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Review

Advances in wearable chemical sensor design for monitoring biological fluids

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ABSTRACT

The state of the art and future challenges related to wearable chemical sensors are addressed within this review. Our attention is focused on the monitoring of biological fluids such as interstitial fluids, breath, sweat, saliva and tears, while aiming at the realization of miniaturized, non-invasive and low cost point of care systems. The development of such sensing devices is influenced by many factors and is usually addressed through the use of "smart materials" such as graphene, carbon nanotubes, poly ionic liquids, etc. These are seen as the pivotal steps towards the integration of chemical sensors within pervasive applications for personal health care.

Contents

1. Introduction

The pronounced increase in incidence of ageing related pathologies observed in the recent years has greatly emphasized the need for a novel class of personalized point of care systems that could be

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extensively and effortlessly integrated into the daily life of a patient in the form of wireless body sensor networks (WBANs) [1]. So far, most research efforts in this direction have been focused on the adaptation of miniaturized wearable designs based on relatively mature technologies such as motion tracking [2], bio-electrical signals analysis [3] and temperature detection [4]. On the other hand, the biochemical analytes contained in biological fluids have been often overlooked as possible sensor targets despite the valuable information they convey about the state of health of an individual. Urine and blood are routinely analyzed through standard analytical techniques such as Atomic Absorption Spectroscopy, Ion Chromatography, and Gas Chromatography, but these methodologies are relatively expensive and can inevitably provide only discontinuous "one shot" measurements of the concentration of an analyte of interest, since they are currently not suitable for miniaturization. While blood is by far the most understood sample for diagnostic measurements, other biological fluids such as sweat, saliva, interstitial fluids, tears and breath are more readily accessible and thus are attractive targets for non/minimally-invasive wearable sensor platforms, as recently described by Bandodkar and Wang [5].

The multiple challenges faced by chemo/bio-sensors during their normal use have been described in detail in a recent review by Diamond et al. [6]. For instance, due to their continuous exposure to the fluid of interest and to wear and tear, portable sensors targeting biological fluid analytes need frequent recalibration to correct for signal drift over time, and in some cases have relatively high energy demands. Recent advances in materials science and microfluidic fabrication techniques [7–9] are opening routes towards enhanced miniaturization and more efficient handling of the liquid substrates, and the more general availability of low-cost, reliable, and wearable "lab-on-a-chip" systems. At the same time, the availability of novel "smart materials" for the realization of both protective and active layers for sensor production promises to greatly increase the performance of sensing devices, and are currently the object of intense research [6]. Finally, the integration of sensors within textiles could provide a much-needed improvement in robustness and mechanical durability of sensors thanks to a better protection of the sensing area. Materials that are currently employed for the embedding of electrochemical sensor are flexible polymers such as Kapton and Mylar, which have excellent thermal properties and stability, and GoreTex, which combines hydrophobic character with water permeable behaviour [10].

Perhaps most striking is the recent dramatic movement by multinational IT companies into the wearable sensor space, including the first signs of attempts to integrate chemo/bio-sensors. For example, Google Glass [11] is a wearable spectacle-based electronics platform that can be used to harvest body sensor data. Google have announced a novel project to integrate a glucose sensor into a contact lens which, due to the close proximity to the spectacles, could be powered inductively to enable glucose measurements and short-range communications [12]. Similarly, it seems that Apple will soon announce iWatch [13], which can be conveniently worn on the wrist and is rumoured to integrate a glucose sensing capability, while IBM [14] and Intel [15] have sponsored several initiatives aimed at promoting the design of novel wearable systems.

In this review, we will provide an overview of some of the advances occurring in the past decade and summarize on-going research activities aimed at integrating chemical sensors into wearable platforms for non/minimally-invasive monitoring of analytes in biological fluids. Particular attention will also be paid to the most promising trends observed in the development of new materials for the enhancement of the performance of wearable chemical sensors.

2. Interstitial fluid monitoring

Except for large molecules (e.g. lipids), the composition of the interstitial fluid is very similar to that of blood in terms of salt, protein, glucose, and ethanol contents. Preliminary studies were carried out on the employment of needle type biosensors to detect cholesterol [16]. However, a better minimally invasive approach was based on the use of micro-needle arrays that allow for the realization of miniaturized, wireless devices with a patch-like configuration. In preliminary studies H_2O_2 [17], pH, glucose [18] and lactate variations [17,18] were monitored using hollow needles filled with carbon paste sensitive materials tested on bench. Validation through animal models and ad hoc miniaturized electronic platforms are still missing. Nevertheless, most of minimally invasive devices already on the market are devoted to glucose monitoring. The correlation with blood glucose levels makes interstitial fluid a convenient target for continuous monitoring of patients affected by diabetes [19,20]. The measurements obtained via subcutaneously implanted devices can then be corrected to take into account the time lag between variations in blood and interstitial glucose levels via validated compartmental models [21].

2.1. Minimally invasive devices for glucose monitoring

Several devices for glucose monitoring are already on the market, and enable continuous detection with no need for repeated finger pricking. All devices employ electrochemical sensors, while different approaches were taken for sampling within the subcutaneous adipose tissue. Some designs rely on a direct implantation through a needle (e.g. Guardian RT by Medtronic) [20] while others employ a microdialysis harvesting approach (e.g. GlucoDay by Menarini [20,22]) correlated with external monitoring of the collected sample [20].

The most popular system is probably the Glucose Free Style Navigator by Abbott, which consists of a disposable sensor delivery unit, a radio frequency transmitter directly connected to the sensor, and a hand held receiver that shows the glucose levels. Abnormal conditions (e.g. hypoglycemia <70 mg dL^{-1} and hyperglycemia >240 mg dL^{-1}) are signalled by an acoustic alarm with high accuracy (96% and 99.7% of cases respectively for hypoglycemia and hyperglycemia) [23]. The minimally invasive nature of such devices could be further enhanced by totally implantable devices inserted at the adipose levels, provided that a battery with sufficient durability and biocompatibility is available. In a 2006 review, Heller [24] described several possible battery configurations where the interstitial fluid is used as the electrolyte between cathode and anode. In particular, the adoption of wired bilirubin oxidase as cathode (where O_2 is electroreduced to water) and glucose oxidase as anode would provide the best biocompatibility for long-term implanted glucose sensors [24]. This approach seems revolutionary, and we expect that several investigations will be driven in this direction in the next coming years.

