

## Review

## Flexible Substrate-Based Devices for Point-of-Care Diagnostics

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**Point-of-care (POC) diagnostics play an important role in delivering healthcare, particularly for clinical management and disease surveillance in both developed and developing countries. Currently, the majority of POC diagnostics utilize paper substrates owing to affordability, disposability, and mass production capability. Recently, flexible polymer substrates have been investigated due to their enhanced physicochemical properties, potential to be integrated into wearable devices with wireless communications for personalized health monitoring, and ability to be customized for POC diagnostics. Here, we focus on the latest advances in developing flexible substrate-based diagnostic devices, including paper and polymers, and their clinical applications.**

### Point-of-Care Diagnostics

**Point-of-care (POC**, see [Glossary](#)) diagnostics play an important role in guiding timely patient care in primary care settings, because they are inexpensive, rapid, simple-to-use, and instrument-independent [1–4]. Owing to the advantages over laboratory-based diagnostics, POC diagnostics are used for the prevention and control of infectious diseases and monitoring of health conditions in non-laboratory settings, where well-trained technicians and sophisticated laboratory infrastructure are not available [5,6]. For example, paper-based POC diagnostics such as glucose strips or pregnancy tests can be used by end users at home, without requiring formal training. Continued research in paper-based diagnostics focuses on enhancing sensitivity, minimizing batch-to-batch variation, and increasing multiplexing capability. Challenges include overcoming considerable variation in liquid evaporation and sample retention, which compromise the sensitivity and reproducibility of paper-based diagnostics. In addition, it is difficult to capture quantifiable information, particularly for multiplexed paper-based POC diagnostics [1,7]. Therefore, a great deal of research effort focuses on overcoming these challenges and creating next-generation POC diagnostic tools to meet the need for patient care and personalized healthcare monitoring in non-hospital and home settings.

Flexible polymers have been recently used in POC diagnostics because of their enhanced and adjustable physicochemical properties such as transparency, heat conductivity, or electrochemical resistance [8–10]. Examples of polymers include polydimethylsiloxane (PDMS), poly(methyl methacrylate) (PMMA), polypropylene (PP), polyimide (PI), hydrogel, and polystyrene (PS), which can be used in optical detection platforms such as photonic crystal-based sensing [11], surface plasmon resonance (SPR) [12], or photonic nanosensing [13]. Other polymers, such as polyethylene terephthalate (PET), are generally stable at temperatures up to 180 °C and have high heat conductivity [14], which is ideal for temperature-controlled biological reactions. Polymers such as polycarbonate (PC), PET, cyclic olefin copolymer

### Trends

**Flexible substrates:** owing to enhanced optical, physical, and chemical properties, as well as affordability and amenity to mass production, flexible polymer-based substrates are increasingly being used to develop next-generation POC diagnostic devices with enhanced performances.

**Wearable devices:** as a result of excellent bending tolerance, flexible polymer substrates are highly attractive for developing wearable diagnostic devices with integrated thermal, electrical, and/or optical sensors for real-time monitoring of health conditions in POC settings.

**Cloud-based POC diagnostics:** mobile- or computer-based wireless systems can be easily integrated with on-body devices to enable real-time personalized healthcare monitoring and information exchange in a remote mode, offering a new, viable, and affordable paradigm for better healthcare delivery at the POC.

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(COC), and poly(methyl methacrylate) (PMMA) are electrochemically inert and can be used for developing electrochemical-based diagnostic devices [15]. Unlike paper, flexible polymers do not absorb liquid and enable more precise delivery of materials for detection. Lastly, and most importantly, flexible polymers can be engineered thin and bendable, potentially allowing for the integration of sensors within wearable devices or textiles so as to achieve conformal contact to the curvilinear surfaces of the human body for personalized healthcare monitoring at the POC [16–19].

In this review, we aim to summarize the latest advances in developing flexible substrate-based devices for POC diagnostics. Paper-based diagnostic devices are also discussed in this review from the perspective of latest advances in multiplexing and enhancing sensitivity for POC diagnostics. Polymer-based flexible devices are discussed in detail with regard to their capability to detect/measure targeted biomarkers from biological fluids or to monitor physiological parameters [16,17,20] and their integration into wearable devices. Emerging smartphone and computer-based wireless technologies offer unprecedented ease of data collection, analysis, storage, and transportation, and they have been increasingly tethered with wearable devices for POC applications. An overview of the latest advances in developing flexible substrate-based POC diagnostics is illustrated in Figure 1.

### Flexible Substrate-Based POC Devices for POC Diagnostics

In this section, we first present recent advances in improving the multiplexing capability and sensitivity of paper-based POC diagnostics, and then we summarize the development of flexible polymer-based POC diagnostics for sensing biological targets, and their potential applications to be developed into wearable devices.

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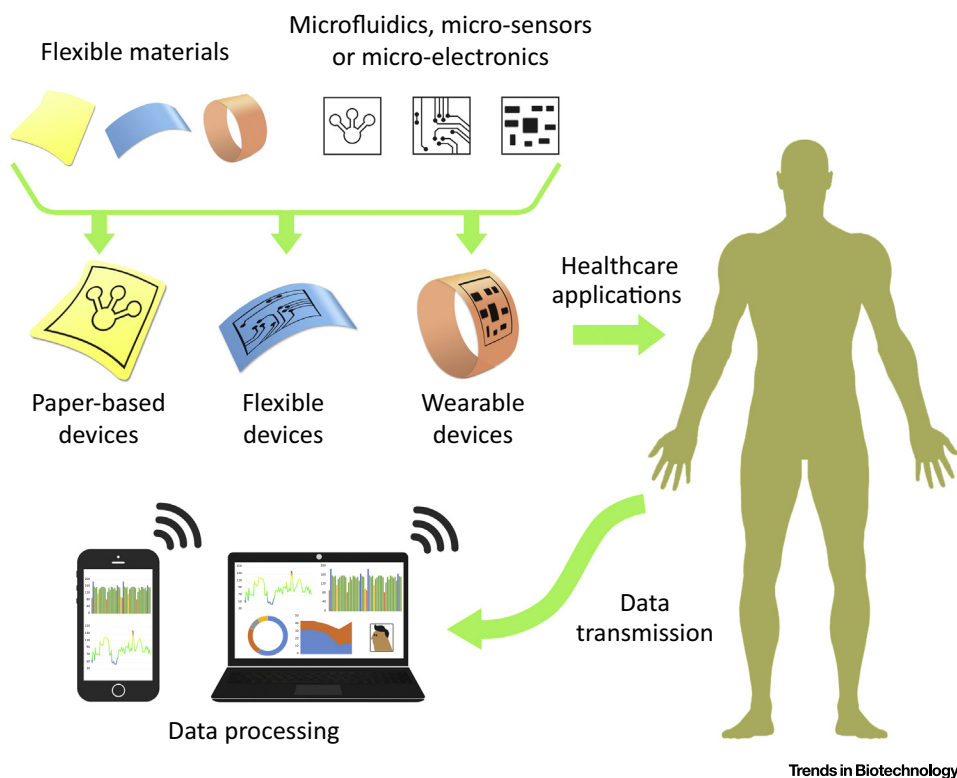


Figure 1. Overview of Flexible Substrate-Based Devices for Point-of-Care Diagnostics.

