Wearable Transdermal Biosensors

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1 Introduction

The twenty-first century is revolutionizing the biomedical industry with myriad innovations from point-of-care diagnosis to telehealth solutions. Among the various fields of biomedical innovations, wearable biosensors have shown noteworthy advancements. The first few generations of wearable transdermal biosensors (WTBs) are capable of monitoring various physiological parameters such as temperature, blood pressure, and electrocardiogram and are restricted to non-invasive physiological parameters that are indicative of overall health status, but cannot provide disease/condition-specific diagnosis or continuous status monitoring. The current generation of WTBs have improved capabilities owing to both technological and biochemical sensing developments. This chapter discusses the concepts and components of WTBs. In general, WTBs have three major components, namely (i) Biorecognition elements (sensing element)—chosen sensing element specific to target analytes; (ii) Detection methods—methods to observe the interactions between biofluids and biorecognition elements; (iii) Biofluids, which are sampled by the wearable device to detect the target analyte(s).

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2 Biorecognition Elements

Biorecognition elements are the component of WTBs responsible for the selective detection of the target analyte(s) by the sensor. Their necessity as a component of the final sensor is dependent on the target analyte. Some small, electroactive compounds can be detected using methods like differential scanning voltammetry or potentiometry purely based on their unique electrochemical properties. When the target analyte(s) are chemically complex, biorecognition elements are necessary for distinguishing the analytes from non-targets. Biorecognition elements rely on steric and intermolecular interactions to specifically bind with a complementary substrate. The binding event is then transduced to a measurable signal that is dependent on the chosen detection method. They can be either native biological macromolecules or biomimetic. Enzymes, antibodies, and aptamers represent the three commonly used biorecognition elements used in WTBs, while molecularly imprinted polymers are an emerging approach.

2.1 Enzymes

Enzymes, as biological catalysts, accelerate chemical reactions. Through billions of years of evolution, enzymes have become highly specific, efficient, and robust. Their active binding sites fit and lock specifically to particular substrates, aided by functional groups that bind or lower activation energy. This specificity makes enzymes ideal for biosensors. Enzymes can also repeatedly catalyze reactions without being consumed. These properties make enzymes suitable for biosensing, including WTBs. Biosensors combine biological components, like enzymes, with transducers to detect and quantify analytes. However, challenges such as enzyme stability, reproducibility, and calibration must be addressed. Ongoing research and technological advancements aim to enhance the performance of enzyme-based biosensors for health care and personal monitoring.

2.2 Antibodies

Antibodies are immune system-produced proteins that target foreign substances called antigens. They identify, neutralize, and eliminate harmful pathogens. Antibodies consist of two identical heavy chains and two identical light chains, forming a Y shape. Their variable regions, known as antigen-binding sites, bind specifically to antigens. Like enzymes, this binding is highly specific, with antibody sites matching antigen structures precisely. Antibodies are classified into different isotypes (IgG, IgM, IgA, IgE, IgD) that function in various immune response stages. The immune system generates new antibodies through V(D)J recombination, in which

gene segments (variable (V), diversity (D), and joining (J) segments) are rearranged, recombined, and imprecisely joined. This process allows for an essentially random and highly diverse array of antibodies that contain antibodies that successfully recognize foreign antigens, which can be separated from self-recognizing antibodies. Antibodies have applications in diagnostic tests but are limited to binding biological materials, not smaller chemical compounds or analytes. Nonetheless, their specificity and engineering potential make them valuable biosensing recognition elements.

2.3 Aptamers

Aptamers are slowly gaining popularity as alternatives to enzymes and antibodies as the chosen biorecognition element in WTBs. Aptamers, also known as oligonucleotides, are short, single-stranded DNA or RNA molecules that exhibit high affinity and selectivity for specific target molecules. They can be chemically modified to enhance stability and prevent degradation. Aptamers, often called "chemical antibodies", have a broad target range beyond biological entities. Their production involves SELEX [1] (Systematic Evolution of Ligands by Exponential Enrichment), a process similar to antibody generation. SELEX includes iterative rounds of selection and amplification to isolate aptamers that bind to the desired target. Aptamers can be designed to bind small molecules, proteins, peptides, nucleic acids, viruses, and cells. Offering advantages such as easy synthesis, chemical modification, and high specificity, aptamers are valuable tools for biomedical research, diagnostics, and therapeutics. Their small size compared to enzymes and antibodies enables greater densities of aptamer probes at the active layer. Consequently, devices that employ aptamers can generally achieve greater analytical sensitivity.

2.4 Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) are synthetic biorecognition elements that aim to emulate the natural formation of biorecognition patterns between antibodies and antigens. They are formed from the polymerization of a chosen monomer and subsequent crosslinking of the resulting polymers in the presence of a target analyte. The target analyte acts like a template which, after removal from the MIP, leaves a cavity that acts as the biorecognition pattern between the analyte and the MIP [2]. Advantages of MIPs over other biorecognition elements are they are generally more cost-effective and can be produced for nearly any target analyte [3]. A disadvantage is the general decrease in selectivity with analyte size. MIPs have thus far been successfully implemented in wearable sweat sensors for detecting non-electroactive species such as cortisol [4–6].

3 Detection Methods

3.1 Electrochemical Detection

Electrochemical detection is widely used in analytical chemistry, biochemistry, and environmental science for its high sensitivity, specificity, simplicity, and affordability. Techniques such as amperometry, voltammetry, potentiometry, and impedance spectroscopy are the most employed detection strategies in WTBs.

Amperometry measures current for measuring analyte concentration. Amperometry is most commonly used in WTBs to measure the enzymatically catalyzed oxidation or reduction of a target molecule. One of the key advantages of amperometry is its high sensitivity, which allows for the detection of very low concentrations of analytes. One of the main limitations is that it is a single-point measurement, hence only provides information about the concentration of the analyte at a specific location. Amperometry is most used in continuous glucose monitors (CGMs) [7] to measure the amount of glucose oxidation occurring at the working electrode.

Voltammetry measures current as a function of applied potential. One of the key advantages of voltammetry is its ability to provide information about the redox behavior of analytes, including the number of electrons involved in the reaction and the standard reduction potential that can be used to identify and quantify the analyte in complex matrices. However, the complexity of the voltammogram can make interpretation difficult, requiring careful analysis of the data.

Potentiometry measures potential difference between electrodes, with high selectivity for ions and small molecules when combined with ion-selective membranes, making it useful in clinical applications. It is relatively simple and inexpensive. It is also a non-destructive technique, which means that the sample can be reused for further analysis. Potentiometry is generally used with WTBs in conjunction with ion-selective membranes for the detection of specific electrolytes in sweat or ISF.

Electrical impedance spectroscopy (EIS) is a method for measuring interfacial changes in capacitance, resistance, and conductance at electroactive surfaces. EIS can be either Faradaic or non-Faradaic. In the former, redox reactions govern changes in the interfacial electrical properties. For non-Faradaic EIS, disruptions of the electric double layer at the electrode-fluid interface is most responsible for measured changes in electrical properties. The latter is particularly useful for detecting non-electroactive analytes and electroactive analytes that cannot be easily distinguished from non-target entities. As such, EIS has been employed in wearable transdermal sensors (WTS) alongside affinity-based biorecognition to detect hydrophobic molecules like cortisol [8–10].

While electrochemical detection in sweat differs from that in ISF, the underlying principles of the various electrochemical approaches remain the same. Amperometry is primarily employed for detecting enzymatically catalyzed redox reactions while potentiometry is mainly used for ion detection. The disparity between electrochemical sensing in sweat and ISF arises from the contrasting compositions, properties, sampling methods, and sensing environments of the two fluids. Electrochemical

approaches involving a transistor as the biosensor or relying on electrochemical impedance spectroscopy (EIS) for analyte detection are not utilized for in situ ISF sampling. However, they find applications in in situ sweat sampling. Transdermal gases are usually detected based on gas-specific optical sensors. For example, transdermal CO_2 is detected using an IR detector that observes the change in the IR absorbance in the sampling chamber specific to CO_2 [11].

