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Nanostructured wearable electrochemical and biosensor towards healthcare management: a review

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In recent years, there has been a rapid increase in demand for wearable sensors, particularly these tracking the surroundings, fitness, and health of people. Thus, selective detection in human body fluid is a demand for a smart lifestyle by quick monitoring of electrolytes, drugs, toxins, metabolites and biomolecules, proteins, and the immune system. In this review, these parameters along with the main features of the latest and mostly cited research work on nanostructured wearable electrochemical and biosensors are surveyed. This study aims to help researchers and engineers choose the most suitable selective and sensitive sensor. Wearable sensors have broad and effective sensing platforms, such as contact lenses, Google Glass, skin-patch, mouth gourds, smartwatches, underwear, wristbands, and others. For increasing sensor reliability, additional advancements in electrochemical and biosensor precision, stability in uncontrolled environments, and reproducible sample conveyance are necessary. In addition, the optimistic future of wearable electrochemical sensors in fields, such as remote and customized healthcare and well-being is discussed. Overall, wearable electrochemical and biosensing technologies hold great promise for improving personal healthcare and monitoring performance with the potential to have a significant impact on daily lives. These technologies enable real-time body sensing and the communication of comprehensive physiological information.

1. Introduction

A wearable electrochemical biosensor (WEB) is an effective point-of-care (POC) diagnostic tool with excellent electrical signal response in normal physiological conditions. A wide range of platforms containing the properties of flexible, convenient, and light weighted materials that are sensitive to detect the target biomolecules have been reported for WEB applications.[1,2] WEB as a POC diagnostic tool has a great demand for its comfort physiological signal sensing of electrolytes, drugs, toxins, metabolites and biomolecules, protein, and immune assay.[3,4] In addition, wearable biosensor platforms can be easily attached to the body by using daily wearable items, such as glasses, clothes, shoes, gloves, and watches.[5–8] Most wearable sensors can be easily controlled using bluetooth-driving smartphones or remote servers.[9–16] Examples of the major wearable platforms are Google Glass, contact lenses, polyethylene terephthalate (PET) contact lenses, mouthguards, cotton underwear, cotton yarn, elastomeric stamps, and temporary tattoos.[17,18] Currently, researchers are actively working on developing wearable biosensors to measure significant small molecules and biomarkers present in common body fluids.[19] Up to date, skin, tears, and sweat-based electrochemical sensors and biosensors have been developed to monitor a few electrolytes and biomolecules with a selective and sensitive detection output.

Bio-receptors are utilized to modify the wearable sensing platforms depending on the physicochemical properties of the targeted analytes. In electrochemical analysis, the current is flown through the sensing platform by signaling for the oxidation or reduction of electroactive bio-nutrients. The modified working electrode is the key component, which is preferably prepared from poly(vinyl alcohol), silicones (*e.g.*, PDMS, Ecoflex, and Solaris), or inert plastics (*e.g.*, PET and PEN)[20] to create a conductive base and then was decorated by using recognition agent to ensure better chemical responses. As an alternative to the flexible base, the metal-decorated conductive base becomes stretchable and bendable.[21] To avoid this barrier, nanoparticles and graphene-processed conductive ink or tunable conductive polymers can be easily printed on a flexible base using conventional printing and binding methods.[22–24] In the potentiometric and impedimetric sensors, no current is flown and

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further, the electrode is usually functionalized with commercial ionophores. Conversely, an enzymatic redox reaction is required to modify the working electrode, which is relatively complex and time-consuming. Besides, enzymes have high reduction potential and hierarchical structure, which is responsible for lower electron transfer from the enzyme pocket to the electrode. A mediator is required to reduce the working potential and increase electron mobility, such as Prussian blue (PB) and tetrathiafulvalene (TTF).[25–27] Due to the toxicity of the mediator, alternatives, such as carbon-based materials, metal nanoparticles, and conductive polymers have been explored to mediate electron transfer in amperometric systems.[28,29]

Although enzymatic-recognized biosensors have a few limitations, they have wide applications in sensing metabolites in human sweat. In immune sensor studies, the bio-recognition elements have a strong affinity towards target analytes, but these bio receptors are not suitable for continuous monitoring in the human body for the lack of their irreversible nature. It is worth mentioning that, the surrounding is the reason for degradation and becoming unable to measure the target analyte.[17] Self-life enzyme-modified electrodes were prepared by selected encapsulation technique and polymer-enzyme composites.[30] Additionally, in wearable biosensors, molecularly imprinted polymers (MIPs) are intriguing replacements for enzymatic recognition elements because they are suitable ligand environments for specific analytes.[31] Nanoparticles and organic frameworks have also been proposed as artificial receptors.[32] In textiles and garments, modified wearable fibers can be used with a three-electrodes system to continuously measure electrochemical signals and avoid intermediate interactions.[33] Furthermore, Wang *et al.* created a fiber-based working electrode by electrodepositing active materials onto carbon fibers and integrating these fibers into a sensing array.[34] In another work, a thin gold fiber was prepared by dry-spinning gold nanowires for glucose sensing.[35] Recently, fiber-based working electrodes and interconnected sensing systems have been reported through versatile research work on carbon fibers,[36] stainless steel yarns,[37] metallic fibers,[38] and silver-coated nylon threads.[39]

This review surveys the modification of wearable electrochemical sensors and biosensors to detect ions, drugs, toxins, metabolite and biomolecules, proteins, and immune assay. The discussion about the previously reported research work along with the working principles, fabrication approaches, and flexible electrochemical biosensors based on nanoparticles and bio receptors to target analyte detection are presented. Furthermore, nanoparticles, MXene, composite materials, polymers, and biological receptors recently created extremely sensitive and selective electrochemical sensing devices and contributed to the modification of electrodes in electrochemical-based research work worldwide. The discussion and analysis in this work will be helpful to reach a concrete decision for the fabrication of wearable biosensor electrodes to detect a definite analyte. Furthermore, the insights are expected to become basic principles for upgrading next-generation wearable electrochemical bio-sensing devices.

2. Wearable sensor and biosensor platform

Body fluids are rich sources of important biomarkers including ions (*e.g.* Na^+, K^+, Ca^+, NH_4^+, and Cl^-), drugs and toxins (*e.g.* levodopa, caffeine, methyl xanthine, *p*-cresol), metabolites and biomolecules (*e.g.* glucose, lactate, uric acid, cortisol, ascorbic acid), protein and immune assay (*e.g.*, AFP, CA125, CA153, ferritin, and *E. coli*), participate in many physiological diseases such as diabetes, gout, Parkinson's diseases, hepatitis, and myopia. The latest wearable electrochemical sensors and biosensors are designed according to their site of application. Joohee Kim *et al.* reported research on multifunctional contact lenses, which are applicable to both *in vivo* and *in vitro* analysis of live rabbits and bovine eyeballs.[40,41] Contact lens would be an ideal vehicle for continuous tear glucose monitoring.[42] Another wearable rapid diagnostic tool (RDT) is Google Glass, which is capable of both qualitative and quantitative analysis based on a hands-free voice-controlled interface and then digitally transmitted to a server for digital processing.[41]

The textile-based screen-printed carbon electrode cotton underwear offers voltammetric and chronoamperometric measurements of 0–3 mM ferrocyanide, 0–25 mM hydrogen peroxide, and 0–100 μM NADH.[43] In sports and military applications, it brings great benefits to make digitalized human power. Another easily worn and replaceable biosensor is the mouthguard.[43] This saliva monitoring biosensor can detect without any interruption or little occurrence. Additionally, conductive yarns can be prepared by simple dyeing with carbon nanotube ink. Afterwards, an ion-selective potentiostat was prepared by coating a polymeric membrane. This potentiometric yarn can easily sense pH, K^+, and $NH4^+$.[44] Furthermore, conductive and insulating ink-mediated elastomeric stamps are well-suited for the formation of electrochemical sensors by following conventional screen-printed techniques.[45] The elastomeric stamp preparation methods can be extended to epidermal electrochemical sensors. Temporary tattoos were used in several parts of the human body with individual platforms such as printed temporary transfer tattoos on the skin to monitor lactate in human perspiration.[46] Windmiller *et al.* demonstrated the use of potentiometric ion-selective electrodes to monitor the pH level.[47] Similarly, two research studies reported the detection of sodium[47] and ammonium[48] in sweat. In addition, a complete self-driven smartwatch can monitor glucose levels in a simple and easy way and further maintains safety and infection risk. It made a correlation between the sweat composition and human body dynamics.[49]

Jungil Choi *et al.* demonstrated the use of a pressure-induced skin-mounted microfluidic network for the quantification of lactate, sodium, and, potassium by chrono-sampling of device.[50] Another soft microfluidic-based research work was performed using a microsystem designed with a biocompatible electronic-based electrochemical biosensor.[51] Reflectance pulse oximetry is a miniature flexible device that can facilitate the mounting on the external (*e.g.* skin) and internal (*e.g.* heart and brain) of the human body. This flexible platform incorporated

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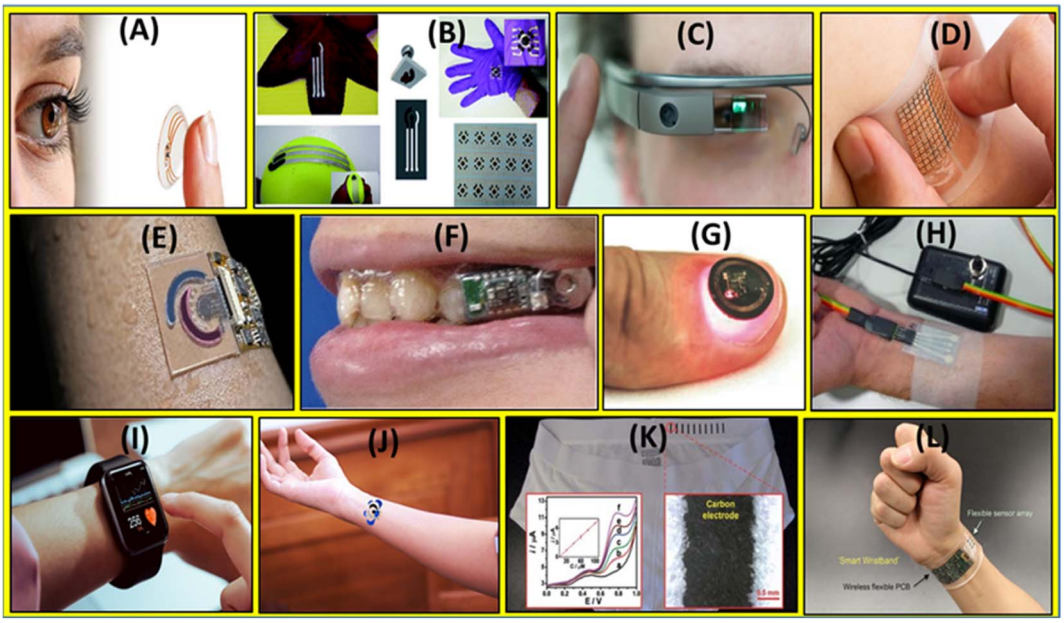
with optoelectronic functionality can be used for wireless capture and transmission of quantitative information on blood oxygenation, heart rate, and heart rate variability.[52] The NFZ-GQDs@GOx platform was fabricated to construct a bio-electronic tongue to continuously monitor glucose with a detection limit of 14 μM[53] (Scheme 1).

