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## Carbon-based electrochemical biosensors as diagnostic platforms for connected decentralized healthcare

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Electrochemical biosensors have the potential to provide rapid and inexpensive diagnostics while moving clinical testing from centralized labs to point-of-care (POC) applications. Conductive materials functionalized with bioreceptors that remain stable and functional for measurements in real-world conditions are essential for the fabrication of electrochemical biosensors, and carbon-based nanomaterials provide the electrical, chemical, structural, and mechanical features that make them suitable for POC devices. This review details the most recent developments in the use of carbon-based nanostructures, with a focus on one-dimensional carbon nanotubes, two-dimensional graphene, and graphene oxide, their interface with biological receptors, deposition on portable, flexible, and wearable substrates, and integration on low-cost platforms for detection of clinical biomarkers. The large-scale manufacturing and implementation of microneedles as implantable and electronic tattoos as wearable devices for on-skin diagnostics, and lab-on-mouth platforms as well as the interface with mobile technologies and their potential implementation for remote POC monitoring and decentralized healthcare through cloud processing and the internet of things (IoT) are discussed with examples of applications. The review concludes with an overview of the regulatory perspectives and future trends, challenges, and opportunities for commercialization and translation of these technologies from the research lab to practice, as useful diagnostic tools for remote monitoring of patient health conditions.

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## 1. Introduction

Electrochemical biosensors are attractive in the biomedical field due to their simple instrumentation, ease-of-use, low cost, and ability to provide healthcare management from laboratory to point-of-care testing (POCT).<sup>1</sup> Wearable sensing systems that collect, measure, and transfer analytical data from various biofluids of the wearer *via* wireless communication have gained increased interest due to their potential to measure biomarkers in real-time and non-invasively. Electrochemical biosensors have made notable progress over modern laboratory-based techniques, *e.g.*, spectrophotometry, chemiluminescence, nuclear magnetic resonance (NMR), colorimetric, fluorimetry, and mass spectroscopy (MS) that involve complex sample processing, skilled lab operators, and lack miniaturization. Unlike conventional methods, electrochemistry is not affected by turbidity or interferences from light-absorbing molecules. Moreover, the use of biomolecular receptors in the biosensing

design provides the desired selectivity for measurements. Electrochemical biosensors can be fabricated at a large scale and easily integrated into POCT devices. However, while various biosensing devices have been reported, several challenges still impede their transition into practice. These include i) deviations from calibration when used in real environments, ii) lack of manufacturing practices to ensure large-scale fabrication with preserved sensing functions, iii) the stability of the bioreceptor and their effective integration with the electronic components, and iv) the availability of flexible conductive materials. A critical need for the development of wearable patient-centered biosensing devices is the availability of conductive materials functionalized with bioreceptors that remain conformable to the body, stable, and functional under strain conditions. At present few materials fulfill this role.

Advancements in nanotechnology have generated a variety of materials with the desired physiochemical properties and conductivity that can be incorporated into wearables. These include carbon-based materials, conductive polymers, metal nanoparticles, and liquid metals. Among these, carbon-based nanomaterials (CBNs) are the most commonly used due to their electrical, optical, mechanical, and thermal properties, cost, and availability.<sup>2</sup> Introduced by RN Adams in 1958,<sup>3</sup>

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carbon-based materials are the dominating class of electrode materials. Their ability to promote electron transfer kinetics, stability, low ohmic resistance, good biocompatibility, and enhanced interfacial adsorption properties compared to many traditional electrochemical materials make them unique for sensing applications.<sup>4</sup> CBNs including one-dimensional CNTs (*i.e.*, single-walled carbon nanotubes (SWCNTs), multiwalled carbon nanotubes (MWCNTs)), two-dimensional graphene (graphene oxide (GO), reduced graphene oxide (rGO), and graphene nanoribbon (GNR)) have improved electrical and mechanical properties making them suitable for electrode modification and fabrication of wearable devices. Others, like quantum dots and fullerenes have also been applied but not as extensively used as 1D and 2D materials.

Improved performance has been achieved by interfacing carbon-based nanostructures with polymers, metallic or catalytic materials to enhance their conductivity and electrocatalytic functions and facilitate stabilization of biomolecules.<sup>5</sup> The rich surface functionalities of hybrid structures enable them to be modified with biomolecules such as aptamers, antibodies, DNA, redox markers, RNA, nanoparticles (NPs), or be deposited in nanocomposite forms on flexible substrates to enable the fabrication of disposable, flexible, and inexpensive devices. CBNs facilitate the electron transfer by increasing the accessible surface area while maintaining the flexibility.<sup>6</sup> Wearable biosensors can provide non-invasive real-time information of dynamically changing biomarker levels in biological fluids such as sweat, interstitial fluid (ISF), tears, and saliva.<sup>7</sup> Significant efforts have been made to effectively integrate these materials and biomolecular receptors within low-cost supporting structures such as paper, textiles, polymer, or ceramic substrates, which is an essential step to building biomolecular structures for field deployment and implementation.<sup>2b</sup> This review focusses on the use and integration of 1D and 2D carbon-based nanomaterial hybrids for constructing wearable biosensing devices as diagnostic tools for connected personalized healthcare.

To date, reviews on CBNs for electrochemical biosensors have provided a summary of new properties of these materials and discussed their implementation in conventional laboratory-based biosensing designs and standard electrodes for personalized applications.<sup>8</sup> Here we focus on multifunctional hybrid carbon-based materials and discuss their functionalization with bioreceptors, and integration within flexible and wearable substrates. We then discuss the possible implementation of these devices as diagnostic tools for remote monitoring of patient health conditions, and connectivity through cloud-based processing and the internet of thing (IoT). We also address manufacturing challenges, a necessary step to achieve the large scale needs to translate biosensing technologies from the lab into the market. In the first part of the review, we first discuss the properties, functionalization, and interfacing of carbon-based nanomaterials (GO, rGO, CNTs) with biological

receptors. We then evaluate their deposition onto flexible and stretchable platforms and their integration with wearable electronic circuitry and the IoT health network. Finally, we discuss manufacturing by inkjet, 2D or 3D printing and the roll-to-roll fabrication on flexible electronics substrates and summarize their capabilities and potential for use at the point of need and decentralized connected healthcare. Table 1 provides examples of recent work on carbon-based materials (*i.e.*, carbon nanotubes, graphene and hybrid carbon composites), their characteristics, applicability and integration in POC devices.

## 2. Carbon nanomaterials and hybrids for electrochemical biosensors

### 2.1. Properties of carbon nanomaterials and hybrids

Commonly used forms of carbon-based materials including porous carbon, graphite, graphene, quantum dots, carbon nanotubes, (*e.g.*, SWCNTs and MWCNTs), graphitic carbon nitride, carbon nanofibers (CNFs), buckminsterfullerene (C60), nanodiamonds (NDs) and carbon nanospheres,<sup>41</sup> summarized in Fig. 1. Their intrinsic conductivity, stability and ease of functionalization makes them ideal for electrochemical biosensors.<sup>42</sup> Graphene and CNTs are the most used forms of carbon for electrochemical biosensing. A summary of their properties is provided below.

**2.1.1. Graphene and graphene derivatives.** Graphene is a monolayer of carbon atoms arranged in a honeycomb-like structure, typically obtained by mechanical (*e.g.*, scotch-tape method), electrochemical or solution-based exfoliation from bulk graphite.<sup>42</sup> Laser irradiation, thermal annealing, photo-reduction, microwave-assisted reduction, and chemical reduction are commonly used methods to prepare graphene and its derivatives; graphene oxide (GO) and reduced graphene oxide (rGO).<sup>43</sup> Two-dimensional sp<sup>2</sup> hybridized carbon network provides unique properties that makes them well suited for wearable electronics, *e.g.*, flexibility, high sensitivity-to-surface-charge distribution, and ultra-small thickness of the layers.<sup>44</sup> Graphene can act as an electron transfer bridge and signal reporter, providing increased sensitivity of biosensors for the detection of biomarkers.<sup>41a,45</sup> Activated graphene provides functional groups for the attachment of biomolecular recognition reagents *e.g.*, enzymes, aptamers, and antibodies (Ab). In the same way, graphene works as an excellent flexible support for the immobilization of Ab, enabling the development of biosensors for SARS-CoV-2 detection.<sup>46</sup> Some common issues associated with this material are limited reproducibility due to large batch-to-batch deviations, difficulties in up-scaling production, and the need to more clearly define its toxicity.<sup>47</sup>

The key to the successful application of graphene in electrochemical biosensors lies in its ability to be interfaced with bioreceptors and be integrated within substrates that can serve as support for electrodes. GO, a highly oxidized form of chemically modified graphene, is commonly used for this purpose due to the presence of carboxylate groups that



**Table 1** Examples of carbon-based POC diagnostic platforms

Modified electrodes	Substrate	Analyte	Sample	Linear range	LOD	Ref.
<b>Carbon nanotubes-based sensors</b>						
MWCNT-COOH-PB	Rubber glove	Uric-acid, glucose	Sweat		3.58 $\mu\text{M}$ , 9.10 $\mu\text{M}$	9
G/CNTs	Textile	Glucose	ISF		0.06 $\mu\text{M}$	10
MWCNT-PB	Cloth-based chip	Glucose	Sweat	0.05–1 mM		11
Carbon paste	Microneedle	Levodopa	ISF	0.5–3 $\mu\text{M}$	0.5 $\mu\text{M}$	12
MCNTs-RGO/CFT	Textile	Glucose	Urine	0–40 mM	3.95 mM	13
MWCNT/PEDOT	Fabric	K <sup>+</sup>	Saliva	1–1000 nM	1 nM	14
<b>Graphene based sensors</b>						
Graphene fiber-PB	Fabric patch	Glucose	Sweat	50 mM–1 M	50 $\mu\text{M}$	15
Graphene ink	Flexible on-chip	Dopamine	Sweat		5 nM	16
PB-RGO nanofilms	Head band (fabric)	Glucose	Sweat		7.94 $\mu\text{M}$	17
Laser induced graphene (LIG)	Filter paper	Uric acid	Urine	10–300 $\mu\text{M}$	3.97 $\mu\text{M}$	18
Elastomer/graphene ink	Wearable	Na <sup>+</sup>	Sweat			19
PEDOT/sulfur-doped graphene (PEDOT-G)	Wearable	Dopamine	Tears		101 $\times$ 10–9 m	20
Graphite nanocrystals with tetrahedral amorphous carbon (GNC-TAC)	Filter paper	Pb <sup>2+</sup>	Urine	0.5–700 $\mu\text{g L}^{-1}$	0.15 $\mu\text{g L}^{-1}$	21
Graphene sponge-chitosan-PB (GS/CTS/PB)	Flexible wearable	Glucose	Sweat	8.17–1000 $\mu\text{M}$	2.45 $\mu\text{M}$	22
Carbon/graphite ink	Tattoo	Vitamin C	Sweat	10–50 $\mu\text{M}$		23
<b>Hybrid carbon-based sensors</b>						
Boron-doped graphene quantum dots anchored to CNTs (BGQDs/CNTs)	Wearable	Uric acid	Sweat	6.10–7.35 $\mu\text{M}$	50 $\mu\text{M}$	24
Carbon nanotubes and gold nanotubes (C-Au NTs)	Flexible wearable	Urea	Sweat	1100 mM	0.1 mM	25
SWCNTs/ferrocene-polyaniline film/Cu (SWCNTs/F-P/Cu)	Non-invasive	Glucose	Sweat		0.081 mM	26
AuNPs-rGo and PtNPs-rGo	Flexible substrates	Dopamine	Urine	0.1–20 $\mu\text{M}$ (Au); 0.1–10 $\mu\text{M}$ (Pt)	75 nM and 62 nM	27
Graphene-gold NPs (GP-Au NPs)	Flexible substrate	Dopamine and uric acid	Cerebrospinal fluid	20 nM–40.76 $\mu\text{M}$ and 20–500 $\mu\text{M}$	10 nM and 1.47 $\mu\text{M}$	28
Nafion and reduced graphene oxide enclosed carbonized silk fabric (Nafion/rGo/CSF)	Wearable electronics	Dopamine	Urine	1 nM–30 $\mu\text{M}$	1 nM	29
Nitrogen doped graphene (NG/PEDOT) film	Flexible wearable	Dopamine		0.2 $\mu\text{M}$ to 90 $\mu\text{M}$	54 nM	30
Nickel nanoparticles decorated laser induced graphene (Ni/LIG)		Glucose		0.50–1666 $\mu\text{M}$	0.29 $\mu\text{M}$	31
MIP modified polyvinylidene fluoride (PVDF)/graphene	Flexible wrist band	Lactate	Sweat	0–20 mM	15 mM	32
Fe/Co/rGo	Flexible wearable	Glucose	Tears	0.1906.4 $\mu\text{M}$	0.07 $\mu\text{M}$	33
NiO/rGO/PtE	Reusable	Linezolid drug	Urine	0.1–90 $\mu\text{M}$	31 $\mu\text{M}$	34
Copper oxide modified carbon nanotubes (CuO@CNTFs)	Flexible microelectrodes	Glucose	Biofluid	13 mM	1.4 $\mu\text{M}$	35
Porous polypropylene/carb on nanotube/polyaniline (p-PP/CNT/PANI)	Flexible mask	Respiratory rate	Human breath	500–70 ppb	500 ppb	36
MXene-MWCNTs	Flexible wearable	Cortisol	Sweat	0.1 fg mL <sup>-1</sup> –1 $\mu\text{g mL}^{-1}$	0.03 fg mL <sup>-1</sup>	37
rGo-Au/SPGE	Non-invasive wearable	Glucose	Sweat	1.25–850 $\mu\text{M}$ 0.85–7.72 mM	1.25 $\mu\text{M}$	38
Carbon fiber based-MXene-MoS <sub>2</sub>	Flexible substrate	AA, DA, UA, microRNA	Human serum urine	10–1000 $\mu\text{M}$ , 0.5–200 $\mu\text{M}$ , 0.5–150 $\mu\text{M}$	0.89 $\mu\text{M}$ , 0.23 $\mu\text{M}$ , 0.35 $\mu\text{M}$ 3.16 aM	39
Au-CNTs-chitosan	Disposable	$\beta$ -Hydroxybutyrate	Saliva	0.1 to 3.0 mM	50 $\mu\text{M}$	40

act as binding sites for biomolecules. The carbon sheet of GO has COOH on the outside along with oxygen functionalities, –OH, and epoxy (–O–) groups inside, which enhance its water solubility. The surface chemistry of GO is negatively charged due to the carboxylate groups, with partially hydrophilic regions with hydrophobic distributions at edges, capable of

hydrogen and electrostatic binding. The reduced form of GO, rGO, is also commonly used due to its ability to restore conductivity and introduce defective structures into the carbon lattice. rGO is produced by treating GO under reducing conditions, through chemical, thermal or electrochemical means. 1) Chemical reduction involves the



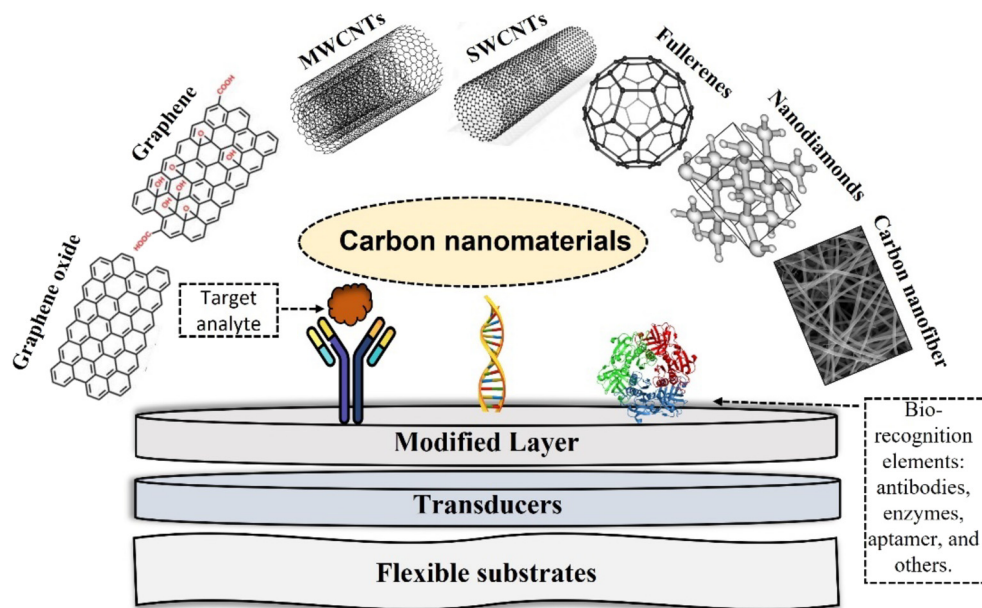


Fig. 1 Summary of different types of carbon nanostructures for electrochemical biosensors and active biofunctionalized surface for sensing design.

