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REVIEW

Flexible Electrochemical Biosensors for Healthcare Monitoring

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As the interest in wearable devices has increased recently, increasing biosensor flexibility has begun to attract considerable attention. Among the various types of biosensors, electrochemical biosensors are uniquely suited for the development of such flexible biosensors due to their many advantages, including their fast response, inherent miniaturization, convenient operation, and portability. Therefore, many studies on flexible electrochemical biosensors have been conducted in recent years to achieve non-invasive and real-time monitoring of body fluids such as tears, sweat, and saliva. To achieve this, various substrates, novel nanomaterials, and detection techniques have been utilized to develop conductive flexible platforms that can be applied to create flexible electrochemical biosensors. In this review, we discussed recently reported flexible electrochemical biosensors and divided them into specific categories including materials for flexible substrate, fabrication techniques for flexible biosensor development, and recently developed flexible electrochemical biosensors to externally monitor target molecules, thereby providing a means to noninvasively examine cells and body fluid samples. In conclusion, this review will discuss the materials, methods, recent studies, and perspectives on flexible electrochemical biosensors for healthcare monitoring and wearable biosensing systems.

1. Introduction

Since the development of smartphones, wearable devices have received considerable attention as they can be utilized for multiple applications by connecting and communicating with a smartphone in real-time.[1] Particularly, communication between wearable devices and smartphones enables the monitoring and analysis of signals obtained from our body and many personalized wearable devices for sports and medical applications have been developed.[2] To improve the detection performance and suitability of wearable devices, flexibility is a key factor for the development of each component, including flexible displays and batteries that can be used in wearable devices.[3,4,5]

Among the many wearable device research fields, biosensor applications have great potential due to the wide possibility for personalized diagnosis and patient-specific analysis[6]. Biosensors are analytical devices that can detect target molecules such as small molecules, chemicals, or harmful biomolecules.[7-9] To detect these target molecules, the interaction or specific binding between target molecules and sensing probes such as enzymes, nucleic acids, or antibodies is analyzed through various approaches such as electrochemical, colorimetric, fluorescent, and plasmonic techniques.[10-13] From a biosensor standpoint, early and precise detection of target molecules is an important goal, which can be achieved and strengthened through the introduction of a wearable device that can be used for personalized diagnosis and patient-specific analyses.[14] Based on this perspective, granting flexibility to biosensors has attracted much attention because it is the basic requirement for wearable biosensor development. However, some limitations may be encountered depending on biosensor types. For example, although most analysis techniques have advantages for biosensor development, biosensors based on surface-enhanced Raman spectroscopy (SERS) have the restriction of not being portable, which is an essential requirement for wearable devices. Moreover, colorimetric biosensors have sensitivity limitations, which makes them unsuitable to identify extremely small amounts of molecules through the naked eye without specialized analysis techniques.[15-17] Due to these limitations, selecting suitable techniques for the development and operation of flexible biosensors is required in order for these technologies to be applied to wearable biosensors in the future.

Among the various types of biosensors, electrochemical biosensors are uniquely suited to meet all the requirements for flexible biosensor development. An electrochemical biosensor detects electrical or electrochemical signals derived from the reaction between target molecules and sensing probes.[18-21] An advantage of electrochemical biosensors is that various electrochemical analysis techniques can be utilized depending on the environment and target molecules such as cyclic voltammetry (CV), differential pulse

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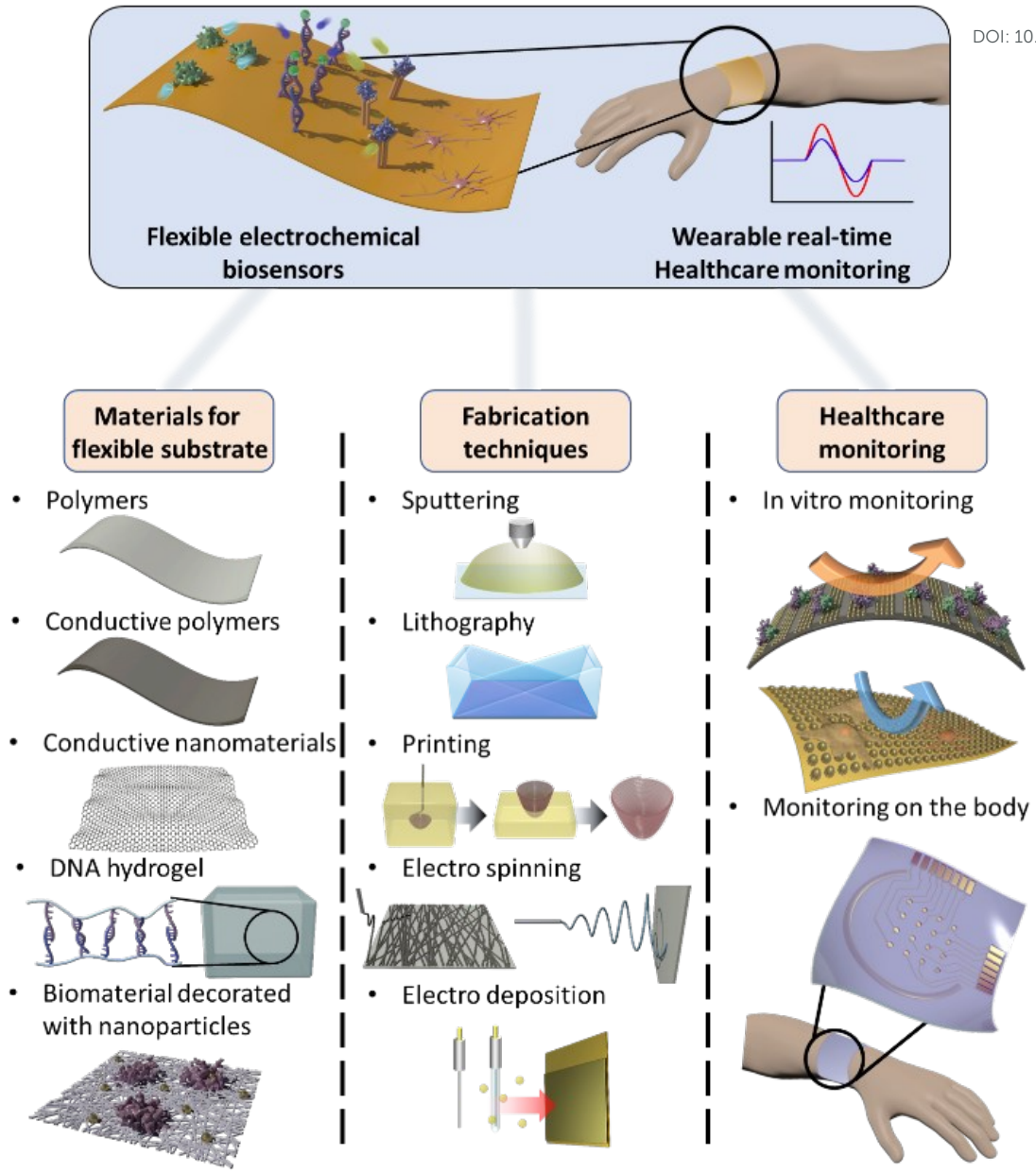

