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(71) Applicant: **UNIVERSITY OF CINCINNATI** [US/US]; 2900 Reading Road, Suite 460, Cincinnati, OH 45206-0829 (US).

(72) Inventor: **HEIKENFELD, Jason, Charles**; 136 Dorsey Street, Cincinnati, OH 45202 (US).

(74) Agent: **JEFFERIES, David, E.** et al.; Wood Herron & Evans LLP, 441 Vine Street, Suite 2700, Cincinnati, OH 45202-2917 (US).

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(54) **Title:** DEVICES FOR INTEGRATED, REPEATED, PROLONGED, AND/OR RELIABLE SWEAT STIMULATION AND BIOSENSING AND FOR REMOVING EXCESS WATER DURING SWEAT STIMULATION

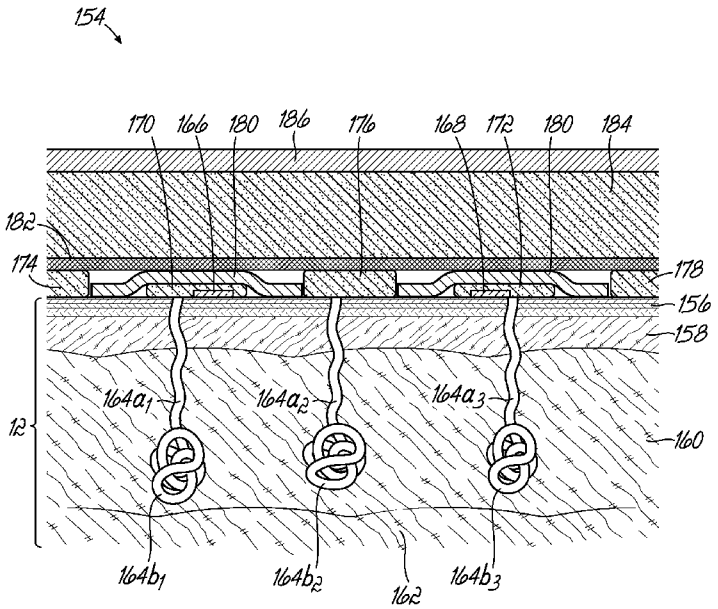


FIG. 12

(57) **Abstract:** A device (154) for sensing sweat on skin (12) includes an analyte- specific sensor (166, 168) for sensing an analyte in sweat; a sweat stimulant reservoir (174, 176, 178) separated from a waste water reservoir (184) by a water-permeable, sweat- stimulant impermeable membrane (182). The waste water reservoir (184) has a wicking force that is not greater than the wicking force of the sweat stimulant reservoir (174, 176, 178). The waste water reservoir (184) removes excess water from sweat to prevent the dilution of the sweat stimulant from the sweat stimulant reservoir (174, 176, 178), thereby maintaining the effectiveness of the sweat stimulant.

[Continued on next page]

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DEVICES FOR INTEGRATED, REPEATED, PROLONGED, AND/OR RELIABLE SWEAT STIMULATION AND BIOSENSING AND FOR REMOVING EXCESS WATER DURING SWEAT STIMULATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/734,462, entitled "Devices for Removing Excess Water During Sweat Stimulation" filed on September 21, 2018, the disclosure of which is hereby incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

[0002] Sweat sensing technologies have enormous potential for applications ranging from athletics, to neonates, to pharmacological monitoring, to personal digital health, to name a few applications. This is because sweat contains many of the same biomarkers, chemicals, or solutes that are carried in blood, which can provide significant information that enables one to diagnose ailments, health status, toxins, performance, and other physiological attributes even in advance of any physical sign. Furthermore, sweat itself, and the action of sweating, or other parameters, attributes, solutes, or features on or near skin or beneath the skin, can be measured to further reveal physiological information. However, obtaining a sweat sample free of contamination is challenging.

[0003] Sweat has significant potential as a sensing paradigm, but it has not emerged beyond decades-old usage in infant chloride assays for Cystic Fibrosis (e.g. Wescor Macroduct system) or in illicit drug monitoring patches (e.g. PharmCheck drugs of abuse patch by PharmChem). The majority of medical literature discloses slow and inconvenient sweat stimulation and collection, transport of the sample to a lab, and then analysis of the sample by a bench-top machine and a trained expert. All of this is so labor intensive, complicated, and costly, that in most cases, one would just as well implement a blood draw since it is the gold standard for most forms of high performance biomarker sensing. Hence, sweat sensing has not achieved its fullest potential for biosensing, especially for continuous or repeated biosensing or monitoring. Furthermore, attempts at using sweat to sense 'holy grails' such as glucose have failed to produce viable commercial products, reducing the publically perceived capability and opportunity space for sweat sensing. A similar conclusion has been made in a substantial 2014

review provided by Castro titled "Sweat: A sample with limited present applications and promising future in metabolomics", which states: "The main limitations of sweat as clinical sample are the difficulty to produce enough sweat for analysis, sample evaporation, lack of appropriate sampling devices, need for a trained staff, and errors in the results owing to the presence of pilocarpine. In dealing with quantitative measurements, the main drawback is normalization of the sampled volume."

[0004] Biosensing using sweat has many drawbacks and limitations that must be resolved in a manner that affordably, effectively, conveniently, intelligently, and reliably brings sweat sensing technology into intimate proximity with sweat as it is generated.

[0005] Many of these drawbacks stated above can be resolved by creating novel and advanced interplays of chemicals, materials, sensors, electronics, microfluidics, algorithms, computing, software, systems, and other features or designs, in a manner that affordably, effectively, conveniently, intelligently, or reliably brings sweat sensing technology into intimate proximity with sweat as it is generated. Sweat sensing therefore becomes a compelling new paradigm that clearly was overlooked in terms of its ultimate potential as a biosensing platform.

[0006] Sweat sensors have many potential advantages over other biofluid sensors. But one potentially confounding factor is that prolonged stimulation of sweat can be problematic as some individuals can be hyper sensitive to prolonged stimulation of sweat or their glands will adapt to sweat stimulation and provide no or reduced response to sweat stimulation by heat, electricity, iontophoresis, or other means. Furthermore, for prolonged stimulation, risk of electrode detachment is a risk, and can even be a risk at the onset of stimulation. Solutions for solving these risks are lacking.

[0007] Another problematic factor is the difficulty in obtaining a sweat sample free of contamination and/or dilution. For example, iontophoretic delivery of a sweat stimulant can be significantly confounded by solutes in sweat (such as ions). And the presence of excess water during and following sweat stimulation can also disrupt biosensing using a sweat source.

[0008] The number of active sweat glands varies greatly among different people, though comparisons between different areas (ex. axillae versus groin) show the same directional changes (certain areas always have more active sweat glands while others always have fewer). The palm is estimated to have around 370 sweat glands per cm^2; the back of the hand 200 per cm^2; the forehead 175 per cm^2; the breast, abdomen, and forearm 155 per cm^2; and the back and legs 60–

80 per cm^2. Assuming use of a sweat gland density of 100/cm^2, a sensor that is 0.55 cm in radius (1.1 cm in diameter) would cover ~1 cm^2 area or approximately 100 sweat glands. According to "Dermatology: an illustrated color text" 5th edition, the human body excretes a minimum of 0.5 liter per day of sweat, and has 2.5 million sweat glands on average and there are 1440 minutes per day. For prepubescent children, these sweat volumes are typically lower. For 2.5 million glands that rate is 0.2 µl per gland per day or 0.14 nl/min/gland. This is the minimum 'average' sweat rate generated per pore, on average, with some possible exceptions being where sweating increases slightly on its own (such as measuring sleep cycles, etc.). Again, from "Dermatology: an illustrated color text" 5th edition, the maximum sweat generated per person per day is 10 liters which on average is 4 µL per gland maximum per day, or about 3 nL/min/gland. This is about 20X higher than the minimum rate.

[0009] According to Buono 1992, J. Derm. Sci. 4, 33-37, "Cholinergic sensitivity of the eccrine sweat gland in trained and untrained men", the maximum sweat rates generated by pilocarpine stimulation are about 4 nL/min/gland for untrained men and 8 nL/min/gland for trained (exercising often) men. Other sources indicate maximum sweat rates of an adult can be up to 2-4 liters per hour or 10-14 liters per day (10-15 g/min•m^2), which based on the per hour number translates to 20 nL/min/gland or 3 nL/min/gland. Sweat stimulation data from "Pharmacologic responsiveness of isolated single eccrine sweat glands" by K. Sato and F. Sato (the data was for extracted and isolated monkey sweat glands, which are very similar to human ones), suggests a rate up to ~5 nL/min/gland is possible with stimulation, and several types of sweat stimulating substances are disclosed. For simplicity, we can conclude that the minimum sweat on average is ~ 0.1 nL/min/gland and the maximum is ~5 nL/min/gland, which is about a 50X difference between the two.