use of strong reducing agents such as hydrazine, sodium borohydride, hydroquinone, gaseous hydrogen, and strong alkaline solutions. 2) Thermal mediated reduction is produced by direct heating of GO at high temperature creating thermodynamically stable carbon oxide species. The heating of GO to 1050 °C causes the release of high temperature carbon dioxide and exfoliation in between the stacked layer of GO platelets. 3) The electrochemical treatment involves the removal of oxygen functionalities in a sodium phosphate buffer solution. Functionalized GO deposited on a variety of electrode substrates (plastic, paper, polymer *etc.*) showed rapid reduction within few seconds during electrochemical measurements.<sup>48</sup> Fig. 2 illustrates the common forms of graphene, GO and rGO. The carboxylate groups on GO are activated using coupling reagents such as 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) for further covalent attachment of biomolecules.<sup>49</sup>

Activated graphene is the most commonly used electrode material in electrochemical biosensors for direct detection of electroactive molecules such as catecholamine neurotransmitters like dopamine. Dong *et al.*, developed a 3-D loofah sponge made of a carbon/graphene aerogel, fabricated *via* one pot hydrothermal method and demonstrated its efficacy for the electrochemical detection of H<sub>2</sub>O<sub>2</sub> and dopamine with detection limits of 1.2 μM and 0.25 μM, respectively.<sup>51</sup> The activated graphene provided a large working surface area, while the sponge-like structure prevented the graphene sheets from aggregation with improved catalytic activity. Zhou *et al.*, utilized a hybrid nitrogen-doped graphene microelectrode and demonstrated its performance for neurotransmitters sensing with high sensitivity and low detection limits of 0.69 nM for dopamine and 6.5 nM for 5-hydroxy tryptamine in real serum samples.<sup>52</sup> The doped material provides enhanced electrocatalytic

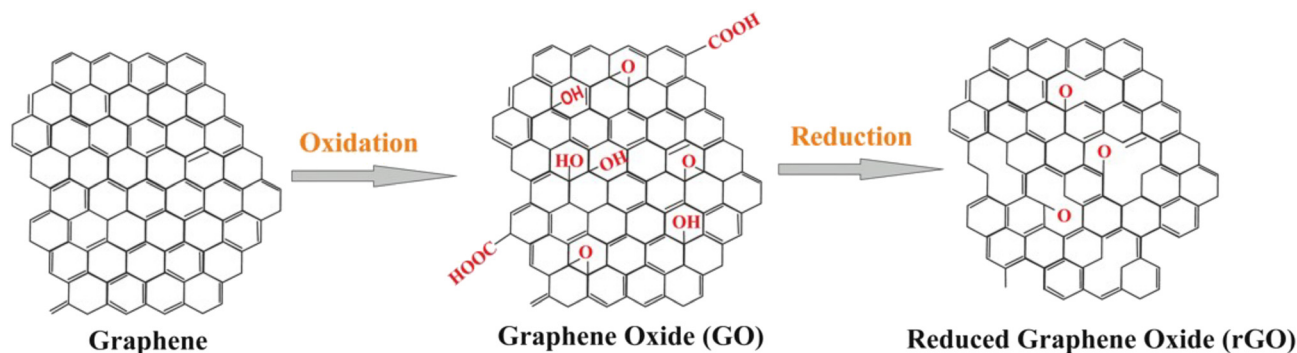


Fig. 2 General structure of graphene, carboxyl-functionalized graphene oxide and reduced graphene oxide nanomaterials (with permission from Springer, copyright [2018]<sup>50</sup>).





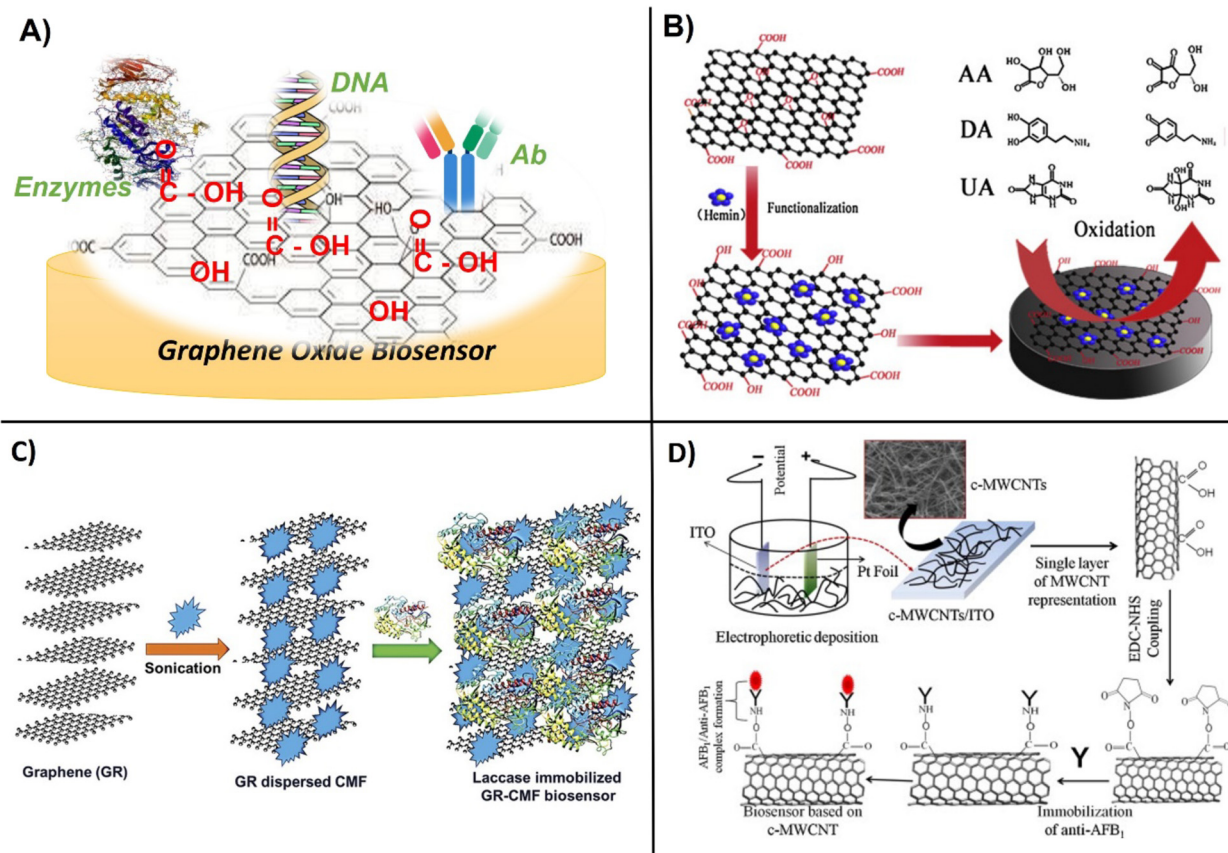
activity which results in increased performance for neurotransmitter detection.

**2.1.2. Carbon nanotubes (CNTs).** CNTs have been explored in the biomedical field for tissue engineering, drug release, gene therapy, and microfluidics diagnostics due to their excellent chemical, physical, structural, thermal, and mechanical properties. In biosensing, CNTs have been used as electrode materials and as a matrix for the immobilization of biomolecules *via* physical and chemical interactions.<sup>53</sup> Single nanotube conducting channels have been used to fabricate field effect transistors for biosensing applications.<sup>54</sup> The hollow structure and the large surface area of CNTs favor adsorption of proteins, DNA, and small molecules. The characteristic Raman signature of CNTs makes them suitable for use as label (tags) in electrochemical biosensing.<sup>55</sup> Covalent modification typically involves oxidation by high acid concentrations to introduce functional groups (*e.g.*, carboxyl) onto the terminal or defect sites of the CNTs, followed by covalent binding of biomolecules using EDC linkers. It is known that acid treatment creates functional groups (–OH, COOH) and surface defects which provide a large number of binding sites for biomolecules.<sup>53</sup> CNTs have small diameters and are considered to be more chemically reactive due to their high aspect ratio.<sup>56</sup> Several

reports have shown that CNTs provide direct electrical wiring between the electrode and the redox center of redox proteins, promoting direct electron transfer.<sup>57</sup> While the drawbacks include possible agglomeration, presence of impurities, and potential toxicity which may affect measurements in biological environments.<sup>58</sup>

## 2.2. Surface activation

The high surface area and abundant functional groups of carbon-based nanomaterials make them attractive platforms for the immobilization of biomolecular receptors such as Ab, enzymes, nucleic acids, and proteins (Fig. 3A). The vast majority of studies have utilized graphene, SWCNTs and MWCNTs, surface-activated or in hybrid-composite forms with polymers and other materials such as metal or metal oxide NPs and ionic liquids to enhance their binding properties and electrochemical performance. Activated carbon has increased porosity and surface area and the surface treatment can be customized by selecting the activating agent to fit the desired application.<sup>59</sup> Typical activation procedures involve the use of strong oxidizing gases and agents like phosphoric acid to produce pores through etching and add oxygen-containing functional groups onto the



**Fig. 3** Schematic showing, A) general design of GO modification with enzymes, DNA and Abs, B) functionalization of graphene oxide with hemin for enhanced oxidation of ascorbic acid, dopamine and uric acid (with permission from Elsevier, copyright [2014]<sup>65</sup>), C) immobilization of laccase within graphene and cellulose microfibers (with permission from Springer, copyright [2017]<sup>70</sup>), and D) carboxylation of CNTs followed by functionalization with monoclonal Abs for the detection of aflatoxin-B1 (with permission from Elsevier, copyright [2013]<sup>69</sup>).



carbon structure, creating active sites that can easily attach biomolecules.<sup>60</sup> Tunable porosity and surface chemistry was achieved by activating hydrochar with phosphoric acid. The procedure involves mixing hydrochar with phosphoric acid for 8 hours at room temperature, drying at 110 °C followed by activation under static air for 1 hour.<sup>61</sup> These materials can be used to create composites for sensing.

Surface activation of CNTs by acid treatment generates functional groups that can further facilitate biomolecule binding.<sup>53</sup> Further, site defects and edge effects provide enhanced reactivity of graphene and CNTs due to higher density of functional groups on their active sites.<sup>62</sup> Surface activation is known to increase the surface/volume ratio and improve the sensing performance. For example, the electrochemical pretreatment of carbon fiber microelectrodes (CFMEs) in 0.1 M NaOH at a potential of 1 V for 600 s was shown to improve the sensitivity of the CFMEs, enabling ultrasensitive measurements of dopamine in the brain of zebrafish embryos in the physiological nanomolar concentration range.<sup>63</sup>

### 2.3. Biofunctionalization

Carbon nanostructures are generally seen as biocompatible and highly suitable for biomolecule grafting. This can be achieved through simple adsorption *via* forces such as weak van der Waals, electrostatic, hydrophobic, and hydrogen bonding, with adsorption varying with the type of receptor molecule.<sup>64</sup> This process is favored by positively charged molecules and those with conjugated  $\pi$ -bonds that can form  $\pi$ - $\pi$  stacking with the aromatic residues of proteins. Zou *et al.*, utilized the  $\pi$ - $\pi$  stacking interaction between graphene oxide and hemin (H-GO) to increase electrochemical performance and enable the simultaneous detection of dopamine (LOD = 0.17  $\mu$ M), ascorbic acid (LOD = 0.3  $\mu$ M) and uric acid (LOD = 0.17  $\mu$ M) respectively (Fig. 3B).<sup>65</sup> Aromatic compounds such as pyrene and perylene tetracarboxylic acid (PTCA) molecules have also been used as anchors for the binding of bioreceptors *via*  $\pi$ - $\pi$ -stacking. PTCA-stacked graphene worked as a redox mediator to facilitate the immobilization of aptamers with increasing sensitivity of electrochemical aptasensors. Yali *et al.*, demonstrated the use of graphene/peryene tetracarboxylic acid (GPD) as a novel redox probe with improved conductivity and electrochemical active area. The GPD probe was shown to improve the electron transfer due to the large delocalized face-to-face surface interaction of  $\pi$ - $\pi$ -stacking. The aptasensor exhibited high sensitivity with a detection limit of 200 fM.<sup>66</sup> Activated forms of carbon are more easily able to form conjugates with biomolecules, but their properties vary with the size, charge, and nature of the biomolecule. In a study comparing binding interactions between GO and DNA, it was found that shorter DNA is adsorbed tighter and more rapidly to graphene than longer DNA and that adsorption is favored at a low pH and a higher ionic strength.<sup>67</sup> Chemically reducing carbon with hydrazine provided enhanced surface area, good reusability and a low detection limit of 0.37 pM of SARS-CoV19, as compared to methods such as electrical, thermal, or catalytic reduction.<sup>68</sup> An

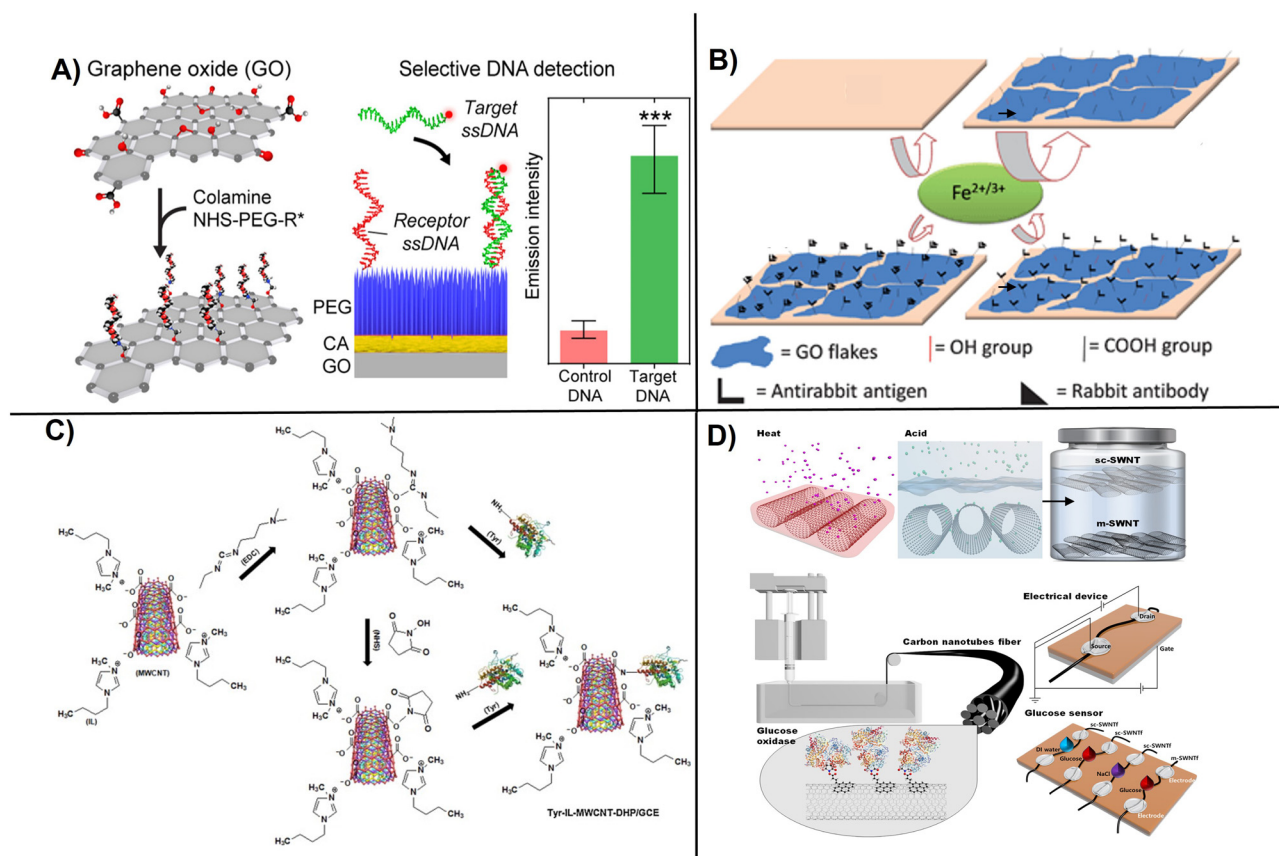
immunosensor based on covalently functionalized MWCNTs deposited on indium tin oxide (ITO) modified with monoclonal aflatoxin-B1 Ab enabled detection of aflatoxin-B1, with a sensitivity of 95.2  $\mu$ A ng<sup>-1</sup> mL cm<sup>-2</sup> and a LOD of 0.08 ng mL<sup>-1</sup> (Fig. 3D).<sup>69</sup>