Fig. 1 Development of flexible electrochemical biosensors for healthcare monitoring.

voltammetry (DPV), amperometric techniques, and electrochemical impedance spectroscopy (EIS).[22,23] Furthermore, these sensors also feature a fast response, high sensitivity, inherent miniaturization, convenient operation, and portability, all of which makes them especially suitable for the development of flexible biosensors.[24-28] However, to develop flexible electrochemical biosensors, conductivity should be considered in addition to flexibility. Moreover, studies on flexible electrochemical biosensors could begin with the fabrication of the flexible conductive substrates themselves. To achieve this, various materials and methods such as polymer substrates coupled with novel nanomaterials, highly conductive polymers, or printing techniques have been proposed to fabricate flexible conductive substrates or develop electrochemical analysis systems on flexible substrates.[29-32] Based on these efforts, several flexible electrochemical biosensors have been recently studied.

In this review, we will discuss flexible electrochemical biosensors along with a selective overview of recent studies. Especially, this review will address the materials used for the development of flexible substrates, techniques for the development of flexible electrochemical biosensors, and recently developed flexible electrochemical biosensors. Moreover, recently reported flexible electrochemical biosensors will be classified and discussed depending on the monitored environments including external monitoring, cells, and samples obtained through the attachment of biosensors directly onto the body.

2. Materials for Flexible Substrate Design

To date, various substrate materials such as gold, silicon, glass, or indium tin oxide (ITO) have been used as biosensor templates.

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However, flexible substrates are essential components for the development of flexible biosensors. Polymer materials have been recognized among the most suitable candidates for flexible substrate development and have been utilized to develop flexible biosensors. Additionally, both conductivity and flexibility are important factors to consider when developing electrochemical biosensor substrates. Notably, recent studies have focused on granting conductivity to flexible substrates to increase electrochemical biosensor sensitivity. To achieve this, various novel materials have been introduced to nonconductive polymer substrates or utilized as flexible conductive substrates on their own. In this chapter, we will discuss the various materials used for flexible substrates by classifying them into polymers, conductive polymers, conductive nanomaterials, and other materials (e.g., biomaterials).

2.1 Polymers

A polymer is a large-sized macromolecule composed of numerous repeating subunits. Although there is a difference in the degree of flexibility depending on the length of the branches, polymer materials generally have flexible characteristics.[33,34] This property is a clear advantage of polymer materials, which clearly distinguishes them from the rigid substrates that were formerly widely used. Moreover, most polymer materials are light-weight and inexpensive, both of which are strongly demanded characteristics in the industry. Therefore, polymer materials have been massively implemented in the field of flexible electronics.[35,36] In this way, the application of polymer materials as substrates has resulted in the development of flexible polymer transistors and flexible diodes.[37,38]

Based on these advantages, polymer materials have been actively studied to be applied to biosensors recently. Many readily available and widely used polymers such as polyethylene terephthalate (PET) and polyimide (PI) are being actively studied as substrates for flexible biosensor fabrication. Optical glucose biosensors have been developed using phenylboronic acid functionalized-hydrogels capable of sensing glucose through their affinity to diol-containing molecules (e.g., cis-diols) in glucose molecules.[39] Therefore, upon glucose addition, a glucose-induced volumetric change occurs in the hydrogel system and is easily detected via optical approaches. Moreover, flexible enzymatic biosensors for lactate detection have been developed using polycarbonate (PC) as substrate (Figure 2a).[40] Furthermore, a flexible SERS sensor was developed using gold nanostar-embedded polydimethylsiloxane (PDMS) film as a highly sensitive flexible substrate.[41]

As mentioned above, various polymer materials have been utilized in addition to the polymer materials mentioned here for the development of flexible biosensors based on diverse techniques including SERS and fluorescence.[42,43] However, most polymer materials have nonconductive properties that hinder the development of flexible electrochemical biosensors by only using polymer materials as substrates. To solve this limitation, synthesis of conductive polymers or the addition of novel conductive materials on the polymer materials, which will be covered later, have been studied to be applied to flexible electrochemical biosensors.

2.2 Conductive Polymers

To compensate for the nonconductive qualities of general polymer materials, conductive polymers have become the focus of increasing attention as an alternative solution for the development of flexible conductive substrates.[44,45] Conductive polymers are organic polymers that contain aromatic rings or double bonds as their main chains,[46] and most of them can be synthesized by chemical synthesis processes or electro-copolymerization.[47] These polymers possess metallic conductivity or semiconducting properties that can be utilized to develop flexible electronics. For example, efficient flexible solar cells were developed using the conductive polymer poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS), and various polypyrrole (PPy)-based electrodes have been reported for energy storage applications.[48-50]

In the biosensor field, conductive polymers have specific advantages for the development of electrochemical biosensors.[51] Conductive polymers provide an effective matrix for immobilization or entrapment of biomolecules, as well as a fast electron transfer mediator. Additionally, conductive polymers provide both conductivity and limited flexibility. Despite their mechanical limitations (e.g., brittleness) compared to normal polymer materials, conductive polymers possess flexibility compared to rigid substrates. Therefore, many conductive polymer-based flexible electrochemical biosensors have been proposed. PEDOT:PSS, one of the most widely used conductive polymers, was used as an electron transfer mediator for flexible fibroin support to develop a flexible electrochemical dopamine biosensor (Figure 2b).[52] Furthermore, an electroconductive film composed of a polyvinylaniline (PVAN) and polyaniline (PANI) bilayer was constructed to develop a flexible electrochemical biosensor for the detection of dopamine released from dopaminergic neurons.[53]

Nonetheless, the use of conductive polymers alone has limitations for the development of highly sensitive flexible electrochemical biosensors because there is a limit to their biosensing capacities. Therefore, to improve the properties of conductive polymers, the introduction of other materials as the supporting materials to the conductive polymers is now under study. For instance, the iron oxide (Fe_2O_3) or nickel oxide (NiO) nanoparticles were used as the supporting materials to be hybridized with conductive polymers to enhance the flexible electrochemical biosensor performance.[54,55] These materials have advantages for combining with conductive polymers because of easy for synthesis and surface modification, and commercialization hugely. Still, countless studies are undergoing to compensate for the mechanical defects of conductive polymers and improve the properties of the conductive polymers for flexible electrochemical biosensor applications.