Several improvements are still needed also in terms of sensor technology in order to enhance durability (currently available sensors must be replaced every 3–7 days) and sensitivity (especially in hypoglycemic and hyperglycemic conditions) [20]. Among the various devices that have been proposed in the last ten years, one of the most original and innovative relied on the integration of the sensor into a polymeric housing endowed with a microperfusion channel for sample collection. A cavity contained the Pt working electrode made of a glucose oxidase (GOx)/agarose/glutaraldehyde sensitive layer covered by a silicon pore membrane (105 pores with 50 μm diameter on an area of 2650 μm^2) that controlled glucose diffusion. At the same time, the membrane protected the sensor from fouling and improved its accuracy and reliability over time. Bench tests showed fast responses to glucose variations (30 s, flow

rate of 0.5 μL min^{-1}) within the range of interest (0.05–20 mM) and stability over time (1 week). The sensor was also tested in a clinical environment showing promising results for its potential application for open flow microinfusion techniques [25]. Other approaches have involved integrating sensors within microfluidic chips, in order to reduce the overall size of the system. This was possible through optimization of the length (1.0 cm) of a microdialysis membrane connected to an enzymatic microreactor endowed with a chaotic mixing channel, which was bonded to a glass chip providing the Pt working electrode. An overoxidised polypyrrole (PPy) layer was employed to avoid Faradaic interferences from other analytes present in the interstitial fluid (such as ascorbic acid, uric acid, acetaminophen) and an Ag/AgCl layer was used as a reference electrode. A constant supply of GOx was provided to the chamber at a flow rate of 1.5 μL min^{-1}. In the chamber, the GOx mixed with the interstitial fluid where it reacted with glucose, generating a linear signal within the range 2.1–20.6 mM (lag time of 18 min). The device was then implanted in the abdominal area of rats and successfully tested after bolus injection and under controlled insulin infusion. Before testing in humans, several improvements were recommended to increase sensor durability from hours (test on rats lasted 4 h) to several days [26].

Despite these advances, it must be appreciated that there are still serious limitations to the in vivo electrochemical sensing of glucose. These include glucose consumption at the electrode surface, suboptimal performance at low glucose levels, loss of electrode materials, degradation of enzymes, interference from other sample components, oxygen deficiency at the electrode, impact of biofilm formation, need for frequent recalibration, and short durability of the sensing strip. Nonetheless, various aspects of the sensor design and materials employed can bring many benefits in terms of sensor performance. For instance, the use of affinity materials can be advantageous to reduce biofouling through modification of the binding time of the molecule of interest. A differential affinity glucose sensor was developed for dielectric and viscometric detection, with the former showing the best performances [27]. This Microelectromechanical System (MEMS) consisted of two connected microchambers, one containing a glucose-sensitive (boronic acid containing the co-polymer poly(*N*-hydroxyethylacrylamide-*ran*-3-acrylamidophenylboronic acid) (PHEAA-*ran*-PAABA)) and the other a glucose-insensitive (poly(acrylamide) (PAA)) material. Two parallel electrodes were also integrated into the system, the lower one being realized on a glass substrate while the upper one made on a perforated parylene diaphragm (sustained by stiff posts to prevent its collapse) that allowed analyte diffusion. In vitro tests of the frequency responses to the electric field were conducted at 32 kHz and showed response (4.9 min) and recovery times (7.8 min) comparable with standard systems used for glucose monitoring within the sensitive range 0–500 mg dL^{-1}, with an accuracy of 1.74 mg dL^{-1} [27,28]. Sensors implanted into the scapular area of mice provided a stable signal for ca. 8 min, as shown through comparison with capillary blood glucose levels measured at the tail region. The recorded time behaviour was similar (after bolus or insulin injection) to that of blood, with a delay of approximately 5–15 min. The results showed good correlation (r^2 = 0.962) and Clarke Error Grid analysis suggested the approach would be suitable for clinical tests (83.6% of the samples in zone A) [28].

New solutions might be also offered by optical systems, but they are still at their infancy. The use of Surface Enhanced Raman Spectroscopy (SERS) and more recently Spatially Offset Raman Spectroscopy (SORS) could indeed revolutionize the approaches for continuous glucose monitoring in interstitial fluids [29]. Raman spectroscopy can detect the unique vibrational signature of molecules whose signal can be enhanced using surfaces covered by a film of silver nanospheres functionalized with a self-assembled monolayer of decanethiol (DT)/6-mercato-1-hexanol (MH) that is able to create a 'dynamic pocket' with the approximate size of a glucose molecule. The tip of an optical fibre was covered with this film, implanted in the abdominal area of rats, and was found to be able to successfully track low glucose levels (31–79 mg dL^{-1}) over a period of 17 days [30].

2.2. Non-invasive devices for glucose monitoring

Completely non-invasive designs for wearable interstitial fluid sensors aim at removing the need for subcutaneous implantation, to find a balance between the need to access a representative sample, and the need for more comfortable wearability and an effective usage model. With the GlucoWatch, realized by Tierney et al. [31], the solution adopted was to bring interstitial fluid through the skin to the external analytical platform using iontophoresis. Although it has been withdrawn from the market due to induced irritation problems in patients [31], the GlucoWatch stimulated intense research and follow up studies. Dachao et al. [32] developed a system that includes SonoPrep, a device that delivers ultrasonic energy at the skin level (frequency 55 kHz on an area of 0.8 cm^2). This also stimulates the release of interstitial fluid, which can be collected in an external glass chamber using a vacuum for up to 15 min. Glucose in the fluid is monitored using a commercial biosensor. The system was validated after administering glucose to healthy subjects and sampling the fluid every 20 min. Glucose variations in the interstitial fluid followed those in blood, and a mathematical model was developed and validated to provide a predictive capability. However, this system was not miniaturized enough to be considered wearable [32].

The GlucoTrack was designed to be positioned at the tip of the earlobe of a user. Three sensors simultaneously monitored physical variations in electric and acoustic impedance and heat transfer for 1 min. The combined measurement allowed the estimation of glucose levels indirectly and with increased accuracy with respect to the single measurements (Clarke Error Grid with a mean ARD value of 15.8%). The system was first calibrated on each user to adjust the correlation parameters, and then measurements were taken at regular intervals (every 30 min) while subjects were carrying out standard daily activities. Clarke Error Grid data showed that the readings (96%) were within the clinically acceptable ranges [33].

Another non-invasive system was based on resonance-enhanced pulsed photoacoustic spectroscopy with a windowless resonator cell positioned on the skin of the fingertip. This open stainless-steel resonator cell was characterized by two perpendicularly connected absorption and resonance cylindrical cavities. The laser beam enters the system irradiating the sample on the far side (energy below 1 mW mm^{-2}, laser pulse width kept at 500 ns), inducing the photoacoustic effect in the absorption cavity. In response to the stimulus, the sample produces an acoustic wave that can be detected by an ultrasound detector (i.e. a microphone), positioned at the end of the resonance cavity. This magnifies the signals, which are then filtered via a lock-in amplifier. Experiments using a glassy carbon layer showed that the best resonance peak (best Q factor and the highest signal-to-noise ratio) was obtained at 51.7 kHz [34]. Principal component analysis of the IR spectra (obtained from the skin) showed that glucose was the first principal component [35]. Improvements in the configuration of the cell and its connection to a real, miniaturized portable instrument are still necessary [34].

