Wearable Biosensors for Non-invasive Sweat Analysis

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Abstract. Wearable biosensors, especially sweat sensors have drawn significant attention in recent years. Sweat is of importance for its wealth of biomarkers about physiological states. It is therefore utilized for health monitoring, exercise monitoring and so on. Physiological states of individuals can be analyzed through non-invasive wearable sweat biosensors. This paper introduces progress made in sweat sensing technology, including soft materials and microfluidic networks that achieved sweat collection without evaporation. Recent research improved sweat capture of rest individuals and led to robust biosensing assays involving the integration of biosensing arrays and flexible substrates. Novel non-invasive wearable sweat biosensors for the analysis of glucose, alcohol, lactate, and urea were also proposed recently, as well as a sweat sensor capable of analyzing these biomarkers simultaneously. These biosensors all had outstanding sensitivity, selectivity, and stability. While existing wearable sweat sensors have multiple challenges, the future of non-invasive sweat sensing is still promising.

Keywords: Wearable Biosensor, Microfluidics, Biosensing Assay, Sweat Analysis.

1. Introduction

In the last few years, wearable bio-sensors have been widely used as a tool for the detection of infectious agents associated with diseases and conditions. These sensors offer a non-invasive and easily extractable source of biomarkers for continuous health monitoring [1]. They have the potential to revolutionize a wide range of applications, such as health surveillance and disease testing, sports monitoring, metabolic surveillance, and alcohol testing.

Among all human fluids, sweat is the most important since it contains a wealth of information about physiological states, including alcohol, lactate, glucose, and urea. One of the main benefits of sweat-based bio-sensors is that they can give you an overview of health at a molecular level. For example, sweat-based glucose monitoring systems have been developed for real-time glucose level detection, offering a potential solution for the painless treatment of diabetes [2]. Researchers have also developed wearable devices that can measure other analytes such as sodium ion (Na+) and pH, enabling comprehensive health monitoring.

Exercise monitoring is another common application of wearable sweat biosensors. These sensors can monitor multiple analytes simultaneously, providing insights into exercise intensity and performance. The sensors are designed to resist the pressures of daily wear and exercise, providing precise and reliable measurements.

Furthermore, wearable sweat biosensors have the potential to monitor stress levels, which is crucial for managing mental health. It has been suggested that a wearable chemical sensor can be used to detect the level of the stress hormone cortisol in perspiration without invasion [2]. This sensor showed a strong relationship between perspiration and circulation of cortisol, providing a quick and reliable way for stress monitoring.

Sweat-based wearable biosensors offer a promising technology for biomonitoring. There are, however, issues to be tackled, including increasing the availability and quality of perspiration biomarkers, securing sustainable collection of samples, and improving energy delivery systems for wearable devices [3]. Despite these challenges, the field of wearable sweat biosensors holds great potential for future research and development.

This review mainly focuses on non-invasive in-situ sweat-sensing technology. In the following sections, microfluidic networks and the corresponding soft material and structures will be discussed

as the technology supporting non-invasive epidermal sweat-sensing. Also, research works of biosensing, mainly focusing on sweat capture and real-time biosensing assays will be discussed. Finally, this paper will go over the applications of sweat analysis using non-invasive epidermal biosensors.

2. Sweat-sensing Technology

Sweat is easy to collect and can be incorporated into non-invasive biosensing measures. However, biomarker detection and direct sampling can hardly be done by sweat sensors without the evaporation of sweat [4]. Therefore, conventional measures of sweat sampling require absorbent pads to collect sweat and require professional handling after the collection. Thus such measures are confined to the laboratory environment which contradicts the requirement of in situ analysis.

A thin and flexible microfluidic system reported in 2016 can accumulate sweat from the skin surface directly and reliably [5]. The prototype directs sweat to multiple channels and reservoirs, enabling the sensing of multiple parameters. The parameters of interest can be analyzed with the method of quantitative colorimetric analysis [6]. With the combination of similar designs and bioelectronics, wearable sensors that collect and analyze sweat can be fabricated with the ability to transmit and exhibit results.

A microfluidic system typically consists of tiny channels, chambers, and other structures etched or fabricated on a small chip or substrate. In the case of wearable sweat sensors, a flexible epidermal microfluidic network is based on a soft substrate.

2.1. Soft Materials and Structures

Over a decade, soft and wearable sensing substrates have been developed for sweat analysis. In sensor platforms, microfluidic channels are incorporated into the substrates. The earliest approach to sensing sweat was based on textiles, including cotton, nylon, and polyester. These materials only had low stretchable performance, low transparency, and low tensile strength.