3. Nanostructured wearable biosensor

3.1 Biosensor for ions

Electrolytes play an important role in the body when they remain in body fluids as charged ions. They regulate the osmotic pressure in cells, help maintain the function of muscle and nerve cells, and transmit neural signals, body water content, and others. The main electrolytes include sodium, chlorine, potassium, calcium, and magnesium. A literature survey summarizing human body fluid concentration measurements is presented in Table 1. Gao *et al.* demonstrated research work for the detection of Na^+ and K^+ ions in human sweat samples using amperometric detection. A Na^+ selective membrane cocktail mainly consisting of Na ionophore X (1% w/w), Na-TFPB (0.55% w/w), PVC (33% w/w), DOS (65.45% w/w), and tetrahydrofuran was used to disperse the cocktail for the purpose of drop casting. The drop casting electrode was denoted as Na^+ selective electrode.[68] On the other hand, the K^+ selective membrane cocktail constructed from a mixture of valinomycin (2% w/w), Na-TPB (0.5%), PVC (32.7% w/w), DOS (64.7%), and cyclohexane was used to disperse and drop cast on a working electrode. Here, PVB modified electrode was used as a reference electrode.[58] In both cases, poly(3,4-ethylenedioxythiophene) PEDOT:PSS polymerized film was first considered as an ion–electron transducer to minimize the potential drifts of the ISEs.[59] Another study of Na^+ selective membrane followed the same procedure with PVB reference electrode. The $FeCl_3$ injected Ag/AgCl electrode was used as a Cl-selective electrode and PEDOT:PSS was chosen as the ion-selective transducer.[60] Furthermore, AuNPs were electro-deposited on chips from 5 mM $HAuCl_4$ and 0.5 M H_2SO_4 mixture at a constant potentiostatic voltage. Thereafter, Na^+ ISE was also prepared from the same procedure reported by Bandodkar *et al.*[57] An advanced-level research work detected the three electrolytes Na^+, K^+, and Ca^+ using carbon nanotube-modified weaving fabric and was reported by Wang *et al.* In this fabric, Na^+-ISE was prepared from a mixture containing Na-TFPB, high molecular weight PVC, DOS, sodium ionophore X in tetrahydrofuran, and Na^+-ISE was prepared by replacing sodium ionophore X with potassium ionophore. The Ca^+-ISE was also prepared following the same way as Na^+ and K^+-ISE, whereas, the ionophore was replaced with Ca ionophore II.[34] The detection ranges of Na^+, K^+, and Ca^+ ions were reported to be 10–160 $\times 10^{-3}$ M, 2–32 $\times 10^{-3}$ M, and 0.5–2.53 $\times 10^{-3}$ M, respectively. This study was conducted using sensing fiber weaving fabric in amperometric detection as represented in Fig. 1.



Scheme 1 (A) Contact lens (https://xtalks.com/postech-researchers-develop-smart-contact-lenses-that-can-diagnose-and-treat-diabetes-2232/), (B) elastomeric stamp,[45] (C) Google Glass (https://time.com/3669927/google-glass-explorer-program-ends/), (D) skin-patch (https://physicsworld.com/a/wearable-patch-could-predict-risk-of-stroke-and-heart-attacks/), (E) microfluidic device (https://innovationtoronto.org/index.php/2022/08/28/new-wearable-microfluidic-sensing-technology-can-provide-continuous-monitoring-for-many-health-conditions/), (F) mouthguard,[54] (G) pulse-oximeter,[52] (H) potentiostat,[55] (I) smart watch,[56] (J) temporary tattoo (https://www.wionews.com/science/tattoo-as-health-monitoring-device-south-korean-scientists-develop-unique-technology-502791), (K) underwear,[43] and (L) wristband (https://www.medicaldesignandoutsourcing.com/wristband-detects-analyzes-real-time-changes-in-sweat-chemical-composition/). Figure is adopted from all reference sources with permission.

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Table 1 A comparative electrolyte measurement study using wearable electrochemical sensors[a]

Sensing material	Analyte	Detection range	Detection limit	Method of detection	Bio-fluid	Ref.
ISE-K^+ membrane	K^+	2–16 × 10^{-3} M	N/A	Amp	Sweat	68
ISE-Na^+ membrane	Na^+	20–120 × 10^{-3} M	N/A	Amp	Sweat	68
ISE-Na^+ membrane	Na^+	10–80 × 10^{-3} M	N/A	Amp	Sweat	60
ISE-Cl^- membrane	Cl^-	10–80 × 10^{-3} M	N/A	Amp	Sweat	60
AuND-ISE-Na^+ membrane	Na^+	0–40 × 10^{-3} M	0.8 × 10^{-6} M	Poten	Sweat	61
Electrochemical fabric-CNT fabric substrate	Na^+	10–160 × 10^{-3} M	N/A	Amp	Sweat	34
Electrochemical fabric-CNT fabric substrate	K^+	2–32 × 10^{-3} M	N/A	Amp	Sweat	34
Electrochemical fabric-CNT fabric substrate	Ca^+	0.5–2.53 × 10^{-3} M	N/A	Amp	Sweat	34
Ag/AgCl	Cl^-	N/A	N/A	Poten	Sweat	55
Bare gold	Electrolytes	N/A	N/A	Conduc	Tears	69
Graphene-doped Au mesh	p^H		N/A	Poten	Sweat	70
Carbon/rGO-Na^+ membrane	Na^+	10–160 mM	N/A	Poten	Sweat	63
Carbon/rGO-K^+ membrane	K^+	2–32 mM	N/A	Poten	Sweat	63
AuNP/PANI	p^H	3–8 mM	N/A	Poten	Sweat	63
PEDOT:PSS/carbon fiber thread	Na^+	0.1–100 mM	N/A	Poten	Sweat	71
PEDOT:PSS/carbon fiber thread	K^+	0.1–100 mM	N/A	Poten	Sweat	72
CNT	NH_4^+	N/A	N/A	Poten	Sweat	73
PANI conducting polymer	p^H	N/A	N/A	Poten	Wounds	65
CNTs	p^H	8.51–2.69	N/A	Poten	Sweat	74
rGO-PANI	p^H	75.09 nm pH^{-1} at pH 11.35	N/A	Poten	Sweat	75
Graphite/Ag/AgCl	p^H	pH range 6–9	N/A	Poten	Sweat	76
Bi	Cd^+	<100 μg L^{-1}	N/A	CV	Sweat and urine	77
Bi, Au	Pb^+	<100 μg L^{-1}	N/A	CV	Sweat and urine	77
Au	Cu^{2+}	100–100 μg L^{-1}	N/A	CV	Sweat and urine	77
Au	Hg^+	<100 μg L^{-1}	N/A	CV	Sweat and urine	77
Ammonia ionophore	NH_4^+	10^{-4} to 0.1 M	N/A	Poten	Sweat	65
Lanthanum fluoride	Fluoride	0.19–1.9 ppm	N/A	Poten	Saliva	78
ISE/fluorinated alkyl silane/GO	K^+	0–6.5 mM	N/A	Poten	Sweat	79
ISE/fluorinated alkyl silane/GO	Na^+	0–49.5 mM	N/A	Poten	Sweat	79
ISE/fluorinated alkyl silane/GO	Cl^-	0–61.4 mM	N/A	Poten	Sweat	79
ISE/fluorinated alkyl silane/GO	p^H	0–6.91 mM	N/A	Poten	Sweat	79
PEDOT	Na^+	1.89–2.97 mM	N/A	Amp	Sweat	2
PEDOT	K^+	3.31–7.25 mM	N/A	Amp	Sweat	2
PEDOT:PSS/Au	Na^+	10–160 mM	N/A	Poten	Sweat	68
PEDOT:PSS/Au	K^+	1–32 mM	N/A	Poten	Sweat	68
Polyaniline	p^H	3–8	N/A	Poten	Sweat	80
PEDOT:PSS	Na^+	45.8 mV dec^{-1}	N/A	Poten	Sweat	81
PEDOT:PSS	K^+	35.9 mV dec^{-1}	N/A	Poten	Sweat	81
PEDOT:PSS	Ca^+	52.3 mV dec^{-1}	N/A	Poten	Sweat	81
PANi/CNT fibre	p^H	N/A	N/A	Poten	Sweat	81

[a] Abbreviations: Amp-amperometric, Pote-potentiometric, Conduc-conduct metric, CV-cyclic voltammetry, ISE-ion selective electrode, CNT-carbon nanotube, rGO-reduced graphene oxide, PANI-polyaniline, PEDOT-poly(3,4-ethylenedioxythiophene), PSS-polystyrene sulfonate.

Further detection of Na^+ and K^+ using all-solid-state ISEs was fabricated using reduced graphene oxide (rGO) as the ion-to-electron transducer between the ionophore and carbon working electrode.[63] The rGO-based electrode showed high stability at low temperature without considering humidity. This sensor fabrication involves a low-cost method.[64] The pH was measured by de-protonation from the surface of polyaniline (PANI).[63] Similarly, pH was also measured from wounds and sweat in the human body.[65] Additionally, Guinovart *et al.* reported a research work to quantify ammonium ions in sweat using a flower shaped potentiometric sensor similar to the tattoo sensor, and the construction is represented in Fig. 2.[65] To make this temporary tattoo, a transparent insulator was printed on the tattoo paper. Thereafter, Ag/AgCl layer with a longer right petal, a carbon layer a let petal, and a surrounding blue insulator were printed. After the tattoo was printed and cured, the reference and ion selective membranes were incorporated *via* drop casting and drying methods.