2.3 Conductive Nanomaterials

Even though their nonconductivity limits the development of flexible electrochemical biosensors, polymer materials are still the best candidates for the development of flexible substrates. Therefore, conductive polymers or combined materials composed of conductive polymers with various conductive nanomaterials have been studied to grant the high conductivity to the polymer materials.[56,57]

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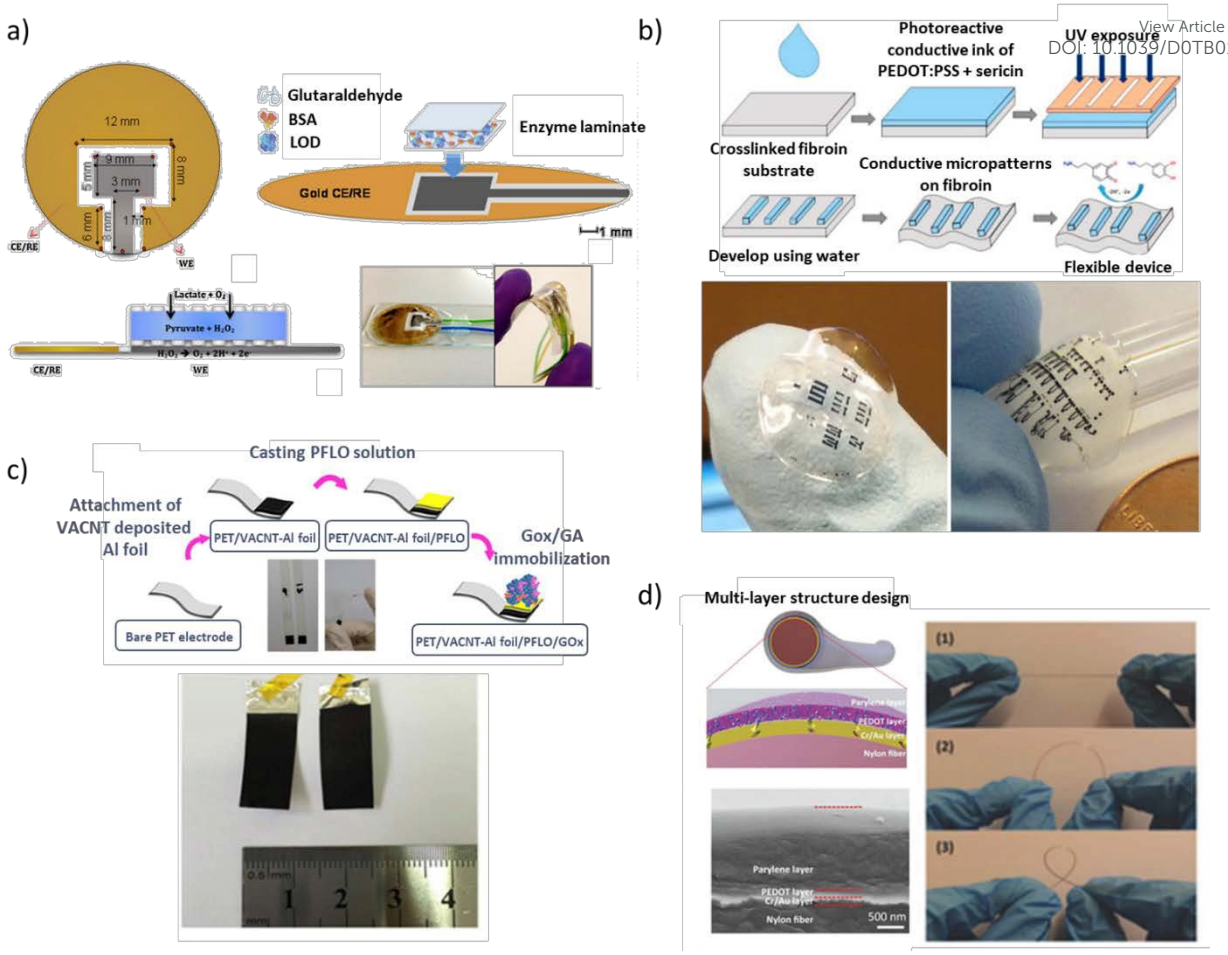

Fig. 2 (a) Schematic diagram and images of a flexible enzymatic lactate biosensor developed on a flexible PC substrate from ref. 40. © 2016 Elsevier B.V. All rights reserved, (b) schematic diagram of the fabrication process of a PEDOT:PSS conductive micropattern on a flexible fibroin support, and image of fabricated flexible micropattern from ref. 52. Copyright © 2016 Elsevier B.V. All rights reserved, (c) schematic diagram of the fabrication process of a flexible enzymatic glucose biosensor prepared by using a hybrid film composed of vertically aligned CNT on a PET substrate, and the photograph of the CNT grown on the flexible substrate from ref. 69. © 2016 Elsevier Ltd. All rights reserved, (d) the overall structure and SEM image of the core-shell structure of conductive nylon fiber, and images of the conductive nylon fiber device with different bending directions from ref. 88. © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Among the various conductive nanomaterials, the conductive nanoparticles including the most widely known gold nanoparticles (GNP) and silver nanoparticles (SNP) have been extensively studied to determine their effectiveness in combination with conductive polymers for the development of flexible electrochemical biosensors by compensating the limitation of conductive polymers.[58,59] In addition to nanoparticles, carbon-based nanomaterials such as graphene and carbon nanotubes (CNT) have the huge potential for developing the flexible substrates with the excellent conductivity for electrochemical biosensors.[60,61] Carbon-based nanomaterials have received attention in biological fields because of their exceptional properties including biocompatibility and conductivity.[62-64] These advantages are also applicable for the development of biosensors, and therefore various types of carbon-based nanomaterials have been used as the core components for developing flexible and conductive substrates. For instance, reduced graphene oxide (GO) was electrodeposited on PEDOT:PSS to create a flexible electrochemical microelectrode,[65] and CNT was coated with PEDOT to develop a superior conductive and flexible layer for biosensor application.[66] Furthermore, to maximize the benefits of conductive polymers, nanoparticles and carbon-based nanomaterials have been recently jointly implemented in conductive polymers.[67,68] In addition, Hybrid films composed of carbon-based nanomaterials and nonconductive polymer materials including vertically aligned CNT on PET (Figure 2c) [69] and CNT networks embedded in PDMS[70] have been reportedly used for the development of flexible electrochemical biosensors.