2.3. Non-invasive monitoring of ethanol

Glucose is not the only analyte of interest that can be detected and continuously monitored in the interstitial fluid samples. For instance, ethanol can be monitored via poration of the stratum

corneum of the skin. This sampling technique is painless and consists of positioning a handheld porator that is able to create micropores roughly of the diameter of a human hair (<100 μm in diameter, open for 3 days). An interstitial liquid harvesting system is positioned at the skin location wherein the pores have been generated. An electromechanical pump (6–9 in. of mercury) allows the fluid collection (10 $\mu L\,h^{-1}$) in the harvesting unit, which also contains an electrochemical sensor with an alcohol oxidase working electrode catalyzing the reaction between ethanol and O_2, producing H_2O_2 which is then reduced to H^+ (see Eqs. (1) and (2))

$$\text{Ethanol} + O_2 \rightarrow \text{acetaldehyde} + H_2O_2 \quad (1)$$

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

The sensor linear range was 0–0.2% ethanol content, with a resolution approaching 0.01%. The typical response delay was 8–12 min from the point of ethanol consumption. The system was shown to be insensitive to common interferents such as uric acid, ascorbic acid and glucose. The main drawbacks of this device were the long time (1–2 h) needed to stabilize the baseline reading during tests and the use of a porator based on a laser source, which can cause burns, scars and facilitate bacterial growth, limiting the access over time to the underlying interstitial fluid for monitoring. A solution might be represented by the use of microneedles integrated on a patch, able to collect, drive and sense the analyte of interest within the sample. However, a really good outcome is represented by the connection of the harvesting/sensing chip to a wireless radio frequency (RF) platform allowing for the realization of a portable, real-time wearable device [36].

3. Breath monitoring

Monitoring of breath vapours allows for completely noninvasive detection of several analytes of interest in clinical diagnosis and therapy [37,38].

Breath monitoring poses challenges across all aspects of the measurement procedure, spanning from the choice of an opportune target bio-marker, to the implementation of the measurement technique [37]. Efficient sampling is also complicated, and because of these difficulties, the overall accuracy of breath-based measurements is generally not as good as one would like. Nonetheless, the convenience in accessing breath vapours has promoted the deployment of several successful sensing devices which are in common use, the most notable examples probably being the portable alcohol analyzers (based on an electrochemical fuel cell) that are used by police officers to estimate (indirectly) ethanol concentration in blood [39]. In the following sections, we provide other examples of devices that have successfully exploited breath to provide an indication on the content of analytes such as nitric oxide and oxygen, ammonia and acetone in the body. However, humidity is a common and serious interferent with in breath-based sensing, as it is with most gas-phase measurements.

3.1. Humidity monitoring in breath

Pronounced variations in humidity occur naturally in the expirate of patients and this can affect sensor response. Regardless of the target analyte, most breath sensing devices need thus to be coupled with miniaturized fast-response humidity sensors to enable opportune bias corrections. Corres et al. [40] exploited the electrostatic self-assembly of super-hydrophilic SiO_2 nanoparticles to build a humidity sensor integrated into an optical fibre. The novel sensor showed good reproducibility and low hysteresis even when exposed to three consecutive human breathing cycles (inspiration and expiration times of 100 ms and 150 ms, respectively) [40]. Even better performances were obtained by exploiting humidity-induced variations in the metachromasy properties of methylene blue (MB). The dye was dip-coated on the tip of an optical fibre connected to a red light emitting diode (λ = 660 nm). Increases in relative humidity reduced light absorbance due to MB dimerization within working ranges of 8–98%, with fast response (0.5 s) and low hysteresis. This sensor was tested during a breathing trial, and was shown to be capable of detecting the characteristic respiration patterns of hyperventilation [41].

Despite their good performances, both these devices could not be categorized as wearable due to the size of the system. On the other hand, a humidity sensor based on a hydrophilic polytetrafluoroethylene membrane (80 μm thick), covered on both sides by a gold layer, appears to offer significant improvements in this regard. The sensitive area was insulated with a biocompatible cyanoacrylate adhesive that was also used to fix the electrode. The sensor was then connected to a miniaturized Inductance, Capacitance and Resistance (LCR) meter able to monitor changes in resistance with humidity levels. Overall the device was soft, flexible, tear resistant, chemically stable, and able to work within a 30–85% humidity range at room temperature (25 °C). Real-time tests on healthy subjects allowed the respiration rate to be tracked with the sensor positioned on the upper part of the mouth or on the fingertip for simultaneous, real time sweat monitoring [42].

Graphene oxide (GO) is a promising material for use in humidity sensors thanks to the interactions between the exposed functional oxygen groups and water. Good results were obtained with 15 nm GO layers deposited by spray-coating on top of Ag screen printed interdigitated electrodes. These sensors were able to follow changes in humidity over the range 10–90%, with fast response (20–30 ms) and recovery (30 ms) times. The fast response behaviour was mainly dictated by the 2D GO flake structures that created a porous layer with randomly connected bi-dimensional domains. The GO based sensing devices were able to not only track breathing, but also to classify different voices, by analysis of whistles tones through principal component analysis [43]. These results are really promising also for eventual integration into smart-fabrics, but they lack essential features for continuous measurements, and results showing the overtime stability of these patterns (for hours or days).

3.2. NO and O_2 monitoring in breath

The detection of NO levels or its related products (NO_2^- and NO_3^-) is important for understanding many biological processes and health conditions. For example, in asthmatic patients, events are signalled by release of NO from inflammatory cells. NO in breath ranges between low ppb to around 100 ppb. Most NO sensors are based on electrochemical techniques, and while these can cover the range of interest [44–46], in general they are not yet integrated into point of care systems for breath analysis. However, a portable hand-held breath analyzer featuring a miniaturized spectrometer was recently realized using a mouth piece, a pressure metre endowed with three LEDs that helped the person to maintain constant exhalation flow, a fluoroplastic tube, and a Teflon piece with a hole characterized by different diameters (allowing different flow rates, keeping the mouth pressure at 10 mbar) connected to a Mylar balloon for sample collection. The sensing part was based on a wavelength modulation spectroscopy NO sensor coupled with a quantum cascade laser used as the source. Sensor performance was assessed through comparison with a Loccioni breath sampler, showing a correlation coefficient of r^2 = 0.993 and a slope of 0.986, with a limit of detection in the range 0–100 ppbv, a response time of 1 s and insensitivity to changes in flow rate [47].