[0010] Based on the assumption of a sweat gland density of 100/cm^2, a sensor that is 0.55 cm in radius (1.1 cm in diameter) would cover ~1 cm^2 area or approximately 100 sweat glands. Assuming a dead volume under each sensor of 50 µm height or $50x10^{-4}$ cm, and that same 1 cm^2 area, provides a volume of 50E-4 cm^3 or about 50E-4 mL or 5 µL of volume. With the maximum rate of 5 nL/min/gland and 100 glands it would require 10 minutes to fully refresh the dead volume. With the minimum rate of 0.1 nL/min/gland and 100 glands it would require 500 minutes or 8 hours to fully refresh the dead volume. If the dead volume could be reduced by 10X to 5 µm roughly, the max and min times would be 1 minute and 1 hour, roughly respectively, but the min rate would be subject to diffusion and other contamination issues (and 5 µm dead volume height could be technically challenging). Consider the fluidic component

between a sensor and the skin to be a 25 μm thick piece of paper or glass fiber with, which at 1 cm^2 equates to a volume of 2.5 μL of volume and if the paper was 50% porous (50% solids) then the dead volume would be 1.25 μL. With the maximum rate of 5 nL/min/gland and 100 glands it would require 2.5 minutes to fully refresh the dead volume. With the minimum rate of 0.1 nL/min/gland and 100 glands it would require ~100 minutes or ~2 hours to fully refresh the dead volume.

[0011] Sweat stimulation is commonly known to be achieved by one of several means. Sweat activation has been promoted by simple thermal stimulation, by intradermal injection of drugs such as methylcholine or pilocarpine, and by dermal introduction of such drugs using diffusion-based delivery or using iontophoresis. Gibson and Cooke's device for iontophoresis, one of the most employed devices, provides DC current and uses large lead electrodes lined with porous material. The positive pole is dampened with 2% pilocarpine hydrochloride, and the negative one with 0.9% NaCl solution. Sweat can also be generated by orally administering a drug. Sweat can also be controlled or created by asking the subject using the patch to enact or increase activities or conditions which cause them to sweat.

[0012] Sweat rate can also be measured real time in several ways. Sodium can be utilized to measure sweat rate real time (higher sweat rate, higher concentration), as it is excreted by the sweat gland during sweating. Chloride can be utilized to measure sweat rate (higher sweat rate, higher concentration), as it is excreted by the sweat gland during sweating. Both sodium and chloride can be measured using ion-selective electrodes or sealed reference electrodes, for example placed in the sweat sensor itself and measured real time as sweat emerges onto the skin. Sato 1989, pg. 551 provides details on sweat rate vs. concentration of sodium & chloride. Electrical impedance can also be utilized to measure sweat rate. Grimnes 2011 and Tronstad 2013 demonstrate impedance and sweat rate correlations. Impedance and Na concentration, and or other measurements can be made and used to calculate at least roughly the sweat pore density and sweat flow rate from individual sweat glands, and coupled with sweat sensing or collection area to determine an overall sweat flow rate to a sensor. More indirect measurements of sweat rate are also possible through common electronic/optical/chemical measurements, including those such as pulse, pulse-oxygenation, respiration, heart rate variability, activity level, and 3-axis accelerometry, or other common readings published by Fitbit, Nike Fuel, Zephyr Technology, and others in the current wearables space, or demonstrated previously in the prior art.

[0013] With reference to Fig. 1A, a prior art sweat stimulation and sensing device 10 is positioned on skin 12 and is provided with some features shown that are relevant to the present invention. The device 10 is adhered to the skin 12 with an adhesive 14 which carries a substrate 13, control electronics 16, at least one sensor 18, a microfluidic component 20, a reservoir or gel with pilocarpine referred to as pilocarpine source 22, an iontophoresis electrode 24, and counter electrode 26. The electrodes 24 and 26 are electrically conductive with and through the skin 12 by virtue of the conductance of materials 22, 20 and 14 and, in some cases adhesive 14 can be locally removed beneath one or more electrodes or sensors to improve conductance with the skin and/or to improve collection or interface with sweat. Adhesives can be functional as tacky hydrogels as well which promote robust electrical, fluidic, and iontophoretic contact with skin (as commercially available examples such as those by SkinTact for ECG electrodes). With reference to Fig. 1B, a top view of connections to the electronics 16 is shown, such connections by example only and not representing a limiting configuration. The electronics 16 can be a simple as a controlled current source and sensing electronics only, or more complex including computing, communication, a battery, or other features. Again, in some embodiments, the electronics may be much simpler or not needed at all.

[0014] With further reference to Fig. 1A and 1B, if the device 10 were to stimulate sweat by virtue of iontophoretically driving a stimulating drug such as pilocarpine from the source 22 into the skin 12, it could conventionally do so for several minutes and stimulate sweat that could be collected for 10-30 minutes by the microfluidic component 20 and flow over the sensor 18 which could detect one or more solutes of interest in the sweat. This conventional stimulation and collection time frame is typical and similar to that broadly used for infant-chloride assays for cystic fibrosis testing, such as found in products by Wescor Corporation. Sweat sensors have advantages over other biofluid sensors, but one potentially confounding factor is that prolonged stimulation of sweat for more than 30 minutes could be problematic as some individuals can be hyper sensitive to prolonged stimulation of sweat or their glands, or will adapt to sweat stimulation and provide no or reduced response to sweat stimulation by heat, electricity, iontophoresis, or other means. Furthermore, for prolonged stimulation, electrode detachment can be a risk, or even be a risk at the onset of stimulation. Solutions for solving these risks are lacking. Furthermore, the stimulation can interfere with the quality of the sensing. Another problematic factor is the difficulty in obtaining a sweat sample free of contamination and/or dilution. As described above, iontophoretic delivery of a sweat stimulant can be significantly confounded by solutes in sweat (such as ions). And the presence of excess water during and

following sweat stimulation can also disrupt biosensing using a sweat source. These drawbacks need to be resolved as well.

SUMMARY OF THE INVENTION

[0015] Certain aspects of the present invention are premised on the realization that sweat can be effectively stimulated and analyzed in a single, continuous, or repeated manner inside the same device. The present invention addresses the confounding factors that result in performance being too poor for many practical uses. More specifically, some aspects of the present invention provide: sweat sampling and stimulation with at least one shared microfluidic component; sweat sampling and stimulation with at least one component or membrane added to mitigate the interference of a sweat stimulating portion of device with the purity of sweat delivery to the sampling portion of the device; multiple stimulation pads and some with their own sensors; timed pulsing of stimulation in some cases allowing areas of skin to rest; detection of a faulty stimulation contact with skin; and parametric specification of pads small enough to reduce irritation during sweat stimulation; and additional alternate embodiments as will be taught in the specifications.

[0016] Further, minimizing dead volume, that is the volume of sweat that must be generated to be detected by an electrode or other type of sensor, can in some cases ease some of the challenges of sweat stimulation. For example, consider a polymer matrix that is porous to sweat with 10% open porosity, and which is tacky and gel like (so it adheres and bonds to skin). If this were 50 μm thick, then the equivalent dead volume would be that of a 5 μm thick dead volume, the max and min times would be 1 minute and 1 hour, roughly respectively, and this is much less technically challenging than a highly open/porous dead volume. Reducing dead volume, isolating sweat pores, minimizing irritation, and other aspects are all desirable for prolonged stimulation of sweat for chronological monitoring applications. If dead volumes are reduced enough, hourly or even once a day readings are highly possible without need for high sweat rates.

[0017] Other aspects of the present invention are premised on the realization that unlike iontophoretic delivery of a sweat stimulant, diffusion-based delivery of a sweat stimulant is not significantly confounded by solutes in sweat (such as ions), so long as excess water can be removed by the waste water reservoir. And so, embodiments of the invention provide a device for sensing sweat on skin. In certain embodiments, a device includes an analyte-specific sensor for sensing an analyte in sweat; a sweat stimulant reservoir including a sweat stimulant; a waste

water reservoir having a wicking force that is not greater than a wicking force of the sweat stimulant reservoir; and a water-permeable, sweat stimulant-impermeable membrane between the sweat stimulate reservoir and the waste water reservoir.

[0018] The objects and advantages of the disclosed invention will be further appreciated in light of the following detailed descriptions and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The objects and advantages of the present invention will be further appreciated in light of the following detailed descriptions and drawings in which:

[0020] Fig. 1A and 1B are side view and top-view diagrams of prior art.

[0021] Fig. 2A is a side view diagram sweat sensor device with multiple sweat stimulation pads.

[0022] Fig. 2B is an overhead view of Fig. 2A, with only circuitry and electrodes shown.

[0023] Figs. 3-7 show one of an alternate arrangement for a plurality of arrangements for sweat stimulation, sweat collection, and sensors.

[0024] Fig. 8 shows an embodiment of the present invention that is able to detect an unreliable contact of a sweat stimulation pad with the skin.

[0025] Figs. 9A and 10A are block diagrams of the functionality of integrated sweat stimulation and sweat sampling.

[0026] Figs. 9B and 10B are cross-sectional representations of the embodiments shown in Figs. 9A and 10A.

[0027] Fig. 11 is a diagrammatic top plan view of the layers used in the device shown in Fig. 10A.

