

FIG. 2

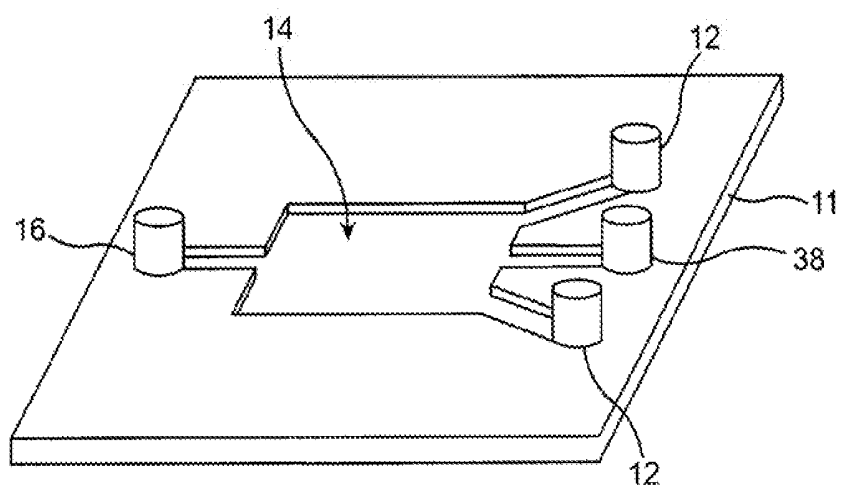


FIG. 3

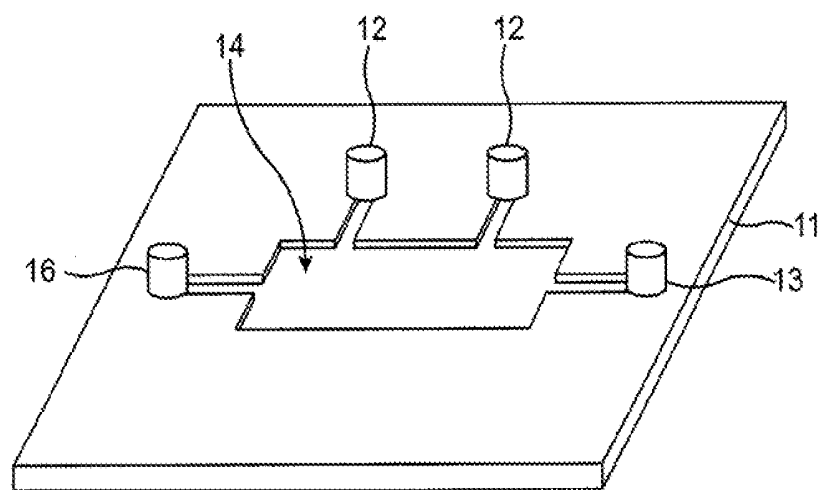


FIG. 4

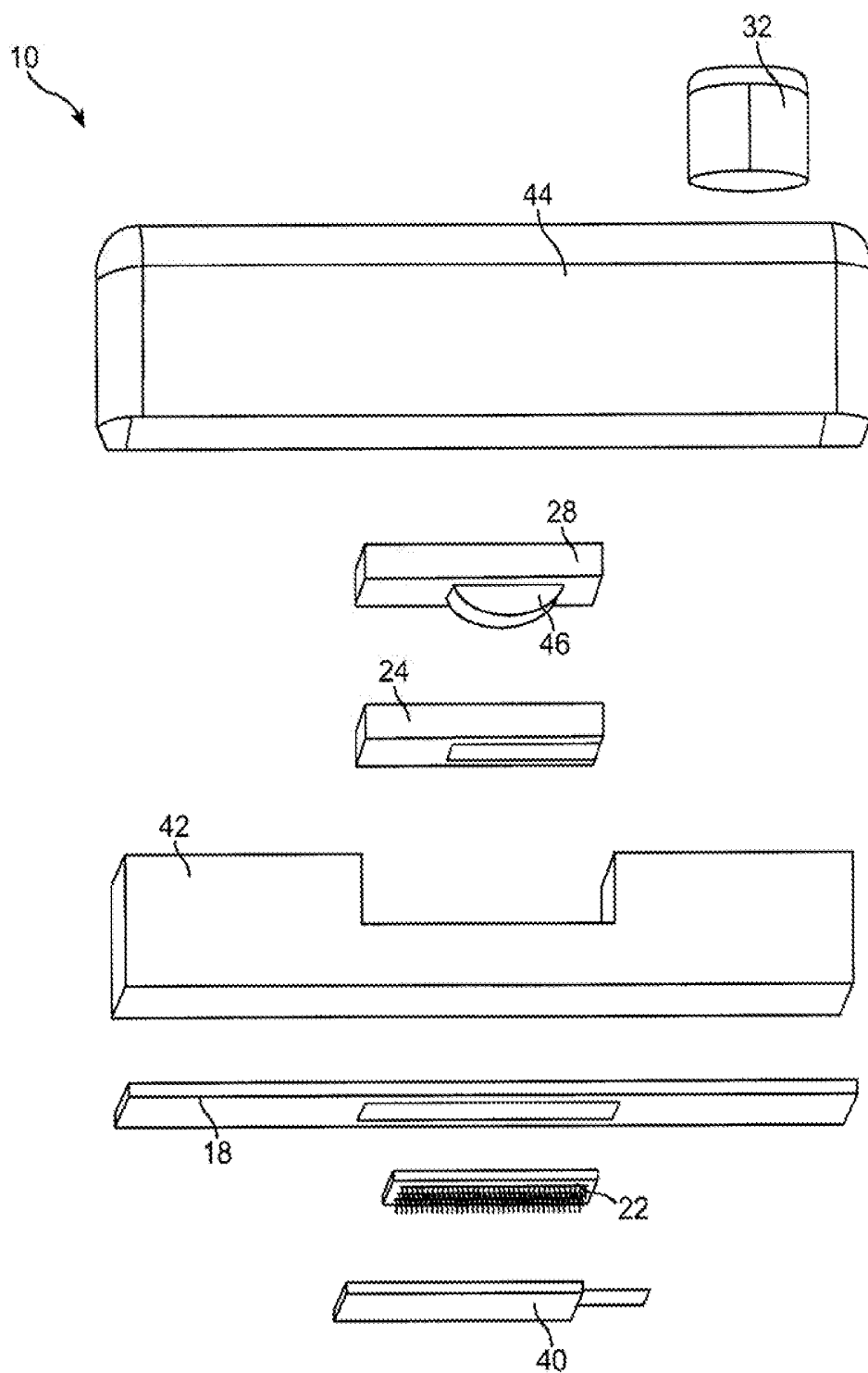


FIG. 5

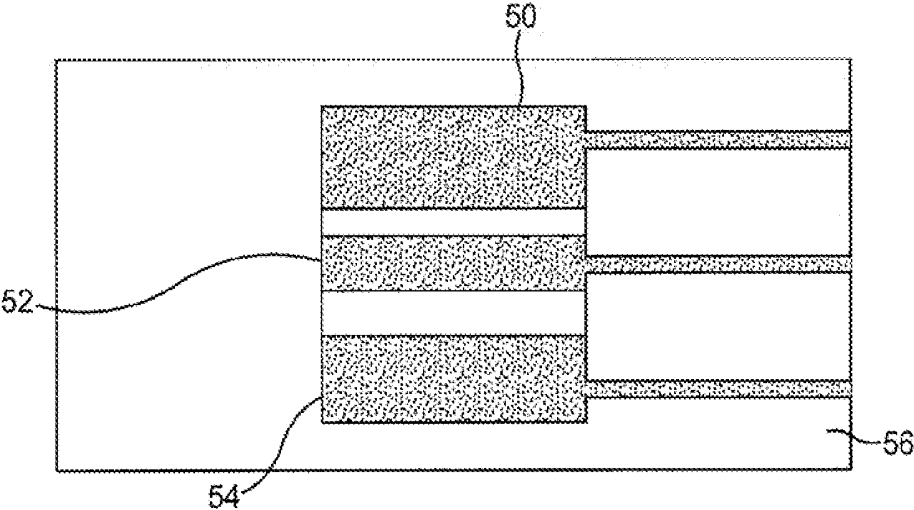


FIG. 6A

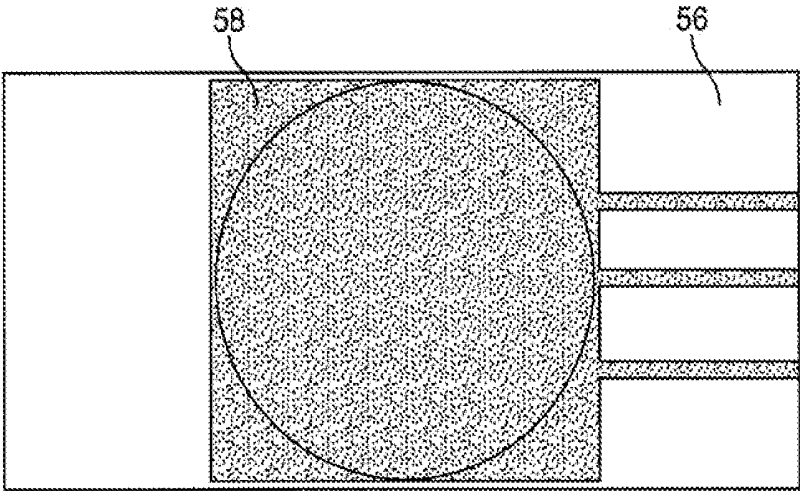


FIG. 6B

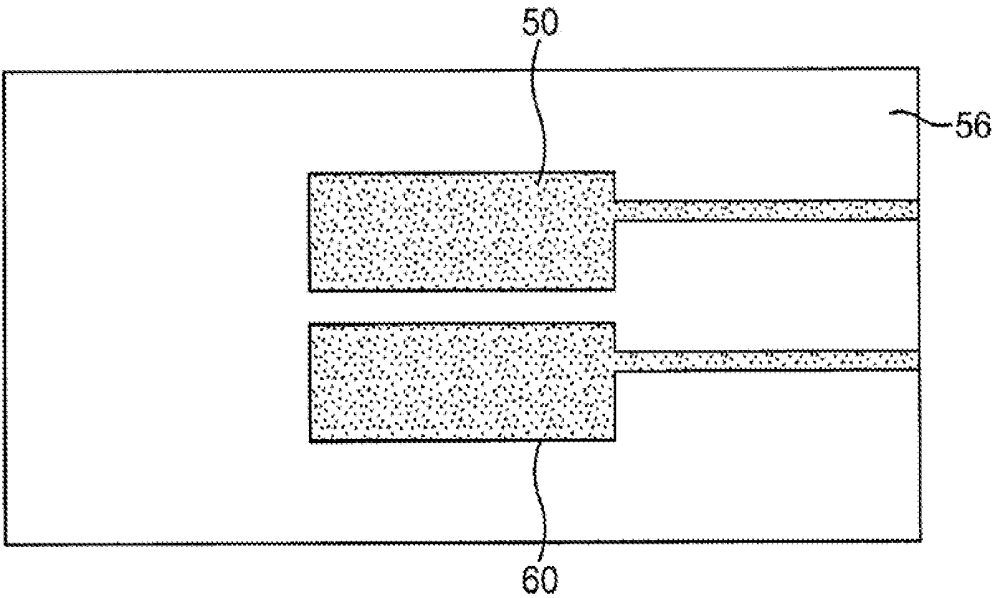


FIG. 7A

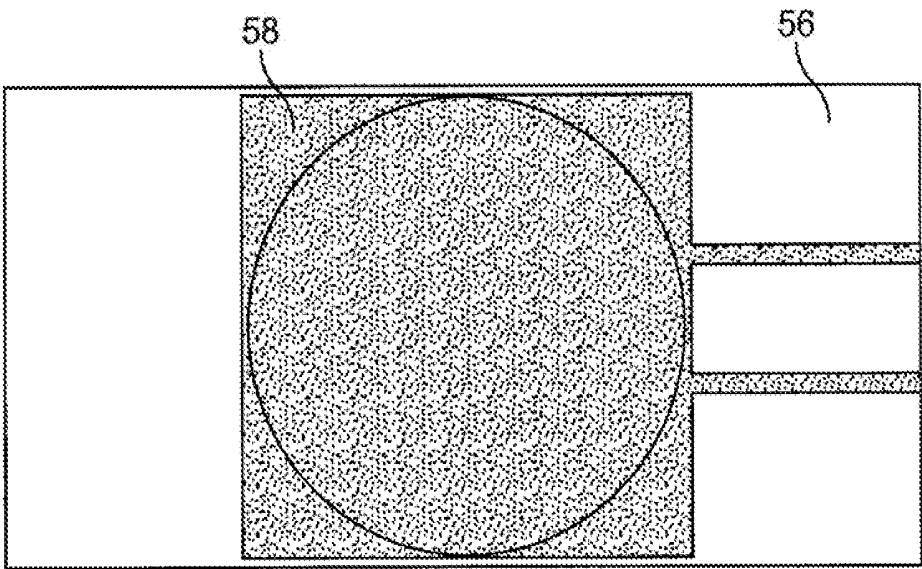