Among the large classes of Na^+, K^+, and Cl^- ion detection methods paper-based ISEs are one of the reported methods. In this study, high-quality graphene was dispersed in ethanol and sprayed onto the modified C_{10}^F paper through a stainless-steel mask. Then, Na^+, K^+, Cl^-, and reference electrode cocktails were dropped on the graphene-modified electrode. After graphene modification, the electrode gained high charge carrier immobility, chemical stability, large surface area, increased

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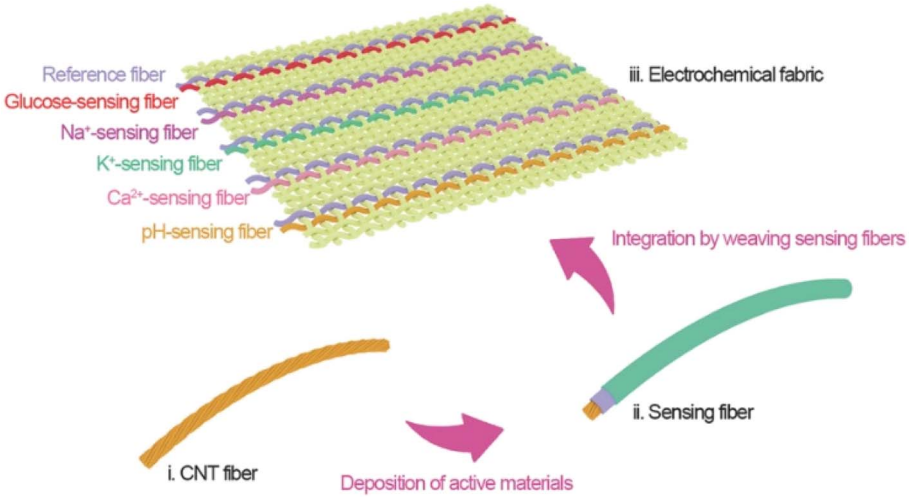

Fig. 1 Weaving carbon nanotube fiber to smart electrochemical fabric. Adopted from ref. [35] with permission from American Chemical Society, Copyright© 2019.

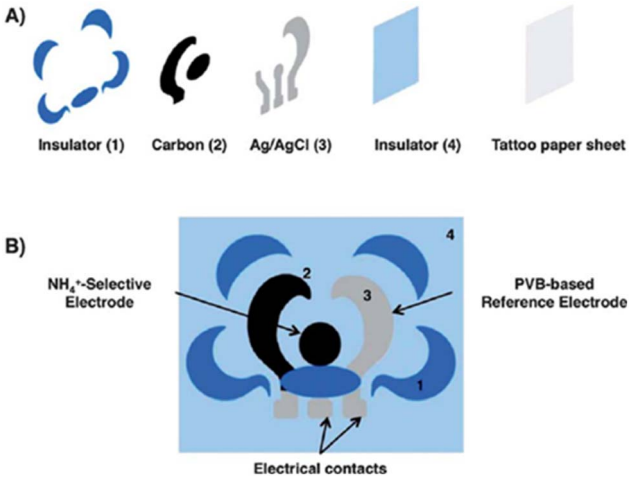

Fig. 2 Stepwise potentiometric tattoo sensor fabrication. (A). Release the fabrication layer by the insulator, carbon, Ag/AgCl, and insulator. (B). Ion-selective and reference electrodes deposited onto the suitable area [adopted from ref. [65] with permission from Wiley, Copyright© 2014].

toughness, and stretchability.[66,67] From the above literature survey, it can be concluded that ISE-based ion detection can play a vital role in the next generation rapid diagnostic tools.

3.2 Biosensor for drugs and toxins

Drug monitoring plays an important role in doping control, at the right dose, for the right patient, and at the right time.[82,83] It helps physicians to obtain information about drug dosages, compliance with prescriptions, and understanding the complex pharmacokinetics of drugs for optimal benefits.[82] Normally, blood is a conventional biofluid source to monitor drugs, and further urine, transdermal, and sweat source are reported alternative sources for wearable electrochemical biosensing platforms.[82] A literature survey for the comparison is summarized in Table 2. A wearable instant kit can also prevent the illegal accident of uptaking drug-related crimes, violence, sexual assaults, infectious diseases, *etc.*[84] The ongoing expectation is that the continuous monitoring of the drug is a mechanism of selective detection in body fluidic samples.

Tai *et al.* reported research work on the fabrication of a PET substrate with Cr and Au by photolithography and evaporation method to detect levodopa, a medication to treat Parkinson's disease.[85] In this study, Au nano-dendrites were grown on the electrode surface *via* a square wave of Gamry electrochemical potentiostat and chloroauric acidic medium. The lowest detected concentration of levodopa after 6000 cycles of Au deposition for 120 seconds was found to be 10 μM. Furthermore, a 1 × 3 pyramid-shaped hollow microneedle of 1500 μm height and 425 μm diameter was fabricated to detect levodopa in artificial ISF using SWV. Two of these electrodes, WE1 and WE2 were modified by carbon paste and the remaining electrode, Ag/AgCl was used as a reference electrode. WE2, the working electrode was further modified by tyrosinase mushroom enzyme.[86] The schematic representation for the detection of levodopa is shown in Fig. 3.

Another research work was performed by fabricating a PET substrate with silver, carbon, and nasion to quantitively measure methyl xanthine drugs and caffeine in sweat samples. In this study, Ag ink was mixed with 10% poly(vinyl butyral) in terpineol and printed at a constant temperature of 23 ± 2 °C and 35 ± 2%. Afterwards, carbon paste with 359 cP of viscosity was printed and dried at 150 °C for 5 seconds. Finally, the insulation was performed using polyethylene resin by annealing at 150 °C for 1 hour.[82] L-Histidine is an essential amino acid and precursor of hormones and metabolites, as well as a drug for eczema. The detection was performed using MOF particles and chitosan-modified electrodes with a detection limit of L-histidine at 5.3 μM.[87]

Alcohol is a psychoactive and toxic substance depending on its producing properties. Ashlesha Bhide *et al.* published

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Table 2 A comparative drug and toxin measurement study using wearable electrochemical and biosensors[a]

Sensing material	Analyte	Detection range	Detection limit	Method of detection	Bio-fluid	Ref.
Au	Levodopa	<10 μM	N/A	Amp	Sweat	85
Carbon paste/tyrosinase enzyme	Levodopa	0.5–3 μM	0.5 μM	SWV	Artificial-ISF	86
Carbon ink/CNT/Nafion	Caffeine	<40 μM	N/A	Amp	Sweat	82
Carbon ink/CNT/Nafion	Methyl xanthine	0–40 × 10^{-6} M	3 × 10^{-6} M	DPV	Sweat	82
Cu-MOF	L-Histidine	N/A	5.3 μM		Living cell	87
Carbon ink/chitosan/BSA/AOx/PB	Alcohol	0–36 × 10^{-3} M	N/A	Amp	Sweat	89
Au–ZnO/AOx	Alcohol	2.17 × 10^{-6} to 43.4 × 10^{-3} M	2.17 × 10^{-6} M	EIS	Sweat	88
Pt wire/Chitosan/AOx	Alcohol	0–80 × 10^{-3} M	N/A	Amp	Transdermal	12
Au-electro needle	*p*-Cresol	1 × 10^{-6} to 1 × 10^{-3} M	1.8 × 10^{-6} M	N/A	Transdermal	90

[a] Abbreviations: Amp-amperometric, SWV-square wave voltammetry, EIS-electrochemical impedance spectroscopy, DPV-differential pulse voltammetry, CNT-carbon nanotube, MOF-a metal–organic framework, BSA-bovine serum albumin, AOx-alcohol oxidase.

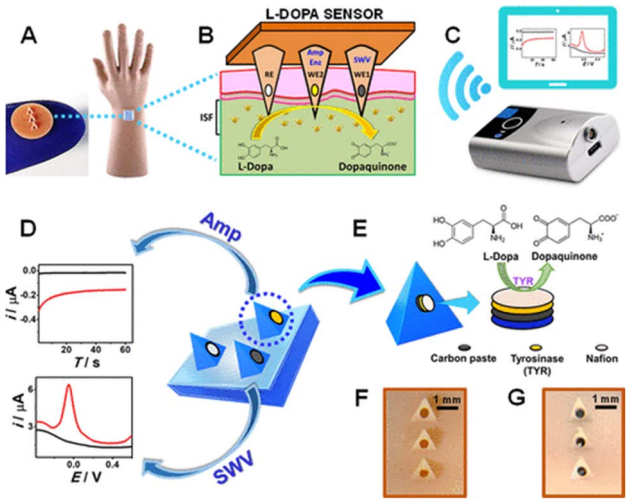

Fig. 3 Schematic representation of levodopa (L-Dopa) detection, (A) hand-wearing mannequin microneedle sensor, (B) ISF levodopa monitoring, (C) wireless electroanalayser, (D) microneedle sensor platform for levodopa sensing using SWV and amperometry, (E) cross-sectional view of CP, tyrosinase and Nafion layer, (F) and, (G) optical image before and after CP packing of microneedles. Adopted from ref. [87] with permission from American Chemical Society, Copyright© 2019.

a research work on the detection of alcohol in sweat samples using alcohol oxidase modified electrode. The gold particle was deposited by Temescal e-beam and ZnO film by AJA Orion RF magnetron and finally with alcohol oxidase.[88] Additionally, Ag/AgCl ink and PB conductive carbon were screen printed on working and counter electrodes by semi-automatic screen printing. A transparent insulator was screen-printed over the surface of the electrode pattern to confirm the electrodes and contact areas. For fabrication, the Ag/AgCl ink was cured at 90 °C for 10 minutes, and then the PB conductive carbon ink was cured at 80 °C for 10 minutes in a convection oven.[89] The schematic representation is illustrated in Fig. 4. Furthermore, medical grade liquid crystal polymer (*O*-phenylene diamine) was poured onto the Pt wire micro-transducer followed by the immobilization of alcohol oxidase where chitosan was an intermediate layer.[12] In this study, the alcohol detection linear range was 0–80 × 10^{-3} M. From an overall literature survey, it was found that levodopa detection with tyrosinase enzyme is suitable, and further, alcohol oxidase is suitable for the detection of alcohol.

3.3 Biosensor for metabolites and biomolecules

In metabolism, the intermediate and final products are the metabolites. These metabolites can be glucose, urea, uric acid, lactates, cholesterol, creatinine, hydrogen peroxide, ketone bodies, hypoxanthine, xanthine, *etc.* There have been some significant breakthroughs *via* electrochemical sensors in clinical applications concerning their measurement in a simple

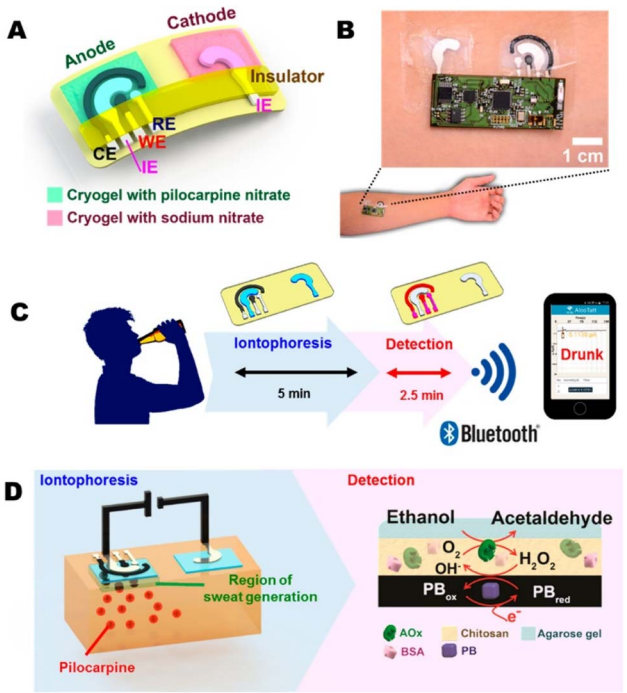

Fig. 4 Transdermal alcohol sensor (A) iontophoretic tattoo electrode, (B) alcohol iontophoretic-sensing tattoo device, (C) diagram of iontophoresis and amperometric detection of alcohol, (D) diagram of iontophoresis system (left) and amperometric system (right). Adopted from ref. [13] with permission from Elsevier, Copyright© 2018.