To improve multiplexing capability, a lateral flow diagnostic microarray was used to enable simultaneous detection of 15 IgE antibodies from human serum [21]. In this array, 15 allergens were well positioned on paper substrates, and their specific IgE antibodies were detected in serum samples from 35 patients with a sensitivity comparable to a commercial test, that is, ImmunoCAP [21]. This strategy overcame the drawback of traditional lateral flow strips for detecting multiple targets due to cross-reaction that causes false positive results. Alternatively, a paper-based vertical flow microarray (VFMA) was developed to improve multiplexing capability [22]. In this report, ten allergens were first microspotted on a porous nitrocellulose membrane, and this special configuration allowed the detection of their allergen-specific IgE antibodies in human serum without signal interference. The VFMA assay had a high concordance with ImmunoCAP with a linearity of 0.89 among 31 patient samples. Recently, fluorescence labeling was used to increase the multiplexing capability as demonstrated in detection of up to 13 types of human papillomavirus from PCR products using a portable fluorescence lateral flow reader [23].

The development of microfluidic paper-based analytical devices ( $\mu$ PADs) also facilitated multiplexed testing using multiple microchannel configurations [24–28]. For instance, a  $\mu$ PAD-based assay was initially developed to detect glucose and bovine serum albumin (BSA) in artificial urine samples with a limit of detection of 5 mM and 0.75  $\mu$ M, respectively [29]. Further, a liver function  $\mu$ PAD was developed to semiquantitate the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) for drug-related hepatotoxicity monitoring in resource-limited settings [26].  $\mu$ PADs are similar to lateral flow assays in that they are inexpensive, equipment-free, and can be used for colorimetric visualization, but also include the capability to multiplex and can be scaled-up with different microfabrication methods such as photolithography [25], inkjet etching [30], plasma etching [31], and wax printing [32]. Although these manufacturing methods seem more complicated than fabrication of lateral low-based assays,  $\mu$ PADs are still regarded as one of the ideal diagnostic platforms for resource-limited settings.

To increase the sensitivity of paper-based POC diagnostics, a variety of nanotechnologies have been employed including **surface-enhanced Raman scattering (SERS)**, surface plasmonic resonance, and others [33–36]. Despite superior sensitivity, SERS has not yet been translated to POC diagnostics potentially due to the complexity in preparing SERS substrates. Recently, an SERS array was created on paper substrates using an ordinary ball pen filled with plasmonic inks consisting of Au nanospheres, Au nanorods, and Ag nanorods [37]. This paper-based SERS array showed high uniformity and long stability. In a real-world application, this sensor detected thiabendazole, a fungicide and parasiticide, at 20 parts per million (ppm). Self-assembled arrays of Au nanoparticles on common chromatographic paper were also developed for SERS [38]. The array of evenly distributed Au nanoparticles was prepared using an oil/water interface and then transferred to paper with a well-designed geometry to create a large number of hot spots at the tip via capillary force. This paper-based SERS array successfully detected 10 fM of malachite green from fishpond water. This POC SERS array is easy-to-prepare, inexpensive (less than 3 cents), and sensitive, showing the potential for environmental and food safety monitoring in the field. In another study, high density of nanowires was patterned on paper substrates for electrical sensing [39].

Flexible polymers, owing to enhanced physicochemical properties, have been increasingly used as substrates for developing POC diagnostics. Table 1 lists some clinical diagnostic applications of flexible substrate-based POC devices. For instance, a sensitive and wearable flexible organic thin-film electrode was reported for analysis of glucose and uric acid in human saliva samples (Figure 2A) [40]. In this study, Pt electrodes and poly(3,4-ethylene dioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) were printed on a PET substrate to form **organic electrochemical transistors (OECTs)**. Pt electrodes were coated with a composite film of graphene, Nafion, and polyaniline

## Glossary

**Point-of-care (POC):** patient care in the emergency room, in primary clinics, at home, or in other non-hospital settings where diagnosis can be made and treatment can be administered.

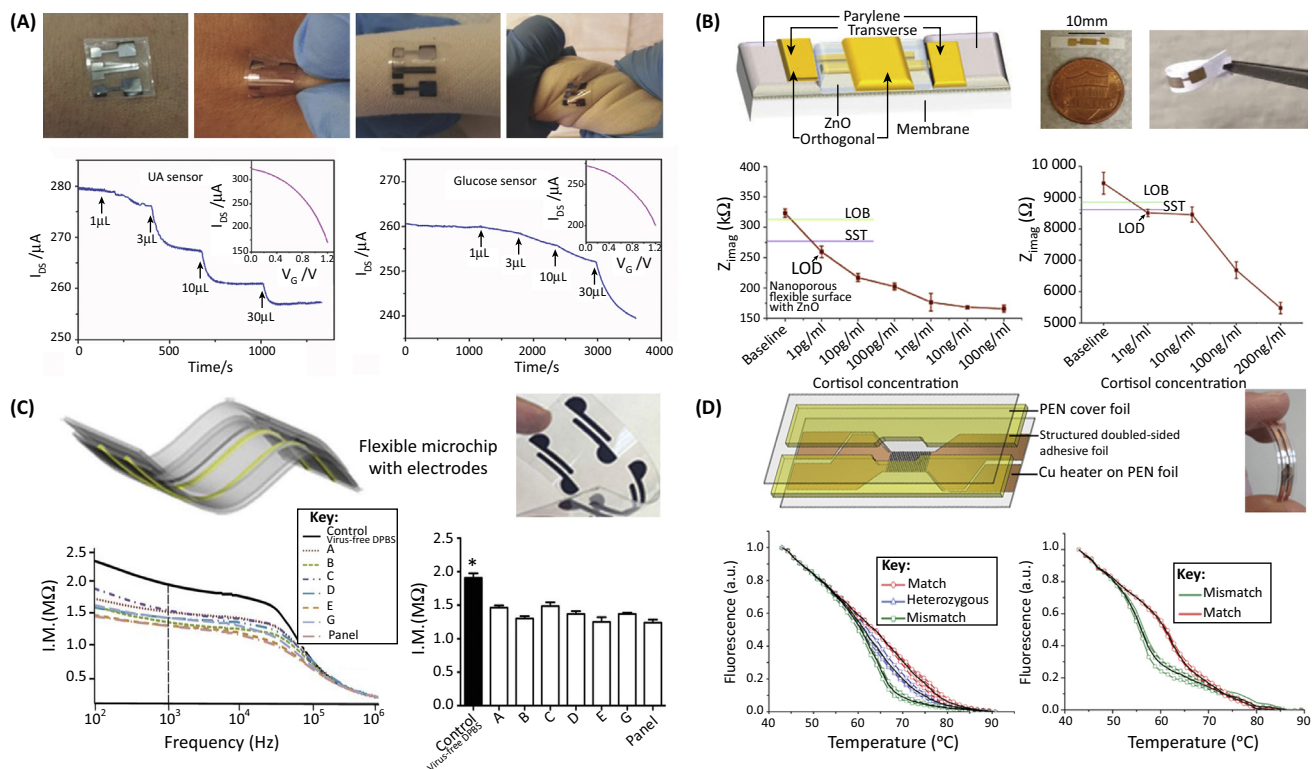
**Surface-enhanced Raman scattering (SERS):** a spectroscopic approach that can detect chemical and biological species down to a single molecule without labeling.