The surface properties, solubility, functionality, and binding efficiency of biomolecules on carbon nanomaterials have been significantly improved by coating or co-entrapment with polymeric layers. The immobilization of polymers and biomolecules on graphene can be achieved by weak noncovalent interactions *via* electrostatic,  $\pi$ - $\pi$  stacking, and van der Waals forces. For example, laccase was effectively immobilized within a graphene-cellulose microfiber composite which enabled detection of catechol in the concentration range 0.2–209.7  $\mu$ M (Fig. 3C).<sup>70</sup>

Covalent attachment of polymers such as chitosan, polyvinyl alcohol (PVA), and polyethylene glycol (PEG) to GO *via* the residual oxygen-containing functional groups has also been demonstrated, shown to improve processing and make nano-GO highly dispersible and chemically stable in physiological solutions. The covalent functionalization of a few-layers graphene or carbon nanosheets with PVA through EDC esterification improves solubility of the graphene nanomaterials<sup>71</sup> and facilitates uniform deposition. PEG-GO is the preferred material for biosensing measurements in biological environments and physiological conditions. The use of PEG layers in a graphene-based field effect transistor with aptamer receptors enabled selective real-time detection of a cancer biomarker, prostate specific antigen, in physiological medium.<sup>72</sup> The aptamer receptor was regenerated for multiple uses. The functionalization of GO with colamine and PEG cross-linker was shown to improve biomolecule adhesion and coating density of DNA for DNA biosensing as compared to a silanization procedure using (3-aminopropyl) triethoxysilane.<sup>73</sup> The multistep functionalization of GO with PEG enhanced the surface coverage and robustness of the biosensor while improving applicability and performance, particularly those designed for *in vivo* applications. A preferred polymer for enhancing enzyme immobilization on carbon nanostructures is chitosan. Yang *et al.*, developed a uric acid biosensor by using a chitosan/CNT dispersion cross-linked with glutaraldehyde and drop casted on a 3D super-aligned CNT array electrode. The advantage of this procedure is that the CNTs in the 3D array maintained their structure and electronic conductivity while providing a high surface area for enzyme immobilization. A detection limit of 1  $\mu$ M uric acid was reported. This platform can be broadly implemented for point-of-care testing of other biomolecules.<sup>74</sup>

Examples of commonly used procedures to attach enzymes, DNA, and Ab to carbon-based nanostructured electrodes though PEG and EDC activation are shown (Fig. 4).<sup>49,73</sup> Polymer-supported rGO prepared using poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) demonstrated high efficiency for the electrochemical detection of DNA hybridization. The PEDOT:PSS/rGO composite was prepared in a lightweight sponge-like structure and was highly conductive. When used as





**Fig. 4** Examples of surface functionalization and electrochemical device fabrication procedures showing: A) PEG-modified graphite layer for DNA coupling (with permission from ACS, copyright [2021]<sup>73</sup>), B) covalently immobilized Ab on GO flakes onto a glassy carbon electrode surface for label-free sensing (with permission from RSC, copyright [2011]<sup>49</sup>), C) functionalization of MWCNTs with tyrosinase using 1-butyl-3-methylimidazolium chloride ionic liquid (with permission from Elsevier, copyright [2013]<sup>78</sup>), and D) fabrication of fiber-based glucose biosensor involving surface activation by acid treatment and immobilization of glucose oxidase on modified SWCNTs for glucose sensing (with permission from Elsevier, copyright [2020]<sup>77</sup>).

electrode material, PEDOT:PSS/rGO composite enabled electrochemical detection of DNA hybridization with a sensitivity of 1 fM.<sup>75</sup> Other procedures work on the modification of GO with proteins such as glutathione peroxidase using EDC and Nafion to construct a glutathione biosensor with an LOD of 0.9 nM as reported.<sup>76</sup> Lee *et al.*, developed semiconducting single-walled carbon nanotubes (sc-SWCNTs) fibers as wearable electrochemical biosensors for glucose monitoring above 0.5  $\mu$ M. The sc-SWCNTs are difficult to work due to needing an improved separation method that limit their practical application.<sup>77</sup>

### 3. Integration in portable low-cost substrates

#### 3.1. Paper-based electroanalytical devices

Paper is an interesting material for POC devices due to its high surface area, porosity, ease of modification, and affordability. Electrochemical paper-based analytical devices (ePDAs) were introduced more than a decade ago, providing several inherent advantages such as ease of use, low volume

per sample, portability, manufacturability, disposability, and low cost.<sup>79</sup> ePADs combine the advantages of paper-based microfluidic devices through capillary action with electrochemical detection. Dungchai *et al.*, 2009 reported the first electrochemical paper-based device by depositing a highly conductive track onto a paper substrate, using a carbon-based ink containing Prussian blue as a mediator.<sup>80</sup> Fabrication of paper electrodes involves modification of the cellulosic structure to impart conductivity and hydrophobicity in order to facilitate migration of reagents and enable monitoring of electrochemical processes on paper. Therefore, the selection of a suitable material to impart conductivity and the functionalization steps are highly important to develop low-cost POC diagnostics. Carbon has been used as a convenient conductive material to create conductive tracks.

Along with carbon, a variety of different materials including metals, polymers, ionic liquids, and metal NPs have also been incorporated to create conductive structures, while maintaining simplicity and low cost. Fabrication of an ePADs typically involves the use of various printing techniques including inject printing, screen printing, stencil

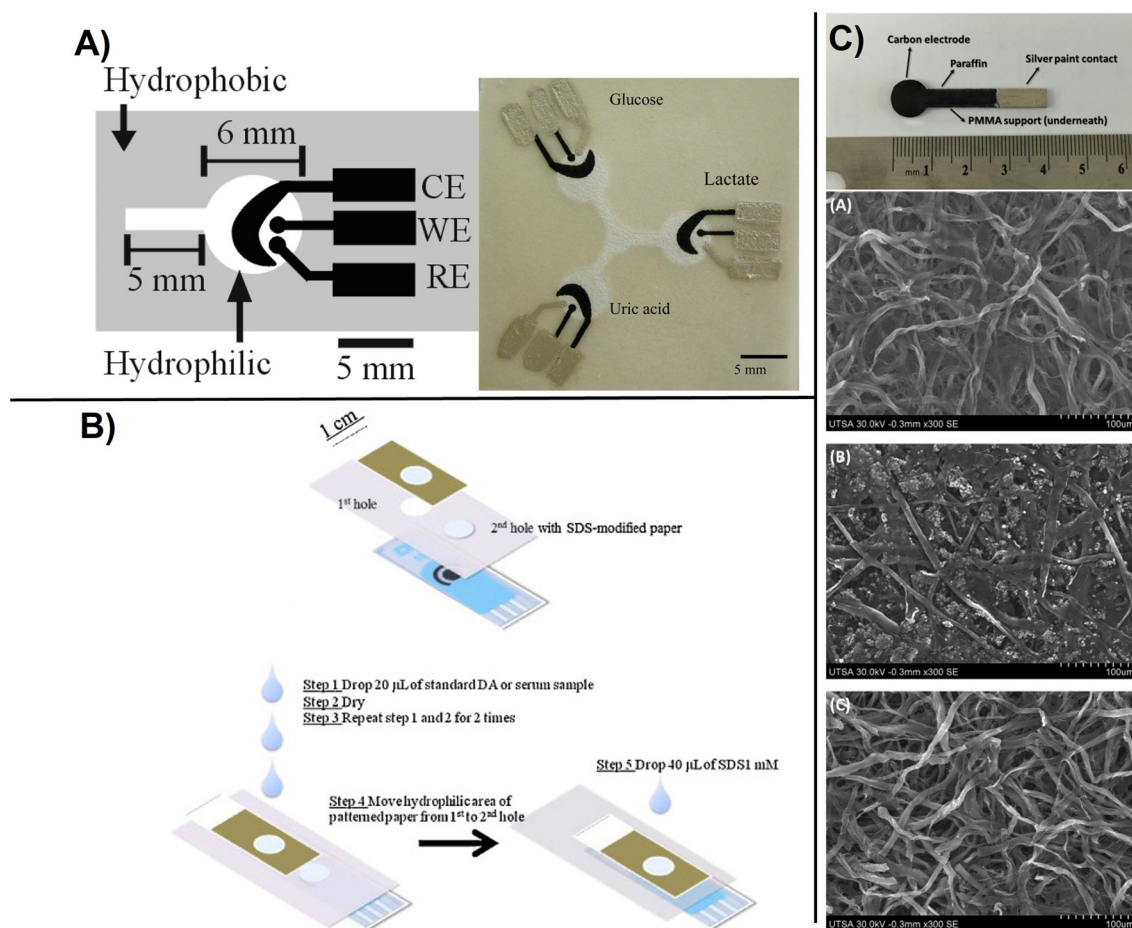




printing and photolithography, many of which require development of biocompatible inks of specific rheology to enable printing. Carbon inks have been prepared by mixing graphite, chitosan, glycerol, and enzyme before screen printing on polyethylene terephthalate (PET) substrate to design a disposable sensor for uric acid and catechol as reported.<sup>81</sup> This section summarizes the recent advancements of carbon-based materials to design conductive ePADs for electrochemical POC testing along with their working performance and applications.

One of the first coupling paper designs of electrochemistry was based on a three-electrode system for simultaneous detection of glucose, lactate, and uric acid. The biosensor was fabricated from a carbon ink deposited on Whatman grade 1 filter paper to create working, counter, and reference electrodes (WE, CE and RE) and their connections (Fig. 5A).

The WE ink contained oxidase enzymes that specifically recognized each target analyte and detection was accomplished by electrochemically measuring the enzymatically produced hydrogen peroxide. The ink contained Prussian blue (PB;  $\text{Fe}^{\text{III}}_4[\text{Fe}^{\text{II}}(\text{CN})_6]_3$ ) as mediator for the reduction of  $\text{H}_2\text{O}_2$ . The RE contained Ag/AgCl as a pseudo-reference.<sup>80</sup> This study demonstrated the successful integration of carbon ink for creating functional conductive ePAD for POC monitoring. The electrochemical sensor was further combined with a colorimetric unit for dual lab-on-a-chip screening of gold and iron.<sup>82</sup> In other configurations, ePADs have been deposited on top of a screen-printed electrode for sample pre-concentration (Fig. 5B). These sensors have demonstrated applicability for the detection of dopamine in serum with the concentration range 1–100  $\mu\text{M}$  and an LOD of 0.37  $\mu\text{M}$  using square-wave voltammetric technique.<sup>83</sup> The



**Fig. 5** Basic design of paper based electrochemical sensors showing: A), printed carbon-based paper electrode with WE, working electrode; RE, reference electrode and CE, counter electrode for detection of glucose, lactate, and uric acid. The silver electrodes and contact pads are made from Ag/AgCl paste with the black electrode portions being the PB-modified carbon electrodes. The device size is 4 cm × 4 cm (with permission from ACS, copyright [2009]<sup>80</sup>), B) ePAD-screen printed electrode configuration for the determination of DA where the patterned paper was used for sample pre-concentration, and improved selectivity using SDS to shift the operating potential to lower values preventing interferences (with permission from Elsevier, copyright [2012]<sup>83</sup>), and C) carbon electrodes made from pyrolyzed paper deposited on paraffin and sealed with a silver paint including SEM micrographs of the working carbon electrodes obtained from pyrolysis of imaging card paper (A), multipurpose printing paper (B), and 3MM chromatography paper and (C), showing a microfibrillar structure (with permission from Elsevier, copyright [2016]<sup>84</sup>).



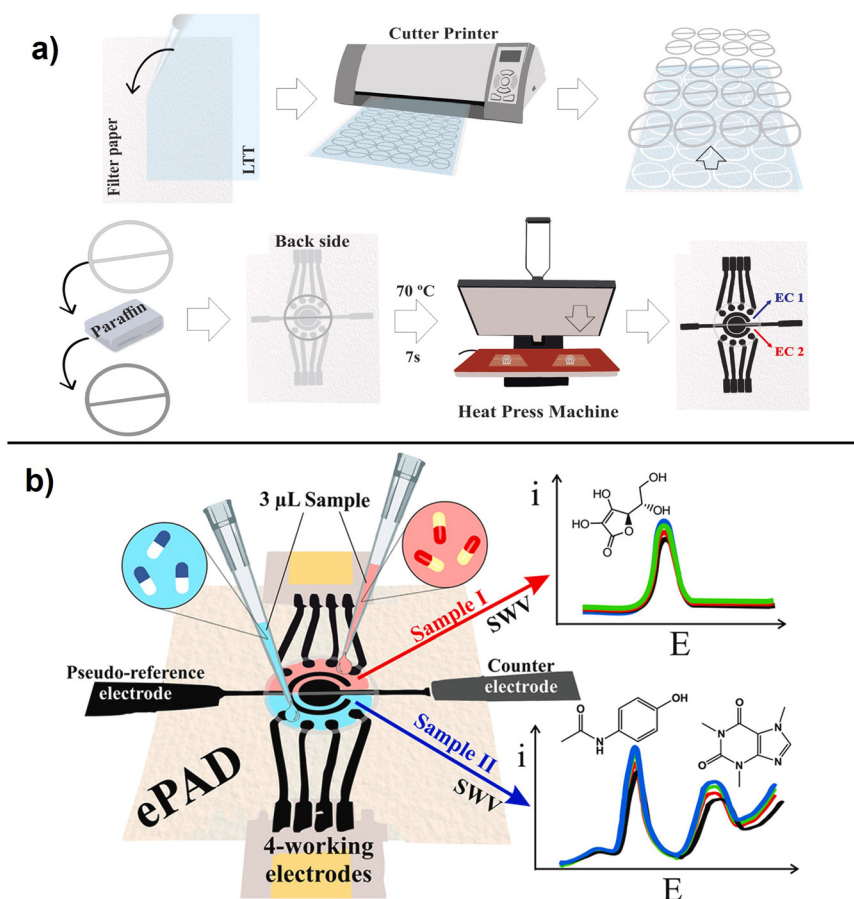


method provided selectivity against ascorbic acid and uric acid by integrating the anionic surfactant of sodium dodecyl sulfate impregnated in one layer of the device which shifts the oxidation peak of dopamine to more negative values. Other works explored the use of pyrolyzed paper as a conductive substrate for ePAD design. A microscopic study of three types of papers, 3 mM chromatography, imaging card, and multipurpose printing paper, demonstrates a fibrous cellulose structure preserved in all cases after pyrolysis (Fig. 5C). The pyrolyzed papers were used as electrode materials and showed high surface area and quasi-reversible behavior for  $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$  redox couple. The largest electroactive area and detection performance was obtained for the 3MM chromatographic paper.<sup>84</sup> The procedure enabled the immobilization of urate oxidase enzyme that catalyzes the oxidation of uric acid to allenoate, reaching detection in the concentration range of 0.001–0.833 mM. Such low-cost platforms can be easily interfaced with cellphone connected electroanalyzers and are suitable for applications in remote and low-resourced communities.