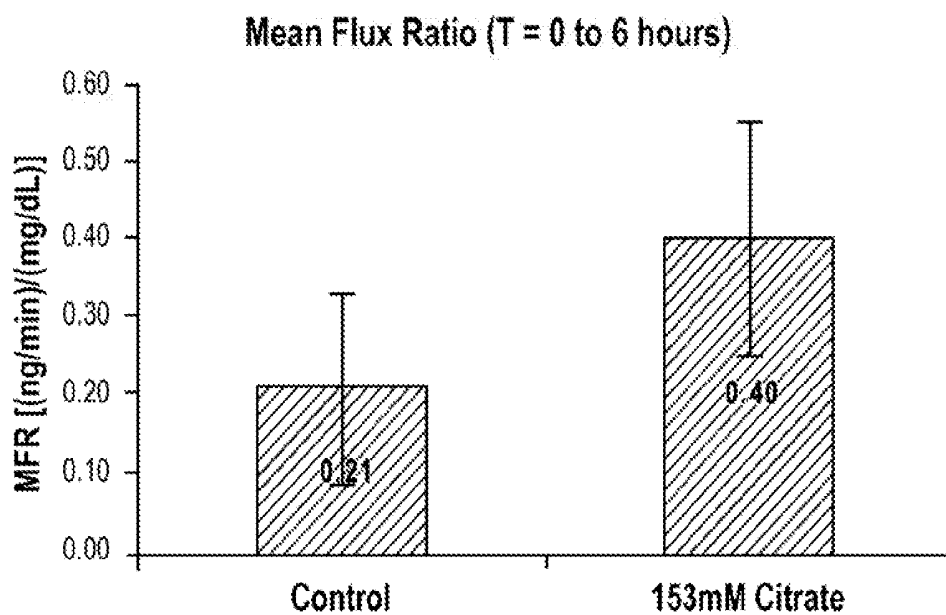
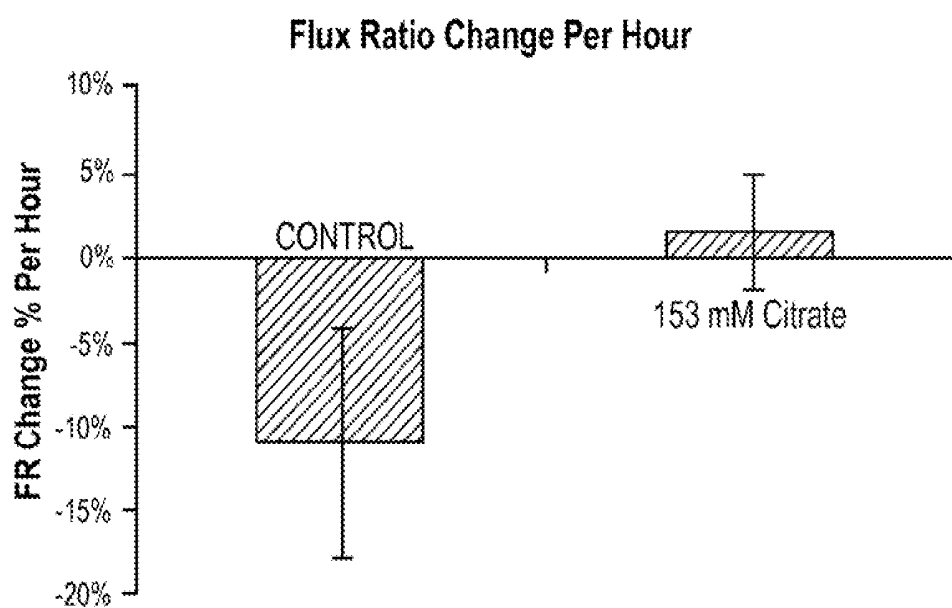
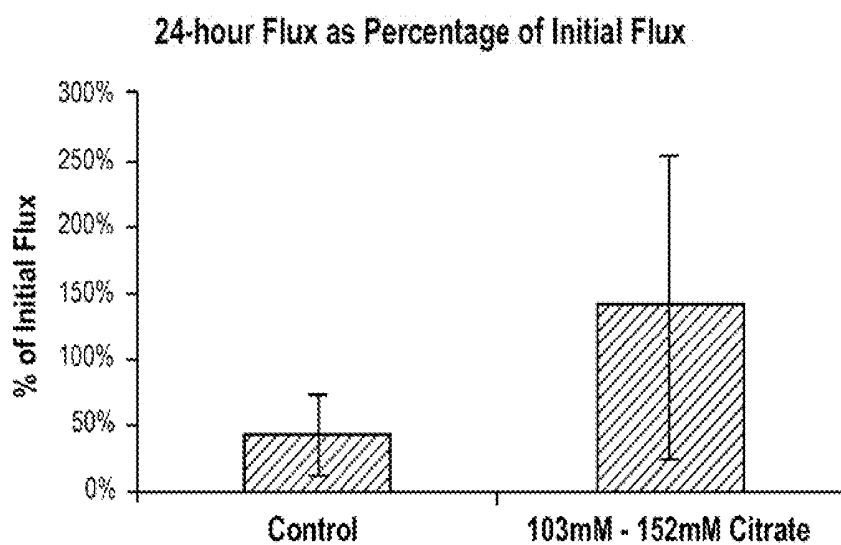
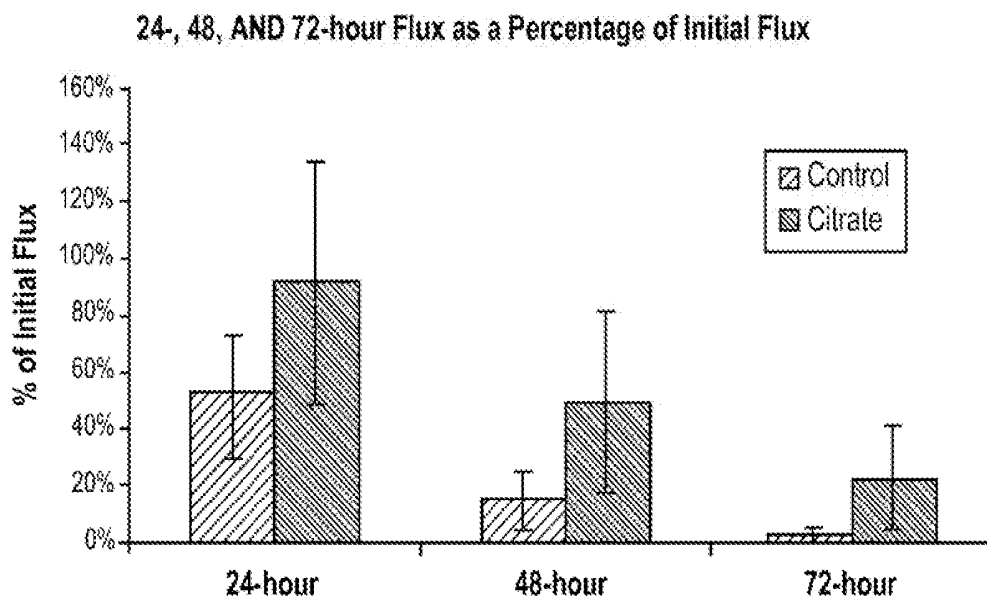
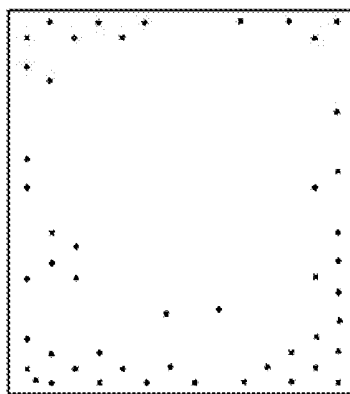
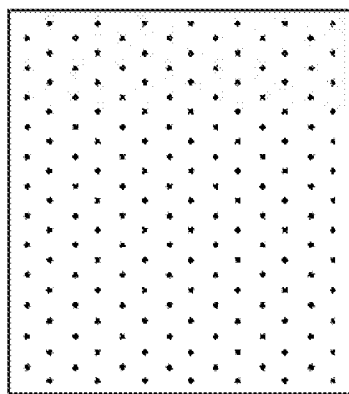
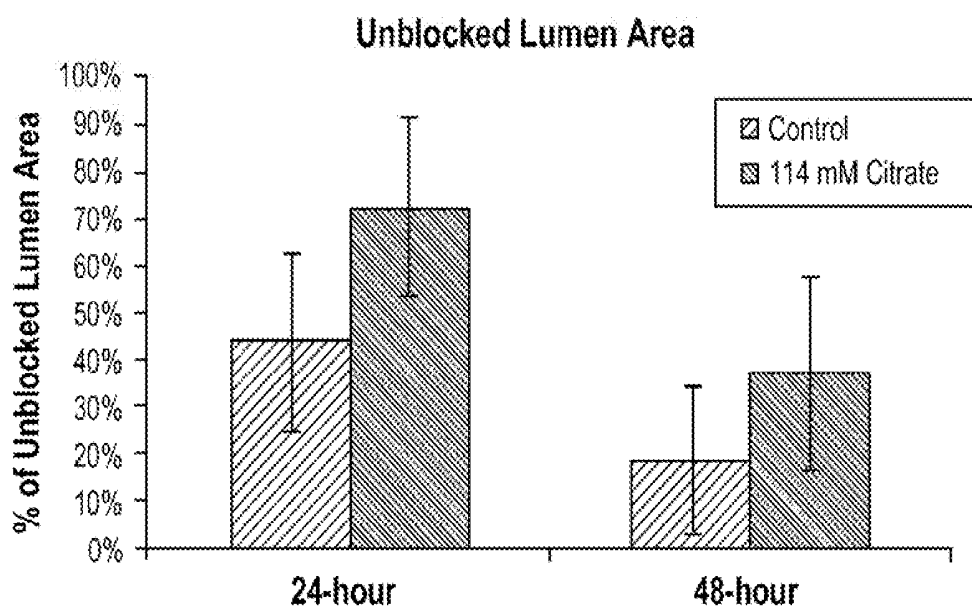
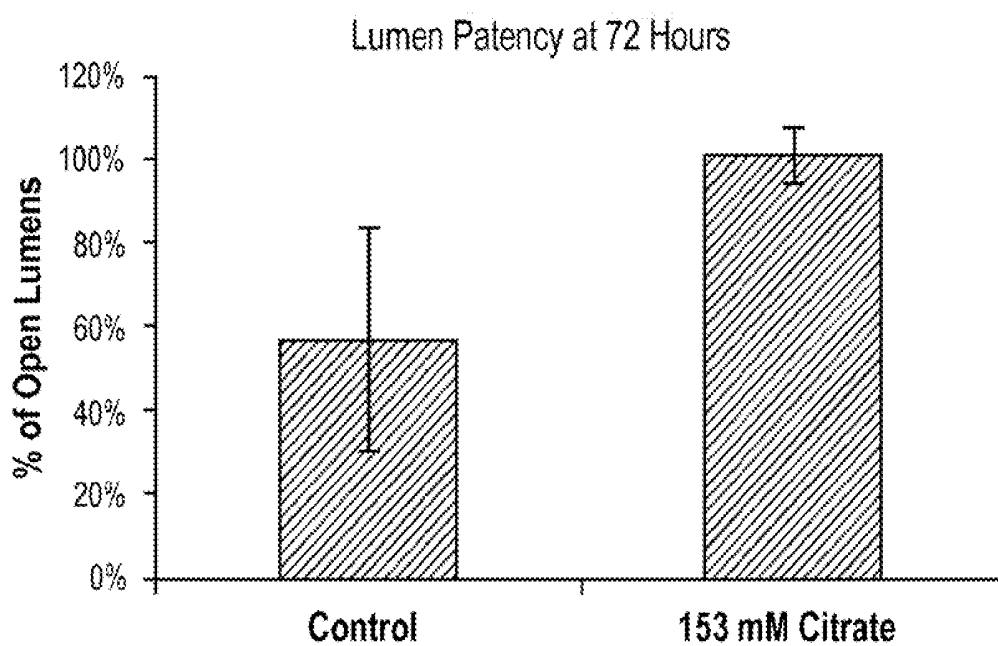