Furthermore, novel conductive nanomaterials have been extensively discovered and studied recently, particularly to improve their intrinsic characteristics beyond those of carbon-based nanomaterials. Among them, transition metal dichalcogenides (TMD) nanomaterials are of interest due to their exceptional properties including their direct bandgap and high conductance, which make them suitable for diverse applications in biosensors and bioelectronics.[71,72] Similarly, TMD nanomaterials have been recently combined with nonconductive polymer materials to improve the flexible substrate conductance. Choi et al. reported flexible electrochemical biosensors based on molybdenum disulfide (MoS_2) nanoparticles modified on PI and PET for the detection of glucose and gp120 protein (i.e., an HIV-1 surface protein).[73,74] Moreover, other types of excellent TMD nanomaterials have been combined

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with polymer materials to develop superior flexible substrates such as tin diselenide ($SnSe_2$)-modified cellulose films and tungsten diselenide (WSe_2)-modified PI films.[75,76]

Additionally, studies on the development of flexible and conductive substrates using exclusively nanomaterials have been recently conducted. To make flexible substrates composed exclusively of conductive nanomaterials, one or more than two different nanomaterials are combined and cross-linked to form networks to create a template.[77,78] For example, flexible and conductive hybrid films composed of CNT and graphene nanoplatelets were prepared by sonication through a vacuum filtration process.[79] The prepared hybrid film without any polymeric substrates exhibited excellent conductivity and flexible characteristics. As discussed here, conductive nanomaterials show a huge potential for the development of flexible substrates such as being able to be combined with polymers or act as flexible electrodes by themselves.

2.4 Other Materials

Unlike the flexible substrates seen so far, fewer studies on the fabrication of novel flexible substrates using biomaterials or organic materials have been conducted. Naturally synthesized biomaterials such as silk fibroin and cellulose have tremendous potential as flexible substrates for flexible electrochemical biosensors due to their unique properties including biocompatibility and mechanical characteristics.[80] Therefore, various novel biomaterial-based flexible substrates have been proposed including engineered protein-based elastin-like substrates and silk fibroin hybridized with Kevlar (i.e., a synthetic polymer) for the creation of flexible nanobiocomposites.[81,82] Among the various biomaterials that could be potentially used as flexible substrates, bacterial cellulose, and nanofibrous biomaterials have been extensively studied due to their advantages, including excellent mechanical properties and production possibilities in various forms.[83] Similarly, in situ self-polymerized dopamine (polydopamine) on bacterial cellulose was proposed as a flexible conductive bioelectrode to monitor electrophysiological signals from the body.[84] Additionally, GNP- and CNT- decorated conductive bacterial cellulose films were reported for electrochemical glucose detection.[85] Moreover, DNA-based hydrogels also have suitable characteristics for the development of flexible substrates by itself.[86] In addition to biomaterials, fibers have also been used as flexible substrates, including synthetic fibers such as nylon or natural fibers such as cotton fabric (Figure 2d).[87,88]

3. Fabrication Techniques for Flexible Electrochemical Biosensing Platforms

In the previous chapter, we discussed the various materials used to create flexible electrodes. However, the fabrication of flexible substrates is just the beginning step in the demonstration of flexible electrochemical biosensing systems. To develop flexible electrochemical biosensors, additional processes are required to achieve a highly efficient flexible electrode conductivity to enhance biosensor sensitivity and the construction of electrode systems without interference between each of the introduced electrodes for the investigation of electrochemical reactions. Furthermore, the introduction of two- or three-electrode systems directly on flexible substrates would facilitate the efficient operation of electrochemical sensing systems to be applied for wearable biosensing systems. To achieve these goals, several delicate techniques have been utilized to create electrochemical biosensing systems, including lithography and printing tools. In this chapter, we will comprehensively discuss various techniques used for the development of electrochemical biosensing systems on flexible electrodes, from simple and easy sputtering techniques to three-dimensional (3D) printing techniques.

3.1 Sputtering

Although various factors are required for the operation of electrochemical biosensors, conferring conductivity is the most important optimization for the implementation of flexible electrochemical biosensors. Among the various methods used to impart conductivity, the sputtering of conductive metals on nonconductive flexible substrates can provide a simple, easy, and effective method to establish an electrochemical biosensor system. The sputtering technique is based on physical phenomena for conductive layer formation. Specifically, conductive metal layers can be uniformly formed on nonconductive substrates by using extracted metal elements from the surface of metal targets through a process of plasma or gas energetic particle bombarding onto the substrate surface.[89,90] This technique has been widely applied to form a thin metallic conductive layer in various fields, including the development of battery electrodes and biosensors.[91-93] In addition to these applications, the sputtering technique has also been introduced to develop efficient electrochemical biosensors with high conductivity via the deposition of novel metals on the electrode.[94,95]

Several studies have employed the sputtering technique to develop flexible electrochemical biosensors due to its simple implementation for the creation of conductive layers compared to other techniques. To develop a highly sensitive and flexible electrochemical biosensor, indium oxide (In_2O_3) was reportedly sputter-deposited on a PET substrate to be utilized for glucose level monitoring in body fluids (Figure 3a) [96]. Another study reported the formation of a nickel oxide (NiO) film on a flexible PET substrate via the sputtering technique to develop a flexible lactate biosensor measured with a potentiometric system.[97] Moreover, flexible electrochemical biosensors for the detection of cortisol in sweat have been constructed by sputter-depositing zinc oxide (ZnO) on a polyamide substrate[98]. Furthermore, other types of biosensors for the detection of glucose based on a vertically aligned nickel-CNT on a flexible graphite substrate were prepared via the sputtering technique.[99] Additionally, Choi et al. performed metal sputtering on nonconductive polymer substrates and simultaneously introduced novel TMD nanomaterials (i.e., MoS_2 nanoparticles). The authors created a sandwich structure composed of sputtered gold layers and a spin-coated MoS_2 layer located between the sputtered gold layers to enhance the electron transfer reaction to achieve high biosensor sensitivity to detect glucose and HIV-1 surface protein.[73,74] Nonetheless, the sputtering technique has limitations when implemented for the introduction of two- or three-electrode systems composed of counter or reference electrodes on flexible and conductive electrodes. This is due to its non-specific (i.e., random) deposition properties on the whole area of the deposited

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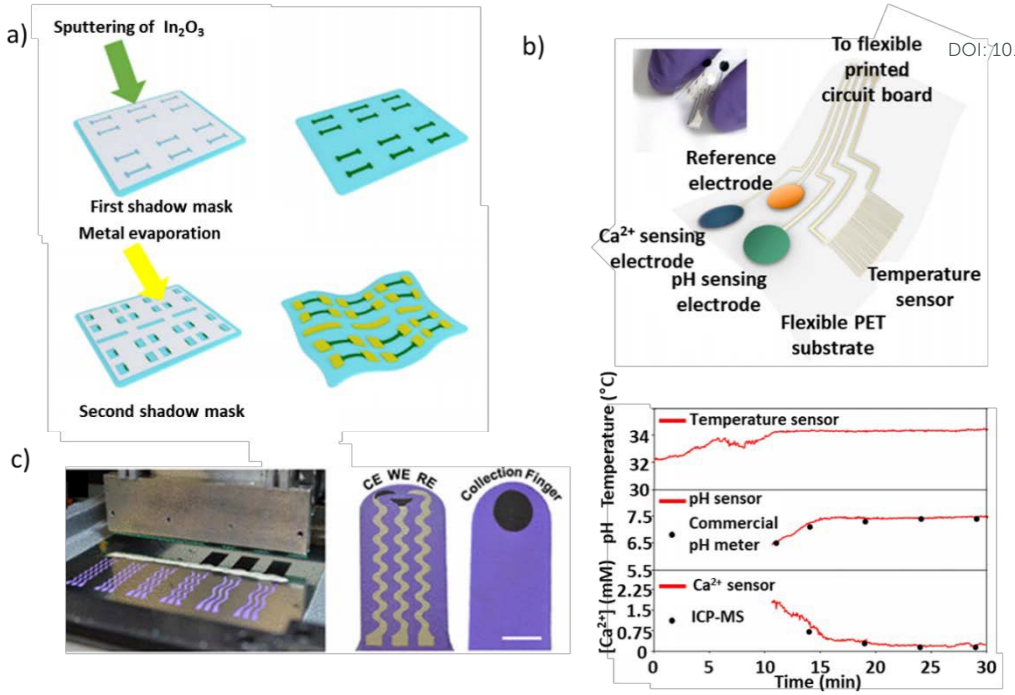