A simple method to fabricate an ePAD with 2 cells and 4-working electrodes on paper surface using an

inexpensive craft cutter was reported for multiplexed determination of pharmaceutical compounds (ascorbic acid, paracetamol, and caffeine), following to the fabrication steps showed in (Fig. 6).<sup>85</sup> The interaction of graphene nanomaterials, including graphene oxide, reduced graphene oxide and few layer graphene, with different biomolecules have been discussed and additional details can be found in ref. 86.

Improved sensing can be achieved by functionalization with conductive polymers, metal oxides and metal sulfides. Flexible self-standing graphene-based paper electrodes have been designed by using GO functionalized with zinc sulfide nanostructures (GO/ZnS) *via* thermal reduction provided increased electrochemical activity for oxidation of dopamine, enabling detection in 0.1–2300  $\mu\text{M}$  concentration range (Fig. 7a).<sup>87</sup> Flexible electrodes have been fabricated by coating graphene paper with electrocatalytic 2D gold nanoclusters. The process involved transfer of 2D nanoclusters at an oil densely packed to a GO paper substrate which led to formation of a uniformly monolayer of AuNPs (Fig. 7b). The performance of these sensors was demonstrated for the electrochemical sensing of glucose and  $\text{H}_2\text{O}_2$  secreted by living cells.<sup>88</sup> These approaches open a new avenue to control



**Fig. 6** Schematic representation of multiplexed (4 WE) ePAD fabrication showing a), steps in the fabrication process using a cutter printer involving wax barrier production and b), illustration of the integrated ePAD for multi-analyte detection using SWV (with permission of Elsevier, copyright [2019]<sup>85</sup>).



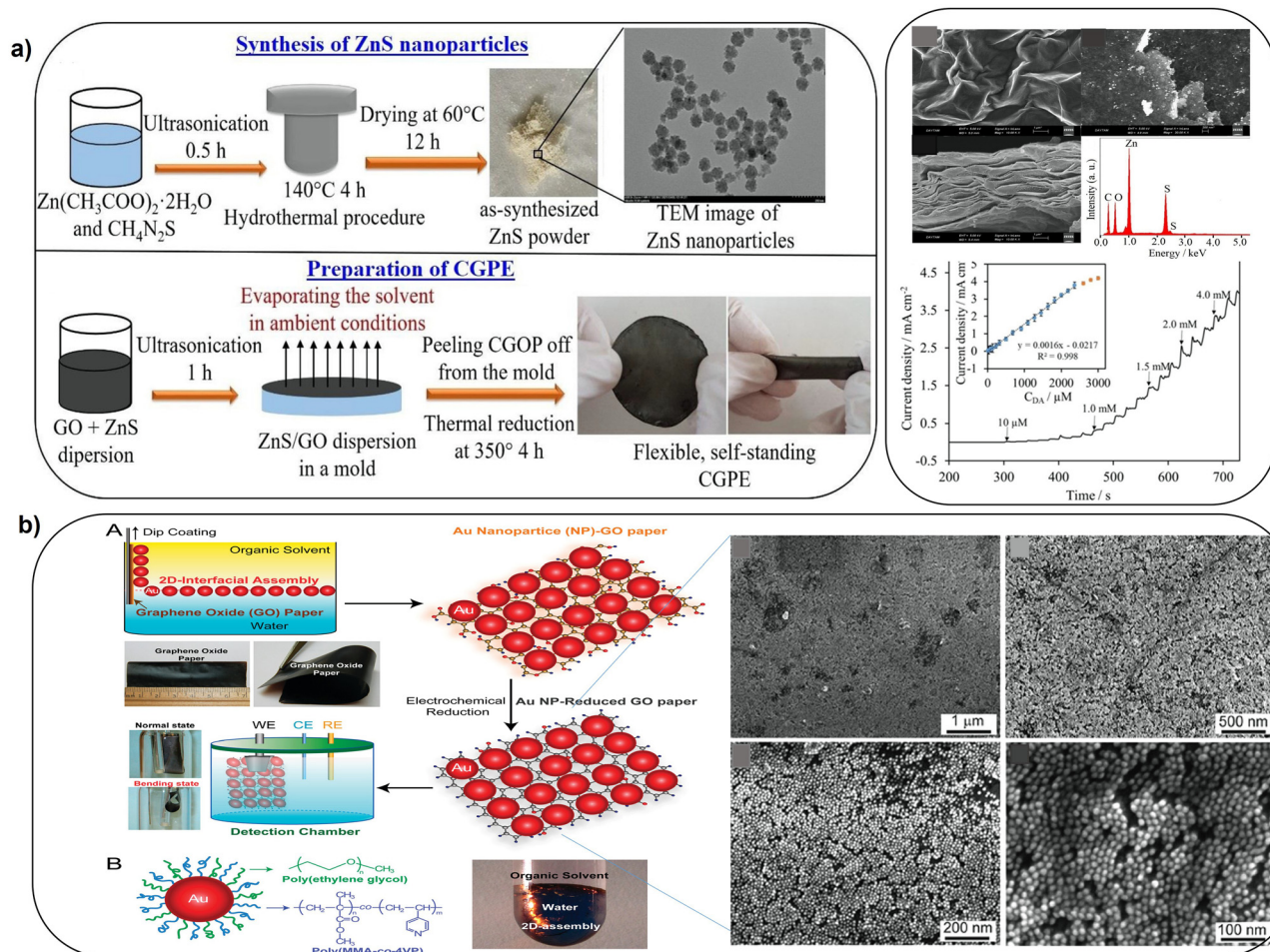


Fig. 7 Schematic illustration of: a), synthesis of ZnS NPs and deposition of GO and ZnO as a flexible standalone CGPE sensor (with permission from Wiley, copyright [2021]<sup>87</sup>) and b), the fabrication of freestanding hybrid electrodes from 2D-assembly of AuNPs and GO-modified paper, with SEM images of 2D-assembly of AuNPs coated on GO paper at different magnifications (with permission from ACS, copyright [2012]<sup>88</sup>).

and systematically study the sensing interface for the next generation miniaturized and flexible freestanding bioelectronics.

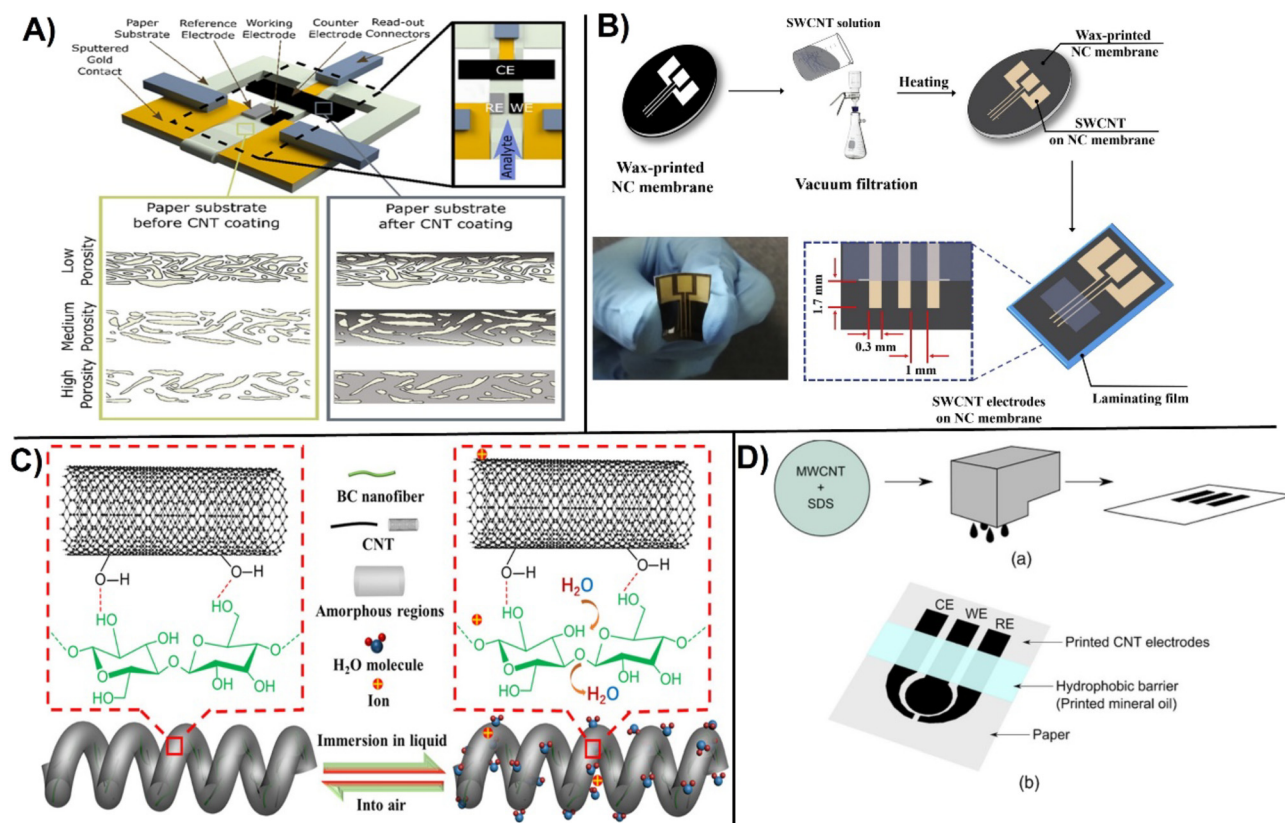
In another work, flexible freestanding rGO-based ePADs were fabricated by direct electrochemical deposition of bimetallic sulfides ( $\text{NiCo}_2\text{S}_4$ ) under vacuum filtration onto the paper substrate, enabling the simultaneous detection of ascorbic acid and folic acid. Bimetallic sulfides can be used for device fabrication due to their porous structure, self-doping ability, high electrochemical activity and active site density, providing superior performance as compared to monometallic sulfides.<sup>89</sup> Flexible  $\text{NiCo}_2\text{S}_4$  based rGO paper electrodes exhibited a very low detection limit (ascorbic acid  $\sim 3.0 \times 10^{-8}$  M, folic acid  $\sim 1.6 \times 10^{-9}$  M) due to its large electrochemically active surface area.<sup>90</sup> Yan *et al.*, designed an ePAD by modifying rGO with thionine and gold nanoparticles (rGO/Thi/Au NPs). The platform was used for the detection of cancer antigen based on the strong immunocomplex formation between the CA125 Ab and CA125 antigen which caused a reduction of current response

of thionine, directly relating with the concentration of analyte. A LOD of  $0.01 \text{ U mL}^{-1}$ , good linearity ( $0.1\text{--}200 \text{ U mL}^{-1}$ ) and good correlation with the traditional Elisa procedure were reported.<sup>91</sup> Sadkate *et al.*, designed a non-enzymatic sensor by modifying a GO-based ePAD with a cobalt phthalocyanine-ionic liquid composite. The sensor was used for the electrochemical detection for glucose oxidation within the  $0.01\text{--}1.3 \text{ mM}$  concentration range with LOD of  $0.67 \mu\text{M}$  respectively.<sup>92</sup>

Performance of ePADs vary with the porosity, material type and the modification procedure. Integration of CNTs for ePADs fabrication takes an advantage of high surface area, improved internal structure, and chemical stability. Valentine *et al.*, studied the effect of the porosity of paper before and after functionalization with CNTs-Nafion using different fabrication methods (*i.e.*, drop casting, laser scrubbing and origami) to establish the effect of network porosity on the sensor performance for the detection of glucose. The results indicate that changing porosity of the paper can lead to an almost two fold increase in sensitivity (Fig. 8A).<sup>93</sup> Novell







**Fig. 8** A) Fabrication of paper-based sensor before and after CNT modification and electrode design studying the influence of porosity on the CNT coating (with permission from ACS, copyright [2020]<sup>93</sup>). B) Micropatterning on SWCNT patterning on NC membrane to fabricate a flexible SWCNT electrodes (with permission from Elsevier, copyright [2018]<sup>95</sup>), and C) immobilization of CNTs on bacterial nanocellulose for creating stretchable helical fibers (with permission from ACS, copyright [2022]<sup>96</sup>), and D) fabrication of inkjet-printed CNT-based paper sensor involving: (a) preparation of CNT ink, followed by inkjet-printing the ink, and (b) printing a hydrophobic barrier on top (with permission from ECS, copyright [2015]<sup>98</sup>).

*et al.*, designed a simple CNTs-paper based potentiometric sensor for the electrochemical detection of  $K^+$ ,  $NH_4^+$ , and pH with performance comparable to classical solid-state ion-selective electrodes.<sup>94</sup> In the same way A non-enzymatic SWCNT-based ePAD for the detection of glucose was fabricated by wax printing and micropatterning on nanocellulose surface. The method provided good electrochemical conductivity and mechanical flexibility due to the strong interconnection between the nanotubes layer and the porous cellulose membrane. To design a hybrid conjugation, gold nanoparticles were further deposited on the SWCNT to enhance the electrocatalytic performance. Designed sensor provided a sensitivity of  $240 \mu A \text{ mM}^{-1} \text{ cm}^2$  with the LOD of  $148 \mu M$  as shown (Fig. 8B).<sup>95</sup> Modification of bacterial cellulose with CNTs provides flexibility and smart stretch-resistant for self-wearable platforms as recently demonstrated with a water sensor for smart diapers (Fig. 8C).<sup>96</sup> Handling of fluid in an ePAD design is very important for accurate measurements. A hydrophobic barrier was drawn by patterning (photolithography, wax printing, inject printing, stamping, screen printing *etc.*) the paper surface to create a barrier for liquid confinement and ensuring controlled flow of reagents to the reaction zone.<sup>97</sup> Another demonstration of

ePAD fabrication involve the development of a CNT-based conductive ink inkjet-printed directly on the paper surface using a standard office-based printer. Additionally, a hydrophobic barrier was created on paper by depositing mineral oil onto the desired surface (Fig. 8D). The printed electrodes were tested for the detection of dopamine and iron.<sup>98</sup> As demonstrated by these examples, carbon based conductive materials have several notable advantages and can be easily incorporated in ePADs using straightforward printing.

### 3.2. Disposable screen-printing platforms

Screen printing is the first, most frequently used method for designing low cost electrodes. Screen printing carbon electrodes (SPCEs) are fabricated by sequentially jetting conductive inks with the desired size and geometry on a low-cost and eco-friendly substrate (*i.e.*, such as silk, nylon or even paper). The sensors are most often fabricated as an integrated three electrodes system of working, counter, and reference electrode. Introducing carbon-based nanomaterials into screen printing inks offers an economical, simple, and reproducible way to fabricate nanostructured sensing





interfaces. The procedure involves optimization of suitable inks and substrate templates as well as deposition conditions to ensure homogeneity and uniformity of the ink on the substrate. The scalability, low cost and the ability to multiplex electrodes for various biomarkers makes screen printing an attractive manufacturing methods for large scale diagnostics, with possibility for implementation at industrial scale.<sup>99</sup> A SPCE electrode fabricated by printing of a graphite ink over a paper adhesive enabled direct measurement of melatonin oxidation in the concentration range between 10–100  $\mu\text{M}$  without any electrode modification.<sup>100</sup> With careful optimization, bioreceptors can be included in printing inks and be deposited automatically by printing.