Optical fibres represent also another inexpensive, real-time monitoring tool that can be exploited to realize portable or wearable devices. For example, this technology was used to realize an

O_2 breath analyzer, using sensors based on Organically Modified SILicate (ORMOSIL) sol–gel with embedded ruthenium O_2 sensitive luminophores [Ru(III)-tris(4,7-diphenyl-1,10-phenanthroline] ($[Ru(ddp)_3]^{2+}$) [48] or the cyclometalated iridium complexes bis(1-phenylisoquinoline)(acetylacetonate)iridium(III) ($[Ir(piq)_2(acac)]$) O_2 sensitive fluorophores, with the latter reporting the best performances [49]. The fluorophore doped ORMOSILs (composed of alkyl ORMOSIL n-propyltrimethoxysilane (n-propyl-TriMOS) and perfluoroalkyl ORMOSIL 3,3,3-trifluoropropyltrimethoxysilane (TFP-TriMOS)) was dip coated on an uncladded optical fibre and subsequently inserted in a glass capillary to form a microchannel acting as a flow cell. An evanescent wave from a blue 475 nm LED was used to excite the fluorophore to produce a fluorescence emission which was quenched after interacting with O_2, decreasing the amplitude of the signal (as recorded by a photodiode). The different parameters were optimized (for a sample volume of 20 μL), and under these conditions, there was minimal influence from temperature changes (within the range 25–45 °C). The device was used to monitor real breath samples, with good response times (typically around 1 s), and agreement with standard analytical techniques (relative error of ca. 1.5%)) [49].

3.3. Ammonia monitoring in breath

Ammonia detection in breath can be used as a diagnostic tool for helicobacter pylori stomach infection, in which excess urea is converted to ammonia and bicarbonate within the acidic stomach environment. Knowledge of ammonia levels can also be helpful during hemodialysis, asthma assessment, diagnosis of hepatic encephalography, and analysis of halitosis. The ammonia diffuses out of the blood into the lungs, thus enabling non-invasive breath monitoring [38]. In addition, ammonia levels can be elevated during exercise, in the order of 0.1–10 ppm. The main features required for ammonia breath-sensing devices are a working range of 50–2000 ppb and response times of less than 1 min, within the temperature range of 20–40 °C [50].

Most recent research has been devoted to the development of electrochemical sensors with conducting polymers as the sensitive layer [50]. For example, a sensing system was reported based on a chamber in which sensors can be tested. It consists of a respiratory air pump (to simulate human ventilation) bringing the air into a humidification chamber, in which the temperature was monitored and varied over the range 35–45 °C. The ammonia level in the humidified air could be varied using flow controller regulators [51]. A disposable, screen-printed interdigitated electrode on a polyethylene terephthalate (PET) substrate covered within the sensitive area by ink-jet PANI nanoparticles was employed. An impedimetric approach was employed using as experimental conditions 962 Hz, 5 mV rms, under which the conductive polymer layer displayed a purely resistive behaviour. The best performance was obtained using a flow rate of 110.8 ± 0.7 L min^{-1} as this prevented humidity accumulation within the system while also facilitating effective ammonia mass transfer at the sensor interface. The sensor was sensitive within the range 40–2993 ppbv (i.e. covering the physiological range 50–2000 ppbv), allowing for 8 sequential breath measurements in 5 min without significant drift over time (2% within 3 weeks when continuously operated) [52]. This setup was interfaced with a laptop and programmed through a Lab View interface [51]. The system was evaluated using breath samples from normal people, and the results compared to a reference photoacustic laser spectroscopic gas analyser (PALS) (slope of 0.93, $r^2 = 0.9705$, $n = 11$). Other trials involved patients undergoing dialysis treatment, checking ammonia levels before and after treatment. The values ranged from an average of 930 ppbv down to 227 ppbv, which was in agreement with typical values previously reported in the literature [52]. However, this system was quite bulky and considerable further development would need to happen to convert it into a portable device.

3.4. Acetone monitoring in breath

Elevated acetone levels in breath are an indication of systemic ketosis, which can occur due to the conversion of fat to ketones. This is associated with people suffering from Alzheimer Disease and children undergoing seizure control treatment. In addition, abnormal acetone levels can also signal hypoglycaemia in diabetic patients, suggesting a possible future non-invasive monitoring approach to track this condition. Acetone was monitored in the breath of diabetic patients via a Pt electrode, with Cr-WO_3 nanopowder deposited on top of a commercial heater. ε-WO_3 is a ferroelectric material with a dielectric moment that is affected by polar acetone molecules, leading to a decrease in resistance. The sensor was integrated within a portable electronic device, insulated by a Teflon casing into which air was conveyed using a mouth-piece. When acetone reached levels of 1.8 ppm or higher (threshold value for diabetes), the resistance dropped from 20 MΩ to 3.5 MΩ activating a warning LED. The device was reported to be insensitive to the main interferents (up to 10 ppm) that are typically found in human breathing (NO, NH_3, CO), except for ethanol and methanol (at 3 ppm) [53]. A similar system was developed using a sensor made