3.2 Optical Detection

Optical detection methods comprise a variety of techniques used to detect and quantify molecules based on their optical properties. They generally involve the use of light to interact with the analyte of interest, which can then be measured and analyzed to determine the concentration of the molecule. There are several types of optical detection techniques that have been utilized in WTSs, including colorimetry, surface plasmon resonance, fluorescence spectroscopy, and surface-enhanced Raman scattering.

Colorimetry involves measuring the color change of a sample solution in response to the presence of an analyte. Certain chemical reactions or interactions between molecules can cause a change in the absorption or reflection of light in the visible spectrum, which are detected using a colorimetric assay. Colorimetric detection is commonly used in various applications, such as clinical diagnostics, environmental monitoring, and food safety testing. It is often preferred due to its simplicity, low cost, and ease of use compared to more complex analytical techniques.

Plasmonic detection utilizes the optical properties of metallic nanoparticles to detect the presence of an analyte [12]. Noble metal nanomaterials show collective oscillations of surface electrons that can interact with the surrounding molecules causing change in their optical properties [13]. These changes are measured using spectrophotometers as absorbance, scattering, or SPR to detect the presence or concentration of the analyte. Plasmonic detection is used in a variety of applications, including biosensing, medical diagnostics, and environmental monitoring. One of the main challenges is the potential for non-specific binding, which can lead to false positives or inaccurate readings.

Fluorometric detection relies on measuring the fluorescence emission of a sample in response to the presence of an analyte. Certain molecules, known as fluorophores, can absorb light at a specific wavelength and emit the absorbed energy as radiative emission at a longer wavelength, which is measured using a spectrophotometer [14]. It is also a versatile technique, allowing for the detection of a wide range of molecules and analytes. Additionally, fluorescence measurements can be affected by factors such as pH, temperature, and sample preparation.

Surface-Enhanced Raman Scattering (SERS) is a detection technique that utilizes the enhanced Raman signal of molecules adsorbed onto a metal surface, typically silver or gold, to detect the presence and concentration of analytes. The enhancement is achieved by the plasmonic resonance of the metal surface, which greatly amplifies

the Raman signal of the analyte. Advantages of SERS-based detection are high sensitivity, label-free technique, simplified assay, and cost reduction. Limitations of SERS are signal interference and variability due to the complex nature of the metal surface and sensitivity to temperature and pH.

Detection in interstitial fluid (ISF) in situ is currently performed exclusively with electrochemical methods. Optical detection methods, such as colorimetry and fluorometry, are difficult to implement because of the low penetration depth of visible light into skin. There are a few exceptions, including the commercially available continuous glucose monitor (CGM), Eversense® [15, 16], developed by Senseonics that uses fluorescence for analyte detection in ISF. However, in such cases, the fluorescence is measured subcutaneously. Sweat, on the other hand, is not limited by the poor penetration depth of light because analysis requires secretion outside the body. Therefore, optical detection methods can be more easily integrated into wearable sweat sensors (WSSs) for in situ analysis than wearable sensors that sample ISF. Optical detection methods have the advantage of not requiring a power supply for their operation, which sets them apart from electrochemical methods. This advantage is most noticeable in WSSs that use colorimetry. When combined with smartphone technology [17], this detection method has shown to be an effective, point-of-care approach to measuring sweat rate and simple analytes like electrolytes and metabolites [18–20] in secreted eccrine sweat.

4 Biofluids

4.1 Interstitial Fluid

Interstitial fluid (ISF) is the aqueous solution that bathes the extracellular space acting as the transport medium for exchange of nutrients and waste products between the capillaries and surrounding tissues; signaling molecules between cells and larger solutes such as protein macromolecules, lipoproteins, antigens, and endogenous vesicles are delivered from the interstitium to the lymphatic system [21, 22]. Ex situ approaches for analyzing ISF involve minimally invasive methods like wicking, microdialysis, suction blisters, etc., which require puncturing the skin, or non-invasive methods like reverse-iontophoresis or sonification to extract ISF for off-body analysis. In situ WTBs provide us with a means to expand the utility of ISF beyond laboratory-based diagnostics and proteomics to healthcare applications that would benefit from rapid, continuous sampling of a target analyte.

4.1.1 Sourcing Interstitial fluid—anatomical Understanding

The skin consists of three layers: the epidermis, dermis, and hypodermis in that order from external to internal. The epidermis (50–150 μm thick), the outermost layer,

includes the stratum corneum and viable epidermis (Fig. 1a). The viable epidermis is avascular and is mainly comprised of special antigen-presenting cells, known as Langerhans cells, and keratinocytes [23]. ISF comprises 15–35% [24] of the volume of the viable epidermis. This ISF is the target of microneedle platforms that are designed to be minimally invasive because of the absence of blood vessels and nerves in the epidermis. The dermis is divided into two essential layers: the papillary dermis (~15 μm thick) and the reticular dermis (~1–4 mm thick) containing the lowest cellular content of the layers, but the highest ISF content [24]. The extracellular matrix (ECM) of the dermis which consists of glycoaminoglycans, collagen, and proteoglycans [25] plays a crucial role in the extraction and sampling of ISF. The hypodermis, composed of adipose tissue, connects the dermis to muscle and is relevant for continuous glucose monitors (CGMs). While WTBs focus on the dermis and epidermis, the hypodermis provides nutrient-rich ISF and allows secure sensor insertion without flexible electronics. Ions and small, non-lipophilic molecules (molecular weight < 3 kDa) freely partition between the blood and dermal ISF via paracellular diffusion through intercellular junctions of the capillary endothelium. Small lipophilic solutes passively diffuse through the cells' lipid bilayers. Solutes with sizes in the range of 3–70 kDa are transported by paracellular and transcellular routes. Which transport mechanism dominates depends on the size [25, 26] of the solute as is illustrated in Fig. 1b.

4.1.2 Sampling Interstitial Fluid

ISF and sweat need different sampling methodologies. One of the early methods used for ISF extraction that is relevant to WTSs is reverse-iontophoresis. It requires the application of a small current across the skin to initiate the electroosmotic flow of ISF from the epidermis and papillary dermis (Fig. 2). Electroosmosis [27, 28] is bulk fluid flow in the direction of counter ion flow due to the application of an electric field across a membrane. Since ISF is an aqueous solution, it is incapable of passing through the lipophilic stratum corneum, and, hence, exits the body through sweat ducts or hair follicles before collecting at the cathode [29]. Concerns that prolonged use or increased current density can cause skin irritation hinder the wider use of reverse-iontophoresis in WTSs. In addition, there is a general lack of validation of correlations between measured concentrations of analytes in iontophoretically extracted ISF and circulating concentrations. Nonetheless, proof of concepts demonstrating its feasibility for ISF extraction has been presented [30–32].