**Organic electrochemical transistors (OECTs):** one type of highly flexible organic thin-film transistors that can be used for potentiometric sensing.

Table 1. Clinical Diagnostic Applications of Flexible Substrate-Based POC Devices for Detection of Targeted Species<sup>a</sup>

Analyte	Dynamic Range	Limit of Detection	Sample Type	Number of Samples	Detection Modality	Substrate	Thickness	Dimension	Refs
Uric acid and glucose	100 nM to 500 $\mu$ M for uric acid; NR for glucose	10 nM and 30 nM for uric acid and glucose, respectively	PBS	NR	Amperometric	Polyethylene terephthalate	50 $\mu$ m	NR	[40]
Glucose	0.05–1.0 mM	30 $\mu$ M	Serum	NR	Amperometric	Flexible graphite	NR	NR	[41]
Cortisol	10–200 ng/ml	1 ng/ml	Sweat	NR	EIS	Polyamide	NR	NR	[42]
Ascorbic acid, dopamine, and uric acid	20–400 $\mu$ M, 0.5–35 $\mu$ M, and 0.5–35 $\mu$ M, respectively	2.0 $\mu$ M, 0.01 $\mu$ M, and 0.02 $\mu$ M, respectively	PBS	Five urine samples	DPV	Flexible graphite	NR	1 $\times$ 3 cm	[44]
HIV-1	NR	10 <sup>6</sup> copies/ml	PBS	NR	Impedance spectroscopy	Polyester film	NR	NR	[45]
<i>Escherichia coli</i>	NR	4.5 $\times$ 10 <sup>7</sup> cfu/ml	Culture media	NR	Impedance spectroscopy	Acetate sheet	NR	NR	[46]
Type 2 diabetes Leu7Pro gene	NR	Single mismatch	PCR DNA	NR	Melting curve analysis	Polyethylene naphthalate	125 $\mu$ m	NR	[47]
Glucose	0.1–0.6 mM	NA	PBS	NR	Amperometric	Polyimide film	~1.5 $\mu$ m	NR	[50]
Lactate	0.1–0.5 mM	NA	Unstimulated human saliva	NR	Amperometric	Polyethylene terephthalate	NR	NR	[51]
Glucose	3–100 $\mu$ M	3 $\mu$ M	Interstitial fluid under the epidermis	7	Amperometric	Tattoo-based paper	NR	NR	[54]
Lactate	1–20 mM	1 mM	Sweat	10	Amperometric	Tattoo-based paper	NR	NR	[55]
pH	4.35–8.00	NR	Interstitial fluid (wound)	NR	Potentiometric	Polyaniline	NR	NR	[56]
NADH	0–100 $\mu$ M	NR	PBS	NR	Chronoamperometry	Textile	NR	1.5 $\times$ 15 mm	[59]
<i>Helicobacter pylori</i>	Up to 10 <sup>6</sup> cells	~100 cells	Tooth enamel	NR	Electrical resistance	Silk fibroin film	50 $\mu$ m	NR	[71]
Sodium	0.1–100 mM	0.1 mM	Sweat	10	Potentiometric	Polyethylene terephthalate	NR	NR	[73]

<sup>a</sup>Abbreviations: NR, not reported; PBS, phosphate-buffered saline; EIS, electrochemical impedance spectroscopy; DPV, different pulse voltammetry; cfu, colony forming unit.



**Figure 2. Flexible Substrate-Based Point-of-Care (POC) Diagnostic Devices.** (A) Flexible organic electrochemical transistors (OECTs) for detection of uric acid and glucose in saliva [40]. (B) Flexible nanoporous polyamide-based electrical sensors with detection of cortisol in sweat [42]. (C) A flexible polyester film-based electrical sensor for detection of HIV-1 [45]. (D) A lab-on-a-foil biosensor for detection of single nucleotide polymorphism in type 2 diabetes Leu7Pro gene via melting curve analysis [47]. (A–D) Adapted, with permission, from [40,42,45,47].

(PANI) for selective detection of hydrogen peroxide. OECTs were employed to detect glucose and uric acid as low as 30 and 10 nM, respectively, and demonstrated biocompatibility, ease of fabrication with different conductive materials, and mechanical robustness during bending tests up to 1000 times. In another study, a carbon nanotube (CNT)-based flexible graphite electrode was developed for detection of glucose with a detection limit of 30  $\mu M$  [41]. The device was robust and unaffected by mechanical stress up to 200 cycles of bending, so it may be suitable for continued development as a sensitive and wearable device for glucose monitoring.

In another study, a flexible nanoporous substrate-based biosensor was developed to detect and quantify the level of cortisol in sweat (Figure 2B) [42]. Cortisol is an important multifunctional hormone in controlling metabolism, bone formation, and the immune system, and its imbalance is highly related to psychological stress and a number of clinical outcomes [43]. This flexible sensor consisted of three electrodes deposited on flexible polyamide substrates with nanoporous structures. The semiconductive nanoporous structures, when interacting with liquid electrolytes, formed an electrical double layer, which significantly improved charge storage capacity, and thus the sensitivity and dynamic range of the electrical sensor. A zinc oxide (ZnO) thin film was also deposited to form an active region for cortisol antibody immobilization. This flexible sensor exhibited a linear dynamic range of 10–200 ng/ml and a detection limit of 1 ng/ml for detecting cortisol in human sweat samples. This flexible sensor can be turned into a wearable device for noninvasively monitoring the level of cortisol in a clinically relevant range. In another report, a flexible graphite electrochemical detection based diagnostic assay was recently developed for the analysis of dopamine and uric acid in urine samples [44].



Flexible substrate-based diagnostic devices have also been developed for analysis of various intact/pathogenic microorganisms. A flexible polyester based diagnostic device was demonstrated for detection of HIV-1 (Figure 2C) [45]. Via impedance magnitude spectra-based electrical sensing, HIV-1 was successfully detected at a level of  $10^6$  copies/ml, showing the feasibility of detecting high HIV-1 viremia at the stage of acute HIV-1 infection. In another study, *Escherichia coli* was detected using an inexpensive, label-free, flexible substrate-based diagnostic device [46]. In this device, graphene was assembled on an acetate film, and interdigitated gold electrodes were deposited on graphene to facilitate impedance spectroscopy. Although no capturing moieties were needed, this device only detected *E. coli* with a limit of detection of  $4.5 \times 10^7$  cfu/ml.

