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Recent Progress on Non-invasive Wearable Epidermal Biosensors

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Abstract. Wearable biosensors have the potential to provide valuable information about our physiological states and transform traditional healthcare. Compared to traditional blood sampling, on-body analysis of non-invasive biofluids can offer continuous and painless monitoring of relevant biomarkers. Recent developments in epidermal sensors feature integrated systems capable of sensing multiple factors while providing easy readout and great skin conformity. Innovative solutions based on advanced material fabrication and novel designs have also emerged to address challenges such as power, sensor sensitivity and selectivity, and communication. As a result, more possibilities have emerged to develop sophisticated integrations with more functionalities, optimized skin conformity, and less disruption of daily routine. While sensing performance and functions continue to improve, attention should also be drawn to practical problems such as biofouling, contamination, and complex composition dynamics. Moreover, although past and current research have highlighted studies investigating the use of sweat in diagnostics, more evidence of correlations between sweat biomarker levels and physiological conditions is needed to promote the utility of these systems.

Keywords: Wearable biosensors, health monitoring, self-powering systems

1. Introduction

Wearable biosensors are portable devices that integrate on-body sensing and readout for health monitoring[1]. They serve as a competitive non-invasive alternative to traditional invasive blood tests, with minimal side effects and the promise of continuous user-friendly monitoring that does not conflict with daily activities[2]. Early advances of such devices were proof-of-concept demonstrations that combined conventional biosensing techniques with flexible substrates[3][4]. During the past decade, major advances in flexible material fabrication and sensing approaches were made, stimulating rapid growth of research that aimed for integrated on-body application[5]. Currently, research has mostly been focusing on exploration and novel sensing methods of new detectable analytes that correlates with physiological activities.

Among the available non-invasive biofluids (tears, saliva, and urine), sweat exhibits several advantages such as a small travel distance from secretion, more available biomarkers that relate to their blood level, and minimal discomfort due to sampling and analysis[6]. To extract reliable information from sweat in-vivo, epidermal swear sensors must acquire proper samples with close

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resemblance to real-time sweat compositions and have high sensitivity and selectivity to accommodate a complex range of chemicals that is present in sweat in minimal quantities. In addition, flexibility, portability, continuous operation, and easy readout should also be considered as key factors in promoting commercialization, with the goal of designing devices that minimally disrupts daily routine and attention. To address these challenges, numerous efforts have been made on developing innovative electrode fabrication and functionalization, integrated with miniaturized self-powering strategies, low-power communication, and microfluidic sampling platform[7][8][9].

The purpose of the review is to provide a systematic view of recent developments in sweat-based wearable biosensors and the principles and motives behind them. Major self-powering strategies are first highlighted and explained. Then, common biomarkers targeted for sensing and health monitoring are introduced, with illustrations of relevant sensing techniques. Subsequently, readout approaches that are utilized in different scenarios are demonstrated. Finally, current challenges and future developments are evaluated.

2. Self-powering strategies

The promise of continuous operation of wearable biosensors lies in the foundation of efficient energy harvesting techniques and miniaturized energy storage devices with optimal flexibility. Currently, most flexible batteries have energy storage capacities lower than 5 mWh cm^{-2} , while microcontrollers in wearable biosensors demand 1-100 mW of power[10]. As an alternative, self-powering devices consisting of flexible materials have been developed as promising energy sources.

2.1. Biofuel cell

Biofuel cells (BFC) utilize molecules that are extensively present in body fluids to generate power through enzyme-based electrochemical redox reactions[11]. A typical BFC adopts cathodic oxygen reduction and anodic enzyme-catalyzed oxidation. The power produced is proportional to the concentration of the target molecule and thus BFC is capable of powering the circuitry and sensors or serve as a direct measurement probe of the target specie.