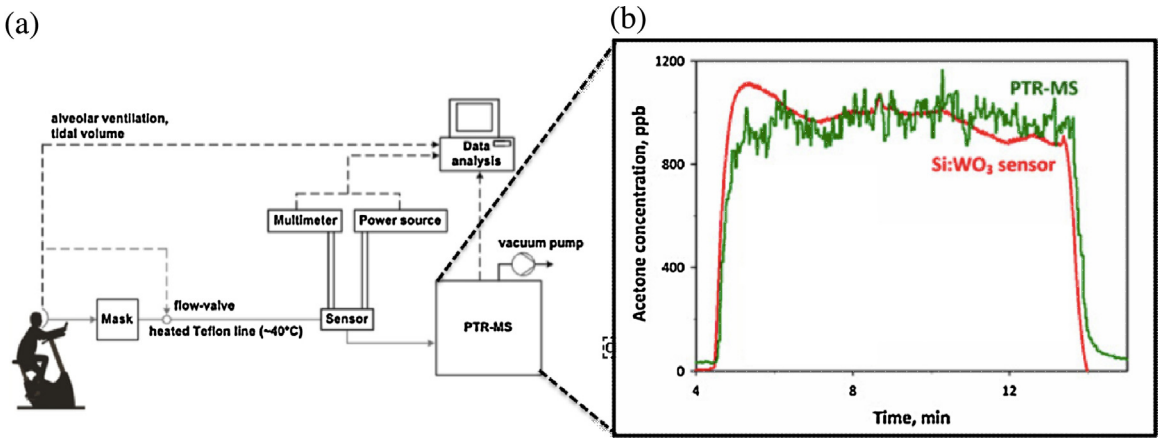

Fig. 1. (a) Experimental setup used to monitor acetone within breath samples. The grey lines represent the breath flow while the dashed lines show the data collected by the computer (adapted from Ref. [54]). (b) Comparison of the acetone variations measured by the Si:WO_3 oxide sensor (red line) and the one monitored by PTR-MS (green line) [54]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of 10% mol Si-doped WO_3 nanoparticles synthesized and deposited by flame spray pyrolysis on a back heated alumina substrate incorporating Pt interdigitated electrodes. Under constant temperature monitoring, the breath sample was conveyed to a heated Teflon line (40 °C) connected to a mask by a flow valve within a T-shaped chamber (Fig. 1a). The sensor was connected to a multimeter that transmitted information to a computer. The sensor was able to discriminate between acetone and ethanol at varying temperature (best results obtained at 365 °C). The sensor response was constant for flow rates larger than 0.2 L min^{-1}, reaction-limited at the sensitive surface, and almost independent of the relative humidity present in the system. Real-time tests were carried out using breath samples from people at rest and under physical activity, showing no changes in the acetone levels. The concentration profiles measured with the sensor and with the standard proton transfer reaction-mass spectrometer (PTR-MS) showed good correlation, with response times around 27 s and a limit of detection of 20 ppb (Fig. 1b) [54]. This system was not used to monitor diabetic patients, although it was potentially suitable for this application, despite its relatively large dimensions [53,54]. In addition, the need to constantly keep the sensors at high working temperatures prevented their use in wearable, miniaturized platforms. These difficulties were overcome using an array of chemo-resistive interdigitated μ-electrodes coated with 11-mercaptoundecanoic acid functionalized gold nanoparticles (MUA-$Au_{2\,nm}$). The electrodes were located within a chamber incorporating six channels, each associated with a separate electrode connected to a computer controlled flow metre or a manual pump used to simulate real breath conditions. The sensor chamber was also interfaced to an electronic circuit for collecting and conditioning the sensor signals. Detection of pure air, acetone in air, human breath and acetone-spiked human breath samples was demonstrated using pattern recognition algorithms, allowing healthy subjects and simulated unhealthy breathing to be discriminated. This new non-invasive device could change the current clinical management of diabetes while, at the same time, differently decorated Au-NPs would allow the monitoring of other analytes [55].

4. Sweat monitoring

There has been a significant increase in sweat analysis in recent years due to its potential for non-invasive monitoring of fluid and electrolyte loss by elite athletes during events (rehydration optimization) [56] and also for improved clinical management of certain pathologies (e.g. Cystic Fibrosis (CF)) [57], where pH, Cl^- and Na^+ concentration monitoring can provide valuable information. Furthermore, changes in NH_4^+ concentrations can indicate switching from aerobic to anaerobic conditions, which can be linked to the breakdown of proteins related to dietary conditions or to hepatic dysfunctions [50].

As per breath monitoring (Section 2.2), humidity can be an issue and the wearability of the system needs to be considered. An early example by Chang et al. [58] was a conductometric sensor based on poly-(2-acrylamido-2-methylpropane sulfonate) spin coated on a commercial interdigitated electrodes, connected to a miniaturized home-made impedance metre, which was used to continuously monitor human perspiration. The device was integrated into a polystyrene mini-chamber and positioned on top of the palm of people tested. The sweat emerging from the skin was absorbed by the polymer leading to an increase in conductivity. The sensor had a time constant of 38 s and activity was recovered after drying. This quite bulky device was characterized by an undefined response time for the entire system, mainly related to all the difficulties associated with accurate sampling. For this reason, it was necessary to replace it with flexible, wearable sensors based on poly-tetrafluoroethylene developed by Miyoshi et al. [42] (see Section 2.2 and Fig. 2a).

Sweat may also have a role in diabetes management through glucose monitoring, although few examples can be found in the literature. The work done by Talary et al. [59] was based on the dielectrical and optical characterization of the skin. Glucose variations were monitored through a multi-device characterized by three fringing field sensors, allowing the penetration of the magnetic field at three different depths (deep, mid and upper). Changes were mainly dictated by the mid and deep depths, while the data coming form the upper region were used to correct for common-mode variations not caused by glucose. The sensors were connected to a Li-Ion Battery (power of 1800 mAh) integrated in an arm-band (see Fig. 2b). Clinical trials were carried out but accuracy and stability evaluations still need to be performed [60]. Recently, sweat was also investigated as a medium to monitor ethanol using a device based on an amperometric biosensor characterized by a graphite electrode with embedded alcohol oxidase, horseradish peroxidase and ferrocene, in the presence of a working solution (0.05 M, pH 7.4), separated by a PTFE membrane from the contacting skin. It was connected to a miniaturized potentiostat and a microprocessor that translated the obtained signal into variation of ethanol concentration in sweat. Sweating was stimulated by pilocarpine iontophoresis and it was claimed that this allowed the concentration of ethanol in blood to be tracked continuously in real-time. The sensors showed high reproducibility (RSD value of 10.5%, $n = 10$), repeatability (RSD value of 9.1%), with a shelf-life of at least 2 months. The linear range (0.0005–0.6 g L^{-1}) covered the legislation limits (0.002–0.03 g L^{-1}) with reduced false results compared to breath analysers. The system was able to detect and monitor ethanol variations 5 min after ingestion [61].

4.1. Monitoring analytes for sports science applications

Measuring biochemical parameters can be useful when monitoring athletes under effort, as this can help to improve their performance. One of these parameters is lactate, as this indicates the switch from aerobic to anaerobic metabolic conditions. For this, an electrogenerated chemiluminescence (ECL) biosensor was realized using a luminol hydrogen peroxide sensitive compound, lactate dehydrogenase and pyruvate oxidase (as catalyst), adsorbed onto a carbon nanotubes layer. The maximum ECL signal was obtained at pH 8, at a temperature of 30 °C, covering the linear range 8.9×10^{-12} to 8.9×10^{-6} mol L^{-1} (standard deviation of 4.13% in six parallel measurements) and a recovery ability of 101.3% after exposure to lactate. As the device was too bulky to be worn, sweat samples were collected from volunteers and brought to the system, which increased risk of sample contamination [62]. Clearly, the ability to perform such measurements at point-of-need would be much more attractive, as this would allow real-time tests, faster access to critical data, and reduced potential for sample contamination.

