406** has a polarity that is opposite to the polarity of the next adjacent electrode panel. This creates a plurality of reaction volumes, which are coupled together in series to form flow path **408**. Each reaction volume is defined by the gap between a respective pair of electrodes **404** and **406**. The serpentine flow path can be used to increase the liquid residence time within reactor **400** and to increase the turbulence of the liquid flow, which may assist in keeping the gas bubbles more evenly distributed and of a small size in the liquid. Any number of reaction volumes can be used in alternative embodiments. For example, reactor **400** can include a single reaction volume such as shown in **FIG. 1**, two reaction volumes that form a U-shaped flow path, or a plurality of reaction volumes as shown in **FIG. 4**. In an alternative embodiment, the individual reaction volumes extend parallel to one another from inlet **401** to outlet **402**.

[0092] **FIG. 5** is a cross-sectional view of a tubular non-thermal plasma reactor **500** according to an alternative embodiment of the present invention. Reactor **500** has a tubular structure, with flow going into or out of the page in **FIG. 5**. Reactor **500** includes a tubular ground electrode **502** and a wire high voltage electrode **504**, which is coaxial with electrode **502**. In an alternative embodiment, electrode **502** is a high voltage electrode and electrode **504** is a ground electrode. Electrodes **502** and **504** are separated by a gap, which defines a reaction volume **506**. Electrodes **502** and **504** are physically and electrically isolated from reaction volume **506** by respective dielectric barriers **508** and **510**. Dielectric barriers **508** and **510** prevent electrodes **502** and **504** from contaminating the liquid being treated and provide electrical isolation that prevents the liquid within reaction volume **506** from shorting electrode **502** to electrode **504**.

[0093] **FIG. 6** is a perspective, schematic view of a non-thermal plasma reactor **600** having narrow strip electrodes **602** and **604**. Electrodes **602** are biased at one polarity, and electrodes **604** are biased at an opposite polarity. Electrode strips **602** and **604** are arranged perpendicular to one another and are spaced about a reaction volume. Each individual electrode **602** and **604** is insulated by a dielectric barrier. For example, all of the electrodes **602** can be embedded within one sheet of dielectric material, and all of the electrodes **604** can be embedded within another sheet of dielectric material. With this type of electrode structure, the local electric fields around electrodes **602** and **604** are greatly enhanced, which ensures discharge takes place easily and effectively in the gas bubbles.

[0094] **FIG. 7A** is a side cross-sectional view of a non-thermal plasma reactor **700** according to another alternative embodiment of the present invention. Reactor **700** includes a housing **702** and at least one "surface" discharge electrode **704**. Housing **702** has a liquid inlet **706**, a liquid outlet **708** and a pair of flow paths **710** extending on either side of surface discharge electrode **704**. Surface discharge electrode **704** includes a plurality of adjacent conductors **712** and **714** having opposite polarity. Conductors **712** and **714** are electrically insulated from flow paths **710** by a dielectric mate-

rial **715**. In one embodiment, conductors **712** and **714** are each individually coated with a dielectric material that forms an electrically insulating sheath. In an alternative embodiment, conductors **712** and **714** are embedded in a dielectric material to form an electrode sheet. Conductors **712** and **714** can have diameters of about 0.1 to about 3.0 millimeters, for example, and are each separated by a gap in the range of 0 to 6 millimeters, for example.

[**0095**] Excitation of conductors **712** and **714** generates micro-current electric field discharge paths **716** along the surfaces of electrode **704**. Electric field discharge through discharge paths **716** generates non-thermal surface plasma species within the liquid being treated, along the surface of electrode **704**. These non-thermal surface plasma species are highly reactive and destroy pathogens living in the liquid, similar to the embodiments discussed above. Electrode **704** can have a variety of shapes, such as planar or tubular. **FIG. 7B** is a plan view of electrode **704** in planar form, which illustrates one possible arrangement of conductors **712** and **714**.

[**0096**] **FIG. 8** is a side view of a non-thermal plasma reactor **800** according to another alternative embodiment of the present invention. Reactor **800** includes fluid inlet **801**, fluid outlet **802**, electrodes **804** and **806** and dielectric barriers **808** and **810**. Electrodes **804** and **806** are separated from one another by a gap, which defines a reaction volume between dielectric barriers **808** and **810**. Reactor **800** further includes a sprayer **812**, which is coupled to fluid inlet **801** for receiving the liquid to be treated. Sprayer **812** spays the liquid through the reaction volume, between dielectric barriers **808** and **810** to form a fine mist within the reaction volume. The treated liquid then exits through liquid outlet **802**. Sprayer **812** assists in generating a gas-liquid mixture within the reaction volume, which helps the non-thermal plasma in destroying pathogens living in the liquid.

[**0097**] **FIG. 9** illustrates an NTP reactor **900** having a set of barriers used to increase the back pressure within the liquid being treated. Briefly referring back to **FIG. 2**, the stream of the gas-liquid mixture from gas mixing device **206** to reactor **208** is of high speed and high pressure. To some extent, the distribution of gas bubbles in the liquid depends on the back pressure of the mixture. The higher the back pressure, the higher the solubility of the gas in the liquid. In one embodiment, a large tank **202** can be used to increase the back pressure in the system.

[**0098**] In the embodiment shown in **FIG. 9**, the arrangement of electrode panels is used to increase the back pressure. As liquid is pumped through tube **901**, gas injector **902** draws gas into gas inlet **903** and produces a gas-liquid mixture at the outlet of the injector. Tube **904** delivers the gas-liquid mixture from gas injector **902** to inlet **908** of NTP reactor **900**. NTP reactor **900** has a plurality of electrode plates **905** and **906**, which are arranged to form a serpentine flow path from inlet **908** to outlet **909** and are arranged perpendicular to inlet **908**. With this arrangement, electrode plates **905** and **906** form barriers to the liquid stream entering from inlet **908** and being passed from one portion of the flow path to the next. These barriers further increase back pressure within the gas-liquid mixture.

[**0099**] **FIG. 10** is a simplified, perspective view of two mesh-type non-thermal plasma electrodes **920** and **922** that can be used for pasteurizing liquids according to another alternative embodiment of the present invention. Electrodes **920** and **922** are each formed of a conductive wire mesh, which has been coated with a dielectric material such that the wire mesh is electrically insulated from the liquid being treated. The dielectric coating is formed so that the area between each conductive segment in the mesh is open to fluid flow. Any coating technique can be used, such as physical vapor deposition (PVD) or chemical vapor deposition (CVD).

[**0100**] The liquid to be treated is passed through electrodes **920** and **922** in the direction of arrow **924**, substantially perpendicular to the planes formed by electrodes **920** and **922**. As the liquid passes through meshes **920** and **922**, electrodes **920** and **922** are electrically coupled to opposite voltage potentials, which creates a non-thermal plasma within gap **926** for treating the liquid present within the gap. If the openings in electrodes **920** and **922** are sufficiently small, the openings can further assist in breaking-up larger gas bubbles and maintaining the gas bubbles in the liquid at a sufficiently small size. Other arrangements can also be used, and meshes **920** and **922** can be non-planar. Also, a series of electrode pairs **920** and **922** can be used, wherein the liquid flows sequentially through each electrode pair for treatment. In an alternative embodiment, a gas injector or diffuser is not used to mix the gas and liquid. Rather, the gas is supplied through a tube into the reactor and is then broken into small bubbles as the gas and liquid are forced through the small openings in the mesh electrodes.