FIG. 7B

**FIG. 8****FIG. 9**

**FIG. 10****FIG. 11**

**FIG. 12A****FIG. 12B****FIG. 13**

**FIG. 14**

DEVICES AND METHODS FOR ENHANCED SKIN PERFORATION FOR CONTINUOUS GLUCOSE MONITORING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a non-provisional of U.S. Provisional Application No. 62/265,319 filed Dec. 9, 2015, the entirety of which is incorporated by reference. This application is also related to U.S. patent application Ser. No. 12/985,228 filed Jan. 5, 2011, now US Publication No. 2011/0257497 which claims reference to U.S. Provisional Application No. 61/326,086 filed Apr. 20, 2010, and U.S. patent application Ser. No. 12/844,710 file Jul. 27, 2010 (now U.S. Pat. No. 7,949,382 issued May 24, 2011) and Ser. No. 11/277,731 filed Mar. 28, 2006 (now U.S. Pat. No. 8,280,476 issued Oct. 5, 2006), the entirety of each of which is incorporated by reference herein.

BACKGROUND OF THE INVENTION

[0002] The invention relates to methods and apparatus for monitoring the presence and/or concentration of an analyte or analytes, such as for monitoring the glucose level of a person having diabetes. More specifically, the invention relates to systems, devices, sensors and tools and methods associated therewith for monitoring analyte levels continuously, or substantially continuously. In some variations, the systems, devices, sensors and tools and methods monitor analyte levels to confirm that there is an uninterrupted analyte flux where the concentration of the analyte may or may not vary during the uninterrupted flux.

[0003] Diabetes is a chronic, life-threatening disease for which there is no known cure at present. It is a syndrome characterized by hyperglycemia and relative insulin deficiency. Diabetes affects more than 120 million people worldwide, and is projected to affect more than 220 million people by the year 2020. There are almost 30 million children and adults in the United States, or 10% of the population, who have diabetes. Of these people, 21.0 million have been diagnosed with the disease, while unfortunately nearly one-third remain undiagnosed. It is estimated that one out of every three children today will develop diabetes sometime during their lifetime. Diabetes is usually irreversible, and can lead to a variety of severe health complications, including coronary artery disease, peripheral vascular disease, blindness and stroke. The Center for Disease Control (CDC) has reported that there is a strong association between being overweight, obesity, diabetes, high blood pressure, high cholesterol, asthma and arthritis. Individuals with a body mass index of 40 or higher are more than 7 times more likely to be diagnosed with diabetes.

[0004] There are two main types of diabetes, Type I diabetes (insulin-dependent diabetes mellitus) and Type II diabetes (non-insulin-dependent diabetes mellitus). Varying degrees of insulin secretory failure may be present in both forms of diabetes. In some instances, diabetes is also characterized by insulin resistance. Insulin is the key hormone used in the storage and release of energy from food.

[0005] As food is digested, carbohydrates are converted to glucose and glucose is absorbed into the blood stream primarily in the intestines. Excess glucose in the blood, e.g. following a meal, stimulates insulin secretion, which pro-

motes entry of glucose into the cells, which controls the rate of metabolism of most carbohydrates.

[0006] Insulin secretion functions to control the level of blood glucose both during fasting and after a meal, to keep the glucose levels at an optimum level. In a non-diabetic person blood glucose levels are typically between 80 and 90 mg/dL of blood during fasting and between 120 to 140 mg/dL during the first hour or so following a meal. For a person with diabetes, the insulin response does not function properly (either due to inadequate levels of insulin production or insulin resistance), resulting in blood glucose levels below 80 mg/dL during fasting and well above 140 mg/dL after a meal.

[0007] Currently, persons suffering from diabetes have limited options for treatment, including taking insulin orally or by injection. In some instances, controlling weight and diet can impact the amount of insulin required, particularly for non-insulin dependent diabetics. Monitoring blood glucose levels is an important process that is used to help diabetics maintain blood glucose levels as near as normal as possible throughout the day.

[0008] The blood glucose self-monitoring market is the largest self-test market for medical diagnostic products in the world, with a size of approximately over \$3 billion in the United States and \$7.0 billion worldwide. It is estimated that the worldwide blood glucose self-monitoring market will amount to \$9.0 billion by 2008. Failure to manage the disease properly has dire consequences for diabetics. The direct and indirect annual costs of diabetes in the United States was more than \$240 billion in 2012.

[0009] There are two main types of blood glucose monitoring systems used by patients: non-continuous systems, also known as single point, discrete or episodic, and continuous systems. Episodic systems consist of meters and tests strips and require blood samples to be drawn from fingertips or alternate sites, such as forearms and legs (e.g. OneTouch® Ultra by LifeScan, Inc., Milpitas, Calif., a Johnson & Johnson company). These systems rely on lancing and manipulation of the fingers or alternate blood draw sites; which can be extremely painful and inconvenient, particularly for children.

[0010] Continuous monitoring sensors are generally implanted subcutaneously and measure glucose levels in the interstitial fluid at various periods throughout the day, providing data that shows trends in glucose measurements over a short period of time. These sensors are painful during insertion and usually require the assistance of a health care professional. Further, these sensors are intended for use during only a short duration (e.g., monitoring for a matter of days to determine a blood sugar pattern). Subcutaneously implanted sensors also frequently lead to infection and immune response complications. Another major drawback of currently available continuous monitoring devices is that they require frequent, often daily, calibrations using blood glucose results that must be obtained from painful fingersticks using traditional meters and test strips. This calibration, and re-calibration, is required to maintain sensor accuracy and sensitivity, but it can be cumbersome and inconvenient.

[0011] Data from various studies such as the Diabetes Control and Complications trial (DCCT) show that frequent testing of blood glucose levels is essential to improve the quality of life for diabetics. However, most diabetics avoid frequent testing because of the inconvenience, fear, and pain

of pricking their fingers or alternate sites to obtain blood samples. Thus there is a need to develop simple glucose monitoring systems that eliminate or minimize these barriers to frequent testing. With some embodiments of the proposed present invention a user or diabetic patient can obtain 20 or more glucose test results over a two or three day period thus allowing frequent measurements on a daily basis.

[0012] Wearable devices are transforming the way millions of people around the world achieve their health and fitness goals. Wearable sensing devices can have diagnostic and monitoring applications. These devices are currently used for physiological and biochemical sensing, as well as motion. Other sensors, depending on the clinical application of interest, can also be incorporated to determine a person's overall health status. There are several commercially available wearable multi-sensor devices that measure galvanic skin response, skin temperature, heart rate and motion. Some companies are believed to be working on wearable devices that may include glucose and other analyte sensors. However, these devices for general health and wellness monitoring, are not yet commercially available.