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way, within the shortest possible time, with high accuracy, selectivity, and stability. The economic and sturdy nature of commercially available electrochemical glucometers is a strong indicator of their success. Here, we will discuss some of the significant developments of biosensors in different metabolites, as summarized in Table 3.

As the biosensors can be prepared by easy procedure with the benefits of low cost, they are efficient and further very easy-to-use with a variety of areas. Moreover, they are popular for the detection of metabolites and biomolecules. Recently, different kinds of biosensors were applied to detect food-born, clinical or environmental pathogens harmful not only to humans but also to animals.

Uric acid (UA). Uric acid (UA), $C_5H_4N_4O_3$, is usually a human body waste product, this planar, heterocyclic compound is usually produced while the metabolic breakdown of purine nucleotides occurs.[91] Glucose oxidase-based $NiCo_2O_4$ nanomaterial shows high sensitivity to the glucose sample with a wide range of concentrations from 0.005 mM to 15 mM with a sensitivity of 91.34 mV per decade and also showed a fast response time.[92] Later, graphene fibers (GFs) with $NiCo_2O_4$ nanowires GF/$NiCo_2O_4$ were also reported for UA detection with a liner range of 10–26 μM and LOD of 0.2 μM.[93] This novel biosensor can be successfully applied directly as a working electrode for the detection of ascorbic acid, AA (liner range of 200–750 μM and LOD of 50 μM) and dopamine, DA (liner range of 1–13 μM and LOD of 0.1 μM). On DPV profiles at GF/$NiCo_2O_4$ electrodes these UA, AA, and DA can be detected at different voltage values.

Laser-scribed graphene, LSG, or LSG with platinum[94] nanomaterials also may be a good promising and excellent electrode for the detection of UA, AA, and DA compound swith a wide range of applications. The measurement of UA is not limited to urine samples only; various reports show that wearable sweat sensors have great potential for the continuous measurements of this sample.[95] They reported a highly sensitive LEG-CS; a laser-engraved graphene-based chemical sensor for the detection of lower concentrations of UA and tyrosine (Tyr). This LEG-CS-based multi-inlet microfluidic module can be an alternative to a wearable microfluidic system, which is made from silicone elastomers and is also complicated and expensive.

For the analysis of uric acid, especially point-of-care (POC) monitoring, M. Yang *et al.* developed a 3D electrochemical biosensor based on super aligned single wall carbon nanotube SWCNT array immobilized with uricase by the means of a precipitation and crosslinking procedure.[96] This biosensor possesses a higher enzyme density, a larger contact area, and showed excellent conductivity after modification with a sensitivity of 518.8 μA $(\text{mM cm}^2)^{-1}$, a wide linear range of 100–1000 μM, and a low limit of detection of 1 μM for uric acid in serum samples.

Here, uric acid is catalyzed by uricase on the working electrode and oxidized into allantoin, while producing carbon dioxide and hydrogen peroxide, this reaction was previously described by Numnuam *et al.*[161] group.

$$\text{Uric acid} + H_2O \xrightarrow{\text{uricase}} \text{allantoin} + H_2O_2 + CO_2 \quad (1)$$

The produced H_2O_2 decomposes on the surface of the electrodes according to eqn (2) and thus the corresponding current is detected.

$$H_2O_2 \rightarrow O_2 + H^+ + 2e^- \quad (2)$$

To date, the development of a biosensor for UA detection offers very high sensitivity and selectivity and is thus highly desirable in different fields of chemistry. Overlapping of the oxidation potentials of different molecules along with providing poor selectivity and reproducibility are major problems facing biosensing detection.[97] To overcome these problems, metal oxides,[98] noble metals,[99] polymers,[100] and carbon materials[101] were also considered as a candidate for electrode modification.

H. Liu *et al.*[102] showed that the polymer of cellulose (CLC), which is dissolved in [BMIM]Cl, and combined with different functional groups such as –NH_2, –SH – grafted poly(3,4-ethylene dioxythiophene) (PEDOT), *i.e.*, PEDOT-MeNH_2/CLC and PEDOT-MeSH/CLC electrode might be very useful for the detection of guanine (G) and uric acid (UA), respectively, with good selectivity and detection limits.

However, the tendency of people to visit any clinic is decreasing, and currently, patients visit doctors only after noticeable symptoms. Therefore, treatment at home using a single device such as a wearable or portable device is becoming popular as they are easy to operate and can measure the specific sample very accurately.

X. Wei *et al.* reported wearable biosensors for non-invasive and real-time monitoring of sweat compositions with high sensitivity and selectively.[62] Their developed method described the fabrication of wearable biosensors for the detection of uric acid in artificial sweat samples as depicted in Fig. 5. In this biosensor, a flexible and conductive CNF-worked electrode was used for the detection of the uric acid molecules in artificial sweat samples with good selectivity. It ensured a significant linear correlation between the current output and the concentration of uric acid.

Ascorbic acid (AA). Ascorbic acid (AA), known as vitamin C, is a readily water-soluble micronutrient that is required for multiple physiological/biological functions in various living organisms. This acid is working as a reducing agent and antioxidant, thus, in cellular metabolism, it can protect cellular components from oxidative damage and various oxidizing free radicals or harmful oxygen-derived species, such as hydroxyl radicals, hydrogen peroxide, and singlet oxygen.[103–106] So, the detection of this AA is very important for clinical application as well as to keep the human body fit.

The literature revealed various reports for the detection of AA, such as electrochemical methods,[107,108] but other molecules such as dopamine can interfere with this type of detection. To solve this problem and for the development of a selective and sensitive method for the determination of AA, conducting polymers[107] is highly desirable for analytical and diagnostic applications. A polymer, polyaniline-based biosensor, and ascorbate oxidase (AsOx) immobilized covalently onto carboxylated multiwalled carbon nanotubes, was reported by Chauhan and his team.[109] This AsOx/c-MWCNT/PANI/Au electrode was

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Table 3 A comparative metabolites and the biomolecules measurement study using wearable biosensors[a]

Sensing material	Analyte	Detection range	Detection limit	Method of detection	Sample	Ref.
PEDOT-$MeNH_2$/CLC/GCE	UA	5−400 μM	0.255 μM	DPV	Blood	102
GA/uricase/chitosan/SWCNT/Pt	UA	100–1000 μM	1.0 μM	CV	Serum samples	161
PEDOT-MeSH/CLC/GCE	UA	0.4–650 μM	0.085 μM	DPV	Blood	102
UOx/Pt NPs/PANI/MEA	UA	0.1–1.2 × 10^{-3} M	4 μM	Amp	Standard sample	162
CoO/N-CS-rGO	UA	1–125 μM	0.22 μM	CV	Standard and human serum	126
Urate oxidase/PLGA/MoS_2-hydrogel system	UA	100–500 μmol L^{-1}	20 μmol L^{-1}	ECL	Serum	163
GF/$NiCo_2O_4$	UA	10–26 μM	0.2 μM	CV and DPV	Serum and urine	93
Pt/LSG	UA	1–63 μM	0.22 μM	CV and DPV	Urine	94
Uricase/ZnONW	UA	0.024–0.101 × 10^{-3} M	10 μM	Piezo	Sweat	137
LEG-CS	UA	N/A	0.74 μM	DPV	Sweat	95
Uricase	UA	O.5–50 μM	N/A	CV and DPV	Wound fluid	164
Nafion/uricase/ZnO/Ag/Si	UA	50–2000 μM	0.019 μM	CV	Serum	165
Uricase/tetrapod-shaped ZnO	UA	0.8–3490 μM	0.8 μM	CV and amp	Standard sample	166
Nafion/uricase/ferrocene/GCE	UA	0.5–60 μM	0.23 μM	Amp and DPV	Blood	167
AsOx/c-MWCNT/PANI/Au	AA	2–206 μM	0.9 μM	Amp	Serum substances	109
AuNPs@PANI/CS/GCE	AA	20–1600 μM	8 μM	CV, amp and DPV	Standard and real sample	110
Ni@poly-1,5 DAN/GC	AA	100–500 μM	0.010	SWV	Serum	168
Pt/LSG	AA	10–890 μM	6.1 μM	CV and DPV	Urine	94
GO/NNO20	AA	30–1100	11.3 μM	CV, chrono amp	Standard and synthetic sweat	111
GO/NNO100	AA	30–1100	3.8 μM	CV, chrono amp	Standard and synthetic sweat	111
GF/$NiCo_2O_4$	AA	200–750 μM	50 μM	CV and DPV	Serum and urine	93
Transfer tattoo	AA	10–50 μM	N/A	CV	Sweat	169 and 170
MNA-PLA/f-MWCNT	AA	0–1 × 10^{-2} M	180 μM	DPV	Dermal	171
Pyox/LSGE	DA	0.01–0.5 and 0.5–10 μM	0.007 μM	DPV	Human serum and tap water	129
PANI-WO_3/GCE	DA	20–300 μM	0.139 μmol L^{-1}	CV, DPV, EIS	Standard	125
CoO/N-CS-rGO	DA	0.5–110	0.15	CV	Standard and human serum	126
PEDOT-G-TYR	DA	N/A	101 × 10^{-9} M	Amp	Tear	124
Graphene/PEDOT/TYR	DA	N/A	101 × 10^{-9} M	Amp	Tear	124
AuNPs@PANI/CS/GCE	DA	10–1700 μM	5 μM	CV, amp and DPV	Standard and real sample	110
Ni@poly-1,5 DAN/GC	DA	100–500 μM	0.00011	SWV	Serum	168
Pt/rGO paper	DA	87 nM to 100 μM	5 nM	DPV	Living cell	172
rGO MEA	DA	N/A	0.1 μM	DPV	Sweat	131
GF/$NiCo_2O_4$	DA	1–13 μM	0.1 μM	CV	Urine	93
Pt/LSG	DA	0.5–56 μM	0.07 μM	CV and DPV	Urine	94
PEDOT/LSG	DA	1–150 μM	0.33 μM	Amp	Rat brain	173
GF/$NiCo_2O_4$	DA	1–13 μM	0.1	CV and DPV	Serum and urine	93
Textile-OECT/PEDOT:PSS	DA	1–10 × 10^{-6}	1 μM	DPV	Sweat	173
Textile-OECT/PEDOT:PSS	AD	10–100 × 10^{-6} M	10 μM	FET	Sweat	173
Transfer tattoo	AA	10–50 μM	N/A	CV	Sweat	169 and 170
SPCE-PPy-urease	Urea	10 μM to 5mM	8 μM	Poten	Sweat	174
AuMNA-P(GMA-*co*-VFc)	Urea	50–2500 × 10^{-3}	2.8 μM	CV	Transdermal	174
rGO MEA	Tyramine	N/A	3.7 μM	DPV	Sweat	175
LEG-CS	Tyramine	N/A	3.6 μM	DPV	Sweat	95
E200acryl-CP/catechol-agar	Tyrosinase	0.1–0.5 mg mL^{-1}	N/A	Amp	Transdermal	176
Au/rGO/Au-Pt NP/GOx/Nafion	Glucose	0–2.4 × 10^{-3} M	5 μM	CV, amp	Sweat	141
GOx/Pt-graphite	Glucose	0–0.9 mM	0.01 mM	Chrono amp	Human perspiration	177
PANI/TEGO/PVA	Glucose	0.2 μM to 10 mM	0.2 μM	CV	Sweat	177
PB/Au-graphene/GOx	Glucose	10 μM to 0.7 mM	10 μM	CV	Sweat	178
Pt/Co/NPG/GO	Glucose	35 μM to 30 mM	5 μM	Amp	Blood	179
PtAu/rGO-CNT-IL/GP	Glucose	0.1–11.6 mM	80 μM	Amp	Blood	180
GO/PB/Gp-hybrid	Glucose	0.01–0.7 mM	10 μM	Poten	Sweat	178
Au/graphene/AuNps/GOD	Glucose	0–40 mg dL^{-1}	0.3 mg dL^{-1}	Amp	ISF	181
LIG/PtNPs	Glucose	300 nM to 2.1 mM	300 nM	Amp	Blood	182