1. Experimental Results

[**0101**] Several experiments were performed to demonstrate the effectiveness of non-thermal plasma in reducing pathogens living in a biological fluid.

[**0102**] For example, the NTP pasteurization system shown in **FIG. 11** was built and used to treat fresh cow blood samples. System **1000** included five NTP reactors **1001** connected together in series with each NTP reactor **1001** having its own source tank **1002**, pump **1003** and gas injector **1004**. The outlet of each NTP reactor **1001** was coupled to the source tank **1002** of the next reactor **1001** in the series. The plurality of gas injectors **1004** ensured that the gas-liquid mixture contained sufficiently fine bubbles throughout the flow. Air was injected through each injector **1004** at 2 cubic feet per hour (CFH). Pumps **1003** pumped the liquid through system **1000** at 30 gallons per hour. The electrical connections to the NTP reactors **1001** were coupled together in parallel with one another and were excited at 20-30 kV alternating-current (AC) voltage.

[**0103**] Each reactor **1001** had a similar structure as that described with reference to **FIG. 4**, but with only two individual reaction volumes coupled in series with one another to form a serpentine flow path. Each electrode panel was formed of a thin, flat solid copper plate, which was attached to the backside of an epoxy resin board such that the copper plate was physically isolated from the liquid being treated. The epoxy resin board served as a dielectric layer, which had a thickness of 1.5 millimeters. The discharge gap between the dielectric layers, across the reaction volume, was 7 millimeters. The effective discharge volume for each leg of the serpentine flow path was 500 millimeters by 10 millimeters by 7 millimeters. These parameters were used as examples only and can be further modified or

optimized for treating a particular biological fluid in a particular manner with one or more NTP reactors for a particular gas, such as oxygen.

[0104] Each test was conducted with a one liter cow blood sample that was inoculated with more than five logs of either *Escherichia coli* or *Salmonella*. The tests were conducted under three conditions: (1) without gas injection; (2) with air injection; and (3) with oxygen injection. The output from each stage was sampled at source tanks **1002** to verify the reduction of bacteria at different stages of the system.

[0105] a. Inactivation of *Escherichia coli* and *Salmonella*

[0106] **FIG. 12** shows the level of inactivation of *Escherichia coli* and *Salmonella* in the blood samples. The test results in **FIG. 12** show that NTP treatment can cause five logs of *Escherichia coli* and *Salmonella* reduction under the condition outlined above. No difference in bacterial inactivation was found between *Escherichia coli* and *Salmonella*. Oxygen as a mixture gas media in biological fluids was more effective than air. One possible explanation is that air has more nitrogen, which requires higher ionization energy than oxygen, and therefore the concentration of energetic radicals in non-thermal plasma would be lower in air than in oxygen.

[0107] b. Change in Hematological Properties of Blood Samples

[0108] Blood is regarded as one of the most delicate biological fluids. Hematology and morphology studies were also performed on the samples to verify that non-thermal plasma treatment does not cause significant changes in the biologically active ingredients in blood. These studies included tests on blood specifications of hematology, such as morphology, blood cell count, blood plasma hemoglobin, and coagulation profile, the results of which are shown in **FIGS. 13-17**. The morphology of blood cells remained normal after treatment.

[0109] **FIG. 13** is a graph illustrating the red blood cell count as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen). **FIG. 14** is a graph illustrating the white blood cell count as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen). From **FIGS. 13 and 14**, it is seen that red blood cell count and white blood cell count did not change significantly. The small variations noticed in the test results may be caused by statistical error and the non-uniform distribution of blood cells.

[0110] **FIG. 15** is a graph illustrating blood plasma hemoglobin in milligrams per deciliter (mg/dl) as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen). The blood plasma hemoglobin increased after treatment. Although the hemoglobin increased after treatment, the blood plasma activated partial thromboplastin time (APTT) and fibrinogen showed no significant difference before and after treatment as shown in **FIGS. 16 and 17**.

[0111] **FIG. 16** is a graph illustrating blood plasma APTT in seconds as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen). Normal APTT for a cow is 6.8 to 9.2 seconds. The test on blood plasma APTT varied from 7.5-8.6 after NTP treatment. They are within the normal range of 6.8 to 9.2 seconds for ATPP in a cow.

[0112] **FIG. 17** is a graph illustrating hematology fibrinogen in grams per deciliter (g/dl) as a function of the number of NTP stages in the system and the type of injected gas (air or oxygen). The hematology fibrinogen values varied from 0.1-0.35 g/dl as a result of NTP treatment, which are also within a normal range of 0.1-0.7 g/dl for cow blood.

[0113] The conclusions from the above-experiments are that a five-log reduction of bacteria (*Escherichia coli* and Salmonellae) inoculated in cow blood can be achieved using the NTP system of **FIG. 11**, and the NTP treatments do not cause significant changes in blood hematology and morphology. Red and white blood cell counts do not change significantly. While the blood plasma hemoglobin increases after treatment, the values of blood plasma fibrinogen and APTT remain within normal ranges after treatment.

2. Coagulation of Blood and Self-Cleaning of NTP System

[0114] Coagulation of biological fluids is a normal phenomenon. After a specified period of use or after a particular treatment or set of treatments, the NTP system should be cleaned to deodorize the NTP system and to prevent different types of biological fluid samples treated by the same system from contaminating each other (i.e., cross contamination). The NTP system shown in **FIG. 11** and the systems of various other embodiments of the present invention lend themselves well to cleaning. The system can be flushed with sterile water to prevent a biological film from forming on the contacting surfaces of the system. During the water cleaning/flushing process, NTP system can be electrically operated to produce ozone and ozonated water within the system which aid in cleaning the reactors, pipes, pumps, etc. in the system. The ozone-aided self-cleaning process has been found to be very effective in cleaning and deodorizing the NTP system and in preventing cross contamination. The entire process of cleaning such blood-circulating apparatus is well-known in the art, having been practiced for many years for hemodialysis. The present invention utilizes the same principles and methods of between-use disinfection.

[0115] Also, the contacting surfaces of the system, such as the electrode surfaces, can be covered with a thin film coating to reduce biological fluid coagulation and biological film formation. Any biologically-safe low-friction coating can be used, such as polytetrafluoroethylene (PTFE) which is available under the tradename TEFLON from E.I. du Pont de Nemours and Company.

3. Separation of Gas From Blood or Other Biological Fluids

[0116] As discussed above, gas injection into the biological fluids being treated is helpful in inducing non-thermal plasma species that react with the pathogenic microorganisms within the fluids. It was shown that the injected gas, especially oxygen, is a helpful agent in promoting bacterial inactivation (See **FIG. 12**). Following NTP treatment it is often desirable for the injected gas to be separated from the treated biological fluid. The gas can be separated with a gas separator.