[0028] Fig. 12 is a cross-sectional view of a sweat sensing device according to an embodiment of the disclosed invention.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The detailed description of the present invention will be primarily be, but not entirely be, limited to subcomponents, subsystems, sub methods, of wearable sensing devices, including devices dedicated to sweat sensing. Therefore, although not described in detail here, other essential features which are readily interpreted from or incorporated with the present invention shall be included as part of the present invention. The specification for the present invention will provides specific examples to portray inventive steps, but which will not necessarily cover all possible embodiments commonly known to those skilled in the art. For example, the specific invention will not necessarily include all obvious features needed for operation, examples being a battery or power source which is required to power electronics, or for example, an wax paper backing that is removed prior to applying an adhesive patch, or for example, a particular antenna design, that allows wireless communication with a particular external computing and information display device. Several specific, but non-limiting, examples can be provided as follows. The application includes reference to PCT/US2013/035092, the disclosure of which is included herein by reference. The present invention applies to any type of sweat sensor device. The present invention applies to sweat sensing devices which can take on forms including patches, bands, straps, portions of clothing, wearables, or any mechanism suitable to affordably, conveniently, effectively, intelligently, or reliably bring sweat stimulating, sweat collecting, and/or sweat sensing technology into intimate proximity with sweat as it is generated. In some embodiments of the present invention the device will require adhesives to the skin, but devices could also be held by other mechanisms that hold the device secure against the skin such as strap or embedding in a helmet. The present invention may benefit from chemicals, materials, sensors, electronics, microfluidics, algorithms, computing, software, systems, and other features or designs, as commonly known to those skilled in the art of electronics, biosensors, patches, diagnostics, clinical tools, wearable sensors, computing, and product design. The present invention applies to any type of device that measures sweat or sweat rate, its solutes, solutes that transfer into sweat from skin, a property of or things on the surface of skin, or measures properties or things beneath the skin.

[0030] The present invention includes all direct or indirect mechanisms or combinations of sweat stimulation, including but not limited to sweat stimulation by heat, pressure, electricity, iontophoresis or diffusion of chemical sweat stimulants, orally or injected drug that stimulate sweat, stimuli external to the body, natural bioactivity, cognitive activity, or physical activity. Any suitable technique for measuring sweat rate should be included in the present invention

where measurement of sweat rate is mentioned for an embodiment of the present invention. The present invention may include all known variations of biosensors, and the description herein shows sensors as simple individual elements. It is understood that many sensors require two or more electrodes, reference electrodes, or additional supporting technology or features which are not captured in the description herein. Sensors are preferably electrical in nature such as ion-selective, potentiometric, amperometric, and impedance (faradaic and non-faradaic), but may also include optical, chemical, mechanical, or other known biosensing mechanisms. Sensors can allow for continuous monitoring of multiple physiological conditions realizing larger arrays of biomarker-specific sensors. The larger arrays can determine physiological condition through semi-specific but distinct sensors by statistical determination, eliminating the need to quantify individual biomarker levels. Sensors can be in duplicate, triplicate, or more, to provide improved data and readings. Many of these auxiliary features of the device may, or may not, also require aspects of the present invention.

[0031] With reference to Fig. 2A, one embodiment of the present invention is designed for prolonged and reliable sweat stimulation and sensing. Arrayed stimulation pads can provide the same net effect as one pad for prolonged stimulation (e.g. one long pad for 12 hours of stimulation can be replaced by an array of 24 pads on the same device of 30 minutes stimulation each). As shown in Fig. 2A, a sweat sensor 28 positioned on skin 12 by an adhesive layer 30 bonded to fluid impermeable substrate 32. Substrate 32 holds electronics 34, one or more sensors 36 (one shown), a microfluidic component 38, coupled to multiple sweat pads 40, 42 and 44. The microfluidic component 38 can continuously pump sweat by evaporating sweat (water) from its exposed surface above sensor 36, or can include an additional continuous pumping mechanism (not shown) such as addition of a dry hydrogel capable of absorbing or wicking sweat for an extended period of time. As sweat generates its own pressure, microfluidic component 38 could also be a simple polymer microchannel, at least partially enclosed, which is pressure driven. Each pad has a source of sweat stimulant such as pilocarpine 46, 48, 50, and independently controlled iontophoresis electrodes 52, 54, 56. There is also one or more counter electrodes 58. To minimize dead volume, these pads 40, 42 and 44 are preferably less than 1 cm^2, for example, less than 5 mm^2 down to about 1 mm^2.

[0032] The electronics 34 further include a timing circuit connected to each electrode 52, 54, 56 via lines 66, 68 and 70 to promote sweat when desired. Thus, in operation, the electronics 34 would activate one of electrodes 52, 54 or 56 for a defined period of time. This will cause generation of sweat, which will be transferred through the microfluidic structure 38, directed to

the sensor 36. After a defined period of time, the electronics 34 will discontinue current to electrode 56 and direct it to electrode 54, again causing sweat generation beneath electrode 54, but not beneath electrode 56. Again, after a period of time, the electronics 34 will discontinue current to electrode 54 and begin passing current to electrode 52, again starting sweat generation beneath electrode 52 and discontinuing sweat generation beneath electrode 54. Each one of these will direct the sweat through the common microfluidic component 38 to the sensor 36, thus providing long-term generation of sweat without stressing any particular location on the skin 12 of the individual.

[0033] The sweat pad 60 shown in Fig. 3 represents the case where each pad 60 would have its own sensor 62 and microfluidic component 64, along with the electrode 61 and pilocarpine source 63. A plurality of these would be connected to a common circuit which would activate each pad according to a selected schedule.

[0034] In one embodiment, sensors could sense biomarkers of the effects and extent of tissue damage at a longer sweat sampling interval than sensors that could sense biomarkers of short term stress or trauma on the body, the trauma sensors having locally higher sweat stimulation than the tissue damage sensors. A higher stimulation would result in a higher sweat rate, and therefore a faster refilling of any dead volume or microfluidic volumes between the skin and sensors, and therefore an effectively shorter sampling interval. Such stimulation could also occur at regular or irregular intervals, as needed for different biomarkers.

[0035] FIGS. 4-6 show different potential configurations of sweat pads, each suitable for use in the present invention.

[0036] Fig. 4 shows one of an alternate arrangement of a sweat stimulation and collection pad 66. The source 46 and the adhesive 30 of FIG. 2 is replaced with a single layer 68, which includes the adhesive, such as a hydrogel based adhesive that contains pilocarpine or other sweat stimulant. This electrode 70 activates the pilocarpine in layer 68, causing sweat generation. The sweat, in turn, flows through microfluidic layer 72 to a sensor (not shown).

[0037] Fig. 5 shows one of an alternate arrangement for a sweat stimulation and collection pads 76. The sensor 78 is immediately adjacent to the skin 12 and therefore eliminated the need for the functionality of a microfluidic component such as microfluidic element 64. For example, the sensor 78 of FIG. 5 could be fabricated on a plastic film substrate and perforated with holes (not shown) that allow for pilocarpine from source 80 to be iontophoretically dosed through the

sensor 78 and adhesive 82 to the skin 12. In this embodiment, electrode 84, when activated, causes sweat generation, which immediately contacts sensor 78. Again, a plurality of three pads could be employed and activated by a common circuit. Adhesive 82 may not be required in all applications. For instance, the collection pad 76 could be a subcomponent of a larger device that is affixed to skin and therefore collection pad 76 is held in adequate proximity with skin, or a band or strap or other mechanism employed to hold collection pad 76 against skin.

[0038] Fig. 6 shows another alternate arrangement for a sweat stimulation and collection pad 86. The pad 86 includes a gate 88 and a sensor 90. The gate 88 is a structure that starts or stops fluid flow and can be a water soluble member which acts as a sweat barrier until sufficient sweat is generated to dissolve the gate to allow fluid flow. Or it can be a water soluble, water permeable member that initially promotes fluid flow and stops fluid flow after a certain amount of sweat has passed. Therefore the gate 88 can open fluid transport of sweat to the sensor 90 only at a time when desired, typically only when sweat stimulation is applied for that pad and sweat flow is robust enough that the local sweat sample is fresh and representative of a good chronological sampling of solutes in sweat. Gate 88 could also be pressure actuated by sweat itself, or activated by means such as electrowetting, thermocapillarity, or any other suitable means. Gate 88 could be reversible, for example, it could open, close, open, and close again. Pad 86 further includes a porous electrode 91, pilocarpine source 93 and adhesive layer 95. This is particularly useful for single-use sensors such as those that are easily disrupted by surface fouling with time or those with such a strong affinity for the biomarker to be detected that they are unable to detect later decreases of the biomarker concentration. Again, for such single use sensors the gating could be a physical gating of a microfluidic component carrying the sweat, or simply the sensors activated as sweat is stimulated in a manner adequate to bring sweat to the sensor. With further reference to Fig. 6 and in combination with other embodiments of the present invention, a device could also consist only of pad for sweat stimulation and with gates which couple sweat stimulation and sweat collection with one or more microfluidic components. For example, one sensor could be fed by multiple microfluidic components which stimulate sweat and collect it as needed. The gates could allow flow of fresh/stimulated sweat while blocking unstimulated sweat. The gates could also not be needed, just allowing sweat to flow freely to the sensor as it is generated by one or more stimulation pads.

[0039] Fig. 7 is a diagram of a portion of components of a device 94, affixed to skin 12 by adhesive 108, similar to device 10 of Fig. 2 arranged in a manner that provides significantly different function and a unique manner of separation of stimulation and collection/sensing

components. In some cases such as sensing ion concentrations, pilocarpine and/or other solutes or solvents or an electric field used for its delivery or purposes could alter the readings of the sensors 96, 98. Therefore the stimulation electrodes 100, 102, their respective sweat stimulations sources with pilocarpine 104, 106, and adhesives 108 are located near but spaced from sensors 96 and 98, as well as any collection pad, if used. The pilocarpine stimulation, if performed by iontophoresis, follows electrical field, a pathway indicated by arrows 112. This can result in stimulation of sweat while not bringing sensors 96 and 98 into concentrated contact with pilocarpine or other chemical sweat stimulant, or if desired reducing electric field or current on or near sensors 96 and 98. In the example embodiment shown in Fig. 7, the sensor 98 will receive significant sweat because the stimulation is occurring beneath as caused by electric field and iontophoretic current applied between electrodes 100 and ground electrode 114. Again, each of the sweat stimulation pads are preferably attached to timing circuitry that permits selective activation and deactivation of each pad.

