Design and Fabrication of Wearable Biosensors: Materials, Methods, and Prospects

Rajendra Kumar Reddy Gajjala, Sara Muñana-González, Pello Núñez-Marinero, Joseba Totoricaguena-Gorriño, Leire Ruiz-Rubio, and Francisco Javier del Campo

Objects and their manufacture are inseparable. You understand a product if you understand how it´s made.
—Jonathan Ive

1 Introduction and Background

Wearable biosensors are analytical devices intended to provide continuous and personalized information on biochemical parameters in real time. Wearable biosensors originated as tools for better health management, particularly for the monitoring of glucose in diabetes management, but have reached other areas such as sports and fitness, military applications, and environmental monitoring.

The development of wearable biosensors is a highly complex task that involves multidisciplinary teams from areas such as materials science, electronics, biomedical engineering, and information technologies. This is why, despite the recent boom and the increasing reports on wearable biosensors, very few devices (see Table 1) have made it to market.

R. K. Reddy Gajjala · P. Núñez-Marinero · J. Totoricaguena-Gorriño · L. Ruiz-Rubio · F. Javier del Campo (✉)
BCMaterials, Basque Center for Materials, Applications and Nanostructures. UPV/EHU Science Park, 48940 Leioa, Vizcaya, Spain
e-mail: Javier.delcampo@bcmaterials.net

S. Muñana-González · L. Ruiz-Rubio
Grupo de Química Macromolecular, Departamento Química Física, Universidad del País Vasco, UPV/EHU, Campus de Leioa, Vizcaya, Spain

F. Javier del Campo
IKERBASQUE, Basque Foundation for Science, 48009 Bilbao, Spain

K. Mitsubayashi (ed.), *Wearable Biosensing in Medicine and Healthcare*,
https://doi.org/10.1007/978-981-99-8122-9_15

Table 1 Summary of main commercial wearable biosensors to date

Year	Device	References
2000	Glucowatch biographer (EU)	[3, 4]
2006	Dexcom seven (US)	[5]
2014	Abbot Freestyle Libre (EU)	[6]
	Dexcom G4 Platinum	[6, 7]
2016	Senseonics Eversense (EU)	[8]
2017	Abbot Freestyle Libre (US)	[9, 10]
	Dexcom G5 mobile	[6]
	Medtronic Guardian Connect	[11]
2018	Dexcom G6	[12–14]
	Senseonics Eversense (US)	
2021	Supersapiens Abbot Libresense glucose sport	[15]

The most important thing about any wearable biosensor is the information they provide. Biosensor data must be accurate, specific, timely, and reliable. This has important implications in terms of sensor placement, power and data management, and device usability. These are all aspects to consider throughout device development but, in addition to usability and functionality concerns, which can be addressed through frameworks such as REASSURED [1], originated in the area of point-of-care devices as an evolution of the ASSURED criteria [2]. ASSURED criteria stressed accuracy, accessibility and affordability, and REASSURED brings this one step forward by considering real-time connectivity enabled by the Internet of things (IoT), and ease of specimen collection and environmental friendliness. The REASSURED framework thus becomes extremely important for the field of wearable biosensors, as not only the body can be regarded as a "low resource setting", but also because of the criticality of having timely information—if continuous, then even better—and also because wearable biosensor design and manufacture are also heavily conditioned by environmental and sustainability issues.

These aspects and many others need to be considered during the design stage and have an impact on materials selection and manufacturing techniques. This chapter aims to provide a broad introduction to the design, materials selection, and fabrication processes involved in wearable biosensors. We begin by looking at the evolution of wearable biosensors, with a focus on commercial devices. Next, the chapter provides an overview of product design, so that newcomers to the field of wearables have a starting point on the main issues to consider in order to make a meaningful contribution. Next, the bulk of the chapter addresses biosensor design from the perspective of wearable devices. This part is written mostly for those without a strong background in (bio)chemistry, but who need to understand the available options when it comes to the transducer and its functionalization, and the way biosensors work. Last, we provide an overview of the most common fabrication techniques used in the study and in the development of wearable devices.

2 Evolution of Wearable Biosensors

Wearable sensors are intended for the continuous monitoring of health parameters, and the development is driven mainly by glucose monitoring for diabetes management. Diabetes mellitus has a very high prevalence, and affects approximately 10% of the world population. This means millions of people worldwide, so improving their life quality therefore represents a huge challenge for scientists and technologists, and an enormous market. In addition to this, glucose monitoring is also of importance in other application domains, such as sports, work safety, and defense.

Glucowatch was the first commercial device for the monitoring of glucose [3]. However, despite gaining FDA approval, it was withdrawn from the market by 2004 because the reverse-iontophoresis method used to induce sweating caused skin rushes on some of its users. It was nevertheless a true technological milestone.

Shortly after, in 2006, Dexcom released their "Dexcom seven" in the US, which was able to monitor glucose for seven days. Abbot released their wearable "Freestyle Libre" system in 2014 in the EU, and in the US in 2017 following FDA approval. In 2021, Abbot announced a new version of the device for athletes under the commercial name of "Supersapiens system". Barring a few exceptions, the wearable biosensor market seems focused on glucose monitoring for diabetes management.

Table 1 summarizes the main commercial devices and their corresponding release dates. Except for the Glucowatch, which was withdrawn from the market, and Senseonics' Eversense, which is an implantable capsule, the rest have a very similar form factor, as wearable patches. The evolution of these devices has been toward more compact and unobtrusive designs, more reliable sensors, and higher levels of integration with other personal smart devices, particularly smartphones, through wireless communication and user-friendly apps.

All these systems have in common that they consist of a piece of instrumentation, and a disposable part. This is because biosensors typically have a shorter lifespan than the electronics and the mechanical parts supporting them. This is the same for any quantitative point-of-care diagnostic system. While the instrumentation can be used many times, the sensing part is discarded after one use. The main limitations of this model are the complexity of the instrumentation, the need to tightly control the cost of the disposable part, and the environmental impact of this disposable part. The evolution of these systems, driven or facilitated in most part by progress in miniaturization and new materials, has been toward integrating the biosensor into a small smart system that works in tandem with a smartphone or a smartwatch to facilitate user interaction and data management. The biosensing smart system, in turn, comprises a microcontroller, a potentiostat, a small memory, short-range communications, and a button battery cell to power the set. Although these products have doubtlessly improved the life quality of millions of diabetic patients worldwide, they are also extremely wasteful. Whether it is the biosensor or the adhesive that limits the lifetime of the disposable component, the fact is that these capsules are disposed of after only a few days, typically 10–14. The longer lasting device is Eversense XL from Senseonics, which can operate for 90–180 days, although it is a subcutaneous

implant, which has a completely different set of issues. When the biosensing capsules are thrown away, the electronics within it and its mechanical parts are in perfectly good order, so the approach is clearly wasteful.

Turning to the academic literature, as Fig. 1 shows, Diamond et al. pioneered research on wearable biosensors in Europe. These workers proposed the integration of biological sensors in the form of wristwatches and in textiles and garments as early as 2005 [16]. Although these early works on wearables focused on textile-based biosensors, his group has also developed wrist-watch biosensor devices, and they have led the field all this time [17–20]. Other researchers have made great progress in this area, mostly in the US. Joseph Wang, at the Center for Wearable Sensors at UCSD, focused on screen-printed biosensors [21, 22] and enzymatic fuel cells [23, 24] for skin tattoos but also interacted in sportswear [25]. These seminal works led Joshua Windmiller to the creation of the spin-off company Biolinq, formerly Electrozyme. More recently, in 2014, Heikenfeld et al. reported an RFID patch for the monitoring of sweat electrolytes [26]. Heikenfeld's approach differed from the rest in that he focused on understanding the mechanism of sweat [27], modeling it [28], and developing systems able to manage the small sweat volumes available, avoiding sensor contamination from the skin itself. Heikenfeld also worked on sweat generation systems by reverse iontophoresis, similar to the Glucowatch. Around that time, Heikenfeld started Eccrine Systems Inc., a company to develop sweat sensors, initially for the military and today focusing on pharmacokinetic studies. In his latest works, Heikenfeld points that the relevant parameter to monitor dehydration is not the level of electrolytes such as Na^+, Cl^-, or K^+, but the fraction of bodyweight lost as sweat during exercise, and has presented a simple colorimetric patch for it [29]. Last, it is worth highlighting the Rogers' group at Northwestern University, whose work on sweat sensing is spectacular. In contrast to the others, Rogers' work has focused on developing stretchable electronics [30, 31]; he has presented stretchable patches integrating wireless capabilities (for power, sensor control, and data communications), improving the microfluidics to drive sweat around the device, as well as form factor design and device functionality. His systems have been demonstrated on babies and triathletes [32]. Some of the latest work by the Rogers Group features a self-powered skin patch that integrates an Mg (anode) and an Ag/AgCl (cathode) battery to power a circuit that monitors heart rate, sweat chloride, and sweat pH, plus an NFC tag to communicate with a smartphone [33]. It is also worth noting that the Rogers Group has also spun out a company, Epicore Biosystems, to commercialize skin patches. However, in contrast with the others, two out of three products currently featured in the company website seem restricted to passive microfluidic devices. One of them, named *Gx Sweat Patch*, has been developed in partnership with Gatorade to prevent dehydration, and consists of an easy-to-use patch that allows you to monitor sweat rate and (sweat) sodium in real time [34, 35]. The patch contains two microchannels impregnated in colorimetric indicators. Color can be read using a mobile phone and a dedicated app. The other two products are a passive patch for sweat collection, and a patch-like device for the monitoring of temperature, sweat rate, and sweat loss [36], with a similar form factor to the systems featured in Table 1.

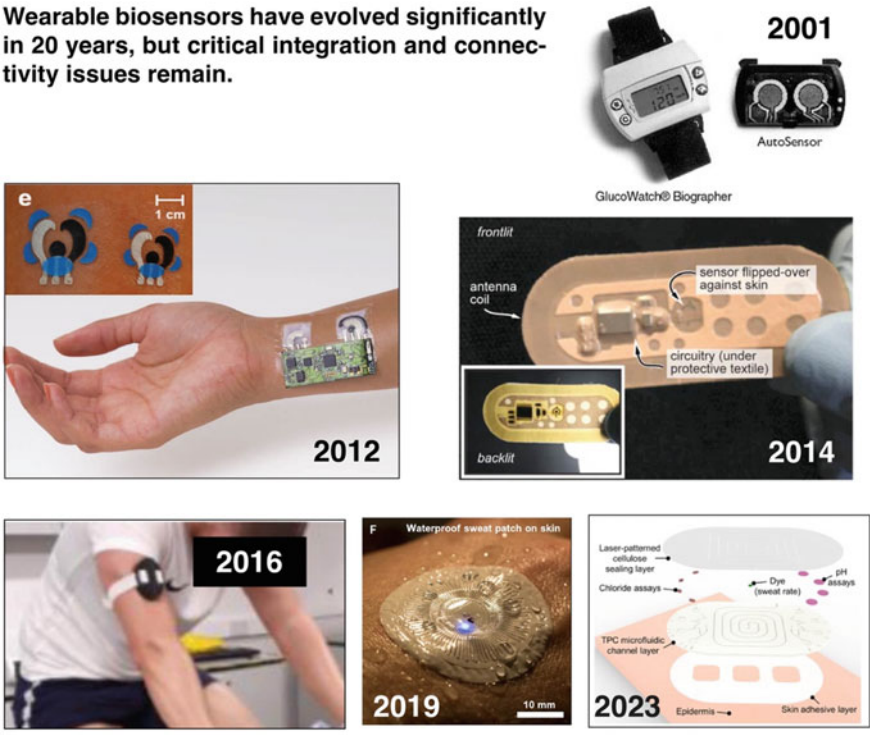

Fig. 1 Key milestones in wearable biosensors to date. 2001—Glucowatch [3]; 2012—Screen-printed biosensing tattoos [21]; 2014—RFID skin patch [26]; 2019—Waterproof skin patch [32]; 2023—Biodegradable skin patch for sweat rate and electrolyte monitoring [35]

In summary, barring the differences mentioned above, which confer a distinct identity to the works of these four leading groups, they all stumble upon the same issues, mainly related to the connectivity between the sensing part (disposable) and the control instrumentation. Wireless connectivity is superior to contact-based connection because it gives more freedom to the user, but it has two important drawbacks. First, the radiofrequency protocols typically used, such as near field communication (NFC), rely on very short-range (<3 cm) radiofrequency, which means that the two communicating nodes need to be aligned and very close to each other. Such short-range protocols can work with low information encryption levels and are in general very safe and energy efficient, but require an excellent coupling between emitter and receptor units. Second, so far, all systems reported which rely on NFC require the integration of an NFC chip and other discrete components on the sensing part. This translates into prohibitive costs of integration into flexible or even elastic substrates. On the other hand, electrochemical chipless devices require a physical connection with the instrumentation, which often makes them uncomfortable and

impractical. This already suggests some areas for research to improve present-day wearable devices, regardless of whether the system mounts a biosensor or a different kind of sensor.

3 Fundamental Aspects of Biosensor Design

As mentioned above, wearable biosensor design involves the consideration of many different aspects beyond aesthetics. Industrial design is responsible for how something works, including every single detail surrounding a new product or service, and not simply how it looks. One way of approaching the subject is to start considering the application environment and the user profile, and then working the way down from the most critical aspects down to the smallest details. The same guiding principles used in the design of any other device apply to wearable biosensors, but additional thought needs to be given to the biosensor itself. The design of wearable biosensor devices should consider at least:

1. Application environment and user profile.
2. Accuracy, specificity, timeliness, and reliability of biosensor data.
3. Sensor placement and design, which can impact accuracy and user comfort.
4. Power and data management, including battery life and wireless connectivity.
5. Usability and user experience, which can affect the adoption and use of the device.
6. Environmental and sustainability issues, including the selection of materials and manufacturing processes that minimize environmental impact and the end-of-life disposal of the device.
7. Regulatory issues.

Design and manufacturability or, better, design for manufacturability, can help overcome technical challenges, comply with regulations, reduce manufacturing costs, and incorporate essential security features to ensure data privacy and protection. However, they are rarely considered in academic environments. The following aims to provide some basic ideas on the process taking wearable biosensors from concept to prototype.

3.1 The (New Device) Design Process: Useful, Usable, Beautiful

Developing a new product from scratch may seem terrifying and extremely difficult. To make it easier, setting specifications correctly is crucial. The first step is to answer a number of questions: why is this new device needed? What problem does it solve? For whom? These questions are intended to clarify the utility of the device. **The first goal has to be to make a useful product.** Additional factors may be considered to complete the specifications. On the technical side, consider application requirements

such as analytical performance, durability, user profile, use environment, etc. Are there any size, weight, and form factor requirements? How about possible constraints on the physical design of the product? Based on this, what are the main features and capabilities to meet the need? Now is the time to think about materials and processes. Are there any specific materials that are necessary or desirable? Any materials that should not be used at all?

At this stage it is quite useful to consider business-related issues. Things like market size (who is the new device solving a problem for?), user profile; whether the user is actually the same person as the buyer, which is not always the case, particularly when dealing with medical products, where the user is a member of the general public, but the purchasing decision is made by the clinician. How about the competition, is there anything like it already in the market? If so, you need to decide how to compete: what will you do that makes your product better than those already in the market? How is technology evolving in this particular area? What are the competing technologies doing, and at what development point are they? These are central strategic questions to ask, although perhaps outside of the scope of the present chapter. The interested reader can find out more about strategic and business-related issues of technology in the works of Peter Drucker [37], Clayton Christensen [38], and Michael Porter [39, 40].

Once we know that the device will be useful, it is time to move to the next step: making it usable.

3.2 On Device Usability: A Heuristics Approach to Product Design

Heuristics are about experience. Heuristics comes from the Greek word meaning "to discover" or "to find". Heuristics are based on intuition and experience, rather than a rigorous analysis. Although heuristics-based design may be prone to errors of interpretation and biases, using heuristics can be extremely helpful during the first stages of the design process. In short, it is about common sense and, in many ways, it means putting yourself in the user's shoes with a little empathy. Above all, it is about trial and error, and how to make a product truly meaningful for the user. Having said this, while heuristics are a powerful tool, their assumptions should be checked and tested with more objective criteria and backed by data, so that the design stage can be more rigorous.

The work of Dan Norman entitled "The design of Everyday Things" [41] is an excellent source of heuristics at work in product design. This book uses great real-life examples of how to use heuristics to come up with excellent products that are both intuitive and easy to use. Another very widely used set of heuristics can be found at the Nielsen Norman Group website (https://www.nngroup.com/articles/ten-usability-heuristics/). In addition to this, it is worth considering also Dieter Rams' ten

Table 2 Dieter Rams' design principles and Norman Nielsen's ten heuristics for new product design

Dieter Rams' design principles	Nielsen's ten heuristics
Good design is innovative	
Good design makes a product useful	Flexibility and efficiency of use
Good design is aesthetic	Aesthetic and minimalist design
Good design makes a product understandable	Match between system and real world Recognition rather than recall Help users recognize, diagnose, and recover from errors
Good design is unobtrusive	User control and freedom
Good design is honest	Visibility of system status
Good design is long lasting	Consistency and standards
Good design is thorough down to the last detail	Error prevention
Good design is environmentally friendly–	
Good design is as little design as possible	Aesthetic and minimalist design

principles of good design.[1] Table 2 lists Dieter Rams' principles along Nielsen's ten heuristics. While there may be slight differences in wording, both approaches have very much in common. Dieter Rams' principles were conceived with physical objects in mind, whereas Nielsen's heuristic finds more application in software user interfaces. Be as it may, they both can be extremely helpful when applied to the design of new wearable biosensors.

Only after usefulness and usability have been addressed is time to work on the device aesthetics. Prototyping offers a systematic way to move from concept to production.

3.3 Prototyping: From Design to Product

At this stage of the work, prototyping techniques play an important role to bridge the gap between the proof of concept and the production line. As Fig. 2 shows, prototyping is an iterative process whose goal is to bring out problems so that they do not appear later, during, or after production. It is generally useful to focus on one or two very specific aspects of the device at a time, and gradually integrate the lessons learned into subsequent prototyping cycles. One round may focus on how process conditions affect materials and functionality. This will help fine-tune the production sequence, to check that the processes are done in the right order, and that the conditions are adequate. It may be possible to identify alternative processes that will facilitate overall yield, or to find that certain design assumptions need to

[1] https://www.vitsoe.com/gb/about/good-design.

be revised. Both hardware and software prototypes can and should be developed. Hardware prototypes should address all aspects of the device: mechanical, optical, electric, etc. Last, these parts need to be integrated into a coherent whole, which can also be addressed through prototypes. Complete functional prototypes can be used to assess user experience and usability: how intuitive and clear is the device? How can we make it more "user-proof"? Generally speaking, how can we improve the user experience without starting all over? In sum, there are many ways in which prototyping is helpful, but in our case we ought to be concerned with materials processability and device functionality. It is important to choose prototyping techniques that are scalable, and relevant to the industrial processes that will be used later but, more importantly, prototyping methods should allow for an easy work cycle. It is important that prototypes can be produced in a short period of time with reasonable resources. The more prototyping cycles that can be performed in a given time, the more robust the final device will be. Although the later sections of this chapter provide an overview of the techniques and processes, prototyping techniques should be industrially relevant, versatile, and able to process different materials in different ways, and easily scaled and combined with other processes.

The following provides an overview of the main fabrication techniques used in the development of new wearable biosensor devices.

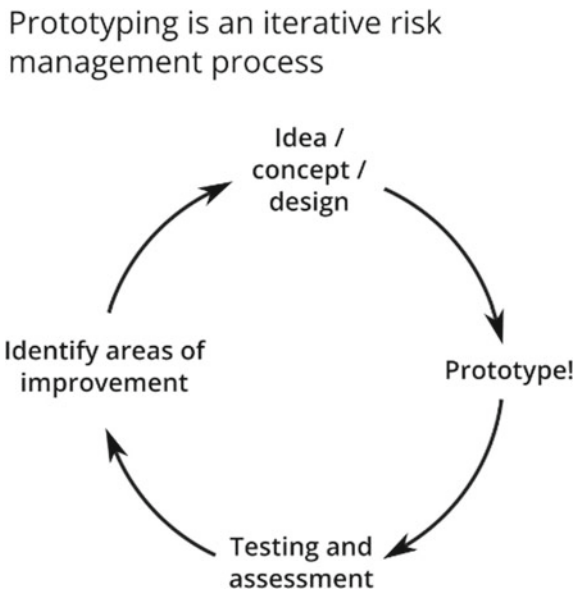

Fig. 2 Prototyping is a key iterative process in product development

4 Fabrication Processes

The production of wearable biosensors involves the combination of several manufacturing processes, from sensor fabrication, device assembly, and packaging. Based on the process results, fabrication techniques or processes could be divided into four main types of processes:

1. Additive processes.
2. Subtractive processes.
3. Pattering techniques.
4. Forming processes.

Additive processes include deposition methods such as thin-film processes like sputtering and other physical vapor deposition methods, chemical vapor deposition methods, electrodeposition, and thick-film processes such as coating methods, printing methods, and replica molding. Subtracting methods include etching methods and cutting and engraving methods. Patterning methods whose function is to transfer functional structures to the device by means of additive or subtractive techniques are mainly based on lithography. This chapter will cover the first three types. Forming processes are those where material is neither added nor removed, but given a particular shape by means of deformation, pressing, calendering, extrusion, thermoforming, and many others. Chris Lefteri provides an excellent overview of manufacturing techniques [42], and the interested reader will find a comprehensive list of industrial manufacturing processes.

The fabrication of wearable biosensors involves a combination of processes and the integration of multiple components, typically through heterogeneous integration processes. Heterogeneous integration consists in the combination of components from a range of different sources into a single working microsystem. The integration process should be oriented toward ensuring higher performance levels, smaller overall size, and low power consumption. Among the processes involved in heterogeneous integration, we can find pick and place techniques, various ways of bonding components to a common substrate, or to other components. Following integration, the device is inserted into its final package, whose main mission is to protect the device and to provide the user interface. In most cases, the case is made of plastic by injection, and assembled mechanically. In the case of wearables, special care needs to be taken to make the device waterproof, for which the IP ratings[2] provide guidance. The following sections describe the main techniques used in the production of wearable biosensors, illustrated with examples from the literature.

[2] https://www.iec.ch/ip-ratings.

4.1 Additive Manufacturing Techniques

Additive manufacturing involves the addition of material on a substrate [43]. There are many ways in which material may be added. Thin-film methods involve the deposition of material layers up to a few hundred nanometres thick. The main thin-film processes are physical and chemical vapor deposition. An important case is that of metal parts, typically gold tracks, pads, and electrodes, which are deposited by physical deposition methods, typically sputtering or evaporation. Thick-film techniques, on the other hand, provide means to deposit layers and structures more than 1 micron-thick. Among thick-layer techniques we find various coating and printing methods. Screen-printing and 3D printing are the most widely adopted.

4.1.1 Coating and Printing Techniques

Broadly, coating refers to covering of a substrate with another material. The coating process is carried out on multiple surfaces or substrates depending on the application. Although there are numerous coating processes, we will focus on those with the greatest interest for the manufacture of wearable biosensors. The coating techniques to be used in each development will vary depending on parameters such as the nature of the inks or coatings, and the substrates, and how these interact. Figure 3 shows a diagrammatic representation of some of the main coating techniques, also used in wearable fabrication.