[0117] **FIG. 18** is a block diagram illustrating an NTP system **2000** having a gas separator according to one embodiment of the present invention. NTP system **2000** includes a raw sample receiving tank **2002**, a pump **2004**, a

gas injector **2006**, a gas source pump **2008**, an NTP reactor **2010**, a power supply and temperature monitor **2012**, gas-liquid separator **2014**, and pasteurized sample tank **2016**. In one embodiment, gas source pump **2008** can include an oxygen generator, for example. Gas separator **2014** can include any suitable liquid/gas separator, and can include a vacuum source if desired for assisting in the removal of gas from the liquid. These small separators are quite common, particularly in the fuel cell industry, where it is necessary to remove carbon dioxide from the electrolyte fluids, or in bioreactors for fermentation. They operate in a variety of ways, depending on the reliability level required: gravity (gas bubbles consolidate and rise to the top), centrifuge, and pressurized types (where a reduction in fluid pressure causes gas bubbles to rise rapidly). A common design is the VIG porous Teflon™ membrane separator for the environmental treatment industry, which uses a filter that will pass only gas through it, leaving the liquid behind. Yokota manufactures a "de-foaming pump" that utilizes a combination of centrifugal and pressure forces to continuously remove high volumes of bubbles from liquid.

4. Alternative Reactor Embodiments

[0118] **FIG. 19** is a diagram, which schematically illustrates a multiple-plate non-thermal plasma reactor **1500** according to another embodiment of the present invention. Reactor **1500** has a liquid source tank **1502** and a liquid outlet tank **1504**. Pump **1506** draws liquid **1508** from tank **1502** through tube **1510** and supplies the liquid to gas injector **1512** through tube **1514**. As liquid **1508** is pumped through gas injector **1512**, gas injector **1512** draws gas into gas inlet **1513** and produces a gas-liquid mixture at the outlet of the injector. Tube **1516** delivers the gas-liquid mixture to valves **1518**, which control flow to a plurality of parallel NTP reactor cells **1520**. The term "tube" as used in the specification and claims can include any conduit or passage formed of any suitable material and having any suitable cross-sectional shape.

[0119] Each cell **1520** has a reaction volume **1522** and a par of oppositely polarized electrodes **1524**, which are electrically and physically isolated from the reaction volume by dielectric barriers **1526**. Tubes **1528** deliver the gas-liquid mixture to reaction volumes **1522** for treatment. Dashed lines **1530** represent the upper surfaces of the gas-liquid mixtures in each reaction volume. Spacers **1527** define the height of reaction volumes **1522**, between opposing surfaces of dielectric barriers **1526**.

[0120] High voltage power supply **1540** delivers electrical excitation energy to electrodes **1524** through conductors **1541** for generating non-thermal plasma within reaction volumes **1522**. In one embodiment, power supply **1540** delivers an AC voltage of 5 kV to 30 kV at a frequency of 1 Hz to 1000 Hz, for example. Other voltages and frequencies can also be used. The treated gas-liquid mixture **1530** is then returned to tank **1504** through tubes **1542**. Electrodes **1524** and dielectric barriers **1526** can have any structure and gap size, such as those disclosed in the present application. Any number of parallel NTP reactor cells **1520** can be used in alternative embodiments of the present invention.

[0121] **FIG. 20** is a diagram that schematically illustrates a two-dielectric barrier NTP reactor **1600** having a discharge initiation region according to another alternative embodiment of the present invention. The same reference numerals are used in **FIG. 20** as were used in **FIG. 19** for the same or similar elements. In this embodiment, a film or plate **1602** divides reaction volume **1522** into a treatment region **1604** and a discharge initiation region **1606**. Film **1602** is suspended in the space between dielectric plates **1526** by spacers **1608**, for example. Tube **1516** delivers the gas-liquid mixture **1530** into treatment region **1604**, and tube **1542** returns the treated gas-liquid mixture to tank **1504**. Film **1602** contains gas-liquid mixture **1530** in treatment region **1604** and prevents the gas-liquid mixture from entering into discharge initiation region **1606**. Discharge initiation region **1606** can be filled with various gases, such as air, another gas or a gas mixture. Discharge initiation region **1606** can also be substantially void of any gas and held under a vacuum at below-normal atmospheric pressure. In this embodiment, electrodes **1524** are parallel plates, and discharge initiation region **1606** and treatment region **1604** are rectangular volumes.

[0122] In one embodiment, film **1602** is formed of a dielectric material, such as a transparent membrane of polytetrafluoroethylene from E.I. du Pont de Nemours and Company. In alternative embodiments, film **1602** can be formed of a transparent epoxy resin or other types of film or sheet materials. Film **1602** has good dielectric properties and allows one or more of the non-thermal plasma species to pass from discharge initiation region **1606** to treatment region **1604**. However, film **1602** should not allow the gas-liquid mixture **1530** to pass into discharge initiation region **1606**. Film **1602** can also be non-dielectric, as long as there is at least one other dielectric barrier between electrodes **1524**. Film **1602** can also include an ion-selective membrane. In one embodiment, film **1602** is made as thin as possible and transparent so as to limit absorption or reflection of the non-thermal plasma species passing through to treatment region **1604**. For example, film **1602** can have a thickness between 0.02 millimeters to 1 millimeter. Smaller or larger thicknesses can also be used. The surfaces of film **1602** can be hydrophilic or hydrophobic. Examples of commercially available films that are effective include CS Hyde 56-10—a Teflon coated fabric that is 10 mils thick and is tan in color, TetraTex Pourous PTFE—a PTFE membrane that is white in color, and a Spetrum Lab filter disk—a 70 mm diameter Teflon mesh filter media that is semi-translucent. Other films can also be used.

[0123] During operation, electrodes **1524** are energized. The resulting electrical field between the electrodes generates non-thermal plasma species within regions **1604** and **1606**. Non-thermal plasma species within region **1606** are easily generated, and the discharge across region **1606** is fairly uniform. This assists in generating more consistent and uniform non-thermal plasma species within treatment region **1604**. Without discharge initiation region **1606**, it has been found that the discharge within the gas-liquid mixture **1530** can be inconsistent or non-uniform, depending on the particular apparatus. The NTP species generated within initiation region **1606** that pass into treatment region **1604** react with the gas-liquid mixture to kill more evenly and consistently pathogens living in the liquid. Film **1602** also protects the upper electrode **1524** and the upper dielectric barrier **1526** from contamination or staining by gas-liquid mixture **1530**.

[0124] In addition, the discharge initiation region **1606** can be used to limit the generation of ozone more easily in applications where ozone is not desired. This region can be filled with a gas other than air, such as nitrogen, carbon dioxide or another gas, and still provide an effective treatment of any live pathogens in the liquid. In these embodiments, gas injector **1512** can be used to inject a gas other than air to further limit the generation of ozone. However, air can also be used if desired. Discharge initiation region **1606** can also be held under a small vacuum to further limit the amount of gas in the region and therefore the amount of ozone that is generated.

[0125] In an alternative embodiment, NTP reactor **1600** further includes a gas source **1620**, which supplies gas to discharge initiation region **1606** through tube **1622**. In addition, a tube **1624** can by coupled between discharge initiation region **1606** and gas inlet **1513** of gas injector **1512**. During operation, gas injector **1512** draws gas containing the non-thermal plasma species from initiation region **1606** into gas inlet **1513** to further enhance the mixture of non-thermal plasma species in the liquid being treated. Gas source **1620** replaces the gas drawn out of discharge initiation region **1606**. In another embodiment the NTP species generated in region **1606** is mixed with the gas-liquid mixture **1530** at the outlet of NTP cell **1520**. Mixing can be accomplished through a gas injector similar to injector **1512**, a diffuser or any other apparatus or method that forces or assists in the NTP species passing through or contacting the treated liquid.