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Table 3 (*Contd.*)

Sensing material	Analyte	Detection range	Detection limit	Method of detection	Sample	Ref.
rGO/AuPtNPs	Glucose	0–2.4 mM	5 μM	Amp	Sweat	141
GOx/Au/MoS_2/Au-nanofilm	Glucose	500–100 nM	10 nM	Amp	Human serum	141
Uricase/ZnONW	Glucose	0.042–0.208 × 10^{-3} M	20 μM	Piezo	Sweat	137
D-Lactate assay kit	Glucose	0–6.3 × 10^{-3} M	N/A	Colo	Sweat	140
PB ink/chitosan/BSA/GOx	Glucose	10–100 × 10^{-6} M	3 μM	Amp	ISF	143
PB ink/agarose/chitosan/GOx	Glucose	0–160 × 10^{-6} M	N/A	Amp	ISF	22
Alcoxysilanes-PB-GOx	Glucose	1 μM-1 mM	1 μM	Amp	Sweat	183
C-PB ink-GOx	Glucose	2–10 × 10–3 M	50 μM	Amp	Sweat	142
rGO/PU-Au	Glucose	500 × 10^{-9} to 10 × 10^{-3} M	500 × 10^{-9} M	CV	Sweat	1
Au/Pt black-Nafion	Glucose	50 × 10^{-6} to 36 × 10^{-3}	50 μM	Amp	ISF	184
GOx/Pt NPs/PANI/MEA	Glucose	2–12 × 10^{-3} M	260 μM	Amp	Standard sample	162
GOD/CMC/Microneedles	Glucose	0–35 × 10^{-3} M	40 μM	Chrono-amp	ISF	185
GOx/PANI-PEO/Pt	Glucose	1–10 × 10^{-3} M	820 μM	CV	Standard sample	186
Nafion/GOx/AuNP-PVP-PANI	Glucose	0.05–2.25 × 10^{-3} M	10 μM	CV	Serum	187
GOD/Pt/MWNT-PANI/GCE	Glucose	0.003–8.2 M	1 μM	Chrono amp	Standard sample	188
Chitosan-PVA/GOx	Glucose	5–50 mg dL^{-1}	N/A	Poten	Tears	189
Chitosan-PVA/GOx	Glucose	0.1–0.6 mM	N/A	Poten	Tears	190
PEGDA	Glucose	0–4 × 10^{-3} M	1 μM	CV	Transdermal	191
Uricase/ZnONW	Lactate	0–20 × 10^{-3} M	0.1 × 10^{-3} μM	Piezo	Sweat	137
PEGDA	Lactate	0–1 × 10^{-3} M	1 μM	CV	Transdermal	191
GP-MoS_2-Cu-LOD	Lactate	5–1775 μM	500 nM	Amp	Sweat	192
CNTs/$Ti_3C_2T_x$/PB/CFM	Lactate	10 μM × 10^{-3} M	0.67 μM	Amp	Sweat	193
BSA-Lox/SPEES/PES	Lactate	0–28 × 10^{-3} M	N/A	Amp	Sweat	194
AuMN/AuMWCNT/MB	Lactate	0.01–0.2 × 10^{-3} M	N/A	Amp	Transdermal	195
E200acryl-filled CP-PEI-LOx	Lactate	0–8 × 10^{-3} M	0.42 M	Amp	Transdermal	196
GTA/BSA/LOx	Lactate	0–1 mM	N/A	Amp	ISF	197
Lactate oxidase	Lactate	0.1–0.5 mM	N/A	Amp	Saliva	198
Cat-Fe_3O_4/rGO	H_2O_2	3.30 μM to 5.56 mM	110 nM	Amp	PBS	199
GF/AuNS	H_2O_2	9.4 μM to 13 mM	1.62 μM	Amp	PBS	200
IL-rGO	H_2O_2	0.1–37.6 μM	0.01 μ	Amp	PBS	200
Au/MnO_2/graphene-coated CP	H_2O_2	0.05–14.2 mM	2 μM	Amp	Cancer cell	176

[a] Abbreviations: Amp-amperometric, Poten-potentiometric, DPV-differential pulse voltammetry, CV-cyclic voltammetry, ECL – electrochemiluminescence, Chrono amp-chrono amperometry, Colo-colorimetric, UA-uric acid, AA-ascorbic acid, DA-dopamine, AD-Adrenaline, PEDOT-poly(3,4-ethylenedioxythiophene), PSS-polystyrene sulfonate, GA-graphene, SWCNT-single-walled carbon nanotube, MWCNT-multiwalled carbon nanotube, CNT-carbon nanotube, GOx-glucose oxidase, UOx-uricase oxidase, PB-Prussian blue, BSA-bovine serum albumin, PVA-polyvinyl acetate, PANI-polyaniline, NW-nanowire, rGO-reduced graphene oxide, CP-carbon paste, PEI-polyethyleneimine, LOx-lactate oxidase, PEG-polyethylene glycol, OECT-organic electrochemical transistors.

employed for the determination of AA in different samples, human serum, fruit juices, and vitamin C tablets without the interference of serum substances. The electrochemical reaction mechanism for the response is explained by the following equation:[109]

$$\text{L-ascorbic acid} + 1/2O_2 \xrightarrow{\text{AsOx}} \text{dehydroascorbic acid} + H_2O$$

$$O_2 + 4H^+ + 4e^- \rightarrow H_2O$$

With this kind of polymer, another biosensor was proposed by L. Yang group.[110] Their developed electrode, AuNPs@PANI/CS/GCE showed very excellent catalytic activity and selectivity to the electro-oxidation of DA and AA with peak currents in the range of 10–1700 and 20–1600 μM and detection limits of 5 and 8 μM, respectively.

In a very recent study, Rossato *et al.* reported[111] that, GO/NNO biosensors might be a suitable candidate for the development of flexible and wearable electrochemical devices to use in AA detection with a very simple assembly, fast response, ultra-sensitivity, and low cost. They showed that a laser-induced graphene (GO) electrode with two different $NaNiO_3$ (NNO) nanotubes, distinctive with their external diameter of 20 nm (NNO20) and 100 nm (NNO100) is a very promising candidate for the detection of AA in synthetic sweat. Both electrodes can detect AA in the linear range of 30–1100 μM^{-1}, while the sensitivity of GO/NNO100 (0.031 μA μM^{-1} cm^{-2}) is significantly better than GO/NNO20 (0.023 μA μM^{-1} cm^{-2}). According to the sensitivity results, the diameters of NTs seem to affect the

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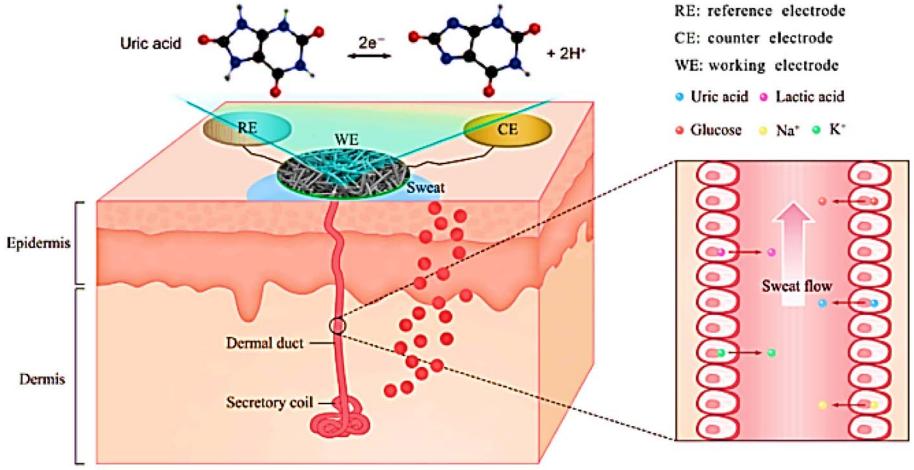

Fig. 5 Schematic illustrations of sweat gland structure, biomarker secretion, and wearable biosensor for uric acid detection in sweat. Adopted from ref. [62] with permission from Elsevier, Copyright© 2021.

sensitivity due to their impact on Ni oxidation, thus affecting the biosensors performance.

Another study by Junlin Ma *et al.*,[112] reported on the development of wearable self-powered textile smart sensors and their effectiveness in smart sensing systems for the portable detection of nutrition with a high sensitivity of 96.6 μA mM^{-1} cm^{-2} and a low LOD of 30 μM. They proposed a novel pH-assisted O/W (oil/water) self-assembly system of the bi-functional PANI/reduced graphene oxide (RGO) composite film. On the O/W interface, the composite film (PANI/RGO) exhibited both good capacitive performance and high-performance biosensing properties for AA detection, enabling it to act as a power source for wearable biosensors.