4.2 Drop Casting

Drop casting is the simplest of the coating techniques, and perhaps the most widely spread. It is used to build the selective layer over a transducer, effectively transforming it into a biosensor. The technique consists of adding a drop of solution, containing the necessary reagents, over the surface to be modified, usually a transducer. After the solvent has evaporated, the solid residue left on the transducer becomes an active part. Thus, enzymes, cross-linking agents, and (semi)permeable membranes can be deposited by drop casting using a micropipette or a spotter. For instance, in a recent work describing a self-powered skin-patch biosensor, glucose oxidase was drop cast over the surface of a screen-printed graphite transducer [44].

4.3 Dip Coating

In Dip coating, the substrate is dipped and lifted out of a cuvette filled with the coating material. The substrate is immersed in the coating material at a constant speed, and

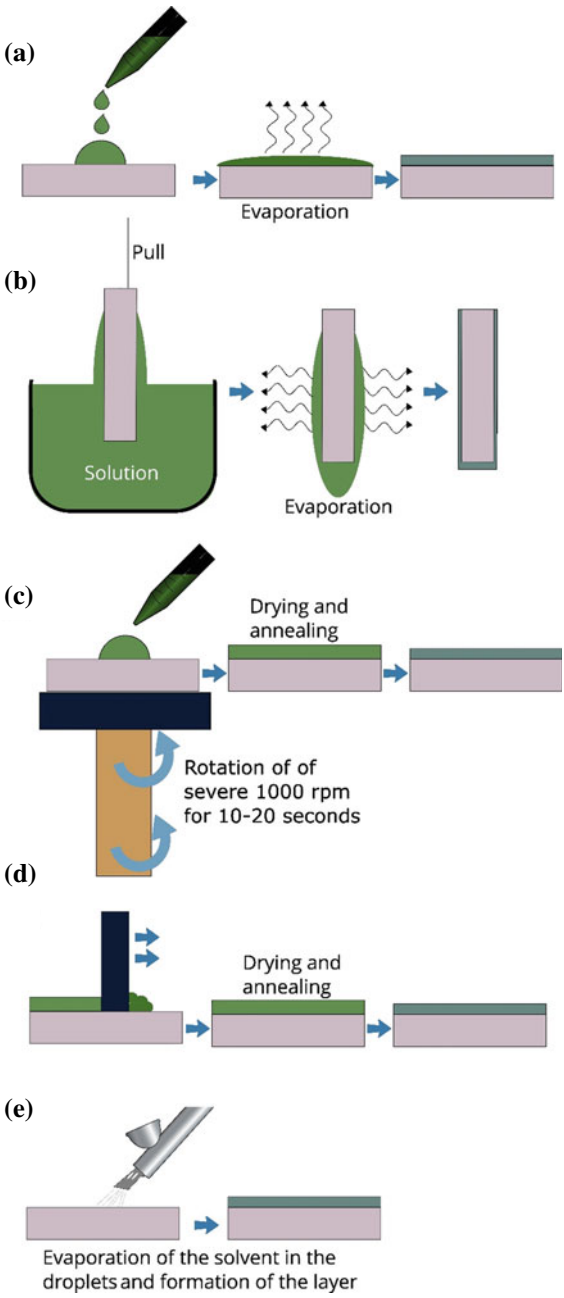

Fig. 3 Diagrammatic representation of the main coating techniques. **a** Drop casting; **b** Dip coating; **c** Spin coating; **d** Blade and Bar coating; **e** Spray coating

after some time the material is lifted carefully, so it does not jolt. The film starts depositing as the substrate exits the solution, and the extraction speed determines the resulting film thickness. Dip coating is used when spin coating or spray coating are not possible.

Dip coating can be used to create different types of functional materials. For example, it was used for the fabrication of a non-enzymatic sweat biosensor [45, 46] a polymeric humidity sensor was fabricated by coating a PDLLA fiber coated in a CNC/PEDOT:PSS. In the two articles different solutions were made to coat the material with the wanted layer, and in both a previous coating was made with a different material to make sure that the wanted layer wouldn't have any problem to stick, this shows a common problem of dip coating, sometimes an extra layer of other material is need.

4.4 Spin Coating

This technique consists of pouring or dispensing a few mL of coating material on top of a substrate that will be spun at a high rotational speed of over several 1000 rpm, and excess material is spun off the substrate. Due to centrifugal force, the dispersed material spreads into a uniform film of desired thickness, typically in the micron range; this technique is used to coat the wanted material with a suspension of other material. This is mostly used in microfabrication of electronics and in lithography creating uniform thin layer with nanoscale thickness.

On the one hand, we have a very fast method, a few seconds, and we can control the thickness of the layer applied and the obtained layers are very smooth. On the other hand, the shape of the substrate is an important factor in this method, square or rectangular shapes have edge beads at the corners of the substrate, and this also can happen with thick films. In addition, the surface of the substrate can affect the homogeneity of the films.

Spin coating is used in different fabrication processes. In [47] they used spin coating to add a layer of RuS2 nanoparticles in the PMDS substrate, this way they obtain a RuS2/PMDS electrode, as they use spin coating to coat the electrode, they could put a smooth layer in a very fast way spinning at 5000 rpm, as the shape is kind of a limiting factor for this technique, previous or subsequent work must have been carried out to give the wanted shape [48].

4.5 Blade and Bar Coating

Blade coating is another very popular method. The coating material is cast on the substrate, which rests on a flat surface, typically a glass tile. Then a bar that is kept parallel to the substrate at a controlled distance, slides over the substrate and spreads the coating material over it, leaving behind a coat of same thickness as the distance

between the bar and the substrate surface. When the solvent evaporates, the remaining film is usually slightly thinner than the height of the blade, but it is possible to predict the final thickness by accounting for the volume of solvent present in the original coating mixture. One way in which bar and blade coating may be very useful is in the elaboration of flexible and elastomeric substrates several hundred microns thick. This gives the freedom to prepare substrates in materials other than the usual PET, polyimide (Kapton), and PDMS. Also, it allows the possibility to prepare functional substrates, if the polymer is combined with a functional component.

4.6 Spray Coating

In spray coating the material is deposited in μm size droplets and lands on the surface of the substrate. If the substrate surface or size does not allow for spin coating with required homogeneity spray coating can be used, because it offers the potential to coat arbitrary shaped substrates. To form the droplet ultrasonic atomization or nitrogen filed nozzle can be used, most of the droplet's land outside of the substrate and due to Micro-turbulences, the center of the substrate is thicker than in the edges. A particular advantage of spray coating is the ability to produce thinner coatings than other methods, such as screen-printing or blade coating. One particular application is in the fabrication of transparent electrodes based on carbon nanotubes. Nanotube suspensions can be sprayed and, on evaporation of the solvent, leave a conducting mesh behind that can be used to produce electrochromic or other electro-optical devices, of high potential interest also in wearables. Asaduzzaman et al. made a 3D graphene-based epidermal patch for glucose and lactate analysis in sweat [49] and Zahid and co-workers made a Strain-responsive wearable based on PEDOT:PSS/graphene [50] using spray coating.

As a final word, spray coating can also be considered one of the non-contact printing methods, as depicted in Fig. 4. Recently, aerosol jet-printing has gained attention because it allows the deposition of very fine and thin structures over 3D objects [51].

4.7 Screen-Printing

Screen-printing or serigraphy is a printing method with a long history and tradition in the graphic arts. In screen-printing, ink is pressed through a mesh screen onto the substrate, transferring the patterns defined on the screen or stencil, as shown in Fig. 5. This technique is one of the most commonly used printing techniques, and possibly the most used technique in the fabrication of wearable devices. Screen-printing is a straightforward and cost-effective way of reproducing patterns. In contrast to inkjet-printing, screen-printing is a very robust technique. It can print materials (inks) with particle size several microns in size (typically less than 10 microns), and widely

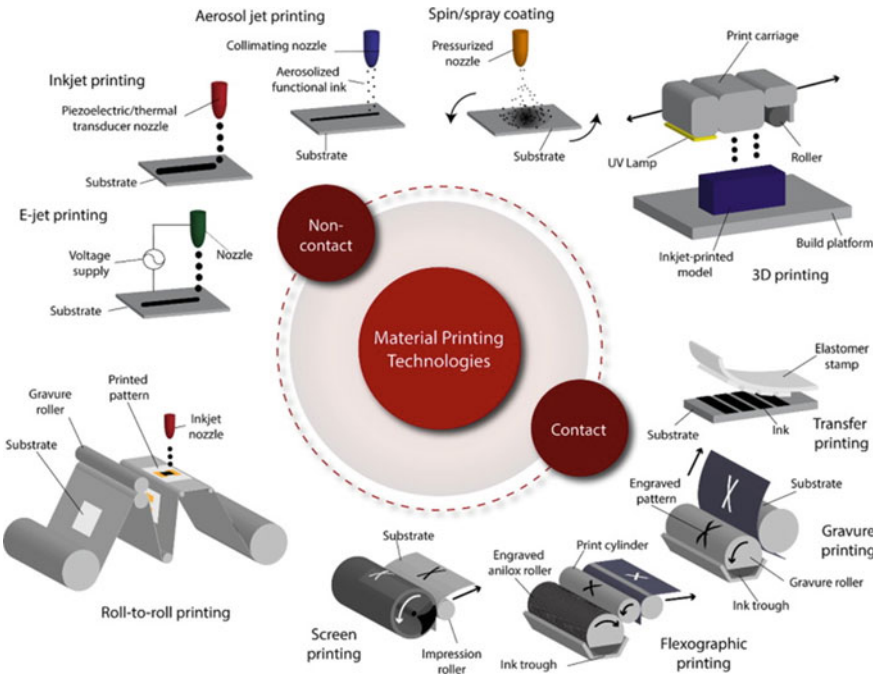

Fig. 4 Overview of contact and non-contact printing techniques [43]

different viscosity and rheological properties. By changing mesh count and squeegee profile, it is possible to control the amount of material transferred, and hence the lateral size and thickness of the features. However, the larger the particle, the larger the necessary opening in the mesh and hence the lower the resolution. Although the state of the art allows the printing of structures with critical dimensions down to few tens of microns, such screens are very costly and are only used in high-end applications and in research environments. Most screens consist of nylon meshes, and mesh counts between 77 and 120 thread cm^{-1} are typical in the area of electrochemical sensors and wearable biosensors. Such mesh counts allow the printing of structures down to 100–150 microns, which is enough in most cases.

Although screen-printing is typically used to print electrodes and dielectric structures, it is in principle possible to print structures up to 100 microns in height, which could lead to printing gaskets, fluidic channels, and membranes. Screen-printing also allows printing virtually any material imaginable, and it enables the fabrication of complete devices if suitable inks are available [44].

Figure 5 depicts the process of screen-printing an electrochemical sensor using three screen levels. First, the conducting pads and tracks are printed on the substrate, followed by the transducer and auxiliary electrodes, which are typically graphite or other carbon allotrope and, last, a dielectric structure is printed to protect conducting

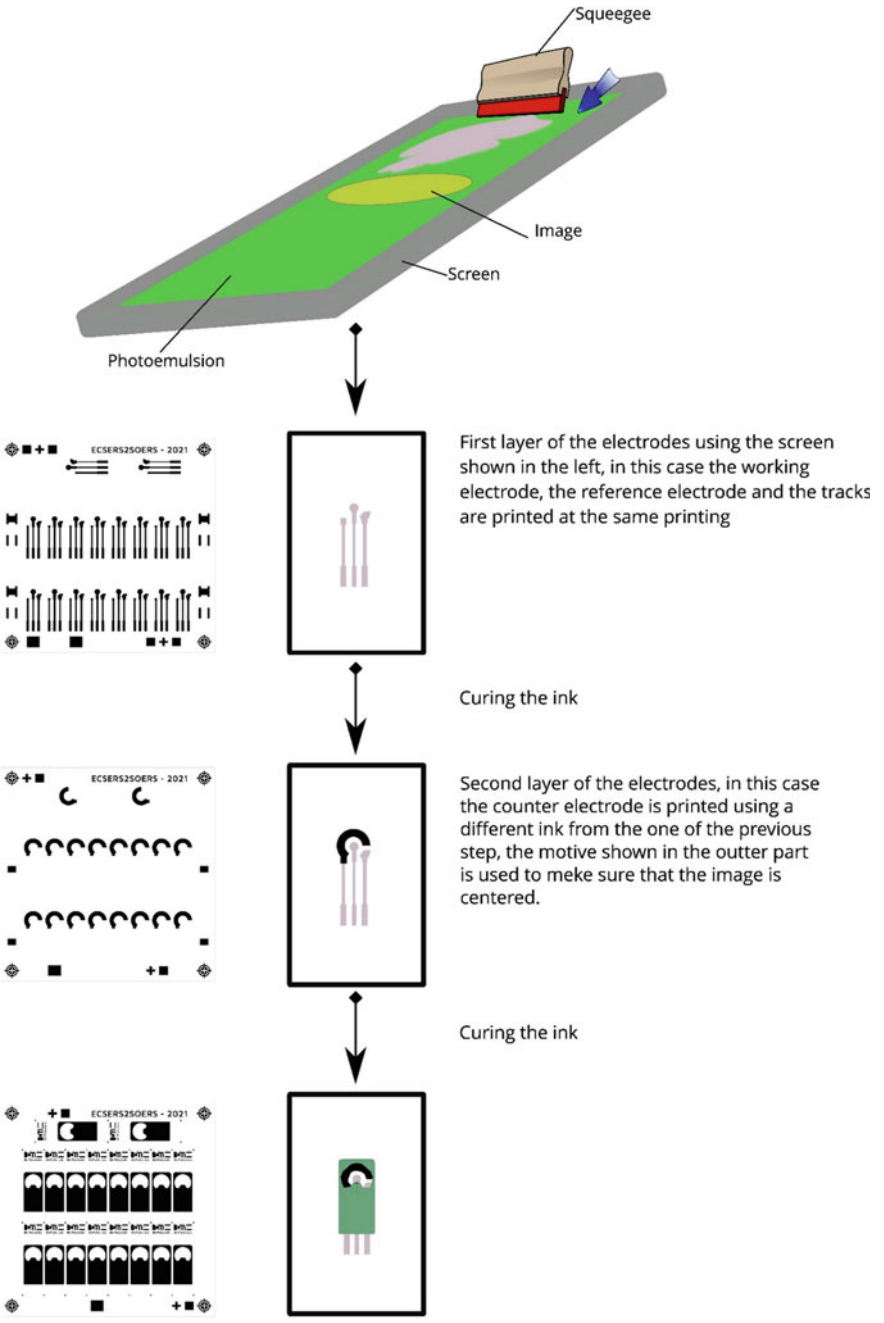

Fig. 5 Representation of the screen-printing process to produce electrodes for electroanalytical devices

tracks and to define the working areas of the electrodes. On the side of each step, film positives are shown.

This technique has a lot of versatility and can be used to fabricate wearables, but often the required functional inks have to be developed specifically. For example, a screen-printed GOx/NQ/MWCNT-based bioanode and a screen-printed GOx/PB/MWCNT-based biocathode were prepared to obtain a self-powered and energy-harvesting biosensing device, these inks were created to function as a single-enzyme-based energy-harvesting device and a self-powered biosensor driven by glucose on bioanode and biocathode [52]. This is a good example of how screen printing allows to obtain different types of devices with diverse types of functions depending on what ink we use. On the other hand, we see that this technique is very useful and very fast, as [53] it replicates a lot of biosensors as a flexible wearable biosensor using screen-printing as a technique. So, this shows that the future of this technique looks like is going to be creating different types of inks with new kinds of materials to obtain different effects in the wearables.

4.8 3D Printing

3D printing is a fabrication process that involves creating three-dimensional objects by adding successive material layers. The different ways to deposit the material, or to grow layer upon layer of material, define 3D printing techniques. Fused filament deposition modeling (FDM) works by extruding melted material through a heated nozzle, and each subsequent layer is printed or deposited on top of the previous one until object is formed. This technique allows the creation of complex objects in a fast and cost-effective process. 3D printing could be used in biosensor fabrication allowing the rapid prototyping, tailor-made sensor designs, and miniaturization. Stereolithography, SLA, is becoming even more popular than FDM because it allows for higher resolutions than the latter, and typical photoresins used are thermally and mechanically more stable than PLA used by filament deposition techniques. In stereolithography, an ultraviolet light source is selectively applied to cure a photocurable resin with a high degree of accuracy. Selective laser sintering, SLS, is a third stereolithographic technique where a laser beam is used to selectively cure a powder into a solid shape. Polymers such as polyamide, and even metal powders can be sintered, which opens a vast number of possibilities.

3D printing has transformed many of the manufacturing sectors as it allows rapid prototyping of devices for many different applications. In this sense, and despite the fact that 3D printing has not been as widely adopted as other techniques in the area of wearable biosensors, even if things may be starting to change [54]. Until now, many of the developments have focused on pressure sensors, in which highly stretchable sensors had created by 3D printing a resistive carbon-based ink onto an elastomeric matrix [55–57]. However, recently Kim et al. presented a wearable sweat sensor by means of 3D microprinting [58]. The authors describe the development of a low-cost, mechanically flexible, all-inclusive integrated wearable patch (AIIW)

containing 3D-printed flexible sensors, which are shown in Fig. 6. In addition, the developed system integrates a microfluidic system. Studies successfully demonstrate the ability to obtain ex situ and in situ measurements of multiple electrolyte levels (Na^+, K^+, and Ca^{2+}) in sweat.

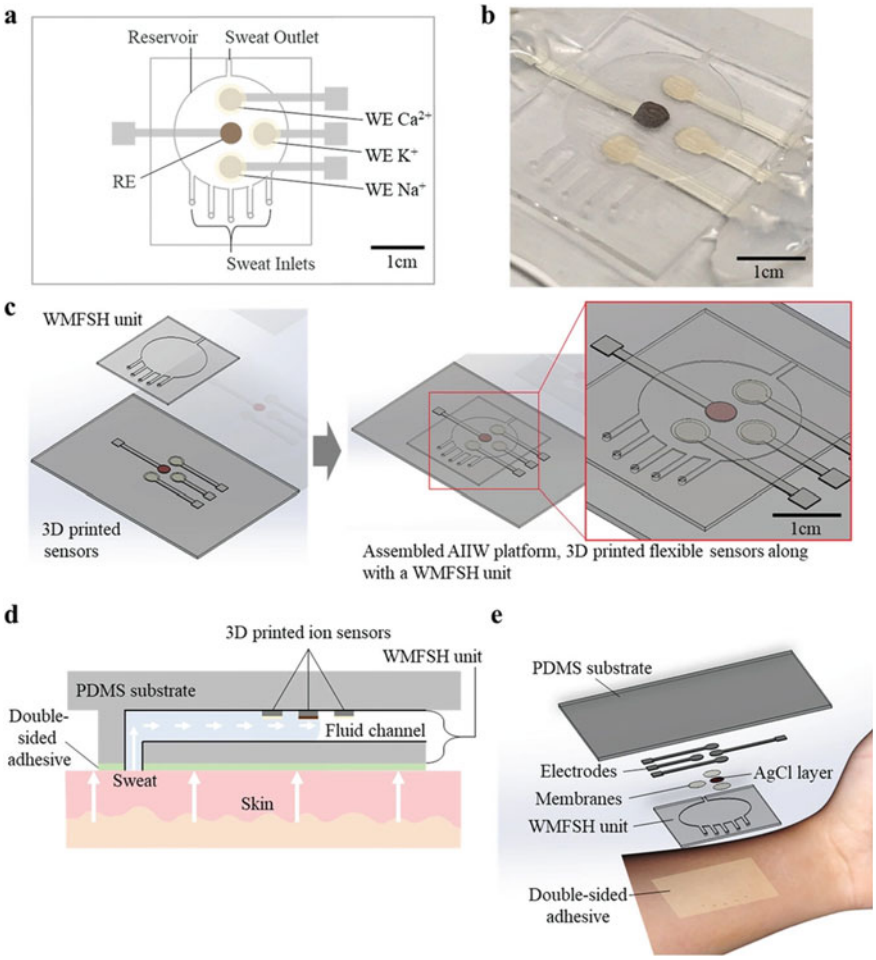

Fig. 6 Scheme of the multisensing integrated flexible device developed by Kim et al. [58] (Reproduced with permission of Wiley)

5 Subtractive Manufacturing Techniques

Subtractive techniques involve the selective removal of material from a substrate. Etching processes are very widely used subtractive techniques in micro-, nanomachining, integrated circuits, and when fabricating wearables. Etching processes may be wet or dry, depending on the phase of the etchant. Wet etching is the chemical removal by the immersion in a solution, and dry etching is the removal of the material by a reactive ion plasma. Etching may be isotropic if the same amount of material is lost vertically and horizontally, or anisotropic, if the etch rate is direction dependent. This section will describe different types of etching processes used in the fabrication of wearable biosensors.

5.1 Etching

Etching is a type of subtractive technique where the material is removed; this removal can happen in a bath with an etchant (wet etching) or in a reactive ion plasma (dry etching).

The most important parameters in etching processes are:

1. Etch rate: The rate at which the material is removed from the substrate. It is typically expressed in depth, D, Å, nm or μm per unit of time, t, and can be calculated using the following formula $ER = \frac{D}{t}$
2. Uniformity: It is defined as the etch rate constancy across the substrate. It may consider other post-etch characteristics such as selectivity and profile.
3. Throughput: Amount of material etched during one process cycle.
4. Directional control: Control of the horizontal and vertical etch rate.
    i. isotropic: the same amount of material from the substrate is removed horizontally and vertically
    ii. Anisotropic: different amount of material is removed from the substrate vertically and horizontally.

Anisotropy can be calculated using this formula $A = 1 - \frac{r_{hor}}{r_{vert}}$ where *rhor* is the horizontal etch rate and *rvert* is the vertical etch rate.

5. Etch selectivity: It is the ratio between two different etch rates, normally the mask and the material being etched. Selectivity can be adjusted through the selection or thickness of the mask, in dry etching we work with plasma and during the etching process part of the mask is removed, understanding the selectivity in this example lets to choose a perfect mask thickness and control the etching results.

5.2 Wet Etching

Wet etching is the chemical removal of the material by immersion of a substrate in a bath with a suitable etchant. Wet etching can be used in microfabrication of pattern metals like aluminum, gold, nickel, and chromium, among others. In addition glass and silicon can be etched using this technique. Due to diffusion controlling of the etch reaction, this type of technique is in most cases isotropic. This has important implications in mask design and the process of critical dimensions, due to the fact that structure with aspect ratio greater than 1:1 is difficult to obtain using wet etching. On the other hand, wet etching can be very selective, uniform, and is used in the removal of thin layers and for cleaning purposes. Wet etching or chemical etching is used a lot in the synthesis of biosensors based in MXenes [59, 60] and Nanoporous metals [61, 62].

5.3 Dry Etching

Dry etching is the removal of the material in a reactive ion plasma, without using wet chemicals or rising, they enter and leave the etching cycle in dry state. In dry etching, a solid surface is etched in the gas or vapor phase physically by ion bombardment. There are 3 different types of etching methods:

1. Physical sputter/ion etching and ion-beam milling etching occurs because of a purely physical effect.
2. In chemical plasma etching, reactive species such as chlorine or fluorine atoms generated in the plasma diffuse to the substrate, where they react to form volatile products with the layer to be removed.
3. In the case of physical/chemical etching (RIE), Line of sight impacting ions damage the surface, inducing highly anisotropic chemical reactions on the surface with plasma neutrals.