[0126] In a further embodiment (not shown in **FIG. 20**), a second dielectric film **1602** is positioned on the other side of treatment region **1604**, between treatment region **1604** and bottom dielectric barrier **1526**. The second dielectric film can be spaced from the bottom dielectric barrier **1526** by a further discharge initiation region **1606**, such that both sides of treatment regions **1604** have a discharge initiation region **1606**.

[0127] One or more of the dielectric barriers **1526** and **1602** can be eliminated as long as there is at least one dielectric barrier between electrodes **1524**. For example, both dielectric barriers **1526** can be eliminated such that dielectric film **1602** serves to separate regions **1604** and **1606** and as the sole dielectric material between electrodes **1524**. In yet a further embodiment, dielectric film **1602** is eliminated and one or both of the dielectric barriers **1526** are spaced from their respective electrodes **1524**. In this embodiment, the liquid being treated will still have no direct contact with electrodes **1530**, and the spaces between dielectric barriers **1526** and their respective electrodes **1524** can be used as discharge initiation regions similar to region **1606**. Also, the gas injector can be eliminated in alternative embodiments of reactor **1600** and in alternative embodiments of the reactors shown in the various other figures or discussed herein.

[0128] **FIG. 21** is a diagram, which illustrates an NTP reactor **1700** according to another alternative embodiment of the present invention. Again, the same reference numerals that are used in **FIG. 21** as were used in **FIGS. 19-20** for the same or similar elements. In this embodiment NTP cell **1520** has a dielectric film **1602**, which separates gas-liquid mixture **1530** from discharge initiation region **1606** and a bare metal electrode **1702**. The upper dielectric barrier **1526** (shown in **FIG. 20**) adjacent the upper electrode **1524** has been removed. In another embodiment, the lower dielectric barrier **1526** can also be removed such that dielectric film **1602** serves as the main dielectric barrier between electrodes **1524**.

[0129] **FIG. 22** is a diagram, which schematically illustrates an NTP reactor **1800** according to another embodiment of the present invention. NTP reactor **1800** is similar to NTP reactor **1600** shown in **FIG. 20**, but has no dielectric film **1602**. Reaction volume **1522** has a height **1802** that exceeds the height **1804** of the gas-liquid mixture **1530** flowing through reaction volume **1522** to create a gap **1806** between the upper surface of mixture **1530** and the bottom surface of the upper dielectric barrier **1526**. As long as the gap **1806** is maintained during operation, the gap can serve as a discharge initiation region. The gap can be maintained by controlling or otherwise setting the volume flow of gas-liquid mixture **1530** through the inlet and outlet of reaction volume **1522** such that the gas-liquid mixture remains confined to the treatment region. Gap **1806** can be filled with air or any other suitable gas.

[0130] **FIGS. 23-25** show the electrode structure of one of the NTP cells **1520** shown in **FIGS. 19-22**, according to one embodiment of the present invention. **FIG. 23** is a top plan view of the NTP cell **1520** in which upper electrode **1524** and upper dielectric barrier **1526** are partially cut-away to expose a portion of bottom dielectric barrier **1526**. **FIG. 24** is a cross-sectional view of NTP cell **1520**, taken along lines **24-24** of **FIG. 23**. **FIG. 25** is a cross-sectional view of NTP cell **1520** taken along lines **25-25** of **FIG. 23**.

[0131] In **FIGS. 23-25**, dielectric film **1602** is removed for clarity. A pair of opposing end spacers **1608** and **1609** and opposing sidewall spacers **1906** define the reaction volume between the upper and lower dielectric barriers **1526** and contain the gas-liquid mixture being treated. End spacer **1608** has a plurality of passages **1902** (shown in dashed lines in **FIG. 23**) for passing the gas-liquid mixture from tube **1516** (shown in **FIGS. 19-22**) to the reaction volume. End spacer **1609** (**FIG. 17**) has similar passages **1902** for passing the treated gas-liquid mixture to tubes **1542** (shown in **FIGS. 19-22**).

[0132] Within reaction volume **1522**, upper surface of the lower dielectric barrier **1526** can include a plurality of raised ridges or separating walls **1910** that maintain a dispersed flow of the gas-liquid mixture through reaction volume **1522**. Separating walls **1910** define a plurality of recessed channels **1912** along which the gas-liquid mixture flows. Separating walls **1910** can have heights that are equal to the height of reaction volume **1522** or less than the height of reaction volume **1522**. Spacers **1608**, **1904**, and **1906** and separating walls **1910** can be formed of the same material as dielectric barrier **1526** or from different material.

[0133] **FIG. 26** is a diagram, which schematically illustrates an NTP reactor **2200** according to another alternative embodiment of the present invention. Again, the same reference numerals are used in **FIG. 26** as were used in **FIGS. 19-25** for the same or similar elements. NTP reactor **2200** has a cylindrical NTP cell **2202** having a central axis **2204**, which is oriented normally (i.e., vertically) with respect to the floor on which reactor **2200** is supported and therefore parallel to the gravitational forces of the earth. NTP cell **2202** has a lower end **2216**, an upper end **2218**, a

cylindrical inner stainless steel ground (or alternatively high voltage) electrode **2206**, a cylindrical inner dielectric barrier **2208** and a cylindrical outer high voltage (or alternatively ground) electrode **2210**. Cell **2202** has an inlet **2212** and an outlet **2214** located at the bottom end **2216** of cell **2202**. The space between the outer diameter of dielectric barrier **2208** and the inner diameter of high voltage electrode **2210** forms a reaction volume **2222** within which gas-liquid mixture **1530** is treated.

[**0134**] Tube **1516** is coupled between valve **1518** and inlet **2212**. The interior of cylindrical ground electrode **2202** and dielectric barrier **2208** serves as a passageway **2220** for delivering gas-liquid mixture **1530** (shown in dashed lines) to top end **2218** of NTP cell **2202**. As gas-liquid mixture **1530** exits the top of passageway **2220**, the gas-liquid mixture falls through reaction volume **2222** due to the force of gravity. The treated gas-liquid mixture **1530** then exits outlet **2214** and returns to tank **1504** through tube **1542**. The falling gas-liquid mixture **1530** maintains the mixture of gas and liquid and increases the surface area of the liquid that is exposed to the NTP species. This can further increase the effectiveness of the NTP treatment. Alternatively, inlet **2212** can be positioned at upper end **2218**.

[**0135**] NTP cell **2202** further includes a cylindrical dielectric film **2230**, which separates reaction volume **2222** into a treatment region **2232** and a discharge initiation region **2234**. Discharge initiation region **2234** can be filled with a gas or a vacuum, as discussed above, and is physically isolated from the gas-liquid mixture being treated in region **2232**. In an alternative embodiment, initiation region **2234** is positioned between treatment region **2232** and electrode **2210**. Additional discharge initiation regions can also be used, as discussed above.