Modification of SPCE electrodes, carbon-based nanostructures, and bioreceptors, particularly enzymes, is well established. Materials such as graphene or CNTs have been reported to enhance the surface area and facilitate enzyme attachment, also enhancing electron transfer and increasing sensitivity and stability.<sup>101</sup> Enzyme immobilization is generally accomplished with the help of conductive polymers or biopolymers, or with chemical immobilization procedures. Most enzyme sensors are dedicated to blood glucose sensing. A glucose SPCE-based biosensor was fabricated by first coating the surface of SPCEs with graphene-poly(3,4-ethylenedioxythiophene): polystyrene sulfonic acid (GP-PEDOT:PSS) nanocomposites, followed by the immobilization of the glucose oxidase (GOD) enzyme with (GP-PEDOT:PSS) by crosslinking with glutaraldehyde. Designed graphene based (GOD/GP-PEDOT:PSS)

conductive electrodes were 13 times more sensitive than the ones without graphene (GOD/PEDOT:PSS) with the LOD of  $\sim 0.3 \mu\text{M}$ .<sup>102</sup>

Using a unique form factor, Hu *et al.*, designed a screen-printed electrode for glucose detection in blood using a porous graphene aerogel composite with Prussian blue (PB) and immobilized glucose oxidase (GOx). The combination of graphene aerogel increased conductivity and catalytic performance, enabling measurements of glucose with a linear range between 0.5–6.0 mM with LOD 0.15 mM. The preparation of the biosensor followed a typical screen-printing process with the last layer being a graphene-PB aerogel with GOx immobilized in chitosan (Fig. 9A).<sup>103</sup> Other approaches involve the use of catalytic GO-based materials for non-enzymatic glucose detection. In recent example, a copper-reduced GO-modified SPCE electrode was designed for non-enzymatic measurement of glucose oxidation, achieving a linearity range between 0.10–12.5 mM, an LOD of 65  $\mu\text{M}$ , and a sensitivity of  $172 \mu\text{A mM}^{-1} \text{cm}^{-2}$  (Fig. 9B).<sup>104</sup> To improve detection sensitivity, MWCNTs can be modified with perylene tetracarboxylic acid to electrochemically amplify the signal as reported.<sup>82</sup>

Recently, an enzyme based electrochemical biosensor was fabricated by electrochemical deposition of hybrid silver-based graphene oxide (Ag-rGO) and used for the detection of urea in urine. The hybrid electrodes offered a 12 folds increase in sensitivity ( $47.598 \mu\text{M}^{-1} \text{M}^{-1}$ ) and a LOD of  $0.1623 \mu\text{M}$ .<sup>105</sup> Given their improved performance, carbon-based hybrid materials have been further employed for the detection of several

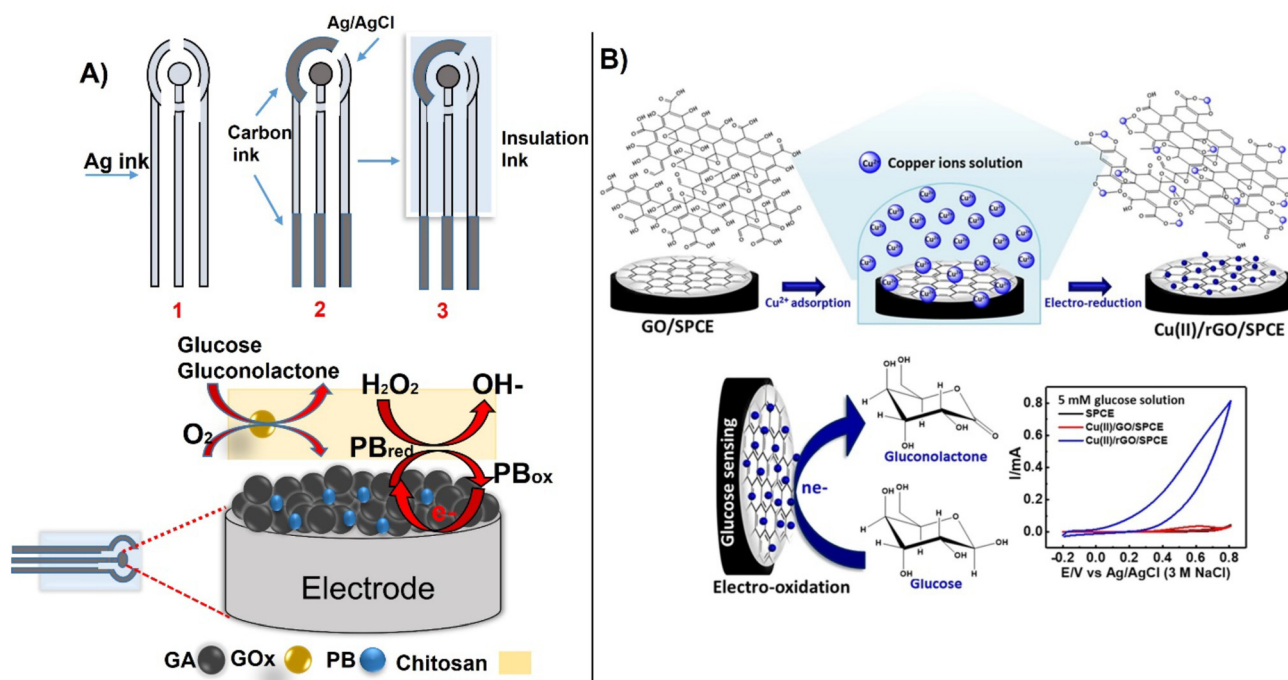


Fig. 9 The sequential process for preparing SPCE electrodes for glucose detection by printing, (A) with an example of functionalization of the working electrode with GOx immobilized within graphene-PB aerogel composites, (reproduced from Springer,<sup>103</sup> copyright [2022]) and (B) non-enzymatic detection using Cu-rGO deposited on SPCE (with permission from Springer, copyright [2021]<sup>104</sup>).

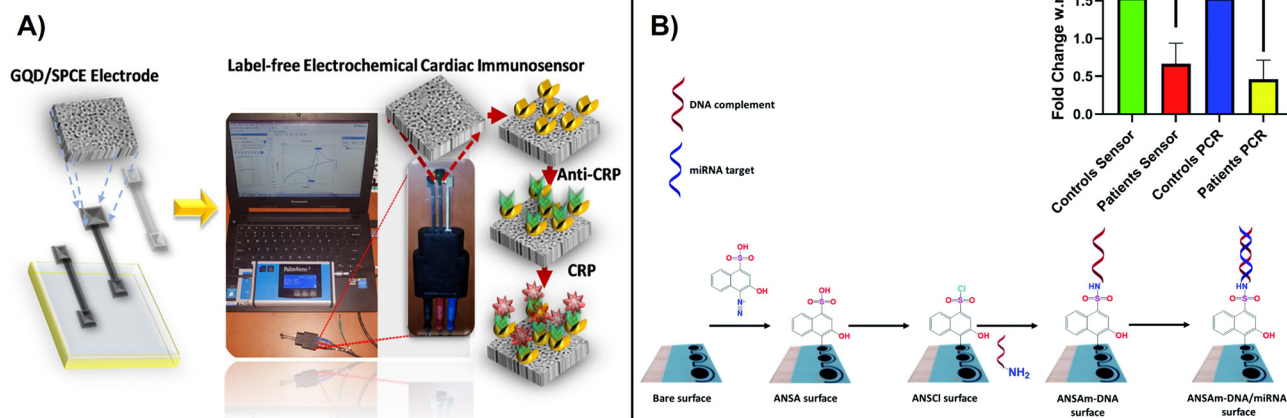


clinically relevant compounds such as salivary uric acid,<sup>106</sup> tyrosine,<sup>107</sup> SARS-CoV-2,<sup>108</sup> viruses,<sup>109</sup> and DNA,<sup>110</sup> respectively. In the same way, these materials are provided useful platforms for the detection of viruses,<sup>111</sup> bacteria,<sup>112</sup> heavy metals<sup>113</sup> and antimicrobial resistance (AMR).<sup>114</sup> Bachmann *et al.*, developed a label-free electrochemical sensor for point-of-care detection of AMR for better management of patient monitoring during antibiotic therapy as an alternative to culture-based methods.<sup>114</sup> The method involves immobilization of peptide nucleic acid probes on the electrodes surface *via* electrochemically reduced diazonium cations activated with EDC/NHS *via* electrochemical impedance spectroscopy. SPCEs are useful platforms for biomarker detection through affinity recognition involving immuno and aptamer recognition, enabling detection of cancer antigens, tumor biomarkers<sup>115</sup> and cardiac diseases like C-reactive protein<sup>116</sup> and troponin.<sup>117</sup> A C-reactive protein (CPR) based on label-free SPCE immunosensing with CPR antibodies displayed a linear range of 0.5–100.03 ng mL<sup>-1</sup> and an LOD of 0.036 ng mL<sup>-1</sup>.<sup>116</sup> A disposable diazo-sulfonamide modified SPCE with DNA enabled measurements of aberrant microRNA expression in urine samples from diabetic kidney disease patients and control subjects based on miR-192 expression relative to miR-191 at levels comparable with the conventional PCR technique (Fig. 10).<sup>118</sup> An SPCE biosensor strip containing  $\beta$ -hydroxybutyrate dehydrogenase (HBD) enzyme, *o*-toluidine blue O mediator, and the nicotinamide adenine dinucleotide (NAD<sup>+</sup>) as an HBD cofactor on an SPCE with CNTs and gold substrate has been developed for non-invasive monitoring of salivary ketone *e.g.*,  $\beta$ -hydroxybutyrate, (HB) and wellness applications.<sup>119</sup> These types of sensors have potential to be used for personalized decentralized measurements of salivary biomarkers for different health applications. More advanced SPCE measurements couple electrochemical detection with

microfluidics. In a recent example, an electrochemical aptasensor integrated within a herringbone-embedded microfluidic chip was designed for the detection of carcinoembryonic antigen (CEA), a widely used clinical tumor marker. The SPCE was modified with a hemin-coated CNT-decorated Ti<sub>3</sub>C<sub>2</sub> MXene nanosheet, where the MXene was used to improve dispersion and maintain electrochemical performance of the CNTs. The functionalized SPCE was embedded into a microfluidic chip to facilitate the interaction between the immobilized aptamer and the CEA in the sample. The platform showed the capability to measure CEA with an LOD of 2.88 pg mL<sup>-1</sup> within the range of 10–1 × 10<sup>6</sup> pg mL<sup>-1</sup>. Such technologies could be adapted for further use in clinical settings.

### 3.3. Electronic tattoos for on skin diagnostics

Electronic diagnostic tattoos (e-tattoos) are a new wave of devices with potential for personalized medicine.<sup>120</sup> Customized e-tattoos are generally made by patterning or printing conductive materials on supporting substrates or as standalone substrate-free films. The main requirements for e-tattoo development are: i) skin compatibility, ii) stretch-resistant electrical conductivity, iii) flexibility, and iv) mechanical durability.<sup>121</sup> Carbon nanostructures have the required characteristics to be used as a conductive interface for epidermal e-tattoos due to their electrical conductivity and high mechanical strength, making them ideally suited for skin-conformed electronics. Most e-tattoos developed to date demonstrate capabilities for monitoring heart rate, temperature, or electrophysiological activity. An example of a CNT-based lightweight and deformable e-tattoo was reported using CNTs in conjunction with porous silk nanofiber. When



**Fig. 10** A) Fabrication of SPCE immunosensor and portable electrochemical detection set-up for detection of C-reactive protein (CRP)(with permission from ACS, copyright [2021]<sup>116</sup>), and B) modification of SPE with diazo-sulfonamide modified and DNA for detection of microRNA expression in samples from diabetic kidney disease patients, as compared to controls (inset shows comparative results between sensor data and RT-qPCR) (with permission from RSC, copyright [2021]<sup>118</sup>).

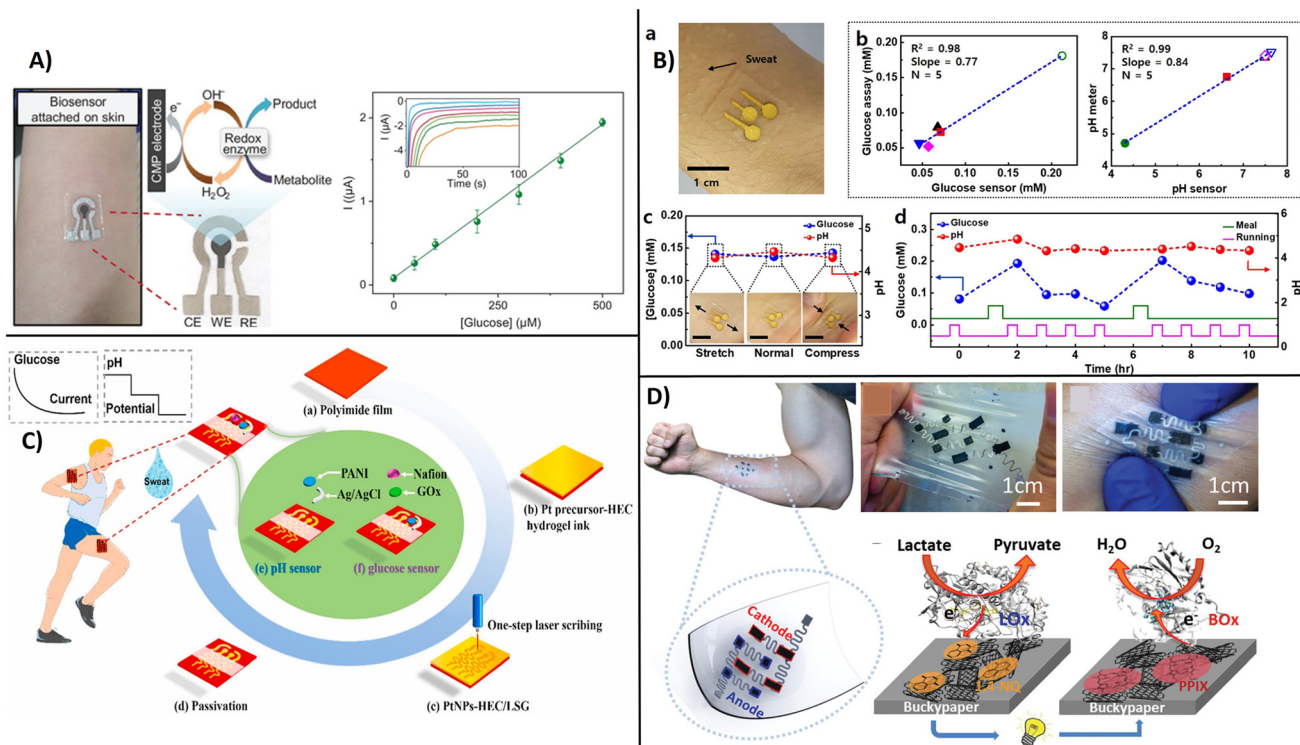


attached to dermal surfaces, these devices enabled temperature monitoring, real-time electrophysiology, and drug delivery.<sup>122</sup> Only a few examples of e-tattoos are developed as biosensors for molecular diagnostics for monitoring disease biomarkers.