Fig. 3 (a) Schematic image of the sputter-deposition of In_2O_3 on the PET, and the optical images of prepared flexible electrochemical glucose biosensor from ref. 96. Copyright © 2018, American Chemical Society, (b) Schematic image of a flexible biosensor for monitoring the multiple targets containing temperature, pH, and ions developed via photolithography, and its real-time monitoring results for detection of multiple targets from ref. 104. Copyright © 2016 American Chemical Society, (c) fabrication of a flexible glove biosensor composed of a three-electrode system via printing technology from ref. 122. Copyright © 2017 American Chemical Society, further permissions related to the material excerpted should be directed to the ACS.

substrate. Therefore, in order to develop flexible electrochemical biosensors to be ultimately applied for wearable biosensors, other novel techniques for direct introduction of electrode systems on flexible substrates are needed even if they are not as simple and convenient as the sputtering technique.

3.2 Lithography

To establish an electrochemical system directly on flexible substrates or to develop multiple-target monitoring systems on a miniaturized chip without interference between each target monitoring system, there is a need for finer techniques capable of exquisitely granting conductivity on flexible substrates beyond the non-specific deposition that results from sputtering. Such implementations include the arrangement of sophisticated conductive nanoelectrodes.[100] The lithography technique, which has been widely used for silicon-based electronic devices, is a technique that satisfies the above-mentioned requirements.[101]

Among the various current lithography techniques, photolithography uses a photo-sensitive photoresistor that can be patterned by laser exposure through the specific blocking of a predesigned photomask to make a pattern on the substrate. Notably, this technique can be applied for the fabrication of delicate conductive electrodes on flexible substrates.[102] For example, micropatterned photo-sensitive conductive polymer electrodes were formed on a flexible substrate for the electrochemical detection of glucose, and patterned conductive polymer electrodes retained their structure and function after bending.[30] In addition to this research, a flexible electrochemical interleukin-10 biosensor based on a three-electrode system (i.e., working-, counter-, and reference- electrodes) was fully integrated on a PI substrate via the photolithography process.[103] Moreover, a flexible multiple-target sensing platform was reported to simultaneously detect ions, temperature, and pH changes with an electrochemical system integrated on a flexible substrate via the photolithography technique (Figure 3b).[104]

In addition to the photolithography technique, various lithography techniques including electron-beam lithography, soft lithography, or electrochemical lithography are also widely used for the development of flexible electrochemical biosensors due to their unique advantages.[105,106] For instance, flexible silver nanowire films prepared by soft lithography were proposed for the detection of hydrogen peroxide (H_2O_2).[107,108] Similar to the aforementioned studies, lithography techniques have been used to develop elaborate flexible electrochemical biosensors with fully integrated electrochemical systems or to develop biosensors capable of simultaneously detecting multiple targets.

Although the highly-accurate lithography technique is one of the most effective tools for the development of flexible electrochemical biosensors to detect multiple targets or to make a fully integrated circuit system, the operation of delicate lithography techniques has some limitations such as complicated processes, expensive equipment, and high costs. Therefore, there is a need for relatively inexpensive, simpler, and more accessible techniques that can be used widely for the development of flexible electrochemical biosensors. Therefore, several studies including the utilization of recent lithography techniques such as the salt impregnated inkjet maskless lithography (SIIML), and etching inkjet maskless lithography (E-IML) are being conducted to compensate for the limitations of

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lithographic techniques and to enhance its performance, thereby further enabling the development of highly efficient flexible electrochemical biosensors. [109,110]

3.3 Printing

Recently, printing has received increasing attention in various fields because it can provide a useful method to effectively fabricate delicate structures on any substrate through the spraying of any material that can exist in solution. Additionally, printing has the advantage of being accessible enough to be widely implemented. As the spraying sophistication and velocity of printing develop, various printing techniques have begun to be applied in the fields of biology and medicine, particularly in the biosensor field.[111,112] Given the possibility for accurate printing of biomolecules and novel nanomaterials on substrates to form biocompatible and functional layers to develop miniaturized biosensors, many biosensors have been created using printing technology.[113,114] For instance, printed microfluidic-based biosensing systems have been developed through the formation of polymer layers on substrates using printing (e.g., inkjet printing).[115]

Particularly, the aforementioned advantages of printing technologies enable the precise control of conductive substrate structure for the development of flexible electrochemical biosensors. Various biomaterials and novel conductive nanomaterials can be printed to grant conductivity or establish electrochemical systems on flexible substrates (e.g., paper-based substrates). In one study, SNP ink was printed on a PET substrate using an inkjet printer to fabricate silver inter-digital electrodes for pathogen detection.[116] In another study, a flexible enzymatic biosensor was reportedly used for continuous glucose monitoring.[117] In this research, platinum nanoparticles and reduced GO were directly and effectively micropatterned on the curved surface of a cylindrical polyetheretherketone (PEEK) substrate with a rotating inkjet printer, resulting in an integrated electrode system on the curved substrate. Moreover, printing provides an efficient method to develop disposable paper-based biosensors which are suitable for point-of-care testing (POCT); this has recently attracted growing attention in biosensor field.[118-120] In addition to paper-based biosensors, conductive three-electrode systems composed of working, counter, and reference electrodes have been developed on flexible gloves for the development of wearable lab-on-a-glove systems (Figure 3c).[121,122] Also, 3D printing technology is being actively studied recently and has been recognized as a novel next-generation printing technique. 3D printing has great potential in the field of biology for several applications including tissue engineering due to its unique capacity to create specific three-dimensional biological structures such as artificial tissues or organs.[123] These advantages of 3D printing could now also be utilized in the development of flexible electrochemical biosensors.[124,125]