Vitamin C (ascorbic acid-AA) is a known nutrient in the human body needed for the formation of blood vessels, cartilage, muscle, and collagen in bones. It is always important to keep track of the nutrients level in the body. Sweat contains rich chemical information, and further is an attractive bio-fluid for routine assessment of nutrient levels of the human body. For nutritional screening and dietary intervention, a wearable sensor that can selectively measure AA concentration in biofluids, including sweat, urine, and blood was developed by Zhao *et al.*[113] On the oral intake of vitamin C, they monitored its concentration increases (compared without intake) in sweat along with urine and blood. They used a conductive polymer, poly (3,4-ethylene dioxythiophene) doped with lithium perchlorate (PEDOT:$LiClO_4$) on the Au surface, encapsulated with Nafion that was characterized by measuring amperometric responses with LOD of ≈4 μm. It showed high selectivity against glucose, lactate, and uric acid in the same measurement. The developed sensor showed a linear response within 0 to 5000 μm with a sensitivity of 1.2 and 2.0 nA μm^{-1} for bare Au and nano-textured electrodes, respectively.

An epidermal noninvasive bioelectronics wearable biosensor was developed[114] for the detection of vitamin C in sweat (AA), realized by immobilizing the enzyme ascorbate oxidase (AAOx) on printable tattoo electrodes. Here, the AAOx enzyme catalyzes the oxidation of AA to dehydroascorbic acid by oxygen. Furthermore, this amount of oxygen consumed by the reaction is directly proportional to the concentration of AA, and the reduction current of the oxygen.[115,116] Co-substrate expresses the changes in the AA concentration level by an amperometric method after the intake of vitamin pills and fruit juices, and, the related chemical reaction already discussed earlier, reported by Chauhan *et al.*[109]

For the real-time and multiplex sweat analysis such as glucose, lactate, ascorbic acid, uric acid, Na^+, and K^+ simultaneously, an integrated sensor was developed by Wenya He and his group.[117] They used silk fabric derived intrinsically from nitrogen (N) doped carbon (graphitic) textile (SilkNCT), as

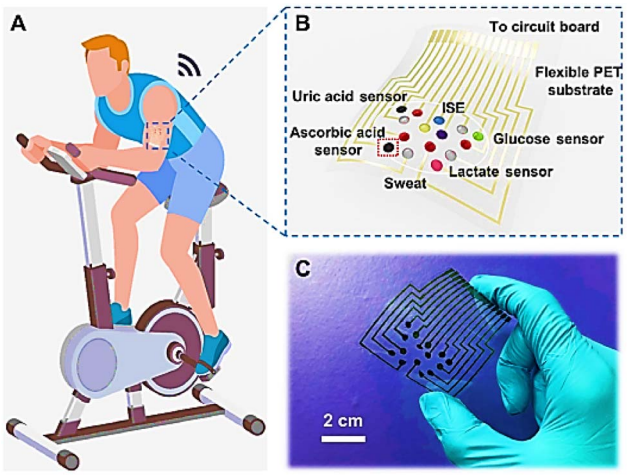

Fig. 6 Wearable sweat analysis patch based on SilkNCT. (A) and (B) Schematic illustration of wearable sweat analysis patch mounted on human skin (A) and the multiplex electrochemical sensor array integrated into the patch (B). (C) Photograph of the wearable sweat analysis patch. Adopted from ref. [117] with permission from the American Association for the Advancement of Science (AAAS), Copyright© 2019.

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illustrated in Fig. 6. For high selectivity glucose and lactate sensors, the working electrode was fabricated by drop-casting of glucose oxidase/chitosan and lactate oxidase/chitosan solution onto the Pt/SilkNCT electrode. The good electrical conductivity (electrochemical activity for redox reactions of the molecules) and rich active sites ensured high sensitivity of the AA and UA sensors made of pristine SilkNCT. The reason for this sensitivity can be attributed to N-doping and the hierarchical structure of SilkNCT. While measuring AA and UA, the sensor showed a linear range of 20 to 300 μM and 2.5 to 115 μM, LOD of 1 and 0.1 μM, and a sensitivity of 22.7 and 196.6 nA μM^{-1}, respectively.

For the measurements of glucose in real time it is always important to measure the accurate amount of glucose. Successive reports were also published reporting different substrates and electrode materials.

Dopamine (DA). Dopamine (DA) is a biomolecule and a key substance, which regulates the body's metabolism, and central neurological systems including mental activity in the human brain and body. If there is any deficiency or excess of DA, it causes various serious disorders and illnesses, including Parkinson's disease, senile, dementia, Harrington's, epilepsy, and schizophrenia.[118–123] There are several methods for the detection of DA in the human body.

W. Zhang and his group[124] showed that the content of DA can be detected by a wearable corneal biosensor. The sensor is prepared by enzyme tyrosinase, and poly (3,4-ethylenedioxythiophene) functionalized with sulfur-doped graphene (PEDOT-G) on a self-designed corneal microelectrode. Here, the sulfur-doped π–π conjugated graphene contributed to a high electroactivity in the resulting microelectrode of PEDOT-G with excellent selectivity and high sensitivity of 12.9 $\mu A \times 10^{-3}$ m^{-1} cm^{-2} and a good LOD of 101×10^{-9} M.

A very recently developed sensor, PANI-WO_3/GCE[125] proved an excellent sensor for the detection of DA with a good linearity of concentration range of 20–300 μM and a detection limit of 0.139 μmol L^{-1} and further showed good selectivity.

Clinically applicable diagnostic graphene-based sensor CoO/N-CS-rGO/GCE was reported[126] to monitor two analytes, dopamine and uric acid (UA) in human serum with high accuracy. It is an ultrasensitive electrochemical sensor, for DA and UA with a sensitivity and linear range of 1378 and 1393 μA mM^{-1}, and 0.5–110 and 1–125 μM, respectively, with satisfactory stability, and a fast sensing process without pretreatment. The possible oxidation reaction can be expressed as follows[127,128]

$$CoO + H_2O \rightarrow CoOOH + H^+ + e^-$$

$$CoOOH + C_8H_{11}NO_2\ (DA) \rightarrow CoO + C_8H_9NO_2\ (\text{dopamine-}o\text{-quinone})$$

$$CoOOH + C_5H_2N_4O_3\ (UA) \rightarrow CoO + C_5H_2N_4O_3\ (\text{dehydrourate})$$

Polypyrrole (PPyox) modified laser scribed graphene electrode (LSGE) for DA sensing was successfully developed,[129] showing a great potential to be applied in flexible wearable biosensors. This modified electrode is very selectivity to DA in the presence of AA with a lower limit of detection of 7 nM and linearity of 0.5–10 μM, which can be employed for human blood serum and tap water samples, with satisfactory recovery values.

Glucose sensor. A glucose sensor based on a platinum wire and perfluorosulfonic acid polymer-coated enzyme electrodes was developed by Harrison *et al.* for blood sample.[130] Their device showed a linear response from 10 nA to 2 μA with lower LOD <2 nA. For the detection and quantification of blood glucose samples, enzyme-based electrochemical sensors were also developed and some of them were based on invasive blood sampling techniques. Examples of these sensors are, screen-printed carbon (SPC) paste electrodes attached with a glucose oxidase immobilizer and hexamine ruthenium(III) chloride $[Ru(NH_3)_6]^{3+}$ containing nitrocellulose electrode,[131] thermal biosensor evaluated for the determination of glucose in whole blood by measuring the heat evolved when the glucose sample passed through in a small column with immobilized glucose oxidase with a directly measurable catalase around 1 μL,[132] and amperometric glucose sensor on a Prussian blue layer developed based on glucose oxidase immobilized by chitosan for blood sample exhibiting an excellent sensitivity of 98 nA M^{-1} with a linear range of 0.1–6.0 mM.[133] For human perspiration, previously used glucose-oxidase immobilized on Pt-decorated graphite exhibited a low LOD of 10 μM with a linear range between 0 μM and 0.9 mM.[134]

Changes in glucose and norepinephrine levels can be monitored in tear fluid samples by an amperometric rolled thick-film biosensor. The glucose oxidase (GOx) with thick-film carbon working electrode showed a sensitivity range of 20–200 μM for glucose solution,[135] while measuring the common electroactive interferences of ascorbic and uric acids can be effectively excluded using polytyramine.[136] For the norepinephrine sample, the flow rolled microsensor of copper-clad polyimide contacted with screen-printed band electrodes displays sharp anodic peaks, *i.e.*, the amperometric signals over the 300–900 ppb range, which reflect the rapid and sensitive response to the sample.[135]

The enzyme/ZnO nanoarray-based piezo biosensor electrode was proposed and named electronic-skin,[137] where the surface of ZnO nanowires was modified with lactate oxidase, glucose oxidase, uricase, and urease. This developed electronic skin was a self-powered biosensor and could detect and monitor lactate, glucose, uric acid, and urea in the perspiration in real-time and continuously.[137] It is noteworthy that selectivity is an important parameter for biosensors.[138]

$$\text{Glucose} + H_2O + O_2 \xrightarrow{GOx} \text{gluconic} + H_2O_2$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \ (\text{ref. } 139)$$

Modifier lactate oxidase (LOx), glucose oxidase (GOx), uricase, and urease showed high selectivity against lactate, glucose, uric acid, and urea in perspiration. Furthermore, piezo-based biosensing performance arises from the coupling effect

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among them. While lactate oxidase can only detect lactate, and the responses against glucose, uric acid, and urea are almost close to zero, which ensures the applications of electronic skin in real samples. The reaction between GOx and glucose can produce gluconic acid and H_2O_2,[138] next H_2O_2 can increase the surface carrier density by producing H^+ and e^- (ref. [139]) thus affecting the piezoelectric effect.

Another wearable microfluidic device was developed and is capable of monitoring human health conditions by the quantitative chemical analysis of sweat. Their microfluidic device can robustly bond to the skin surface (a small set of sweat glands such that perspiration spontaneously initiates routing of sweat) without mechanical and chemical irritation, which approaches based on a sporadic assessment of blood samples containing glucose or lactate.[140]

A wearable low-cost electrochemical glucose biosensor comprising a hybrid working electrode Au/rGO/AuPtNP/GOx/Nafion,[141] was successfully developed for the determination of glucose levels/concentrations in human sweat by amperometric analysis. The Au and Pt nanoparticles modified on the Au/rGO surface contribute to the increase of the electroactive surface area of the electrode, resulting in the acceleration of electron transfer between the redox probe and the electrode. This sensor showed a good analytical sensitivity of 82 μA mM^{-1} cm^{-2} actively with a linear range of 0.1–2.3 mM, demonstrating a sufficient range for glucose sweat detection[142] (Fig. 7).