An e-tattoo developed using Pt-decorated CNTs deposited on a gallium-based liquid metal composite demonstrated potential as a skin-attached wearable biosensor for measuring oxidase enzyme substrates, *i.e.*, lactate, glucose, and ethanol (Fig. 11A).<sup>120</sup> Another example of skin-attachable electrochemical sensor for glucose and pH in human perspiration has been developed by coating CoWO<sub>4</sub>/CNT and polyaniline/CNT nanocomposite onto CNT-AuNS electrodes with a chlorinated silver nanowire as a reference electrode.<sup>123</sup> To obtain a skin attachable device, the patterned electrodes were encapsulated within a sticky silbione led. Silbione was found to provide superior adhesion behavior, high biocompatibility and mechanical stability, which makes it an ideal material for wearables. Sensitivities of 10.89 mA mM cm and 71.44 mV pH<sup>-1</sup> for glucose and pH with a stability of 10 days and 30% stretchability were reported (Fig. 11B). Wang *et al.*,<sup>124</sup> reported a flexible and wearable biosensor design based on one-step laser synthesis and functionalization of platinum nanostructures within 3D

porous graphene for multiplexed analysis of glucose and pH for *in situ* perspiration to facilitate diabetes management in a non-invasive manner (Fig. 11C). The dual functional biosensor provided a sensitivity of 67.64  $\mu\text{A mM}^{-1} \text{cm}^{-2}$  for glucose and pH 72.4 mV pH<sup>-1</sup>.<sup>124</sup>

Platforms such as this enable personalized diagnostics and physiological monitoring. Further development and implementation of e-tattoos can advance patient specific on-skin diagnostics. In the future, several improvements are needed to enable autonomous and continuous operation such as the development of sampling collection and integration of energy harvesting systems. An example of a sweat collection patch has been reported recently, consisting of an analysis chamber for measurements of sweat conductivity and the [Na<sup>+</sup>] and [Cl<sup>-</sup>] of samples.<sup>125</sup> With respect to energy harvesting, most currently developed e-tattoos rely on power sources using conventional energy devices to enable operation. A new direction to realize truly autonomous wearables is to couple the sensing system with a bioenergy microgrid such as those relying on human activity to harvest energy input, creating an autonomously integrated on-body wearable.<sup>126</sup> In this example, the energy requirements for the microgrid e-textile are harvested from the sliding motion between the arms. Several examples of



**Fig. 11** Example of smart headband for *in situ* measurement of perspiration, A) e-tattoo biosensor on skin for measuring glucose, ethanol, and lactate with example of chronoamperometric measurements and calibration curve for glucose (with permission from Wiley, copyright [2022]<sup>120</sup>), B) electrochemical sensor attached to the skin for glucose and pH measurements (a and b), over time and under mechanical deformation (c), before and after meal (d), (with permission from ACS, copyright [2018]<sup>123</sup>), C) flexible design of electrochemical biosensor attached to the skin for glucose and pH measurements by using one step scribing design of multi-layered working electrode (with permission from Elsevier, copyright [2023]), and D) flexible CNTs-based buckypaper lactate biofuel cell for autonomous wearable electronics (with permission from Wiley, copyright [2019]<sup>127</sup>).





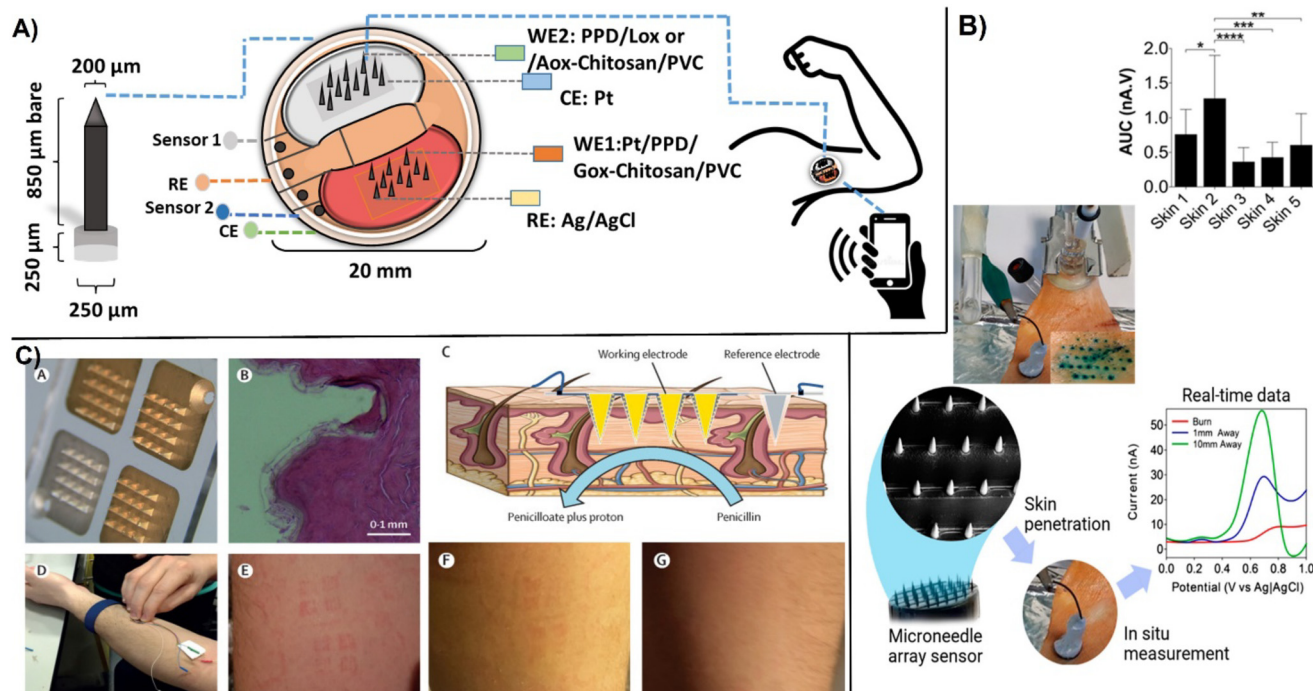
printed carbon-based nanostructured patches that function as an integrated biofuel cell for self-sustained power have been developed and can be found in literature, such as a stretchable lactate/O<sub>2</sub> biofuel cell using buckypaper composed of CNTs as electrode material (Fig. 11D).<sup>127</sup> However, it must be noted that development of most of these devices is restricted to “proof-of-concept” measurements with little or no market or clinical validation. Reasons for the lack of commercial success are the difficulties in continuous access of bio-fluids, variations in flow rates and measurements parameters, lack of biofluid replenishment rate at the sensor surface, and possible biofouling or contamination. Fig. 11 provides an example of various wearable tattoos for monitoring of different biomarkers in sweat. The next section discusses the design of CBMs and their implementation in implantable microelectrodes and for personalized healthcare.

### 3.4. Carbon-based wearable microneedles and implantable microelectrodes

Wearable micro-electrochemical sensors are well suited for non-invasive monitoring and personalized healthcare.<sup>128</sup> A recent development in this field is to use micro-sized array

electrodes and microneedles that penetrate skin and can measure biomarkers in interstitial fluid. Compared to analysis of biomarkers on the surface of skin, measurements in the interstitial fluid using microneedles provides continuous monitoring and higher accuracy measurements, *e.g.*, as compared to analysis of surface biofluids like sweat. The interstitial fluid in the subcutaneous tissue can be easily accessed and measured through painless insertion of microneedles. The interstitial fluid contains biologically relevant biomarkers found in blood, providing an alternative measure to direct blood analysis. Another benefit is the reduced biofouling, although mechanical friction and potential damage of the sensing layer can occur during skin penetration.<sup>128b</sup>

The small size characteristics of the microneedles functioning as epidermal patches allows them to be applied on different locations of the body and be multiplexed for measuring multiple analytes.<sup>129</sup> However, the microneedle size and shape relate to performance and conform to wearer. Direct correlation was reported between the tip length and the number of microelectrodes inserted and pain/discomfort to patient.<sup>128a</sup> Typical size dimensions of microelectrode arrays are tip radius of 5–80  $\mu\text{m}$  with a conical, base diameter between 100–200  $\mu\text{m}$  with a cylindrical or pyramidal



**Fig. 12** Examples of microneedle microarray biosensors: A) images of microneedle tip used for *in vivo* monitoring of glucose, lactate, and ethanol in the interstitial fluid. The sensor patch was placed to the arm of the wearer (reproduced from Springer ref. 129 copyright [2022]); B) *ex vivo* electrochemical measurements in the skin of excised porcine showing penetration of the microneedles visualized by methylene blue staining by DPV measurements. Linear time-dependent plot at 0.7 V and variation of the oxidative peak across five skin samples with statistical measurements one-way ANOVA with Tukey's multiple comparisons test were made for  $n = 5$  animals (with permission from ACS, copyright [2019]<sup>133</sup>); C) microneedle array created by metalized electrodes consisting of three independent Au-coated working electrodes (150 nm) and Ag/AgCl (150 nm) functioning as the reference electrode (A) showing base of microneedles used, (B), showing cross-section of human skin after application (C), for penicillin measurement, (D) and application to the forearm under pressure, (E) time lapsed of left marks after wearing microneedles and, (F) after 1 h removal and, (G) 12 h after removal (with permission from Elsevier, copyright [2019]<sup>132</sup>).

geometry to facilitate the insertion, and tip lengths  $\sim 600\ \mu\text{m}$ . An integrated wirelessly operated microneedle array enabled continuous real-time monitoring of glucose, lactate, and alcohol with LODs of 0.32, 0.15 and 0.5 mM respectively, through the epidermic-inserted microneedle tip.<sup>129</sup> The structure of the microarray electrode for the real-time monitoring of lactate and ethanol with the sensor patch placed on the arm of the wearer are shown in (Fig. 12A). This low-cost sensor was constructed from nine distinct sub-components that included a disposal sensing unit with oxidase enzymes immobilized within poly-*o*-phenylenediamine (POPDA) and a reusable electronic unit.

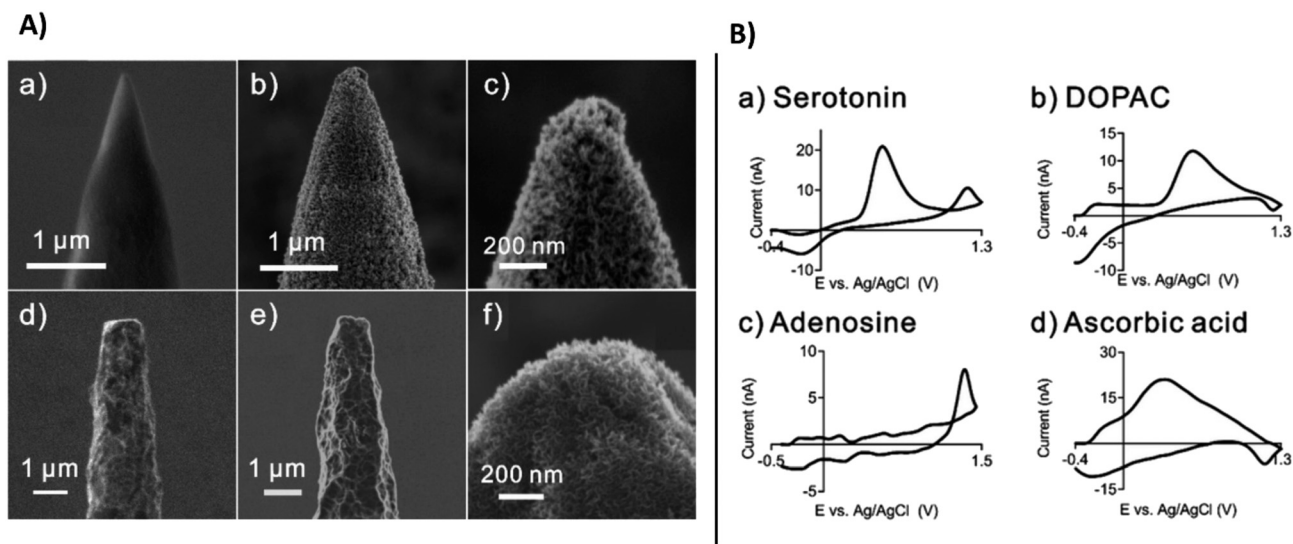
Enzyme-based electrochemical sensors require efficient immobilization and connectivity between the enzyme and electrodes. Many electrochemical enzyme biosensors are second generation devices that involve the use of electron mediators to facilitate the contact between the active sites and the electrode surface. In wearable devices, mediators are ideally immobilized on the electrode surface along with the enzyme. In the reported work, POPDA had a unique ability to work as a redox mediator and enzyme immobilization material, catalyzing the oxygen reduction without the use of additional mediators.<sup>130</sup> Designed sensor demonstrated on-body performance for monitoring the three analytes with accuracies falling within 20–30% of reference methods. In microarray sensors, sensing elements typically consist of polymeric layers such as PPD, biopolymers like agarose, or poly-lactic acid that contain bioreceptor molecules deposited onto conductive micro-tip arrays. Carbon nanostructures have been used as conductive materials for dermal biosensing. Poly-lactic acid loaded with 6% wt. MWCNTs on conical microneedles with a tip diameter of  $40\ \mu\text{m}$ , a base diameter of  $250\ \mu\text{m}$ , and an accessible height of  $870\ \mu\text{m}$  was found as a suitable material for the construction of a microneedle array produced by micro modeling (Fig. 12B). The biosensor enabled real-time monitoring of ascorbic acid with an LOD of  $180\ \mu\text{M}$  by using DPV and its functionality was effectively demonstrated in a burn wound model.<sup>131</sup> The first evaluation of microneedle biosensors in human volunteers was reported in 2019.<sup>132</sup> Two microneedle  $\beta$ -lactam biosensors (one control) were applied to the participant's forearms to measure phenoxymethylpenicillin to obtain real-time individualized antibiotic monitoring. The microelectrode array consisted of four independent electrodes system. After metallising the electrodes with an adhesion coating of chromium. Three of them are coated with Au to work as a working electrodes while fourth electrode is metallized with Ag and chloridised to Ag/AgCl to work as a reference electrode (Fig. 12C). The working electrodes were modified by electrodeposition with iridium oxide (IrOx) to measure pH changes with a hydrogel layer containing  $\beta$ -lactamase from *Enterobacter cloacae*. Prior to application, the electrodes were sterilized with cobalt-60 gamma radiation. The volunteers wore the microneedle biosensor for up to 6 h while being dosed with phenoxymethylpenicillin. The results of in-human trial

demonstrated that the microneedle biosensors can continuously monitor antibiotic concentrations at comparative levels with conventional microdialysis standard monitoring technology.<sup>132</sup> More details on the current development status and challenges in developing microneedle-based electrochemical sensors can be found in several recent reviews.<sup>128,133</sup>

Along with microneedle technologies, a large variety of carbon-based microelectrodes have been developed for monitoring neurotransmitters. The wide majority are carbon fiber microelectrodes used to detect neurotransmitters to understand their role and evaluate treatment of diseases such as cancer, Parkinson's, and Alzheimer's,<sup>134</sup> which destroy the tissue of the brain, impairing movement and memory.<sup>135</sup> Modification of microelectrodes with carbon nanomaterials has been shown to improve the sensitivity of these microelectrodes, enhance electron transfer rate, and increase conductivity.<sup>136</sup> The performance of these electrodes can be enhanced by carefully selecting the required customized shape and size of carbon nanostructures, *e.g.*, nanotips, cavity nanopipettes, *etc.*<sup>137</sup> Nano-sized electrodes can be prepared by nanoprinting method *via* flame etching, designed electrodes can be inserted in the synapses for studying cell exocytosis.<sup>138</sup> Growing carbon nanospikes on tungsten and niobium metal wires enabled mass production of nano-tip electrodes for sensitive detection of dopamine, serotonin, ascorbic acid, and 3,4-dihydroxyphenylacetic acid (DOPAC) (Fig. 13A) shows the SEM images of tungsten and niobium wires with carbon nanospikes (CNSs) modification. Selected CNSs exhibited dense and defect rich surface behaviour and can be easily functionalized. Layers of CNSs were grown on metal wired by using plasma enhanced chemical vapor deposition. The etched tungsten wire displayed tapered conical tips while niobium wires were thin and long. Both showed excellent sensitivity for real time detection of neurotransmitters. Fast scan cyclic voltammetric (FSCV) measurements were demonstrated for ascorbic acid, DOPAC, serotonin and adenosine (Fig. 13B). This method has potential for mass production of CNS based microelectrodes for neurotransmitter monitoring.