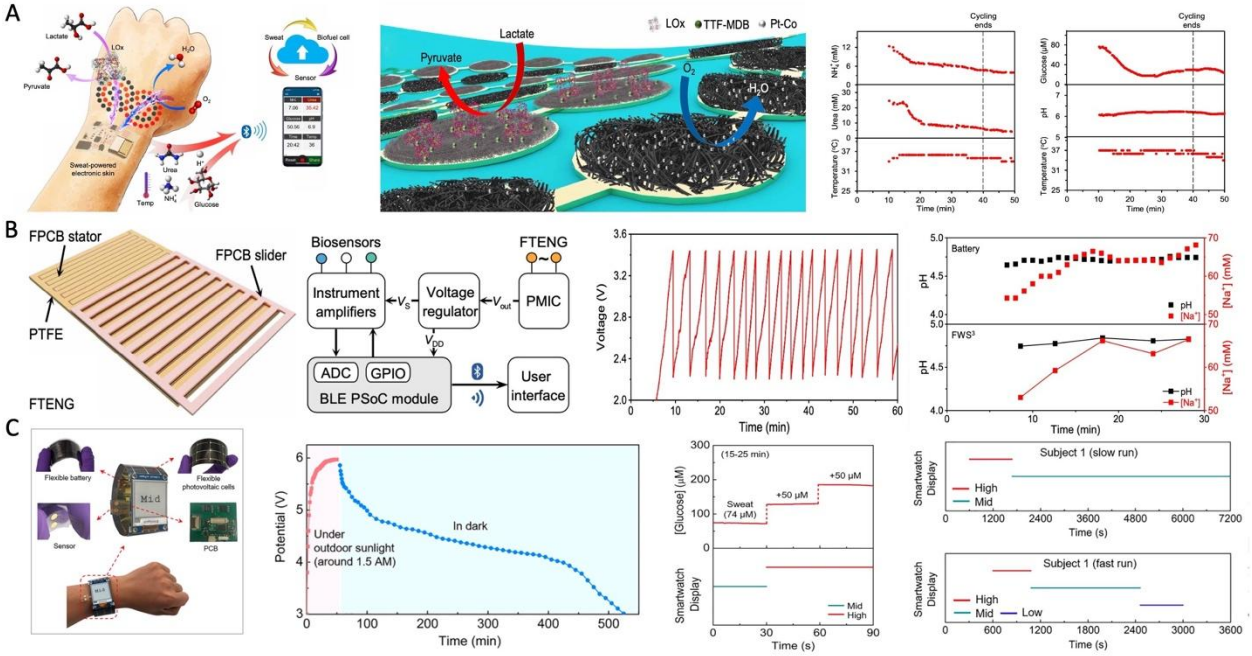

Figure 1. Common self-powering strategies (A) Lactate BFC-based sweat metabolite sensor for continuous monitoring [12]. (B) Triboelectric nanogenerator-powered sweat sensor integrated with wireless communication [13]. (C) Sweat glucose sensor powered by solar cells and integrated with ink display [14].

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A novel fully perspiration-power electronic skin based on BFCs has been reported that supports measurement of several important metabolites such as urea, ammonium ion and glucose, and other physical parameters including temperature and strain while allowing distant communication via Bluetooth (Figure.1A)[12]. The lactate biofuel cell anode consisted of Ni, reduced graphene oxide films, and modified carbon nanotubes building on Au electrode array and was capable of oxidizing lactate to pyruvate. The oxygen reduction cathode was constructed from modified carbon nanotube networks functionalized by Pt-based nanoparticles at the surface. Integration of such 0D-3D nanomaterials on BFC electrodes provided tremendously large electrochemically active surface area, yielding high power density ($3.5\ \mathrm{mW\ cm^{-2}}$) in real sweat samples, and with optimized energy management could support additional sensing and communication functionalities. The developed e-skin could also see potential in prosthesis control due to enabled real-time strain response, which could be used for rehabilitation or assistive walking.

Material selection of biofuel cells is key to improving power output but is often limited due to requirements of skin conformity and flexibility. Under such limitations, optimization of the approach to surface modification such as enzyme immobilization is essential to enhance electron conduction and prolong electrode stability[11]. The evolution of wearable BFC is still at an early stage and other challenges such as non-specific biofouling and mediator leaching also need to be addressed.

2.2. TENG

Triboelectric nanogenerators (TENGs) utilize contact electrification and electrostatic induction to generate current pulses[15]. When two materials are in contact and then separated, charges are transferred based on affinity to the electrons, causing potential differences that induce currents. TENG is an energy harvesting technique independent of outside input and is often cost-effective and flexible due to the wide range of choice of materials. Currently, four different modes are implemented in TENG, including single electrode, lateral sliding, freestanding triboelectric-layer mode, and vertical contact separation mode, with differences in stability, power output, and fabrication process.