As such, the detection of glucose in sweat samples is becoming popular. Among these, interstitial fluid (ISF) recently received great attention in connection to the management of diabetes.[143–147] For the first time, dual epidermal fluid sampling and detection methods integrated on a single conformal wearable platform[22] based on PB ink/agarose/chitosan/GOx, (Prussian blue (PB)) were reported. Furthermore, it showed a wide range of concentration linearity around 0–160 × 10^{-6} M with 20 × 10^{-6} M increments and a very selective response to glucose sample concentrations against the electroactive interference compounds. Their developed concept and designed device are capable of noninvasive glucose and alcohol analysis in healthy humans and it further showed excellent correlation to commercial blood glucometer subjecting consumption of food and drink.

Several attempts were reported for the evaluation of glucose concentration detection with painless, convenient, and automated capabilities. For the glucose self-monitoring systems, proposed and developed methods include contact lenses, watches, tattoos, and patches, which collect their information from tears, interstitial fluid, or sweat. Patch-type wearable glucose sensor systems, which are wearable, can be mounted to the human body, and are able to determine the glucose levels in sweat or in ISF fluids with sensing continuously and non-invasively.

Biosensors can be used for the detection of female sex hormone named 17β-estradiol, which is known as a natural and bio-identical form of estrogen. A recent report by Bacchu *et al.*,[148] showed that the biosensor, g-C_3N_4/APTES/SPE (g-C_3N_4 = graphitic carbon nitride, APTES = 3-aminopropyltriethoxysilane, SPE = screen-printed electrode) is very effective and can detect estradiol with a wide range of linearity from 1 × 10^{-6} to 1 × 10^{-18} mol L^{-1}, LOD of 9.9 × 10^{-19} mol L^{-1}, high selectivity, and stability. Other biosensors, such as aptamer-based label-free biosensors,[149–153] near-infrared (NIR) phosphorescence aptasensor,[154] split aptamer regulated CRISPR/Cas12a (CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats, Cas12a = RNA-guided endonuclease) biosensor,[155,156] Hydrogel optical waveguide spectroscopy a label-free biosensor,[157] highly sensitive laccase-based biosensor, *i.e.*, Lac/rGO-RhNP/GCE electrode,[158] Lac/PLLY/CA-GR/GCE,[159], (Lac = Laccase, CA-GR = critic acid@graphene, PLLY = poly l-lysine), Impedance-based E-screen cell biosensor[160] were reported with excellent performance for different kinds of samples with 17β-estradiol.

From the survey of metabolites and biomolecules, it was found that uric acid detection is more priority on the uricase enzyme modified surface by amperometric technique, ascorbic acid on MWCNT modified surface by differential pulse voltammetric (DPV) technique, dopamine on graphene surface by

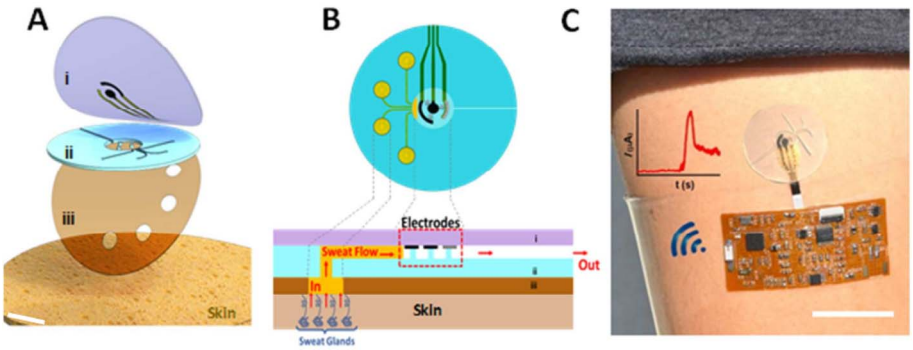

Fig. 7 Microfluidic device design and operation. The soft epidermal microchip device conforms to the skin and routes the sampled sweat toward the electrochemical detector. (A) Schematic representation of layered microfluidic device configuration on skin composed of (i) top PDMS layer with incorporated sensor electrodes, (ii) PDMS microfluidic device, and (iii) adhesive layer on the skin. (B) Schematic representation of microfluidic device sweat collection and operation on the skin in top-down and cross-sectional views. (C) Photograph of microfluidic device integrated with wireless conformal electronics on skin with lithography-based gold current collectors and screen-printed silver–silver chloride (RE) and Prussian blue (WE and CE). Adopted from ref. [142] with permission from American Chemical Society, Copyright© 2019.

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DPV technique, glucose on glucose oxidase surface by chronoamperometric technique and lactate on lactate oxidase by amperometric technique. Thus, the modified surface materials or receptors and the detection techniques can be counted as selective detections of relative biomolecules.

3.4 Biosensor for protein and immune assay

The key to the innate immune response is the recognition of danger signals or damaged tissue by receptors modified sensor on the surface of the sensor.[201] It is the first line of the host defense mechanism against pathogens and harmful substances.[202] Recently, it was reported that wearable biosensor receptors can continuously monitor and patrol the host tissues for microbes, damage, and stress, searching for signals of danger and damage.[201,203] A literature survey on protein and immune assay is summarized in Table 4.

Nah *et al.*, developed a wearable immunosensing path with a microfluidic system for cortisol biomarker detection. They successfully incorporate Ti_3C_2Tx MXene nanosheets into the porous structure of laser-burned graphene.[204] This wearable path system exhibited dynamic range and limit of detection of 0.01–100 nM and 88 pM, respectively. Additionally, conductive carbon yarn (CCY) and Fe_2O_3 materials were directly deposited onto the working electrode to improve flexible electrochemical biosensors. In this study, anti-C_{mab} was covalently bound onto the Fe_2O_3/CCY surface using EDC as a coupling agent and NHS as an activator. The fabricated BSA/anti-C_{mab}/Fe_2O_3/CCY electrode was used to measure cortisol concentration from 1 fg to 1 μg in PBS (pH 7.0) by the CV technique.[36] Furthermore, highly specific single-stranded DNA (ssDNA) was immobilized onto the ZnO active region to construct a label-free electrochemical sensor for the real-time monitoring of cortisol levels. Here, cortisol is bound with the captured probe, changing the confirmation of the electrochemical signal.[205,206] Another contact lens-controlled cortisol monitoring in tears was reported for the mobile-controlled electrochemical platform.[207] Where monoclonal antibody was immobilized on the surface of graphene to construct a FET sensor. This process was able to measure low concentrations of cortisol. The schematic representation of the smart contact lens packaging is shown in Fig. 8.

The aptamer-based field effect transistor was developed using graphene-Nafion composite film to detect the presence and quantification of cytokine levels by immune sensing human interferon-gamma (IFN-γ). This inflammatory cancer biomarker was collected by adhesive and disposable membrane capsule in sweat.[209] Another report demonstrated aptamer functionalized graphene-based field effect transistor (GFET) for immune sensing IFN-α, and IFN-γ. In this study, a 2.5 μm Mylar film was deposited on a glass slide and then the drain, source, and gate electrodes were patterned by lithography process. Thereafter, a graphene sheet in polymethyl methacrylate (PMMA) was transferred onto the electrode, and after dissolving the PMMA layer the graphene was functionalized with the corresponding aptamer of IFN-α, and IFN-γ.[210] Additionally, polyaniline was electrodeposited on graphene screen printed paper to increase surface area and immobilize the IFN-γ antibody. The remaining two electrodes were carbon ink and Ag/AgCl ink screened.[208] The systematic representation is shown in Fig. 6. The other research work was done with an aerosol-jet-printed graphene-based immunosensor capable of monitoring two distinct cytokines enzymes, IFN-γ and IL-10 with heavy-ranging sensitivity. Here, the IFN-γ and IL-10 antibodies were covalently linked with graphene[211] (Fig. 9).

Cancer biomarker detection using the paper-based differential pulse voltammetry method was a unique reported work in which the electrode was modified using the corresponding antibodies. In this study, GO was first modified by drop casting method, and then chitosan was dropped on GO film and finally incubated in glutaraldehyde solution to be ready for antibody immobilization. To immobilize the antibody, AFP, CEA, CA125, and CA153 captured antibodies were applied to a glutaraldehyde-modified electrode and after drying, they were ready to be applied in the corresponding antigen in PBS solution.[213] Furthermore, the NH_2-G/Thi/AuNPs nanocomposite was modified on a carbon working electrode and then anti-CEA was fabricated on an amine-functionalized modified electrode.[214]

Pancreatic polypeptide Neuropeptide Y (NPY) detection is certainly vital for immune sensing because it maintains essential biological processes in the human body. Kodjo *et al.* reported the detection of the neuropeptide Y using anti-neuropeptide Y-modified electrodes in sweat samples.[212] The modified electrode showed an ultrasensitive linear range of 10–500 pg mL^{-1}. Also, a graphene-based field effect transistor (GFET) was another electrode functionalized with anti-NPY and finally was tested in sweat and saliva samples.[215] Additionally, the gold working electrode was carboxylate functionalized to immobilize the TNF-α antibody and thereafter trialed in PBS and sweat sample to measure TNF-α protein.[216,217] In this literature survey, it was concluded that antigen detection showed an excellent response with the corresponding antibody-modified electrode.

4. Present challenges and future prospects

Over the last few decades, carbon nanomaterials, metal nanomaterials, polymer nanomaterials, and bio-recognized modified electrodes brought great attention to electrochemical sensors and biosensors in human body fluidic components. There are several challenges that need to be addressed before the realization of wearable flexible electronics, especially for the physical and chemical sensors.