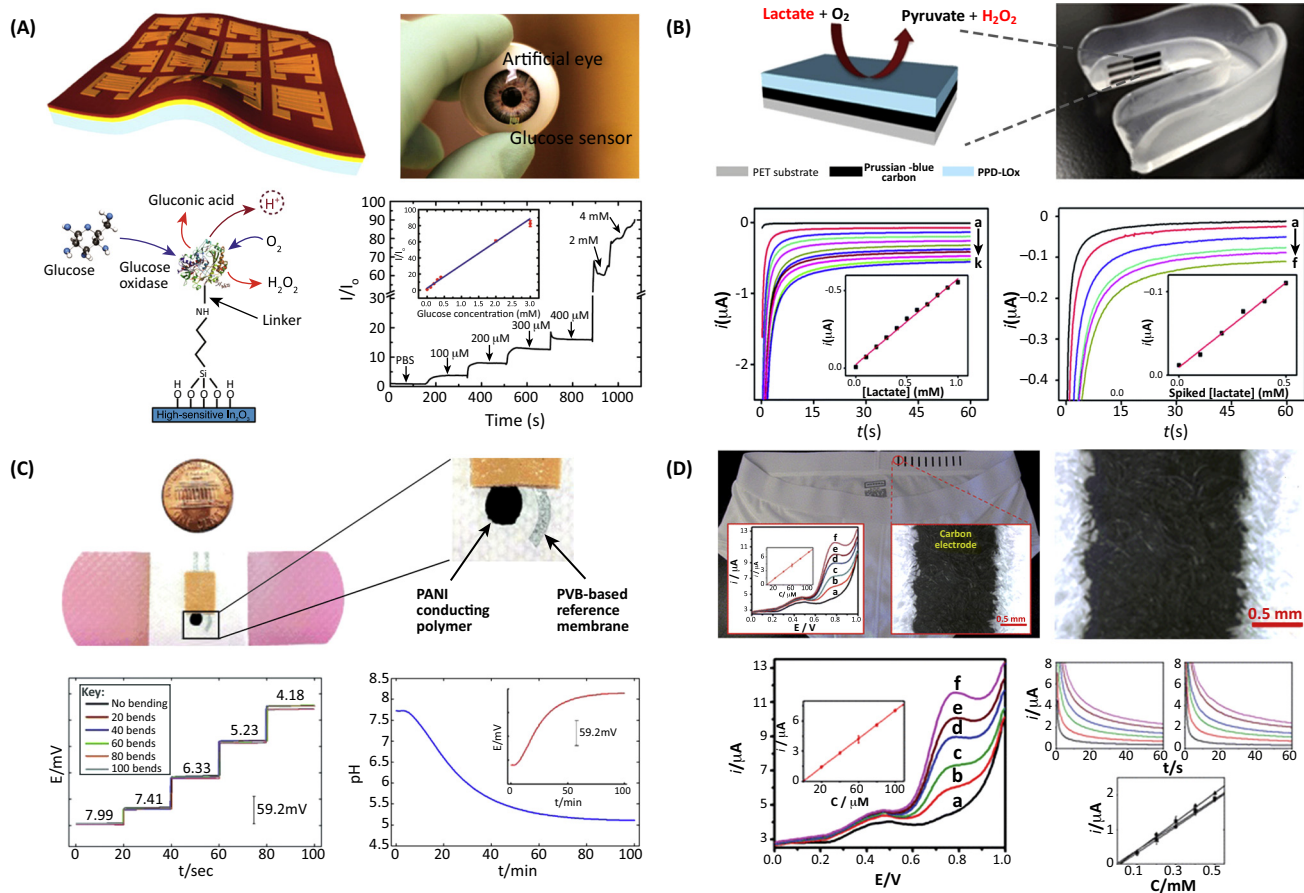
DNA analysis has also been miniaturized into flexible substrate-based POC diagnostics. For example, a flexible lab-on-a-foil microfluidic device was developed for the detection of SNP in type 2 diabetes biomarker Leu7Pro, based on melting curve analysis (MCA) (Figure 2D) [47]. The device was made of polyethylene naphthalate (PEN) foil with an integrated Cu thin-film mesh heater, which was coated with parylene C. After PCR amplification, fluorescence-labeled single-stranded DNA amplicons hybridized to their specific probes, which were immobilized on the Cu thin-film mesh heater. An increase in temperature on the mesh heater dissociated fluorescent DNA amplicons from their probes, leading to reduction of fluorescence intensity and detection of SNP in Leu7Pro. However, the gene of Leu7Pro was amplified using a conventional PCR instrument prior to MCA analysis on-chip, making this device unsuitable for POC testing [47]. On-chip amplification of bacterial DNA was also demonstrated on a flexible cassette [48]. The flexible cassette consisted of three layers of polyethylene ribbons with punched holes. These layers were bonded together via double-sided adhesive, and white paper was attached to the bottom of the flexible device to form sample reservoirs and to facilitate subsequent colorimetric detection. The flexible cassette containing bacteria was rolled onto a collector reel for loop-mediated isothermal amplification (LAMP) at  $65^\circ\text{C}$  in less than 1 h. This flexible cassette detected *E. coli* and *Staphylococcus aureus* at 30 and 200 cfu/ml, respectively.

### Wearable Devices for Personalized Healthcare Monitoring

In this section, we divide wearable device-based POC diagnostics into the two groups: (i) wearable devices for detection of targeted species (e.g., glucose, pH, metabolites, electrolytes, and pathogens) from biological fluids (e.g., tear, saliva, and sweat), and (ii) wearable devices for monitoring physiological parameters such as temperature, motion, and stress.

For detection of glucose in tears, a flexible amperometric sensor integrated in a contact lens was reported [49]. This glucose sensor was microfabricated by depositing Ti/Pd/Pt metal electrodes on a PET film and then shaped into a contact lens. Subsequently, a titania sol-gel film was applied on the surface to immobilize glucose oxidase, and a Nafion film was used to remove interfering agents such as ascorbic acid, lactate, and urea. This contact lens-shaped wearable glucose sensor exhibited detection of limit of 0.01 mM and a good linearity over a clinically relevant range of 0.1–0.6 mM in tears. In another study, a printable ultrathin conformal biosensor was fabricated by coating  $\text{In}_2\text{O}_3$  solutions on ultrathin polyimide films (Figure 3A) [50]. The ultrathin polyimide film allowed for highly conformal contact to curvilinear surfaces, and high-density  $\text{In}_2\text{O}_3$  enabled chemical modification with target-specific functional groups. This hybrid material-based flexible field-effect transistor showed current responses to 0.1–0.4 mM of glucose. These two wearable glucose sensors showed great potential to noninvasively measure glucose levels in tears for management of patients with diabetes at the POC.

Saliva testing shows great promise for the detection of metabolites such as glucose, lactate, uric acid, and cholesterol for personalized healthcare monitoring. Recently, a wearable biosensor was integrated into a mouthguard for detection of lactate in saliva (Figure 3B) [51]. This



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**Figure 3. Wearable Devices for Measuring Physiological Bioanalytes.** (A) An ultrathin  $\text{In}_2\text{O}_3$ -based conformal glucose biosensor [50]. (B) A polyethylene terephthalate (PET)-based mouthguard lactate biosensor [51]. (C) A bandage-based wearable pH sensor [56]. (D) A textile-based amperometric sensors [59]. (A–D) Adapted, with permission, from [50,51,56,59].

mouthguard biosensor consisted of three electrodes printed on a flexible PET substrate. The working electrode was coated with a poly(o-phenylenediamine) film to immobilize lactate oxidase. Lactate was oxidized by lactate oxidase, and the resultant hydrogen peroxide was oxidized by a Prussian blue transducer. Chronoamperometric measurements were made to quantify the concentration of lactate in human saliva, exhibiting a linear dynamic range of 0.1–0.5 mM. A similar mouthguard-based biosensor was subsequently developed for detection of salivary uric acid [52].