This technique is anisotropic because the high directionality of the electric field inside the reaction chamber makes the etch rate of the direction perpendicular to the substrate surface much higher than etching in the lateral. Structures with very high aspect ratios can be obtained by controlling the plasma conditions.

Figure 7 shows the etching of a substrate using a mask. High aspect-ratio structures typically involve a series of steps of etching and masking, as in the Bosch process, to ensure verticality of the structures, as there is always some degree of lateral etching [63].

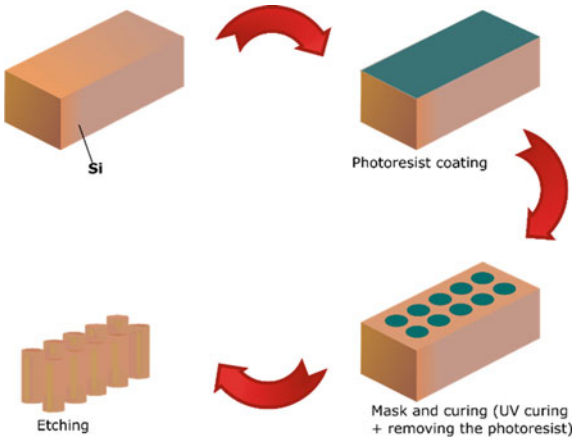

Fig. 7 Selective etching using a mask

5.4 Milling

In milling, a rotatory cutting tool like a milling cutter or drill is used to carve structures from a block of material, chipping it off. Milling can be used to make components ranging from fluidic channels to molds to cast different types of materials such as PDMS. The most important parameters during milling processes are the spindle rotation speed and the material feed rate. Although tool manufacturers provide approximate work conditions, these values are generally a starting point, and conditions need to be adapted as each material will behave differently.

To calculate them you can use the following formulas in decimal metric system.

Cutting speed (m/min):

$$Vc = \frac{(\pi \times DC_{ap} \times n)}{1000}$$

where *DCap* is Cutting diameter at cutting depth ap (mm) and *n* Spindle speed in rpm.

Feed rate (per revolution mm/r):

$$F_n = \frac{V_f}{n}$$

where *Vf* is the table feed (mm/min) and *n* Spindle speed rpm.

Milling is used to produce objects from a few hundred microns up to meters. In the case of wearable devices, milling can be used to produce mm-size objects with sub-millimetric features, such as molds and casings. Although the versatility and increasingly high resolution of stereolithographic 3D printing methods offer an easy alternative, the fact is that milling still provides far superior finish quality. A

smart approach is to combine both techniques. Using a 3D-printed part as the starting point, and ending the part by milling. This results in less wasted material, and very high-quality finished parts.

5.5 Laser Cutting

Laser cutting and etching works by ablating the material; that is, by sublimating the material where the laser energy is focused. An advantage of laser cutting compared to milling or other mechanical cutting techniques is that the material does not suffer mechanical stress during the cutting because this is a "contactless" technique. On the one hand, this technique is faster than milling, but on the other hand, the high temperatures during the cutting can lead to problems such as the cracking of brittle materials like ceramics or the formations of burr in polymeric materials. In addition, as with milling the optimal conditions for the cutting has to be obtained with experience.

In addition to cutting, laser can also be used to transform materials. For instance, it is possible to produce graphene structures from Kapton, a polyimide, simply by using the right laser conditions [64].

5.6 Patterning Methods: Lithography

It is difficult to classify lithographic techniques as either additive (a photoresist material is added onto a substrate) or subtractive (photoresist is selectively removed during development following UV insolation). However, it is easy to agree that the main function of lithography is to transfer patterns over a substrate. This is the reason why we have decided to separate it from the other two big families of techniques.

The term lithography comes from the Greek words Lithos (stone) and Graphein (write), it was discovered by Aloys Senefelder when he found that the limestone properly inked and treated with chemicals was able to transfer a carved image onto paper. Lithographic methods, developed in the microelectronics industry, are widely used in biosensor production, since complex micro/nanoscale structures with high-resolution topography can be produced from it. Lithography offers the capability to fabricate sensors in a microscale, reaching sub-micrometric resolution.

Lithography encompasses a wide range of surface fabrication methods (photolithography, electron/ion-beam lithography, soft lithography, etc.) and selecting the most appropriate technique for a specific application involves a compromise between resolution, performance, and cost considerations [65]. In the area of wearable or flexible devices, lithographic techniques are also limited due to the resolution that can be achieved on flexible substrates compared to silicon substrates.

The most used lithography method is photolithography. In photolithography, patterns transfer from masks onto substrates using photomasking and chemical

processing, as schematized in Fig. 1. Photolithography relies on three key components: a photoresist, a suitable light source, and a photomask. However, direct writing and projection methods are gradually gaining ground as means to insolate the resist and transfer the desired patterns to the photoresist.

A photoresist is a light-sensitive polymeric material that protects the underlying substrate, providing a selective barrier to the processes following lithography. The chain length of the resin molecule determines the properties of the photoresist; long chains have better stability against thermal rounding while shorter chains improve adhesion to the substrate. Also, depending on how they react when exposed to UV light, we can differentiate two types of photoresists, positive resists and negative resists. In positive resists, the insolated areas are removed during the development stage. When the photoresist is exposed to the UV light, the chemical structure changes and becomes more soluble in the solvent, these exposed areas are washed away with the photoresist developer solvent, leaving the underlying material. In contrast, in the case of negative resists, insolated are not removed in the development stage. With negative resists, exposure to UV light causes the chemical structure of the photoresist to polymerize, instead of becoming more soluble, negative photoresists become extremely difficult to dissolve. As a result, the UV exposed negative resist remains on the surface while the photoresist developer solution works to remove the unexposed areas.

Conventionally, photoresists are insolated through photomasks, although direct-write laser systems are becoming more popular recently as they facilitate prototyping and changing designs on the fly. Photomasks are typically chromium or soda lime glass featuring a patterned chromium layer that block light during substrate exposure. If photoresists were either positive or negative, photomasks may be bright-field or dark-field depending on whether chromium patterns are the positive or the negative images of the desired structures.

The set of process steps to transfer a pattern in photolithography are the following, summarized in Fig. 1:

1. A wafer is coated with a photoresist layer (typically between 0.5 and 10 μm thickness).
2. Insolation, using UV light of suitable energy, through a photomask or by direct writing to activate exposed resist areas.
3. Resist development to remove resist from unwanted areas.
4. Deposition or etching step.
5. Resist removal and substrate clean-up.

This technique can be used to fabricate different types of electronics [66]. Photolithography is widely used in industry for the fabrication of devices that play an important role in the miniaturization of next-generation electronics. While photolithography patterns are commonly performed on rigid semiconductors such as silicon and glass wafers, the demand for micropatterned flexible substrates has significantly raised, leading to the emergence of new photolithographic techniques. As an example, Ma et al. [1] has developed a new photolithographic method to create micropatterned metal arrays on flexible substrates. In this work, they successfully

fabricated a flexible glucose sensor with an LOD of 1.2 μM, a dynamic range from 0.05 to 0.30 mM, and good stability and reproducibility. Photolithography can be an interesting technique for manufacturing self-healing sensors. Thus, for example, Kim et al. fabricated pressure sensors via photolithography using polyurethane substrate. This sensor presents a chemical resistance and self-healing capability due to the polymeric substrate and the photolithography patterning allowed for the precise and controlled fabrication of the sensor arrays, demonstrating high potential application in the development of skin-attachable flexible devices [67].

6 Materials in Wearables

Materials play a crucial role in the functionality, durability, and overall performance of biosensors. As described so far, only the biosensor incorporates several different materials, ranging from the substrate on which they are placed, the transducer, the biorecognition element, any protective coating to preserve the biosensor functionality, and to provide an effective interface with the body.

Manufacturing processes not only define the device architecture. They also have a strong influence on the final properties that materials display in the device. Materials and fabrication processes are intimately related. Once the desired function has been identified, materials are selected that can meet the desired requirements in terms of functionality but biocompatibility, cost, and sustainability issues. These materials then need to be processed by suitable manufacturing methods that will transform them into their final forms in the device. However, devices display the properties of the processed materials, so it is important to know the effects of the manufacturing process on the desired material properties involved. For instance, high process temperatures, pressures, or harsh chemical conditions can be either beneficial or damaging. For instance, polyvinylidene fluoride, PVDF, is a fluoropolymer, it is elastic, and it is also piezoelectric. It can be processed in a number of different ways, such as by using from solution, for instance using casting and printing methods to produce coatings or specific geometries selectively, it can be turned into fibers and non-woven fabrics by electrospinning, or it can be turned into membranes and films that can subsequently be cut by different means. The precise technique of choice will be dictated by the function that PVDF needs to fulfill in a device. Thus, if PVDF is to be used as a mere substrate, casting into films and subsequent slot-die or laser cutting can be perfectly fine, and then other criteria will be needed to select the most adequate process. Typically, one looks at the processes upstream and downstream in the production line to ensure that the process does not spoil any of the work from the previous processes, but also that any subsequent processes will be able to deal with the processed parts.

Going back to the PVDF example, if the reason to use this material is its piezoelectricity, then it is important that the processes involved result in the correct orientation of its polymer chains and that the thickness of the material will allow the observation of the piezoelectricity. This sets important temperature constraints for any

process downstream in the chain, as temperatures above PVDF Curie temperature will result in loss of piezoelectricity. One last possible application of PVDF in a wearable biosensor is as matrix of solid electrolytes, in combination with organic salts and ionic liquids. In this case, high transparency may be the desired property, and the process of choosing a printing technique such as screen-printing [44]. To produce an ink, PVDF needs to be added to a suitable solvent, and heated while stirred. However, most PVFD forms will turn yellow if heated above 70 ºC, so the temperature of any processes downstream ink formulation needs to be kept below this temperature.

This is only an example of how the material influences the choice of manufacturing process and process conditions.

The substrate material is critical in wearable biosensors because it supports the sensor and provides mechanical flexibility. Flexible substrates, such as silicon and polyurethane, facilitate the conformability of the device to the user's body. Flexible substrates enhance comfort and facilitate the necessary contact to ensure that the sample, typically sweat or interstitial fluid, reaches the sensor in optimal conditions.

The materials used in the fabrication of wearable biosensors also include adhesives, sealants, and coatings. These materials are used to ensure that the biosensor remains securely attached to the skin and to protect the sensor and substrate from damage due to exposure to the environment or sweat. Although adhesives are carefully chosen, cases of allergies and skin irritation are occasionally reported [68].

One significant issue with wearable biosensors is their disposable nature, which raises sustainability issues. Wearable biosensors are designed to be used once, or for a few days at best, and then discarded. This generates a significant amount of waste. There are two main strategies to prevent this. One is to develop more durable wearable biosensors, which involves biorecognition element engineering. The other way consists of using more sustainable materials and manufacturing processes. Using biodegradable or compostable materials in the fabrication of wearable biosensors can alleviate the environmental impact. In addition to this, exploring ways to recycle or repurpose used biosensors is also needed.

All this has become very important in recent years and sustainability has become one of the most important issues in today's society. Moreover, since 2015, the member states of the United Nations and the United Nations Framework Convention on Climate Change (UNFCCC) adopted the Sustainable Development Goals (SDGs), also known as Agenda 2030. The SDGs consist of 17 goals and 169 targets that aim to end poverty, protect the planet, and ensure prosperity for all [69]. As a result, the materials used in the manufacture of wearables have evolved over the last few years and more advanced materials and generally manufacturing methods are being used that have less impact on the environment. Personalized medicine is also of great importance in the SDGs, so wearable biosensors help to better monitor health but have to be environmentally friendly to follow the SDGs.

The wearable-body interface, sensors and actuators, substrates, electronics, and encapsulation and packaging (Fig. 2).

Poitout et al. showed a blood glucose sensor implanted in the subcutaneous area with a wearable unit control [70]. In this case, the sensor consists of a Teflon coated

platinum anode except for the end which is functionalized with glucose oxidase. This glucose oxidase in turn is coated with polyurethane. Around the Teflon is an Ag/AgCl cathode. The size of the control unit is 6 × 12 × 18 cm and it has a memory which is able to store the information as well as a display which shows it. The information in the memory can be transferred to a PC so that it can be read out. Although it was one of the first wearables to monitor glucose in humans, it was a very large device and it was an invasive monitoring system because it involved subcutaneous measurements.

In 2006, Kudo et al. [71] presented a flexible and functional wearable glucose sensor based on polymers. Soft-microelectromechanical systems were used to fabricate the sensor. In this case it is a two-electrode system as it is possible to see in Fig. 3. The working electrode is a Pt electrode with two poly phospholipid polymer membranes copolymerized with dodecyl methacrylate (PDM) and one of them is functionalized with glucose oxidase. The auxiliary/reference electrode is Ag/AgCl coated with a PDM layer. The substrate is also polymeric because it is hydrophobic polydimethyl siloxane (PDMS). The Pt and Ag electrode were deposited by ion-beam sputtering obtaining a thickness of 200 nm and 300 nm respectively. Subsequently, the Ag was electrochemically chlorinated and the whole was coated with PDM by dip coating. Glucose oxidase was immobilized by solution casting of PDM.

Mannoor et al. [72] showed a graphene biosensor printed on silk fibroin. In this case the biosensor is placed in the patient's mouth but the silk fibroin is 100% biocompatible. This biosensor is used to detect bacteria by bio-functionalizing antimicrobial peptides in graphene-modified silk tattoos. These measurements are made by changing the resistance of the sensor. One of the major advantages of this sensor is that it is wireless, which means better use of energy and no batteries and less environmental impact.

Kim et al. in 2014 reported one of the first oral biosensor for continuous lactate monitoring [25], This wearable is non-invasive because it is a mouthguard where the three-electrode electrochemical cell has been printed on a polyethylene terephthalate (PET) film and is therefore flexible. These materials, being flexible, adapt perfectly to the shape of the person's teeth and at the same time take up very little space in the mouth, making it a comfortable device for the end user. The reference electrode is made of Ag/AgCl while the auxiliary electrode and the working electrode are made of Prussian blue-graphite ink. The electrode was attached to the mouthguard by using double-sided tape. To make the working electrode lactate selective it was functionalized by means of a lactate oxidase immobilized with an electropolymeric entrapment coating in a poly(*o*-phenylenediamine) (PPD).

In 2015, Rose et al. demonstrated an RFID adhesive patch which could be used for the co-concentration of sodium ions in sweat. In this case, they claim it can be used to measure other ionic solutes in sweat, Cl^-, K^+ and others [26]. Although this device can only measure ions, it is battery-free which makes it interesting because of its energy efficiency. They made two different sizes of devices, one 25 mm × 60 mm for the arm and one 40 × 70 mm for the leg. This device uses an RFID tag which makes the device very interesting because the sensor is powered wirelessly and without a battery. For this purpose, the printed circuit board (PCB) is a combination of flexible, conformal polyimide and a thin copper foil. For the adhesion to the body,

they use a double-sided medical adhesive and a medical textile for the encapsulation. The working and reference electrodes have Pd and Ag electrodeposited on top of the copper. The reference electrode has had the Ag layer chlorinated. To detect the Na^+ ions, an ISE layer has been applied, in this case the ionophore membrane establishes a difference in potential across the electrode-ionophore barrier corresponding to the Na^+ concentration.

In 2016, Gao et al. presented a device with an array of 5 sensors which were able to detect lactate, glucose, temperature, Na^+, and K^+ in sweat [73]. The substrate of this wearable device is PET on which a flexible printed circuit board (FPCB) is printed. After that, a parylene layer is used to passivate the circuit. The auxiliary electrode is Ag/AgCl, so to obtain it, first the parylene is removed from the electrode by O_2 etching and Ag patterning and then chlorinating it. Finally, the working electrodes have been modified. For the glucose sensor, Prussian blue was deposited on the gold electrode. Finally, by drop casting the sensor was functionalized with a solution of glucose oxidase, chitosan, and carbon nanotubes. For the $Na^+$and K^+ sensors, the reference electrode was coated with polyvinyl butyral in order to stabilize the potential. The working electrodes, in this case, were made of poly(3,4-ethylenedioxythiophene) polystyrene sulfonate, PEDOT:PSS, and ISE membranes were applied by drop casting. The temperature sensor is a Cr/Au nanowire resistor.

In 2022, Huang et al. demonstrated an ultra-thin, flexible, cotton-based microelectronic device powered by sweat-activated battery for real-time monitoring of sweat [74]. This wearable device, represented in Fig. 4, is a big step forward because it does not use any conventional batteries or power transmission as it is powered by sweat. Instead, a sweat-activated battery generates a maximum power density of 3.17 mW cm^{-2}. The battery size is 3.5 cm $\times$ 1 cm so it produces a maximum power of 11,095 mW. This wearable can measure Na^+ concentration, pH, and skin impedance. In this case the electronics are mounted on a polyimide substrate. The conducting material is a 10 cm copper coated with 50 nm gold. The impedance sensors are two gold electrodes. For the Na^+ sensor PEDOT:PSS was electrodeposited and then an Na^+ selective layer was applied. The working electrode of the pH sensor was obtained by electrodeposition of a polyaniline layer (PANI). The pseudo-reference electrodes for the Na^+ and pH sensors are made of Ag/AgCl obtained by Ag deposition and subsequent chlorination. For the Na^+ sensor a PVB layer was applied on the Ag/AgCl electrode. The SAB has been fabricated by homogeneously dispersing graphene on absorbent paper and Mg on each side of the electrode. Next, by putting obtained graphene/paper and Mg sheet side by side on the air-permeable cloth and finally, attaching a thin layer of cotton with KCl powders on it to the electrodes.

As it has been possible to observe over the years there has been an evolution in materials. Nowadays, more miniaturized devices are being used and they are based more on energy efficiency, avoiding the use of conventional batteries. This also comes from the evolution of electronics which has allowed electronic components to be printed on flexible substrates, with these complex circuits feeding and obtaining information from sensors.

7 Biosensors and Wearables

7.1 Overview

Biosensors are miniaturized analytical devices. Their fundamental feature, in contrast to chemical sensors, is that a biological recognition element (also known as a bioreceptor) is intimately coupled to a transducer, enabling the selective detection of a particular target analyte [75]. Bioreceptors are functionally active molecules, and this functionality is usually maintained over a predefined range of certain parameters such as pH, temperature, and ion concentration, which presents some limitations from the design point of view that must be considered when developing wearable biosensing devices [76].

Figure 5 is a schematic representation of the basic structure of a biosensor. Typically, biomolecules are attached or immobilized onto a biocompatible surface in the body-wearable interface. A transducer coupled to that biocompatible layer converts the biochemical responses coming from the interaction between analytes and bioreceptors into a readable/measurable signal (optical, electrical, thermal…) that can be amplified and processed for analyte concentration monitoring.

When designing a wearable biosensing device, it is also important to consider biosensor placement, and the fluid or matrix where the target analyte is measured, as that will determine the required sensitivity of the biosensor as well as possible interferences or incompatibilities. For example, as skin covers most of the body surface, it seems reasonable to consider sweat as an easily available biological fluid to measure various analytes of interest. Biomarkers available to be measured in sweat are well documented, and some analytes exhibit strong correlation between sweat and blood concentrations. This is usually the case for small lipophilic analytes such as hormones (cortisol, testosterone…) and various drugs (ethanol, methylxanthine, levodopa…) that are known to travel transcellularly through the lipophilic cell membranes [77]. However, bigger, or more hydrophilic compounds that enter the sweat through alternative routes (paracellular, vesicular…) experience a considerable dilution during this process, decreasing their measurable concentration. This is notably the case for glucose, which is found in sweat over 50 times more diluted than in blood at concentration values around 0.06 to 0.2 mM, corresponding to 3.3 and 17.3 mM of blood glucose respectively [78]. Other interesting analytes available in sweat that lack blood-sweat correlation are lactate and urea [79]. This underlines the need for highly sensitive and selective detection systems, and the use of bioreceptors such as enzymes, antibodies, aptamers, or DNA (that bind selectively to specific target analytes), combined with more efficient sampling methods, could be a way to solve some of these problems and could play a key role in the evolution of wearable monitorization devices.

This section will mostly focus on the recognition sites of the biosensing device, looking at the various types of bioreceptors and their incorporation into wearables. Some examples of recent advances in this field will help understanding the role of biomolecules in fully functional wearable devices and provide an overview of

the main advantages and disadvantages of each biomolecule and the techniques employed to immobilize them for repeated usage.

7.2 Enzymatic Biosensors

Enzymatic biosensors have been extensively used for the fabrication of wearables and monitorization devices for direct detection of small target analytes (glucose, urea, catechol, glutamate…) or inhibition-based indirect detection of heavy-metals and other pollutants. These types of sensors are based on the biocatalytic ability of enzymes to convert target analytes at a high rate, which allows for the measurement of changes caused by that reaction (H^+ concentration, electron transfer, heat, or light emission…) [80]. The main advantage of enzymes is their great selectivity due to their unique 3D structure. However, this high selectivity comes at the expense of relatively high production costs and structural instability under certain pH and temperature conditions that can lead to loss of activity, limiting their repeatable usage. For that reason, immobilization of enzymes on electrode surfaces is a prominent area of research nowadays, as it is a way to extend the lifespan and reduce the degradation of these bioreceptors [81]. The focus is on developing strategies that allow for the enzyme mobility required for catalysis (folding and conformational changes), while keeping them uniformly immobilized on the substrate, avoiding denaturing and aggregation to allow for catalytic activity under various reaction conditions. Figure 6 summarizes the main approaches for enzyme immobilization: physical adsorption, covalent binding, entrapment, and cross-linking. These techniques have been used for the fabrication of enzymatic biosensors as well as for devices that use other types of bioreceptors (e.g., antibodies, DNA, or aptamers).

7.2.1 Physical Adsorption

Physical adsorption is a simple and quick method for manufacturing enzymatic biosensors. Although this method has the benefit of speed and simplicity, unfavorable orientations and decreased functionality are likely. Usually, this method is carried out by some sort of prior modification of the surface of the electrode in order to provide the functionalization that allows for non-covalent interactions (Van der Waals forces, electrostatic interactions, H-bonding…). After a proper functionalization of the working surface, enzymes can be adsorbed by simply dipping the material into a solution containing the bioreceptor. An example of this could be the reduction of gold nanoparticles (AuNPs) with a negatively charged ligand like citrate, allowing for electrostatic interactions between the positively charged amino residues of enzymes and the now negatively charged surface of AuNPs [82].

Nanomaterials seem to be the preferred option for surface functionalization. Nanoparticles (NPs), nanotubes (NTs), and nanoarrays (NAs) have distinct properties due to their reduced size and unique morphology [83]. Some nanomaterials,

such as the aforementioned AuNPs and carbon nanotubes (CNTs) are popular choices for biosensor electrode functionalization, as their great chemical stability, relative non-toxicity, high surface area, and good electron transfer capabilities, make them compatible for biomolecule immobilization, and at the same time, act as the electrochemical or optical (in case of AuNPs) transducer of the biosensor [84]. For example, Yanyan Niu et al. used star-shaped AuNPs and horseradish peroxidase (HRP) for biosensor electrode modification with enhanced electrocatalytic activity [85]. Fang et al. combined 3D nanoarchitecture ZnO with gold nanoparticles for successful immobilization of GOx enzyme on a high-performance glucose-sensing electrode, achieving satisfactory low detection limits (0.02 mM) and an acceptable linear range (1–20 mM) [86].