Most recent wearable electrochemical and bio-sensing devices can measure a limited range of biomarkers. The multi-analyte measuring is essential for tracking health under several dynamic conditions, and up to date, all efforts are continued toward single and simultaneous monitoring. Additionally, the most accurate and reliable electrochemical responses are the desires of modern electroanalytical chemistry. Accuracy often hinders bio-fouling effects through non-specific binding, potential contamination from the surroundings, and signal drift (sensor calibration). Another common wireless

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Table 4 A comparative protein and immune assay study by using wearable electrochemical and biosensor[a]

Sensing material	Analyte	Detection range	Detection limit	Method of detection	Bio-fluid	Ref.
Ti_3C_2Tx MXene/LBG/PDMS	Cortisol	0.01–100 nM	88 pM	EIS	Sweat	204
CCY-Fe_2O_3-anti-C_{mab}	Cortisol	2.75×10^{-15} to 2.75×10^{-6} M	1.38×10^{-17} M	CV	Sweat	36
ZnO SAM/DTSS/ssDNA	Cortisol	1–256 ng mL^{-1}	N/A	EIS	Sweat	206
Graphene/C-Mab	Cortisol	1–40 ng mL^{-1}	10 pg mL^{-1}	Conduc	Tears	207
Graphene-Nafion	Cytokines (IFN-γ)	0.015–250 nM	740 fM	FET	Sweat	209
Graphene/PMMA/PASE	Cytokines (IFN-α, IFN-γ)	N/A	2.75 PM and 2.89 PM	FET	Artificial tears	210
PANI paper based electrode	IFN-γ	500–20000 pg mL^{-1}	106 pg mL^{-1}	EIS	Serum	208
PANI/G-paper based electrode	IFN-γ	5–1000 pg mL^{-1}	3.4 pg mL^{-1}	EIS	Serum	208
GFET/PASE or Nafion	IL-6, TNF-α, IFN-γ	N/A	IL-6: 6.11 fM; TNF-α: 6.08 fM., IFN-γ: 4.76 fM	FET	Sweat, tears, saliva, serum, urine	209, 210, 218 and 219
AJP graphene IDE/PI	IFN-γ and IL-10	0.1–5 ng mL^{-1}	25 pg mL^{-1}	EIS	Serum	211
		0.1–2 ng mL^{-1}	46 pg mL^{-1}			
Graphene/AgNWs/IgG	MMP-19	N/A	0.74 ng mL^{-1}	FET	Tears	220
AJP graphene IDE/PI	Histamine	56.25 μM to 1.8 mM	30.7 μM	EIS	PBS	221
GO film	Rotavirus	10^3–10^5	10^3	CV	PBS	222
Ag/GOx	Influenza A	10 ng mL^{-1} to 10 μg mL^{-1}	10 ng mL^{-1}	Amp	Sweat	223
Au/MoS_2/Au/PET	Gp 120	0.1 pg mL^{-1} to 10 mg mL^{-1}	0.066 pg mL^{-1}	SWV	PBS	224
Anti-AFP/chitosan/rGO	AFP	0.001–100 ng mL^{-1}	0.001 ng mL^{-1}	DPV	PBS	213
Anti-CA125/chitosan/r-GO	CA125	0.001–100 ng mL^{-1}	0.001 ng mL^{-1}	DPV	PBS	213
Anti-CA153/chitosan/r-GO	CA153	0.005–100 ng mL^{-1}	0.005 ng mL^{-1}	DPV	PBS	213
rGO/Thi/Au NPzs	CA125	0.1–200 UmL	0.01 U mL	DPV	Serum	213
Anti-CEA/chitosan/r-GO	CEA	0.005–100 ng mL^{-1}	10 pg mL^{-1}	DPV	PBS	213
(NH_2-G)/thionine/Au NPs	CEA standard	50 pg mL^{-1} to 500 ng mL^{-1}	10 pg mL^{-1}	DPV	PBS	214
BSA/ANTI-FTH/GO/SPGE	Ferritin	1–1000 pg mL^{-1}	0.19 ng mL^{-1}	DPV	Serum	225
TR-GO	Anti-IgG	0.3–7 μg mL^{-1}	10 μg mL^{-1}	EIS	PBS	226
rGO/Au NPs	*E. coli*	1.5×10^2 to 1.5×10^7 cfu mL^{-1}	1.5×10^2 cfu mL^{-1}	EIS	PBS	227
Graphene/AMP	*E. coli*	N/A	Single bacterium	N/A	Saliva	228
SPE/DTSSP/antibodies	IL-1β, IL-6, IL-8, IL-10, TNF-α, CRP	0.2–200 pg mL^{-1}	N/A	EIS	Sweat	217
SPE/DSP/anti-NPY	Neuropeptide Y	10–500 pg mL^{-1}	N/A	EIS	Sweat	212
GFET/Pi stacked PBASE	Neuropeptide Y	1 pM to 10 μM	N/A	FET	Sweat	215 and 229
Au/TNF-α	Peptides	0.1 pM to 00.1 μM	N/A	Amp	Human serum	216
Textile/Zn NRs/PANI	Pesticide	N/A	N/A	Poten	Body fluid	217

[a] Abbreviations: Amp-amperometric, EIS-electrochemical impedance spectroscopy, CV-cyclic voltammetry, Conduc-conductometric, FET-field effect transistor, Poten-potentiometric, rGO-reduced graphene oxide, PANI-polyaniline, NRs-nanorods, SPE-screen printed electrode, BSA-bovine serum albumin, NW-nanowires, LBG-laser-burned graphene, PDMS-polydimethyl siloxane, IgG-immunoglobulin G, PASE-1-pyrenebutanoic acid succinimidyl ester, PMMA-polymethyl methacrylate, ssDNA-single-stranded DNA.

electronic printed circuit board is used as a flexible wireless platform. Update is necessary and it can be performed by establishing a high level of integration with the biosensor platform. Furthermore, according to the market analysis report (report ID: GVR-2-68038-154-2), the global wearable sensor market value will reach $2.86 billion by 2025. This market price

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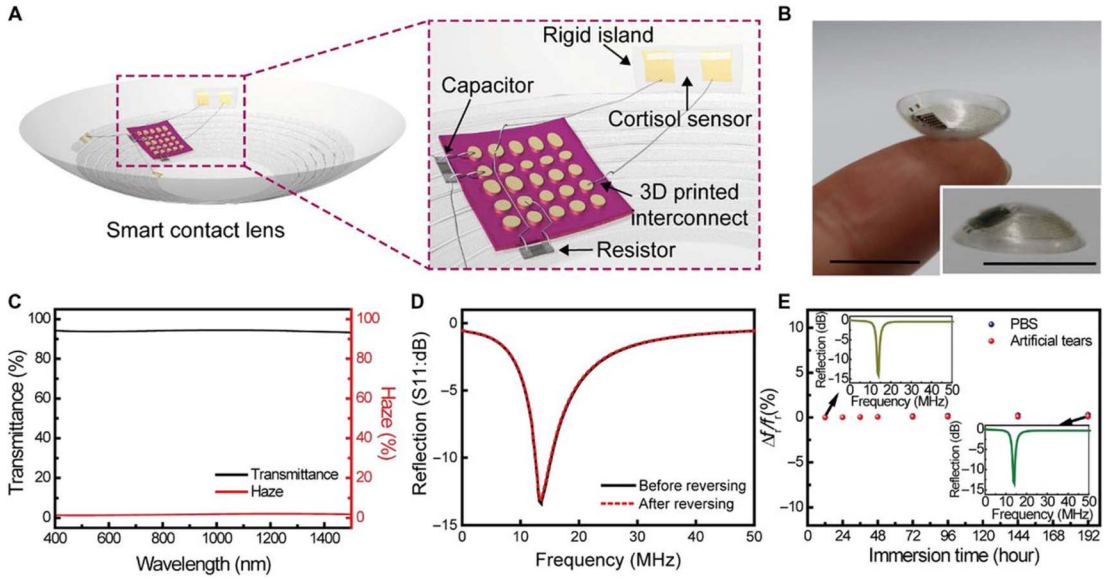

Fig. 8 Wearable contact lens packaging, (A) smart contact lens integrated with three-dimensional interconnects, the sensor on the rigid island. A capacitor and resistor were interconnected for resonance frequency and reference resistance. (B) Fabricated smart contact lens, (C) optical transmittance and haziness of hybrid material, (D) after and before radiation characteristics of the stretchable antenna, (E) relative resonance frequency in PBS and artificial tears up to 192 hours (inset: radiation characteristics of antenna in artificial tears for 12 and 192 hours). Adopted from ref. [208] with permission from Elsevier, Copyright© 2019.

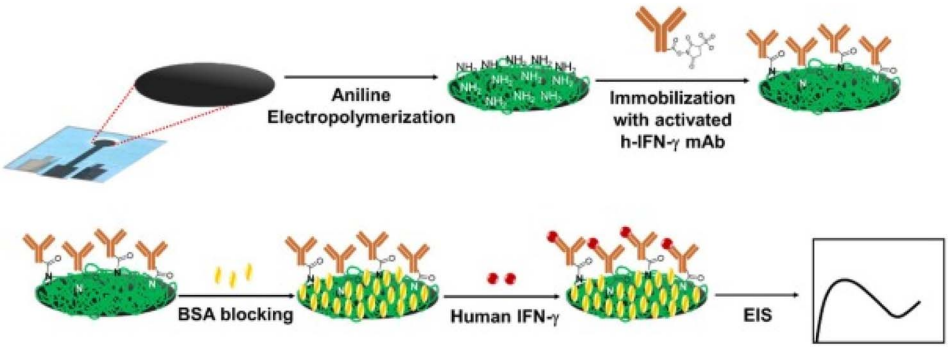

Fig. 9 Fabrication of Human Interferon-gamma (IFN-γ) immunosensor for the detection in serum samples. The figure is adopted from [212] with permission from The Royal Society of Chemistry, Copyright© 2020.

majorly depends on the wearable biosensor platform successfully integrated with the electrode modification material selection and finding out proper fabrication routes.

Proper installation of mobile devices and smartphone-based wireless platforms, including algorithm-based applications, is expected to facilitate the successful translation of wearable biosensors and proof-of-concept demonstration. Moreover, wearable biosensor technologies can improve human health and performance by monitoring and taking physiological treatment in human dynamic life. Furthermore, radiofrequency or bluetooth-based identification with wearable physical and chemical sensors will facilitate data transmission from users to cellphones/computers.[230–232] The goal is to make a wearable flexible, decomposable, low-cost, high-performance, reliable, catalytic, highly conductive, and porous nanostructured working electrode with a suitable wireless installation that can address the demand of the next generation. Such future wearable electrochemical sensors and biosensors will non-invasively monitor a wide range of biomarkers including ions, drugs and toxins, metabolites and biomarkers, proteins and immune assays. These advances will reach a multidisciplinary collaboration in nano-engineering, bioengineering, electronics, and medical communications.

5. Conclusions

Wearable sensor technology is evolving in a remarkable way. The ability of these devices to extract quantitative and innovative information in real-time, especially selective detection with limited funds, enhanced the advancements in fields such as nanotechnology and internet-based point of care, revolutionizing user health, well-being, and safety practices. In

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electrochemical sensors, the production of these wearable platforms has drawn significant attention to wearable approaches. This is due to the fact that such techniques enable the production of fully integrated devices in a miniature, adaptable, and durable manner, enabling direct analysis of the human body and processing the wireless data transmission to a portable device. Although wearable sensors have largely been developed for health and fitness purposes, these technologies are crucial for many different scientific and industrial domains. From the perspective of practical applications and commercialization, little attention has been given to crucial and fundamental processes that will lead to the device's ultimate goal of leading smart life.

Conflicts of interest

There is no conflict of interest.

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