3.4 Other Techniques

In addition to the three representative fabrication techniques discussed earlier, various other efficient and creative techniques are used for the creation of flexible electrochemical biosensors, although not used as much as the techniques mentioned above. Electrodeposition is a widely used technique that is often used to form highly conductive layers on the surface of conductive or semiconductive substrates by using metal ion solutions and simple electrochemical techniques. Using this approach, platinum nanoparticles were electrodeposited on a flexible graphene-modified PI substrate for the development of an ultrasensitive glucose biosensor.[126] Additionally, iridium oxide (IrOx) was also electrodeposited on electropolymerized PPy film to develop a flexible electrochemical multiparameter sensing platform.[127] Moreover, electrospinning has also been used to form patterned polymer membranes on selected substrates.[128] By coupling this tool with spray-based layer-by-layer deposition techniques, polysulfone fiber mats were developed to serve as flexible substrates, and a CNT-based conductive layer was formed on these flexible mats for biosensor development.[129] As another example, nitrogen-doped carbon fibers were developed to form a free-standing flexible and conductive substrate for glucose monitoring.[130] To develop this platform, polyacrylonitrile (PAN) nanofibers were prepared using electrospinning and carbonized thereafter. Various other novel techniques have been successfully implemented to date to develop flexible biosensors, including electrochemical/mechanical exfoliation and chemical vapor deposition (CVD) .[131-134]

4. Current Applications of Flexible Electrochemical Biosensors

By implementing the novel materials and fabrication techniques discussed in the previous chapters, numerous flexible electrochemical biosensors have been developed and reported to date. The reported flexible electrochemical biosensors are divided and discussed in this chapter depending on the types of target molecules and the operating environment of the biosensors. More specifically, the studies are divided into two categories: 1) flexible electrochemical biosensors for *in vitro* molecule monitoring and 2) body-attached biosensors for direct monitoring.

4.1 Flexible Electrochemical Biosensors for *In Vitro* Monitoring of Environmental Molecules

The ultimate goal of flexible electrochemical biosensor research is to be applied for the development of wearable biosensors. Once the wearable biosensors are applied to directly monitor target molecules on the body, the noise signals derived from the surroundings and other undesired molecules should be removed, and the selective detection of target molecules should be achieved through compensation by taking advantage of the high sensitivity of the biosensor. Therefore, current studies on flexible electrochemical biosensors largely focus on sensitivity and selectivity maximization, as well as retention of stability and flexibility in various physical conditions. The main target molecules of flexible electrochemical biosensors are small biological molecules that can adversely affect health (e.g., glucose and lactate) and are associated with specific diseases. So far, many externally measurable glucose biosensors

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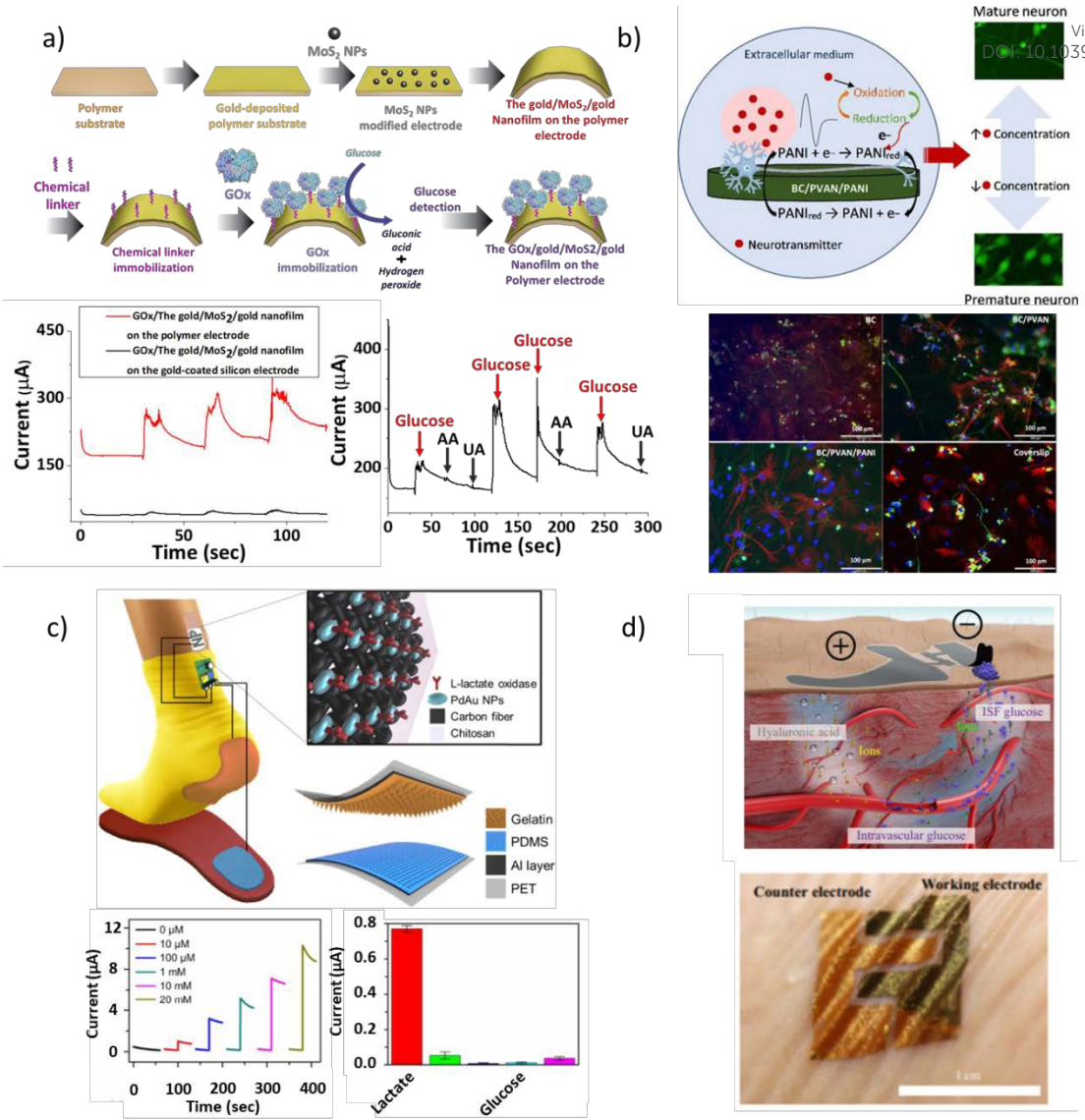

Fig. 4 (a) Fabrication process of a glucose biosensor based on a sandwich structure composed of gold/MoS_2/gold nanofilms on a PI substrate via sputtering and spin-coating technologies and the glucose sensing performance of the proposed flexible biosensor from ref. 73. © 2019 Elsevier B.V. All rights reserved, (b) conductive PVAN/PANI bilayer-modified flexible bacterial cellulose film for the monitoring of neurotransmitters secreted from neural stem cells during stem cell differentiation, and fluorescence images of differentiated cells grown on flexible substrates from ref. 53. Copyright © 2019, American Chemical Society, (c) schematic images of a self-powered electrochemical lactate biosensor composed of two different flexible electrochemical systems (one system for energy harvesting and the other one for lactate detection), and its superb lactate sensing performance with high selectivity from ref. 146. © 2017 Elsevier Ltd. All rights reserved, (d) schematic process of the ETC system for glucose monitoring in the blood, and a picture of the developed wearable biosensor on the body from ref. 151. Copyright © 2017 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works. Distributed under a Creative Commons Attribution Noncommercial License 4.0 (CC BY-NC).