Hydrophobic domains of proteins and enzymes have a natural affinity toward carbon nanotubes, which allows for spontaneous adsorption of the biomolecules [82]. In some cases, is also possible to modify the enzyme as well as the transducer surface to include functional groups for non-covalent interactions. Holzinger et al. immobilized cyclodextrin-tagged glucose oxidase (CD-GOx) and histidine-tagged glucose oxidase (His-GOx) via coordination and host–guest interactions on adamantane-modified single-wall carbon nanotubes (SWCNT) [87]. However, despite advances on surface modification, enzyme immobilization through physical adsorption still faces issues with durability and repeated usage. For that reason, even if it has been used in non-wearable or single use biosensing devices, there is a lack of reported wearable biosensors for continuous analysis and monitorization.

In contrast to the soft approach of physical adsorption, **covalent bonding** provides a strong and effective binding of the enzyme to the substrate or transducer surface. It is one of the most used enzyme immobilization techniques, creating a usually uniform and stable biomolecule layer, that allows for increased lifetime and reusability of the biosensor [88]. Side chain amino acids (e.g., lysine, cysteine, aspartic acid, glutamic acid…) of enzymes and other proteins have reactive functional groups such as -OH, -NH_2, -COOH, or -SH that can be used for covalent bond formation [89]. It is crucial, however, that the amino acids involved in the immobilization process are different from the ones on the active site of the enzyme, as mistakenly binding the active site amino acids to the substrate can lead to severe conformational changes that result in denaturization of the enzyme and its loss of catalytic activity [75]. Despite these drawbacks, and the large amount of bioreagent required, the high uniformity of the covalently bonded monolayer and the good control over the amount of immobilized enzyme are solid advantages of this immobilization method [89].

7.2.2 Cross-Linking

This is another enzyme immobilization technique that has the advantage of providing strong chemical binding between the biomolecules using relatively simple and fast methods such as drop casting and dip coating. Enzymes are cross-linked to each other using bifunctional reagents (glutaraldehyde, glyoxal, or hexamethylenediamine, for example). This allows for greater stability for repeated usage, but it comes at the

expense of possible distortion of the enzyme 3D structure during cross-linking reaction and its consequential loss of activity, although inert proteins such as gelatine or bovine serum albumin (BSA) are often added during the process to minimize enzyme damage [90]. Cross-linking of enzymes is sometimes combined with electropolymerization of biocompatible polymers used for protein stabilization such as polyethyleneimine (PEI) [91].

Following this approach, enzymatic biosensors have successfully been incorporated into wearable devices for the detection of small analytes in different matrices during the last decade. For example, in 2015, Kim et al. integrated cross-linked uricase enzyme on a screen-printed electrode system for a mouthguard wearable device for the detection of uric acid in saliva [92] and in 2017 Tur-García et al. prepared an enzyme laminate of cross-linked lactate oxidase/BSA placed between two polycarbonate membranes to fabricate a flexible wearable sensor for lactate analysis in sweat [93].

A widely used enzyme immobilization method in biosensor fabrication is the **entrapment** of the enzymes in a semipermeable or porous system where ions, electrons, and small molecules can freely flow. This is usually achieved by the use of membranes, polymeric or inorganic 3D networks, and hydrogels.

The first biosensor using a semipermeable membrane for enzyme entrapment was developed in 1962 by Leland Clark and Champ Lyons for a glucose detection electrode immobilizing glucose oxidase (GOx) enzyme [94]. In recent times, polymeric films in biosensors are used for enzyme immobilization as well as protection of the transducer material. This becomes relevant following the recent advances in electrochemical sensing materials that convert common enzyme-catalyzed reaction products (e.g., H_2O_2) into measurable electrical signals. Some examples of these materials are conductive carbon inks and Prussian Blue inks, which exhibit excellent electrochemical performance with relatively low toxicity, but are unstable over extended periods of time and that limits their use in wearable devices [95].

Hydrogels are a popular choice of material for the entrapment approach. Being an insoluble 3D network that can hold fluids provides a few advantages. Hydrogels establish a liquid contact between the biosensor and the body surface, which allows for analytes to flow through. They also create a viscous environment that protects the three-dimensional conformation of the enzyme, minimizing the loss of activity. They can also serve as skin contact material and extraction site, as a reservoir, or as an electrolyte for the transducing system [96]. Some biopolymers extracted from natural resources, such as chitosan or alginate, can form gels by cross-linking or complexation, and have the advantage of being biocompatible and inert, making them suitable for direct contact with the body [97].

Hydrogels were used as enzyme entrapment matrices for biosensing purposes all the way back to the mid-60 s, with Updike and Hicks' glucose-sensing electrode using GOx immobilized in an acrylamide gel [98]. A famous example of the use of hydrogels for this purpose has already been mentioned in this chapter: the Glucowatch, the first commercial glucose monitorization device [3]. Nowadays there is still a prominent use of hydrogels in wearable enzymatic biosensors, now taking advantage of modern manufacturing techniques and materials. Gun Jin Kim and Kyu Oh

Kim used electrospinning to create hydrogel nanofibers immobilizing GOx enzyme for the fabrication of a flexible and transparent glucose-responsive sweat patch [99]. Jayoung Kim et al. created a flexible tattoo-like alcohol biosensor via screen-printing of a Prussian Blue electrode immobilizing alcohol-oxidase enzyme in a chitosan matrix with an agarose gel cover [100].

A drawback of the entrapment approach is that, even though analytes can flow through the hydrogel matrix, diffusion is still restricted, which affects the sensitivity and response time of the biosensor [101]. Nagamine et al. developed a non-invasive enzymatic sweat-lactate biosensor employing an agarose gel touch pad as enzyme immobilization and sweat extraction site [102]. They pointed out that the signal detected did not reflect the actual lactate concentration in sweat, but the concentration extracted in the agarose gel. On top on that, since analytes diffuse directly into the gel, there is no control over the volume of sweat extracted, so the amount of lactate detected depends not only on the concentration in sweat but also on the perspiration rate.

An alternative to conventional cross-linked polymer hydrogel entrapment could be inorganic sol–gel matrices. These porous materials are chemically inert and can be formed under mild conditions and maintain enzyme structure. The key differences when comparing to their organic counterparts are the mechanical stability and neglectable swelling of the sol–gel matrices. This restricts undesired movement and conformational changes of the trapped enzymes and results in good preservation of the chemical and bioactive properties. However, the rigidity of the matrix makes its use limited for wearable applications that require flexibility, and even though the synthesis conditions are mild, and the method is relatively simple, sol–gel process can be exceedingly long sometimes [103].

8 Immunosensors

Antibodies are specialized immunoglobulins, a type of glycoproteins produced by lymphocyte cells in response to the presence of a foreign species known as antigen. Among immunoglobulins, the most widely used for biosensing (IgG) have a Y-shaped structure where two identical pairs of light chains (25,000 Da) and heavy chains (50,000 Da) are linked together by disulphide and hydrogen bonds [104]. Antibodies have been used as bioreceptors for a long time due to their broad application range, strong antigen–antibody interactions, and high specificity and sensitivity. Biosensors based on this type of interactions are called immunosensors [88].

In a similar manner to other biomolecule-based sensing devices (enzymatic, DNA…), immobilization of the bioreceptor on the surface of the transducer material is a crucial step in the fabrication if immunosensors. Surface coverage, binding stability, or orientation of the immobilized antibodies influence the detection performance of the system and need to be considered during the manufacturing process.

Table 3 DNA-based wearable biosensors

S. No	Probe	Immobilization method	Transduction method	Mechanism	Analyte	Limit of detection	Ref.
1	Aptamer/ SiO_2microneedle arrays-based skin patch	EDC/NHS-based Covalent immobilization	Optical/ Colorimetric	SiO_2 nanoparticle hydrogen swells upon the aptamer analyte interaction and changes its color due to the photonic nanocrystals	Histamine in rat muscle	2 μg/mL	[183]
2	dCAS9/Graphene/ Gold microneedle skin patch	EDC/NHS-based Covalent immobilization	Electrochemical	CRISPR/dCAS9-based detection for continuous measurement	Universal cell-free DNA	1.1 fM	[148]
3	5'NH-ssDNA/P-Si electrode	APTES/ Glutaraldehyde cross-linking	Optical	Hybridization of 5´NH-ssDNA with HPV complimentary DNA	Human papilloma virus	6,700 copies	[152]
4	Thiolated MB-modified DNA aptamer/Gold microelectrode	Thiolated aptamer immobilization on gold electrode surface	Electrochemical	Aptamers undergo reversible binding induced conformational changes upon interaction with the analyte molecules that affect electron transfer (eT) between the reporter and the gold needles	Tobramycin, Irinotecan, and Doxorubicin	Tobramycin = 1 μM, Irinotecan = 10 nM Doxorubicin = 10 nM	[120]
5	6-FAM-Biotin-SS DNA probe	Freeze dried in the μ-PAD system integrated into face mask	Optical	CRISPR-Cas12a-SHERLOCK-based 6-FAM-Biotin-SS DNA cleavage	SARS-CoV-2	17aM/500 copies	[182]
6	6-FAM-5' Iowa Black® FQ quenched ssDNA fluorescent reporter	Quickly deposited in-fabric to be snap-frozen and then lyophilized for 4–8 h within the device	Optical	CRISPR-Cas12a-based 6-FAM-5' Iowa Black® FQ quenched ssDNA cleavage	*mec*A/*spa*/ *erm*A genes	2.7 fM	[182]

(continued)

Table 3 (continued)

S. No	Probe	Immobilization method	Transduction method	Mechanism	Analyte	Limit of detection	Ref.
7	Methylene blue tagged aptamer	Thiolated aptamer immobilized on gold nano particle modified surgical microneedle patch implanted in rat for the measurement of administered drugs in blood	Electrochemical	Aptamer undergoes conformational change from linear upright to bent position upon interaction with analyte	Tobramycin and vancomycin	20 mg/kg of Tobramycin and 40 mg/kg of vancomycin	[184]
8	MB tagged poly-thymine (T-12) pseudoknot aptamer	MB tagged thiolated aptamer self-assembled on gold screen-printed electrode	Electrochemical	Pseudoknot unwinds bringing the MB redox probe close to the gold electrode surface upon cortisol interaction	Cortisol in sweat	0.2 pM	[169]
9	Guanine-rich VR11 aptamer	Aptamer was cross-linked to graphene surface via 1-Pyrenebutanoic acid succinimidyl ester (PBASE)	Field effect transistor	Change in charge carrier concentration due to confirmation switch of the aptamer to G-quadraplex upon interaction with the analyte	TNF-α	26 pM	[173]

Physical adsorption is a fast and simple strategy, as it was for enzyme immobilization. Functionalized surfaces and nanomaterials are used to create a self-assembled monolayer (SAM) of antibodies via non-covalent interactions such as H-bonding and electrostatic interactions. Due to the difference in the functional groups present in the F_c section of the bioreceptor (abundance of −COOH groups) and the F_{ab} sections, where the active antigen binding sites are found (abundance of −NH2 groups), changing some parameters such as pH can favor certain biomolecule orientations when forming the monolayer. This phenomenon was studied, for example, to immobilize anti-horseradish peroxidase antibodies (anti-HRP) on gold nanoparticles (AuNPs), finding that pH 7.5 provided more favorable orientations, with the active sites of the F_{ab} sections facing outwards, than pH 8 or higher [105]. However, physical adsorption still faces some limitations, as, because of the weak interactions, the final concentration of the immobilized antibodies is usually low. Some other factors that can affect the quality of an adsorption-based immunosensor are surface contamination at a low concentration of antibody, blocking active sites when the adsorption takes place near to the substrate surface, leading to loss of binding capacity, or partial denaturation of the immunoglobulin [104].

Covalent bonding is a more popular approach for antibody immobilization, and it has been used for immunosensors that have been successfully incorporated in working wearable devices. Jingwei et al. developed a multifunctional biosensing patch for simultaneous detection of various biomarkers. Enzymatic detection of ascorbic acid via hydrogel-entrapped ascorbic acid oxidase (AAOx) was complemented with the detection of neuropeptide Y (an important biomarker related to cardiovascular diseases) using its complementary antibody NPY-Ab. Immobilization of the antibody on the surface-modified Au transducer was carried out via amide-bond formation from the reaction of a self-assembled monolayer of carboxylic acid groups and the amine groups of the antibody. This covalent approach, as we have previously seen for enzyme immobilization ensures strong binding and proper orientation of the bioreceptor [106].

A popular strategy is to first form a SAM on the electrode surface and then covalently bind the antibody to the monolayer. SAM-forming alkanethiols are commonly used for covalently immobilization of antibodies on the surface of electrodes since they offer good surface coverage, stable covalent binding, and the possibility of controlling the orientation and distribution, of the sensing element while reducing non-specific interactions [107]. Functionalization of gold surfaces with highly ordered monolayer of alkyl thiols can be efficiently achieve via strong S–Au bond [107]. This combined approach allowed Han-Byeol et al. (2020) to build a stretchable lab-on-patch immunosensor that used an Au-ZnO nanostructured electrode with a di(N-succinimidyl)3,3'-dithiodipropionate (DSP) monolayer to covalently bind an antibody probe biomolecule for selective cortisol detection [108]. Alternatively, antibodies can be engineered to contain modifications at site-specific locations to control orientation upon immobilization. However, even though it is effective for that purpose, simpler modification methods need to be developed, as they currently involve complex conjugate synthesis with additional steps or require advanced protein engineering [105].

8.1 DNA-Based Biosensors

DNA-based biosensors have become increasingly popular due to their ability to detect and quantify biological and chemical analytes with high sensitivity and specificity. One of the most significant applications of DNA-based biosensors is the detection of DNA association with small molecules, such as drugs and chemicals [109]. These biosensors can be utilized in various fields, including pharmaceuticals and environmental monitoring, to determine the presence and concentration of small molecules of interest. Another application of DNA-based biosensors is the investigation of DNA interactions with proteins [110]. Biosensors can detect catalytic activities of DNA-processing enzymes and study affinity interactions between DNA and proteins, providing valuable insights into various cellular processes [111].

Aptamers are single-stranded DNA or RNA molecules that can bind to specific target molecules with high affinity and specificity. They are often referred to as "chemical antibodies" due to their ability to recognize and bind to targets with high specificity and affinity, similar to antibody-antigen interactions. Aptamers are selected from a large pool of oligonucleotides through a process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment). During SELEX, a random pool of oligonucleotides is incubated with the target molecule of interest, and the oligonucleotides that bind to the target are isolated and amplified by polymerase chain reaction (PCR). This process is repeated several times to enrich for oligonucleotides with high affinity and specificity for the target [112]. There have been significant development over microfluidic electrochemical aptamer-based sensors. These sensors offer a promising approach for non-invasive and real-time monitoring of biomarkers in different biofluids such as blood, sweat, and interstitial fluid [113]. As an example, Ferguson et al. developed an ex vivo microchip that accesses the bloodstream via catheters, allowing continuous and sensitive detection of drug concentrations. By integrating an electrochemical aptamer-based (E-AB) sensor with a continuous-flow diffusion filter, the system effectively prevents biofouling and interference from blood-borne substances. The sensor achieved a low limit of detection (LOD) for the chemotherapy drug doxorubicin and successfully monitored its concentration over an extended period in live animal models [114]. Another area of focus is the use of wearable sweat sensors for continuous non-invasive monitoring of biochemical biomarkers. Human sweat, which contains valuable diagnostic information, serves as an attractive medium for capturing molecular data [115, 116]. Researchers have developed wearable, flexible sensing platforms that can detect multiple sweat metabolites, electrolytes, and even drug concentrations. For example, a study where a wearable sweat sensor successfully monitored caffeine levels in real time during exercise [117, 118]. This concept has been expanded to continuously collect other drugs, such as levodopa, in sweat for personalized management of conditions like Parkinson's disease [119]. Another important application of wearable microneedle aptasensors for continuous monitoring of chemical biomarkers in interstitial fluid. Microneedle arrays penetrate the outermost layer of the skin, allowing minimally invasive and rapid sensing [120].

Lastly, DNA-based biosensors have been used for in vitro DNA damage detection, which can provide crucial information for assessing the genotoxicity of various compounds [121]. There is wide spectrum of transducing methods such as optical, acoustic, gravimetric, electrical, and electrochemical techniques have been employed in DNA-based biosensors, making them versatile tools for wearable sensor applications [122–126].

Integration of nucleic acid amplification methods with wearable biosensors for improved sensitivity in DNA-based sensing. Traditional amplification techniques like PCR, which require thermal cycling, are not suitable for wearable devices. Therefore, isothermal nucleic acid amplification includes Nucleic Acid Sequence-based Amplification, Loop-mediated Isothermal Amplification, Strand Displacement Amplification, Rolling Circle Amplification, and Recombinase Polymerase Amplification (RPA) [127]. RPA, in particular, stands out due to its fast reaction time and near-physiological temperature (37 °C). It has been successfully employed in wearable devices as it is highly sensitive and can utilize the heat generated by the human body as a heat source. The use of such techniques enables point-of-care testing with improved detection capabilities, making wearable biosensors valuable tools in various diagnostic and monitoring applications [128].

This section is focused to discuss the different immobilization methods which are suitable for the wearable biosensor with examples and the changes in DNA which leads to signal generation after binding to the analyte of interest. This includes DNA hybridization, DNA conformational changes, and CRISPR-CAS (clustered regularly interspaced short palindromic repeats—with CRISPR-associated Protein)-based detection methods.

Figure 7 is schematic diagram which shows a wearable biosensor with plausible mechanisms and components of wearable DNA. There are several wearable biosensors mentioned in the Table 1, which covers detection mechanism, immobilization technique for the probe molecule on the transducer surface and detection limits.

8.1.1 Immobilization Methods

Functionalization of transducer surface with the DNA probe depends on the choice of application, which in turn decides the sensitivity, stability, and minimizes the cross reactivity of the sensor. There were different types of immobilizations of DNA probes on the functional surfaces such as adsorption, covalent immobilization, avidin–biotin interactions, and entrapment.

8.1.2 Adsorption

DNA oligomers, aptamers, and SS-DNA can be immobilized on transducer surfaces by adsorption. Negatively charged DNA strands can be adsorbed onto positively charged surfaces via electrostatic interactions. The adsorbed DNA is

oriented randomly, presenting different directions without uniformity. Cationic polymers such as Poly(L-Lysine), chitosan, polyethyleneimine, and poly(3-(3′-N,N,N-triethylamino-1′-propyloxy)-4-methyl-2,5-thiophene) (PMNT) were used to demonstrate for the DNA immobilization for biosensing applications [129–132]. FAST™ slides are a kind of glass slides with microporous nitrocellulose surface was used as microarray technology. The FAST™ surface can be modified with DNA molecules, which adsorbs on the polymer surface irreversibly. These slides were employed for fluorescent and chemiluminescence-based assay [133]. DNA can be immobilized via physical adsorption on surface such as pyrolytic graphite electrode, glassy carbon electrode, and gold electrode [134]. FAM (fluorescein) labeled DNA oligonucleotide was immobilized on 13 nm sized gold nanoparticle. The FAM labeled DNA incubated with gold nanoparticles at lower pH for a duration of 5 to 8 min followed by centrifuging and washing steps in PBS at neutral pH condition [135]. Wang et al. demonstrated metal–organic frameworks (MOFs) with different metal compositions such as Zr, Cr, Fe, and Al were successfully adsorbed with DNA oligonucleotides with rich phosphorous moieties for the successful demonstration of measuring and manipulating intracellular processes [136]. Sun et al. successfully developed colorimetric sensor array by adsorbing thiolate SS-DNA gold nanoparticles on zirconium metal–organic frameworks (Zr-MOFs) for the determination of semen quality of humans [137]. Xiong et al. developed a MOF, heme-like ligand FeTCPP into commonly used MOFs (UiO-66) (FeTCPP $\subset$ UiO-66) and modified by incubating 6-carboxy fluorescein (FAM)-labeled SS-DNA for a duration of 10 min. DNA physically adsorb on the MOF surface with one among the electrostatic, π-stacking, hydrogen-bonding, and coordination interactions and was used to perform dual fluorescence resonance energy transfer (FRET)-based fluorescence quenching assay and peroxide mimicking colorimetric assay for the determination of AFB1 (Aflatoxin B1) [138].

Though several biosensors were designed based on DNA physical adsorption on the substrates such as gold nanoparticles, polymers, MOFs, gold and graphite electrodes their use in the design of real-time biosensor will be limited due to non-specific, disoriented immobilization on the surfaces [139, 140]. Moreover the adsorption of DNA is susceptible to varying conditions such as pH, salt concentrations, non-specific desorption, etc. [141].

8.1.3 Covalent Immobilization

Covalent immobilization methods are based on oligonucleotide covalently binding to the immobilizing surfaces, which offers good stability, flexibility, binding strength, and provides a suitable vertical orientation to the detecting probe [142].

The oligonucleotide typically binds covalently to the amine or carboxy terminated self-assembled monolayers on the gold surfaces at the 3′ or 5′ end. Thiols show a strong affinity to the gold surface, also referred chemisorption, attaching covalently forming a self-assembled layer.

Glenn et al. conjugated oligonucleotide with gold nanoparticles by mixing 1:20 volume ratio of 100 μM thiolated oligonucleotide with gold nanoparticles and incubated at 37 °C for 30 min, followed by addition of 150 μL of 1 M NaCl/100 mM phosphate buffer of pH 7.0 and incubated at 37 °C for aging. Excess oligonucleotides were removed by centrifuging the volume, and resuspending into 0.3 M NaCl/ phosphate buffer at pH 7.0 for several cycles [143]. Ahmadi et al. prepared thiolated oligonucleotides by adding nucleotide to 0.1 N dithiothreitol (DTT) and incubated for 15 min. Excess DTT was removed by washing thrice with ethyl acetate. The thiolated DNA-gold nanoparticle mixture was used as colorimetric sensor for the detection of Klebsiella pneumoniae [144]. Self-assembly of the 32-mer-5′ oligonucleotide on Nano-Au electrode surface achieved by adding thiolated DNA to the electrode surface and incubating at –4 °C for 6 h. The developed DNA-based electrochemical biosensor is used for DNA-based bioassay of legionella pneumonia pathogen [145].

Covalent attachment of modified probe on functionalized surface can be achieved by self-assembled monolayers on the transducer surface. As it is well known thiol show strong affinity toward gold surfaces, several alkane thiols, dithiols, and thiol containing amino acids [146, 147]. These self-assembled monolayers mostly terminated with carboxyl functional groups which can be attached to the amine terminated oligonucleotide probe via carbodiimide chemistry, which also referred as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxy succinimide coupling (EDC-NHS coupling). EDC enables coupling carboxylic groups to primary amines of the linking molecules by forming and amine-reactive O-acylisourea intermediate. NHS mediates to form active amine ester which will efficiently couples the EDC mediated reaction.