[**0136**] **FIG. 27** is a cross-sectional view of a cylindrical NTP cell **2300** according to an alternative of the present invention. **FIG. 28** is a cross-sectional view of NTP cell **2300** taken along lines **28-28** of **FIG. 27**. The same reference numerals are used in **FIGS. 27 and 28** as were used in **FIG. 26** for the same or similar elements. NTP cell **2300** is similar to NTP cell **2202**, but further includes an outer cylindrical dielectric barrier **2302** positioned between reaction volume **2222** and the inner diameter of outer electrode **2210**.

[**0137**] **FIG. 29** is a diagram, which schematically illustrates an NTP reactor **2500** in which NTP cell **2300** (shown in **FIGS. 27 and 28**) can be used. Again, the same reference numerals are used in **FIG. 29** as were used in **FIG. 26** for the same or similar elements.

[**0138**] **FIG. 30** is a diagram, which illustrates a conical NTP reactor **2600** according to another alternative embodiment of the present invention. Reactor **2600** includes a conical NTP cell **2600** having a conical inner electrode **2604**, a conical inner dielectric barrier **2606**, a conical outer dielectric barrier **2608** and a conical outer electrode **2610**. The space between the outer diameter of dielectric barrier **2606** and the inner diameter of dielectric barrier of **2608** defines a reaction volume **2612** through which gas-liquid mixture **1530** passes for treatment. NTP cell **2602** has a central axis **2614**, which is aligned vertically similar to the NTP cells shown in **FIGS. 26-29**. Inlet **2212** is positioned at the base of cell **2602**, and includes a passage **2614**, which extends through the interior of conical electrode **2604** to the top of reaction volume **2612**. In an alternative embodiment, inlet **2212** is positioned at the top of NTP cell **2602**. Dielectric barriers **2606** and **2608** isolate electrodes **2604** and **2610** from the gas-liquid mixture **1530** within reaction volume **2612**.

[**0139**] In alternative embodiments, the cylindrical or conical NTP cells shown in **FIGS. 26-30** can further include one or more dielectric films and discharge initiation regions similar to those shown or described with reference to **FIGS. 20 and 21**. Also, the cylindrical or conical dielectric barriers can be spaced from their respective electrodes to provide one or more discharge initiation regions between the electrodes and dielectric barriers.

[**0140**] **FIG. 31** illustrates a non-thermal plasma reactor **2700** in which the liquid is sprayed into the reaction volume, according to another alternative embodiment of the present invention. Reactor **2700** has an NTP cell **2702**, which includes vertically aligned electrode plates **2704** and **2706**, dielectric barriers **2708** and **2710** and reaction volume **2712**. A spraying nozzle **2714** is positioned at a top end **2716** of reaction volume **2712** as is coupled to valve **1518** through tube **1528**. Spraying nozzle **2714** sprays the liquid **1508** through reaction volume **2712**, between dielectric barriers **2708** and **2710** to form a fine mist **2718** within the reaction volume. Gravity pulls the liquid droplets in mist **2718** downward toward outlet **2720** at which the liquid droplets are returned to tank **1504**.

[**0141**] Any of the reactor cell structures discussed in the present application can be used in the NTP reactor **2700** in alternative embodiments of the present invention. NTP cell **2702** can have parallel plate electrodes or concentric cylindrical electrodes, for example, and can have one or more discharge initiator regions as discussed above.

[**0142**] **FIG. 32** is a diagram, which illustrates an NTP reactor **2800** according to another alternative embodiment of the present invention. The same reference numerals are used in **FIG. 32** as were used in **FIG. 31** for the same or similar elements. Similar to the embodiment shown in **FIG. 31**, NTP reactor **2800** includes an NTP cell **2802** having vertically aligned electrode plates **2704** and **2706**, dielectric barriers **2708** and **2710** and reaction volume **2712**. In addition, NTP cell **2802** includes a pair of dielectric films **2804** and **2806**, which separate reaction volume **2712** from dielectric barriers **2708** and **2710**, respectively. The space between dielectric film **2804** and dielectric barrier **2708** forms a discharge initiation region **2808**. Similarly, the space between dielectric film **2806** and dielectric barrier **2710** forms a discharge initiation region **2810**.

[**0143**] NTP cell **2802** further includes a thin curtain-forming tube **2812**, which is coupled to tube **1528** at the top end **2816** of cell **2802**. As tube **1528** delivers liquid **1508** to curtain-forming tube **2812**, the liquid falling from tube **2812** forms a "curtain"**2820** of liquid through reaction volume **2712**. The curtain of liquid **2820** significantly increases the surface area of the liquid that is exposed to the NTP species and encourages mixing of the liquid with the surrounding gas in reaction volume **2712**. The treated liquid returns to tank **1504**. Curtain forming tube **2812** can include a horizontal tube with holes in the bottom or with overflow openings along the sides of the tube to form the curtain of liquid. Other structures can also be used to form a continuous or intermittent liquid "curtain".

5. Further Experimental Results

[0144] Further experiments were conducted on animal blood samples using a variety of the further reactor embodiments discussed above, and with various operating conditions. For example in one experiment, NTP system **3300** shown in **FIG. 33** was used as a test apparatus. System **3300** included a liquid source tank **3302**, a pump **3302**, NTP reactor **3306** and power supply **3308**. Tube **3310** coupled tank **3302** to pump **3304**, and tube **3312** coupled pump **3304** with an inlet of reactor **3306**. Tube **3314** coupled an outlet of reactor **3306** with tank **3302**.

[0145] Reactor **3306** had a pair of oppositely polarized electrodes **3320** and **3322**, which were separated by a reaction volume **3326**. Dielectric barriers **3328** and **3330** electrically and physically isolated electrodes **3320** and **3322** from reaction volume **3326**. Electrodes **3320** and **3322** had an area of 920 mm×52 mm, and were separated by a discharge gap of 16 mm. Dielectric barriers **3328** and **3330** had thicknesses of 1.5 mm.

[0146] Pump **3304** drew liquid **3336** from tank **3302** through tube **3310** and passed the liquid to reaction volume **3326** through tube **3312**. Tube **3314** returned the treated liquid to tank **3302**. The upper surface of liquid **3336** in reaction volume **3326** is shown by the dashed line. The volume above the dashed line served as a discharge initiation region. In this experiment, reaction volume **3326** did not include a membrane for separating the discharge initiation region. Also, a gas injector was not used for creating a liquid-gas mixture. However, other experiments were performed with these additional elements, and achieved positive results.

[0147] During the experiment, three separate tests were performed. For each test a sample of six hundred milliliters (ml) of fresh cow blood was placed in tank **3302**. Each sample included 10 standard units per milliliter (u/ml) of heparin anti-coagulant, and was inoculated with *E. coli* 933 prior to treatment. Each sample had a different total bacteria count. Power supply **3308** energized electrodes **3320** and **3322** with an AC voltage of 16-22 kV at a frequency of 60 Hz. Pump **3304** passed the sample through reactor **3306** at a flow rate of 250 milliliters per minute (ml/min).

[0148] The samples were treated for up to three hours, which deviated from the experiments discussed above. In those experiments, a shorter treatment time and a higher electric field were used. It was found that when a high electric field (such as 30 kV) was used, the blood samples coagulated very fast, especially along the surface layer of the sample. In the present experiment, lower energy and longer treatment times were used, which were expected to reduce any adverse impacts on the quality of the treated samples.