Microneedles constitute an approach to sampling ISF that has the advantage of direct sampling, while being less invasive than the probes used with commercial glucose sensors. Generally, microneedles refer to needles with lengths of 50–2000 μm, base widths of 25–500 μm, and tip diameters of 1–100 μm [33, 34]. Microneedle platforms can be categorized as either ex situ or in situ [35]—referring to off-body and on-body detection, respectively. Most advances in microneedle technology for transdermal biosensing can be classified as in situ. Microneedles for

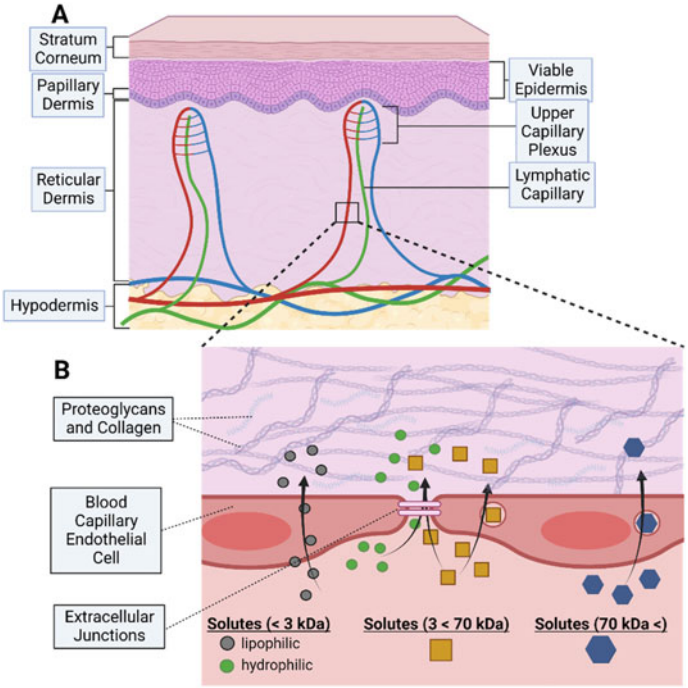

Fig. 1 **a** An illustration showing the layers of skin. **b** Illustration of the partitioning of solutes between the blood and dermal ISF

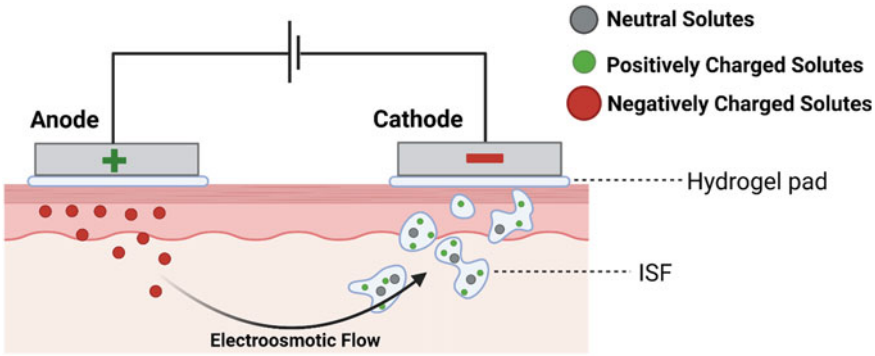

Fig. 2 An illustration of the principle of reverse-iontophoresis

in situ, in vivo sensing come in four basic configurations (Fig. 3): hollow microneedles with the sensor situated ex vivo, hollow microneedles where the sensor is inserted or packed into the tip, solid-tip microneedles where the tips are dry electrodes, and solid-tip microneedles where the sensor is fabricated on the tip [36]. The hollow microneedles, not interfaced with the sensor element, aid in extracting ISF to the

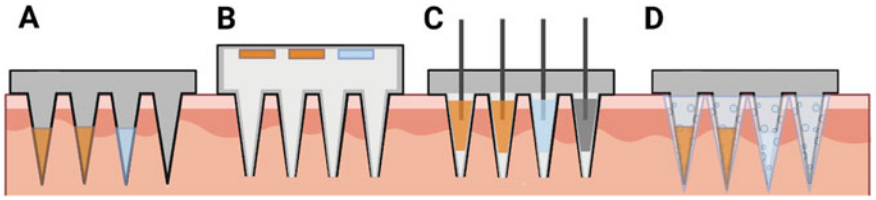

Fig. 3 Illustrations of conventional microneedle configurations for transdermal sensing. Orange denotes the working and counter electrodes and blue denotes the reference electrode of a typical three-electrode electrochemical cell. **a** Solid-tip microneedles. **b** Hollow microneedles with an ex vivo sensor. **c** Packed-hollow-tip microneedles. Wires are imbedded from the backside of the sensor

biosensor. The high fluidic resistance of the dermis makes this method impractical for in situ monitoring; however, the configuration of hollow microneedles packed with the biosensor has been demonstrated [37] as a possible way to circumvent the complexity of the dermal interstitium. Thus far, microneedles for in situ monitoring have been demonstrated for metabolites [37–40], drugs [41–44], and electrolytes [45–47]. However, few have been clinically validated.

4.2 Biofluids

4.2.1 Sweat

Sweat is our bodies' natural way to regulate internal temperature for maintaining thermal homeostasis. Sweat secretion is controlled by the sympathetic nervous system and is initiated through either thermal or psychological stimulation. Sweat is easy to access, naturally secreted, and contains a cornucopia of biomarkers also found in ISF and blood. Above all, sweat can be sampled non-invasively. However, wearable sweat sensor devices still have not seen the same level of commercial or clinical success as their ISF sampling counterparts due to operational challenges such as variations in composition and rate of secreted sweat. Rate of secretion due to environmental and physiological factors requires consideration of not only the biosensor, but also how the sweat is being sampled. Ultimately, development of a wearable sweat sensor amounts to a uniquely interdisciplinary problem.

4.2.2 Sourcing sweat—anatomy of Eccrine Sweat Gland

Eccrine sweat glands are the smallest, present in whole body, most numerous, and contribute the most to the total volume of sweat secreted [48]. The other two sweat gland types, apocrine and apoeccrine, are primarily found in regions less accessible for sweat sampling. Sweat secreted from apocrine and apoeccrine glands is also

comparatively more complex [49]. For these reasons, eccrine sweat is mainly chosen for sweat analysis.

The anatomical structure (Fig. 4) of the eccrine sweat gland extends from the reticular dermis to the stratum corneum. In the dermis are the two main functional units, the secretory coil and the dermal duct. The secretory coil is composed of three cell types: clear, dark, and myoepithelial cells. Clear cells are responsible for primary sweat secretion as is evidenced by the cells' system of intercellular canaliculi, glycogen, large amount of mitochondria, and high Na–K-ATPase activity [50]. The function of the dark cells is not entirely understood; however, they are known to secrete glycoproteins, dermicidin, and sialomucin and are necessary for proper sweat secretion [51, 52]. The function of myoepithelial cells is primarily as structural support for the secretory coil [50]. The dermal duct is composed of basal and luminal epithelial cells. The function of the duct is reabsorption of Na^+ and Cl^- as secreted sweat flows through the duct [49, 50].

Solutes that comprise secreted sweat's composition include metabolites like glucose, lactate, urea, amino acids, and creatinine; micronutrients like Mg^{2+}, Ca^{2+}, iron, zinc, and vitamins like ascorbic acid; adrenal steroids like cortisol and dehydroepiandrosterone (DHEA); neurotransmitters like serotonin and dopamine; and cytokines like IL-6, IL-8, IL-10, IL-31, and TNF-α [49, 53, 54]. Mechanisms and correlations with blood plasma concentrations have not been fully established for most of these solutes. Complicating these deficits in data are physiological factors like sweat rate and sweat pH [52, 55], which can vary with sex, age, diet, weight, temperature, etc. If they are not properly accounted for, these variabilities can negatively affect the accuracy of measurements depending on the analyte. As such, WSSs may require sensors for measuring sweat rate, pH, skin temperature, and ion concentration alongside the biosensor for the analyte.