One example of mechanical energy harvesting in wearable sensors utilized TENG fabricated by flexible printed circuit board (FPCB) technology to sustain sensing and communication (Figure 1B)[13]. The device consisted of copper and polytetrafluorethylene triboelectric materials, which were patterned into gratings through photolithography. The structure generated pulses of current during movement-induced sliding motion due to the triboelectric effect, which was then stored in a capacitor for a timely release. A power density of $416\ \mathrm{mW\ m^{-2}}$ was accomplished, which is much smaller compared to biofuel cells and thus might require large patches embedded in textiles. Through efficient power management such as shutdown modes, FPCB-enabled TENG reported could sustain low-power biosensors and Bluetooth-based communication during vigorous exercise and obtain 5 recordings in a 30-minutes exercise. Stable response and good accuracy were produced during in-situ experiments, with insignificant noise due to intensive mechanical deformations.

In addition to skin-interfaced TENG, the device can also be integrated into textiles using embroidery, which offers greater skin conformity and better user experience but inferior electrical performances[15]. TENG still faces challenges of size and output stability optimization and low power density.

2.3. Photovoltaic cells

Solar power, as one of the most abundant energy sources on earth, can be harvested by solar cells in an efficient and portable way[16]. The existing technology for solar power harvesting is relatively mature and illustrates high energy conversion efficiency. However, the power density of solar cells largely depends on the illuminance level of the environment and thus might require an additional storage device to compensate for light variation and unstable power density[7].

Figure 1C introduced a integration of solar power harvesting and storage, glucose sensing, and wearable watch with simple display[14]. Flexible amorphous silicon-based photovoltaic cells and Zn-MnO2 batteries were used for energy harvesting and storage, integrated with an enzyme-based glucose

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sensor and electronic ink display. The solar cells with an area of 28.44 cm^2 and 3.2% power conversion efficiency allowed charging up to 6V in 1 hour under outdoor sunlight, which could maintain the system for 8 hours. The device was able to operate in situ and display glucose levels continuously in three states, with a requirement of 10-minute warm-up time for adequate sampling of sweat.

The application of solar cells in wearable technologies faces different challenges than conventional uses. The flexibility and durability of the materials selected should be carefully evaluated to improve user experience and stability under various working environment[16]. Temperature is also an important factor that can greatly affect performance and should be managed properly in wearable systems.

3. Biomarker sensing

Sweat is a very complicated mixture containing numerous chemicals including amino acids, electrolytes, wastes, proteins, and even toxicants[17]. Sweat is secreted by sweat glands distributed over the epidermis and contains extensive information due to its correlation with serum and physiological processes. Therefore, real-time analysis of sweat can provide continuous monitoring of changes in the body as a result of diet, disease, and deficiencies, in connection to various healthcare applications. Compared with conventional invasive approach for continuous monitoring such as micro-needles, sweat sampling and sensing enables non-invasive and painless detection which promotes user experience and a range of applications[2]. Such continuous on-body measurement technique faces more challenges than lab-based biosensors and activity-tracking devices and numerous efforts have been made to offer miniaturized and flexible solutions while improving sensitivity and stability. Sensors commonly implemented for biochemical include enzymatic and ion-selective sensors based on potentiometry, voltammetric sensors based on pulsing techniques, and bioaffinity sensors measuring target-bioreceptor interactions[18].