A wearable platform for monitoring pH was recently reported. It employed a textile-based fluid handling system made of a moisture wicking material (mixture of polyester (92%) and lycra (8%)), and an optical pH sensor based on bromocresol purple (Fig. 3a), with the optical detection system shown in Fig. 3b. The sensor worked over the range 4–7, suitable for monitoring pH in sweat, and was characterized by good repeatability (within 2%, $n = 2$), without dye leaching during the experiments. The sensor was positioned on the body at the lower back, and held in place by a waist band. The pH values recorded with the fabric sensor were compared to parallel values obtained with a miniaturized pH electrode sited on-body close to the textile based sensing platform [63]. The same device incorporated sensors for monitoring sodium concentration (first tested connecting the Ion Selective Electrodes

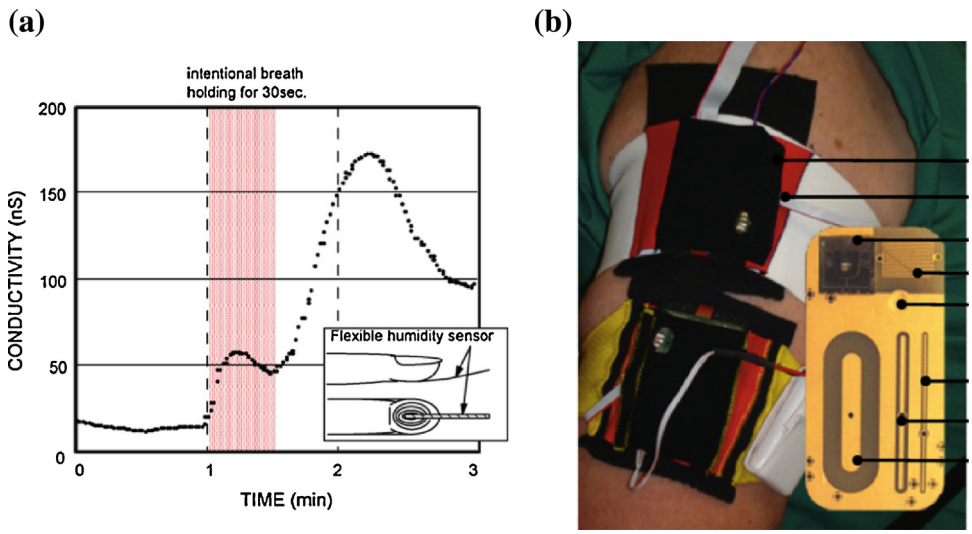

Fig. 2. (a) Changes into sweat rate measured at a fingertip using a wearable miniaturized flexible humidity sensor (adapted from Ref. [42]). (b) A completely wearable system used to monitor glucose in sweat (adapted from Ref. [60]).

(ISEs) to a portable multimeter [64]) and conductivity (shown in Fig. 3c), with the data collected and recorded on a SD card enclosed into the wearable electronic control unit. The time needed to collect the minimal amount of fluid for an accurate analysis was about 35 min [65], due to the delay in onset of sweat generation during exercise, and to the dead volume of the platform. In an attempt to overcome such issues, Curto et al. [66] realized an integrated microfluidic device with a small sensitive area (5 mm in diameter) containing an absorbent material impregnated with the pH sensitive dye bromocresol purple (Fig. 4a). The detection system was characterized by a surface-mount LED and light detector working in transmission mode, which responded to colorimetric changes of the pH indicator (see Fig. 4b). Real-time tests were conducted on samples collected directly from the lower back region of athletes during training. The results were found to be in broad agreement with reference values obtained from a flat pH electrode [66]. A further reduction of the device dimensions was deemed necessary to allow for more convenient wearable applications.

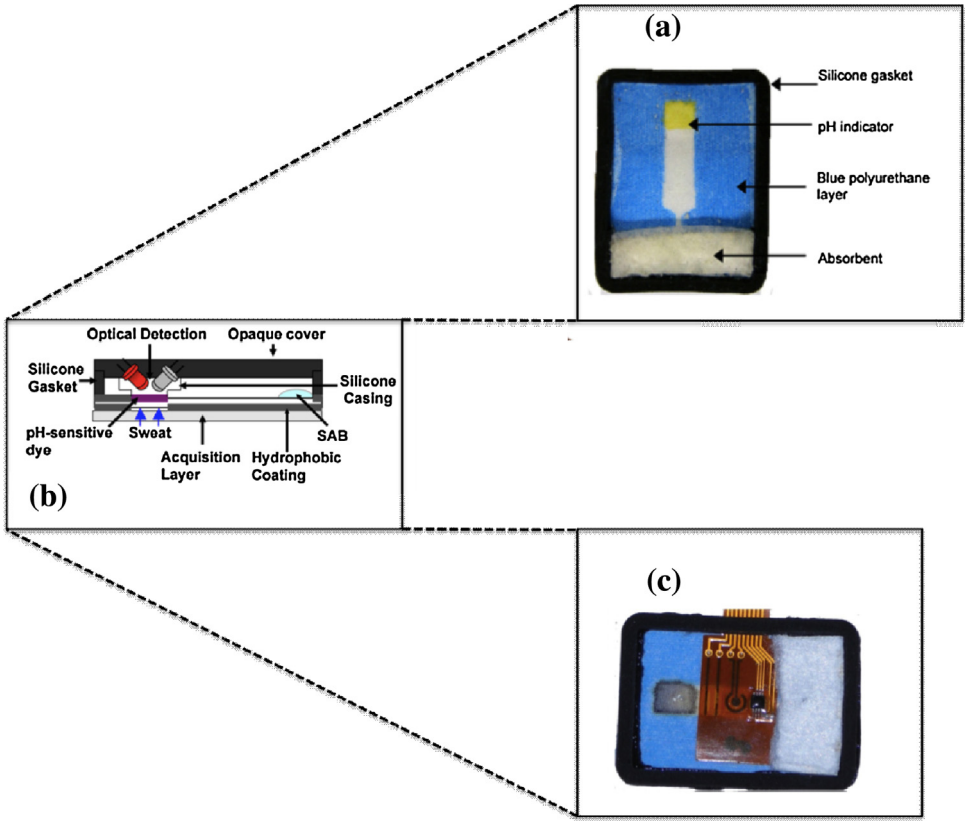

Fig. 3. (a) Top view of a textile-based passive pump with an embedded pH optical sensor. (b) Side view of the sensing area and detection system used (adapted from Ref. [63]). (c) Integration of a multisensor strip consisting of a pH indicator, conductivity, sodium and temperature sensors in contact with the fluidic handling system (adapted from Ref. [65]).