Silicon and polymer materials with enhanced stretchability similar to human epidermis have been developed and implemented using screen-printing technology. Polyethylene erephthalate(PET) and polyethylene naphthalate(PEN) and polydimethylsiloxane(PDMS) have high tensile strength, high transparency, and high Young's modulus. An integrated sweat sensor for in situ analysis using PET was developed and gained the benefits of reliable signal processing performance, practical wearability, and mechanical flexibility [7].

As for long-term use, the electronics of the sensor require enhanced tensile strength and stretchability, which can be achieved with innovative structure designs. One innovative 2D design is the Kirigami–inspired design, with the back of paper sliced with symmetrical slits of the fish-scale Kirigami network. The devices based on this design can retain electrical conductivity when stretched and are capable of tolerating stretches up to 2000% [8]. Another design is an open-hole grid construction that greatly increases the deformation and sensitivity of the machine compared to the conventional foam-based design. In addition, the mesh-based transducer also showed fast response rates (< 1ms), suggesting that it can be integrated into machine learning algorithms [9].

Despite the significant improvements in stretchability with the 2D structure design, due to its physical characteristics, 2D structures, if compared with 3D structures, still have notable limitations in elasticity. Researchers reported an interlocked 3D structure integrating two hierarchical layers of mesh structure [10]. The interlocked sensor was constructed using PDMS, allowing for improvements in the deformation and recovery of the micropillar structures. Besides the elastic properties, the intermeshed structure has a quicker response time of under 19 ms and a lower detecting limit of approximately 1.0 Pa as compared with 2D structures.

2.2. Microfluidic Networks

In sweat sensors, the microfluidic network is a thin, soft, "skin-like" platform designed to collect and store sweat sequentially. Microfluidics are micro-fluidics and capillary rupture valves (CBVs) that allow for passive flow of perspiration across the system.

Micro reservoirs in their fluidic network enable the collecting and storing of perspiration. The sweat glands of the skin generate pressure that drives the flow of sweat through the microchannels. The CBVs inside these microchannels are set to be opened at varying pressures, which results in a gradual flow of perspiration across the system. This sequential filling capability enables the analysis of variations of the biomarkers present in sweat.

A typical microfluid network is built with PDMS stacked on a medical-grade acrylic glue membrane [11] (Fig 1a). The upper layer includes microfluidic channels with CBVs that lead to separate chambers. Each chamber is equipped with a vent, which is intended to relieve air and avoid back pressure, which might prevent perspiration from entering the room. The chambers are designed to be filled in order to enable perspiration samples to be collected and analyzed at various times (Fig 1b). A second layer serves as the structural foundation for the device and provides the necessary support for the upper layers. The bonding layer creates an attachment to the skin and provides an aperture that allows perspiration to flow into the microfluidic system, enabling perspiration to be collected from the targeted areas of the skin.

It has been demonstrated that this type of microfluidic network is able to collect perspiration in sequence with no undesirable rupture of CBVs. The flow characteristics of the microchannels guarantee the laminar flow and avoid undesired mixing. The network is also stable under mechanical perturbations, such as stretching, flexing, and twisting (Fig 1c).

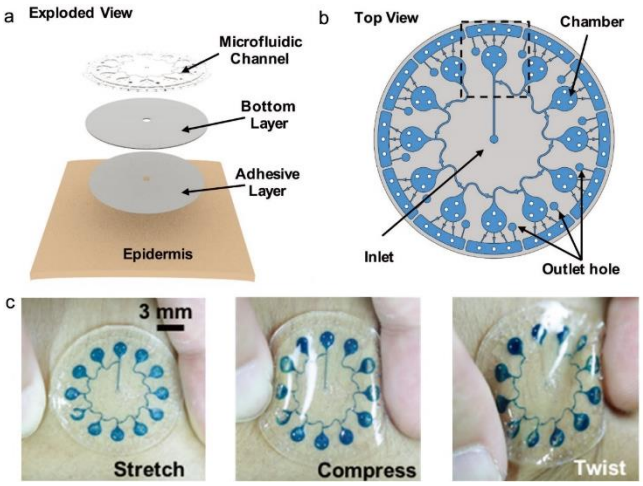


Figure 1. Microfluidic network. a Structure of the microfluidic network. b Top view of the microfluidic layer. c Epidermal microfluidic network withstanding the modes of mechanical deformation and movement [11]

3. Research Works of Sweat Biosensors

3.1. Sweat capture

In addition to the evaporation of sweat, there are other difficulties in sweat collection. Although sweat collection can be easily achieved from the exercises of the individual, it may not be practical to achieve continuous sweat monitoring with this method. And for individuals at rest, sweat production is unpredictable and limited.