have been commercialized, but there remains a need for flexible glucose biosensors that can be used for real-time monitoring of glucose levels in the body to prevent diabetes mellitus. Therefore, various flexible glucose biosensors have been recently reported.[57,117] To greatly enhance flexible biosensor sensitivity, Choi et al. introduced MoS_2 nanoparticles to form a sandwich structure composed of gold/MoS_2/gold nanofilms on a PI substrate.[73] Sputtering was used to easily and rapidly construct the sandwich structural film, which resulted in gold layers and a spin-coating MoS_2 layer (Figure 4a). After the formation of the sandwich film, GOx was modified on the film to serve as a glucose-sensing probe. The prepared flexible biosensor exhibited an excellent glucose sensing performance (detection limit: 10 nM) with high selectivity and retained its structure and function after repeated bending. Moreover, carbon nanomaterial-based flexible electrochemical glucose biosensors have been recently developed by taking advantage of the highly conductive properties of carbon nanomaterials such as graphene and CNT.[126,135] Additionally, flexible electrochemical biosensors based on hybrid nanomaterials composed of platinum nanoparticles and 3D porous graphene were developed to simultaneously monitor multiple targets (e.g., glucose, pH, and electrocardiographic signals), which can be applied to the

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creation of wearable smart devices.[136] In addition to glucose biosensors, a flexible electrochemical lactate biosensor was also developed by implementing LOx immobilized on a flexible graphene nanowall-modified copper foil with an integrated three-electrode system.[137] To develop this integrated system, the CVD technique was introduced to make the graphene nanowall-modified copper foil on the PET substrate, and printing was used to create the three-electrode system directly on the flexible substrate. The newly developed lactate biosensor exhibited excellent stability even after bending and twisting the biosensor. Moreover, a flexible electrochemical alcohol biosensor has been reportedly utilized in many situations.[138] This biosensor could even distinguish between the alcohol contents of different beers with high sensitivity.

Until now, in most studies, target molecules of flexible electrochemical biosensors have been restricted to the glucose and lactate because these molecules can be easily accessed and monitored on the body by using the wearable biosensor which is the permanent goal of flexible electrochemical biosensors. However, to diversify the target molecules variously, studies to develop the flexible electrochemical biosensors for *in vitro* monitoring the other molecules have been conducted recently which can diversify the target molecules of wearable biosensors in the future.

For example, the flexible electrochemical biosensor was reported for monitoring the heavy metal ions including the lead (Pb), and cadmium (Cd) in the food.[139] As another example, flexible electrochemical biosensor was proposed for detection of Parkinson's disease biomarkers, the dopamine and the Parkinson's disease protein 7 (PARK7/DJ-1).[140] In addition, the flexible electrochemical H_2O_2 biosensor was developed based on the laser scribed graphene and SNP hybrid composite by laser irradiation.[141]

In addition to these researches for monitoring the other target molecules, flexible electrochemical biosensors can be applied to monitor the states of living cells *in vitro*. Living cells can be affected by their surrounding conditions such as the microevironment and niches that influence the interaction between cells and substrate, as well as secreted substances from affected cells. Therefore, flexible substrates that can finely control the surface environment are needed to investigate the response from living cells[142]. To this end, some studies have implemented flexible electrochemical sensing to detect cell-secreted molecules such as dopamine and the tumor necrosis factor-α (TNF-α) cytokine[143,144]. Recently, a conductive PVAN/PANI bilayer-modified flexible bacterial cellulose film was developed to monitor neurotransmitters secreted by neural stem cells under differentiation grown on a flexible substrate.[53] Based on the dual functionality of this flexible biosensor for cell growth and monitoring, the differentiation of neural stem cells was electrochemically monitored directly on the flexible biosensor (Figure 4b).

As discussed above, various studies on flexible electrochemical biosensors are currently being actively conducted, and these studies are expected to be utilized as the core technology for the development of highly sensitive wearable biosensing systems in the future.

4.2 Flexible Electrochemical Biosensors for Target Molecule Monitoring on the Body

To date, a few flexible electrochemical biosensors capable of monitoring target molecules directly on the body have been reported. Flexible biosensors specifically developed to be worn directly on the body must be biocompatible and noninvasive. Additionally, a superior sensitivity is required to monitor target molecules in body secretions such as blood, tears, and sweat. Therefore, these biosensors have been built into specific wearable structures such as gloves, mouthguards, wristbands, and adhesive plasters that can be easily attached to the body with high sensitivity and biocompatibility.[122, 145] In addition, due to the current limitation of molecules available as the target molecules included in real samples such as tears and sweat, most target materials of flexible electrochemical biosensors for monitoring the targets on the body are restricted to the most accessible and easily measurable targets such as the glucose and lactate in most studies.

One research group reported a wearable and disposable glove-based electrochemical biosensor to detect fentanyl and synthetic opioids.[122] To develop this biosensor, the authors used 3D-printed molds to create a three-electrode system on the glove, and using screen printing technology, a three-electrode system composed of a reference electrode (printed Ag/AgCl ink layer), a counter electrode (printed carbon ink layer), and a working electrode (printed carbon ink layer) was fabricated. Then, a nanomaterial composite made of CNT, polyethyleneimine (PEI), and an ionic solution (IL, 4-(3-Butyl-1-imidazolio)-1-butanesulfonate) was printed on the working electrode to enhance the sensitivity of the biosensor. The developed biosensor exhibited excellent and rapid fentanyl detection performance (detection limit: 10 μM) prepared in both powder and liquid forms. Similar to this research but with the addition of a self-supplying energy system, a self-powered electrochemical lactate biosensor was developed and hybridized into socks to act as a sensor directly on feet.[146] To demonstrate this novel system, authors developed two different flexible electrochemical systems, one of which was composed of gelatin, PDMS, and an aluminum layer on a PET substrate to harvest biochemical energy from human walking or running when worn directly on the foot, and the other was composed of LOx, palladium-gold (PdAu) bimetallic nanoparticles, carbon fibers, and chitosan for lactate sensing connected to an energy harvesting system (Figure 4c).