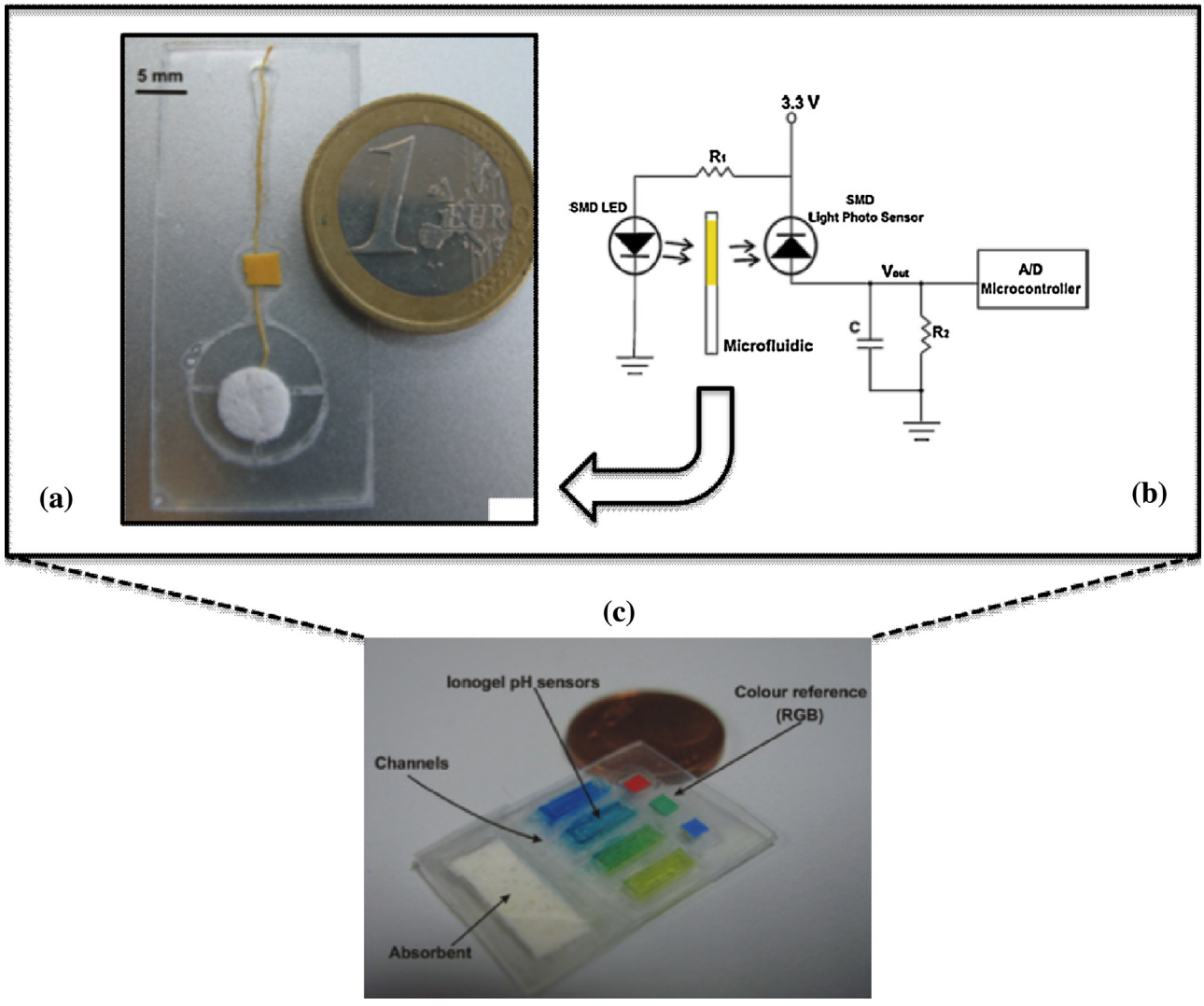

Fig. 4. (a) Microfluidic chip with an integrated pH optical sensor. (b) Electronic circuitry used as the detection system (adapted from Ref. [66]). (c) Microfluidic platform with four reservoirs containing a poly-IL, incorporating four different dyes used as pH indicators (adapted from Ref. [67]).

Improvements were needed in terms of fluidic transportation, better positioning of the components, and shielding of the detectors from external lighting effects. Improvements in performance were offered by patch worn during exercise (Fig. 4c). The device was based on cross-linked polymer gels (N-isopropylacrylamide and N,N-methylene-bis(acrylamide)) incorporating an ionic liquid (trihexyltetradecylphosphonium dicyanoamide) integrated within 4 reservoirs, each of which also contained a different pH dye (methyl red, bromocresol green, bromocresol purple and bromothymol blue) to cover the pH range 4–8, i.e. to overlap the range that can be physiologically found in sweat. Fluid movement through the device was induced by a wicking effect driven by a highly absorbent material, which allowed a continuous stream of fresh sweat to be in contact with the sensitive area, and an operational life-time of approximately 135 min. During an exercise period, images of the sensor array were captured at 10 min intervals, and processed to extract the analytical information. The difference in measurements by this method, with respect to a glass pH meter, was found to be less or equal to 0.49 of a pH unit [67].

4.2. Fabric and tattoos technologies for sweat monitoring

The implementation of wearable chemical sensors would be greatly advanced by the realization of sensing elements embedded in cotton yarns. A recent paper on this topic reported the use of potentiometric fibres, made conductive after repeated immersions in carbon nanotube ink (until a resistance of 500 Ω for a length of 1 cm was reached), partially coated with an ion-selective membrane (from 4 to 5 dips into a modified PVC solution) for pH, K^+ and NH_4^+. The sensors were able to function within the required physiological range (pH 3–11, NH_4^+ 10^{-6}–10^{-2} M, K^+ 10^{-5}–10^{-1} M) and were insensitive to mechanical stresses such as bending or stretching, thus preserving the cotton properties (softness, texture, etc.). They were then integrated into a garment, in a configuration that did not affect their performance, and tested using simulated sweat solutions. The concentration of the ion of interest was varied by changing the fluid that contacted the electrodes through a cellulose acetate layer covering the sensitive area. It is hoped that such strategies will pave the way for mass produced wearable chemical sensors [68]. The pioneering work carried out by the Rogers research group at the University of Illinois at Urbana-Campaign on "epidermal electronic systems" was based on standard lithographic techniques to monitor physical parameters [69–71]. A natural evolution of this approach brought to the adaptation of electronic tattoos to the detection of chemical analytes. Screen-printed technology was used to realize electrodes that could be transferred onto the skin via a tattoo process [10]. So far, potentiometric sensors for ammonium (10^{-4} to 10^{-1} M) [72], pH (3–7) [73] and Na^+ (0.1–100 mM) [74] and