Huang *et al.*, produced a flexible enzyme-based electrode with MWCNTs with immobilized glucose oxidase for blood glucose monitoring with a sensitivity of  $25\ \text{nA mM}^{-1}$  for diabetes management. Designed electrodes showed improved electron transport efficiency, excellent stability though entrapment, and no enzyme leaching.<sup>134</sup> Using two-photon lithography followed by pyrolysis, 3D-printed carbon spheres and cones with electroactive surfaces enabled detection of dopamine at levels as low as  $11 \pm 1\ \text{nM}$  and  $10 \pm 2\ \text{nM}$ , respectively. This pretreatment procedure allows for customizable geometry of the electrode while maintaining high resolution, electroactive surfaces due to pyrolyzed carbon, allowance of a free-standing structure without the need for a large base, and great reproducibility.<sup>140</sup> Xiao *et al.*, developed a  $7\ \text{mm}$  by  $25\ \mu\text{m}$  Pt nanoparticle with a reduced graphene oxide-functionalized microelectrode array for





**Fig. 13** A), SEM images of tungsten and niobium wires before and after modification with CNSs: (a) the etched tungsten wire, (b) CNSs grown on the tungsten wire, (c) enlarged image of the CNSs on the tungsten wire, (d) the etched niobium wire, (e) the etched niobium wire, and (f) a more enlarged image of the CNSs on the niobium wire. B), fast scan cyclic voltammetric FSCV measurements of neurochemicals using CNS nanoelectrodes, (a) serotonin, (b) DOPAC, (c) adenosine and (d) ascorbic acid (with permission from RSC, copyright [2022]<sup>139</sup>).

dopamine monitoring during deep brain stimulation in Parkinson disease rat models with a detection limit of 50 nM and sensitivity of  $8.251 \text{ pA } \mu\text{M}^{-1}$ . Such biosensing tools have the potential to provide a better understanding of the mechanism with increased therapeutic efficacy.<sup>141</sup>

Other types of microbiosensors have been designed for detection of cytokines which are small soluble proteins involved with inflammatory response and cell proliferation. These devices can be used for diagnosis and monitoring of cancers and other inflammatory diseases. Qi *et al.*, designed an electrochemical sensor using graphene oxide nanosheets covalently bonded to a gold surface and 4-aminophenyl phosphoryl choline as an antifouling agent for detection of cytokine interleukin-6 (IL-6). The immunosensor enabled *in vivo* monitoring of IL-6 with an LOD of  $1 \text{ pg mL}^{-1}$  in both RAW cells and live mice.<sup>45b</sup> Shen *et al.*, developed an electrochemical sensor using a glassy carbon rod for measuring multiple cytokines (IL-1 $\beta$ , IL-6, and TNF- $\alpha$ ) within Parkinson disease mice models. An LOD of  $5 \text{ pg mL}^{-1}$  for each protein was reported. This sensor allowed for obtaining quantifiable data on inflammatory cytokines comparable to ELISA, showing better sensitivity and deploy ability, opening possibilities for the development of brain chips for early detection of different biomarkers.<sup>135</sup>

CFMEs have been used for measuring rapid changes in neurotransmitters because of their high sensitivity, small size, and excellent electrochemical behavior. The most popular application of CFMEs is for direct detection of neurotransmitters, and associated processes within the brain. Carbon fiber has several advantages such as small size and compatibility with biological compounds. Carbon fibers have less than 10  $\mu\text{m}$  in diameter are extremely sensitive for measurements in implantable conditions, also causing less

tissue damage as compared to other conventional electrodes.<sup>142</sup> Detection of the dopamine neurotransmitter *in vivo* has also been achieved with a CFMEs bundle functionalized with tyrosinase and catalytically active oxidase mimetic nanoparticles, enabling the detection of dopamine at levels as low as 1 nM with a linear range of 0.01–220  $\mu\text{M}$ , sensitivity of  $14.2 \text{ nA } \mu\text{M}^{-1}$ , and a response time of less than 8 seconds.<sup>143</sup> The procedure has been extended to monitoring lactate and glutamate for understanding their behavior in ischemia/reperfusion studies.<sup>144</sup> Other microbiosensors were functionalized with aptamers for detection through bioaffinity recognition. Hou *et al.*, developed an alkyl chain-functionalized CFMEs with non-covalently immobilized aptamer cholesterol amphiphiles for detection of neurotransmitters *in vivo*.<sup>145</sup> In the same way Seven *et al.*, developed a nanoporous CFE through heat treatment for the electrochemical detection of  $\text{H}_2\text{O}_2$  and dopamine in tissue analysis with the potential for use as probes for *in vivo* studies due to catalytic activity and low LODs of 0.57  $\mu\text{M}$  and 35.6 nM, respectively.<sup>146</sup> Chang *et al.*, modified CFMEs with electrodeposited graphene oxide for detection of dopamine (LOD = 11 nM, sensitivity =  $41 \pm 2 \text{ nA } \mu\text{M}^{-1}$ ) and tested the functionality of the electrode in electrostimulated brain slices of mice as a demonstration of use in tissue. This was a first demonstration of electrodeposited graphene oxide onto the CFMEs to enhance the sensitivity for *in vivo* measurements.<sup>147</sup> The achieved high carbon-to-oxygen ratio favors conductivity and electron transfer rate due to more adsorption sites with enhanced oxygen functionalities<sup>147</sup> to produce a concentration-dependent current change.<sup>146</sup> The simultaneous detection of serotonin and dopamine *in vivo* via DPV was performed using a CFMEs array functionalized with diazonium salts and SWCNTs,





which improved selectivity for pharmacological and physiological applications.<sup>148</sup>

### 3.5. Non-invasive lab-in-a-mouth biosensors for salivary biomarkers

Several examples of POC-based electrochemical biosensors were developed for the non-invasive monitoring of biomarkers in saliva. The concept called lab-in-a mouth, or cavitas sensors is a relatively new development to the field promising real time monitoring of biochemical information from the oral cavity.<sup>149</sup> An example is a cell-phone connected pacifier-type biosensor connected to wireless electronics was reported for measurements of salivary biomarkers.<sup>150</sup> The biosensor consisted of a screen printed electrode modified with chitosan and glucose oxidase. The electrode was connected to the pacifier using a 3D printed customized cell. In addition to glucose bioelectronics pacifiers have also been developed to real time monitoring of salivary electrolytes.<sup>151</sup> This device consisted of ion-selective sensors and flexible microfluidic channels. Other devices have been developed in form of a mouthguard biosensor, placed on a tooth with the help of a cellulose acetate membrane and used for the *in vivo* measurement of glucose in saliva.<sup>152</sup> The biosensor measured glucose concentrations within the range of 1.75–10 000  $\mu\text{M}$ , which covers the typical levels in saliva, between 20–2002  $\mu\text{M}$ . Using a similar concept but with the biosensor attached to a toothbrush, Liu *et al.* detected glucose in the concentration range from 0.18 mM to 5.22 mM within 5 min.<sup>153</sup> The biosensor was fabricated using a carbon graphite ink with glucose oxidase (immobilized using 2% glutaraldehyde) as working electrode and a Ag/AgCl ink as reference electrode painted on a toothbrush. More details on micro/nanodevices for biomarkers detection in saliva and applications in stomatology can be found in recent reviews.<sup>149,154</sup>

## 4. Large scale manufacturing of low-cost carbon-based diagnostic devices

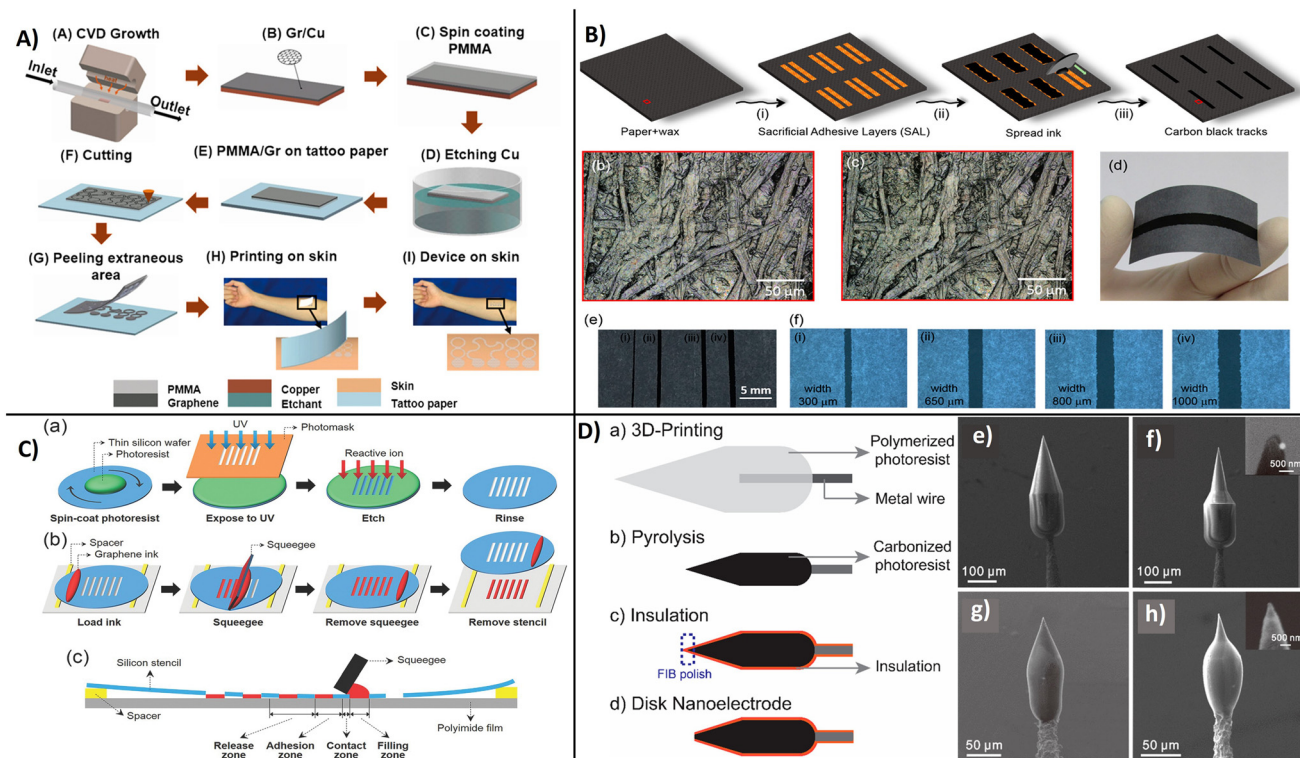
One important requirement for the implementation of carbon-based wearables on a commercial scale is the ability to manufacture high quantity devices at a low cost and with high reproducibility for consumer use. The success of manufacturing relies on the selection of suitable materials and substrates as well as the method of device fabrication. As discussed earlier, carbon nanostructures have great potential to be used as a platform for bioelectronic devices design. Wearable POC device can be fabricated on flexible and inexpensive substrates like plastic,<sup>155</sup> textiles,<sup>156</sup> paper tattoos,<sup>157</sup> and elastomers,<sup>157,158</sup> which have the capability to directly contact human skin. Key design requirements and benefits offered by carbon nanostructures are the material compatibility, mechanical and structural stability, scalability for large scale application, as well as the ability to be functionalized with receptor molecules for biomarker

detection. To ensure functionality and accuracy of analysis, the manufacturing protocol should be made without affecting the intrinsic properties or the recognition and detection functions of the active interface. Carbon-based nanostructures, particular CNTs and graphene, can be deposited on flexible platforms such as fabric, paper, or skin-conformable tattoos. They can be used in conjunction with other materials such as fibers of bacterial nanocellulose,<sup>96</sup> ZnS,<sup>87</sup> or AuNPs<sup>88</sup> and be interfaced with biomolecules to create multifunctional hybrid films. These multicomponent structures are relatively complex and their fabrication on an industrial scale is still challenging. Methods such as printing, dipping, and drying, chemical vapor deposition (CVD), and photolithography have been explored to manufacture low-cost biosensors. Several examples are provided in this section.

A CVD-based fabrication procedure used to create transparent, stretchable, and wearable graphene e-tattoo sensors is shown in Fig. 14A. The procedure involves a series of steps starting with the growth of graphene on copper foil by atomic pressure chemical vapor deposition (CVD) followed by dry patterning and coating of poly-methyl methacrylate (PMMA) polymer precursor and baking it to obtain a film of  $\sim 460$  nm on the graphene area.<sup>159b</sup> The copper layer was further etched away to create a graphene film on PMMA that constitutes the actual substrate of the e-tattoos. The sensors have been fabricated as an open mesh structure to facilitate long-term conformability with skin for applications as an e-tattoo for the noninvasive monitoring of skin temperature, hydration, electrocardiogram (ECG), electromyogram (EMG), and electroencephalogram (EEG). Similar designs have been reported by combining carbon-based nanomaterials with Ag nanoparticles to effectively increase conductivity and enable measurements of ECG and EMG.<sup>160</sup> In this case, polyisobutylene-*b*-poly(oxyethylene)-*b*-polyisobutylene triblock copolymer was used as a dispersant for AgNPs and different shapes of carbon nanomaterials: carbon black, CNTs, and graphene. The sensor displayed high stability even after 5000 repetitions of 50% tension–tension fatigue testing and low resistance of  $4.1 \times 10 \Omega \text{ sq}^{-1}$ . Kirev *et al.*, reported a multistep fabrication protocol for manufacturing GETs<sup>161</sup> as a solution for ‘high end low-cost’ wearables. The method involves transfer of graphene onto tattoo paper by CVD growth and formation of a multilayer graphene stack followed by contact and transfer of the GET from paper to skin using a soft adhesive conductive tape. The sensor placed on skin was tested for monitoring skin hydration, temperature, EEGs, EMGs, and ECGs. Shirhatti *et al.*, fabricated a flexible wearable sensor using a laser-etch process to pattern gold interdigitated electrodes drop-cased with an ultrathin layer of graphene nanosheets and demonstrated its use for monitoring heart rate, hydration, temperature, and breathing rate when the sensor was placed on skin.<sup>162</sup>

A straightforward fabrication procedure is to use printing, *e.g.*, inkjet or screen printing *via* deposition of inks on substrates. The benefit of using printing is the low-cost mass





**Fig. 14** Examples of manufacturing procedures for carbon-based electrodes illustrating: A) CVD-based fabrication procedure of on-skin graphene e-tattoos (GET) showing CVD disposition of graphene on copper foil (A and B), followed by PMMA coating (C), copper etching (D) and cutting (E), and transfer of the graphene/PMMA on tattoo paper substrate (F and G), peeling (H) and placement on the skin (I) (with permission from ACS, copyright [2017]<sup>159a</sup>). B) Screen printing fabrication of a fully printed carbon black-based wearable sensor by: addition of sacrificial adhesive layers (i), deposition and spread of the carbon black ink (ii), and the cure of the layers at 60 °C (iii), followed by the removal of the adhesive layers; with scanning microscope images (b) before and (c) after the removal of the sacrificial adhesive layer and photographs of the flexible paper-based device (d–f) with different widths (with permission from ACS, copyright [2017]<sup>165</sup>). C) Fabrication of high resolution thin silicon stencil using a conventional photolithography technique showing the screen printing process with deposition of graphene ink (a and b) and cross-sectional picture of the printed electrode (c) (with permission from Wiley, copyright [2014]<sup>164</sup>), and D) fabrication of 3D-printed carbon nanoelectrodes (a–d). With SEM images of printed structures, before pyrolysis of 3D-printed electrodes with normal conical geometry (e) and sharper conical geometry (f), and after pyrolysis: carbonized electrodes (g and h) (with permission from ACS, copyright [2020]<sup>170</sup>).