A lactate biosensor composed of the two aforementioned systems showed a superb lactate sensing performance by taking advantage of the electricity generated from body activity. This self-powered biosensing system represents a creative approach to develop minimized wearable sensing devices capable of long-term operation, biocompatibility, and without the need for an additional energy supply device on the system. Moreover, tattoo-type wearable biosensors can be easily attached to the body and have been

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reported to be effective for direct and real-time monitoring of important bioindicators.[147,148] Among the various types of structures that can be applied to wearable devices, bandage- or patch-shaped wearable electrochemical biosensors that can be directly and noninvasively attached anywhere on the body are the most actively studied in recent research.[149,150] Among these studies, wearable biosensors based on electrochemical twin channels (ETC) were reported to noninvasively monitor blood glucose.[151] This biosensor was composed of multilayers of the GOx, Prussian blue, gold, PI, and poly(methyl methacrylate) (PMMA). By using the proposed ETC system, which could penetrate hyaluronic acid and withstand glucose refiltration and outward transportation, intravascular blood glucose could be driven out from the vessel, and transported to the surface of the skin where the biosensor was worn (Figure 4d). Based on the advantages of this ETC system, including glucose transportation and noninvasive sensitive monitoring, this biosensor provided an effective means for continuous glucose monitoring for noninvasive clinical applications. In addition to this research, a bandage-shaped biosensor was developed to detect tyrosinase, a cancer biomarker related to melanoma, by using modified microneedle array sensors on the bandage.[152]

In this chapter, we discussed the many types of flexible electrochemical biosensors worn on the body for direct monitoring of target molecules. However, many obstacles must be overcome prior to commercialization of various flexible electrochemical biosensors worn on the body for direct monitoring of target molecules. For example, to diversify the target molecules beyond the glucose and lactate, there is a need to develop more sophisticated and noninvasive flexible electrodes capable of body penetration and achieving the high sensitivity with high signal-to-noise ratio. Nevertheless, because research to improve the sensitivity of *in vitro* flexible electrochemical biosensors are being continuously conducted and research on wearable biosensing systems is ongoing, it is predicted that excellent wearable electrochemical biosensors may be commercialized in the near future.

Table 1 Summary of materials, fabrication techniques, and developed flexible electrochemical biosensors discussed in this review.

Materials	Advantages	Disadvantages	Types	Reference
Polymers	Excellent flexibility, light-weight, inexpensive	Nonconductivity	PC / PDMS / PI / PET	40 / 41 / 73 / 74
Conductive polymers	Flexibility, conductivity, or semiconductivity	Brittleness, limitations in their ability to deliver both excellent conductivity and flexibility	PEDOT:PSS / PPy / PANI	48 /49 /53
Conductive nanomaterial	Confers excellent conductivity to nonconductive polymers	Restricted to being used as the flexible substrate itself	Metal NPs / graphene / CNT / MoS_2 / graphene nanoplatelet	58 / 62 / 69 / 73 / 79

Fabrication techniques	Advantages	Disadvantages	Ref.
Sputtering	Simple, easy, and effective approach to grant conductivity	Non-specific deposition	89, 93, 96
Lithography	Highly-specific patterning, suitable for biosensor miniaturization	High cost, complex processes, requires expensive equipment	104, 106
Printing	Easily accessible, precise control of the 2D/3D structures of the conductive substrates	Applicable only for soluble materials	112, 115, 118, 125

Monitoring condition	Target	Composition of sensor	Fabrication techniques	Substrates	Ref.
In vitro monitoring	Glucose	GOx/Au/MoS_2/Au	Sputtering and spin-coating	PI substrate	73
	Lactate	Lox/graphene nanowall	CVD and screen printing	PET substrate	137
	Stem cell differentiation	PVAN/PANI	Chemical synthesis	Bacterial cellulose membrane	53
Real-time monitoring on the body	Lactate	Lox/PdAu bimetallic nanoparticles	Electrochemical deposition and chemical modification	Carbon fiber/chitosan layers	146
	Glucose	GO/Prussian blue/gold/PMMA	Transfer-printing and electrochemical deposition	PI substrate	151

5. Conclusions and Future Perspectives

Given the widespread interest in wearable devices, including smart devices, wearable biosensing systems have become an important milestone for next-generation biosensors and possess enormous potential for application in the fields of wearable healthcare devices such as electrocardiogram measurement smartwatches and POCT systems. This potential could provide a means to achieve a personalized diagnosis and patient-specific analysis, thereby achieving the fundamental goals of biosensors, that is, the early, precise, and personalized detection of target molecules.

Among the various current biosensor measurement techniques, electrochemical approaches are uniquely suited for biosensing purposes given that they meet all the requirements for flexible biosensor development. Given the variety of electrochemical techniques, including CV, DPV, and EIS, it is possible to select and apply suitable techniques according to the intended application. Additionally, electrochemical techniques provide several benefits, including a rapid response, high sensitivity and selectivity, inherent miniaturization, convenient operation, and portability, all of which make these sensors uniquely suited for the development of flexible biosensors. As a result, several flexible electrochemical biosensors have been extensively studied recently to be applied as wearable biosensor technologies. Several essential requirements must be met to further develop flexible electrochemical biosensors, such as outstanding flexibility and the capacity to impart conductivity onto the flexible substrate. Therefore, studies on the development of flexible conductive substrates have been extensively conducted using novel nanomaterials and fabrication techniques.

This review provides a summary of the current state of flexible electrochemical biosensor technology, including materials, fabrication techniques, and recently reported flexible electrochemical biosensors, along with a selective overview of recent relevant studies, as summarized in Table 1.

Although each material and fabrication technique discussed here has exceptional advantages for flexible electrochemical biosensors, they also have disadvantages that should be overcome as summarized in Table 1. In the case of materials, the low conductivity, brittleness, or hard for achieving both excellent conductivity and flexibility should be solved by studying and developing the novel substrates having both excellent conductivity and flexibility. For this, through the synthesis of new and novel materials and the fusion of materials being studied, it is thought that the advantages of each material can be maximized and the disadvantages can be compensated. Also, in the fabrication techniques, rather than fabricating a substrate by using only one fabrication technique, it is considered that a new fabrication technique can be developed to compensate for the disadvantages of each technique through the simultaneous utilization of two or more fabrication techniques. These approaches will be helpful to overcome and solve the current limitations and challenges.

In addition, even if the most ideal materials and fabrication techniques are developed for substrates, there are still challenges to be solved including the development of adequate energy supply solutions and the capacity of biosensors to operate on the body without the need for additional equipment. These challenges should be solved to develop practical wearable biosensors in the future. To solve these challenges, converging research in the biosensor field and other relevant fields such as electronics and energy may provide ways to overcome these limitations soon. Besides, for commercialization, the mass production system of flexible conductive substrates should be achieved by minimizing the complex synthesis process for novel materials and simplifying the complicated fabrication techniques for biosensors. If these challenges are succeeded, it is believed that wearable electrochemical biosensors can be utilized for personalized POCT systems.

In conclusion, this review provides important insights into the development of flexible electrochemical biosensors and suggests creative guidelines and inspiration to develop accurate, flexible, and practical electrochemical biosensors.

Conflicts of interest

The authors do not have any conflicts of interest to declare.

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