[0013] Personal lifestyle and wellness monitors currently on the market measure such parameters as heart-rate, temperature, movement, etc., and calculate from these measurements physiological parameters such as calories burned and quality of sleep. Absent from these fitness tracking devices, however, is the direct measurement of glucose levels, a key physiological parameter for those people who are interested in monitoring their glucose levels or users with metabolic syndrome or pre-diabetes. A device which could provide glucose level measurements, along with other physiological measurements, would be much more useful and beneficial to users who are keen to modify their lifestyles to attain better health.

[0014] Continuous glucose monitoring (CGM) has been shown to be a useful tool in improving average blood glucose levels and reducing glycemic excursions in persons with diabetes (1). While usage of continuous glucose monitors has increased over the past decade, their adoption by the wider diabetic population has been limited, especially in patients with Type 2 diabetes and persons who are pre-diabetic. Over 86 million people in the U.S. over age 20 have pre-diabetes with blood sugar levels that are higher than normal, but are not high enough to be classified as diabetes. The pain and inconvenience associated with the implantation and wearing of commercially available needle sensor CGM devices has been shown to be one of the factors cited which hinders wider adoption (2). That some patients do not tolerate the device is substantiated by the high drop-out rate in clinical trials (3). Much work has been done on the development of minimally invasive glucose sensors (4) on the premise that a device that is less painful and less obtrusive to apply and wear would be more attractive to a greater fraction of the diabetic and general populations.

[0015] Hence, the development of accurate, minimally invasive continuous glucose monitoring (CGM) devices has been the subject of much work by several groups. Currently available glucose monitoring technologies such as fingerstick whole blood testing and invasive needle sensor based CGM devices or technologies are not suitable or convenient for daily use by people who are interested in monitoring their glucose levels to make behavioral and lifestyle changes. Consequently, there is a clear and an unmet need to develop convenient health and wellness monitoring devices

that contain glucose monitoring technologies or solutions which are minimally invasive and can be incorporated into easy to use wearable devices.

[0016] The wearable monitoring device using a MicroTip or microneedle-based CGM technology could be a valuable tool for persons who are interested in monitoring their overall health and wellness, including persons with metabolic syndrome and pre-diabetes. With advances in sensor technologies and the advent of convenient wearable devices for consumers, minimally invasive CGM technology can be also coupled with multiple sensor enabled health and wellness monitoring devices. These devices will provide meaningful and useful integrated actionable data structured to facilitate behavioral and lifestyle changes for improving blood glucose levels and overall health and wellness. In addition, the wearable device platform will enable helps people become more active, exercise more, sleep better, eat smarter, and manage their weight through the use of mobile apps, data analytics, motivational and social tools.

[0017] Most commercially available wearable devices automatically track users' daily steps, calories burned, distance traveled, floors climbed, and display real-time feedback to encourage them to become more active in their daily lives. A wearable device capable of measuring and tracking glucose levels will enable users to understand the consequences of their food in-take on their glucose levels and allow them thus to make desired lifestyle changes not only to improve their fitness state but also manage their glucose levels. Users of our wearable device would range potentially from people interested in improving their health and fitness through everyday activities to individuals who may be prediabetic.

BRIEF SUMMARY OF THE INVENTION

[0018] The efficacy of tissue piercing elements in the area of transdermal drug delivery is well-documented. Multiple studies have shown that enhancement of skin permeation via creation of microscopic pores in the stratum corneum can greatly improve the delivery rates of drugs. However, skin perforation with tissue piercing elements is not the only factor affecting the rate of drug transport. Other factors including such as the formulation of the drug and rate of closure of the micropores closure also need to be considered. Similarly micro tissue piercing elements have been used with less success by several workers for continuous glucose monitoring.

[0019] One aspect of the invention is a method of in vivo monitoring of an individual's interstitial fluid glucose concentration comprising inserting a plurality of tissue piercing elements with a simple applicator through a stratum corneum layer of an area of the individual's skin. The tissue piercing elements are immediately removed and a flexible glucose sensor gel patch impregnated with one or more a chelating agents is applied to the perforated area on the skin. To minimize the closure of the micropores within the perforated area, biochemical inhibitors can be incorporated in the reagent gel matrix. These biochemical inhibitors prevent skin healing by limiting the synthesis of essential lipids. Keeping the micropores open allows consistent glucose flux thus minimizing the need for fingerstick calibrations.

[0020] Another aspect of the invention is a method of in vivo monitoring of an individual's interstitial fluid glucose concentration comprising inserting a plurality of tissue

piercing elements through a stratum corneum layer of an area of the individual's skin. The tissue piercing elements each comprise a distal end in fluid communication with interstitial fluid of the individual, and a proximal end in fluid communication with a sensing zone located outside of the patient's body. An interior space extends between the distal and proximal ends of the tissue piercing elements. A sensing fluid fills substantially the entire interior space and the sensing fluid concentration comprises a concentration of chelating agents in a buffer solution.

[0021] One aspect of the invention is a method of in vivo monitoring of an individual's interstitial fluid glucose concentration comprising inserting a plurality of tissue piercing elements with a simple applicator through a stratum corneum layer of an area of the individual's skin. The tissue piercing elements are immediately removed and a flexible glucose sensor gel patch impregnated with one or more chelating agents is applied to the perforated area on the skin.

[0022] One aspect of the invention is a method of in vivo monitoring of an individual's interstitial fluid glucose concentration comprising inserting a plurality of tissue piercing elements through a stratum corneum layer of an area of the individual's skin. The tissue piercing elements each comprise a distal end in fluid communication with interstitial fluid of the individual, and a proximal end in fluid communication with a sensing zone located outside of the patient's body. An interior space extends between the distal and proximal ends of the tissue piercing elements. A sensing fluid fills substantially the entire interior space and the sensing fluid concentration comprises a concentration of citrate in a buffer solution. The concentration of citrate may range from 100 mM to 200 mM, preferably 135-165 mM.

[0023] Addition of citrate to a buffer solution in the sensing fluid of a glucose monitoring device has shown to provide at least the following benefits. First, transdermal glucose flux is increased through the tissue piercing elements immediately after application of the tissue piercing elements to the skin. Second, a decrease in inhibition of transdermal glucose flux several hours after application occurs. Third, a decrease in inhibition of transdermal glucose flux up to several days after application also occurs.

[0024] One aspect of the invention is a method of in vivo monitoring of an individual's interstitial fluid analyte concentration. The method comprises inserting a plurality of tissue piercing elements through a stratum corneum layer of an area of the individual's skin to create a plurality of fluid paths, said fluid paths each comprising a distal end in fluid communication with interstitial fluid of the individual, a proximal end in fluid communication with a sensing zone located outside of the patient's body, an interior space extending between the distal and proximal ends of the fluid paths, and a sensing fluid filling substantially the entire interior space. The method also comprises allowing at least one analyte to passively diffuse from the patient's interstitial fluid through the tissue piercing elements and into the sensing zone. The method further comprises sensing a concentration of the at least one analyte in a sensing fluid within the sensing zone using a sensor located at least partially in the sensing zone, wherein the sensing fluid comprises a concentration of an agent (such as, e.g., citrate) adapted to immediately increase an analyte flux through the tissue piercing elements and to mitigate a decrease over time of the analyte flux through the tissue piercing elements.

[0025] In the method, the sensing fluid concentration may comprise a sufficient concentration of citrate or other agent to mitigate a decrease of the analyte flux through tissue piercing elements for several days. The concentration of citrate may range from 100 mM to 200 mM. The citrate or other agent concentration may be such that at least 70% of the interior spaces remain unblocked after 24 hours. Also, the citrate or other agent concentration may be such that at least 40% of the interior spaces remain unblocked after 48 hours.

[0026] Also, in the method, the sensing fluid may comprise a phosphate buffered saline solution. The analyte may be glucose.

[0027] Another aspect of the invention is an analyte monitor. The analyte monitor comprises a plurality of fluid paths (defined, e.g., by a plurality of tissue piercing elements), each fluid path comprising a distal opening adapted to be disposed on one side of a stratum corneum layer of a user's skin, a proximal opening adapted to be disposed on another side of the stratum corneum, and an interior space extending between the distal and proximal openings. The analyte monitor comprises a sensing zone in fluid communication with the proximal openings of the fluid paths. Also, the analyte monitor comprises a sensing fluid extending from the sensing zone into substantially the entire interior space of the fluid paths, wherein the sensing fluid comprises an agent (such as, e.g., citrate) adapted to increase an analyte flux through the fluid paths and to mitigate a decrease over time of the analyte flux through the fluid paths. The analyte sensor is adapted to detect a concentration of analyte in the sensing fluid within the sensing zone.

[0028] With regard to the analyte monitor, the concentration of citrate may range from 100 mM to 200 mM. The sensing fluid may comprise a phosphate buffered saline solution and the analyte may be glucose.

[0029] Another aspect of the invention is to incorporate the flexible glucose sensor gel pad after removal of the tissue piercing elements into a convenient wearable wellness monitor to enable glucose monitoring for 24 to 72 hrs as shown in FIG. 1

[0030] Another aspect of the invention is the wearable device technology platform as conceptualized below will be used to enhance the health and wellness monitoring experience by enabling all types of persons to make desired lifestyle changes not only to improve their fitness state but also manage their glucose levels. Our users would range potentially from people interested in improving their health and fitness through everyday activities to individuals who may be prediabetic.

[0031] Yet another aspect of the invention is a method of in vivo monitoring of an individual's interstitial fluid glucose concentration comprising inserting a plurality of tissue piercing elements with a simple applicator through a stratum corneum layer of an area of the individual's skin. The said tissue-piercing element can be hollow, solid or planar (where there is a protrusion on the planar substrate, the protrusion penetrates tissue and an individual's interstitial fluid glucose concentration is monitored through the planar substrate). Specifically for application with the use of the wearable wellness monitor the tissue piercing elements will be planar elongated tips about 200 microns in length fabricated out of metal or elongated tips fabricated out of plastic materials.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0032] The novel features of the invention are set forth with particularity in the claims that follow. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0033] FIG. 1 is a perspective view of one embodiment of the analyte monitor of this invention.

[0034] FIG. 2 is a cross-sectional view of the analyte monitor shown in FIG. 1 showing tissue piercing elements piercing through the patient's skin.

[0035] FIGS. 3 and 4 illustrate embodiments in which the analyte monitor comprises a plurality of calibration fluid reservoirs and a sensing fluid reservoir.

[0036] FIG. 5 shows an exploded view of an analyte monitor according to one embodiment of the invention.

[0037] FIGS. 6A and 6B are a schematic representative drawing of a three electrode system for use with the analyte sensor of one embodiment of this invention. FIG. 6A shows electrodes on a substrate, and FIG. 6B shows the electrodes and a portion of the substrate covered with a reagent.