Tattoo-based wearable devices have been developed for sensing targeted species from sweat on skin or interstitial fluids [53]. Recently, a wearable amperometric biosensor was integrated into temporary tattoos for noninvasively measuring interstitial glucose in human skin [54]. In this tattoo-based glucose meter, an additional pair of iontophoresis electrodes were utilized to extract interstitial glucose to the epidermis, where interstitial glucose was amperometrically measured between a glucose oxidase-modified working electrode and a reference/counter electrode. For device fabrication, a conductive silver/silver chloride (Ag/AgCl) ink, a Prussian blue conductive carbon ink, and a transparent insulator ink were sequentially printed on a tattoo base substrate. Glucose oxidase was then functionalized on the surface of Prussian blue electrodes. The flexible tattoo-based electrode detected interstitial glucose at a limit of 3  $\mu\text{M}$ . Although this tattoo-based glucose meter was noninvasive, it took 10 min for reverse

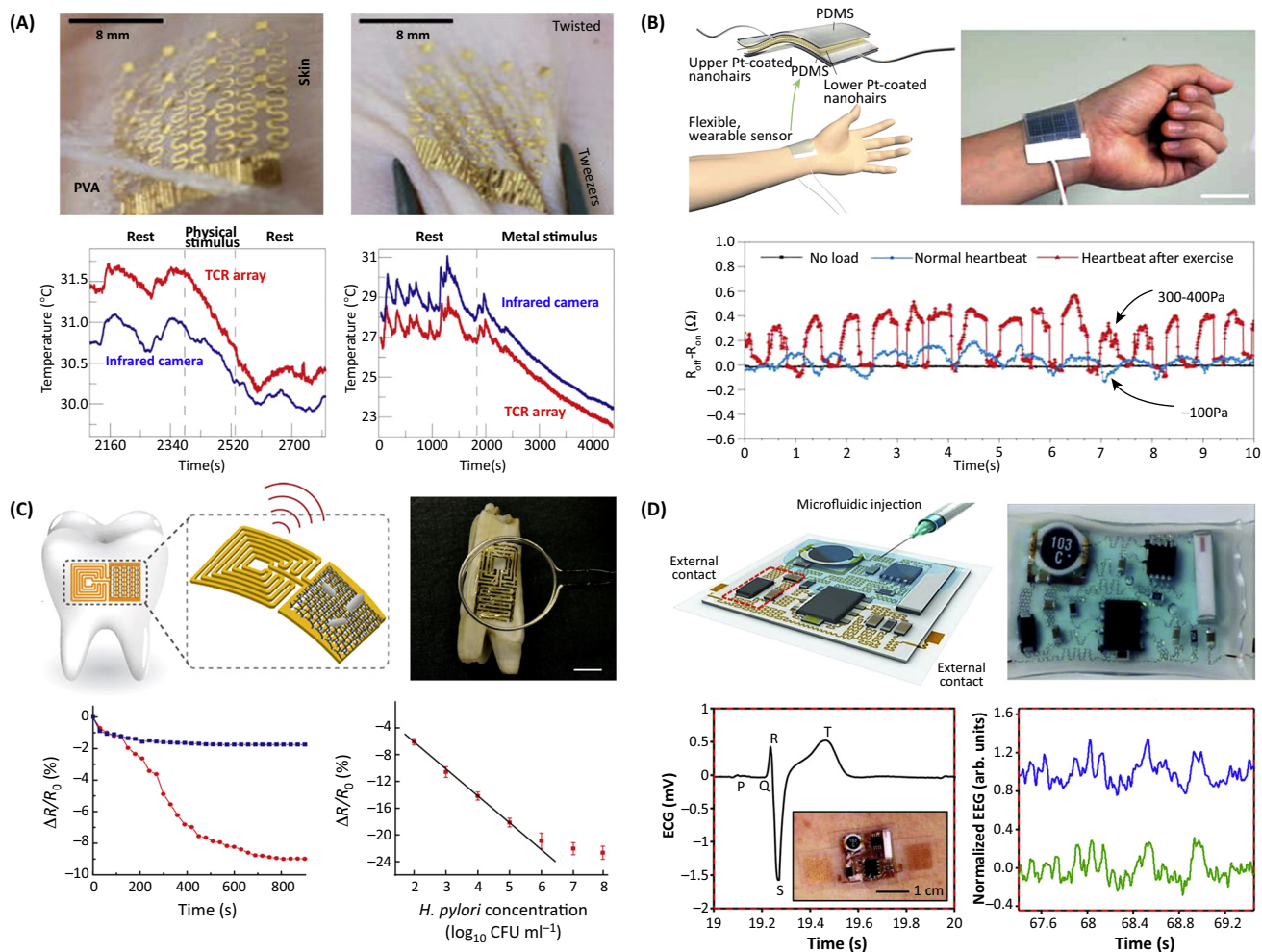
iontophoresis and 5 min for measurement, which is longer and less user-friendly for POC testing than a finger prick-based glucose meter. Similarly, a wearable tattoo-based electrochemical enzymatic biosensor was developed for monitoring the level of lactate in human sweat during physical activity [55].

Recently, wearable pH sensors have been developed to assess chronic wound healing. For example, a bandage-based wearable sensor was constructed by screen-printing one Ag/AgCl reference electrode and one carbon electrode on a conductive polyaniline membrane and polyvinyl butyral (PVB) polymer, respectively [56]. These screen-printed electrodes were assembled on a commercial adhesive bandage (Figure 3C). This wearable potentiometric sensor exhibited a Nernstian behavior to pH ranging from 4.35 to 8.0 with good reproducibility against mechanical stress. In another report, a flexible pH electrochemical sensor array was developed on polyaniline, and it showed a linear dynamic range of pH from 4 to 10 [57]. Recently, a flexible hydrogel fiber-based dressing has been developed for monitoring pH during wound healing [58]. In this study, mesoporous microparticles loaded with pH-sensitive dyes were incorporated into flexible hydrogel fibers via spin coating. The pH hydrogel fibers were then attached to a transparent medical tape to create a wearable pH sensor, which exhibited responses to pH ranging from 5.5 to 9.0. Clearly, these studies demonstrated the feasibility of developing wearable pH sensors for monitoring the wound healing process at the POC.

Flexible biosensors have also been integrated into textiles. Amperometric sensors fabricated by screen-printing technology on the elastic waist of underwear were reported (Figure 3D) [59]. This textile-based sensor not only demonstrated satisfactory mechanical robustness but also showed good sensing capability to detect ferrocyanide, hydrogen peroxide, and nicotinamide adenine dinucleotide reduced dipotassium salt (NADH). Recently, a new way of fabricating flexible electrochemical sensors using textile weaving was performed [60]. The electrochemical sensors were made of silk yarn, which was precoated with conducting inks and reagents. The modified silk yarn was woven into textile forming different electrodes. In contrast to traditional screen-printing technology, fabrication of electrochemical sensors using a simple handloom significantly lowered the cost of reagent consumption and requirement for manufacturing facilities, and thus the cost of sensors (less than 2 cents per test). As demonstrated, the electrochemical sensor detected blood glucose and hemoglobin within clinically relevant ranges using chronoamperometry and differential pulse voltammetry, respectively. However, the requirement for sample preprocessing and data recording significantly limits their POC application for detection of blood glucose and hemoglobin as wearable devices.