production capability as well as the high resolution and versatility of the process. Sensors can be printed at ambient temperature which is compatible with biomolecules.<sup>163</sup> Printing requires the development of inks of characteristic formulation and viscosities. Carbon nanomaterials such as graphene and CNTs are well suited for creating conductive inks due to their mechanical and electrical properties. In ink-jet printing, a single droplet of conductive ink can be printed very precisely on a suitable substrate with low material consumption, enabling low-cost high-volume printing of wearable sensors.<sup>164</sup> Santhiago *et al.*, reported a scalable fully printed paper-based wearable sensor using a conductive ink based on carbon black and Prussian blue as an electron mediator (Fig. 14B). The printed pattern had a resolution of 500  $\mu\text{m}$  in width and high folding stability of over 20 000 cycles.<sup>165</sup> Hyun *et al.*, achieved increased resolution patterning of graphene with shapes as narrow as 40  $\mu\text{m}$  by screen printing using a silicon stencil and viscosity-controlled inks. This strategy, summarized in Fig. 14C, enables manufacturing of flexible electrodes for printed electronics.<sup>164</sup> Most reported sensors are used to monitor

physiochemical signals including breathing, hearth rate, blood pressure, *etc.* Examples of physiochemical signals of pulse and breathing rate monitoring by a wearable strain sensor were reported with functionalized carbon nanostructures,<sup>166</sup> CNTs,<sup>167</sup> and graphene.<sup>168</sup> It's evident that carbon-based wearable electrodes demonstrate viability for biomonitoring (EEG, EMG, ECG, *etc.*)<sup>169</sup> but the selection of suitable materials, flexible substrates, and their effective structural design is highly important for high-performance diagnostics for healthcare applications. A new approach for producing microelectrode sensors is to use 3D printing by direct laser writing which enables fabrication of customized implantable electrodes.<sup>170</sup> The group of J. Venton has reported a method to 3D printing free-standing carbon nanoelectrodes for *in vivo* monitoring of neurotransmitters.<sup>170</sup> The fabrication process, illustrated in Fig. 14D, involves deposition of IP-S photoresist on a metal wire immobilized on a silicon chip, pyrolysis of the polymer structure producing a glassy carbon-like structure followed by insulation with a layer of  $\text{Al}_2\text{O}_3$  (100 nm) by atomic layer deposition (ALD) and milling of the end of the tip by focused



ion beam (FIB), resulting in a disk shape carbon electrodes. The diameter of the pyrolyzed tip was  $\sim 260$  nm, while the disk electrode was  $\sim 600$  nm with geometries shown in the SEM images in Fig. 14D. The nanoelectrode was able to determine dopamine within a linear range of 10–50  $\mu\text{M}$  and functionality was demonstrated with the electrode implanted in adult fruit fly.

## 5. Biosensing connectivity for remote monitoring and decentralized healthcare

With a few exceptions, the clinical diagnostic system is largely centralized and provides limited options for personalized testing and therapy monitoring. Modern healthcare indicates a clear trend towards decentralized healthcare by promoting mobile, individualized, and predictive medicine. The concept of mobile health – mHealth or eHealth, using portable biomedical devices, represents a paradigm shift from the current practice. In mHealth, a portable sensing device is connected to a smart wireless communication system such as a cell phone. The integrated sensor should be able to monitor and continuously provide health-related signals and track physiological parameters related to motion, physical activity, or biochemical markers of disease. While several existing technologies and on-body wearables measure signals such as heart rate, temperature, or hydration levels, devices that can detect biological signals and provide continuous monitoring of disease biomarkers for health monitoring are still limited, despite the massive wearable technology market. Some of the existing challenges are related to the rigidity/bulkiness of electronic transducers, while others are due to the stability of bioreceptors, or the functionality of the measuring system. Biological molecules are affected by the environment, pH, temperature, and storage conditions, and biosensors can lose their functionality over time. Other limitations are related to calibration accuracy and reliability of measurements, e.g., possible drifts in calibration, particularly

when these are placed in real-world conditions and are intended for long-term use.

Integration of portable biosensing systems with the cloud-based processing and the internet of things (IoT) is important for advancing the concept of smart and connected health.<sup>171</sup> The IoT enables device communication and use of data to coordinate decisions by sharing information.<sup>172</sup> A challenge in mobile crowdsensing for ensuring reliable IoT in healthcare is ensuring tamper resistance of the biosensed data to ensure data integrity, privacy, and trustworthiness.<sup>173</sup> Smartphones and tablets can be interfaced with low-cost portable sensing devices for monitoring biomarkers in biological fluids. Communication through mobile apps empowers patients to monitor medical conditions and treatment, and the medical professionals to manage and make decisions about the patient's health from any location. However, the ability of the system to be used as a diagnostics tool for eHealth relies on the successful integration of the sensing and data processing components to enable automated data collection, transmission, processing, and visualization for health management purposes. The main components of a typical remote patient monitoring system are illustrated in Fig. 15 which include: 1) the sensing system with data acquisition and transmission capabilities, 2) data processing and cloud computing unit with stored calibrations and health-related correlations, and 3) data visualization and diagnostics. Another requirement for the successful implementation of mobile eHealth systems also requires the use of low power sources and communication protocols, which accounts for a significant power consumption in sensing devices.<sup>174</sup> Development and effective implementation of such systems involves strong collaboration between biosensing experts with data/computer scientists and system engineers. In the future, biosensors for eHealth could be used as diagnostic support tools, adding to the already available clinical decision infrastructure in healthcare to improve diagnostic and patient outcomes.<sup>175</sup>

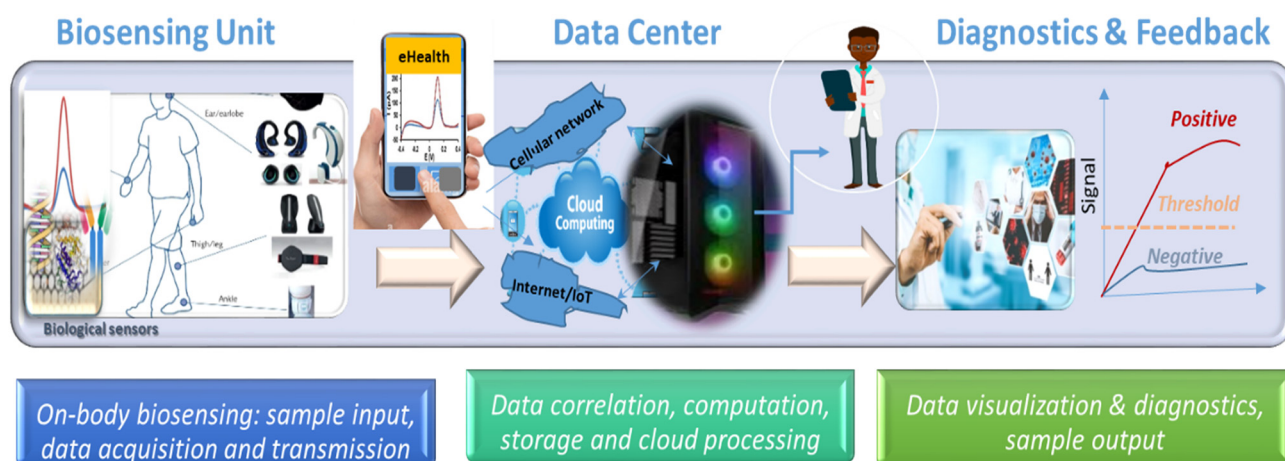


Fig. 15 Components of an electronic remote patient monitoring system (eHealth): biosensing unit, data center and diagnostic and outcome.





## 6. Considerations for commercialization and translation to practice

To be widely adopted, biosensors must meet several criteria: i) meet quality standards in accordance with FDA criteria, ii) be inexpensive, accessible, and scalable, iii) easy to use to allow medical professionals to quickly interpret the results and provide timely intervention, iv) provide accurate and reliable data. In addition, v) such devices should have no or limited sample preparation and (vi) require no reagents, or reagents should be integrated within the sensing unit. To address this requirement, efforts have been made to couple microfluidic platforms, biochips or fluidic disks containing cartridges with stored reagents and rotating channels to precisely mix reagents and perform chemical reactions in confined volumes, automating sample preparation, and minimizing reagents cost.<sup>176</sup> The large scale production of single use disposable POC devices still requires improvement in the material design, particularly with respect to biocompatibility. Given the potential large scale use of these devices, development on novel biocompatible and sustainable materials is still needed to prevent the generation of harmful waste.<sup>177</sup> Lastly, transition of mobile biosensors into real-world applications is subject to FDA approval<sup>178</sup> and policy considerations regarding the design, performance, data security, and confidentiality.<sup>179</sup> The World Health Organization (WHO) has a set of recommended criteria for developing low-cost diagnostics to be “affordable, sensitive, specific, user-friendly, rapid, robust, and equipment free” (ASSURE), and most such emerging technologies follow these criteria.

The translation of these biosensors into a commercial product requires, in addition to technology development, several market and business considerations, specifically: 1) market need identified based on whether the technology offers a solution to an existing need or an improvement over existing products, 2) market size and share determined by the number of potential users, and the competitive landscape, 3) readiness for commercialization determined by a technology readiness level (TRL) done at several critical points during the technology development and testing. POC devices also require manufacturing, storage, and packaging. It should be noted that the development of most POC devices is realized in academic labs, while transfer of the technology into industry requires business, intellectual property protection, collaboration with industry and entrepreneurial endeavors. The framework for market entry readiness involves all aspects of technology development as well as business including: technology, market, regulatory, commercial and management readiness. One of the biggest challenges of translating POCs from labs to industry is the gap between academic research and industry, with most innovations in academic labs being at the proof of concept and TRL levels. Most commercial POC biosensors today are

lateral flow test strips with nucleic acids and antibodies-based detection. The most successful electrochemical biosensor remains the glucometer, whose success has been driven by the large and growing diabetes market need and size.<sup>180</sup> While advances in wearable, subcutaneous and microanalysis-based POC designs have been made and some are beginning to be commercialized, such systems still require improvements in accuracy and reliability for long term monitoring. The status of the commercial POC platforms for diabetes monitoring has been discussed and can be consulted in a recent review.<sup>180</sup>

## 7. Conclusions and future outlook

This review has provided an overview of the status of carbon-based electrochemical biosensors as diagnostic platforms and POC devices for healthcare. Carbonaceous-based materials have been widely used to design cost-effective devices and their integration with wireless communication devices has provided exciting opportunities for biomarker detection for decentralized healthcare.<sup>181</sup> From the material perspective, carbon materials and nanostructures like graphene are some of the most suitable for high performance POC and wearable biosensors due to their flexibility and sub-nanometer thickness, conforming to the curvature of skin.<sup>161</sup> Significant progress has been made in manufacturing electrochemical biosensors based on active and functional carbon nanostructures. Functionalizing carbon nanomaterials and creating hybrid structures with biomolecules has improved sensitivity and selectivity while lowering the detection limits and providing a good foundation for measuring relevant biomarkers levels, advancing their use for biomedical applications. Their integration in portable low-cost and wearable substrates has also been demonstrated with additive manufacturing methods such as printing, enabling large-scale production with increased reproducibility. This review summarized the various designs and performances reported over the last 3–5 years focusing on paper-based devices, electronic tattoos, implantable and wearable microelectrodes, and interstitial microneedles. The continuous innovations in materials design and additive manufacturing have enabled the development of a wide variety of carbon-based devices that are flexible and adaptable for biomarker detection in human biofluids (e.g., sweat, tears).<sup>44b,182</sup> Moreover, the growing electronic health network and computing capabilities have advanced the development of apps-based monitoring and connectivity for eHealth.

Existing commercially available eHealth-connected devices are physical sensors that measure physical health indicators such as heart rate and blood oxygen. Despite significant progress, few electrochemical biological sensors have been implemented into clinical practice (with the exception of the widely used glucometers); many still require development to enhance their stability, selectivity and detection performance in realistic environments to meet the needs of accurate and



effective healthcare in modern society. Their capabilities to measure health indicators, diagnose diseases, and provide real-time feedback to optimize therapy makes them particularly suited for personalized healthcare. However, future commercial implementation requires strong partnerships with industry and advancement of the technology to more mature readiness levels, beyond proof-of-concept (TRL2–3) to prototype testing and validation (TRL5–6), and ultimately their proven operation in the clinical field (TRL9). The recent successful implementation of POC for rapid diagnostics of COVID-19 (ref. 183) demonstrate that such technologies are needed and can be successfully deployed when there is a strong societal, market and technology need.

With the emergence of digital health and communication networks and the focus of healthcare towards decentralization, we envision that a large range of low-cost diagnostic devices will be needed to provide real-time monitoring for health management in the future. However, their widespread adoption still requires further development and testing for improving accuracy and performance when used on patients. The future developments and challenges concerning biosensors for digital health will include:

(1) Further optimization to increase stability and functionality from hours to several months or years with on-body sensors tested in realistic monitoring conditions.

(2) Strategies to address biofouling issues by developing novel electrode materials and coatings.

(3) Selective multi-analyte detection protocols and strategies to remove interferences to enable reliable and selective measurements in complex biofluids.

(4) Development of calibration protocols for inclusion in electronic software. As with other biosensing technology, the calibration is essential in order to ensure accuracy of analysis and mitigate calibration challenges due to changes in mass transfer conditions, calibration drifts with sequential measurements, and possible alterations of the electrode surface that are common with wearable and implantable biosensors. Performing pre-post analysis calibration, validation strategies with established methods and designing protocols that take into account potential drifts are recommended to ensure accuracy of analysis. Other approaches could be to use self-reference sensors to compensate for fouling or fluctuations that could affect the accuracy of the signals. The adoption of chemometrics and machine learning could provide the necessary data analytics to improve accuracy and reliability of measurements, especially for multiplexed detection.

(5) Manufacturability, biocompatibility, stability of use, and cost considerations by incorporating criteria for material selection, large scale fabrication, and adaptability in early prototype development. The ability to adjust the design ‘on-the-fly’ and adapt protocol to address individual needs is essential, particularly for personalized bioelectronics<sup>133</sup> such as wearables, microneedle-based interstitial sensors, and e-tattoos. Biocompatibility, mechanical durability, and

intrinsic conductivity are additional criteria that should be delivered to meet FDA requirements. Biointegration, stretchability, and safe contact with body tissues is highly desirable for implantable and on-body sensors for achieving compliant human-sensing interfaces. Further developments in flexible electronics, the use of additive manufacturing methods, and appropriate tools are envisioned to improve fabrication and commercialization at a large scale.

(6) Integration of sensing systems with existing electronic infrastructure in a single platform to enable connectivity with the IoT/cloud computing networks with the possibility to be accessible from any location remotely. These can facilitate data collection, transmission, and diagnostics by monitoring the evolution of specific biomarkers at relevant levels for disease diagnostics, treatment, or health conditions. Real-time capabilities could be connected with drug delivery systems to provide personalized feedback on therapy, such as achieving a closed-loop glucose-insulin for diabetes management.

(7) Large scale clinical trials with independent multi-laboratory validations and comparability using conventional clinical analysis laboratories is needed to establish performance in relevant conditions and accelerate adoption.

In conclusion, carbon-based materials are important to the advancement of bioelectronic devices for decentralized health. This review showed that carbon-based electrochemical biosensors provide great selectivity and sensitivity for biomarker detection while providing advantages such as ease-of-use, low cost, and adaptability to be used as on-body wearables compared to other methods such as spectroscopy or chromatography. Further development needs include improving robustness, accuracy, long term operability, manufacturability, and stability to provide real-time specific and effective data for disease monitoring. Due to the wide range and interdisciplinary, further developments require convergent research and collaboration across chemistry, biology, data analytics, engineering, biomedical, and clinical fields. Collaboration between researchers, business, entrepreneurs, regulatory agencies and investors is also needed to advance commercialization and transfer these devices from the lab to the consumer for everyday use. These developments could open up a new approach to medical biosensing and eHealth to improve healthcare, enable connectivity, and reduce healthcare costs for managing health conditions in the community.

## Conflicts of interest

There are no conflicts to declare.

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