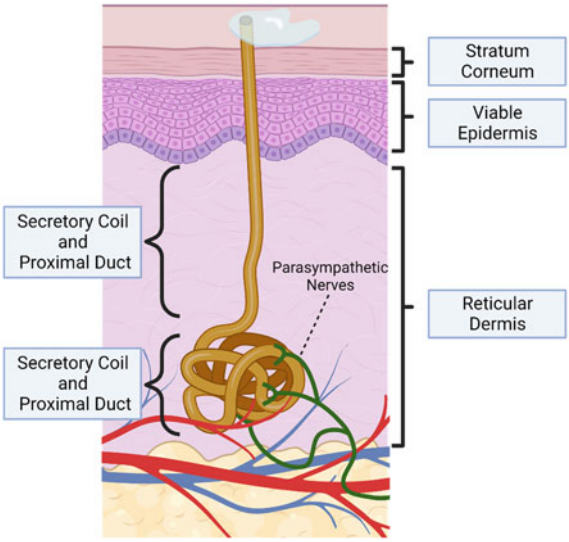

Fig. 4 An illustration showing the basic anatomy of an eccrine sweat gland

4.2.3 Sampling Sweat

Traditional sweat sampling methods include the whole body-washdown technique, patches, polymer bags/films, and macroducts. The limited-use whole body-washdown technique collects all sweat runoff to determine whole body-sweat electrolyte loss [56]. Using absorbent patches is flexible and can be applied to specific sites for sweat accumulation, but they may overestimate analyte concentration and have time-consuming application processes [57]. Polymer bags/films and macroducts [58, 59] offer alternative collection methods but are also associated with limitations such as sweat composition changes and inhibiting sweat evaporation. Current approaches to sweat sampling for wearable transdermal biosensing aim to actively collect secreted sweat for on-body analyte monitoring. These methods aim to enable real-time, continuous sweat sensing with improved reliability and sensitivity. Absorbent materials integrated with wearable sensors, microfluidic devices with superhydrophobic/superhydrophilic surfaces, fabrics with moisture-wicking properties, and controlled sweat secretion via the iontophoretic delivery of sweat-stimulating drugs are emerging techniques that address some of the limitations of traditional sampling methods [33].

4.3 Transcutaneous Gases

Despite the benefits offered by sweat-based analysis, the successful diagnosis of the related problem is still a big challenge, sometimes resulting in misleading information if a standalone sensor shows a signal change. For example, sensing potassium levels in the sweat may provide incomplete status as they are invariant with sweat rate and with normal physiological changes in the body. While change in sodium, lactate, or glucose signals and the potassium signal holds steady, multiple sensor could provide a solution for monitoring a real physiological event [60]. Alternative solutions involve the monitoring of transcutaneous gases that include volatile organics and gases emanating through human skin (skin gas). Skin emanation gas was firstly proved by Hirabayashi et al. with the releasing hydrogen gas and acetone vapor from skin [61].

4.3.1 Volatile Organic Compounds (Vocs)

The commonly studied VOCs include acetone, ethanol, and ethylene having insight into the health state of the human body. Diabetic patients have shown high acetone concentration in their breath that can be used for diagnosis. Prompted by this, Kondo et al. measured skin acetone concentration and its correlation with blood glucose for regular examination of diabetes to avoid later complications [62]. However, this can only be applied to patients with insulin therapy and not in regular patients on oral medication or with controlled diet. Although the results have large deviations, they

proved that the acetone concentration varied with the diabetic condition of the patients diagnosed with diabetes. In other recent study, Misra et al. fabricated a wrist-worn system to monitor skin acetone with output represented by the resistance change [63]. Their sensing system comprised commercial off the shelf components along with a microcontroller and a rechargeable Li ion battery. They made a wristband form factor constituting an array from four sensors (n-type metal oxide semiconductor-based gas sensor, BME680) operated at different temperatures as the sensing modality. Due to the reducing effect of acetone on the metal oxide, a decrement in the resistance was detected. The acetone reacts with oxygen anions of metal oxide and increases the carrier concentration thereby declining the resistance of the sensor. Besides the instrumentation evaluation of the fabricated wrist-worn system, they provided real-time analysis showing larger changes in the subjects with intermittent fasting than the normal subjects.

Other volatile organic compounds (VOCs) such as ethanol and acetic acid are also analyzed in recent studies and correlated with different health conditions for example psoriasis [64]. One such study reported wearable e-nose constituting an array of chemical sensors integrated in a wireless communication system [65]. The array comprised eight elements formed by varying the printing patterns and sensing materials (MWCNT/polymer films) to produce the cross responses toward different analytes. The applicability was demonstrated with fabricated armband and corresponding monitoring of ammonia, acetic acid, acetone, and ethanol. The electrical responses of the fabricated system yielded discrete patterns to ammonia and acetic acid unlike the similar patterns for acetone and ethanol. Another study reported the skin gas sensor and named bio-sniffer for measurement of ethanol by using alcohol dehydrogenase (ADH) [66]. It measured the change in fluorescence intensity of nicotinamide adenine dinucleotide (NADH), as a result of oxidation of ethanol by ADH. Later, it was modified as sniff-cam or skin gas cam extending the bio-sniffer toward the measurement of the time-dependent spatial distribution of ethanol [67].

4.3.2 Trace Gases

Apart from the VOCs, skin gas also contains important trace gases such as NH_3, NO_2, and CO_2 which have particular benefits in healthcare systems [68]. For example, measurement of skin CO_2 can provide ventilation status of newborn infants along with respiratory and cardiac status [69]. They constructed a wearable prototype for monitoring transcutaneous CO_2 by employing polymer films (Poly(propyl methacrylate), PPMA) with pH-sensitive fluorescent dye (8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt or HPTS). Their sensor is based on the fluorescence changes of a pH indicator while protonation or deprotonation. The fabricated device comprised a microcontroller along with other electronics. The fluorescence response of the film is monitored via intensity of the sensing film on interaction with CO_2 skin gas and was found to be highly sensitive in the physiological CO_2 range. They proposed that their future work will aim to make a CO_2 permeable scattering layer which will not require heating for transcutaneous applications. Another study reported a

new approach based on the initial pseudo steady state diffusion rate monitoring for non-invasive measurement of transcutaneous partial pressure of carbon dioxide ($tcpCO_2$) [70]. They provided additional reliable data to compare their sensing device with commercially available equilibrium-based transcutaneous monitor, Radiometer TCM4, used for CO_2 monitoring in hospitals [71]. Radiometer TCM4 requires the start-up at 37 °C and took 85 min before to provide meaningful results. Even at a higher temperature of 42 °C, it requires 20 min to stabilize. Unlike this, their device can provide immediate reliable data, almost independent of temperature by using a rate-based approach.

Recently, Ahuja et al. reported the room temperature measurement of skin CO_2 gas with an air permeable transcutaneous sensor comprising of polyaniline (PANI)-based sensing material as shown in Fig. 5 [72]. Porous sugar-templated polydimethylsiloxane (PDMS) elastomer sponge (PP) was used as a matrix for PANI-based ink (oxSWCNT-PANI) and fabricated using a dip coating method (Fig. 5a). The dispersant (Zn-Al) used for ink fabrication offered uniform active sites for the analyte (transdermal CO_2 gas) as shown by TEM analysis (Fig. 5b). The interaction of CO_2 with the sensor (partial extraction of electrons from PANI) resulted in an augmented charge transfer interaction leading to increased response of the sensor. Besides the sensor's fast response and recovery (Fig. 5c), it can selectively detect transcutaneous CO_2 at room temperature, unlike the commercial devices operated at a high temperature. Noteworthily, the deformability tolerance capability of the sensor opens up novel opportunities for skin-compliant wearable systems.

5 The Case of Continuous Glucose Monitors

The Center for Disease Control and Prevention (CDC) reported [73] in 2022, that, in the United States, 37.3 million people have diabetes and 96 million people aged 18 or older are prediabetic. In the late twentieth century until the beginning of the twenty-first century, personal management of one's glycemic level exclusively required pricking a finger to draw blood, then testing the glucose concentration of the blood sample with a disposable electrochemical sensor integrated with a digital-readout device. In the year 1999, Medtronic's Minimed Gold became the first continuous glucose sensor (CGM) to be approved by the FDA for market distribution [74]. The device still required daily calibration via finger pricking; however, the decrease in frequency of the pricking was a dramatic improvement to patients' quality of life and demonstrated the future healthcare potential of wearable biosensors.