[0038] FIGS. 7A and 7B are a schematic representative drawing of a two electrode system for use with the analyte sensor of one embodiment of this invention. FIG. 7A shows electrodes on a substrate, and FIG. 7B shows the electrodes and a portion of the substrate covered with a reagent.

[0039] FIG. 8 illustrates an average ratio of transdermal glucose flux to reference blood glucose measurement for sensing blood glucose concentration.

[0040] FIG. 9 illustrates change in ratio of transdermal glucose flux to reference blood glucose per hour for sensing blood glucose concentration.

[0041] FIG. 10 illustrates percentage of initial transdermal glucose flux after 24 hours of glucose monitoring device wear.

[0042] FIG. 11 illustrates percentage of initial glucose flux after 24, 48, 72 hours of glucose monitoring device wear.

[0043] FIG. 12a illustrates the proximal ends of tissue piercing elements, many of which are blocked, after use with a control solution.

[0044] FIG. 12b illustrates the proximal ends of tissue piercing elements after use with a buffer solution comprising citrate.

[0045] FIG. 13 illustrates percentage of unblocked tissue piercing element lumen area within 24 and 72 hours when citrate concentration is added to a sensing fluid.

[0046] FIG. 14 illustrates percentage of open tissue piercing element lumens within 72 hours when citrate concentration is added to a sensing fluid.

DETAILED DESCRIPTION OF THE INVENTION

[0047] While many of the exemplary embodiments disclosed herein are described in relation to monitoring glucose levels in people with diabetes, it should be understood that aspects of the invention are useful in monitoring glucose levels in people without diabetes, or for monitoring an analyte or analytes other than glucose. For example, the present invention may be used in monitoring the concentration, or presence, of other analytes such as lactate, acetyl

choline, amylase, bilirubin, cholesterol, chorionic gonadotropin, creatine kinase (e.g., CK-MB), creatine, DNA, fructosamine, glutamine, growth hormones, hematocrit, hemoglobin (e.g. HbA1c), hormones, ketones, lactate, oxygen, peroxide, prostate-specific antigen, prothrombin, RNA, thyroid stimulating hormone, troponin, drugs such as antibiotics (e.g., gentamicin, vancomycin), digitoxin, digoxin, drugs of abuse, theophylline, and warfarin. Accordingly, while the invention will be described in connection with glucose monitoring, it should be understood that the invention may be used to monitor other analytes as well.

[0048] The present invention provides a significant advance in biosensor and analyte monitoring technology. According to various aspects of the invention, a glucose monitoring system may be constructed to be portable, painless, virtually non-invasive, self-calibrating, integrated and/or have non-implanted sensors which continuously indicate the user's glucose concentration, enabling swift corrective action to be taken by the patient. The invention may also be used in critical care situations, such as in an intensive care unit to assist health care personnel. The sensor and monitor of this invention may be used to measure any other analyte as well, for example, electrolytes such as sodium or potassium ions. As will be appreciated by persons of skill in the art, the glucose sensor can be any suitable sensor including, for example, an electrochemical sensor or an optical sensor.

[0049] One aspect of the invention is a glucose monitor. The glucose monitor may comprise a plurality of tissue piercing elements or fluid paths, a sensing zone in fluid communication with the plurality of tissue piercing elements or fluid paths, a plurality of calibration reservoirs each adapted to house a calibration fluid and in fluid communication with the sensing zone, and a sensor configured to detect glucose and provide an output indicative of the glucose concentration of the fluid in the sensing zone.

[0050] FIGS. 1-2 illustrate one embodiment of the present invention. Glucose monitor 10 includes a fluidic network in which a calibration reservoir 12 is in fluid communication with sensing zone 14 and waste reservoir 16 to allow for the movement of calibration fluids from the reservoirs through sensing zone 14 and into the waste reservoir 16. Glucose monitor 10 includes an adhesive pad or seal 18 which is coupled to substrate or chip 20 which comprises a plurality of tissue piercing elements 22 forming and defining fluid paths.

[0051] Glucose monitor 10 includes a sensing layer 11 with a fluidic network having a calibration reservoir 12 in fluid communication with a calibration fluid channel 13 adapted to receive calibration fluid from the calibration fluid reservoir. Calibration fluid channel 13 is in fluid communication with a sensing zone or sensing channel 14. Sensing zone 14 is fluidly connected (optionally via a check valve, not shown) to a waste channel 15 in fluid communication with a waste reservoir 16. As shown, substrate 20 is coupled to an optional adhesive pad 18 for attachment to a user's skin. When in use, the tissue piercing elements 22 each have an interior space defining a fluid path that passes through the stratum corneum 26 of the skin with a distal opening at its distal end 21 in fluid communication with the user's interstitial fluid and a proximal opening at its proximal end 23 in fluid communication with sensing zone 14 and with sensor 24.

[0052] While not shown in FIGS. 1-2, at least one pump and at least one check valve can be incorporated into the

glucose monitor to facilitate or control the flow of fluid unidirectionally from the calibration fluid reservoir into the sensing zone. Also not shown in FIGS. 1-2 is an actuator which can be manually or automatically actuated and can be configured to work in conjunction with a pump and/or series of valves to initiate the flow of fluid from the calibration fluid reservoir. The channels shown in FIG. 1 are intended to be optional in the glucose monitor, as the calibration fluid can flow directly from the calibration fluid reservoir into the sensing zone (passing through valves), and further directly into the waste reservoir. One or more waste reservoirs may be incorporated into the glucose monitor.

[0053] Alternatively, the embodiment in FIG. 1 may include a plurality of calibration reservoirs. The calibration reservoirs may include a plurality of calibration fluids. The calibration fluid which may be the sensing fluid, for example, the calibration fluid does not include glucose.

[0054] In one embodiment, sensing zone 14 and the tissue piercing elements or fluid paths 22 are pre-filled with sensing fluid prior to the first use of the device. The sensing fluid may also be filled upon application to the user's skin. Thus, when the device is applied to the user's skin and the tissue piercing elements or fluid paths may pierce the stratum corneum and the epidermis, there is substantially no net fluid transfer from the interstitial fluid into the tissue piercing elements or fluid paths. Rather, glucose diffuses from the interstitial fluid into the fluid within the tissue piercing elements or fluid paths, as described below.

[0055] Exemplary tissue piercing elements or fluid paths that can be used with the present invention include microneedles described in Stoeber et al. U.S. Pat. No. 6,406,638; US Patent Appl. Publ. No. 2005/0171480; and US Patent Appl. Publ. No. 2006/0025717. Tissue piercing elements or fluid paths and microneedles described in co-assigned U.S. patent application Ser. No. 11/642,196, filed Dec. 20, 2006 may also be used. Any other tissue piercing elements or fluid paths or needle arrays that can penetrate into the epidermis layer and allow glucose to diffuse from the interstitial fluid into the sensing zone of the present invention may also be incorporated into the embodiments described herein.

[0056] Disposed above and in fluid communication with sensing zone 14 is sensor 24. In some embodiments, the sensor is an electrochemical glucose sensor that generates an electrical signal (current, voltage or charge) whose value depends on the concentration of glucose in the fluid within sensing zone 14. Details of sensor 24 are discussed in more detail below.

[0057] Electronics element 28 is configured to receive an electrical signal from sensor 24. In some embodiments, electronics element 28 uses the electrical signal to compute a glucose concentration and display it. In other embodiments, electronics element 28 transmits the electrical signal, or information derived from the electrical signal, to a remote device, such as through wireless communication. Electronics element 28 can comprise other electrical components such as an amplifier and an A/D converter which can amplify the electrical signal from the sensor and convert the amplified electrical signal to a digital signal before, for example, determining a glucose concentration or transmitting the signal to an external device which can then determine a glucose concentration.

[0058] Glucose monitor 10 can be held in place on the patient's skin by one or more adhesive pads 18.

[0059] The glucose monitor has a built-in calibration system. As shown in FIG. 1, the glucose monitor includes one or more calibration reservoirs each adapted to house a calibration fluid. The one or more calibration reservoirs are in fluid communication with the sensing zone. A glucose monitor with two or more calibration fluids can have a sensor that can be calibrated at two or more different glucose concentrations, which allows for a multi-point calibration curve during the sensor calibration. This can provide a more accurate calibration curve which in turn can enable a more accurate glucose concentration determination.

[0060] The calibration fluids in each of the different calibration fluid reservoirs have known glucose concentrations, and can be different known glucose concentrations. For example, in some embodiments a first calibration fluid in a first calibration fluid reservoir has a glucose concentration of between about 0 mg/dl and about 100 mg/dl, and a second calibration fluid in a second calibration fluid reservoir has a glucose concentration of between about 100 mg/dl and about 400 mg/dl. The ranges of glucose concentrations in the different calibration fluid reservoirs may, however, be different. When more than one calibration fluid reservoir is used, the calibration fluids in each reservoir may have, however, substantially the same or similar glucose concentrations.

[0061] In some embodiments, one of the reservoirs can be filled with a sensing or washing fluid which does not comprise glucose and which is not used to calibrate the glucose sensor. The sensing or washing fluid can comprise, for example, de-ionized water, buffer, surfactants and preservative. More information about the sensing fluid is provided later in the description. In embodiments in which there are two reservoirs and one comprises sensing fluid and the other comprises calibration fluid, the calibration fluid may have a glucose concentration between about 0 mg/dl and about 400 mg/dl, and is used to generate a one-point calibration curve for the sensor. In some embodiments, however, the glucose monitor comprises two or more calibration fluids reservoirs in addition to a sensing or washing fluid reservoir.

[0062] Monitoring a subject's interstitial fluid glucose concentration is further described. The method can include calibrating the glucose sensor with one or more different calibrating fluids with different known glucose concentrations. A calibration fluid of known glucose concentration is moved into the sensing zone. This can be done, for example, during manufacture of the monitor, prior to the first use by the patient, or any subsequent time when it may be desirable to recalibrate the sensor. The glucose sensor senses glucose in the calibration fluid in the sensing zone and generates an output signal associated with the known glucose concentration. This information can be used to calibrate (or recalibrate) operation of the glucose sensor.

[0063] In some embodiments, any actuating technique described herein may then be used to move an optional second calibrating fluid with a second known glucose concentration from a second calibration fluid reservoir into the sensing zone, displacing the first calibration fluid into the waste area. The sensor then senses the glucose from the second calibration fluid in the sensing zone and generates an output signal associated with the second known glucose concentration. Using these one or more at least two associations of known glucose concentration to glucose sensor output, a calibration curve or plot can be used to associate

glucose concentration to the output of the glucose sensor, which can then be used to determine glucose concentration of the glucose that diffuses into the sensing zone from the patient's interstitial fluid. Any number of calibration fluids, and thus calibration points, can be used to calibrate the glucose sensor. The calibrated sensor is then ready to sense glucose in the sensing zone which has diffused from the patient's interstitial fluid.