[0149] Another factor in the present experiment was that the sample was circulated many times through reactor **3306**, which inevitably caused recontamination. This, in turn, prolonged the needed treatment time for a given reduction in bacterial count and resulted in unnecessary multiple exposures of the samples to the treatment, which increased potential damage due to the treatment. Although this kind of treatment may not be the most desirable for a practical embodiment, it allowed for a relatively simple and small experimental setup. If re-circulation and re-contamination can be avoided, the treatment time would be substantially reduced. For example, the reactor can be made with a longer reaction path for conducting one-pass experiments.

[0150] a. Inactivation of *Escherichia coli* (*E. coli*)

[0151] The following table shows the level of *E. coli* bacterial reduction for the present experiment as a function of treatment time for each sample.

	ORIGINAL BACTERIAL COUNT	REDUCTION AFTER 1 HOUR	REDUCTION AFTER 2 HOURS	REDUCTION AFTER 3 HOURS
SAMPLE 1	1.1×10^6	75%	99%	99.5%
SAMPLE 2	1.76×10^6	86%	98.5%	99.6%
SAMPLE 3	6.85×10^5	—	—	99.8%

[0152] **FIG. 34** is a graph illustrating bacterial inactivation for each sample as a function of treatment time in hours. These results show that a 3-log reduction of *E. coli* bacteria inoculated in cow blood can be achieved using the simple test apparatus shown in **FIG. 33**.

[0153] b. Change in Hematological Properties of Blood Samples

[0154] **FIG. 35** is a graph illustrating concentration of blood plasma hemoglobin in the blood samples as a function of treatment time for the apparatus shown in **FIG. 33**. The concentration of blood plasma hemoglobins increased with treatment time.

[0155] **FIG. 36** is a graph illustrating concentration of white blood cells (WBCs) in the blood samples as a function of treatment time, and **FIG. 37** is a graph illustrating concentration of red blood cells (RBCs) in the blood samples as a function of treatment time. The white and red blood cell concentrations remained within the normal reference ranges for these properties following treatment.

[0156] **FIG. 38** is a graph illustrating concentration of Total Plasma Protein (TPP) in the blood samples as a function of treatment time. The Total Plasma Protein concentration changed very little after treatment, and varied only from 7.2 g/dL to 8.0 g/dL, which is within the normal range of 6.8 g/dL to 9.2 g/dL for cow blood.

[0157] **FIG. 39** is a graph illustrating concentration of fibronigen in the blood samples as a function of treatment time. Again, the fibronigen concentration varied within the allowed range, except after three hours of treatment.

[0158] c. Treatment of a Virus

[0159] The test apparatus shown in **FIG. 33** was also used successfully to treat a virus in water samples. The water samples contained 5.5×10^3 units per milliliter of Infection Bovine Rhinotracheitis (IBR) virus. A first water sample was passed through NTP reactor **3306** four times, without being re-circulated through a common tank as in **FIG. 33**. No virus could be detected in the first water sample after the treatment. A second water sample was circulated through the reactor and a common tank **3302** for a period of 40 minutes. Again, no virus could be detected in the second water sample after the treatment. The NTP treatments were therefore effective for killing the IBR virus, even with very brief treatment as in the case of the first water sample.

[0160] Non-thermal plasma can therefore be used for at least partially disinfecting biological fluids, such as human and animal blood while leaving most of the hematological properties of the blood within normal ranges following treatment. Any adverse affects on these properties can be reduced by further optimization of the system and its operating parameters.

[0161] Since there is substantially no ohmic heating, energy consumption during non-thermal plasma treatment is small, and there is no need to cool the liquid being treated. This allows the system to be easily scaled-up accommodate a very large treatment volume. The desired treatment time can be obtained by passing the liquid through multiple NTP reactors connected together in series with one another, by increasing the reaction volume length, decreasing the flow rate, or by cycling the liquid through the same reactor multiple times. Also, the number of series-connected reaction volumes in the same reactor can be increased or decreased. Because of the non-thermal nature of the system, the system preserves the quality and other heat-sensitive attributes of the liquid. Also, since each electrode is physically and electrically isolated from the liquid being treated, the electrodes do not act as a source of contaminants to the liquids.

[0162] In effect, the non-thermal plasma utilizes several different species to inactivate pathogen, including highly reactive species that stabilize immediately upon removing the electric field, some ozone, ultraviolet, photons, and the like. These species do not appear to lead to unsafe excess concentration levels, which would be the case with exclusive exposure to higher levels of any one of these inactivation mechanisms alone. Although ozone is one of the highly reactive species that is generated, the concentration is more limited and the species are very short-lived as compared to more traditional ozonation systems. Lingering ozone may be an advantage for purifying bottled water, but in biological fluids, control of all biochemical process is critical. Non-thermal plasma species virtually all resolve themselves and lose reactivity immediately upon removal of the electric field, making it essentially totally controllable by the treating medical specialist. This allows non-thermal plasma to be used for more time-sensitive treatments of biological fluids where long time periods are not generally available for reducing excess ozone levels in the treated fluid.

[0163] Non-thermal plasma can therefore be an effective method of disinfecting biologic fluids using processes that are either replacements for or adjunctive to traditional antibiotic and anti-viral therapies. For example, blood or cerebrospinal fluid can be treated with non-thermal plasma to eliminate bacteria such as *staphylococcus* or viruses without toxicity to the characteristics of the fluids. Another clinical application would be to couple an NTP system in series with a renal dialyzer. Non-thermal plasma can be used to treat severe blood-borne infections when drugs are not indicated, or to disinfect donated blood in blood bank storage, increasing the usable supplies. Treatment can be performed as the blood is drawn from the donor, at the blood bank, or as the blood is transfused into the receiving patient. It is also possible that non-thermal plasma can potentially be used as an adjunctive (supporting) therapy to assist with the treatment of bacterial meningitis, where there is a need to stay ahead of the rapid bacterial reproduction and spread without poisoning the patient with the bacterial toxins.

[0164] Further examples of possible non-thermal plasma treatments include: 1) treatment of peritonitis (quasi-dialyzing by injecting sterile water or other dialysate compositions into the peritoneal cavity and cycling the water through an endoscope or catheter having an associated internal or external NTP reactor); 2) treatment of Septicemia (e.g., by coupling an NTP reactor in series with a hemodialysis apparatus); 3) treatment of a liver or gall bladder abcess endoscopically, such as by cycling bile, etc. through a lumen in the endoscope and treating the bile with non-thermal plasma; 4) treating hepatitis by injecting sterile saline or other fluidic agents into the liver and processing liver fluids in vivo with non-thermal plasma; 5) treating viral or bacterial meningitis; and 6) treating lymph by processing the lymph with non-thermal plasma and feeding it back into the bloodstream through the thoracic duct, for example. Numerous other applications exist.

[0165] For example, when blood is treated using the hemodialysis-type blood pasteurization system described by **FIGS. 2, 18** and **42**, for example, which could utilize any of the different described NTP reactor chambers, in conjunction with the other circulating and filtering mechanisms shown, the blood is diluted for improved flow and clotting resistance. After treatment, the diluted blood is applied to a semi-permeable membrane having openings for filtering-out the diluent, common blood-born bacteria and/or other toxins in the blood, as described in more detail with respect to **FIGS. 18 and 42**.