Yang et al. developed graphene oxide decorated gold micro needle electrode to demonstrate CRISPR-dCAS9-based skin-patch biosensor for long-term capture and real-time monitoring of universal cell-free DNA. 1-pyrenebutanoic acid (PBA), binds to graphene surface with π–π interaction was used to establish EDC-NHS coupling between COOH of PBA and dCAS9. 1:1 ratio of 4 mM EDC and 11 mM NHS mixture in 50 mM 2-morpholinoethanesulfonic acid buffer was added and incubated for a duration of 60 min. 1% of BSA was used as blocking agent to avoid non-specific binding [148]. 1-pyrenebutanoic acid succinimidyl ester (PBASE) is analogue to PBA and can bind to graphene-modified surfaces in the similar fashion. The advantage of using PBASE is EDC-HNS activation is no longer required, since succinimide ester already exist on PBASE. Kusku et al. modified graphene surface with PBASE by adding 0.2 mM PBASE/N, N Dimethylformamide (DMF). The PBASE modified graphene surface was directly immobilized with probe DNA and incubated at 4 °C overnight. The developed sensor was used as DNA-graphene field effect transistor (GFET) as a proof of concept for the microfluidic-graphene-based molecular communication receiver for Internet of Nano Things (IoNT) [149].

Glutaraldehyde is a widely used alternative as a fixative and cross-linking agent in biological assays. It is a dialdehyde whose aldehydic groups are highly reactive and can form covalent bonds with functional groups such as amines, thiols, phenols, hydroxyl, and imidazoles.

Most commonly glutaraldehyde was employed in coupling reaction between two amine terminated chemical species via its two aldehyde groups, forming water molecule as a by-product, also referred as aldehyde-ammonia condensation reaction. Chitosan-multiwalled carbon nanotunes (CS-MWCNT) modified glassy carbon electrode (GC) was incubated with 1% glutaraldehyde for 2 h followed by cross-linking amine terminated aptamer for the electrochemical detection of tetracycline with detection limit of 5.6 fM [150]. ~ 60 nm thick YbTixOy on silica wafer with ~ 400 nm-thickness Al film at the bottom was used as transducer for the electrochemical biosensor. 3-aminopropyl triethoxysilane (APTES) was immobilized on sensor surface which provide hydroxyl groups. Amine groups pf APTES SAM layer were modified with 2.5% glutaraldehyde solution overnight followed by addition of ssDNA probe for the electrochemical detection of KRAS and BRAF gene mutations in colorectal cancer [151]. 5'Amine C12 modified ssDNA was used as probe molecule and APTES was used as SAM layer on electrochemically etched boron doped porous silica substrate. Glutaraldehyde cross-linking agent was used to bridge the ssDNA probe and APTES molecules. The developed sensor was used as optical sensor utilizing the porous structure of silica for the detection of human papilloma virus (HPV) detection [152]. Owing to the excellent cross-linking ability of the glutaraldehyde as across-linking agent it also offers high molecular weight and hydrophobicity due to the replacement of amino group with aldehyde group. This may affect the confirmations of the probe/analyte molecule confirmation, in turn affecting the sensitivity of the detection system [153].

8.1.4 Avidin (Streptavidin)–Biotin Interactions

Biotin, a vitamin B7 derivative, exhibits exceptionally strong and specific binding to avidin and streptavidin proteins. This characteristic has made the avidin–biotin and streptavidin–biotin interactions a cornerstone of molecular biology and biochemistry research, enabling the development of a wide range of applications such as protein purification, cell labeling, and cross-linking. In cross-linking applications, biotinylated molecules are attached to either avidin or streptavidin, which in turn bind specifically to the other protein or molecule of interest. The resulting complex forms a stable, non-covalent interaction that can be utilized to link enzymes, antibodies, or DNA. The tetrameric avidin protein, isolated from egg whites, and the homotetrameric streptavidin protein, derived from the bacterium Streptomyces avidinii, each have four high-affinity binding sites for biotin. These strong and specific interactions have dissociation constants in the femtomolar range, making them ideal for applications requiring high affinity and specificity. Streptavidin with an isoelectric point (pI) equal to 5.0 is thus preferably used over avidin, which has a pI of 10.5, to avoid non-specific interactions.

5′ biotinlyted DNA probe was immobilized on glassy carbon electrode by cross-linking the DNA probe to the avidin immobilized GCE. The avidin was immobilized on GC electrode by modifying the GC electrode with COOH terminated 4-carboxyphenyl diazonium salt. COOH groups of the GC electrode were activated by

carbodiimide chemistry. The avidin–biotin interaction was achieved by immersing electrode in 0.1 M PBS at pH 7.0, which contains 10 pM biotinylated DNA probe molecules and incubated for one hour at room temperature followed by washing the electrode with 0.1/ SDS to remove unbound DNA probes [154].

Liu et al. designed biotinylated gold electrode sensor array by forming a mixed self-assembling monolayer (SAM) with 11-mercaptoundecanoic acid (MUA) and 11-mercapto-1-undecanol (MU) and activating the COOH with EDC-NHS chemistry to cross-link with biotin. A dual labeled the stem-loop probe oligonucleotide (oligos 1 and 6), has a 5′-digoxigenin (DIG) affinity label and a 3′-biotin was cross-linked to biotinylated electrode surface via biotin-avidin–biotin cross-linking. As a result, the DIG label becomes available by the anti-DIG-HRP, and the target hybridization event can be efficiently transduced via the enzymatically amplified faradaic electrochemical electron transfer phenomenon to sensitively detect femtomolar detection of sequence specific DNA [155]. Pan et al. conducted similar studies, where gold coated Indium tin oixide glass substrate was passivated with mixed thiols of 2-mercaptoethanol (2-ME) and 11-mercaptoundecanoic acid (11-MUA) and activated carboxylic group of 11-MUA by carbodiimide chemistry. The activated COOH group was covalently bound with streptavidin, was meant to bind with 5´ biotinylated probe sequence SS-DNA. The detection event was performed by exposing the mixture of DNA samples to the electrode surface, the hybridization event was transduced into electrochemical event in terms of increment in the charge transfer resistance (Rct). The detection limit was noted up to picomolar of concentration of DNA [156].

8.1.5 DNA Probe-Analyte Interactions

DNA undergoes physical changes which leads to signal generation after binding to the analyte of interest. Based on the changes post to the DNA probe and analyte interaction DNA biosensor can be broadly divided into two types (1) DNA biosensors based on hybridization and (2) DNA biosensors based on conformational changes.

8.1.6 DNA Biosensor Based on Hybridization

DNA biosensor based on the hybridization were studied extensively. Hybridization-based detection mechanism can be broadly divided into two types (1) Label free DNA detection and (2) Labeled DNA detection.

Label free detection of DNA is mostly based on electrochemical detection utilizing the redox properties of DNA base pairs. Purines such as Adenine and Guanine can undergo electrooxidation at lower potentials than that of pyrimidines, moreover purine attracted the focus due to their ability to bind with various labeling molecules such as redox probes, fluorescent probes and dyes, etc. [141, 157]. The principle behind the label free electrochemical DNA biosensors is interaction of guanine with its complementary molecule cytosine and adenine with thiamine during DNA hybridization process leaving few free adenine/guanine moieties for electrochemical

oxidation. As a result, the faradaic current response due to adenine/guanine decreases. Jalit et al. reported electrooxidation of nucleic acids at Poly-L-lysine-MWCNT modified glassy carbon electrode. Single-stranded electrodes were deposited electrostatically on the Poly-L-lysine modified multiwalled carbon nanotubes by immersing in a PBS solution with 50 mg/mL DNA. In this study guanine was monitored for the electrooxidation studies using adaptive stripping voltammetry. The results showed free guanine undergo electrooxidation resulting high current response, whereas, current response decreased as hybridization process increases [158]. The main disadvantage of label free DNA detection is sluggish electron transfer rates and poor current response. To circumvent the problem, redox probe-based electron mediators were introduced to improve the electron transfer kinetics and improved current responses. These redox mediators can either labeled on the ssDNA/dsDNA or can be used without labeling [159]. $Ru(bpy)_3^{2+}$ metal ion complex was widely used as label free redox indicator for the electrochemical detection of DNA hybridization. It was shown in Fig. 8 Guanine can undergo oxidation by reducing the oxidized ruthenium bipyridine $Ru(bpy)_3^{3+}$ to $Ru(bpy)_3^{2+}$[160] also used as probe molecule for the DNA hybridization detection by generating electrochemiluminescence (ECL) signal. Electrochemically oxidation of $Ru(bpy)_3^{2+}$ to $Ru(bpy)_3^{3+}$ can be reduced by co-reactant guanine nucleotide forming excited $Ru(bpy)_3^{2+*}$. ECL signal was generated when $Ru(bpy)_3^{2+*}$ relaxed to ground state form $Ru(bpy)_3^{2+}$. Wei et al. reported MWCNT/Nafion modified glassy carbon electrode for the detection of DNA hybridization process in P53 gene using label free $Ru(bpy)_3^{2+}$-based ECL detection. It was found that the ECL signal generation is sensitive up to 39.3 nM concentration and able to change the signal with single base pair mismatch during the hybridization process [159]. Another redox probe for label free DNA redox mediator is the widely studied $Fe(CN)_6^{3-/4-}$, which do not bind to the DNA due to the electrostatic repulsion of DNA, (depicted in Fig. 8) can be used to quantify the amount of DNA present before and after the hybridization process.

Labeled DNA hybridization-based electrochemical biosensors were reported. The labels are redox responsive which were attached to the single-stranded DNA probe molecules to monitor the DNA hybridization process [161]. The redox labels can bind to the DNA with simple immersion method for a certain period of time. Interaction event can take place between DNA and probe molecule via different modes such as electrostatic, affinity toward a specific nucleotide base, groove binding and intercalation, etc. For example, $Ru(NH_3)_6^{3+}$, a metal complex, can bind electrostatically to negatively charged phosphate of hybridized DNA, was widely used to evaluate the DNA modified surface to understand the surface coverage [121, 162]. Other redox probes such as doxorubicin, an anticancer drug also was used as a labeled indicator for the detection of DNA hybridization. Ferrocene another important redox probe binds specifically to major grove of dsDNA and perform redox reaction on the electrode surface [163, 164]. Ethidium bromide is also one of the important redox indicators for DNA hybridization, show affinity to the guanine-cytosine pair and intercalate between them.

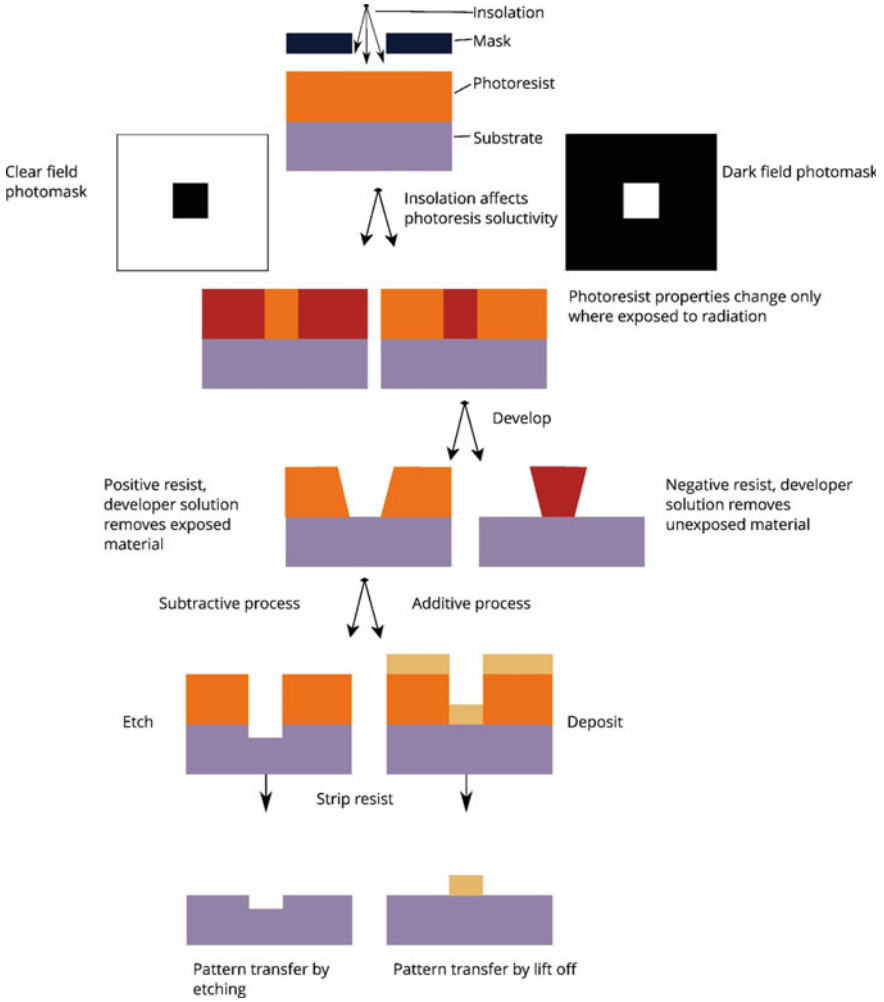

Fig. 8 Schematics summary of the photolithographic process from the wafer with photoresist to the final product

8.1.7 DNA Biosensor Based on Conformational Changes

A DNA biosensor based on conformational change leverages the inherent structural dynamics of DNA molecules to detect and analyze target molecules. The biosensor comprises a DNA probe engineered to selectively interact with the target molecule of interest. Binding or interaction between the target molecule and the DNA probe induces a conformational rearrangement within the DNA structure, which can be exploited for detection and quantification. These conformational changes can be interrogated through various sensing methods such as electrochemical, fluorescence,

and surface plasmonic resonance (SPR) techniques. The detection technique purely depends on the type of detection probe tangled to the DNA and detection event happens once the DNA probe bind to the target molecule, changing the DNA's confirmation which in turn leads to change in the either of the fluorescent/electrochemical/SPR signals providing the qualitative or quantitative information of the analyte of interest.

There are several common types of DNA conformational changes widely used for detection purposes (Fig. 9). These include:

1. DNA Hairpin-based detection
2. DNA pseudoknot-based detection
3. DNA G-quadruplex-based detection.

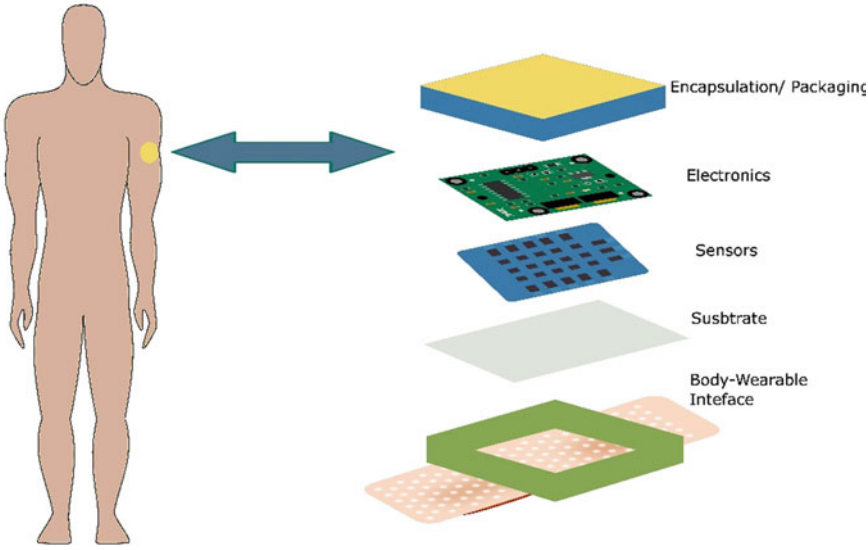

Fig. 9 Schematic representation of a wearable device

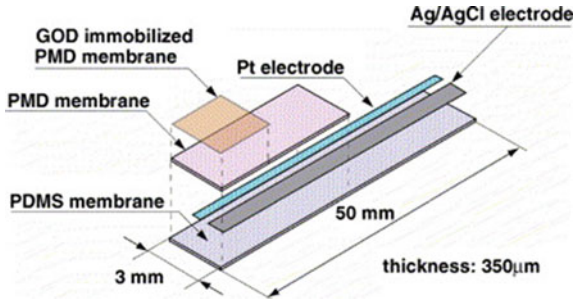

Fig. 10 Schematic representation of the flexible glucose sensor based on polymers by Kudo et al. [71]

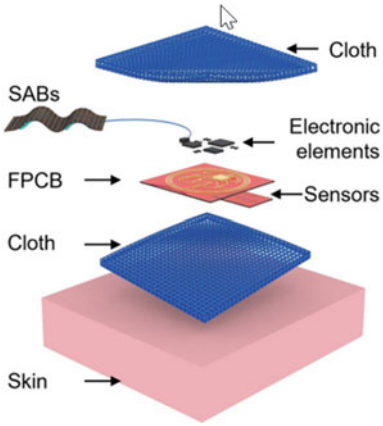

Fig. 11 Schematic illustration of a cotton-fabric-based wearable device [74]

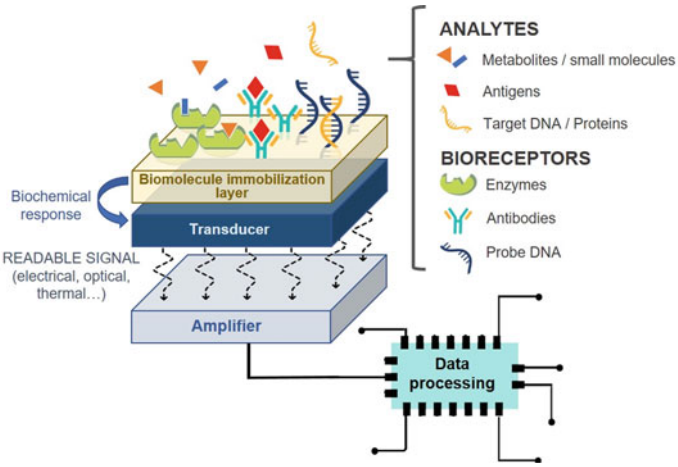

Fig. 12 Schematic representation of the components of a biosensor

8.1.8 DNA Hairpin-Based Detection

A hairpin bend DNA-based biosensor utilizes the conformational changes that occur in hairpin-structured DNA molecules for biosensing applications. Hairpin DNA structures consist of a single DNA strand that folds back on itself, forming a stem-loop structure. The stem region is composed of complementary base pairs, while the loop region contains non-complementary bases.

In a hairpin bend DNA-based biosensor, the DNA probe is designed with a specific recognition sequence for the target molecule of interest, which is typically located within the loop region. The binding or interaction of the target molecule with the

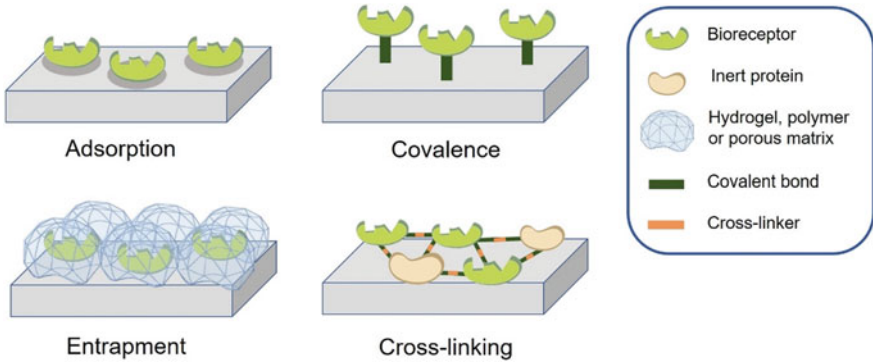

Fig. 13 Diagrammatic representation of the four main enzyme immobilization strategies for transducer functionalization

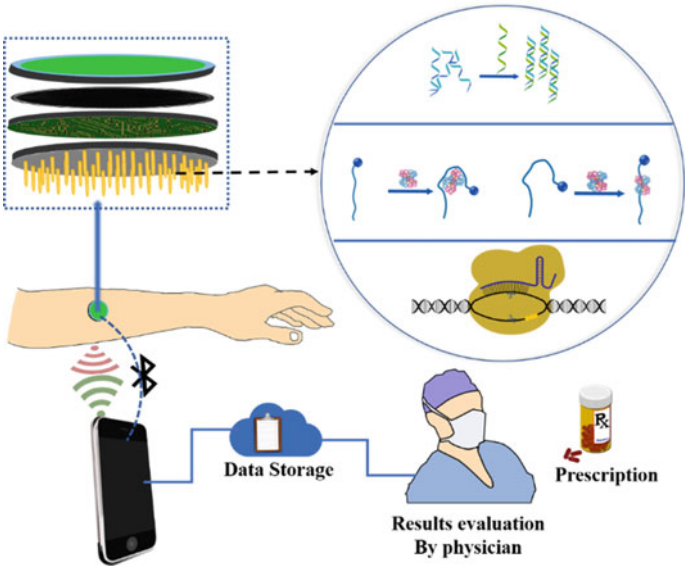

Fig. 14 Scheme of DNA biosensor depicting the different types of DNA detection mechanisms plausible for the wearable DNA biosensors and cell phone-based readout, storage, results evaluation, and prescription by a physician

DNA probe induces a conformational change in the hairpin structure, leading to the bending or distortion of the stem region.

The detection of the hairpin bend conformational change can be achieved through various techniques such as electrochemical and fluorescence techniques. In fluorescent-based detection method, the fluorescent labels or dyes can be attached

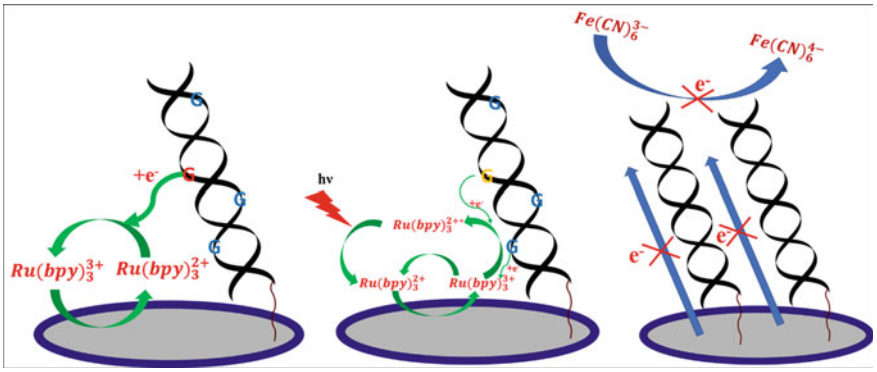

Fig. 15 Mechanism of electron exchange between the redox probes such as $Ru(bpy)_3^{2+}$ and $Fe(CN)_6^{3-/4-}$ with the DNA on the electrode surface

Fig. 16 Three dimensional conformational changes of aptamers. Reprinted (adapted) with permission from [179]. Copyright (2016) American Chemical Society

to the ends of the hairpin structure, such that in the folded or unbent state, the fluorescence signal is quenched due to close proximity or efficient energy transfer between the labels. Upon target molecule binding, the hairpin structure undergoes a conformational change, resulting in the separation or alteration of the fluorophores, leading to an increase in fluorescence signal, which can be measured and quantified [165]. Farjami et al. reported DNA beacons modified gold electrodes with their hair pin folded states through the alkanethiol linker at the 3′ end, while the 5′ end was labeled with a methylene blue (MB) redox probe. The MB lies proximity with the electrode surface enabling the faster electron transfer in the absence of analyte, whereas during the biorecognition event, the electron transfer rate was lowered due to loop unfolding upon hybridization process moving the redox probe away from the electrode surface. This mechanism was successfully employed to construct DNA-based electrochemical detection of cancer biomarker-TP53 gene [166].