Sonner (et al.) suggested integrating the sudomotor axon reflex perspiration stimulus for continuous perspiration analytes in resting subjects [12]. The investigators demonstrated in vivo verification of the effectiveness of the method employing iontophoretic transport of carbachol with ion selectivity and impedance sensors. Through the integration of perspiration modeling and sensing,

they have been able to overcome their past limitations by improving their calibration, precision, and noise reduction.

However, these demonstrations were restricted to about an hour long, inadequate to the requirement of continuous sensing for 24 hours. Long and local perspiration stimulation was obtained by iontophoretic transfer of the cholinergic drug carbachol [13]. The researchers reported in vivo testing results that demonstrated stimulation of carbachol. During the test, sweat glands were stimulated for the generation of sweat, providing more than 24 hours of sweat access. They also demonstrated reduced iontophoretic dosages for sweat stimulation, increasing the viability of repeated dosing.

3.2. Real-time Biosensing Assays

Real-time biosensing assays include reversible biological sensors that allow continuous detection of the target species, with no need for separation, washing, or external agents. Biological sensor arrays and flexible substrates are integrated into wearable bio-sensor devices to detect biomarkers in biological fluid without damage. The latest progress in the field of chemistry biology has enabled us to develop a reliable method for detecting biological analytes over time, which can be used in sportswear.

Recently, a new kind of high-stretch fabric base potential sensor for simultaneous multi-ion perspiration analysis was proposed [14]. Based on the combination of polyurethane ion selective film and ink, the paper has developed a new type of sensor. The PU has enhanced biocompatibility and the ability to withstand mechanical stress, making it fit for wearable devices. The transducer showed a neuroreaction with a linear range of Na and K ions, suggesting high sensitivity and selectivity. The sensor can endure the most intense mechanical loads, including tension, bending, and cleaning, while not affecting its analysis capability.

Hao et al. put forward an aptamer-based graphene nanosensor for continuously detecting cytokine biomarkers [15]. Researchers have created a FET nano-sensor based on FET that can reliably and consistently detect cytokine markers in body fluids like perspiration. The nanosensor was characterized for its suitability in wearable applications, including the effects of substrate bending on the equilibrium dissociation constant and graphene transconductance. This nano-sensor can measure the expression of TNF α (TNF-α), a biomarker of inflammation. It was found that this kind of soft nano-sensor can be used for detecting TNF-α in 5 minutes.

4. Sweat Analysis using Sweat Biosensors

4.1. Alcohol Detection

Recently, a wearable tattoo-based sweat sensor system was proposed for non-invasive alcohol monitoring [16]. The system integrates a temporary tattoo with iontophoresis and amperometric detection to measure ethanol levels in induced sweat. The tattoo platform includes iontophoretic electrodes, an alcohol-oxidase enzyme, and a Prussian Blue electrode transducer. The wearable sensor is coupled with a flexible electronics board that controls the iontophoresis and amperometry operations and transmits data wirelessly via Bluetooth. To the tested blood alcohol concentration (BAC) level, the current responses rise linearly. This system has been studied in people and has shown marked differences between pre-alcoholic and post-alcoholic reactions with a BAC of 0.000%. This system enables non-invasive monitoring of drinking in practice and can be extended to other analyses.

4.2. Lactate Monitoring

A new type of wearable electrochemical sensor for perspiration was presented, which was made up of a kind of conductive wire coated with zinc oxide nanowire. Biosensors built into a wearable headpiece can precisely and wirelessly detect lactic acid and sodium in people's sweat [17]. The Limit of Detection of the biosensor was 3.61mM, and the linearity of the Lactate was 0 to 25mM. Lactic

acid and sodium levels in perspiration are detectable at clinically significant levels, which are reliable and give precise test results.

A new type of sweat sensor was developed to detect lactic acid. This biosensor was a wearable textile electrobiosensor made by an adry-spinning process [18]. The elastic, tensile, and high conductivity of the gold fibers can be made into a latitudinal, a reference, and a counter. A linearity relation was observed in the range 0 to 30 mM. The sensitivity in the range of 25-100% was found to be 7.5% in the low-concentration region (0 - 5 mM) and 5.9% in the high-concentration region (10 to 30mM). The gold fiber-based textile biosensors demonstrated excellent sensitivity and high stretchability compared to other reported lactate biosensors.