[0166] Non-thermal plasma can also be used in conjunction with a single, double or triple lumen probe or catheter, for example, with non-thermal plasma electrodes on the distal end or positioned along one or more of the lumens. These probes can be inserted endoscopically into a diseased organ, an artery or vessel, for example, and local fluids can be treated with non-thermal plasma by positioning the electrode adjacent the area in the body to be treated or by pumping bodily fluids through reaction volume within a lumen to destroy undesirable elements, in place of open surgery. The electrodes can be placed directly on inflamed tissue to shower the tissue with reactive species (e.g., oxygenated radicals, etc.) while they are still reactive. For example, a surface non-thermal plasma electrode (with alternating-polarity conductors along its surface) can be used to generate a surface non-thermal plasma that can be used to treat the tissue. This surface non-thermal plasma electrode can be positioned on the distal end of an instrument, for example, which is inserted into the body. Other structures and treatment methods can also be used.

[0167] **FIG. 40** is a diagram, which schematically illustrates an elongated probe **4000** that can be used for in-vivo NTP treatment according to one embodiment of the present invention. The shaft can include any medical instrument, catheter or endoscope-type device, with or without an internal lumen, which can be used to access an internal cavity, such as the interior of a hollow organ or lumen, of a mammal. In this example, probe **4000** includes a proximal end **4002**, a distal end **4004**, an NTP reactor **4006** carried along the shaft, and a pump **4008**. When the shaft of probe **4000** is inserted into an organ, artery or other region of a patient, pump **4008** draws fluid from inlet **4010** and passes the fluid through NTP reactor **4006** to outlet **4012**. The treated fluid is then returned to the patient from outlet **4012**. Inlet **4010** and outlet **4012** can be positioned anywhere along

probe **4000**, and the particular arrangement would depend on the procedure being performed and the desired flow pattern into and out of the shaft. Similarly, NTP reactor **4006** and pump **4008** can be positioned anywhere on or in probe **4000** or external to shaft **4000**. Also, pump **4008** can be positioned anywhere along the fluid travel path between inlet **4010** and outlet **4012**. Probe **4000** can be a stand-alone device for NTP treatment or can be combined with the function of another medical instrument. Probe **4000** can have one or more lumens for passing the fluid to be treated and can have additional lumens or elements as desired.

[**0168**] **FIG. 41** is a diagram, which schematically illustrates a probe **4100** that can be used for in-vivo NTP treatment according to another embodiment of the present invention in which no pump is needed. Probe **4100** includes a shaft with a proximal end **4102**, a distal end **4104** and an NTP reactor **4106**. Similar to the embodiment shown in **FIG. 16**, for example, reactor **4106** includes oppositely polarized electrodes **4108** and **4110**, a discharge initiation region **4112**, a treatment region **4114** and a separating film **4116**. However, electrode **4110** is formed of a wire mesh, which is supported on the distal end of the shaft by one or more support beams **4118**, for example, to promote the flow of fluid into and out of treatment region **4114**. With this structure, the perimeter of treatment region **4114** is open to an exterior of probe **4100**. Electrode **4110** can be bare conductive material or coated with a dielectric material, for example. Film **4116** isolates discharge initiation region **4112** from treatment region **4114**. In one embodiment, discharge initiation region **4112** is bounded by electrode **4108**, film **4116** and the sidewall of probe **4100**. Fluid entering treatment region **4114** is exposed to the NTP species generated across treatment region **4114** and those species that are generated in initiation region **4112** and pass through film **4116**. During treatment, the distal end **4104** of probe **4100** can be moved in and around the area of the body to be treated. For example, the distal tip can be swept through a locally infected area such as a liver, lung, or abdomen for killing sufficient concentrated pathogens to reduce the viral load to a level that is within the body's immune system capacity. Such an instrument could also process blood flowing into the vena cava from the lymph system.

[**0169**] Numerous other instrument structures can be used for facilitating in-vivo NTP treatment in further alternative embodiments of the present invention. The fact that the NTP species only remain active during roughly the time when the electrical field is powered can help to limit adverse effects of the treatment.

[**0170**] **FIG. 42** is a diagram illustrating one system and method **4200** for ex-vivo disinfecting blood of a live human or animal patient using non-thermal plasma according to another embodiment of the present invention. System **4200** is a circulating ex-vivo hemodialysis-type of apparatus, which draws blood from the circulatory system of a live patent **4201** through one or more arteries, for example, treats the blood with non-thermal plasma and returns the disinfected blood to the patient. The NTP system can achieve substantial reduction in bacterial or viral loads with fewer attendant complications, such as clotting, excess destruction of desired blood fractions, antibiotic resistance, etc. This ex vivo blood treatment could filter out bacterial toxins released before returning the blood to the body because of the disparity in size between bacteria and its products, and the essential solid blood fractions. After treatment, any additional required fluids and lipids can be provided via intravenous (I.V.) application.

[**0171**] NTP system **4200** includes a blood pump **4202** for drawing blood from patient **4201** through a shunt **4204** and a tube **4206**. An arterial pressure monitor **4208** can be coupled to tube **5206** for monitoring pressure within the patient's circulatory system during the procedure. A further pump **4210** pumps the blood drawn from patient **4201** by blood pump **4202** to a reservoir tank **4212** through tubes **4214** and **4216**. In one embodiment, a temperature-conditioned diluent **4220** is added to the blood along tube **4214** until the blood is translucent, for example. Diluent **4220** can include sterile water, an anti-coagulant (such as heparin) and other elements as needed. Reservoir tank **4212** stores the diluted blood for treatment. The diluent makes the fluid less opaque so that photons generated in reactor **4230** can have better effect, and also improves the flow characteristics and reduces potential clotting levels within the apparatus.

[**0172**] The diluted blood passes from tank **4212** to NTP reactor **4230** through tube **4232** and gas injector **4234**. Gas injector **4234** injects a gas into the diluted blood as described above with respect to **FIGS. 2 and 3**, for example. NTP reactor **4230** can have any suitable structure. In one embodiment, NTP reactor **4230** is similar to reactor **1600** shown in **FIG. 22**, which has separate treatment and discharge initiation regions. The treatment region of NTP reactor **4230** can have multiple parallel paths for the diluted gas-blood mixture to optimize throughput and can have consecutive chambers to increase disinfection in each cycle. As many consecutive chambers can be used as are safe and efficacious. NTP reactor **4230** is driven by a continuous A.C. power supply **4236** having an output voltage of 5 kV to 35 kV and a frequency of 1 Hz to 1000 Hz, for example, to generate cool non-thermal plasma as the diluted gas-blood mixture passes through the reactor.