[0064] Describing the method in relation to FIG. 2, upon manual or automatic actuation of actuator 32, fresh calibration fluid is forced from calibration fluid reservoir 12 (only one reservoir is shown) through check valve 34, such as a flap valve, into sensing zone 14. Any fluid within the sensing zone is generally displaced through second check valve 36 into waste reservoir 16. Check valves or similar gating systems can also be used to prevent contamination.

[0065] It may be advantageous to retain a calibration fluid with the lower glucose concentration (such as a first concentration between about 0 mg/dl and 100 mg/dl) in the sensing zone after the calibrating step, to provide for faster response times for the glucose sensing. In the method described above where a second calibration fluid has a higher glucose concentration, it may be advantageous to move a volume of the fresh first lower concentration calibration fluid into the sensing zone after the glucose sensor has been calibrated. This would move the second sensing fluid from the sensing zone into waste reservoir. Alternatively, calibrating can comprise calibrating the sensor with a calibration fluid with a higher glucose concentration followed by calibrating the sensor with a calibration fluid with a lower glucose concentration.

[0066] Glucose monitors with more than one or more calibration reservoirs have been described. In such embodiments, at least one reservoir can be adapted to house a sensing or washing fluid which does not have any glucose, such as, for example, a buffer, preservative, or de-ionized water. As used herein, "sensing fluid" and "washing fluid" may be used interchangeably. Sensing fluid as used herein can be a special case of calibrating fluid with zero glucose concentration. Sensing fluid can be used to displace calibration fluid from the sensing zone after the calibration step. Glucose would then diffuse from the patient's interstitial fluid into the sensing fluid which does not contain glucose.

[0067] Embodiments in which there are a plurality of calibration fluid reservoirs as well as at least one sensing fluid reservoir are shown in FIGS. 3 and 4. In FIG. 3, glucose monitor 10 is shown comprising two calibration fluid reservoirs 12 and one sensing fluid reservoir 38. All three reservoirs are in fluid communication with the sensing zone. An actuator or actuators (not shown in FIGS. 3 and 4) can be configured to move fresh fluid from the reservoirs into the sensing zone.

[0068] In some embodiments the sensor is calibrated with any number of calibration fluids as described herein. The actuator can then move sensing fluid from a sensing fluid reservoir into the sensing zone, displacing a calibration fluid. In other embodiments, the sensor may be calibrated with one calibration fluid and then sensing fluid may be moved into the sensing zone, followed by a second calibration fluid being moved into the sensing zone, displacing the sensing fluid and calibrating the sensor with the second calibrating fluid. Fresh sensing fluid can then be actuated into the sensing zone, readying the monitor for diffusion and glucose detection. In this method, there is a "wash" step between

calibrating the sensor with fluids of different known glucose concentrations. In yet another embodiment the sensor can be factory calibrated thereby eliminating the need of any calibration fluids within the device.

[0069] In some embodiments at least one finger-stick calibration may optionally be performed or may be required to be performed at any point during the use of the monitors described herein.

[0070] Waste reservoirs may be or include an absorption device such as a wicking material to absorb waste fluids. In such embodiments the waste reservoir may not necessarily be an enclosed structure, but may simply be a wicking material or substance in fluid communication with the sensing zone so that it can wick waste fluids as they are moved from the sensing zone.

[0071] While in some embodiments the glucose monitor may be manually actuated to initiate the calibrating procedure, the glucose monitor can also be self-calibrating or self-actuating. For example, the glucose monitor can include a programmable component, such as a timer, that is programmed to automatically activate an actuator, such as a pump and valve system, to initiate the flow of fresh fluid from any of the fluid reservoirs into the sensing zone. The timer can be preprogrammed, or in some embodiments the monitor also includes a remote device that is separate from the sensor that can display a glucose concentration. The remote device can be adapted such that it can program the programmable component. For example, a patient may want to program the monitor to calibrate itself at certain times during the day. The monitor can include a timer that can be programmed, reprogrammed by the patient, and/or automatically reprogrammed. The remote device can be adapted for manual programming.

[0072] In some embodiments the glucose monitor includes a body and sensing zone temperature sensor, which is more fully described in co-assigned U.S. patent application Ser. No. 11/642,196, filed Dec. 20, 2006.

[0073] In some embodiments the glucose monitor includes a vibration assembly adapted to ease the penetration of the needle into the stratum corneum of the skin. Description of exemplary vibration assemblies are described in co-assigned U.S. patent application Ser. No. 11/642,196, filed Dec. 20, 2006.

[0074] In some embodiments the monitor can include an applicator to apply the sensor pad or adhesive pad to the skin. The applicator may be part of the sensor device or when the monitor includes separate components, it may be included in any of the different components. The applicator may also be a separate component.

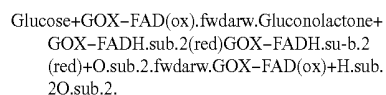
[0075] In some embodiments, the tissue piercing elements or fluid paths, fluid reservoirs, sensing zone, sensor, and optional adhesive pads are contained within a sensing structure separate from a reusable structure comprising the electronics element and actuator. This configuration permits the sensing structure, comprising the sensor, sensing fluid and tissue piercing elements or fluid paths to be discarded after a period of use (e.g., when the fluid reservoirs are depleted) while enabling the reusable structure comprising the electronics and actuator to be reused. A flexible covering (made, e.g., of polyester or other plastic-like material) may surround and support the disposable structure. In particular, the interface between an actuator and a fluid reservoir permits the actuator to move fluid out of the reservoir, such as by deforming a wall of the reservoir or forcing the fluid out of

the reservoir using a pressurized mechanism, such as a piston. In these embodiments, the disposable sensing structure and the reusable structure may have a mechanical connection, such as a snap or interference fit. Any of the monitor components described herein may, however, be located in the reusable structure or the sensing structure. For example, the tissue piercing elements or fluid paths could be configured to be located in the reusable structure. As another example, one or more fluid reservoirs may be located in the reusable structure and may be refillable, emptyable or separately replaceable from other disposable structures.

[0076] FIG. 5 shows an exploded view of another embodiment of the invention. This figure shows a removable seal 40 covering the distal end of tissue piercing elements or fluid paths 22 and attached, e.g., by adhesive. Removable seal 40 retains the fluid within the tissue piercing elements or fluid paths and sensing zone prior to use and is removed prior to placing the glucose monitor 10 on the skin using adhesive seal 18. In this embodiment, tissue piercing elements or fluid paths 22, the fluid and waste reservoirs, sensing zone 14 and sensor 24 are contained within and/or supported by sensing structure 42 which can be a disposable portion of the monitor. Reusable structure 44 comprises or supports electronics element 28 and actuator 32 that can be used to move sensing fluid out of the fluid reservoirs, through the sensing zone into the waste reservoir. Electrical contacts 46 extend from electronics element 28 to make contact with, for example, electrodes in glucose sensor 24 when the device is assembled.

[0077] The following is a description of glucose sensors that may be used with the glucose monitors of this invention. In 1962, Clark and Lyons proposed the first enzyme electrode (that was implemented later by Updike and Hicks) to determine glucose concentration in a sample by combining the specificity of a biological system with the simplicity and sensitivity of an electrochemical transducer. The most common strategies for glucose detection are based on using either glucose oxidase or glucose dehydrogenase enzyme.

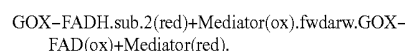
[0078] Electrochemical sensors for glucose, based on the specific glucose oxidizing enzyme glucose oxidase, have generated considerable interest. Several commercial devices based on this principle have been developed and are widely used currently for monitoring of glucose, e.g., self testing by patients at home, as well as testing in physician offices and hospitals. The earliest amperometric glucose biosensors were based on glucose oxidase (GOX) which generates hydrogen peroxide in the presence of oxygen and glucose according to the following reaction scheme:



[0079] Electrochemical biosensors are used for glucose detection because of their high sensitivity, selectivity and low cost. In principal, amperometric detection is based on measuring either the oxidation or reduction of an electroactive compound at a working electrode. A constant potential is applied to that working electrode with respect to another electrode used as the reference electrode. The glucose oxidase enzyme is first reduced in the process but is reoxidized again to its active form by the presence of any oxygen resulting in the formation of hydrogen peroxide. Glucose sensors generally have been designed by monitoring either the hydrogen peroxide formation or the oxygen consumption.

The hydrogen peroxide produced is easily detected at a potential of 0.0 volts, 0.1 volts, 0.2 volts, or any other fixed potential relative to a reference electrode such as a Ag/AgCl electrode. However, sensors based on hydrogen peroxide detection are subject to electrochemical interference by the presence of other oxidizable species in clinical samples such as blood or serum. On the other hand, biosensors that monitor oxygen consumption are affected by the variation of oxygen concentration in ambient air or in any of the fluids used with the monitors as described herein. In order to overcome these drawbacks, different strategies have been developed and adopted.

[0080] Selectively permeable membranes or polymer films have been used to suppress or minimize interference from endogenous electroactive species in biological samples. Another strategy to solve these problems is to replace oxygen with electrochemical mediators to reoxidize the enzyme. Mediators are electrochemically active compounds that can reoxidize the enzyme (glucose oxidase) and then be reoxidized at the working electrode as shown below:



[0081] Organic conducting salts, ferrocene and ferrocene derivatives, ferricyanide, quinones, and viologens are considered good examples of such mediators. Such electrochemical mediators act as redox couples to shuttle electrons between the enzyme and electrode surface. Because mediators can be detected at lower oxidation potentials than that used for the detection of hydrogen peroxide the interference from electroactive species (e.g., ascorbic and uric acids present) in clinical samples such as blood or serum is greatly reduced. For example ferrocene derivatives have oxidation potentials in the +0.1 to 0.4 V range. Conductive organic salts such as tetrathiafulvalene-tetracyanoquinodimethane (TTF-TCNQ) can operate as low as 0.0 Volts relative to a Ag/AgCl reference electrode. Nankai et al., WO 86/07632, published Dec. 31, 1986, discloses an amperometric biosensor system in which a fluid containing glucose is contacted with glucose oxidase and potassium ferricyanide. The glucose is oxidized and the ferricyanide is reduced to ferrocyanide. This reaction is catalyzed by glucose oxidase. After two minutes, an electrical potential is applied, and a current caused by the re-oxidation of the ferrocyanide to ferricyanide is obtained. The current value, obtained a few seconds after the potential is applied, correlates to the concentration of glucose in the fluid.