[0040] Fig. 8 is applicable to any of the devices of Figs. 2-7 or other embodiments of the present invention. If stimulation electrode/pad contact to the skin is inadequate, this can be detected as an increase in impedance and that pad can be deactivated for purpose of skin safety and/or inadequate stimulation. The sweat sensing device 116 affixed to skin 12 by adhesive 117, as shown in Fig. 8, senses impedance of the contact of the electrode 118 (with pilocarpine source 119 and microfluidic component 121) with the skin 12 and/or the contact of counter electrode 120 with the skin 12 where 'contact' refers to direct contact or indirect contact but which has adequate and/or uniform electrical conduction with the skin. Inadequate contact can cause insufficient sweat stimulation, an increase in current density and additionally therefore cause irritation, damage, burns, or other undesirable effects with the skin or with the function of the device. Measurement of electrical impedance includes obvious related measures such as capacitance, voltage, or current which also give a measure of impedance. If the impedance exceeds a preset limit by circuit 122, the electrode 118 can be deactivated. This reduces the likelihood of burning the skin. Furthermore, if sweat stimulation pads are redundant (one or more), the embodiment illustrated in Fig. 7 can allow the present invention to select the 'adequate' or 'best' ones for use with one or more embodiments of the present invention. In an alternate embodiment of the present invention, inadequate stimulation can also be measured by one or more known means of measuring sweat rate, such as impedances, lactate concentration, or sodium or chloride concentration.

[0041] In an alternate embodiment, each counter electrode and iontophoresis electrode of the embodiments of the present invention can be placed close to each other and/or controlled in conjunction with each other. To allow prolonged sweat stimulation but to limit areas of skin to shorter term stimulation, each sweat stimulant source and electrode could be utilized sequentially. For example, if a safe protocol for stimulation was found to be up to 1 hour, but 24 hours of stimulation and sensing is needed, then 24 sets of electrodes and sources could be used sequentially. Also, after a period of time, stimulation can be reactivated under a given electrode and source (for example, sweat generation could become 'tired' and after 'resting' for some time, be enacted again at the same time). Therefore multiple sequences or timings of stimulations and collections are possible, to enact sampling of sweat at multiple intervals or continuously for a longer period of time than is conventionally possible. Multiple microfluidic components could be associated with one-way flow valves as well, reducing fluid flow contamination or confusion between multiple fluidic pathways or elements. The time scales listed herein are examples only, and stimulation for less regular, more short, or even longer total durations are possible.

[0042] In an alternate embodiment, each stimulation pad, even if with or without a microfluidic component, can have a volume between skin and sensor such that reduced stimulation is allowed while still providing adequate chronological resolution (sampling interval). Conventional sweat stimulation requires >1 nL/min/gland flow of sweat to allow a proper sampling volume. The present invention allows the sweat stimulation to be reduced to <2 nL/min/gland, preferably < 0.5 nL/min/gland using sweat stimulation concentrations/dosages as found in the literature (e.g. Buono 1992, J. Derm. Sci. 4, 33-37) appropriate for such reduce stimulation and sweat rates. Such an alternate embodiment can be desirable, because it can reduce one or more of the undesirable aspects or side-effects of sweat stimulation or prolonged sweat stimulation. Enabling calculations for reduced stimulation, sweat rates, volumes and areas, were provided in the background section.

[0043] For sensors located on the palms or soles the skin is very thick and if becomes wet for prolonged periods of time the sweat can slow unacceptably or stop altogether as skin swells to the point where sweat ducts become pinched off. Such state is visibly noticeable as 'wrinkling of the skin' after the skin is exposed to water for a longer period of time. Therefore for prolonged sensing, a dessicant, hydrogel, or other absorbent material can be placed over top or adjacent to the sensors of the present invention to enable longer term viability of sensing of the palm or sole with reduced concern of skin swelling/wrinkling and reduced sweat flow rate either natural or stimulated.

[0044] With reference to Figures 9A, 9B, 10A, and 10B, an alternate embodiment of the present invention is shown using block diagrams to convey function of a more advanced example subset of components of devices of the present invention. The components shown for the device 124 in Figs. 9A and 9B have a pilocarpine source reservoir 126 which contains a sweat stimulating compound such as pilocarpine, a fluidic component 128, and a sampling component 130, all of which are integrated in a device resting on skin 12. Fluidic component 128 and sampling component 130 could also be one and the same where sampling component 130 is just an extension of the fluidic component 128. In an example embodiment, an electrode 132 is provided to reservoir 126, thus enabling iontophoretic dosing of pilocarpine through fluidic component 128 and into skin 12 as indicated by arrow 134. This dosing of pilocarpine generates sweat, which is initially wetted into fluidic component 128 and then transported into sampling component 130 as indicated by arrow 136. The sweat may travel along a partially separate flow path than the pilocarpine to minimize interaction between the sweat and the pilocarpine, but full separation is not required. The above example could be achieved by using pilocarpine placed into a hydrogel which forms the reservoir 126, stacked onto a thin piece of paper or other fluid porous material for the fluidic component 128, which is then connected to another piece of paper or tube for the sampling component 130. The sampling component 130, or even fluidic component 128, may contain or be in fluidic contact one more sensors (not shown), or may simply store sweat for later analysis by a sensor external to the device 124. In further examples, the iontophoresis could be continuous, and allow continuous sampling of non-charged biomarkers or solutes in sweat, or the iontophoresis could be intermittent and between dosing by iontophoresis both charged and non-charged biomarkers or solutes in sweat could be sampled.

[0045] Components 126 and 128 in alternate designs could also be one and the same, as could also be true for components 128 and 130. To minimize sweat solute diffusion into or out of the reservoir 126, the reservoir 126 may be made of a material such as a gel that is slow to diffusion of solutes but fast in allowing iontophoretic transport of solutes. A non-limiting example would be an ion-selective membrane with selectivity partial to pilocarpine or substances with charge or makeup similar to pilocarpine.

[0046] Figures 10A and 10B show a sweat sensing device 138 with similar features as Figs. 9A and 9B, but also includes a membrane 140 and a storage component 142. The storage component 142 may simply collect and store sweat as it is sampled through the sampling component 146. The storage component 142, could for example, be a hydrogel which swells and increases in volume as it takes up a fluid like sweat. The sampling component 146 can include

one more sensors providing a chronological measure of biomarker concentration in sweat instead of a time-integrated measure that would occur if the sensor were instead placed in the storage component 142. Sensors could also be placed at or near the location of fluidic component 144, or at or near the skin 12, as described for previous embodiments of the present invention. The membrane 140 is any component that allows pilocarpine or other compound diffusion or iontophoresis through the membrane 140, but which reduces or prevents diffusion of biomarkers or solutes in sweat through the membrane 140 and back into the pilocarpine reservoir 148. Furthermore, membrane 140 can serve as a barrier to fluidic contact between reservoir 148 and other components of the device 138 of the present invention to increase storage life as pilocarpine gels typically are hydrated and can diffuse out pilocarpine over time into other porous media they are brought into contact with. Reservoir 148 and membrane 140 could also be one and the same, with membrane having selective transport for sweat stimulating substance. For example, selective membranes or materials that are partial to transport sweat stimulant can be known membranes partial to transport of only one type of ion polarity (for example for favoring the charge of the sweat stimulant ions) or partial transport to molecules as small as but not substantially larger than the sweat stimulation molecule through simple principles of size exclusion. Further examples can be found through literature on 'selective molecular sieves'.

[0047] As a result, sweat stimulation and sampling can be integrated in the same device with less interference between the two. For example, the membrane 140 could be a track-etch membrane with 3% porous open area, and the pilocarpine concentration and iontophoretic driving voltage increased on the reservoir 148 such that the amount of pilocarpine dosed can be similar or equal in effectiveness to that that of a reservoir 148 placed directly against the skin 12. Because the membrane 140 only has 3% porous area, diffusion of solutes in sweat into the reservoir 148 is reduced substantially up to 30X. The fluidic component 144 may be adequately thick that any pilocarpine coming through holes or pores in the membrane 140 would have adequate distance before reaching the skin to spread out into a more even concentration and current density into the skin. Membrane 140 could be any material, film, ion-selective gel, or other component which transports a sweat stimulating component such as pilocarpine, but which minimizes the transport of other all or particular sweat solutes back into the reservoir 148. Membrane 140 therefore could also be a fluidic or ionic switch or valve, which is opened during a short period of time for iontophoresis of pilocarpine, but closed once an adequate pilocarpine dose has been released from the reservoir 148. Furthermore, membrane 140 can serve as a barrier to fluidic contact between reservoir 148 and other components of the devices of the present invention to increase storage life as pilocarpine gels typically are hydrated and can

diffuse out pilocarpine over time into other porous media they are brought into contact with. For cases where the membrane 140 is a fluidic switch an electrode may be provided with the fluidic component 144 to complete iontophoresis of pilocarpine even after the fluidic switch 140 is closed to pilocarpine transport. Example fluid switches include those actuated by electrowetting, switchable selective ion channels, and other means achieving the same desired functionality.