[**0173**] After NTP treatment application by reactor **4230**, the diluted gas-blood mixture is filtered to remove the diluent and the physically small toxin molecules while preserving the red and white blood cells and other beneficial blood components. For example, the diluted gas-blood mixture can be pumped under pressure through tube **4240** and past a semi-permeable membrane within filter **4242**. The membrane passes some of the diluent, which carries with it small particles such as bacteria and toxic protein molecules but not all blood plasma and not the larger molecules such as erythrocytes, leukocytes, platelets, etc. The pore size and other characteristics of the membrane can be selected as desired for the particular procedure being performed. In one embodiment, the pores range in size from 0.1 to 2 micrometers, for example, which are capable of passing the most common blood-borne bacteria. *Staphylococcus* molecules are spherically-shaped, and about 0.75 micrometers in size, while *E-coli* are elongated "rounded cylinders" about 0.5 micrometers in diameter by 2 micrometers in length. The toxin proteins and other molecules they release upon cell lysis are much smaller, allowing passage through the filter spaces in the membrane. Solid blood fractions, on the other hand, are much larger than bacteria or proteins: RBCs (erythrocytes) are 6-8 microns in diameter, and WBCs range in size from 6 to 16 microns, preventing them from passing through with the waste and excess water. Some platelets, sized between 1 and 4 microns, may be lost with the excess

water and toxic waste, but most will be retained. The optimal opening size can be determined based on desired final treated blood composition.

[0174] After filtering, the blood can then be tested for pathogen concentration and either membrane-filtered again or passed to de-aeration module **4244** for removing the gas from the gas-blood mixture.

[0175] The de-gassed, re-concentrated blood can then be returned to the patient's body. In one embodiment, the de-gassed, re-concentrated blood first passes through a gas detector **4250** and venous pressure monitor **4252** before being returned to a vein of patient **4201** through a shunt **5254** and a tube **4256**. If any gas remains in the blood, clamp **5258** can be closed automatically to prevent the gas from being delivered to the patient. Also, clamp **4258** can be used to maintain a desired venous pressure within patient **4201** as measured by monitor **5252**. As the blood is returned to the body, it can also be tested for electrolyte and lipid levels and those levels enhanced as needed.

[0176] The process shown in **FIG. 42** can involve multiple chambers in reactor **4230** or multiple passes through the body and back through system **4200** as to best cleanse the blood. System **4200** can have various arrangements and elements in alternative embodiments of the present invention. The gas composition, level of dilution, and flow rates can be traded off with electric field strength to optimize the system performance with regard to time, pathogen kill-rate, and patient tolerance of the process.

[0177] A variety of diseases or pathogens could be treated with NTP using an apparatus similar to those discussed herein or modified to suit the particular treatment. These diseases include any fluid-borne pathogen that can be destroyed with non-thermal plasma generated by one or more electrodes that are electrically isolated from the treated liquid by a dielectric. Different types of gasses can be introduced to the liquid being treated through the gas source (e.g., bubbler or gas injector), depending on desired inactivation effect (target pathogen). In some embodiments, the liquid being treated is not mixed with a gas. Also, certain selected human or other animal blood characteristics can be modified as desired through the use of NTP, such as the variance of white blood cell count.

[0178] In addition, embodiments of the present invention provide a pasteurization process that is able to cycle fluids such as blood through an apparatus outside the body to destroy bacterial infection, filter out the toxins released in the process, and then return the cleansed biologic fluid back to the patient in a process that is reasonably compatible with circulatory system time cycles. This process has the potential to provide significant anti-infective benefit, whether as a primary therapy or as an adjuvant supporting treatment used in conjunction with modified dosages of drugs or in cases where the patient's immune system cannot tolerate the bacterial toxin releases that accompany drug therapy.

[0179] Although the present invention has been described with reference to preferred embodiments, workers skilled in the art will recognize that changes may be made in form and detail without departing from the spirit and scope of the invention.

What is claimed is:

1. A method of at least partially disinfecting biological fluid of a mammal, which comprises pathogens, the method comprising:

(a) drawing the biological fluid from the mammal;

(b) contacting a non-thermal plasma with the biological fluid to kill at least a portion of the pathogens within the biological fluid;

(c) filtering toxins from at least some components of the biological fluid after contacting in step (b); and

(d) returning the biological fluid to the mammal after filtering the toxins in step (c).

2. The method of claim 1 wherein:

step (a) comprises drawing the biological fluid from a blood circulatory system of the mammal; and

step (d) comprises returning the biological fluid to the blood circulatory system.

3. The method of claim 1 wherein:

step (a) comprises drawing the biological fluid from an organ of the mammal; and

step (d) comprises returning the biological fluid to the organ.

4. The method of claim 1 and further comprising:

(e) introducing gas bubbles into the biological fluid to generate liquid-gas mixture; and

(f) contacting the liquid-gas mixture with the non-thermal plasma during step (b).

5. The method of claim 4 and further comprising:

(g) removing the gas bubbles from the liquid-gas mixture after contacting in step (b) and before returning in step (d).

6. The method of claim 5 and further comprising:

(h) passing the biological fluid through a gas detector after step (g) and prior to returning the biological fluid to the mammal in step (d).

7. The method of claim 1 and further comprising:

(e) introducing a diluent into the biological fluid to generate a diluted biological fluid; and

(f) treating the diluted biological fluid with the non-thermal plasma during step (b); and

(g) filtering some of the diluent from the diluted biological fluid in step (c).

8. The method of claim 1 wherein step (c) comprises passing the biological fluid along a semi-permeable membrane having pore sizes that filter toxins having selected sizes from larger components of the biological fluid.

9. The method of claim 8 wherein the semi-permeable membrane comprises pore sizes of approximately 0.1 to 2 micrometers.

10. The method of claim 1 wherein step (c) comprises filtering at least some blood-borne bacteria from at least some components of the biological fluid.

11. A method of at least partially disinfecting biological fluid of a mammal, which comprises pathogens, the method comprising:

(a) contacting a non-thermal plasma with the biological fluid to kill at least a portion of the pathogens within the biological fluid; and

(b) filtering toxins from at least some components of the biological fluid after contacting in step (a).

12. The method of claim 11 wherein:

step (a) releases toxins from at least some of the pathogens; and

step (b) comprises filtering the toxins released in step (a) from at least some components of the biological fluid.

13. The method of claim 11 wherein step (b) comprises passing the biological fluid along a semi-permeable membrane having pore sizes that filter toxins having selected sizes from larger components of the biological fluid.

14. The method of claim 13 wherein the semi-permeable membrane comprises pore sizes of approximately 0.1 to 2 micrometers.

15. The method of claim 11 wherein step (b) comprises filtering at least some blood-borne bacteria from at least some components of the biological fluid.

16. The method of claim 11 and further comprising:

(c) drawing the biological fluid from the mammal prior to step (a); and

(d) returning the biological fluid to the mammal after filtering the toxins in step (b).

17. The method of claim 16 wherein:

step (c) comprises drawing the biological fluid from a blood circulatory system of the mammal; and

step (d) comprises returning the biological fluid to the blood circulatory system.

18. The method of claim 16 wherein:

step (c) comprises drawing the biological fluid from an organ of the mammal; and

step (d) comprises returning the biological fluid to the organ.

19. The method of claim 11 and further comprising:

(c) introducing gas bubbles into the biological fluid to generate liquid-gas mixture prior to step (a);

(d) contacting the liquid-gas mixture with the non-thermal plasma during step (a); and

(e) removing the gas bubbles from the liquid-gas mixture after contacting in step (a).

20. The method of claim 11 and further comprising:

(c) introducing a diluent into the biological fluid to generate a diluted biological fluid;

(d) treating the diluted biological fluid with the non-thermal plasma during step (a); and

(e) filtering some of the diluent from the diluted biological fluid in step (b).

21. A liquid-gas mixture comprising a mammalian biological liquid, gas bubbles, and a non-thermal plasma.

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