Gao et al. reported a flexible multiplexed aptamer biosensor for the insitu wound monitoring of mice model. The sensor designed to detect inflammatory mediators

such as inflammatory mediators [tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8, and transforming growth factor-β1] and microbial load of Staphylococcus aureus. Hair pin bend aptamer was designed with thiol group for the self-assemble on the gold electrode surface and methylene blue was tagged for the faradaic reaction. In the absence of the analyte the hairpin structure of aptamer keeps the methylene blue redox probe close to the electrode surface resulting high faradaic response, whereas in the presence of the analyte, upon aptamer bind to the analyte and undergo conformational change to upright position. This makes redox move away from the electrode surface lowering the current response [113].

8.1.9 DNA Pseudoknot-Based Detection

A DNA pseudoknot-based biosensor utilizes the formation and disruption of pseudoknot structures within DNA molecules for sensing applications. Pseudoknots are intricate three-dimensional structures formed by specific folding patterns of nucleic acids. They consist of regions where a single-stranded DNA segment forms a loop by base-pairing with another region of the same strand, creating a structure resembling a knot. In a DNA pseudoknot-based biosensor, the DNA probe is designed to contain a target recognition sequence, a loop region, and complementary regions that can form the pseudoknot structure. The presence of the target molecule induces the formation of the pseudoknot structure by bringing the complementary regions together. This conformational change results in a detectable signal that can be measured to indicate the presence or concentration of the target molecule [167].

The detection of the pseudoknot formation can be achieved through various techniques. For instance, fluorescence-based methods involve incorporating fluorophores or quenchers within the DNA probe. The pseudoknot formation alters the proximity or accessibility of these fluorophores or quenchers, leading to changes in the fluorescence signal. Electrochemical methods rely on changes in the electrical properties of the DNA probe upon pseudoknot formation, which can be measured as an electrical signal. Other techniques, such as surface plasmon resonance (SPR) or quartz crystal microbalance (QCM), can also be utilized to monitor the mass or refractive index changes associated with the pseudoknot formation.

The advantage of DNA pseudoknot-based biosensors lies in their ability to achieve highly specific and sensitive target detection. The conformational changes resulting from pseudoknot formation offer a robust and tunable mechanism for signal transduction. These biosensors have been utilized in various applications, including the detection of nucleic acids, proteins, small molecules, and pathogens.

Heeger et al. reported an electrochemical pseudoknot-based DNA sensor for the detection of DNA in blood serum. Pseudoknot DNA structure attached with methylene blue (MB) responds to the analyte and undergoes conformational change, bringing it near to the electrode surface, which supports electron transfer. The electrochemical DNA biosensor was reported to be able to detect the analyte as low as 2 nM with a dynamic linear concentration range between 2 and 200 nM [168].

Singh et al. developed aptamer wearable biosensor for the continuous measurement of stress levels by estimating the cortisol concentrations in human sweat. The developed device consists of a poly-thymine (T-12) pseudoknot aptamer with methylene blue tag immobilized on gold screen-printed electrode and a pH sensor to measure and correct the generated signal accordingly. Upon the cortisol interaction with the aptamer the pseudoknot switches its shape enabling the methylene blue redox probe reach the electrode surface to enhance the faradaic current response. The lower detection was reported to be 0.2 pM with the linear concentration range of 1 pM–1 μM [169].

8.1.10 DNA G-Quadruplex-Based Detection

G-quadruplexes are stable secondary structures formed by guanine-rich DNA sequences. These structures consist of stacked planar arrangements of guanine tetrads stabilized by Hoogsteen base-pairing [170]. The formation of G-quadruplexes can induce substantial conformational changes in DNA. Biosensors have exploited the conformational changes associated with G-quadruplex formation for the detection of specific DNA sequences, as well as the binding of small molecules or metal ions. The design of a DNA G-quadruplex biosensor typically involves incorporating a G-quadruplex-forming DNA sequence into a sensing platform, which can be a DNA probe, aptamer, or other DNA-based recognition element. The biosensor takes advantage of the specific binding affinity of G-quadruplexes to various target molecules, such as small molecules, proteins, or nucleic acids, for detection purposes.

Tan et al. reported response of a G-quadruplex of human telomere sequence $(TTAGGG)_4$ to ionnic concentrations of K^+ and Na^+. During their study, they observed that the association and dissociation rate of G-quadruplex with its complimentary DNA were lowered with the concentrations of K^+ and Na^+, while in the presence of K^+ ion kinetics of association and dissociation was lowered compared to Na^+ ion. This study was useful to understand telomere length homeostasis in telomerase-positive cells, such as germ line and cancer cells [171]. AS114q is a G-rich quadraplex 3D structured aptamer with unique property, and has selective affinity to the Cu^{+2} ions. This property of AS114q was employed to design AS114q dependent fluorescent aptasensor for the detection of Cu^{+2}, in which gel red was used as fluorescent dye. Cu^{+2} ions showed the high affinity, supressing the complimentary DNA and bind to the AS114q resulting in weaker fluorescence [172].

Graphene-modified flexible SiO_2 coated polyethylene napthalate (PEN)-based graphene field effect transistor (GFET) was designed by Hao et al. for the wearable biosensor application to detect cytokines biomarker-tumor necrosis factor (TNF-α) from human sweat. TNF-α-specific aptamer, a guanine-rich VR11 upon binding to the TNF-α, changes its confirmation to a compact G-quadruplex structure. The charge carrier concentrations were altered due to interaction dependent conformational change to close proximity of the negatively charged base pairs to the graphene surface. Lower detection limit was reported to be 26 pM with a detection time of 5 min [173].

Travascio et. al. first reported a G-quafraplex-hemen complex showed enhanced peroxidase activity than the individual iron porphyrin containing pristine hemin [174]. This mechanism was widely used for the detection of nucleases, screening G-quadruplex ligands (potential anticancer reagents), heterocyclic compounds such as pentamethine, symmetric trimethine cyanines, micro-RNA, etc. [175–178].

8.1.11 CRISPR-Cas-Based Biosensors

Clustered regularly interspaced short palindromic repeats (CRISPR) are short DNA sequences found in bacterial and archaeal genomes that are part of an adaptive immune system. Cas (CRISPR-associated) proteins are enzymes that use CRISPR sequences to target and cleave foreign DNA, such as viral genomes [180]. A CRISPR-Cas biosensor is a molecular tool that detects specific DNA sequences using the CRISPR-Cas system.

In a CRISPR-Cas biosensor, the CRISPR-Cas system is used to detect specific DNA sequences in a sample. The biosensor consists of a CRISPR RNA (crRNA) that is designed to recognize the target DNA sequence and a Cas enzyme that cleaves the target DNA in the presence of the crRNA. The biosensor also includes a reporter molecule, such as a fluorescent, colorimetric dye, or a redox probe that is activated when the Cas enzyme cleaves the target DNA [181].

To use a CRISPR-Cas biosensor, the crRNA is first designed to specifically target the DNA sequence of interest. The biosensor is then introduced to a sample containing the target DNA, and if the target DNA is present, the crRNA will hybridize to the target DNA and activate the Cas enzyme to cleave the DNA. The reporter molecule will then signal the presence of the target DNA by generating a detectable signal. CRISPR-Cas biosensors have numerous applications in medical diagnostics. They offer a rapid and sensitive method for detecting specific DNA sequences with high specificity and accuracy.

Peter et al. reported face mask integrated sensor with microfluidic paper-based analytical device (μ-PAD) containing lateral flow assay (LFA) strip. The sensor is incorporated with freeze-dried lysis and detection molecules. The molecules get activated upon interaction with sample containing fluid by wicking action and show the response. The sensor is designed based on CRISPR-Cas12a mechanism, in the presence of sample, i.e., SARS-CoV-2, the virus undergoes lysis in the presence of lysis reagent, exposing the SARS-CoV-2 S gene. The SARS-CoV-2 S gene triggers the function of CAS-12a enzyme, which is already paired reverse transcription-recombinase polymerase amplification (RPA) to produce amplicons. The presence of amplicons triggers the cleavage of 6-FAM-(TTATTATT)-Biotin ssDNA probe, leading to emission of fluorescence at 495 nm. This phenomenon is called CRISPR-based specific high-sensitivity enzymatic reporter unlocking (SHERLOCK). The sensor can give results in 1 h 30 min with a limit of detection of 500 copies/17aM. A similar mechanism was integrated into a multi-sensor wearable freeze-dried cell-free (wFDCF)-CRISPR-based sensor for the detection of *mec*A/*spa*/*erm*A genes of methicillin-resistant *Staphylococcus aureus* (MRSA). The sensor works based on

the wicking liquid sample of the fiber optic embedded fabric which is integrated in a jacket. The fiber optic embedded sensor functions upon rehydration of the fabric and measures the changes in the excitation event due to the changes in the molecular interaction cascade during the CRISPR-Cas mechanism [182].

Table 2 shows that the Relation of Various DNA-Based Wearable Biosensors

9 Challenges and Opportunities for Wearable Biosensors

Wearable biosensors are becoming increasingly important not only in healthcare, but also in other areas such as sports, work safety, and defense. The main reason why one uses wearable devices, and particularly wearable biosensors, beyond the ability to obtain physiological information in real time, is their convenience. Wearable devices should push Dieter Rams' idea of unobtrusiveness to the extreme where users become unaware that they are being monitored. This is extremely difficult to accomplish, given the relatively short lifespan of chemical sensors and biosensors, which requires the sensing part to be replaced with certain frequency. Wearable biosensors face other challenges in common to other wearable devices, such as accuracy, reliability. Power management, data security, and user acceptance. In this sense, something worth remembering about wearables in general, is that the users will find it very hard to wear anything they are not used to wearing already. The exceptions to this, of course, stem out of need. Obviously, the patient suffering from a disease such as diabetes mellitus will wear a skin patch if this improves their life quality, or a worker will wear a certain garment or accessory if it improves their safety. But designing wearables for the broader population can pose significant cultural challenges but represents enormous opportunities.

This chapter has focused on the design and fabrication of wearable biosensors, and has provided many examples of different wearable biosensors taken from the academic literature. The design should begin with the user and the use environment, and then work its way down to the smallest detail. This includes not only the perspective of the user, but also of manufacturing. In this sense, fabrication technologies and materials available today make almost anything possible. In the case of wearable biosensors, the disposable nature of the sensing part has driven many a worker to explore the construction of biosensors using biodegradable or absorbable materials that can reduce or eliminate the environmental impact of disposing of a packaged device with relatively high frequency (every 2 to 3 weeks at best in the case of state-of-the-art commercial devices). Another interesting direction for future development is not to make short-lived devices less environmentally damaging, but to make them much longer lasting. However, this not only involves developing new materials or new synthetic receptors, but also new ways to measure. Self-calibrating or calibration-less systems are perhaps a Holy Grail in electroanalytical science, but one that will eventually be found.

References

1. Land, K.J., Boeras, D.I., Chen, X.-S., Ramsay, A.R., Peeling, R.W.: REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat. Microbiol. **4**, 46–54 (2019). https://doi.org/10.1038/s41564-018-0295-3
2. Mabey, D., Peeling, R.W., Ustianowski, A., Perkins, M.D.: Diagnostics for the developing world. Nat. Rev. Microbiol. **2**, 231–240 (2004). https://doi.org/10.1038/nrmicro841
3. Tierney, M.J., Tamada, J.A., Potts, R.O., Jovanovic, L., Garg, S.: Clinical evaluation of the GlucoWatch® biographer: a continual, non-invasive glucose monitor for patients with diabetes. Biosens. Bioelectron. **16**, 621–629 (2001). https://doi.org/10.1016/S0956-5663(01)00189-0
4. Tierney, M.J., Tamada, J.A., Potts, R.O., Eastman, R.C., Pitzer, K., Ackerman, N.R., Fermi, S.J.: The GlucoWatch ® biographer: a frequent, automatic and noninvasive glucose monitor. Ann. Med. **32**, 632–641 (2000). https://doi.org/10.3109/07853890009002034
5. Ellis, S., Naik, R., Gemperline, K., Garg, S.: Use of Continuous Glucose Monitoring in Patients with Type 1 Diabetes. Curr. Diabetes Rev. **4**, 207–217 (2008). https://doi.org/10.2174/157339908785294370
6. Christiansen, M., Bailey, T., Watkins, E., Liljenquist, D., Price, D., Nakamura, K., Boock, R., Peyser, T.: A New-Generation Continuous Glucose Monitoring System: Improved Accuracy and Reliability Compared with a Previous-Generation System. Diabetes Technol. Ther. **15**, 881–888 (2013). https://doi.org/10.1089/dia.2013.0077
7. Garcia, A., Rack-Gomer, A.L., Bhavaraju, N.C., Hampapuram, H., Kamath, A., Peyser, T., Facchinetti, A., Zecchin, C., Sparacino, G., Cobelli, C.: Dexcom G4AP: An Advanced Continuous Glucose Monitor for the Artificial Pancreas. J. Diabetes Sci. Technol. **7**, 1436–1445 (2013). https://doi.org/10.1177/193229681300700604
8. Kropff, J., Choudhary, P., Neupane, S., Barnard, K., Bain, S.C., Kapitza, C., Forst, T., Link, M., Dehennis, A., DeVries, J.H.: Accuracy and Longevity of an Implantable Continuous Glucose Sensor in the PRECISE Study: A 180-Day, Prospective, Multicenter. Pivotal Trial. Diabetes Care. **40**, 63–68 (2017). https://doi.org/10.2337/dc16-1525
9. Laffel, L.M., Aleppo, G., Buckingham, B.A., Forlenza, G.P., Rasbach, L.E., Tsalikian, E., Weinzimer, S.A., Harris, D.R.: A Practical Approach to Using Trend Arrows on the Dexcom G5 CGM System to Manage Children and Adolescents With Diabetes. J. Endocr. Soc. **1**, 1461–1476 (2017). https://doi.org/10.1210/js.2017-00389
10. Aleppo, G., Laffel, L.M., Ahmann, A.J., Hirsch, I.B., Kruger, D.F., Peters, A., Weinstock, R.S., Harris, D.R.: A Practical Approach to Using Trend Arrows on the Dexcom G5 CGM System for the Management of Adults With Diabetes. J. Endocr. Soc. **1**, 1445–1460 (2017). https://doi.org/10.1210/js.2017-00388
11. Yeoh, E., Png, D., Khoo, J., Chee, Y.J., Sharda, P., Low, S., Lim, S.C., Subramaniam, T.: A head-to-head comparison between Guardian Connect and FreeStyle Libre systems and an evaluation of user acceptability of sensors in patients with type 1 diabetes. Diabetes Metab. Res. Rev. 38, (2022). https://doi.org/10.1002/dmrr.3560
12. Guillot, F.H., Jacobs, P.G., Wilson, L.M., Youssef, J.E., Gabo, V.B., Branigan, D.L., Tyler, N.S., Ramsey, K., Riddell, M.C., Castle, J.R.: Accuracy of the Dexcom G6 Glucose Sensor during Aerobic, Resistance, and Interval Exercise in Adults with Type 1 Diabetes. Biosensors **10**, 138 (2020). https://doi.org/10.3390/bios10100138
13. Davis, G.M., Spanakis, E.K., Migdal, A.L., Singh, L.G., Albury, B., Urrutia, M.A., Zamudio-Coronado, K.W., Scott, W.H., Doerfler, R., Lizama, S., Satyarengga, M., Munir, K., Galindo, R.J., Vellanki, P., Cardona, S., Pasquel, F.J., Peng, L., Umpierrez, G.E.: Accuracy of Dexcom G6 Continuous Glucose Monitoring in Non-Critically Ill Hospitalized Patients With Diabetes. Diabetes Care **44**, 1641–1646 (2021). https://doi.org/10.2337/dc20-2856
14. Joseph, J.I.: Review of the Long-Term Implantable Senseonics Continuous Glucose Monitoring System and Other Continuous Glucose Monitoring Systems. J. Diabetes Sci. Technol. **15**, 167–173 (2021). https://doi.org/10.1177/1932296820911919

15. Abbot: Perform Stronger and Recover Faster with CGM, https://www.supersapiens.com
16. Brady, S., Dunne, L.E.E., Lynch, A., Smyth, B., Diamond, D.: Wearable sensors? what is there to sense? IOS Press, Adaptive Information Cluster, National Centre for Sensor Research, Dublin City University, Ireland (2005)
17. Coyle, S., Morris, D., Lau, K.-T., Diamond, D., Moyna, N.: Textile-based wearable sensors for assisting sports performance. Presented at the , Clarity: The Centre for Sensor Web Technologies, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland (2009)
18. Coyle, S., Diamond, D.: Medical applications of smart textiles. In: Multidisciplinary Know-How for Smart-Textiles Developers. pp. 420–443. Elsevier Ltd, CLARITY: Centre for Sensor Web Technologies, National Centre for Sensor Research, Dublin City University, Glasnevin, Dublin 9, Ireland (2013)
19. Morris, D., Coyle, S., Wu, Y., Lau, K.T., Wallace, G., Diamond, D.: Bio-sensing textile based patch with integrated optical detection system for sweat monitoring. Sens. Actuators B Chem. **139**, 231–236 (2009). https://doi.org/10.1016/j.snb.2009.02.032
20. Glennon, T., O'Quigley, C., McCaul, M., Matzeu, G., Beirne, S., Wallace, G.G.G., Stroiescu, F., O'Mahoney, N., White, P., Diamond, D.: 'SWEATCH': A Wearable Platform for Harvesting and Analysing Sweat Sodium Content. Electroanalysis **28**, 1283–1289 (2016). https://doi.org/10.1002/elan.201600106
21. Windmiller, J.R., Bandodkar, A.J., Valdés-Ramírez, G., Parkhomovsky, S., Martinez, A.G., Wang, J.: Electrochemical sensing based on printable temporary transfer tattoos. Chem. Commun. **48**, 6794 (2012). https://doi.org/10.1039/c2cc32839a
22. Bandodkar, A.J., Jia, W., Wang, J.: Tattoo-Based Wearable Electrochemical Devices: A Review. Electroanalysis **27**, 562–572 (2015). https://doi.org/10.1002/elan.201400537
23. Wang, J., Windmiller, J.R., Jia, W.: Printed biofuel cells, (2012)
24. Jia, W., Valdés-Ramírez, G., Bandodkar, A.J., Windmiller, J.R., Wang, J.: Epidermal biofuel cells: Energy harvesting from human perspiration. Angew. Chem. - Int. Ed. **52**, 7233–7236 (2013). https://doi.org/10.1002/anie.201302922
25. Kim, J., Valdés-Ramírez, G., Bandodkar, A.J., Jia, W., Martinez, A.G., Ramírez, J., Mercier, P., Wang, J.: Non-invasive mouthguard biosensor for continuous salivary monitoring of metabolites. Analyst **139**, 1632–1636 (2014). https://doi.org/10.1039/c3an02359a
26. Rose, D., Ratterman, M., Griffin, D., Hou, L., Kelley-Loughnane, N., Naik, R., Hagen, J., Papautsky, I., Heikenfeld, J.: Adhesive RFID Sensor Patch for Monitoring of Sweat Electrolytes. IEEE Trans. Biomed. Eng. **9294**, 1–1 (2014). https://doi.org/10.1109/TBME.2014.2369991
27. Hou, L., Hagen, J., Wang, X., Papautsky, I., Naik, R., Kelley-Loughnane, N., Heikenfeld, J.: Artificial microfluidic skin for in vitro perspiration simulation and testing. Lab Chip **13**, 1868 (2013). https://doi.org/10.1039/c3lc41231h
28. La Count, T.D., Jajack, A., Heikenfeld, J., Kasting, G.B.: Modeling Glucose Transport From Systemic Circulation to Sweat. J. Pharm. Sci. **108**, 364–371 (2019). https://doi.org/10.1016/j.xphs.2018.09.026
29. Zhao, F.J.J., Bonmarin, M., Chen, Z.C.C., Larson, M., Fay, D., Runnoe, D., Heikenfeld, J.: Ultra-simple wearable local sweat volume monitoring patch based on swellable hydrogels. Lab Chip **20**, 168–174 (2020). https://doi.org/10.1039/c9lc00911f
30. Kim, D.-H., Lu, N., Ma, R., Kim, Y.-S., Kim, R.-H., Wang, S., Wu, J., Won, S.M., Tao, H., Islam, A., Yu, K.J., Kim, T., Chowdhury, R., Ying, M., Xu, L., Li, M., Chung, H.-J., Keum, H., McCormick, M., Liu, P., Zhang, Y.-W., Omenetto, F.G., Huang, Y., Coleman, T., Rogers, J.A.: Epidermal Electronics. Science **333**, 838–843 (2011). https://doi.org/10.1126/science.1206157
31. Kim, J.U., Seo, S.G., Rogers, J.A.: Compound semiconductor devices for the skin. Nat. Mater. (2022). https://doi.org/10.1038/s41563-022-01441-9
32. Reeder, J.T., Choi, J., Xue, Y., Gutruf, P., Hanson, J., Liu, M., Ray, T., Bandodkar, A.J., Avila, R., Xia, W., Krishnan, S., Xu, S., Barnes, K., Pahnke, M., Ghaffari, R., Huang, Y., Rogers, J.A.: Waterproof, electronics-enabled, epidermal microfluidic devices for sweat collection,