Flexible materials are attractive for developing epidermal electronics to measure electrophysiological activities and other physiological parameters. For example, a flexible, thin ( $\sim 50 \mu\text{m}$ ) polyester sheet-based sensor was used to record electroencephalograms (EEGs), electrocardiograms (ECGs), and electromyograms (EMGs) [61]. The flexible substrate provided conformal contact to the skin via van der Waals forces, and the contact was not affected by skin motion. Furthermore, an ultra-thin and wearable device was developed for continuously measuring skin temperature to facilitate health/wellness assessment (Figure 4A) [62]. In this study, microscale temperature sensors simultaneously acted as microheaters (actuators) that were integrated in elastomeric PI in an array format with either thin Au (50 nm in thickness) or PIN diodes embedded in a silicon membrane (320 nm in thickness). The integrated PI device offered mechanical properties and device geometries that not only matched human skin for soft and comfortable contact but also reduced measurement artifacts due to relative motion between sensors and the skin. These wearable temperature sensors had a measurement precision comparable to infrared cameras, enabling close monitoring of temperature-related physiological changes due to mental activity or physical motion, as well as monitoring thermal conductivity associated with hydration and/or perspiration at the POC.





Trends in Biotechnology

**Figure 4. Wearable Devices for Measuring Physiological Parameters.** (A) An ultrathin conformal thermal sensor for detection of skin temperature at rest and during mental and physical stimulation [62]. (B) A flexible stain-gauge sensor with reversible interlocking nanofibers for detection of human heartbeat [63]. (C) A graphene-based electrical sensor for detection of *Helicobacter pylori* with wireless recording [71]. (D) A flexible biointegrated electronic device for on-skin acquiring electrocardiograms (ECGs) and electroencephalograms (EEGs) signals [72]. (A–D) Adapted, with permission, from [62,63,71,72].

To reduce design complexity, a wearable skin-like strain-gauge sensor with a configuration of two interlocked arrays was developed for highly sensitive measurement of pressure, shear, and torsion (Figure 4B) [63]. The flexible device was fabricated by sandwiching two layers of identical nanohairs, consisting of Pt-coated nanofibers at a high density, into two thin PDMS substrates (~500 μm). These two layers of nanofibers were reversely bonded via an interlocking mechanism for sensing mechanical stimuli. Upon mechanical stress, such as pressure, shear, or torsion, the two layers of nanofibers generated an electrical resistance signal, which was measured with a strain-gauge sensor. The inexpensive flexible strain-gauge sensor accurately measured pulses with robust tolerance to bending up to 10 000 cycles.

As another example, a wearable biointegrated system was developed for temperature and mechanical sensing, as well as temperature-controlled drug delivery [64]. This multifunctional system consisted of a resistive random access memory (RRAM) array, a temperature sensor, strain sensors, electroresistive sensors, and mesoporous silica nanoparticles loaded with drugs on a flexible hydrocolloid patch. These modules enable sensing of movement such as tremors

(e.g., Parkinson's disease and epilepsy), data storage and analysis, and feedback therapy. Clearly, wearable electronics hold great promise for monitoring health conditions in real time and can be integrated with feedback therapy to enable treatment in POC settings.

### Wireless Communication for Wearable Devices

Smartphone and computer-based wireless electronics have been tethered with wearable devices to acquire sensing signals, analyze data, and report and transfer clinical results for POC applications [58,65–70]. For example, a wearable biosensor attached to tooth enamel was recently developed for detection of bacteria in saliva (Figure 4C) [71]. This wearable biosensor consisted of graphene-based interdigitated electrodes and an inductive coil antenna printed onto silk thin-film substrates. Dissolution of silk thin-film allowed intimate attachment of the flexible biosensor to irregular surfaces of tooth enamel. The capture of bacteria by antimicrobial peptides on the sensor surface resulted in change in conductivity of the graphene film, which was monitored using an inductively coupled radio frequency readout unit. The wireless electronics-based wearable sensor successfully detected ~100 *Helicobacter pylori* in 1  $\mu$ l of human saliva. This wearable graphene sensor offered sensitive, specific, and label-free detection of bacteria on non-planar surfaces with wireless sensing capability, which can be used as POC diagnostics for continuous monitoring bacterial infection in a noninvasive mode.

For continuous monitoring of physiological activities on skin, a sophisticated biointegrated flexible device was developed with the capability to transmit radio frequency data (Figure 4D) [72]. This ultrathin skin-like device was based on a silicon oligomer Sylgard 184, consisting of microfluidic channels, circuits, sensors, and radios in a multilayer interconnected network to filter, amplify, and transmit electrophysiological signals. As demonstrated, this biointegrated device successfully recorded the QRS complex in ECG signals with high single-to-noise ratio in a wireless mode, which was comparable to standard wired connections. This device also successfully documented low frequency EEG signals during mental math performed by a subject counting backwards from 200 by sevens with eyes closed. This device is small ( $1.9 \times 2.9 \text{ cm}^2$ ) and can be integrated with thermal and motion sensors, and thus can be potentially used as POC diagnostics for monitoring neonates in intensive care units or home settings.

Recently, a tattoo-based wearable device with real-time wireless signal transduction was developed for continuously monitoring the level of sodium on the skin [73]. The concentration of sodium in sweat is indicative of electrolyte imbalance under physiological or pathological conditions. This tattoo-based potentiometric sensor had two electrodes and one custom-designed transceiver for wireless signal transduction. Nernstian responses were observed for physiological concentrations of sodium in sweat ranging from 30 to 110 mM. For on-body characterization, this sensor clearly demonstrated real-time sodium dynamics during exercise. In another study, the same group developed a wearable mouthguard biosensor with an integrated Bluetooth low energy (BLE) chipset for continuous monitoring of uric acid in saliva [52]. The demonstrated device was fabricated by using a screen-printed flexible PET electrode that was integrated with a potentiostat, microcontroller, and miniaturized on-chip BLE communication system for low-power operation. The developed platform transferred sensing data through wireless transmission to standard smartphones, laptops, and other consumer electronics for on-demand data processing. These studies clearly demonstrated the feasibility of integrating wireless electronics with wearable devices for continuously monitoring physiological conditions.

### Concluding Remarks and Future Perspectives

So far, flexible substrates with enhanced physiochemical properties have been increasingly integrated into flexible biosensors and wearable devices to allow for detecting/measuring targeted biomarkers from biological fluids and physiological parameters for personalized

### Outstanding Questions

How can the characteristics of flexible substrate materials be leveraged to overcome the limitations of paper as a substrate for developing POC devices?

What substrate materials and device architecture are able to provide sample-to-answer capability for POC testing?

How can an integrated wearable device be developed at low cost for real-time monitoring of human health conditions?

How can mobile-based telediagnosis facilitate clinical decisions on-site and establish disease surveillance networks?

healthcare monitoring at the POC. Flexible substrate-based POC diagnostics, particularly with integrated graphene-based biosensors and OECTs, not only significantly enhance sensitivity but also allow for continuous on-body health monitoring in a noninvasive mode. Improved human–device interfaces are becoming possible as a result of the incorporation of mobile-based technologies for signal acquisition, data analysis, and result reporting directly at the POC without specialized equipment [76]. Moreover, integration of flexible sensors into textiles incorporated into wireless communication data collection (e.g., Bluetooth technologies) will create unprecedented opportunities for real-time, continuous on-body monitoring of physiological parameters during daily activities. Furthermore, back-end cloud services allow for long-term signal collection and data mining, which would be helpful for advanced medical research such as early diagnosis of cancer, Parkinson's disease, and other chronic diseases. Thus, we envision that next generation flexible substrate-based POC devices will leverage mobile health (mHealth) technologies and fast-advancing nanotechnologies to achieve early diagnosis, treatment monitoring, and disease surveillance in the future establishing a better baseline for humans at a personalized level.