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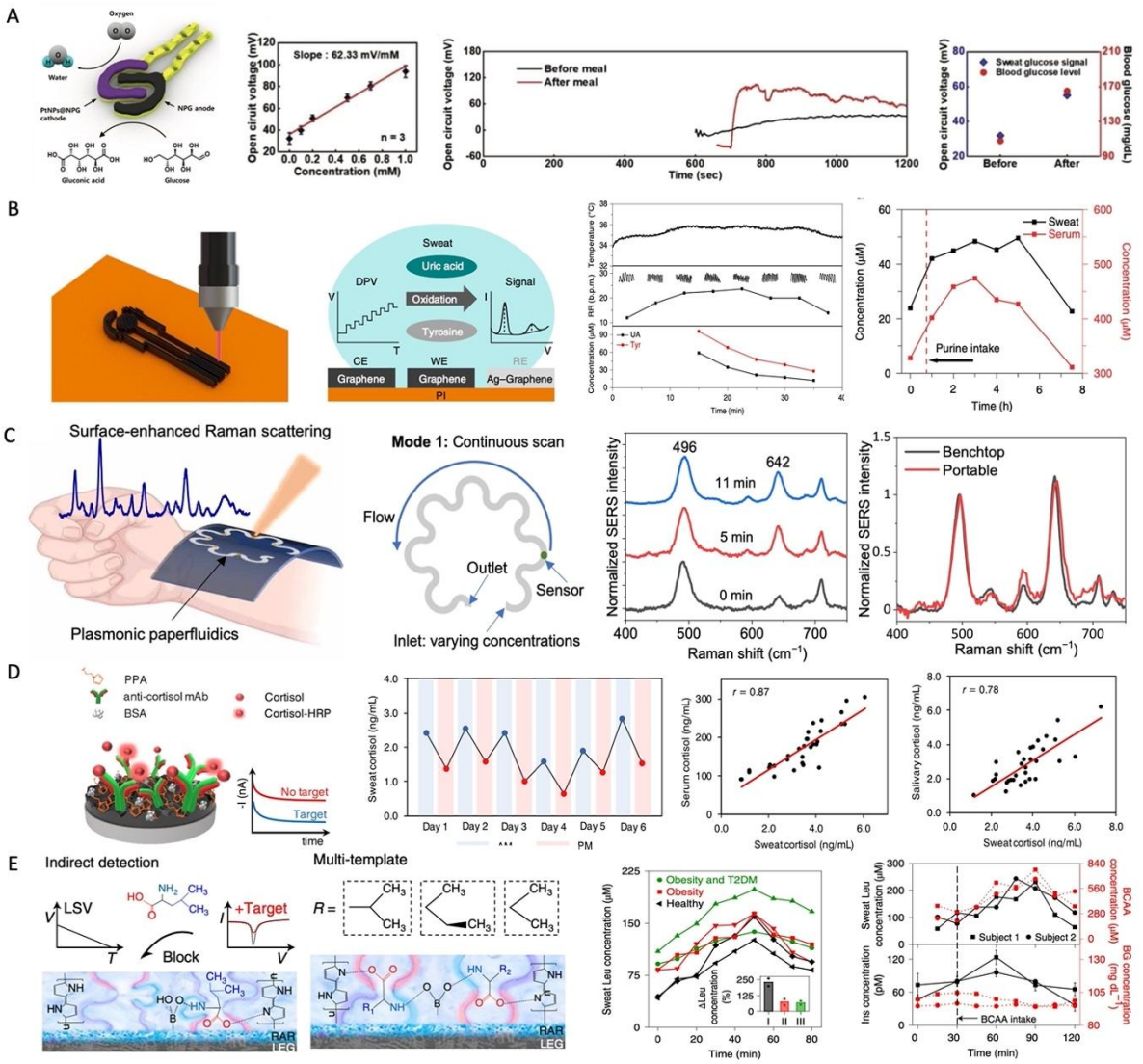

Figure 2. Sensing techniques for biomarkers in sweat (A) Self-powered glucose sensor patch consisting of nanoporous gold electrodes[19] (B) Laser-engraved graphene-based sensor for uric acid and Try detection[20]. (C) Portable Raman spectroscopy for on-body measurement of sweat uric acid[21]. (D) Bioaffinity sensor for monitoring sweat cortisol levels and rhythms[22]. (E) Nutrient detection using molecularly imprinted polymer-based antibodies and electrochemical assay[23].

3.1. Metabolites

Metabolites such as glucose, lactate, and uric acid are present in sweat in various quantities, mostly coming from blood [24]. The levels of these metabolites in blood are great indicators of health status and have already been practically used in many scenarios, such as monitoring of diabetes and gout, and design of exercise programs for athletes. Although detailed mechanisms still need further studies to elucidate, correlations have been found between glucose levels in sweat and blood, indicating a potential alternative to invasive blood glucose monitoring[25]. Meanwhile, additional studies are required to establish clear relations between sweat and blood levels of other metabolites. Sensing of glucose and lactate can be achieved by self-powered BFC, while other common metabolites can be monitored using enzymatic and bioaffinity-based sensors.