4.3. Glucose Monitoring

A non-invasive wearable sweat biosensor for glucose monitoring was proposed for early therapy in diabetics [19]. The biosensors were manufactured with a graphene quantum dot/polyaniline nanocomposite layer. Compared with pure polyaniline, the H2O2 assay showed a higher sensitivity of 68.1s ± 1.11 and 44.06 ± 2.1μA·mM-1·CM-2. The biosensors showed good properties of detecting glucose in man-made perspiration after the immobilization of glucose oxidase. This biosensor can reliably detect the levels of glucose in people's sweat over a long period.

Another flexible electrochemical biosensor was developed for the detection of glucose and lactate in sweat [20]. A new type of sensitive and flexible sensor was developed, which made it possible to enhance the sensitivity of the biosensors. The sensor chip was fabricated using a standard semiconductor laboratory, and enzyme immobilization was carried out using a cross-linker poly(ethylene glycol) diglycidylether. The sensitivity of the transducer was 54μm, and the detection limit was reached at 7 μmol·L-1 for glucose. The biosensor exhibited low detection limits, great selectivity, reproducibility, and stability. The sensor has proved its potential use as a wearable skin sensor for the measurement of glucose and lactic acid.

4.4. Urea Analysis

A portable potential biosensor was fabricated for measuring urea content in perspiration, which can be used to measure urea at various pH levels. [21]. A biosensor comprising a pH sensor, a polyaniline ink, a urease bioink, and a polyvinyl-chloride film can be used for the detection of urea within the 5-200mM range at pH 7.0, which includes the concentration of urea in the human sweat. This kind of biosensor can respond quickly without being disturbed by other materials, and can't be influenced by the bending strain. Furthermore, the possibility of large-scale production has been studied and proved utilizing a roll-to-roll technology.

4.5. Metabolites Analysis

Recent proposals have been made to develop a wearable system with a flexible sensor array for noninvasive and permanent tracking of human metabolites [22]. It consists of signal conditioning, processing, and transmitting components that continuously measure glucose, lactic acid, pH, and temperature. The system runs on three separate electrochemical cells and draws 15mA during continuous operation when supplied with a 3.7v 150 mAh battery. The analog front end of the electrochemistry unit has 4 potentiostats and 3 multiplexers for multiplex and parallel reading from 12 working electrodes. The performance of the system was demonstrated in vitro by measuring glucose, lactic acid, pH, and skin temperature. The sensitive values of the sensors with customized wear were 0.84 ± 0.03 mV·μM-1·cm-2 for glucose, 31.87 ± 9.0 mV·μM-1·cm-2 for lactic acid, and 57.18 ± 1.43 mV·pH-1·cm-2 for pH. The miniaturized non-invasive wearable system gave insights into future sweat biomarkers analysis with excellent performance.

5. Conclusion

Wearable biosensors, especially sweat sensors, have gained significant attraction recently. Biological parameters of interest in health and exercise monitoring can be analyzed through sweat analysis. Sweat is easy to collect yet evaporation can hardly be avoided through conventional methods. To achieve sweat collection without evaporation, microfluidic networks were introduced. A typical microfluidic system is stable under mechanical perturbations and has a microfluidic layer for sweat collection and analysis, a bottom layer allowing for the sequential collection of sweat, and an adhesive layer establishing adhesion to the epidermis.

Despite the evaporation, continuous sweat monitoring of individuals at rest is another problem. Researchers have suggested long and local perspiration stimulation via iontophoretic transport of the cholinergic substance carbachol, which allows for over 24 hours of perspiration, thus breaking past restrictions.

Robust real-time biosensing assays can then be achieved with the integration of biosensing arrays and flexible substrates. Textile-based potentiometric sensors and aptamer-based graphene nanosensors were proposed and exhibited outstanding performance in stability, selectivity, and sensitivity, making them suitable for wearable epidermis sweat analysis.

Various novel sweat biosensors were recently proposed and introduced in this review, including a wearable tattoo-based sweat sensor for alcohol detection, stretchable electrochemical sweat biosensors for lactate monitoring, non-invasive flexible biosensors for glucose monitoring, and a potentiometric biosensor monitoring urea in sweat. Furthermore, a noninvasive wearable system with a flexible sensor array has been developed that can analyze the metabolism of human blood, such as glucose, lactic acid, PH, and temperature. The biosensors mentioned above all exhibited outstanding sensitivity and stability in the test using human sweat.

However, there are still challenges yet to be addressed in sweat biosensing technology. The availability and quality of biomarkers in sweat still need to be improved, as well as sustained sample sources and power supply methods. Despite the existing challenges, the prospect of non-invasive wearable biosensors for sweat analysis is promising and holds great potential for future development and research.

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