[0048] In an alternate embodiment of the present invention, with further reference to Figures 10A and 10B, reservoir 148 will include an electrode to drive electrophoresis (not shown), and the electrode may also be utilized to measure sweat rate by electrical impedance with skin 12. In one example embodiment, to allow proper measurement of sweat rate by impedance, the electrical impedance of membrane 140 should be similar to or preferably less than the electrical impedance of skin 12 (these two impedances being in series, such that skin impedance dominates and improves the quality of sweat rate measurement by impedance). Using first principles, this is easily achieved assuming electrical conductivity of fluids in the device 138 to be roughly equal, and the sum of the electrical resistance of pores in membrane 140 to be less than the sum of the electrical resistance due to sweat ducts in skin 12. Therefore, membrane 140 could be selected such that it has a low enough porosity to help prevent contamination between reservoir 148 and fluidic component 144, but also having high enough porosity such that it does not block proper impedance measurement of skin 12. Fortunately, impedance can be measured using a small signal AC waveform, which results in little or no net migration of pilocarpine or other charged sweat stimulant.

[0049] In an alternate embodiment of the present invention, with further reference to Figures 9A, 9B, 10A, and 10B, the reservoir of pilocarpine and fluidic component can also be switched in locations (trading locations as illustrated and described, but retaining their primary functionalities and the advantages/features as described for embodiments of the present invention).

[0050] For the embodiments of Figures 9A, 9B, 10A, and 10B, it is desirable for some applications that the fluid capacity, or volume, of the fluidic and sampling components 144 and 146 be as small as possible. This is important, because if the fluidic and sampling components 144 and 146 have a large volume, the components will effectively integrate the concentrations of solutes in sweat over a longer period of time, and limit the ability to achieve a time-resolved measurement of solutes in sweat. Furthermore, any delay on transporting sweat from the skin 12 to a sensor can cause degradation in concentrations of some biomarkers or solutes in sweat, and

therefore minimum volume of the fluidic and sampling components 144 and 146 is also desirable.

[0051] An example stack-up of an embodiment of the components comprising the device 138 is shown in Fig. 11. As represented by arrow 150 of Figs. 10A and 10B, sweat will flow along sampling component 146 to storage component 142 while pilocarpine flows directly to the skin 12 as shown by arrow 152.

[0052] Sweat stimulation can be applied continuously or repeatedly over long periods of time so long as the currents utilized for iontophoresis and total doses are properly controlled. In yet another embodiment of the present invention devices can include controllers which allow sweat stimulation for periods of hours to potentially more than a day in duration.

[0053] In some cases, even with careful electrical controllers and microfluidic design, skin irritation could occur, and in these cases in yet another alternate embodiment of the present invention includes sweat stimulation pads that are <50 mm^2 in order to reduce perceived irritation by the user, even less than 10 mm^2 or less than 2 mm^2. These ranges for the present invention are much smaller than the commercial Wescor product, which has a stimulation pad that is >1 cm^2 (>100 mm^2), because large amount of sweat needs to be collected given the highly manual nature of the sweat collection and sensing. Assuming ~100 sweat glands/cm^2, a 50 mm^2 stimulation pad could collect sweat from on average 50 glands, 10 mm^2 on average 10 glands. If the stimulation pad is placed in regions where sweat gland density is >350 glands/cm^2 then a 2 mm^2 stimulation pad could cover on average >6 glands and most likely at least one gland always with careful placement. The present invention may also use much larger sweat stimulation pads, if it is acceptable for the application and/or other embodiments of the present invention are utilized to suitably reduce irritation caused by sweat stimulation.

[0054] In some cases, even with careful electrical controllers, reduced stimulation area, and advanced microfluidic design, skin irritation could occur, and in these cases in yet another alternate embodiment of the present invention, the pilocarpine reservoir can also contain an iontophoretically transported or diffusing anti-inflammatory, numbing agent, or pain-relieving agent (hydrocortisone, for example, or other iontophoretically delivered pain relieving agents). This could allow longer stimulation and usage than otherwise deemed acceptable by the user. Ideally, the anti-inflammatory or pain relieving / numbing agent delivered will have properties such as: (1) not interfering with sweat stimulation (not suppressing it); (2) have a similar charge polarity as the sweat stimulating substance and be co-delivered to the same site with it. For

example, deliver combinations of stimulant or anti-inflammatory/numbing agents, such as "name (example charge polarity)": (1) stimulants – Pilocarpine (+), Acetylcholine (+), Methacholine (+), Phenylephrine Hydrochloride (+), Isoproterenol (+); (2) anti-inflammatories/numbing agents - such as Dexamethasone (-), Hydrocortisone (+ or – depending on compound), Salicylate (-), Lidocaine. Several of such substances or molecules can also be altered in charge to work with positive or negative polarity. Furthermore, even oppositely charged substances could be co-delivered to the same location as sweat extraction takes place, for exampling, using an electrode arrangement using features similar to that shown in Fig. 7 where the numbing agent would be delivered using electrodes that are side by side with electrodes delivering sweat stimulant, and in between such electrode pairs sweat would be collected. Furthermore, agents to reduce pain or irritation or swelling could be allowed to penetrate by diffusion over time (charged or uncharged), as some agents such as hydrocortisone work well based on diffusion alone and do not need to penetrate overly deeply into the skin. Numerous such combinations are possible, the key requirement being delivery either simultaneously or at other times which allow both sweat stimulation and chemical or pharmalogical reduction of irritation, pain, or inflammation. An excellent reference, included herein, is Coston and Li, Iontophoresis: Modeling, Methodology, and Evaluation, *Cardiovascular Engineering: An International Journal, Vol. 1, No. 3, September 2001 (*C ° *2002).*

[0055] The reservoir may also contain a surfactant or other substance that can cause cell death, cell rupture, or increase skin cell membrane permeability, in order to facilitate biomarker release from the body into the sweat being sampled. The reservoir may also contain solvents known to increase the effectiveness of iontophoretic delivery. Furthermore, techniques such as electro-osmosis can be used continuously or intermittently to promote extraction of biomarkers from the cells surrounding a sweat duct or from the skin directly. Also, for long duration sweat stimulation, the iontophoresis could potentially cause electrolysis of water and therefore high concentrations of acids or bases at the two or more electrodes required for iontophoresis. Therefore in yet another alternate embodiment of the present invention, the electrodes contacting components, such as that contacting the reservoir or electrode, may also be equipped with buffering agents, or the electrodes themselves undergo oxidation or reduction in order to suppress undesirable side-effects of water electrolysis and/or pH changes.

[0056] With further reference to the example embodiments of the present invention, sweat generation rate could also be actively controlled to decrease, by iontophoresis of a drug which reduces sweating, such as *anticholingerics* including glycopyrrolate, oxybutynin, benztropine,

propantheline. For example, a sweat retarding chemical could replace pilocarpine in reservoir 126 of FIG. 9A. Sweat generation rate could also be reduced by administering a solvent to the skin such as glycols which can swell the top layer of skin and pinch off the sweat ducts such that sweat generation rate is reduced by constriction of flow of sweat to the surface of skin. Other antiperspirant compounds or formulations, such as Aluminum chloride are possible as well. Why would one want to slow the sweat generation rate? Two non-limiting examples include the following. Firstly, some sensors or subcomponents could foul or degrade in performance more quickly as fresh sweat is brought to them, or the general maximum usage time of the patch decrease as a result of a sweat generation rate that is too high. Second, some solutes or properties of sweat could be read more reliably at lower sweat generation rates, in particular low concentration solutes could have more time to diffuse into slowly flowing sweat inside the sweat gland/duct and therefore a lower sweat generation rate could produce a higher concentration which could be more easily sensed by a sensor. Furthermore, some solutes are generated by the sweat gland itself during high levels of sweat generation (such as lactate) and could interfere with sensors for other solutes or sensors trying to sense lactate diffusing into sweat from blood.

[0057] With reference to Fig. 12, a sweat sensing device 154 according to another embodiment of the disclosed invention is shown. This embodiment is premised on the realization that, unlike iontophoretic delivery of a sweat stimulant, diffusion-based delivery of a sweat stimulant is not significantly confounded by solutes in sweat (such as ions), so long as excess water can be removed. The device 154 of this embodiment is positioned on skin 12 composed of the stratum corneum 156, the epidermis 158, the dermis 160, and layers of skin 162 below the dermis 160. The skin 12 contains multiple sweat glands, each having a ductal lumen 164a1, 164a2, 164a3 and secretory coil 164b1, 164b2, 164b3. The device 154 is capable of indirect and/or direct sweat stimulation and includes at least one sensor specific to at least one analyte in sweat (e.g., at least one of sensors 166, 168). As described further below, the device includes a collection/sensing area that is fluidically isolated from the stimulation area. The collection/sensing area of the device 154 may comprise one or more sweat collectors 170, 172 and optionally one or more sensors 166, 168. Sweat collectors 170, 172 can remove excess sweat to ensure sensors 166, 168 can always receive fresh sweat, for example, by being connected to a separate wicking waste sweat reservoir (not shown). In the illustrated embodiment, the sensors 166, 168 are contained inside a corresponding sweat collector 170, 172. In another embodiment, the sensors 166, 168 may be located away from the skin 12, and sweat brought to the sensors 166, 168 by the sweat collectors 170, 172.