biomarker analysis, and thermography in aquatic settings. Sci. Adv. 5, eaau6356 (2019). https://doi.org/10.1126/sciadv.aau6356
33. Bandodkar, A.J.J., Lee, S.P.P., Huang, I., Li, W., Wang, S., Su, C.-J., Jeang, W.J.J., Hang, T., Mehta, S., Nyberg, N., Gutruf, P., Choi, J., Koo, J., Reeder, J.T., Tseng, R., Ghaffari, R., Rogers, J.A.A.: Sweat-activated biocompatible batteries for epidermal electronic and microfluidic systems. Nat. Electron. **3**, 554–562 (2020). https://doi.org/10.1038/s41928-020-0443-7
34. Baker, L.B., Model, J.B., Barnes, K.A., Anderson, M.L., Lee, S.P., Lee, K.A., Brown, S.D., Reimel, A.J., Roberts, T.J., Nuccio, R.P., Bonsignore, J.L., Ungaro, C.T., Carter, J.M., Li, W., Seib, M.S., Reeder, J.T., Aranyosi, A.J., Rogers, J.A., Ghaffari, R.: Skin-interfaced microfluidic system with personalized sweating rate and sweat chloride analytics for sports science applications. Sci. Adv. 6, eabe3929 (2020). https://doi.org/10.1126/sciadv.abe3929
35. Liu, S., Yang, D.S., Wang, S., Luan, H., Sekine, Y., Model, J.B., Aranyosi, A.J., Ghaffari, R., Rogers, J.A.: Soft, environmentally degradable microfluidic devices for measurement of sweat rate and total sweat loss and for colorimetric analysis of sweat biomarkers. EcoMat. 5, (2023). https://doi.org/10.1002/eom2.12270
36. Kwon, K., Kim, J.U., Deng, Y., Krishnan, S.R., Choi, J., Jang, H., Lee, K., Su, C.-J., Yoo, I., Wu, Y., Lipschultz, L., Kim, J.-H., Chung, T.S., Wu, D., Park, Y., Kim, T., Ghaffari, R., Lee, S., Huang, Y., Rogers, J.A.: An on-skin platform for wireless monitoring of flow rate, cumulative loss and temperature of sweat in real time. Nat. Electron. **4**, 302–312 (2021). https://doi.org/10.1038/s41928-021-00556-2
37. Drucker, P.F.: Innovation and entrepreneurship: practice and principles. HarperBusiness, New York, NY (2006)
38. Christensen, C.M.: The innovator's dilemma: the revolutionary book that will change the way you do business ; [with a new preface]. Harper Business, New York, NY (2011)
39. Porter, M.E.: Competitive strategy: techniques for analyzing industries and competitors. Free Press, New York London Toronto Sydney (2004)
40. Porter, M.E.: Competitive advantage: creating and sustaining superior performance. Free Press, New York, NY (2004)
41. Norman, D.A.: The design of everyday things. Tantor Media Inc., Old Saybrook, Ct (2011)
42. Lefteri, C.: Making It: Manufacturing techniques for product design. Laurence King Publishing, London (2019)
43. Sui, X., Downing, J.R., Hersam, M.C., Chen, J.: Additive manufacturing and applications of nanomaterial-based sensors. Mater. Today **48**, 135–154 (2021). https://doi.org/10.1016/j.mattod.2021.02.001
44. Santiago-Malagón, S., Río-Colín, D., Azizkhani, H., Aller-Pellitero, M., Guirado, G., del Campo, F.J.: A self-powered skin-patch electrochromic biosensor. Biosens. Bioelectron. **175**, 112879 (2021). https://doi.org/10.1016/j.bios.2020.112879
45. Wang, Z., Gui, M., Asif, M., Yu, Y., Dong, S., Wang, H., Wang, W., Wang, F., Xiao, F., Liu, H.: A facile modular approach to the 2D oriented assembly MOF electrode for non-enzymatic sweat biosensors. Nanoscale **10**, 6629–6638 (2018). https://doi.org/10.1039/C8NR00798E
46. Cheng, Y.-T., Chen, L.-C., Wang, W.-C.: Development of a fiber shape polymeric humidity sensor. Presented at the SPIE Smart Structures and Materials + Nondestructive Evaluation and Health Monitoring , Portland, Oregon, United States April 17 (2017)
47. Veeralingam, S., Khandelwal, S., Badhulika, S.: AI/ML-Enabled 2-D - RuS_2 Nanomaterial-Based Multifunctional, Low Cost, Wearable Sensor Platform for Non-Invasive Point of Care Diagnostics. IEEE Sens. J. **20**, 8437–8444 (2020). https://doi.org/10.1109/JSEN.2020.2984807
48. Jiang, D., Xu, C., Zhang, Q., Ye, Y., Cai, Y., Li, K., Li, Y., Huang, X., Wang, Y.: In-situ preparation of lactate-sensing membrane for the noninvasive and wearable analysis of sweat. Biosens. Bioelectron. **210**, 114303 (2022). https://doi.org/10.1016/j.bios.2022.114303
49. Asaduzzaman, M., Zahed, M.A., Sharifuzzaman, M., Reza, M.S., Hui, X., Sharma, S., Shin, Y.D., Park, J.Y.: A hybridized nano-porous carbon reinforced 3D graphene-based epidermal patch for precise sweat glucose and lactate analysis. Biosens. Bioelectron. **219**, 114846 (2023). https://doi.org/10.1016/j.bios.2022.114846

50. Zahid, M., Papadopoulou, E.L., Athanassiou, A., Bayer, I.S.: Strain-responsive mercerized conductive cotton fabrics based on PEDOT:PSS/graphene. Mater. Des. **135**, 213–222 (2017). https://doi.org/10.1016/j.matdes.2017.09.026
51. Fisher, C., Skolrood, L.N., Li, K., Joshi, P.C., Aytug, T.: Aerosol-Jet Printed Sensors for Environmental, Safety, and Health Monitoring: A Review. Adv. Mater. Technol. n/a, 2300030. https://doi.org/10.1002/admt.202300030
52. Veenuttranon, K., Kaewpradub, K., Jeerapan, I.: Screen-Printable Functional Nanomaterials for Flexible and Wearable Single-Enzyme-Based Energy-Harvesting and Self-Powered Biosensing Devices. Nano-Micro Lett. **15**, 85 (2023). https://doi.org/10.1007/s40820-023-01045-1
53. Smith, A.A., Li, R., Tse, Z.T.H.: Reshaping healthcare with wearable biosensors. Sci. Rep. **13**, 4998 (2023). https://doi.org/10.1038/s41598-022-26951-z
54. Kalkal, A., Kumar, S., Kumar, P., Pradhan, R., Willander, M., Packirisamy, G., Kumar, S., Malhotra, B.D.: Recent advances in 3D printing technologies for wearable (bio)sensors. Addit. Manuf. **46**, 102088 (2021). https://doi.org/10.1016/j.addma.2021.102088
55. Muth, J.T., Vogt, D.M., Truby, R.L., Mengüç, Y., Kolesky, D.B., Wood, R.J., Lewis, J.A.: Embedded 3D Printing of Strain Sensors within Highly Stretchable Elastomers. Adv. Mater. **26**, 6307–6312 (2014). https://doi.org/10.1002/adma.201400334
56. Yi, Q., Najafikhoshnoo, S., Das, P., Noh, S., Hoang, E., Kim, T., Esfandyarpour, R.: All-3D-Printed, Flexible, and Hybrid Wearable Bioelectronic Tactile Sensors Using Biocompatible Nanocomposites for Health Monitoring. Adv. Mater. Technol. **7**, 2101034 (2022). https://doi.org/10.1002/admt.202101034
57. Zhou, L., Gao, Q., Fu, J., Chen, Q., Zhu, J., Sun, Y., He, Y.: Multimaterial 3D Printing of Highly Stretchable Silicone Elastomers. ACS Appl. Mater. Interfaces **11**, 23573–23583 (2019). https://doi.org/10.1021/acsami.9b04873
58. Kim, T., Yi, Q., Hoang, E., Esfandyarpour, R.: A 3D Printed Wearable Bioelectronic Patch for Multi-Sensing and In Situ Sweat Electrolyte Monitoring. Adv. Mater. Technol. **6**, 2001021 (2021). https://doi.org/10.1002/admt.202001021
59. Xin, M., Li, J., Ma, Z., Pan, L., Shi, Y.: MXenes and Their Applications in Wearable Sensors. Front. Chem. **8**, 297 (2020). https://doi.org/10.3389/fchem.2020.00297
60. Bhardwaj, S.K., Singh, H., Khatri, M., Kim, K.-H., Bhardwaj, N.: Advances in MXenes-based optical biosensors: A review. Biosens. Bioelectron. **202**, 113995 (2022). https://doi.org/10.1016/j.bios.2022.113995
61. Hondred, J.A., Johnson, Z.T., Claussen, J.C.: Nanoporous gold peel-and-stick biosensors created with etching inkjet maskless lithography for electrochemical pesticide monitoring with microfluidics. J. Mater. Chem. C. **8**, 11376–11388 (2020). https://doi.org/10.1039/D0TC01423K
62. Wang, C., Zhu, S., Liang, Y., Cui, Z., Wu, S., Qin, C., Luo, S., Inoue, A.: Understanding the macroscopical flexibility/fragility of nanoporous Ag: Depending on network connectivity and micro-defects. J. Mater. Sci. Technol. **53**, 91–101 (2020). https://doi.org/10.1016/j.jmst.2020.04.010
63. Sánchez-Molas, D., Esquivel, J.P., Sabaté, N., Muñoz, F.X., del Campo, F.J., Esquivel, J.P.: High Aspect-Ratio, Fully Conducting Gold Micropillar Array Electrodes: Silicon Micromachining and Electrochemical Characterization. J. Phys. Chem. C **116**, 18831–18846 (2012). https://doi.org/10.1021/jp305339k
64. Chyan, Y., Ye, R., Li, Y., Singh, S.P., Arnusch, C.J., Tour, J.M.: Laser-Induced Graphene by Multiple Lasing: Toward Electronics on Cloth, Paper, and Food. ACS Nano **12**, 2176–2183 (2018). https://doi.org/10.1021/acsnano.7b08539
65. Fruncillo, S., Su, X., Liu, H., Wong, L.S.: Lithographic Processes for the Scalable Fabrication of Micro- and Nanostructures for Biochips and Biosensors. ACS Sens. **6**, 2002–2024 (2021). https://doi.org/10.1021/acssensors.0c02704
66. Cao, R., Pu, X., Du, X., Yang, W., Wang, J., Guo, H., Zhao, S., Yuan, Z., Zhang, C., Li, C., Lin Wang, Z.: Screen-Printed Washable Electronic Textiles as Self-Powered Touch/Gesture Tribo-Sensors for Intelligent Human−Machine Interaction Article. ACS Nano **12**, 38 (2018). https://doi.org/10.1021/acsnano.8b02477

67. Ma, D., Chon, S., Cho, S., Lee, Y., Yoo, M., Kim, D., Lee, D.Y., Lim, J.K.: A novel photolithographic method for fabrication of flexible micro-patterned glucose sensors. J. Electroanal. Chem. **876**, 114720 (2020). https://doi.org/10.1016/j.jelechem.2020.114720
68. Oppel, E., Högg, C., Oschmann, A., Summer, B., Kamann, S.: Contact allergy to the Dexcom G6 glucose monitoring system—Role of 2,2′-methylenebis(6-tert-butyl-4-methylphenol) monoacrylate in the new adhesive. Contact Dermatitis **87**, 258–264 (2022). https://doi.org/10.1111/cod.14141
69. Jones, P., Wynn, M., Hillier, D., Comfort, D.: The Sustainable Development Goals and Information and Communication Technologies. Indones. J. Sustain. Account. Manag. 1, 1 (2017). https://doi.org/10.28992/ijsam.v1i1.22
70. Poitout, V., Moatti-Sirat, D., Reach, G., Zhang, Y., Wilson, G.S., Lemonnier, F., Klein, J.C.: A glucose monitoring system for on line estimation in man of blood glucose concentration using a miniaturized glucose sensor implanted in the subcutaneous tissue and a wearable control unit. Diabetologia **36**, 658–663 (1993). https://doi.org/10.1007/BF00404077
71. Kudo, H., Sawada, T., Kazawa, E., Yoshida, H., Iwasaki, Y., Mitsubayashi, K.: A flexible and wearable glucose sensor based on functional polymers with Soft-MEMS techniques. Biosens. Bioelectron. **22**, 558–562 (2006). https://doi.org/10.1016/j.bios.2006.05.006
72. Mannoor, M.S., Tao, H., Clayton, J.D., Sengupta, A., Kaplan, D.L., Naik, R.R., Verma, N., Omenetto, F.G., McAlpine, M.C.: Graphene-based wireless bacteria detection on tooth enamel. Nat. Commun. **3**, 763 (2012). https://doi.org/10.1038/ncomms1767
73. Gao, W., Emaminejad, S., Nyein, H.Y.Y., Challa, S., Chen, K., Peck, A., Fahad, H.M., Ota, H., Shiraki, H., Kiriya, D., Lien, D.-H., Brooks, G.A., Davis, R.W., Javey, A.: Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. Nature **529**, 509–514 (2016). https://doi.org/10.1038/nature16521
74. Huang, X., Liu, Y., Zhou, J., Nejad, S.K., Wong, T.H., Huang, Y., Li, H., Yiu, C.K., Park, W., Li, J., Su, J., Zhao, L., Yao, K., Wu, M., Gao, Z., Li, D., Li, J., Shi, R., Yu, X.: Garment embedded sweat-activated batteries in wearable electronics for continuous sweat monitoring. Npj Flex. Electron. **6**, 10 (2022). https://doi.org/10.1038/s41528-022-00144-0
75. Rasitanon, N., Ittisoponpisan, S., Kaewpradub, K., Jeerapan, I.: Wearable Electrodes for Lactate: Applications in Enzyme-Based Sensors and Energy Biodevices. Anal. Sens. (2023). https://doi.org/10.1002/anse.202200066
76. Sadani, K., Nag, P., Thian, X.Y., Mukherji, S.: Enzymatic optical biosensors for healthcare applications. Biosens. Bioelectron. X. **12**, 100278 (2022). https://doi.org/10.1016/j.biosx.2022.100278
77. Heikenfeld, J., Jajack, A., Feldman, B., Granger, S.W., Gaitonde, S., Begtrup, G., Katchman, B.A.: Accessing analytes in biofluids for peripheral biochemical monitoring. Nat. Biotechnol. **37**, 407–419 (2019). https://doi.org/10.1038/s41587-019-0040-3
78. Zafar, H., Channa, A., Jeoti, V., Stojanović, G.M.: Comprehensive Review on Wearable Sweat-Glucose Sensors for Continuous Glucose Monitoring. Sensors. **22**, 638 (2022). https://doi.org/10.3390/s22020638
79. Chung, M., Fortunato, G., Radacsi, N.: Wearable flexible sweat sensors for healthcare monitoring: a review. J. R. Soc. Interface. **16**, 20190217 (2019). https://doi.org/10.1098/rsif.2019.0217
80. Bucur, B., Purcarea, C., Andreescu, S., Vasilescu, A.: Addressing the Selectivity of Enzyme Biosensors: Solutions and Perspectives. Sensors. **21**, 3038 (2021). https://doi.org/10.3390/s21093038
81. Lipińska, W., Grochowska, K., Siuzdak, K.: Enzyme Immobilization on Gold Nanoparticles for Electrochemical Glucose Biosensors. Nanomaterials **11**, 1156 (2021). https://doi.org/10.3390/nano11051156
82. Cabaj, J., Sołoducho, J.: Nano-Sized Elements in Electrochemical Biosensors. Mater. Sci. Appl. **05**, 752–766 (2014). https://doi.org/10.4236/msa.2014.510076
83. Skaria, E., Patel, B.A., Flint, M.S., Ng, K.W.: Poly(lactic acid)/Carbon Nanotube Composite Microneedle Arrays for Dermal Biosensing. Anal. Chem. **91**, 4436–4443 (2019). https://doi.org/10.1021/acs.analchem.8b04980

84. Lawal, A.T.: Synthesis and utilization of carbon nanotubes for fabrication of electrochemical biosensors. Mater. Res. Bull. **73**, 308–350 (2016). https://doi.org/10.1016/j.materresbull.2015.08.037
85. Niu, Y., Liu, J., Chen, W., Yin, C., Weng, W., Li, X., Wang, X., Li, G., Sun, W.: A direct electron transfer biosensor based on a horseradish peroxidase and gold nanotriangle modified electrode and electrocatalysis. Anal. Methods **10**, 5297–5304 (2018). https://doi.org/10.1039/C8AY01980K
86. Fang, L., Liu, B., Liu, L., Li, Y., Huang, K., Zhang, Q.: Direct electrochemistry of glucose oxidase immobilized on Au nanoparticles-functionalized 3D hierarchically ZnO nanostructures and its application to bioelectrochemical glucose sensor. Sens. Actuators B Chem. **222**, 1096–1102 (2016). https://doi.org/10.1016/j.snb.2015.08.032
87. Holzinger, M., Baur, J., Haddad, R., Wang, X., Cosnier, S.: Multiple functionalization of single-walled carbon nanotubes by dip coating. Chem. Commun. **47**, 2450–2452 (2011). https://doi.org/10.1039/C0CC03928D
88. Faculty of Military Health Sciences, University of Defence, Trebesska 1575, 50001 Hradec Kralove, Czech Republic, Martinkova, P.: Main streams in the Construction of Biosensors and Their Applications. Int. J. Electrochem. Sci. 7386–7403 (2017). https://doi.org/10.20964/2017.08.02
89. Nguyen, H.H., Lee, S.H., Lee, U.J., Fermin, C.D., Kim, M.: Immobilized Enzymes in Biosensor Applications. Materials. **12**, 121 (2019). https://doi.org/10.3390/ma12010121
90. Sassolas, A., Blum, L.J., Leca-Bouvier, B.D.: Immobilization strategies to develop enzymatic biosensors. Biotechnol. Adv. **30**, 489–511 (2012). https://doi.org/10.1016/j.biotechadv.2011.09.003
91. Salazar, P., Martín, M., O'Neill, R.D., González-Mora, J.L.: Glutamate microbiosensors based on Prussian Blue modified carbon fiber electrodes for neuroscience applications: In-vitro characterization. Sens. Actuators B Chem. **235**, 117–125 (2016). https://doi.org/10.1016/j.snb.2016.05.057
92. Kim, J., Imani, S., de Araujo, W.R., Warchall, J., Valdés-Ramírez, G., Paixão, T.R.L.C., Mercier, P.P., Wang, J.: Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics. Biosens. Bioelectron. **74**, 1061–1068 (2015). https://doi.org/10.1016/j.bios.2015.07.039
93. Tur-García, E.L., Davis, F., Collyer, S.D., Holmes, J.L., Barr, H., Higson, S.P.J.: Novel flexible enzyme laminate-based sensor for analysis of lactate in sweat. Sens. Actuators B Chem. **242**, 502–510 (2017). https://doi.org/10.1016/j.snb.2016.11.040
94. Clark, L.C., Lyons, C.: ELECTRODE SYSTEMS FOR CONTINUOUS MONITORING IN CARDIOVASCULAR SURGERY. Ann. N. Y. Acad. Sci. **102**, 29–45 (2006). https://doi.org/10.1111/j.1749-6632.1962.tb13623.x
95. Jiang, Y., Yang, Y., Shen, L., Ma, J., Ma, H., Zhu, N.: Recent Advances of Prussian Blue-Based Wearable Biosensors for Healthcare. Anal. Chem. **94**, 297–311 (2022). https://doi.org/10.1021/acs.analchem.1c04420
96. Herrmann, A., Haag, R., Schedler, U.: Hydrogels and Their Role in Biosensing Applications. Adv. Healthc. Mater. **10**, 2100062 (2021). https://doi.org/10.1002/adhm.202100062
97. Lipińska, W., Siuzdak, K., Karczewski, J., Dołęga, A., Grochowska, K.: Electrochemical glucose sensor based on the glucose oxidase entrapped in chitosan immobilized onto laser-processed Au-Ti electrode. Sens. Actuators B Chem. **330**, 129409 (2021). https://doi.org/10.1016/j.snb.2020.129409
98. Updike, S.J., Hicks, G.P.: The Enzyme Electrode. Nature **214**, 986–988 (1967). https://doi.org/10.1038/214986a0
99. Kim, G.J., Kim, K.O.: Novel glucose-responsive of the transparent nanofiber hydrogel patches as a wearable biosensor via electrospinning. Sci. Rep. **10**, 18858 (2020). https://doi.org/10.1038/s41598-020-75906-9
100. Kim, J., Jeerapan, I., Imani, S., Cho, T.N., Bandodkar, A., Cinti, S., Mercier, P.P., Wang, J.: Noninvasive Alcohol Monitoring Using a Wearable Tattoo-Based Iontophoretic-Biosensing System. ACS Sens. **1**, 1011–1019 (2016). https://doi.org/10.1021/acssensors.6b00356

101. Kemp, E., Palomäki, T., Ruuth, I.A., Boeva, Z.A., Nurminen, T.A., Vänskä, R.T., Zschaechner, L.K., Pérez, A.G., Hakala, T.A., Wardale, M., Haeggström, E., Bobacka, J.: Influence of enzyme immobilization and skin-sensor interface on non-invasive glucose determination from interstitial fluid obtained by magnetohydrodynamic extraction. Biosens. Bioelectron. **206**, 114123 (2022). https://doi.org/10.1016/j.bios.2022.114123
102. Nagamine, K., Mano, T., Nomura, A., Ichimura, Y., Izawa, R., Furusawa, H., Matsui, H., Kumaki, D., Tokito, S.: Noninvasive Sweat-Lactate Biosensor Emplsoying a Hydrogel-Based Touch Pad. Sci. Rep. **9**, 10102 (2019). https://doi.org/10.1038/s41598-019-46611-z
103. Liu, J., Zhang, L., Fu, C.: Os-complex-based amperometric bienzyme biosensor for continuous determination of lactate in saliva. Anal. Methods **7**, 6158–6164 (2015). https://doi.org/10.1039/C5AY01110H
104. Mollarasouli, K.: Ozkan: The Role of Electrochemical Immunosensors in Clinical Analysis. Biosensors **9**, 86 (2019). https://doi.org/10.3390/bios9030086
105. Ruiz, G., Tripathi, K., Okyem, S., Driskell, J.D.: PH Impacts the Orientation of Antibody Adsorbed onto Gold Nanoparticles. Bioconjug. Chem. **30**, 1182–1191 (2019). https://doi.org/10.1021/acs.bioconjchem.9b00123
106. Wei, J., Zhang, X., Mugo, S.M., Zhang, Q.: A Portable Sweat Sensor Based on Carbon Quantum Dots for Multiplex Detection of Cardiovascular Health Biomarkers. Anal. Chem. **94**, 12772–12780 (2022). https://doi.org/10.1021/acs.analchem.2c02587
107. Haji-Hashemi, H., Norouzi, P., Safarnejad, M.R., Ganjali, M.R.: Label-free electrochemical immunosensor for direct detection of Citrus tristeza virus using modified gold electrode. Sens. Actuators B Chem. **244**, 211–216 (2017). https://doi.org/10.1016/j.snb.2016.12.135
108. Lee, H.-B., Meeseepong, M., Trung, T.Q., Kim, B.-Y., Lee, N.-E.: A wearable lab-on-a-patch platform with stretchable nanostructured biosensor for non-invasive immunodetection of biomarker in sweat. Biosens. Bioelectron. **156**, 112133 (2020). https://doi.org/10.1016/j.bios.2020.112133
109. Bagni, G., Osella, D., Sturchio, E., Mascini, M.: Deoxyribonucleic acid (DNA) biosensors for environmental risk assessment and drug studies. Instrum. Methods Anal. -IMA **2005**(573–574), 81–89 (2006). https://doi.org/10.1016/j.aca.2006.03.085
110. Lymperopoulos, K., Crawford, R., Torella, J.P., Heilemann, M., Hwang, L.C., Holden, S.J., Kapanidis, A.N.: Single-Molecule DNA Biosensors for Protein and Ligand Detection. Angew. Chem. Int. Ed. **49**, 1316–1320 (2010). https://doi.org/10.1002/anie.200904597
111. Choi, J.-H., Lim, J., Shin, M., Paek, S.-H., Choi, J.-W.: CRISPR-Cas12a-Based Nucleic Acid Amplification-Free DNA Biosensor via Au Nanoparticle-Assisted Metal-Enhanced Fluorescence and Colorimetric Analysis. Nano Lett. **21**, 693–699 (2021). https://doi.org/10.1021/acs.nanolett.0c04303
112. Mukherjee, M., Gajjala, R.K.R., Gade, P.S., Bhatt, P.: 3.42 - Aptasensors: Paradigm Shift for Detection of Food Toxins. In: Knoerzer, K. and Muthukumarappan, K. (eds.) Innovative Food Processing Technologies. pp. 712–730. Elsevier, Oxford (2021)
113. Gao, Y., Nguyen, D.T., Yeo, T., Lim, S.B., Tan, W.X., Madden, L.E., Jin, L., Long, J.Y.K., Aloweni, F.A.B., Liew, Y.J.A., Tan, M.L.L., Ang, S.Y., Maniya, S.D., Abdelwahab, I., Loh, K.P., Chen, C.-H., Becker, D.L., Leavesley, D., Ho, J.S., Lim, C.T.: A flexible multiplexed immunosensor for point-of-care in situ wound monitoring. Sci. Adv. 7, eabg9614. https://doi.org/10.1126/sciadv.abg9614
114. Ferguson, B.S., Hoggarth, D.A., Maliniak, D., Ploense, K., White, R.J., Woodward, N., Hsieh, K., Bonham, A.J., Eisenstein, M., Kippin, T.E., Plaxco, K.W., Soh, H.T.: Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals. Sci. Transl. Med. 5, 213ra165–213ra165 (2013). https://doi.org/10.1126/scitranslmed.3007095
115. Raju, K.S.R., Taneja, I., Singh, S.P.: Wahajuddin: Utility of noninvasive biomatrices in pharmacokinetic studies. Biomed. Chromatogr. **27**, 1354–1366 (2013). https://doi.org/10.1002/bmc.2996
116. Tsunoda, M., Hirayama, M., Tsuda, T., Ohno, K.: Noninvasive monitoring of plasma l-dopa concentrations using sweat samples in Parkinson's disease. Clin. Chim. Acta **442**, 52–55 (2015). https://doi.org/10.1016/j.cca.2014.12.032