CGM devices that sample ISF are currently the most commercially successful type of WTB available on the market—being capable of long-term continuous monitoring of their target analyte with analytical sensitivity and selectivity great enough [75] for the devices to be used as either adjuncts to fingerstick blood glucose monitors (BGMs) or replacements of fingerstick BGMs. A CGM [76] is any device that can automatically track glucose levels throughout the day and night. Current, commercially available CGMs use a small probe (Fig. 6), either inserted or implanted

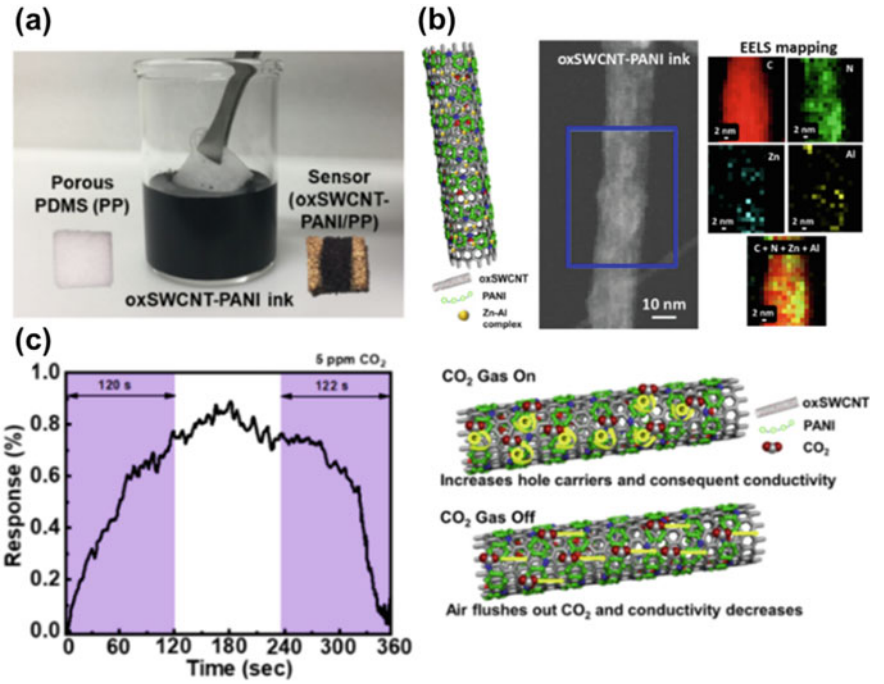

Fig. 5 **a** Photograph of the porous PDMS sponge (PP), oxSWCNT-PANI ink and fabricated sensor (oxSWCNT-PANI/PP); **b** ADF-STEM micrograph of the oxSWCNT-PANI ink with schematic of the fabricated sensor. EELS mapping of particular region in blue box. **c** Response and recovery time of the oxSWCNT-PANI/PP sensor in CO_2 gas sensing (5 ppm). **d** Schematic representation of the probable CO_2 sensing mechanism of the sensor

deep in the dermis or into the hypodermis. The typical glucose sensor relies on the oxidation of glucose catalyzed by glucose-oxidase after the molecule binds with the enzyme. First-generation glucose monitors use oxygen as an electron acceptor/redox mediator by using the electron from the oxidation of glucose to reduce oxygen to hydrogen peroxide. Then the electron obtained from the oxidation of hydrogen peroxide to water by the working electrode is detected by the change in current at the working electrode. This change in current is subsequently correlated to the blood-glucose concentration. These data are wirelessly transmitted to a handheld device, thus providing the patient with real-time changes of their blood glucose levels throughout the wear period. Some commercial CGMs still rely on first-generation glucose sensing. Second-generation CGMs, that use artificial electron acceptors or redox mediators, and third-generation CGMs, that use modified enzymes instead of mediators, are becoming more prevalent in CGM technology [7].

CGMs share four key components: an enzymatic biorecognition element, an electrochemical transduction element, a glucose limiting membrane, and interference rejection [7]. Most CGMs use glucose oxidase (GOx) as the enzyme, which catalyzes the oxidation of glucose. The generated electron participates in a redox reaction

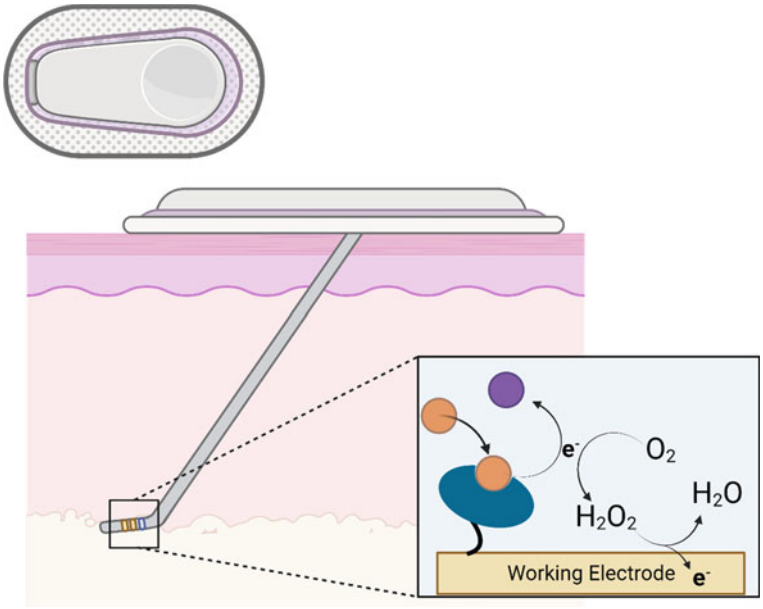

Fig. 6 Illustration of a continuous glucose monitor and first-generation detection of glucose. The orange circle represents glucose, which is oxidized by glucose oxidase into glucono-δ-lactone, represented by the purple circle. The electron removed from glucose is ultimately used to reduce oxygen dissolved in ISF to hydrogen peroxide, which is subsequently oxidized by the working electrode to water

with a mediator molecule near the transduction element, detected amperometrically. First-generation sensors relied on the H_2O_2–O_2 redox reaction but had issues with unintended oxidation of other molecules. Second-generation sensors used artificial mediators or glucose dehydrogenases (GDHs) as replacements but face stability and toxicity challenges. Recent advancements aim to improve enzyme stability, electron transfer rate, or eliminate redox mediators in the reaction. Incorporating GDHs into commercial CGMs is still pending.

6 Discussion and Concluding Remarks

Within the past 5 years, WTB technology has slowly branched away from glucose monitoring for diabetes management toward other analytes and applications. The success of CGMs using ISF as the sampling fluid punctuates the viability of the biofluid for other transdermal sensing applications and sets them as the gold standards which other WTBs aim to emulate regarding sensitivity, selectivity, and longevity. However, many aspects of their designs do not necessarily integrate well with alternative sampling methods or can be easily repurposed for the detection of biomarkers

dissimilar to glucose. Sweat has been extensively investigated as a non-invasive alternative to ISF for glucose monitoring. Correlations between sweat-glucose and blood-glucose have been validated; however, they are still issues with reliable and continuous sampling throughout the day due to uncontrollable environmental and physiological factors—issues which extend to other wearable sweat sensing applications. To this point, monitoring transcutaneous gases has a clear advantage; however, the technology is still in a nascent phase. The number of validated biomarkers that can be detected as gases is currently limited. Regarding dissimilar biomarkers, enzymatic recognition and subsequent amperometric detection is not always a viable strategy. Two examples of such biomarkers, whose continuous monitoring has been repeatedly demonstrated and whose WTB had recent clinical success, are electrolytes and hormones. A recent example of the former case, a zero-power, flexible microfluidic patch [18] containing colorimetric assays, was shown to be effective at monitoring Cl^- and sweat rate for sports-related applications under various environmental conditions. For the latter case, a wearable collection unit [77] connected to a portable liquid-chromatographer/mass spectrometer was able to continuously monitor changes in the concentrations of multiple adrenal steroids in ISF throughout patients' daily activities.

WTBs represent an emerging and variable class of medical devices that have viable potential for improving point-of-care medicine. Their successful development requires careful consideration of the interactions between the sensor and the biofluid containing the target analyte. WTBs most often target sweat and ISF because they contain a plethora of biomarkers and can be minimally or non-invasively sampled. On-body detection of the target analyte(s) is normally done in situ using electrochemical detection methods. Access to these biofluids for continuous, on-body monitoring, however, is not trivial. WTBs for transcutaneous gases are an emerging technology that, in comparison to ISF and sweat WTBs, have the advantage of continuous monitoring with minimal sensor degradation. However, there are few transcutaneous gases that have been identified as viable biomarkers. So far, CGMs have been the most successful WTBs, but that same success has yet to be replicated. However, recent successes highlight the prospective benefits of the WTBs and herald its continuing evolution into a staple of modern medical technology.