[0058] Still referring to Fig. 12, the device 154 further comprises a stimulant reservoir, such as a series of stimulant gels 174, 176, 178 and a sweat impermeable material 180 or other sweat blocking material such as a sweat impermeable adhesive, a wax, petroleum jelly, an air gap, or other suitable material. This sweat impermeable material 180 isolates the sweat that is received by sensors 166, 168 from the stimulant gels, else cross-contamination of the sweat sample could occur. The device 154 also includes a membrane 182 that is permeable to water but not permeable to the sweat stimulant (e.g. a water filtration membrane, dialysis membrane, reverse or forward osmosis membrane, or other suitable membrane). Sweat stimulants may include carbachol, pilocarpine, methacholine, or other cholinergic agents. The stimulant gels 174, 176, 178 contain the sweat stimulant and may also contain a transdermal permeability enhancer such as glycols, surfactants, chelating agents, or other suitable permeability enhancing agents. Above the membrane 182 is a waste water reservoir 184 and a sealing polymer film 186 such as PET. The waste reservoir 184 could be a hydrogel like that used in diapers, paper, silica powder or fumed silica, or other suitable wicking material that is able to wick in water but is similar or weaker in wicking force than stimulant gels 174, 176, 178 such that at least some water remains in stimulant gels 174, 176, 178 at all times during use of the device 154. As a result, the water content of stimulant gels 174, 176, 178 is regulated by the physical volume of the stimulant gels 174, 176, 178 (e.g. if they were made of a non-swellable hydrogel, fumed silica, textile, or other suitable material) or osmotic pressures or other factors. For example, waste water reservoir 184 could have a total volume of 100 μL and the sweat generation rate could be 1 nL/min/gland with 100 glands/cm^2, and the area of sweat stimulant reservoirs 174, 176, 178 on skin was 1 cm^2 and with a total volume of 10 μL, then a sweat rate of 100 nL/min would be received by waste water reservoir 184 such that the device 154 could operate reliably for 1000 minutes. In an embodiment, the waste water reservoir has a volume that is at least 10X greater than the volume of said sweat stimulant reservoir.

[0059] Still referring to Fig. 12, as sweat emerges from the sweat glands, excess water is pulled through membrane 182 into waste reservoir 184, and as a result, the concentration of sweat stimulant is not significantly diluted. Not significantly diluted means that an adequate concentration gradient of sweat stimulant between stimulant gels 174, 176, 178 and the dermis 160 is maintained such that diffusion of sweat stimulants into the dermis is also maintained. One could argue that if stimulant gels 174, 176, 178 were large enough in volume that water removal would not be needed, but a particular advantage of the present invention is that total amount of stimulant placed onto the body is more limited (safety, cost, etc.). After the stimulant diffuses into the dermis and stimulates sweat, that sweat and it solutes (analytes therein) can be detected

by sensors 166, 168. Again, sweat that wets the stimulant gels 174, 176, 178 will have its water extracted on through the membrane 182 and into waste reservoir 184. At some point, the solutes in sweat could remain behind in the stimulant gels 174, 176, 178 causing retention of water by osmotic pressure and dilution of the stimulant. However, membrane 182 could also have a molecular weight cutoff such that small solutes (such as ions associates with pH, salinity, etc. including salts, H+, and OH- ions) could pass through membrane 182, thereby limiting the effects of solute build up and osmotic pressure. If membrane 182 is semi-porous in this manner, then the waste water reservoir 184 could also contain solvents such as propylene glycol which enhance diffusion of sweat stimulant into the skin 12.

[0060] One skilled in the art will recognize that the various embodiments may be practiced without one or more of the specific details described herein, or with other replacement and/or additional methods, materials, or components. In other instances, well-known structures, materials, or operations are not shown or described in detail herein to avoid obscuring aspects of various embodiments of the invention. Similarly, for purposes of explanation, specific numbers, materials, and configurations are set forth herein in order to provide a thorough understanding of the invention. Furthermore, it is understood that the various embodiments shown in the figures are illustrative representations and are not necessarily drawn to scale.

[0061] For example, features of the embodiment of Fig. 12 are largely directed to diffusive delivery of a sweat stimulant to the dermis (while many of embodiments of Figs. 2A-11 are largely directed to iontophoretic delivery). As described above, the present invention (and thus all exemplary embodiments) may be directed to any direct or indirect mechanism or combination of sweat stimulation (including iontophoresis and/or diffusion). And so, features of the embodiment of Fig. 12 can be applied to other embodiments described herein. And, as a further example, other features of the embodiment of Fig. 12 – such as the use of a water-permeable and sweat stimulant-impermeable membrane to prevent dilution of stimulant (and thus maintain the concentration of stimulant delivered to dermis and sweat glands) – are applicable to embodiments using iontophoretic-based delivery (as well as those using diffusion-based delivery). And so, each of the embodiments shown in Figs. 2A-11 may include an alternative version that includes diffusive delivery of sweat stimulant (as opposed to the use of electrodes for iontophoretic delivery). Further, an alternative version of each of the embodiments of Figs. 2A-11 may also include a water-permeable and sweat stimulant-impermeable membrane to prevent dilution of stimulant (and thus maintain the concentration of stimulant delivered to dermis and sweat glands). Such diffusive delivery and membrane that may be used in this

embodiment are described in greater detail with respect to Fig. 12, and are applicable to those alternative versions of embodiments shown in Figs. 2A-11.

[0062] And so, while specific embodiments have been described in considerable detail to illustrate the disclosed invention, the description is not intended to restrict or in any way limit the scope of the appended claims to such detail. The various features discussed herein may be used alone or in any combination. Additional advantages and modifications will readily appear to those skilled in the art. The invention in its broader aspects is therefore not limited to the specific details, representative apparatus and methods and illustrative examples shown and described. Accordingly, departures may be made from such details without departing from the scope of the general inventive concept.

WHAT IS CLAIMED IS

1. A device for sensing sweat on skin comprising:

a sweat collector,

an analyte-specific sensor associated with the sweat collector, the analyte-specific sensor for sensing an analyte in sweat;

a sweat stimulant reservoir containing a sweat stimulant that is coupled into skin;

a waste water reservoir; and

a water-permeable, sweat stimulant-impermeable membrane between the stimulating component and the waste water reservoir.

2. The device of claim 1, wherein the sweat stimulant reservoir has a first wicking force, and the waste water reservoir has a second wicking force not greater than the first wicking force.

3. The device of claim 1, wherein the sweat stimulant reservoir is a sweat stimulant gel.

4. The device of claim 3, wherein the sweat stimulant gel is selected from a hydrogel, fumed silica, or textile.

5. The device of claim 1, wherein the sweat stimulant is selected from carbachol, pilocarpine, methacholine, and combinations thereof.

6. The device of claim 1, wherein the sweat stimulant is a cholinergic agent.

7. The device of claim 1, further comprising at least one sweat collector that couples sweat to at least one sensor specific to an analyte in sweat.

8. The device of claim 7, further comprising a sweat impermeable material between said sweat collector and the sweat stimulant reservoir.

9. The device of claim 1, wherein the water-permeable, sweat stimulant-impermeable membrane is selected from the group consisting of a water filtration membrane, dialysis membrane, reverse or forward osmosis membrane, and combinations thereof.

10. The device of claim 1, wherein the waste water reservoir is selected from a hydrogel, a paper, or combinations thereof.

11. The device of claim 1 where said waste water reservoir has a volume that is at least 10X greater than the volume of said sweat stimulant reservoir.

12. The device of claim 1 further comprising at least one skin permeability enhancing agent that resides at least in the sweat stimulant reservoir.

13. The device of claim 1 where in the sweat stimulant-impermeable membrane is also permeable to salts, H+ and OH ions, and other small solutes in sweat but not to the sweat stimulant.