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3.1.1.Glucose. Glucose levels can be readily obtained using glucose oxidase-based BFCs and such glucose sensors are often integrated with a variety of sensors as power sources or to achieve multiplexed sensing[8][12]. These sensors are both sensitive and selective but suffer from performance degradation due to biofouling and immobilization issues[11]. As an alternative, non-enzymatic fuel cell-based sensors were reported for self-powered glucose monitoring (Figure.2A)[19]. To achieve stretchability and high electrochemical activity in electrodes, Nanoporous gold (NPG) was introduced for anodic glucose oxidation and NPG was coated with Pt nanoparticles for cathodic oxygen reduction. In-vitro detection of glucose concentration through open circuit voltage measurement showed a sensitivity of 62.33 "mV " 〖"mM" 〗^(-1), with minimal performance degradation after repeated stretching. An on-body evaluation was performed after the addition of a cotton-based microfluidic component and illustrated a good correlation between sensor voltage and blood glucose concentration as well as a significant change in glucose level after food intake. Although the sensitivities obtained were several times lower than those in enzyme-based glucose sensors, such sensors might hold promise as a solution to performance instability.

3.1.2.Uric acid. Blood uric acid level has long been identified as a risk factor for conditions such as gout and cardiovascular diseases[26][27]. However, the association between uric acid in sweat and diseases has not been established and thus requires further research[17]. Figure 2B illustrated a multiplexed sensing patch prepared by laser-engraved-graphene (LEG) sensors and a microfluidic module for highly responsive detection of uric acid (UA) and tyrosine (Tyr) in sweat[20]. By measuring electrochemical oxidation response using differential pulse voltammetry, UA and Tyr could be detected at sensitivities of 3.50 "μA " 〖"μM" 〗^(-1) 〖"cm" 〗^(-2)and 0.61 〖"μA μM" 〗^(-1) 〖"cm" 〗^(-2), with low detection limits of 0.74 "μM" and 3.6 "μM" . Monitoring of vital signs was also implemented by utilizing a LEG strain sensor that responded to slight mechanical deformation from respiration and heartbeat. Further experiments identified higher UA levels in subjects with gout or hyperuricemia and that UA and Tyr levels fluctuated with diet in both serum and sweat, suggesting the potential use of the wearable biosensor in risk factors monitoring and disease management.

A novel approach that combined a paper-based microfluidic system and ramen spectroscopy was implemented to measure concentrations of uric acid in sweat without the use of electrochemical reactions (Figure 2C)[21]. The wearable device was composed of chromatography paper channels, integrated plasmonic nanosensors, and a PDMS encapsulation layer that enabled measurement of sweat rate, sweat loss, and uric acid concentration as low as 1μM through surface-enhanced Ramen spectroscopy (SERS). Compared with traditional microfluidics, the paper-based microfluidic device does not rely on pressure from sweat glands but rather capillary forces in porous papers, which allows well-defined active transport and is stretchable and disposable. Utilization of SERS eliminated the need for real-time calibration due to mechanical strain and temperature and provided a much longer device lifetime compared with enzyme-based sensors. However, such method requires a portable Ramen spectrometer which is currently bulky and not well-developed for such purposes.

3.1.3.Cortisol. Figure 2D presented a pilot study that constructed a wireless sweat-based cortisol sensing patch while demonstrating the viability of using sweat to monitor blood cortisol levels. The sensing component was constructed on a polyimide substrate using laser-engraved graphene as the transducer. The surface was then modified by polymerization of PPA, subsequent activation by EDC and Sulfo-NHS, and functionalized by anti-cortisol monoclonal antibody. Amperometry response was measured when cortisol and HRP-labeled cortisol competed for binding in the presence of cathodic hydrogen peroxide reduction. An assay time under 1 minute was obtained and sensor accuracy was confirmed by results from enzyme-linked immunosorbent assay with correlation factor $r = 0.973$. Additional experiments demonstrated successful monitoring of the cortisol variation from morning to evening and as a response to stress with human sweat samples, which proved the potential of non-invasive sweat cortisol monitoring with wearable biosensing devices.

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3.2. Amino acids

Trace amounts of amino acids can appear in sweat as combined results of filtration from plasma, selective reabsorption, and diffusion from the skin[24]. The relation between plasma and sweat might indicate the use of amino acids in sweat as a potential biomarker. Detection of amino acids can be achieved using non-specific voltammetric sensors to measure oxidation response[20].