References

1. Zhuo, Z., Yu, Y., Wang, M., Li, J., Zhang, Z., Liu, J., Wu, X., Lu, A., Zhang, G., Zhang, B.: Recent advances in selex technology and aptamer applications in biomedicine. IJMS **18**, 2142 (2017). https://doi.org/10.3390/ijms18102142
2. Morales, M.A., Halpern, J.M.: Guide to selecting a biorecognition element for biosensors. Bioconjugate Chem. **29**, 3231–3239 (2018). https://doi.org/10.1021/acs.bioconjchem.8b00592
3. BelBruno, J.J.: Molecularly imprinted polymers. Chem. Rev. **119**, 94–119 (2019). https://doi.org/10.1021/acs.chemrev.8b00171

4. Parlak, O., Keene, S.T., Marais, A., Curto, V.F., Salleo, A.: Molecularly selective nanoporous membrane-based wearable organic electrochemical device for noninvasive cortisol sensing. Sci. Adv. 4, eaar2904 (2018). https://doi.org/10.1126/sciadv.aar2904
5. Mugo, S.M., Robertson, S.V., Lu, W.: A molecularly imprinted electrochemical microneedle sensor for multiplexed metabolites detection in human sweat. Talanta **259**, 124531 (2023). https://doi.org/10.1016/j.talanta.2023.124531
6. Mugo, S.M., Lu, W., Robertson, S.: A wearable, textile-based polyacrylate imprinted electrochemical sensor for cortisol detection in sweat. Biosensors **12**, 854 (2022). https://doi.org/10.3390/bios12100854
7. Lee, I., Probst, D., Klonoff, D., Sode, K.: Continuous glucose monitoring systems – Current status and future perspectives of the flagship technologies in biosensor research. Biosens. Bioelectron. **181**, 113054 (2021). https://doi.org/10.1016/j.bios.2021.113054
8. Ganguly, A., Lin, K.C., Muthukumar, S., Prasad, S.: Autonomous, real-time monitoring electrochemical aptasensor for circadian tracking of cortisol hormone in sub-microliter volumes of passively eluted human sweat. ACS Sens. **6**, 63–72 (2021). https://doi.org/10.1021/acssensors.0c01754
9. Pali, M., Jagannath, B., Lin, K.-C., Upasham, S., Sankhalab, D., Upashama, S., Muthukumar, S., Prasad, S.: CATCH (Cortisol Apta WATCH): 'Bio-mimic alarm' to track anxiety, stress immunity in human sweat. Electrochim. Acta. **390**, 138834 (2021). https://doi.org/10.1016/j.electacta.2021.138834
10. Upasham, S., Osborne, O., Prasad, S.: Demonstration of sweat-based circadian diagnostic capability of SLOCK using electrochemical detection modalities. RSC Adv. **11**, 7750–7765 (2021). https://doi.org/10.1039/D0RA10561A
11. Grieshaber, D., MacKenzie, R., Vörös, J., Reimhult, E.: Electrochemical biosensors - sensor principles and architectures. Sensors **8**, 1400–1458 (2008)
12. Rai, B., Malmberg, R., Srinivasan, V., Ganesh, K.M., Kambhampati, N.S.V., Andar, A., Rao, G., Sanjeevi, C.B., Venkatesan, K., Ramamurthy, S.S.: Surface plasmon-coupled dual emission platform for ultrafast oxygen monitoring after SARS-CoV-2 infection. ACS Sens. **6**, 4360–4368 (2021). https://doi.org/10.1021/acssensors.1c01665
13. Srinivasan, V., Ramamurthy, S.S.: Purcell factor: a tunable metric for plasmon-coupled fluorescence emission enhancements in cermet nanocavities. J. Phys. Chem. C **120**, 2908–2913 (2016). https://doi.org/10.1021/acs.jpcc.5b11311
14. Srinivasan, V., Badiya, P.K., Ramamurthy, S.S.: Low-dimensional carbon spacers in surface plasmon-coupled emission with femtomolar sensitivity and 1000-fold fluorescence enhancements. Chem. Commun. **51**, 7809–7811 (2015). https://doi.org/10.1039/C5CC01040C
15. Boscari, F., Vettoretti, M., Cavallin, F., Amato, A.M.L., Uliana, A., Vallone, V., Avogaro, A., Facchinetti, A., Bruttomesso, D.: Implantable and transcutaneous continuous glucose monitoring system: a randomized cross over trial comparing accuracy, efficacy and acceptance. J. Endocrinol. Invest. **45**, 115–124 (2022). https://doi.org/10.1007/s40618-021-01624-2
16. Joseph, J.I.: Review of the long-term implantable senseonics continuous glucose monitoring system and other continuous glucose monitoring systems. J. Diabetes Sci. Technol. **15**, 167–173 (2020). https://doi.org/10.1177/1932296820911919
17. Ghaffari, R., Rogers, J.A., Ray, T.R.: Recent progress, challenges, and opportunities for wearable biochemical sensors for sweat analysis. Sens. Actuators, B Chem. **332**, 129447 (2021). https://doi.org/10.1016/j.snb.2021.129447
18. Baker, L.B., Model, J.B., Barnes, K.A., Anderson, M.L., Lee, S.P., Lee, K.A., Brown, S.D., Reimel, A.J., Roberts, T.J., Nuccio, R.P., Bonsignore, J.L., Ungaro, C.T., Carter, J.M., Li, W., Seib, M.S., Reeder, J.T., Aranyosi, A.J., Rogers, J.A., Ghaffari, R.: Skin-interfaced microfluidic system with personalized sweating rate and sweat chloride analytics for sports science applications. Sci. Adv. 6, eabe3929 (2020). https://doi.org/10.1126/sciadv.abe3929
19. Koh, A., Kang, D., Xue, Y., Lee, S., Pielak, R.M., Kim, J., Hwang, T., Min, S., Banks, A., Bastien, P., Manco, M.C., Wang, L., Ammann, K.R., Jang, K.-I., Won, P., Han, S., Ghaffari, R., Paik, U., Slepian, M.J., Balooch, G., Huang, Y., Rogers, J.A.: A Soft, Wearable Microfluidic Device for the Capture, Storage, and Colorimetric Sensing of Sweat. Sci Transl Med. 8, 366ra165 (2016). https://doi.org/10.1126/scitranslmed.aaf2593