117. Tai, L.-C., Gao, W., Chao, M., Bariya, M., Ngo, Q.P., Shahpar, Z., Nyein, H.Y.Y., Park, H., Sun, J., Jung, Y., Wu, E., Fahad, H.M., Lien, D.-H., Ota, H., Cho, G., Javey, A.: Methylxanthine Drug Monitoring with Wearable Sweat Sensors. Adv. Mater. **30**, 1707442 (2018). https://doi.org/10.1002/adma.201707442
118. Gal, P.: Caffeine Therapeutic Drug Monitoring Is Necessary and Cost-effective. J. Pediatr. Pharmacol. Ther. **12**, 212–215 (2007). https://doi.org/10.5863/1551-6776-12.4.212
119. Tai, L.-C., Liaw, T.S., Lin, Y., Nyein, H.Y.Y., Bariya, M., Ji, W., Hettick, M., Zhao, C., Zhao, J., Hou, L., Yuan, Z., Fan, Z., Javey, A.: Wearable Sweat Band for Noninvasive Levodopa Monitoring. Nano Lett. **19**, 6346–6351 (2019). https://doi.org/10.1021/acs.nanolett.9b02478
120. Wu, Y., Tehrani, F., Teymourian, H., Mack, J., Shaver, A., Reynoso, M., Kavner, J., Huang, N., Furmidge, A., Duvvuri, A., Nie, Y., Laffel, L.M., Doyle, F.J.I., Patti, M.-E., Dassau, E., Wang, J., Arroyo-Currás, N.: Microneedle Aptamer-Based Sensors for Continuous, Real-Time Therapeutic Drug Monitoring. Anal. Chem. **94**, 8335–8345 (2022). https://doi.org/10.1021/acs.analchem.2c00829
121. Mousavisani, S.Z., Raoof, J.B., Ojani, R., Bagheryan, Z.: An impedimetric biosensor for DNA damage detection and study of the protective effect of deferoxamine against DNA damage. Bioelectrochemistry **122**, 142–148 (2018). https://doi.org/10.1016/j.bioelechem.2018.03.012
122. Parab, H.J., Jung, C., Lee, J.-H., Park, H.G.: A gold nanorod-based optical DNA biosensor for the diagnosis of pathogens. Biosens. Bioelectron. **26**, 667–673 (2010). https://doi.org/10.1016/j.bios.2010.06.067
123. Zhang, H., Wang, R., Tan, H., Nie, L., Yao, S.: Bovine serum albumin as a means to immobilize DNA on a silver-plated bulk acoustic wave DNA biosensor. Talanta **46**, 171–178 (1998). https://doi.org/10.1016/S0039-9140(97)00271-3
124. Afzal, A., Mujahid, A., Schirhagl, R., Bajwa, S.Z., Latif, U., Feroz, S.: Gravimetric viral diagnostics: QCM based biosensors for early detection of viruses. Chemosensors. **5**, 7 (2017)
125. Hu, L., Hu, S., Guo, L., Shen, C., Yang, M., Rasooly, A.: DNA Generated Electric Current Biosensor. Anal. Chem. **89**, 2547–2552 (2017). https://doi.org/10.1021/acs.analchem.6b04756
126. Han, S., Liu, W., Zheng, M., Wang, R.: Label-Free and Ultrasensitive Electrochemical DNA Biosensor Based on Urchinlike Carbon Nanotube-Gold Nanoparticle Nanoclusters. Anal. Chem. **92**, 4780–4787 (2020). https://doi.org/10.1021/acs.analchem.9b03520
127. Kim, E.R., Joe, C., Mitchell, R.J., Gu, M.B.: Biosensors for healthcare: Current and future perspectives. Trends Biotechnol. (2022)
128. Yang, B., Kong, J., Fang, X.: Bandage-like wearable flexible microfluidic recombinase polymerase amplification sensor for the rapid visual detection of nucleic acids. Talanta **204**, 685–692 (2019). https://doi.org/10.1016/j.talanta.2019.06.031
129. Galandová, J., Ovádeková, R., Ferancová, A., Labuda, J.: Disposable DNA biosensor with the carbon nanotubes–polyethyleneimine interface at a screen-printed carbon electrode for tests of DNA layer damage by quinazolines. Anal. Bioanal. Chem. **394**, 855–861 (2009). https://doi.org/10.1007/s00216-009-2740-x
130. Suginta, W., Khunkaewla, P., Schulte, A.: Electrochemical Biosensor Applications of Polysaccharides Chitin and Chitosan. Chem. Rev. **113**, 5458–5479 (2013). https://doi.org/10.1021/cr300325r
131. Di Iorio, D., Marti, A., Koeman, S., Huskens, J.: Clickable poly-l-lysine for the formation of biorecognition surfaces. RSC Adv. **9**, 35608–35613 (2019). https://doi.org/10.1039/C9RA08714A
132. Zhang, P., Lu, C., Niu, C., Wang, X., Li, Z., Liu, J.: Binding Studies of Cationic Conjugated Polymers and DNA for Label-Free Fluorescent Biosensors. ACS Appl. Polym. Mater. **4**, 6211–6218 (2022). https://doi.org/10.1021/acsapm.2c00986
133. Stillman, B.A., Tonkinson, J.L.: FASTTM Slides: A Novel Surface for Microarrays. Biotechniques **29**, 630–635 (2000). https://doi.org/10.2144/00293pf01
134. Sassolas, A., Leca-Bouvier, B.D., Blum, L.J.: DNA Biosensors and Microarrays. Chem. Rev. **108**, 109–139 (2008). https://doi.org/10.1021/cr0684467

135. Zhang, F., Wang, S., Liu, J.: Gold Nanoparticles Adsorb DNA and Aptamer Probes Too Strongly and a Comparison with Graphene Oxide for Biosensing. Anal. Chem. **91**, 14743–14750 (2019). https://doi.org/10.1021/acs.analchem.9b04142
136. Wang, S., McGuirk, C.M., Ross, M.B., Wang, S., Chen, P., Xing, H., Liu, Y., Mirkin, C.A.: General and Direct Method for Preparing Oligonucleotide-Functionalized Metal-Organic Framework Nanoparticles. J. Am. Chem. Soc. **139**, 9827–9830 (2017). https://doi.org/10.1021/jacs.7b05633
137. Sun, Z., Wu, S., Ma, J., Shi, H., Wang, L., Sheng, A., Yin, T., Sun, L., Li, G.: Colorimetric Sensor Array for Human Semen Identification Designed by Coupling Zirconium Metal-Organic Frameworks with DNA-Modified Gold Nanoparticles. ACS Appl. Mater. Interfaces **11**, 36316–36323 (2019). https://doi.org/10.1021/acsami.9b10729
138. Xiong, D., Cheng, J., Ai, F., Wang, X., Xiao, J., Zhu, F., Zeng, K., Wang, K., Zhang, Z.: Insight into the Sensing Behavior of DNA Probes Based on MOF–Nucleic Acid Interaction for Bioanalysis. Anal. Chem. **95**, 5470–5478 (2023). https://doi.org/10.1021/acs.analchem.3c00832
139. Weizmann, Y., Chenoweth, D.M., Swager, T.M.: Addressable Terminally Linked DNA−CNT Nanowires. J. Am. Chem. Soc. **132**, 14009–14011 (2010). https://doi.org/10.1021/ja106352y
140. Vittala, S.K., Han, D.: DNA-Guided Assemblies toward Nanoelectronic Applications. ACS Appl. Bio Mater. **3**, 2702–2722 (2020). https://doi.org/10.1021/acsabm.9b01178
141. Rashid, J.I.A., Yusof, N.A.: The strategies of DNA immobilization and hybridization detection mechanism in the construction of electrochemical DNA sensor: A review. Sens. Bio-Sens. Res. **16**, 19–31 (2017). https://doi.org/10.1016/j.sbsr.2017.09.001
142. Papadopoulou, E., Gale, N., Thompson, J.F., Fleming, T.A., Brown, T., Bartlett, P.N.: Specifically horizontally tethered DNA probes on Au surfaces allow labelled and label-free DNA detection using SERS and electrochemically driven melting. Chem. Sci. **7**, 386–393 (2016). https://doi.org/10.1039/C5SC03185K
143. Goodrich, G.P., Helfrich, M.R., Overberg, J.J., Keating, C.D.: Effect of Macromolecular Crowding on DNA: Au Nanoparticle Bioconjugate Assembly. Langmuir **20**, 10246–10251 (2004). https://doi.org/10.1021/la048434l
144. Ahmadi, S., Kamaladini, H., Haddadi, F., Sharifmoghadam, M.R.: Thiol-Capped Gold Nanoparticle Biosensors for Rapid and Sensitive Visual Colorimetric Detection of Klebsiella pneumoniae. J. Fluoresc. **28**, 987–998 (2018). https://doi.org/10.1007/s10895-018-2262-z
145. Mobed, A., Hasanzadeh, M., Babaie, P., Agazadeh, M., Mokhtarzadeh, A., Rezaee, M.A.: DNA-based bioassay of legionella pneumonia pathogen using gold nanostructure: A new platform for diagnosis of legionellosis. Int. J. Biol. Macromol. **128**, 692–699 (2019). https://doi.org/10.1016/j.ijbiomac.2019.01.125
146. Tour, J.M., Jones, L.I., Pearson, D.L., Lamba, J.J.S., Burgin, T.P., Whitesides, G.M., Allara, D.L., Parikh, A.N., Atre, S.: Self-Assembled Monolayers and Multilayers of Conjugated Thiols, .alpha.,.omega.-Dithiols, and Thioacetyl-Containing Adsorbates. Understanding Attachments between Potential Molecular Wires and Gold Surfaces. J. Am. Chem. Soc. 117, 9529–9534 (1995). https://doi.org/10.1021/ja00142a021
147. Reddy Gajjala, R.K., Gade, P.S., Bhatt, P., Vishwakarma, N., Singh, S.: Enzyme decorated dendritic bimetallic nanocomposite biosensor for detection of HCHO. Talanta **238**, 123054 (2022). https://doi.org/10.1016/j.talanta.2021.123054
148. Yang, B., Kong, J., Fang, X.: Programmable CRISPR-Cas9 microneedle patch for long-term capture and real-time monitoring of universal cell-free DNA. Nat. Commun. **13**, 3999 (2022). https://doi.org/10.1038/s41467-022-31740-3
149. Kuscu, M., Ramezani, H., Dinc, E., Akhavan, S., Akan, O.B.: Fabrication and microfluidic analysis of graphene-based molecular communication receiver for Internet of Nano Things (IoNT). Sci. Rep. **11**, 19600 (2021). https://doi.org/10.1038/s41598-021-98609-1
150. Guo, Y., Shen, G., Sun, X., Wang, X.: Electrochemical Aptasensor Based on Multiwalled Carbon Nanotubes and Graphene for Tetracycline Detection. IEEE Sens. J. **15**, 1951–1958 (2015). https://doi.org/10.1109/JSEN.2014.2370051

151. Pan, T.-M., Liao, P.-Y.: High sensitivity and rapid detection of KRAS and BRAF gene mutations in colorectal cancer using YbTixOy electrolyte-insulator-semiconductor biosensors. Mater. Today Chem. **25**, 100979 (2022). https://doi.org/10.1016/j.mtchem.2022.100979
152. Rodríguez-Montelongo, S.A., Moreno-Gutiérrez, D.S., Terán-Figueroa, Y., Gómez-Durán, C.F.A., Bañuelos-Frías, A., Palestino, G.: Porous Silicon-Based DNA Biosensor for Human Papillomavirus Detection: Towards the Design of Fast and Portable Test. SILICON **15**, 2371–2383 (2023). https://doi.org/10.1007/s12633-022-02179-4
153. Capo, C., Bongrand, P., Benoliel, A., Depieds, R.: Non-specific recognition in phagocytosis: ingestion of aldehyde-treated erythrocytes by rat peritoneal macrophages. Immunology **36**, 501 (1979)
154. Chung, D.-J., Kim, K.-C., Choi, S.-H.: Electrochemical DNA biosensor based on avidin–biotin conjugation for influenza virus (type A) detection. Appl. Surf. Sci. **257**, 9390–9396 (2011). https://doi.org/10.1016/j.apsusc.2011.06.015
155. Liu, G., Wan, Y., Gau, V., Zhang, J., Wang, L., Song, S., Fan, C.: An Enzyme-Based E-DNA Sensor for Sequence-Specific Detection of Femtomolar DNA Targets. J. Am. Chem. Soc. **130**, 6820–6825 (2008). https://doi.org/10.1021/ja800554t
156. Pan, S., Rothberg, L.: Chemical Control of Electrode Functionalization for Detection of DNA Hybridization by Electrochemical Impedance Spectroscopy. Langmuir **21**, 1022–1027 (2005). https://doi.org/10.1021/la048083a
157. Deng, C., Xia, Y., Xiao, C., Nie, Z., Yang, M., Si, S.: Electrochemical oxidation of purine and pyrimidine bases based on the boron-doped nanotubes modified electrode. Biosens. Bioelectron. **31**, 469–474 (2012). https://doi.org/10.1016/j.bios.2011.11.018
158. Jalit, Y., Moreno, M., Gutierrez, F.A., Sanchez Arribas, A., Chicharro, M., Bermejo, E., Zapardiel, A., Parrado, C., Rivas, G.A., Rodríguez, M.C.: Adsorption and Electrooxidation of Nucleic Acids at Glassy Carbon Electrodes Modified with Multiwalled Carbon Nanotubes Dispersed In Polylysine. Electroanalysis **25**, 1116–1121 (2013). https://doi.org/10.1002/elan.201200622
159. Zhang, S., Ding, Y., Wei, H.: Ruthenium Polypyridine Complexes Combined with Oligonucleotides for Bioanalysis: A Review. Molecules **19**, 11933–11987 (2014). https://doi.org/10.3390/molecules190811933
160. Drummond, T.G., Hill, M.G., Barton, J.K.: Electrochemical DNA sensors. Nat. Biotechnol. **21**, 1192–1199 (2003). https://doi.org/10.1038/nbt873
161. Wu, N., Gao, W., He, X., Chang, Z., Xu, M.: Direct electrochemical sensor for label-free DNA detection based on zero current potentiometry. Biosens. Bioelectron. **39**, 210–214 (2013). https://doi.org/10.1016/j.bios.2012.07.038
162. Yu, H.-Z., Luo, C.-Y., Sankar, C.G., Sen, D.: Voltammetric Procedure for Examining DNA-Modified Surfaces: Quantitation, Cationic Binding Activity, and Electron-Transfer Kinetics. Anal. Chem. **75**, 3902–3907 (2003). https://doi.org/10.1021/ac034318w
163. Ilkhani, H., Hughes, T., Li, J., Zhong, C.J., Hepel, M.: Nanostructured SERS-electrochemical biosensors for testing of anticancer drug interactions with DNA. Biosens. Bioelectron. **80**, 257–264 (2016). https://doi.org/10.1016/j.bios.2016.01.068
164. Ribeiro Teles, F.R., França dos Prazeres, D.M., De Lima-Filho, J.L.: Electrochemical Detection of a Dengue-related Oligonucleotide Sequence Using Ferrocenium as a Hybridization Indicator. Sensors. 7, 2510–2518 (2007). https://doi.org/10.3390/s7112510
165. Huang, J., Wu, J., Li, Z.: Biosensing using hairpin DNA probes. **34**, 1–27 (2015). https://doi.org/10.1515/revac-2015-0010
166. Farjami, E., Clima, L., Gothelf, K., Ferapontova, E.E.: "Off−On" Electrochemical Hairpin-DNA-Based Genosensor for Cancer Diagnostics. Anal. Chem. **83**, 1594–1602 (2011). https://doi.org/10.1021/ac1032929
167. Fu, L., Zhuang, J., Tang, D., Que, X., Lai, W., Chen, G.: DNA pseudoknot-functionalized sensing platform for chemoselective analysis of mercury ions. Analyst **137**, 4425–4427 (2012). https://doi.org/10.1039/C2AN35662G
168. Xiao, Y., Qu, X., Plaxco, K.W., Heeger, A.J.: Label-Free Electrochemical Detection of DNA in Blood Serum via Target-Induced Resolution of an Electrode-Bound DNA Pseudoknot. J. Am. Chem. Soc. **129**, 11896–11897 (2007). https://doi.org/10.1021/ja074218y

169. Singh, N.K., Chung, S., Chang, A.-Y., Wang, J., Hall, D.A.: A non-invasive wearable stress patch for real-time cortisol monitoring using a pseudoknot-assisted aptamer. Biosens. Bioelectron. **227**, 115097 (2023). https://doi.org/10.1016/j.bios.2023.115097
170. Majee, P., Kumar Mishra, S., Pandya, N., Shankar, U., Pasadi, S., Muniyappa, K., Nayak, D., Kumar, A.: Sci. Rep. **10**, 1477 (2020). https://doi.org/10.1038/s41598-020-58406-8
171. Zhao, Y., Kan, Z., Zeng, Z., Hao, Y., Chen, H., Tan, Z.: Determining the Folding and Unfolding Rate Constants of Nucleic Acids by Biosensor. Application to Telomere G-Quadruplex. J. Am. Chem. Soc. 126, 13255–13264 (2004). https://doi.org/10.1021/ja048398c
172. Bahreyni, A., Ramezani, M., Alibolandi, M., Hassanzadeh, P., Abnous, K., Taghdisi, S.M.: High affinity of AS1411 toward copper; its application in a sensitive aptasensor for copper detection. Anal. Biochem. **575**, 1–9 (2019). https://doi.org/10.1016/j.ab.2019.03.016
173. Hao, Z., Wang, Z., Li, Y., Zhu, Y., Wang, X., De Moraes, C.G., Pan, Y., Zhao, X., Lin, Q.: Measurement of cytokine biomarkers using an aptamer-based affinity graphene nanosensor on a flexible substrate toward wearable applications. Nanoscale **10**, 21681–21688 (2018). https://doi.org/10.1039/C8NR04315A
174. Travascio, P., Witting, P.K., Mauk, A.G., Sen, D.: The Peroxidase Activity of a Hemin−DNA Oligonucleotide Complex: Free Radical Damage to Specific Guanine Bases of the DNA. J. Am. Chem. Soc. **123**, 1337–1348 (2001). https://doi.org/10.1021/ja0023534
175. Wang, Z., Zhao, J., Bao, J., Dai, Z.: Construction of Metal-Ion-Free G-quadruplex-Hemin DNAzyme and Its Application in S1 Nuclease Detection. ACS Appl. Mater. Interfaces **8**, 827–833 (2016). https://doi.org/10.1021/acsami.5b10165
176. Cheng, X., Liu, X., Bing, T., Cao, Z., Shangguan, D.: General Peroxidase Activity of G-Quadruplex−Hemin Complexes and Its Application in Ligand Screening. Biochemistry **48**, 7817–7823 (2009). https://doi.org/10.1021/bi9006786
177. Xu, J., Yan, C., Wang, X., Yao, B., Lu, J., Liu, G., Chen, W.: Ingenious Design of DNA Concatamers and G-Quadruplex Wires Assisted Assembly of Multibranched DNA Nanoarchitectures for Ultrasensitive Biosensing of miRNA. Anal. Chem. **91**, 9747–9753 (2019). https://doi.org/10.1021/acs.analchem.9b01353
178. Owens, E.A., Huynh, H.T., Stroeva, E.M., Barman, A., Ziabrev, K., Paul, A., Nguyen, S.V., Laramie, M., Hamelberg, D., Germann, M.W., Wilson, W.D., Henary, M.: Second Generation G-Quadruplex Stabilizing Trimethine Cyanines. Bioconjug. Chem. **30**, 2647–2663 (2019). https://doi.org/10.1021/acs.bioconjchem.9b00571
179. Tello, A., Cao, R., Marchant, M.J., Gomez, H.: Conformational Changes of Enzymes and Aptamers Immobilized on Electrodes. Bioconjug. Chem. **27**, 2581–2591 (2016). https://doi.org/10.1021/acs.bioconjchem.6b00553
180. Sorek, R., Lawrence, C.M., Wiedenheft, B.: CRISPR-Mediated Adaptive Immune Systems in Bacteria and Archaea. Annu. Rev. Biochem. **82**, 237–266 (2013). https://doi.org/10.1146/annurev-biochem-072911-172315
181. Wan, Y., Zong, C., Li, X., Wang, A., Li, Y., Yang, T., Bao, Q., Dubow, M., Yang, M., Rodrigo, L.-A., Mao, C.: New Insights for Biosensing: Lessons from Microbial Defense Systems. Chem. Rev. **122**, 8126–8180 (2022). https://doi.org/10.1021/acs.chemrev.1c01063
182. Nguyen, P.Q., Soenksen, L.R., Donghia, N.M., Angenent-Mari, N.M., de Puig, H., Huang, A., Lee, R., Slomovic, S., Galbersanini, T., Lansberry, G., Sallum, H.M., Zhao, E.M., Niemi, J.B., Collins, J.J.: Wearable materials with embedded synthetic biology sensors for biomolecule detection. Nat. Biotechnol. **39**, 1366–1374 (2021). https://doi.org/10.1038/s41587-021-00950-3
183. Lu, M., Zhang, X., Xu, D., Li, N., Zhao, Y.: Encoded Structural Color Microneedle Patches for Multiple Screening of Wound Small Molecules. Adv. Mater. n/a, 2211330 (2023). https://doi.org/10.1002/adma.202211330
184. Lin, S., Cheng, X., Zhu, J., Wang, B., Jelinek, D., Zhao, Y., Wu, T.-Y., Horrillo, A., Tan, J., Yeung, J., Yan, W., Forman, S., Coller, H.A., Milla, C., Emaminejad, S.: Wearable microneedle-based electrochemical aptamer biosensing for precision dosing of drugs with narrow therapeutic windows. Sci. Adv. 8, eabq4539. https://doi.org/10.1126/sciadv.abq4539