Figure 2E demonstrated a specific voltammetric sensor capable of detecting trace amounts of multiple species continuously in situ for monitoring nutrition, metabolic syndrome risk factors, and COVID-19 infection[23]. To produce effective binding sites, target molecules were first bound with functional monomers and crosslinkers, which were then polymerized on laser-engraved graphene (LEG), forming molecularly imprinted polymers (MIP) embedding the targets. Active sites complementary to the target molecules were exposed after the targets were extracted. Electroactive molecules could be detected by applying differential pulse voltammetry (DPV) to selectively oxidize targets at specific potentials. On the other hand, non-electroactive metabolites could be measured by introducing a third redox-active nanoreporters (RAR) layer between MIP and LEG that competed with target molecules on binding sites and by subsequent DPV. The former direct approach thus produced peak current height proportional to the logarithm of analyte concentration in DPV voltammograms while in the latter indirect approach peak current decreased with increased concentration. By reconfiguring the electrode material, the LEG-MIP approach enabled the sensitive detection of various biomolecules including essential amino acids, vitamins, hormones, and drugs, while minimizing disturbances in data due to undesired species. Sensitivities of 0.63 $\mu A\,\mu M^{-1}cm^{-2}$ and 0.71 $\mu A\,\mu M^{-1}cm^{-2}$ were achieved by Tyr and Trp sensors. The total concentration of multiple nutrients such as BCAA could also be measured by employing multi-template MIP in a single sensor. The sensors were reusable as bound targets could be unbound by applying constant potential. Integration of the sensing array with iontophoresis-based sweat induction, microfluidic collection chamber, signal processing, and wireless transmission provided reliable continuous monitoring of the relevant biomarkers with easily accessible readout through mobile devices. Another advantage of the developed device was the calibration of sensor response in real-time based on temperature and ion sensor readings which improved stability and accuracy in different environments. Furthermore, the study identified observable differences in sweat leu and BCAA levels between healthy and obese/T2DM individuals, and differences in sweat leu levels of COVID-19 positive and negative individuals, which indicated the potential use of the wearable sensor for management and monitoring of metabolic syndrome risk and COVID-19. Reconfiguration of such devices enables the detection of a variety of biomarkers and potentially offers personalized prevention and diagnostics.

4. Low-power readout

As a final communication step to the user, a simple and explicit readout is essential to the successful commercialization of wearable biosensors[4]. However, efficient readout circuits usually require high power output, which could not be afforded in miniaturized and flexible devices. As a result, most integrated devices utilize either a colorimetric display or low-power passive or active wireless electromagnetic communication via smartphones. Depending on the application, the biosensing module can also be integrated into wearable devices such as a smartwatch, eliminating the need to include a separate readout component. With proper power management designs, advanced signal and data processing techniques could be implemented for calibration and filtration of noise.

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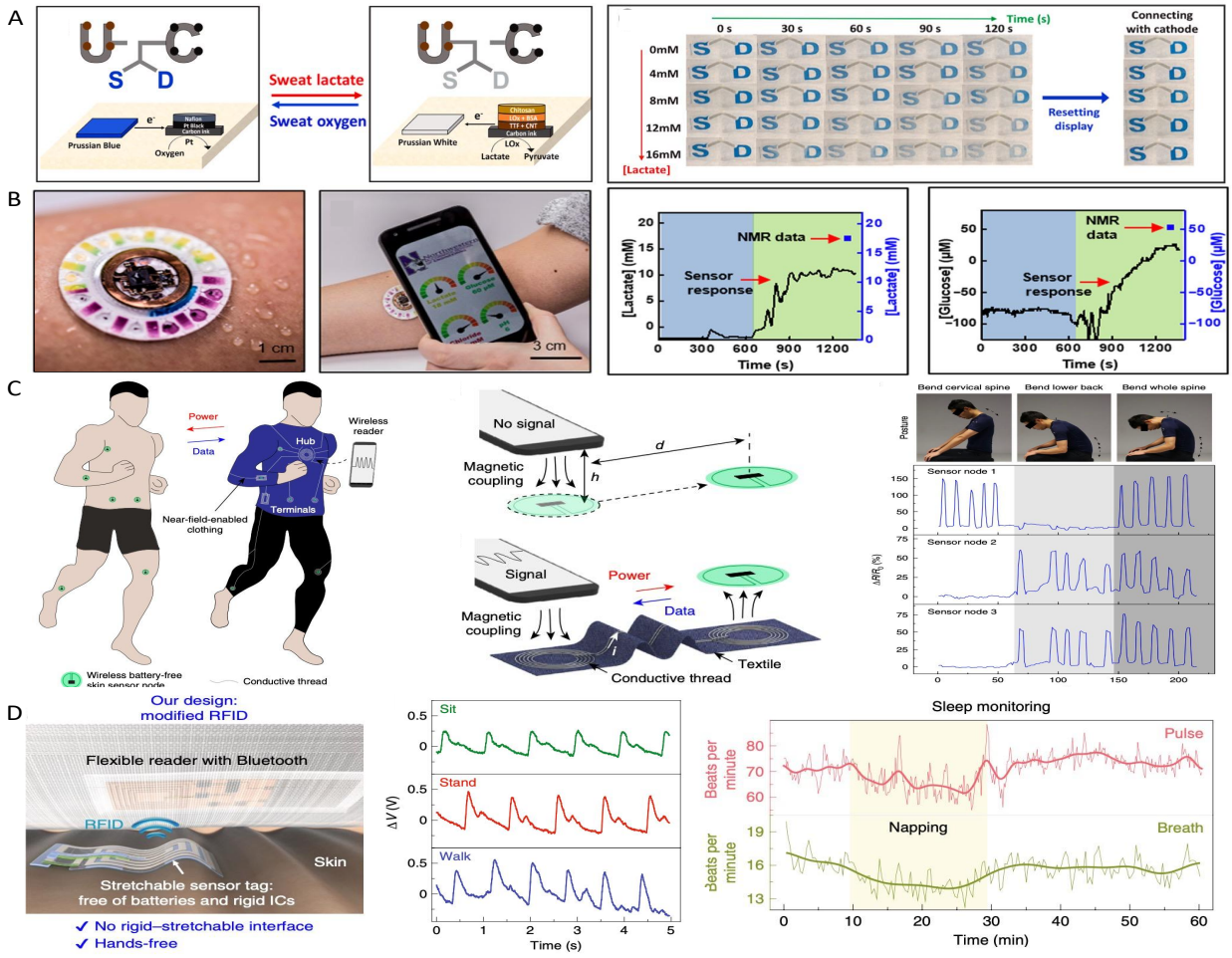