20. Choi, J., Bandodkar, A.J., Reeder, J.T., Ray, T.R., Turnquist, A., Kim, S.B., Nyberg, N., Hourlier-Fargette, A., Model, J.B., Aranyosi, A.J., Xu, S., Ghaffari, R., Rogers, J.A.: Soft, skin-integrated multifunctional microfluidic systems for accurate colorimetric analysis of sweat biomarkers and temperature. ACS Sens. **4**, 379–388 (2019). https://doi.org/10.1021/acssensors.8b01218
21. Trevaskis, N.L., Kaminskas, L.M., Porter, C.J.H.: From sewer to saviour—targeting the lymphatic system to promote drug exposure and activity. Nat. Rev. Drug Discov. **14**, 781–803 (2015). https://doi.org/10.1038/nrd4608
22. Wiig, H., Swartz, M.A.: Interstitial fluid and lymph formation and transport: physiological regulation and roles in inflammation and cancer. Physiol. Rev. **92**, 1005–1060 (2012). https://doi.org/10.1152/physrev.00037.2011
23. Kabashima, K., Honda, T., Ginhoux, F., Egawa, G.: The immunological anatomy of the skin. Nat. Rev. Immunol. **19**, 19–30 (2019). https://doi.org/10.1038/s41577-018-0084-5
24. Groenendaal, W., von Basum, G., Schmidt, K.A., Hilbers, P.A.J., van Riel, N.A.W.: Quantifying the composition of human skin for glucose sensor development. J. Diabetes Sci. Technol. **4**, 1032–1040 (2010)
25. Friedel, M., Thompson, I.A.P., Kasting, G., Polsky, R., Cunningham, D., Soh, H.T., Heikenfeld, J.: Opportunities and challenges in the diagnostic utility of dermal interstitial fluid. Nat. Biomed. Eng. (2023). https://doi.org/10.1038/s41551-022-00998-9
26. Heikenfeld, J., Jajack, A., Feldman, B., Granger, S.W., Gaitonde, S., Begtrup, G., Katchman, B.A.: Accessing analytes in biofluids for peripheral biochemical monitoring. Nat. Biotechnol. **37**, 407–419 (2019). https://doi.org/10.1038/s41587-019-0040-3
27. Pikal, M.J.: The role of electroosmotic flow in transdermal iontophoresisq. Adv. Drug Delivery Rev. 25 (2001)
28. Giri, T.K., Chakrabarty, S., Ghosh, B.: Transdermal reverse iontophoresis: a novel technique for therapeutic drug monitoring. J. Control. Release **246**, 30–38 (2017). https://doi.org/10.1016/j.jconrel.2016.12.007
29. Bakshi, P., Vora, D., Hemmady, K., Banga, A.K.: Iontophoretic skin delivery systems: success and failures. Int. J. Pharm. **586**, 119584 (2020). https://doi.org/10.1016/j.ijpharm.2020.119584
30. Cheng, Y., Gong, X., Yang, J., Zheng, G., Zheng, Y., Li, Y., Xu, Y., Nie, G., Xie, X., Chen, M., Yi, C., Jiang, L.: A touch-actuated glucose sensor fully integrated with microneedle array and reverse iontophoresis for diabetes monitoring. Biosens. Bioelectron. **203**, 114026 (2022). https://doi.org/10.1016/j.bios.2022.114026
31. Yang, B., Fang, X., Kong, J.: Engineered microneedles for interstitial fluid cell-free DNA capture and sensing using iontophoretic dual-extraction wearable patch. Adv. Func. Mater. **30**, 2000591 (2020). https://doi.org/10.1002/adfm.202000591
32. Kim, J., Sempionatto, J.R., Imani, S., Hartel, M.C., Barfidokht, A., Tang, G., Campbell, A.S., Mercier, P.P., Wang, J.: Simultaneous monitoring of sweat and interstitial fluid using a single wearable biosensor platform. Adv. Sci. **5**, 1800880 (2018). https://doi.org/10.1002/advs.201800880
33. Liu, C., Xu, T., Wang, D., Zhang, X.: The role of sampling in wearable sweat sensors. Talanta **212**, 120801 (2020). https://doi.org/10.1016/j.talanta.2020.120801
34. Le, Z., Yu, J., Quek, Y.J., Bai, B., Li, X., Shou, Y., Myint, B., Xu, C., Tay, A.: Design principles of microneedles for drug delivery and sampling applications. Mater. Today. S1369702122002772 (2022). https://doi.org/10.1016/j.mattod.2022.10.025
35. Iitani, K., Ramamurthy, S.S., Ge, X., Rao, G.: Transdermal sensing: in-situ non-invasive techniques for monitoring of human biochemical status. Curr. Opin. Biotechnol. **71**, 198–205 (2021). https://doi.org/10.1016/j.copbio.2021.08.005
36. Teymourian, H., Tehrani, F., Mahato, K., Wang, J.: Lab under the skin: microneedle based wearable devices. Adv. Healthcare Mater. **10**, 2002255 (2021). https://doi.org/10.1002/adhm.202002255
37. Jina, A., Tierney, M.J., Tamada, J.A., McGill, S., Desai, S., Chua, B., Chang, A., Christiansen, M.: Design, development, and evaluation of a novel microneedle array-based continuous glucose monitor. J. Diabetes Sci. Technol. **8**, 483–487 (2014). https://doi.org/10.1177/1932296814526191

38. Miller, P.R., Skoog, S.A., Edwards, T.L., Lopez, D.M., Wheeler, D.R., Arango, D.C., Xiao, X., Brozik, S.M., Wang, J., Polsky, R., Narayan, R.J.: Multiplexed microneedle-based biosensor array for characterization of metabolic acidosis. Talanta **88**, 739–742 (2012). https://doi.org/10.1016/j.talanta.2011.11.046
39. Freeman, D.M.E., Ming, D.K., Wilson, R., Herzog, P.L., Schulz, C., Felice, A.K.G., Chen, Y.-C., O'Hare, D., Holmes, A.H., Cass, A.E.G.: Continuous measurement of lactate concentration in human subjects through direct electron transfer from enzymes to microneedle electrodes. ACS Sens. **8**, 1639–1647 (2023). https://doi.org/10.1021/acssensors.2c02780
40. Yang, J., Gong, X., Chen, S., Zheng, Y., Peng, L., Liu, B., Chen, Z., Xie, X., Yi, C., Jiang, L.: Development of smartphone-controlled and microneedle-based wearable continuous glucose monitoring system for home-care diabetes management. ACS Sens. **8**, 1241–1251 (2023). https://doi.org/10.1021/acssensors.2c02635
41. Mishra, R.K., Goud, K.Y., Li, Z., Moonla, C., Mohamed, M.A., Tehrani, F., Teymourian, H., Wang, J.: Continuous opioid monitoring along with nerve agents on a wearable microneedle sensor array. J. Am. Chem. Soc. **142**, 5991–5995 (2020). https://doi.org/10.1021/jacs.0c01883
42. Vinu Mohan, A.M., Windmiller, J.R., Mishra, R.K., Wang, J.: Continuous minimally-invasive alcohol monitoring using microneedle sensor arrays. Biosens. Bioelectron. **91**, 574–579 (2017). https://doi.org/10.1016/j.bios.2017.01.016
43. Rawson, T.M., Gowers, S.A.N., Freeman, D.M.E., Wilson, R.C., Sharma, S., Gilchrist, M., MacGowan, A., Lovering, A., Bayliss, M., Kyriakides, M., Georgiou, P., Cass, A.E.G., O'Hare, D., Holmes, A.H.: Microneedle biosensors for real-time, minimally invasive drug monitoring of phenoxymethylpenicillin: a first-in-human evaluation in healthy volunteers. Lancet Digital Health **1**, e335–e343 (2019). https://doi.org/10.1016/S2589-7500(19)30131-1
44. Goud, K.Y., Moonla, C., Mishra, R.K., Yu, C., Narayan, R., Litvan, I., Wang, J.: Wearable electrochemical microneedle sensor for continuous monitoring of levodopa: toward Parkinson management. ACS Sens. **4**, 2196–2204 (2019). https://doi.org/10.1021/acssensors.9b01127
45. Yuan, D., Anthis, A.H.C., Ghahraman Afshar, M., Pankratova, N., Cuartero, M., Crespo, G.A., Bakker, E.: All-solid-state potentiometric sensors with a multiwalled carbon nanotube inner transducing layer for anion detection in environmental samples. Anal. Chem. 87, 8640–8645 (2015). https://doi.org/10.1021/acs.analchem.5b01941
46. Parrilla, M., Cuartero, M., Padrell Sánchez, S., Rajabi, M., Roxhed, N., Niklaus, F., Crespo, G.A.: Wearable all-solid-state potentiometric microneedle patch for intradermal potassium detection. Anal. Chem. **91**, 1578–1586 (2019). https://doi.org/10.1021/acs.analchem.8b04877
47. Molinero-Fernández, Á., Casanova, A., Wang, Q., Cuartero, M., Crespo, G.A..: In vivo transdermal multi-ion monitoring with a potentiometric microneedle-based sensor patch. ACS Sens. **8**, 158–166 (2023). https://doi.org/10.1021/acssensors.2c01907
48. Taylor, N.A., Machado-Moreira, C.A.: Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. Extrem. Physiol. Med. **2**, 4 (2013). https://doi.org/10.1186/2046-7648-2-4
49. Baker, L.B., Wolfe, A.S.: Physiological mechanisms determining eccrine sweat composition. Eur. J. Appl. Physiol. **120**, 719–752 (2020). https://doi.org/10.1007/s00421-020-04323-7
50. Baker, L.B.: Physiology of sweat gland function: The roles of sweating and sweat composition in human health. Temperature **6**, 211–259 (2019). https://doi.org/10.1080/23328940.2019.1632145
51. Cui, C.-Y., Schlessinger, D.: Eccrine sweat gland development and sweat secretion. Exp. Dermatol. **24**, 644–650 (2015). https://doi.org/10.1111/exd.12773
52. Min, J., Tu, J., Xu, C., Lukas, H., Shin, S., Yang, Y., Solomon, S.A., Mukasa, D., Gao, W.: Skin-interfaced wearable sweat sensors for precision medicine. Chem. Rev. acs.chemrev.2c00823 (2023). https://doi.org/10.1021/acs.chemrev.2c00823
53. Steckl, A.J., Ray, P.: stress biomarkers in biological fluids and their point-of-use detection. ACS Sens. 32 (2018)
54. Upasham, S., Churcher, N.K.M., Rice, P., Prasad, S.: Sweating out the circadian rhythm: a technical review. ACS Sens. **6**, 659–672 (2021). https://doi.org/10.1021/acssensors.0c02622