However, flexible substrate-based POC diagnostics and wearable devices are still at an early stage of development, and key challenges remain to be addressed (see Outstanding Questions). For example, current flexible polymer-based devices are generally coupled with sophisticated optical or electrical setups for sensitive detection, which is less ideal for POC testing. Although emerging smartphone and computer-based wireless technologies have significantly improved the portability and human–device interface of flexible polymer-based POC diagnostics, technical challenges such as signal drift, variation in lighting, device miniaturization, and body temperature fluctuation need to be addressed for electrochemical sensing and optical sensing. More importantly, commercialization challenges remain a significant hurdle for translating microfluidics-based wearable devices into clinical practice such as lack of standardization on materials, poor integration from sample preparation to result report, and uneven regulatory requirement for POC device approval [74,75]. Limited funding and insufficient access to clinical samples also constrict product refinement and systematic clinical validation of flexible substrate-based POC diagnostics and wearable devices prior to entry to the market. Therefore, joint efforts are needed to advance POC diagnostics and wearable devices to be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end users (ASSURED) [2].

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### Disclaimer Statement

U.D. is a founder of and has an equity interest in: (i) DxNow Inc., a company that is developing microfluidic and imaging technologies for point-of-care diagnostic solutions, and (ii) Koek Biotech, a company that is developing microfluidic *in vitro* fertilization (IVF) technologies for clinical solutions. U.D.'s interests were viewed and managed in accordance with the conflict of interest policies.

### References

1. Wang, S. *et al.* (2016) Advances in addressing technical challenges of point-of-care diagnostics in resource-limited settings. *Exp. Rev. Mol. Diagn.* 16, 449–459
2. Mabey, D. *et al.* (2004) Diagnostics for the developing world. *Nat. Rev. Microbiol.* 2, 231–240
3. Gubala, V. *et al.* (2012) Point of care diagnostics: status and future. *Anal. Chem.* 84, 487–515
4. Yager, P. *et al.* (2008) Point-of-care diagnostics for global health. *Annu. Rev. Biomed. Eng.* 10, 107–144
5. Yetisen, A.K. *et al.* (2013) Paper-based microfluidic point-of-care diagnostic devices. *Lab. Chip* 13, 2210–2251
6. Tian, T. *et al.* (2016) Distance-based microfluidic quantitative detection methods for point-of-care testing. *Lab. Chip* 16, 1139–1151
7. Hu, J. *et al.* (2014) Advances in paper-based point-of-care diagnostics. *Biosens. Bioelectron.* 54, 585–597
8. Focke, M. *et al.* (2010) Lab-on-a-foil: microfluidics on thin and flexible films. *Lab. Chip* 10, 1365–1386