Figure 3. Readout approaches for wearable sensor (A) Reversible lactate-powered sensor with colorimetric display[28]. (B) Integrated sensing system for colorimetric and electrochemical detection of sweat metabolites with near-field communication (NFC)[8]. (C) body network of battery-free sensors with NFC[29]. (D) body sensor network with stretchable sensor tags and textile-embedded reader[30].

4.1. Low-Power bluetooth

Low-Power Bluetooth (BLE) has emerged in the past decade as a low-power solution to close-range communication and control[31]. The energy consumption of BLE depends on various factors such as distance, latency requirement, and duration. Utilization of such technology in integrated self-powering biosensors have already been demonstrated[12][13]. Even in low power generation techniques such as TENG, with the introduction of efficient power management approaches, BLE functionalities could be normally supported and consumed only about 330 μA of current with 510 ms operation time each cycle. And in lactate-based BFC systems, with the addition of 5 to 20 mM lactate solution, BLE can be continuously powered along with sensing and processing functionalities.

4.2. Colorimetric

Colorimetric assays based on chemical reactions or target-receptor binding are commonly used in both real-life and lab settings as an affordable and rapid detection technique. Wearable biosensors integrated with both electrochemical and colorimetric assays were also reported[8]. However, such approaches are irreversible and are only for single use. In **Figure 3A**, a sweat-based fully printed sensor was demonstrated that paired lactate oxidation with Prussian Blue (PB) reduction reaction,

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forming a battery-free biofuel cell system that served as both sensor and reversible colorimetric display[28]. Lactate concentration could thus be quantified by the rate of the color change of PB from blue to white, with a limit of detection of 3.7 mM in vitro using RGB image analysis, which was below the range of lactate concentration in sweat (4-80mM). The process was reversible by introducing an additional oxygen reduction electrode to reset the PB display by switching connections. On-body experiments showed slightly decreased performance but proved the potential for a practical lactate monitoring device during exercise, owing to the simplicity of read-out and mass production through solution-processed screen printing.

4.3. NFC

Another approach to address signal transmission and readout in self-powered devices is the coupling of biofuel cell-based sensors with near-field communication technology (NFC), which requires virtually no power input on the sensor side. An additional NFC-enabled electronic device can establish close-range wireless communication with the NFC module that transmits signals from the biosensor. As shown in Figure 3B, biofuel cell-based lactate and glucose sensors were paired with an NFC module that allowed passive communication of sensor responses and was integrated with chloride and pH colorimetric assays[8]. A Zero-crossover operational amplifier was implemented NFC to increase ed tolerance against supply voltage disturbance due to variations in BFC sensors. The sensor response could be readily recorded using a configured reader for up to 38 mm. Additionally, a separate microfluidic component was connected to the electronics through a releasable interface, which enabled reliable sweat sampling with minimal fouling, simultaneous sweat rate measurement, and replacement. The resulting sensor was cheaper, lighter, and smaller with minimal compromise in performance, data reliability, and skin conformity.