55. Sonner, Z., Wilder, E., Heikenfeld, J., Kasting, G., Beyette, F., Swaile, D., Sherman, F., Joyce, J., Hagen, J., Kelley-Loughnane, N., Naik, R.: The microfluidics of the eccrine sweat gland, including biomarker partitioning, transport, and biosensing implications. Biomicrofluidics **9**, 031301 (2015). https://doi.org/10.1063/1.4921039
56. Shirreffs, S.M., Maughan, R.J.: Whole body sweat collection in humans: an improved method with preliminary data on electrolyte content. J. Appl. Physiol. **82**, 336–341 (1997). https://doi.org/10.1152/jappl.1997.82.1.336
57. Baker, L.B., Stofan, J.R., Hamilton, A.A., Horswill, C.A.: Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. J. Appl. Physiol. 107 (2009)
58. van Heyningen, R., Weiner, J.S.: A comparison of arm-bag sweat and body sweat. J. Physiol. **116**, 395–403 (1952)
59. Ladell, W.S.S.: The measurement of chloride losses in the sweat. J. Physiol. **107**, 465–471 (1948)
60. Heikenfeld, J.: Non-invasive analyte access and sensing through eccrine sweat: challenges and outlook circa 2016. Electroanalysis **28**, 1242–1249 (2016). https://doi.org/10.1002/elan.201600018
61. Naitoh, K., Tsuda, T., Nose, K., Kondo, T., Takasu, A., Hirabayashi, T.: New measurement of hydrogen gas and acetone vapor in gases emanating from human skin. Instrum. Sci. Technol. **30**, 267–280 (2002). https://doi.org/10.1081/CI-120013506
62. Yamane, N., Tsuda, T., Nose, K., Yamamoto, A., Ishiguro, H., Kondo, T.: Relationship between skin acetone and blood β-hydroxybutyrate concentrations in diabetes. Clin. Chim. Acta **365**, 325–329 (2006). https://doi.org/10.1016/j.cca.2005.09.016
63. Lee, B., Lim, M., Misra, V.: Wearable skin vapor sensing system for continuous monitoring of various health and lifestyles. In: 2021 IEEE Sensors. pp. 1–4 (2021)
64. Farkas, RpD, Kemny, L., Szll, M., Dobozy, A., Bata-Csrgo, Z.: Ethanol and acetone stimulate the proliferation of HaCaT keratinocytes. Arch. Dermatol. Res. 295, 56–62 (2003). https://doi.org/10.1007/s00403-003-0399-2
65. Lorwongtragool, P., Sowade, E., Watthanawisuth, N., Baumann, R., Kerdcharoen, T.: A novel wearable electronic nose for healthcare based on flexible printed chemical sensor array. Sensors **14**, 19700–19712 (2014). https://doi.org/10.3390/s141019700
66. Arakawa, T., Sato, T., Iitani, K., Toma, K., Mitsubayashi, K.: Fluorometric biosniffer camera "Sniff-Cam" for direct imaging of gaseous ethanol in breath and transdermal vapor. Anal. Chem. **89**, 4495–4501 (2017). https://doi.org/10.1021/acs.analchem.6b04676
67. Iitani, K., Toma, K., Arakawa, T., Mitsubayashi, K.: Transcutaneous blood VOC imaging system (Skin-Gas Cam) with real-time bio-fluorometric device on rounded skin surface. ACS Sens. **5**, 338–345 (2020). https://doi.org/10.1021/acssensors.9b01658
68. Schmidt, F.M., Vaittinen, O., Metsälä, M., Lehto, M., Forsblom, C., Groop, P.-H., Halonen, L.: Ammonia in breath and emitted from skin. J. Breath Res. **7**, 017109 (2013). https://doi.org/10.1088/1752-7155/7/1/017109
69. Cascales, J.P., Li, X., Roussakis, E., Evans, C.L.: A Patient-ready wearable transcutaneous CO_2 sensor. Biosensors **12**, 333 (2022). https://doi.org/10.3390/bios12050333
70. Chatterjee, M., Ge, X., Kostov, Y., Tolosa, L., Rao, G.: A novel approach towards noninvasive monitoring of transcutaneous CO_2. Med. Eng. Phys. **36**, 136–139 (2014). https://doi.org/10.1016/j.medengphy.2013.07.001
71. Ge, X., Adangwa, P., Lim, J.Y., Kostov, Y., Tolosa, L., Pierson, R., Herr, D., Rao, G.: Development and characterization of a point-of care rate-based transcutaneous respiratory status monitor. Med. Eng. Phys. **56**, 36–41 (2018). https://doi.org/10.1016/j.medengphy.2018.03.009
72. Ahuja, P., Ujjain, S.K., Kukobat, R., Urita, K., Moriguchi, I., Furuse, A., Hattori, Y., Fujimoto, K., Rao, G., Ge, X., Wright, T., Kaneko, K.: Air-permeable redox mediated transcutaneous CO2 sensor. Chem. Eng. J. **457**, 141260 (2023). https://doi.org/10.1016/j.cej.2022.141260
73. National Diabetes Statistics Report | Diabetes | CDC, https://www.cdc.gov/diabetes/data/statistics-report/index.html

74. Block, L., MS, RD, CDE: Continuous Glucose Monitoring: CGMS System Gold, https://www.diabetesselfmanagement.com/diabetes-resources/tools-tech/continuous-glucose-monitoring/
75. Bailey, T.S., Alva, S.: Landscape of continuous glucose monitoring (CGM) and integrated CGM: accuracy considerations. Diab. Technol. Therap. 23, S-5-S-11 (2021). https://doi.org/10.1089/dia.2021.0236
76. Continuous Glucose Monitoring—NIDDK, https://www.niddk.nih.gov/health-information/diabetes/overview/managing-diabetes/continuous-glucose-monitoring
77. Upton, T.J., Zavala, E., Methlie, P., Kämpe, O., Tsagarakis, S., Øksnes, M., Bensing, S., Vassiliadi, D.A., Grytaas, M.A., Botusan, I.R., Ueland, G., Berinder, K., Simunkova, K., Balomenaki, M., Margaritopoulos, D., Henne, N., Crossley, R., Russell, G., Husebye, E.S., Lightman, S.L.: High-resolution daily profiles of tissue adrenal steroids by portable automated collection. Sci. Transl. Med. 15, eadg8464 (2023). https://doi.org/10.1126/scitranslmed.adg8464