WO 2020/061460 PCT/US2019/052163

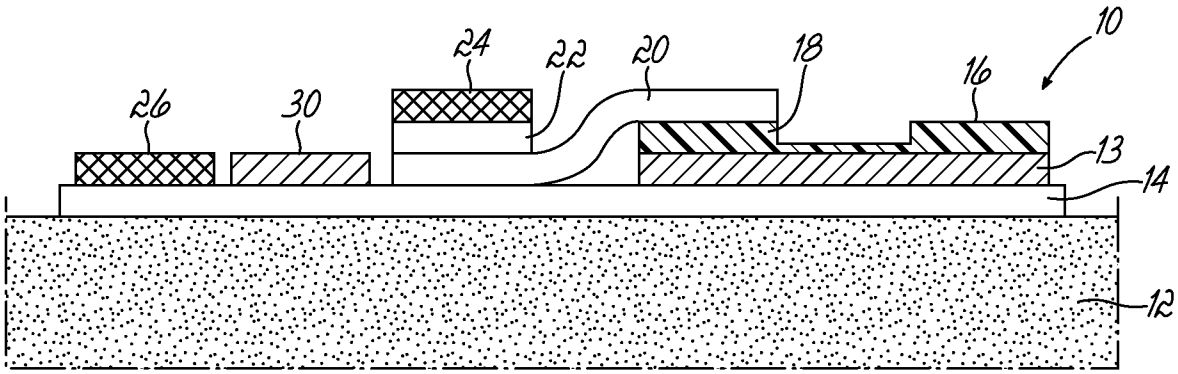


FIG. 1A
PRIOR ART

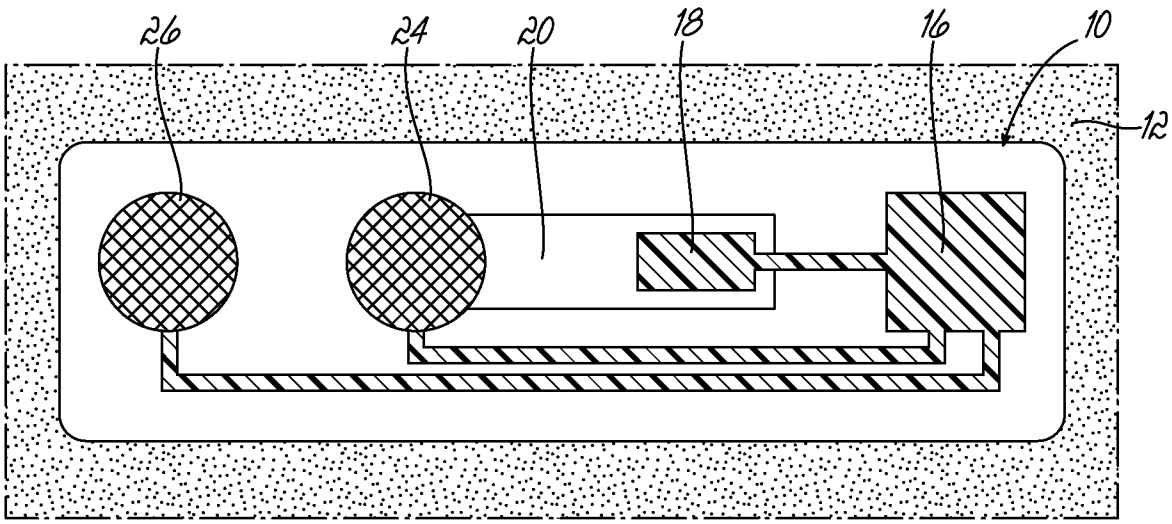


FIG. 1B
PRIOR ART

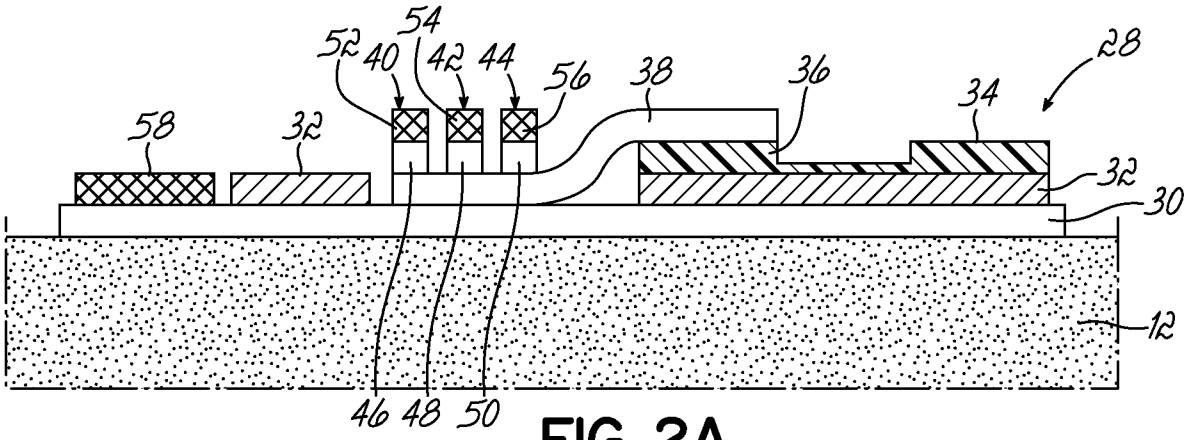


FIG. 2A

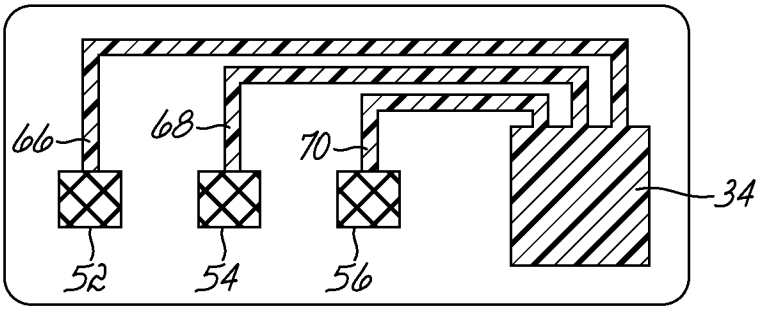


FIG. 2B

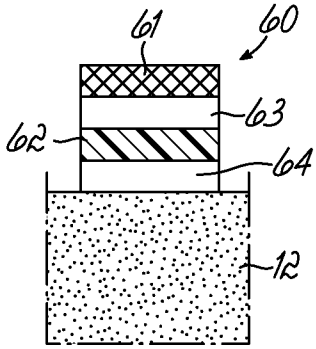


FIG. 3

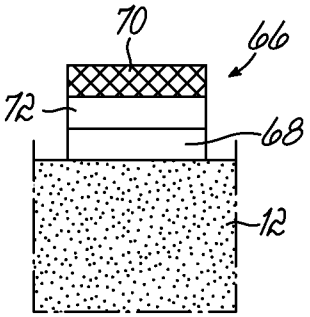


FIG. 4

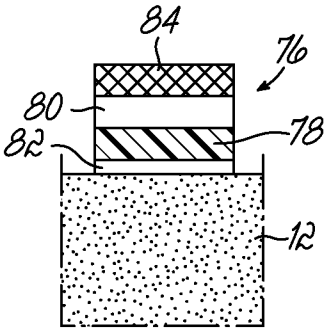


FIG. 5

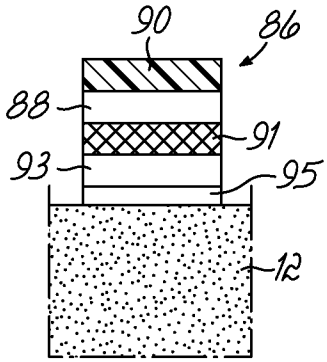


FIG. 6

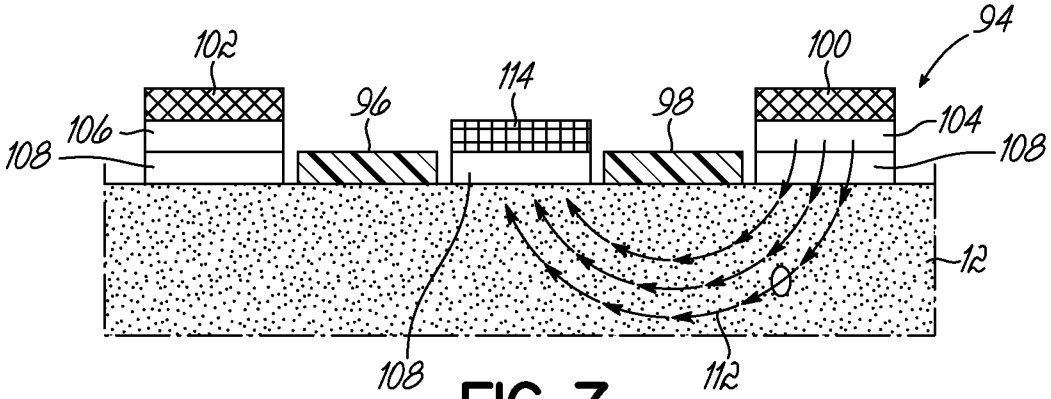


FIG. 7

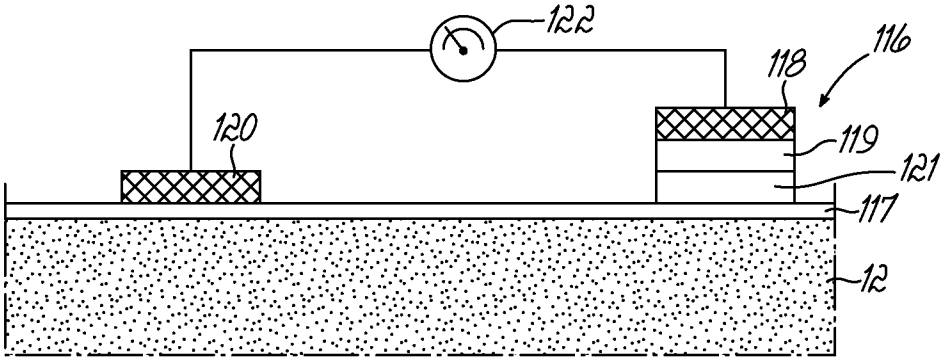


FIG. 8

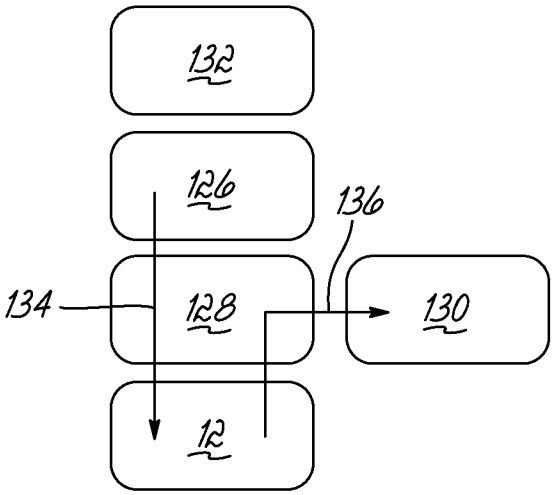


FIG. 9A

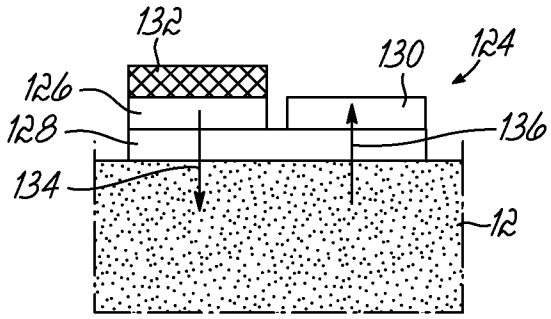


FIG. 9B

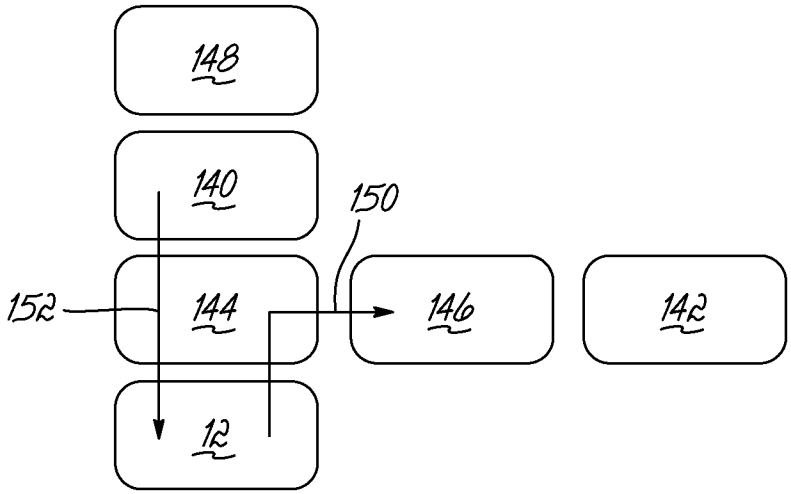


FIG. 10A

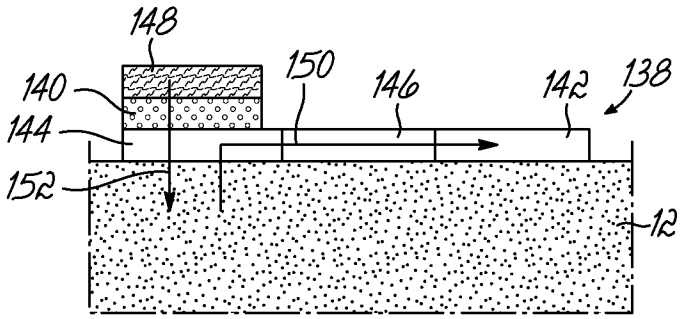


FIG. 10B

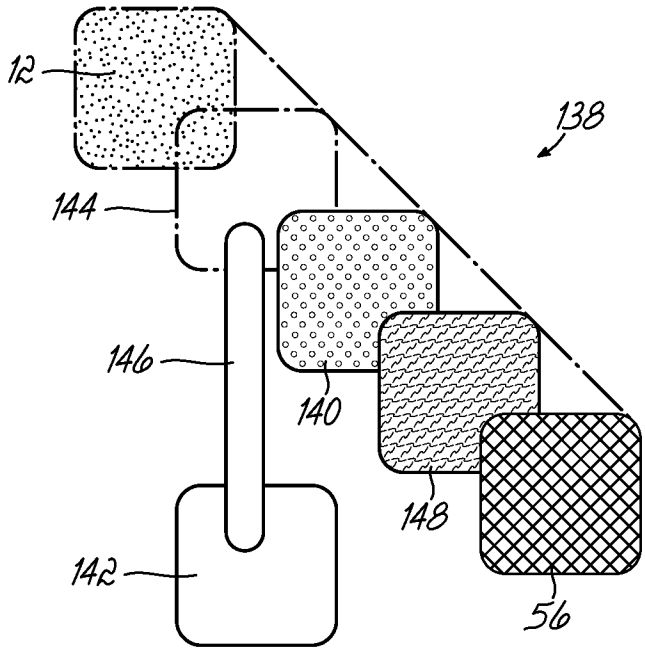


FIG. 11

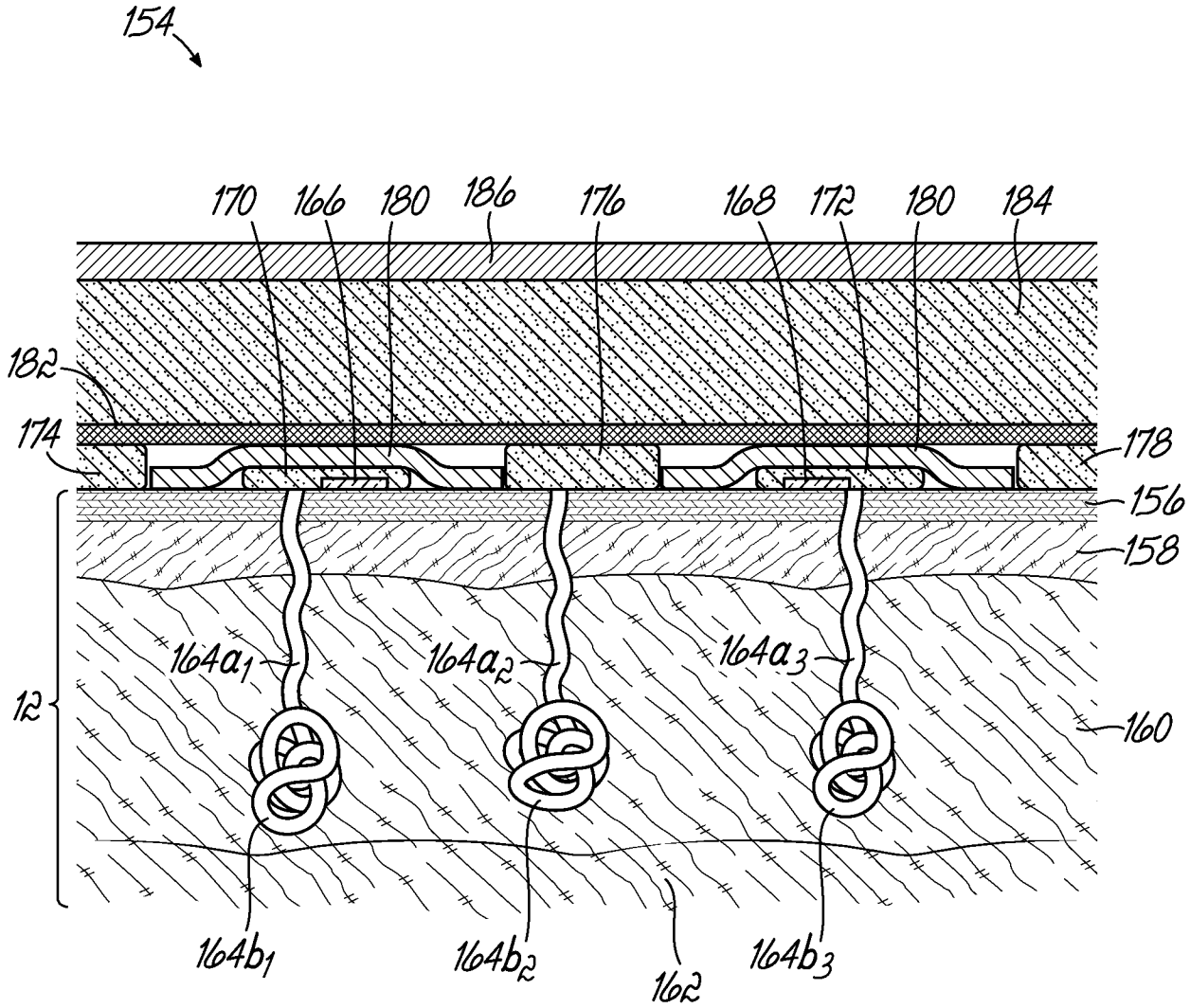

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164b3

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/52163

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61B 5/145, A61B 10/00 (2019.01)

CPC - A61B 5/14521, A61B 5/4266, A61B 10/0064, A61B 5/6832

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/005438 A1 (UNIVERSITY OF CINCINNATI) 04 January 2018; paragraphs [0011]-[0013]	1-10
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Y		12
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A		11, 13
Y	US 2017/0354808 A1 (UNIVERSITY OF CINCINNATI et al.) 14 December 2017; paragraph [0024]	12
A	US 2016/0374598 A1 (ECCRINE SYSTEMS, INC.) 29 December 2016; paragraph [0037]	11, 13
A	US 2017/0095233 A1 (UNIVERSITY OF CINCINNATI) 6 April 2017; paragraph [0108]	11, 13

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

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 November 2019 (12.11.2019)	03 DEC 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300