[0082] There are multiple glucose sensors that may be used with this invention. In a three electrode system, shown in FIGS. 6A and 6B a working electrode 50, such as Pt, C, or Pt/C is referenced against a reference electrode 52 (such as Ag/AgCl) and a counter electrode 54, such as Pt, provides a means for current flow. The three electrodes are mounted on an electrode substrate 56 as shown in FIG. 6A, then covered with a reagent 58 as shown in FIG. 6B.

[0083] FIGS. 7A and 7B show a two electrode system, wherein the working and auxiliary electrodes, 50 and 60 respectively, are made of different electrically conducting materials. Like the embodiment of FIGS. 6A and 6B, the electrodes are mounted on a flexible substrate 56 (FIG. 7A) and covered with a reagent 58 (FIG. 7B). In an alternative two electrode system, the working and auxiliary electrodes are made of the same electrically conducting materials, where the reagent exposed surface area of the auxiliary electrode is slightly larger than that of the working electrode

or where both the working and auxiliary electrodes are substantially of equal dimensions.

[0084] In amperometric and coulometric biosensors, immobilization of the enzymes is also very important. Conventional methods of enzyme immobilization include covalent binding, physical adsorption or cross-linking to a suitable matrix may be used. In some embodiments the reagent chemistry can be deposited away from the electrodes using various different dispensing methods.

[0085] The glucose sensor can be constructed by immobilizing glucose oxidase enzyme on top of the electrode by using a proprietary cross linker and a coating membrane. The cross linker will hold the enzyme on top of the sensor, and the thin layer membrane (e.g., Nafion, cellulose acetate, polyvinyl chloride, urethane etc) will help the long term stability of the glucose sensor. In the presence of oxygen the glucose oxidase will produce hydrogen peroxide. The hydrogen peroxide can be readily oxidized at the working electrode surface in either two or three electrodes systems.

[0086] In some embodiments, the reagent is contained in a reagent well in the biosensor. The reagent includes a redox mediator, an enzyme, and a buffer, and covers substantially equal surface areas of portions of the working and auxiliary electrodes. When a sample containing the analyte to be measured, in this example glucose, comes into contact with the glucose biosensor the analyte is oxidized, and simultaneously the mediator is reduced. After the reaction is complete, an electrical potential difference is applied between the electrodes. In general the amount of oxidized form of the redox mediator at the auxiliary electrode and the applied potential difference must be sufficient to cause diffusion limited electrooxidation of the reduced form of the redox mediator at the surface of the working electrode. After a short time delay, the current produced by the electrooxidation of the reduced form of the redox mediator is measured and correlated to the amount of the analyte concentration in the sample. In some cases, the analyte sought to be measured may be reduced and the redox mediator may be oxidized.

[0087] In the present invention, these elements may be satisfied by employing a readily reversible redox mediator and using a reagent with the oxidized form of the redox mediator in an amount sufficient to insure that the diffusion current produced is limited by the oxidation of the reduced form of the redox mediator at the working electrode surface. For current produced during electrooxidation to be limited by the oxidation of the reduced form of the redox mediator at the working electrode surface, the amount of the oxidized form of the redox mediator at the surface of the auxiliary electrode exceeds the amount of the reduced form of the redox mediator at the surface of the working electrode. Importantly, when the reagent includes an excess of the oxidized form of the redox mediator, as described below, the working and auxiliary electrodes may be substantially the same size or unequal size as well as made of the same or different electrically conducting material or different conducting materials. From a cost perspective the ability to utilize electrodes that are fabricated from substantially the same material represents an important advantage for inexpensive biosensors.

[0088] As explained above, the redox mediator must be readily reversible, and the oxidized form of the redox mediator must be of sufficient type to receive at least one electron from the reaction involving enzyme, analyte, and oxidized form of the redox mediator. For example, when

glucose is the analyte to be measured and glucose oxidase is the enzyme, ferricyanide or quinone may be the oxidized form of the redox mediator. Other examples of enzymes and redox mediators (oxidized form) that may be used in measuring particular analytes by the present invention are ferrocene and or ferrocene derivative, ferricyanide, and viologens. Buffers may be used to provide a preferred pH range from about 4 to 8. In one embodiment, the pH range is from about 6 to 7. The buffer may be phosphate (e.g., potassium phosphate) and may be in a range from about 0.01M to 0.5M, such as about 0.05M. (These concentration ranges refer to the reagent composition before it is dried onto the electrode surfaces.) More details regarding glucose sensor chemistry and operation may be found in: Clark L C and Lyons C, "Electrode Systems for Continuous Monitoring in Cardiovascular Surgery," *Ann NY Acad Sci*, 102:29, 1962; Updike S J, and Hicks G P, "The Enzyme Electrode," *Nature*, 214:986, 1967; Cass, A. E. G., G. Davis. G. D. Francis, et al. 1984. Ferrocene—mediated enzyme electrode for amperometric determination of glucose. *Anal. Chem.* 56:667-671; and Boutelle. M. G., C. Stanford. M. Fillenz. et al. 1986. An amperometric enzyme electrode for monitoring brain glucose in the freely moving rat. *Neurosci lett.* 72:283-288.

[0089] With the above overview, another embodiment of the glucose monitoring device will be described. The glucose monitor of this embodiment comprises a plurality of fluid paths, each fluid path comprising a distal opening, a proximal opening and an interior space extending between the distal and proximal openings. The glucose monitor further comprises a sensing zone in fluid communication with the proximal openings of the fluid paths. Further, the glucose monitor comprises a sensing fluid extending from the sensing zone into substantially the entire interior space of the fluid paths. The sensing fluid comprises a concentration of citrate. Yet further, the glucose monitor comprises a glucose sensor adapted to detect a concentration of glucose in the sensing fluid within the sensing zone.

[0090] Next, methods of measuring continuous glucose concentration are described below. The methods may be applied to any of the above embodiments of the glucose monitoring device.

[0091] Glucose is transported from blood to interstitial fluid. Glucose then diffuses from the interstitial fluid in the individual's skin to sensing fluid in lumens in the tissue piercing elements or to other fluid paths. Glucose further diffuses through the lumens or fluid paths into the sensing zone filled with sensing fluid. As explained earlier, glucose reacts with the sensor chemistry to make hydrogen peroxide. Hydrogen peroxide is detected at an electrochemical sensor, producing an electrical current signal.

[0092] One aspect of the invention is a method of in vivo monitoring of an individual's interstitial fluid glucose concentration comprising creating a plurality of fluid paths through a stratum corneum layer of an area of the individual's skin. The method may also comprise inserting tissue piercing elements through the stratum corneum layer of an area of the individual's skin. The tissue piercing elements may be solid or hollow. The tissue piercing elements may then be removed, leaving voids that form fluid paths directly through the stratum corneum layer. Alternately, the tissue piercing elements may be left in place in the stratum corneum, such that fluid paths are formed through or around the tissue piercing elements.

[0093] As indicated above, the fluid paths may be created by piercing the user's skin. The fluid paths may also be created by removing layers of the individual's skin or by placing holes or pores through the individual's skin. Further, the fluid paths may be created with laser, abrasion or electroporation.

[0094] The fluid paths each comprise a distal end in fluid communication with interstitial fluid of the individual, a proximal end in fluid communication with a sensing zone located outside of the patient's body. An interior space extends between the distal and proximal ends of the fluid path. The interior space may also be referred to as a lumen area in the tissue piercing elements or fluid paths. A sensing fluid fills substantially the entire interior space and the sensing fluid concentration comprises a concentration of citrate. In some embodiments, the concentration of citrate ranges from 100 milli-Moles per liter (mM) to 200 mM. In other embodiments, the concentration of citrate ranges from 135 to 155 mM. In another variation the concentration of citrate ranges from 0.1 mM to 250 mM, where the concentration can be adjusted based on the desired amount of time to monitor an analyte in the fluid.

[0095] A citrate can refer to citric acid or any conjugate base of citric acid (e.g., $\text{C}_3\text{H}_5\text{O}(\text{COO})_3^-$).

[0096] The sensing fluid concentration also comprises a buffer formulation. The buffer formulation may be comprised of phosphate and citrate or other formulations.

[0097] The method allows at least one analyte to passively diffuse from the patient's interstitial fluid through the tissue piercing elements or fluid paths and into the sensing zone. The analyte is glucose and/or another agent that is being transdermally monitored through the fluid paths or tissue piercing elements.

[0098] In addition an additional, the methods and devices can include allowing at least one analyte to passively diffuse from the patient's interstitial fluid through micropores created by tissue piercing elements, where the piercing elements have been removed. The micropores are in fluid communication with the sensing zone and remain in fluid communication due to the concentration of citrate discussed above.

[0099] In particular, some embodiments of the method increase initial transdermal glucose flux and inhibit a decrease over time in transdermal glucose flux or of another agent that is being transdermally monitored through the tissue piercing elements or fluid paths. Flux enhancement may be achieved by addition of citrate to a saline phosphate buffered solution (PBS) in the sensing fluid. Measurement of the transdermal glucose flux can be used to determine whether the micropores remain open. Failure to detect glucose can indicate that the micropores are closed or obstructed. Accordingly, the systems and devices can be configured to monitor the glucose flux to determine that it remains uninterrupted.

[0100] The method may then mitigate a decrease of the transdermal analyte fluid flux through the fluid paths after the step of increasing the transdermal analyte fluid concentration. The mitigation may last up to 72 hours or more

[0101] The method further comprises sensing a concentration of the at least one analyte in a sensing fluid within the sensing zone using a sensor located at least partially in the sensing zone. In particular, glucose concentration is sensed using the sensor.

[0102] Addition of citrate to the PBS buffer results in the following benefits compared to the non-citrate PBS: 1) increased transdermal flux through the tissue piercing elements or fluid paths immediately after application of the tissue piercing elements or fluid paths to the skin, 2) inhibition of a decrease in transdermal flux several hours after application, and 3) inhibition of a decrease in transdermal flux up for several days after application.

[0103] FIG. 8 illustrates an average ratio of transdermal glucose flux to reference blood glucose measurement for sensing blood glucose concentration. As illustrated in FIG. 8, results were taken as tissue piercing elements of glucose monitoring devices were applied to the skin and samples were collected in 75-100 uL sensing zone. Eight control devices without citrate sensing fluid concentration in the sensing zone and eight devices with citrate sensing fluid concentration in the sensing zone were provided. Specifically, the sensing zone was filled with either 300 mM Phosphate PBS (Control) or 300 mM Phosphate PBS+153 mM Citrate. Samples of glucose concentration were taken every 20 minutes for six hours. Reference blood glucose measurements were taken at the same intervals of every 20 minutes for six hours. For each sample, the ratio of transdermal glucose flux through the tissue piercing elements to the corresponding reference blood glucose measurement was calculated. Flux ratios were averaged over the 6-hour sampling period by each glucose monitoring device. Mean flux ratios averaged by buffer condition are shown in FIG. 8. As illustrated, the addition of citrate resulted in a statistically significant increase in transdermal glucose flux, the value being 0.4 of mean flux ratio compared to the control of 0.2 mean flux ratio ($p=0.002$).