Figure 3C features a design of a battery-free temperature and strain sensor network based on interconnected near-field communication embedded in clothing[29]. Electromagnetic responsive patterns integrated into clothing through computer-controlled embroidery could extend effective NFC distance from a few centimeters to near meter-scale of sensor networks distributed among the body surface. Therefore, only one central hub area was needed to access all sensor information using smart devices. The near field enabling clothing was able to monitor body gestures and temperatures without a power source and was compatible with daily wear and washing, allowing application in physical therapy and assisted living.

4.4. Body network

Besides performance and accuracy, successful commercialization is largely dependent on user experience and reliability. Although stretchable sensors with good skin conformity could be achieved, the presence of high-performance rigid silicon components for power, signal processing, and communication is still incompatible with soft human skin, frequent movement, and strain. Currently, no other materials could replace silicon in highly demanding tasks with minimized size. An alternative approach was reported where stretchable sensors integrated with passive tags communicated wirelessly with signal processing and conditioning circuits attached to textiles, and a third smart device was used to establish a connection with the circuits through Bluetooth (Figure 3D)[30]. Compared to traditional NFCs and Bluetooth, the physical separation of sensor and signal processing circuit provided improved wear comfort while allowing communication with multiple sensors simultaneously without frequent user actions such as tapping smartphones. The tag and sensor were prepared with intrinsically stretchable materials and designed by adapting passive radiofrequency identification (RFID) technology, where several properties such as inductance, capacitance, and internal resistance were tuned to enhance strain-induced disturbances. The sensors were able to pick up small mechanical signals and demonstrated high durability against cyclic uniaxial strain and maintained effectiveness at >50% strain. By attaching a network of sensors to the human body, respiration, pulse, and body movements could be monitored continuously and accurately.

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5. Conclusion

To widely implement non-invasive sensing techniques, strong correlations must be established between the biomarkers in non-invasive biofluids and relevant physiological activities and disruptions. Currently, the mechanisms involved in the transport and production of these molecules and their correlations to various activities are still poorly understood. Expanding detectable species and improving device performance have been the major focus while the validity of the underlying basis is still unknown. Many investigations were dedicated to proving the relationship between sweat composition and various factors including disease, nutritional deficiencies, and diet, but flaws could often be identified as a result of undetected confounding factors, insufficient data size, and improper measurement and sampling. The sweat sampling process is highly subjective to various contaminations and errors and is largely affected by the collection and storage technique, indicating that there might be a huge gap between the final analyzed sample and the fluid of interest and between in-vitro and on-body sensing. Additionally, large variations of sweat biomarker concentrations and conflicting trends are usually found among different individuals in different studies, and the reasons behind this are unclear. These challenges impose great difficulties in analyzing relationships and mechanisms. To address them, it might be possible to employ developed wearable biosensor prototypes integrated with efficient microfluidic modules in relational studies to continuously acquire reliable data in large quantities, which cannot be achieved previously. Commercialization and investigation of correlations might be intertwined processes toward an upward spiral, where the promotion of wide use expands the available database, and with the aid of complex data processing techniques, it might provide a more sophisticated understanding of the underlying relations, further broadening the applications.

Another important issue is performance degradation due to biofouling, bioreceptor instability, and dynamic environments. During on-body applications, sensors are constantly subjected to dynamic movements and complex biofluid compositions, which can affect sensing response and long-term performance. Therefore, attention should also be focused on the vulnerability of biosensors to various fluctuations in conditions. To address this, further integration of sophisticated calibration and optimization of functionalization techniques are required. Another important functionality is the ability to cleanse the sensor surface and clear bound species to cope with biofouling and increase reusability. Current technologies still struggle to maintain such functionalities and advanced material fabrication techniques and innovative structural design are needed. It is also possible to design disposable sensor components, at the expense of increased cost.

Overall, the design of wearable sensor devices must take considerations from complex real-world scenarios to accommodate a wider range of applications. Integrated devices with balance and compromise among each aspect of the sensor are highly favorable for the promotion of personalized healthcare. Extensive practical studies should be conducted before advantages can be critically reviewed.

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