9. Pang, C. *et al.* (2013) Recent advances in flexible sensors for wearable and implantable devices. *J. Appl. Polym. Sci.* 130, 1429–1441
10. Ren, K.N. *et al.* (2013) Materials for microfluidic chip fabrication. *Acc. Chem. Res.* 46, 2396–2406
11. Shafiee, H. *et al.* (2014) Nanostructured optical photonic crystal biosensor for HIV viral load measurement. *Sci. Rep.* 4, 4116
12. Tokel, O. *et al.* (2015) Portable microfluidic integrated plasmonic platform for pathogen detection. *Sci. Rep.* 5, 9152
13. Yetisen, A.K. *et al.* (2014) Reusable, robust, and accurate laser-generated photonic nanosensor. *Nano Lett.* 14, 3587–3593
14. Holden, M.T. *et al.* (2015) Photolithographic synthesis of high-density DNA and RNA arrays on flexible, transparent, and easily subdivided plastic substrates. *Anal. Chem.* 87, 11420–11428
15. Lutz, S. *et al.* (2010) Microfluidic lab-on-a-foil for nucleic acid analysis based on isothermal recombinase polymerase amplification (RPA). *Lab. Chip* 10, 887–893
16. Bandothkar, A.J. and Wang, J. (2014) Non-invasive wearable electrochemical sensors: a review. *Trends Biotechnol.* 32, 363–371
17. Hammock, M.L. *et al.* (2013) 25th anniversary article: the evolution of electronic skin (E-Skin): a brief history, design considerations, and recent progress. *Adv. Mater.* 25, 5997–6037
18. Liao, C.Z. *et al.* (2015) Flexible organic electronics in biology: materials and devices. *Adv. Mater.* 27, 7493–7527
19. Vilela, D. *et al.* (2016) Flexible sensors for biomedical technology. *Lab. Chip* 16, 402–408
20. Xu, T.B. *et al.* (2015) High resolution skin-like sensor capable of sensing and visualizing various sensations and three dimensional shape. *Sci. Rep.* 5, 12997
21. Chinnasamy, T. *et al.* (2014) A lateral flow paper microarray for rapid allergy point of care diagnostics. *Analyst* 139, 2348–2354
22. Chinnasamy, T. *et al.* (2014) Point-of-care vertical flow allergen microarray assay: proof of concept. *Clin. Chem.* 60, 1209–1216
23. Xu, Y. *et al.* (2014) Fluorescent probe-based lateral flow assay for multiplex nucleic acid detection. *Anal. Chem.* 86, 5611–5614
24. Martinez, A.W. *et al.* (2008) Three-dimensional microfluidic devices fabricated in layered paper and tape. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19606–19611
25. Martinez, A.W. *et al.* (2010) Diagnostics for the developing world: microfluidic paper-based analytical devices. *Anal. Chem.* 82, 3–10
26. Pollock, N.R. *et al.* (2012) A paper-based multiplexed transaminase test for low-cost, point-of-care liver function testing. *Sci. Transl. Med.* 4, 152ra129
27. Guan, L. *et al.* (2014) Barcode-like paper sensor for smartphone diagnostics: an application of blood typing. *Anal. Chem.* 86, 11362–11367
28. Dungchai, W. *et al.* (2009) Electrochemical detection for paper-based microfluidics. *Anal. Chem.* 81, 5821–5826
29. Martinez, A.W. *et al.* (2007) Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angew. Chem. Int. Ed.* 46, 1318–1320
30. Abe, K. *et al.* (2008) Inkjet-printed microfluidic multianalyte chemical sensing paper. *Anal. Chem.* 80, 6928–6934
31. Li, X. *et al.* (2008) Paper-based microfluidic devices by plasma treatment. *Anal. Chem.* 80, 9131–9134
32. Lu, Y. *et al.* (2009) Rapid prototyping of paper-based microfluidics with wax for low-cost, portable bioassay. *Electrophoresis* 30, 1497–1500
33. Polavarapu, L. *et al.* (2014) Optical sensing of biological, chemical and ionic species through aggregation of plasmonic nanoparticles. *J. Mater. Chem. C* 2, 7460–7476
34. Polavarapu, L. and Liz-Marzan, L.M. (2013) Towards low-cost flexible substrates for nanoplasmonic sensing. *Phys. Chem. Chem. Phys.* 15, 5288–5300
35. Zhuo, Y. and Cunningham, B.T. (2015) Label-free biosensor imaging on photonic crystal surfaces. *Sensors Basel* 15, 21613–21635
36. Gaster, R.S. *et al.* (2011) Quantification of protein interactions and solution transport using high-density GMR sensor arrays. *Nat. Nanotechnol.* 6, 314–320
37. Polavarapu, L. *et al.* (2014) Pen-on-paper approach toward the design of universal surface enhanced Raman scattering substrates. *Small* 10, 3065–3071
38. Zhang, K. *et al.* (2015) Multifunctional paper strip based on self-assembled interfacial plasmonic nanoparticle arrays for sensitive SERS detection. *ACS Appl. Mater. Interf.* 7, 16767–16774
39. Mostafalu, P. and Sonkusale, S. (2015) A high-density nanowire electrode on paper for biomedical applications. *RSC Adv.* 5, 8680–8687
40. Liao, C. *et al.* (2015) Flexible organic electrochemical transistors for highly selective enzyme biosensors and used for saliva testing. *Adv. Mater.* 27, 676–681
41. Kim, W.-S. *et al.* (2014) A flexible, nonenzymatic glucose biosensor based on Ni-coordinated, vertically aligned carbon nanotube arrays. *RSC Adv.* 4, 48310–48316
42. Munje, R.D. *et al.* (2015) Flexible nanoporous tunable electrical double layer biosensors for sweat diagnostics. *Sci. Rep.* 5, 14586
43. Kaushik, A. *et al.* (2014) Recent advances in cortisol sensing technologies for point-of-care application. *Biosens. Bioelectron.* 53, 499–512
44. Cai, W. *et al.* (2014) Electrochemical determination of ascorbic acid, dopamine and uric acid based on an exfoliated graphite paper electrode: a high performance flexible sensor. *Sens. Actuat. B Chem.* 193, 492–500
45. Shafiee, H. *et al.* (2015) Paper and flexible substrates as materials for biosensing platforms to detect multiple biotargets. *Sci. Rep.* 5, 8719
46. Basu, P.K. *et al.* (2014) Graphene based *E. coli* sensor on flexible acetate sheet. *Sens. Actuat. B Chem.* 190, 342–347
47. Ohlander, A. *et al.* (2013) Genotyping of single nucleotide polymorphisms by melting curve analysis using thin film semi-transparent heaters integrated in a lab-on-foil system. *Lab Chip* 13, 2075–2082
48. Safavieh, M. *et al.* (2014) A simple cassette as point-of-care diagnostic device for naked-eye colorimetric bacteria detection. *Analyst* 139, 482–487
49. Yao, H. *et al.* (2011) A contact lens with embedded sensor for monitoring tear glucose level. *Biosens. Bioelectron.* 26, 3290–3296
50. Rim, Y.S. *et al.* (2015) Printable ultrathin metal oxide semiconductor-based conformal biosensors. *ACS Nano* 9, 12174–12181
51. Kim, J. *et al.* (2014) Non-invasive mouthguard biosensor for continuous salivary monitoring of metabolites. *Analyst* 139, 1632–1636
52. Kim, J. *et al.* (2015) Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics. *Biosens. Bioelectron.* 74, 1061–1068
53. Bandothkar, A.J. *et al.* (2015) Tattoo-based wearable electrochemical devices: a review. *Electroanalysis* 27, 562–572
54. Bandothkar, A.J. *et al.* (2014) Tattoo-based noninvasive glucose monitoring: a proof-of-concept study. *Anal. Chem.* 87, 394–398
55. Jia, W. *et al.* (2013) Electrochemical tattoo biosensors for real-time noninvasive lactate monitoring in human perspiration. *Anal. Chem.* 85, 6553–6560
56. Guinovart, T. *et al.* (2014) Bandage-based wearable potentiometric sensor for monitoring wound pH. *Electroanalysis* 26, 1345–1353
57. Rahimi, R. *et al.* (2016) A low-cost flexible pH sensor array for wound assessment. *Sens. Actuat. B Chem.* 229, 609–617
58. Tamayol, A. *et al.* (2016) Flexible pH-sensing hydrogel fibers for epidermal applications. *Adv. Healthc. Mater.* 5, 711–719
59. Yang, Y.L. *et al.* (2010) Thick-film textile-based amperometric sensors and biosensors. *Analyst* 135, 1230–1234
60. Choudhary, T. *et al.* (2015) Woven electrochemical fabric-based test sensors (WEFTS): a new class of multiplexed electrochemical sensors. *Lab. Chip* 15, 2064–2072
61. Kim, D.H. *et al.* (2011) Epidermal electronics. *Science* 333, 838–843
62. Webb, R.C. *et al.* (2013) Ultrathin conformal devices for precise and continuous thermal characterization of human skin. *Nat. Mater.* 12, 938–944

63. Pang, C. *et al.* (2012) A flexible and highly sensitive strain-gauge sensor using reversible interlocking of nanofibres. *Nat. Mater.* 11, 795–801
64. Son, D. *et al.* (2014) Multifunctional wearable devices for diagnosis and therapy of movement disorders. *Nat. Nanotechnol.* 9, 397–404
65. Coskun, A.F. *et al.* (2013) A personalized food allergen testing platform on a cellphone. *Lab. Chip* 13, 636–640
66. Wang, S. *et al.* (2011) Integration of cell phone imaging with microchip ELISA to detect ovarian cancer HE4 biomarker in urine at the point-of-care. *Lab. Chip* 11, 3411–3418
67. Yetisen, A.K. *et al.* (2014) A smartphone algorithm with inter-phone repeatability for the analysis of colorimetric tests. *Sens. Actuat. B Chem.* 196, 156–160
68. Steinhubl, S.R. *et al.* (2015) The emerging field of mobile health. *Sci. Transl. Med.* 7, 283rv3
69. Preechaburana, P. *et al.* (2014) Biosensing with cell phones. *Trends Biotechnol.* 32, 351–355
70. Wang, S. *et al.* (2014) Micro-a-fluidics ELISA for rapid CD4 cell count at the point-of-care. *Sci. Rep.* 4, 3796
71. Mannoor, M.S. *et al.* (2012) Graphene-based wireless bacteria detection on tooth enamel. *Nat. Commun.* 3, 763
72. Xu, S. *et al.* (2014) Soft microfluidic assemblies of sensors, circuits, and radios for the skin. *Science* 344, 70–74
73. Bandodkar, A.J. *et al.* (2014) Epidermal tattoo potentiometric sodium sensors with wireless signal transduction for continuous non-invasive sweat monitoring. *Biosens. Bioelectron.* 54, 603–609
74. Volpatti, L.R. and Yetisen, A.K. (2014) Commercialization of microfluidic devices. *Trends Biotechnol.* 32, 347–350
75. Chin, C.D. *et al.* (2012) Commercialization of microfluidic point-of-care diagnostic devices. *Lab. Chip* 12, 2118–2134
76. Gao, W. *et al.* (2016) Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. *Nature* 529, 509–514