[0104] FIG. 9 illustrates change in ratio of transdermal glucose flux to reference blood glucose per hour for sensing blood glucose concentration. Eight control devices without citrate sensing fluid concentration in the sensing zone and eight devices with citrate sensing fluid concentration in the sensing zone were provided. Specifically, the sensing zone was filled with either 300 mM Phosphate PBS (control device) or 300 mM Phosphate PBS+153 mM Citrate. Signal decay was measured as the change in flux ratio per hour as a percentage of the initial flux ratio. Flux ratio change per hour by condition is shown in FIG. 9. The addition of citrate resulted in a statistically significant inhibition of signal decay compared to the control devices ($p<0.0001$).

[0105] FIG. 10 illustrates percentage of initial transdermal glucose flux after 24 hours of glucose monitoring device wear. Ten control devices without citrate sensing fluid concentration in the sensing zone and thirty one devices with citrate sensing fluid concentration in the sensing zone were provided. Flux at 24 hours as a percentage of initial flux (within the first two hours) is shown in FIG. 10 averaged for the two buffer conditions, with and without citrate concentrations. The addition of 103-153 mM citrate resulted in a statistically significant increase in percentage of initial flux at 24 hours compared to the control devices ($p=0.01$).

[0106] FIG. 11 illustrates percentage of initial glucose flux after 24, 48, 72 hours of glucose monitoring device wear. Eight control devices without citrate sensing fluid concentration in the sensing zone and eight devices with citrate sensing fluid concentration in the sensing zone were provided. Flux at 24, 48 and 72 hours as a percentage of initial flux (within the first two hours) is shown in FIG. 11 averaged for each of the two buffer conditions, with and without

citrate concentrations. The addition of 153 mM citrate resulted in a statistically significant increase in percentage of initial flux at 24 hours ($p=0.035$), 48 hours ($p=0.009$) and 72 hours ($p=0.006$) compared to the control devices.

[0107] After glucose monitoring device removal from the stratum corneum layer of an area of the individual's skin, blockage of the tissue piercing elements lumens was characterized. Tissue piercing elements were placed on a light source. Imaging of the transmitted light on the other side of the tissue piercing elements was performed.

[0108] FIGS. 12a and 12b illustrate images of tissue piercing element lumens after use with sensing fluid for 72 hours. FIG. 12a shows lumens used with a control sensing fluid having no citrate added. FIG. 12b shows lumens used with a sensing fluid having citrate added to the buffer solution. As shown in FIGS. 12a and 12b, glucose monitoring devices with citrate added to the buffer had significantly less lumen occlusion than the control device without the citrate.

[0109] In FIGS. 12a and 12b, lumen dimensions are approximately 50.times.50 microns. "Occlusion" as measured by these photographs may mean filled with opaque material so that light may not be transmitted. In terms of flux, "occlusion" may mean filled with material so that transport of the analyte through the lumen is substantially hindered or blocked. Occlusion may be caused by protein adsorption in the lumen or near the distal end of the lumen, deposition of material via a clotting mechanism, fibrin deposition, precipitation, etc. In FIGS. 12A and B, the citrate condition has 98% open lumens (i.e., 2% occluded) and the control condition has 24% open lumens (i.e., 76% occluded).

[0110] FIG. 13 illustrates percentage of unblocked tissue piercing element lumen area within 24 and 72 hours when citrate concentration is added to the sensing fluid. A series of studies was performed investigating the effect of the addition of citrate on tissue piercing element lumen blockage. Glucose monitoring devices were applied and removed at 24 hours or 48 hours. The results are reported as a percentage of the expected total lumen area, i.e. measured light. Higher percentages of unblocked lumen area were observed at both 24 hours ($p=0.0024$) and 48 hours ($p=0.10$) for the citrate devices.

[0111] FIG. 14 illustrates percentage of open tissue piercing element lumens within 72 hours when 153 mM citrate is added to the sensing fluid. A series of studies was performed investigating the effect of the addition of citrate on tissue piercing elements lumen blockage. Devices were applied and removed at 72 hours. The results are reported as a percentage of the expected number of open lumens. Addition of citrate resulted in significantly higher number of open lumens compared to the control ($p=0.0004$). At 72 hours, citrate devices had all or nearly all lumens with unblocked area.

[0112] While exemplary embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims

define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

We claim:

1. A method of in vivo monitoring of an individual's interstitial fluid analyte concentration comprising:

creating plurality of micropores through a stratum corneum layer of an area of the individual's skin, where the plurality of micropores forms a plurality of fluid paths having a distal end in fluid communication with interstitial fluid of the individual and a proximal end in fluid communication with a sensing zone located outside of the patient's body, where the fluid paths comprise an interior space extending between the distal and proximal ends;

delivering a sensing fluid filling within the interior space of the fluid paths;

allowing at least one analyte to passively diffuse from the patient's interstitial fluid through the fluid paths and into the sensing zone; and

sensing a concentration of the at least one analyte in a sensing fluid within the sensing zone using a sensor located at least partially in the sensing zone, wherein the sensing fluid formulation comprises a chelating agent and or biochemical inhibitors adapted to keep the micropores, created by the tissue piercing elements, open for extended period to maintain an un-interrupted analyte flux through the fluid paths and to mitigate a decrease over time of the analyte flux through the fluid paths.

2. A method of in vivo monitoring of an individual's interstitial fluid analyte concentration comprising:

creating a plurality of micropores through a stratum corneum layer of an area of the individual's skin, where the plurality of micropores define a plurality of fluid paths in communication with an interstitial fluid of the individual, a proximal end of the fluid paths being in fluid communication with a sensing zone located outside of the patient's body; and

where the sensing zone comprises a flexible sensor laminated to a buffered gel allowing at least one analyte to passively diffuse from the patient's interstitial fluid through the fluid paths and into the sensing zone wherein the sensing gel formulation comprises a chelating agent and or biochemical inhibitors adapted to keep the micropores, created by the tissue piercing elements, open for extended period to maintain an uninterrupted analyte flux through the fluid paths and to mitigate a decrease over time of the analyte flux through the fluid paths.

3. The method of claim 1 wherein the chelating agent comprises a sufficient concentration of the agent to keep the skin micropores open to maintain a constant analyte flux through the fluid paths for one and or several days.

4. The method of claim 2 wherein the chelating agent in the gel formulation comprises a sufficient concentration of the agent to keep the skin micropores open to maintain a constant analyte flux through the fluid paths for one and or several days.

5. The method of claim 1 wherein the agent comprises citrate ions and the concentration of citrate ion ranges from 0.1 mM to 250 mM.

6. The method of claim 1 or 2 wherein the sensing fluid comprises a phosphate buffered saline solution.

7. The method of claim 1 or 2 wherein the analyte is glucose.

8. The method of claim 1 or 2 wherein the analyte is other than glucose for example cholesterol, electrolytes or proteins.

9. The method of claim 1 or 2 further comprising inserting tissue piercing elements through the stratum corneum layer using a simple insertion applicator.

10. An analyte monitor comprising:

a plurality of fluid paths, each fluid path comprising a distal opening adapted to be disposed on one side of a stratum corneum layer of a user's skin, a proximal opening adapted to be disposed on another side of the stratum corneum layer and an interior space extending between the distal and proximal openings;

a sensing zone in fluid communication with the proximal openings of the fluid paths; and

sensing fluid extending from the sensing zone into substantially the entire interior space of the fluid paths, wherein the sensing fluid comprises one or more chelating agent adapted to keep the micropores or microchannels open to maintain a constant analyte flux through the fluid paths and an analyte sensor adapted to detect a concentration of analyte in the sensing fluid within the sensing zone.

11. An analyte monitor comprising:

a plurality of fluid paths via micropores through a stratum corneum layer of an area of the individual's skin, said fluid paths are in communication with interstitial fluid of the individual, a proximal end in fluid communication with a sensing zone located outside of the patient's body; and

where the sensing zone comprises a flexible sensor laminated to a buffered gel allowing at least one analyte to passively diffuse from the patient's interstitial fluid through the fluid paths and into the sensing zone wherein the sensing gel formulation comprises a chelating agent adapted to keep the micropores, created by the tissue piercing elements, open for extended period to maintain a constant analyte flux through the fluid

paths and to mitigate a decrease over time of the analyte flux through the fluid paths and an analyte sensor adapted to detect a concentration of analyte in the sensing fluid within the sensing zone.

12. The analyte monitor of claim 10 wherein the agent comprises a concentration of citrate in a range from 1.0 mM to 250 mM.

13. The analyte monitor of claim 10 wherein the sensing fluid comprises a phosphate buffered saline solution.

14. The analyte monitor of claim 10 wherein the analyte comprises glucose.

15. The analyte monitor of claim 10 wherein the analyte is other than glucose for example cholesterol, electrolytes or proteins.

16. The analyte monitor of claim 10 wherein the fluid paths each comprise a tissue piercing element.

17. The analyte monitor of claim 10 wherein the fluid paths do not comprise a tissue piercing element.

18. The analyte monitor of claim 1 wherein the flexible electrochemical gel pad can be adapted and integrated into a simple easy to use wearable wellness monitor for making healthy lifestyle decisions

19. The analyte monitor of claim 1 wherein the flexible electrochemical gel pad can be adapted to be integrated into a wearable wellness monitor containing other sensing devices such to measure for example galvanic skin response, skin temperature, heart rate and motion

20. The method of claim 1 and 10 wherein the digital graphical feedback provided to the user is the sum total of all parameters.

21. The analyte monitor of claim 1 and 10 wherein the analyte monitor provides information regarding a general physiological condition of the patient.

22. The wearable wellness monitor of claim 1 and 10 wherein the analyte monitor is not used for the diagnosis of diabetes or any other medical disease.

23. The wearable wellness monitor of claim 1 and 10 wherein the wellness monitor is suitable for use by all individuals regardless of their health status.

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专利名称(译)	用于增强皮肤穿孔的装置和方法，用于连续葡萄糖监测		
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摘要(译)

组织穿刺元件在透皮药物递送领域的功效已被充分证明。多项研究表明，通过在角质层中产生微孔来增强皮肤渗透可以大大提高药物的递送速率。然而，具有组织刺穿元件的皮肤穿孔不是影响药物运输速率的唯一因素。还需要考虑其他因素，例如药物的配方和微孔封闭物的封闭速率。类似地，几个工人使用微组织刺穿元件用于连续葡萄糖监测的成功率较低。