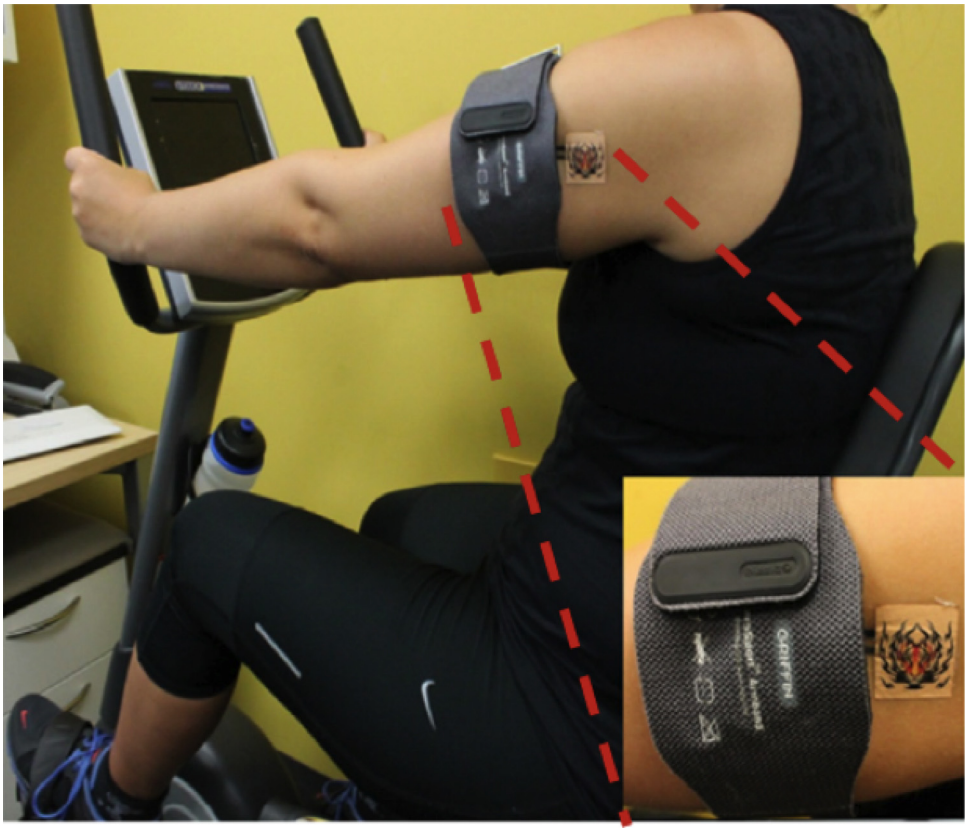

Fig. 5. Example of a wearable sensor for sweat monitoring: skin tattoo connected to a bluetooth electronic platform for sodium monitoring. Adapted from Ref. [74].

an amperometric sensor for lactate (1–20 mM) have been reported based on this approach [75]. The tattoo sensors were able to withstand mechanical tests (stretching and bending) when applied on a Goretex substrate, simulating stress conditions for on-skin measurements. They were also employed during real time tests on volunteers. However, the tattoo sensors were normally connected to standard miniaturized electrochemical commercial potentiometers, except in the case of the Na^+ sensor, which was linked to a Bluetooth wireless wearable transceiver [74], as shown in Fig. 5.

5. Saliva monitoring

Saliva is another valuable source of biochemical information accessible in a non-invasive fashion. While sweat production is primarily an inductive process, saliva is more readily available and therefore possesses advantages for patients suffering from conditions that inhibit sweat production. Saliva is even more readily available and real-time monitoring within the mouth can be extremely beneficial to detect the presence of drugs [76], monitoring healthy mouth conditions [77], and detecting Gastroesophageal Reflux Disease (GERD) events [78].

A number of reports have described the development of SERS biosensor assays. The attention was mainly focussed on the detection of a good indicator for stress such as cortisol [79] and the hormone testosterone [80]. In the first case, the biosensor was characterized by a limit of detection of 49 pg mL^{-1} within undiluted saliva samples, and found to be highly correlated with radioimmunoassay measurements [79]. In contrast, for testosterone monitoring, saliva samples were stripped (i.e. treated with activated charcoal, then centrifuged to remove the charcoal) because of its lower concentration when compared to other analytes. The system was characterized by a limit of detection of 23 pg mL^{-1}, with a suitable sensitivity (range 25–250 pg mL^{-1}) for a good resolution in the range of interest. In both cases the measurements were completed in nearly 10 min, allowing for an almost real-time monitoring system [80]. However, in this case, a benchtop instrument was used, and the approach was not suitable for home-based and wearable monitoring devices where additionally biocompatibility requirements for all the employed reagents must be taken into account.

The need for miniaturized devices is therefore driving attention towards the employment of other techniques such as the development of chemical sensors based on novel fluorescent materials. For example, a new polyfluorene derivative, poly(9,9-bis(6′-benzimidazole)hexyl) fluorene-*alt*-1,4-phenylene (PBP), was developed to detect Fe^{3+} and inorganic phosphate, based on the metal binding (Fe^{3+}) benzimidazole group which is capable of inducing large fluorescence quenching (97%) of the compound. Inorganic phosphate ($H_2PO_4^{2-}$:$H_2PO_4^{-1}$) was able to dequench the Fe^{3+}/PBP complex due to the ability of phosphate to displace the attached Fe^{3+}, showing a recovery of 106%. This assay was used to monitor phosphate in saliva with a dequenching ability of 94% and a limit of detection of 1.44 mmol L^{-1}. Absorbance (280 nm) and fluorescence (340 nm) peaks obtained in saliva did not overlap those of the PBP [81]. This is just one example of a new class of optical sensors that may well gain more popularity in the coming years.

Up to now, however, electrochemical sensors have been much more popular than optical approaches. In the work presented by Kwan et al., phosphate levels were detected through the use of an amperometric screen printed biosensor based on pyruvate oxidase (PyOD) immobilized within a Nafion matrix and covered by a poly(carbamoyl) sulfonate hydrogel as the working electrode. The increase in anodic current at 420 mV versus Ag/AgCl (reference electrode) was caused by the oxidation of H_2O_2 (generated by PyOD

in the enzyme layer), which was proportional to the phosphate concentration. The process was diffusion controlled and the sensor was characterized by a sensitive range of 7.5–625 μM (limit of detection 3.6 μM), a fast response time (2 s), but slow recovery time (2 min). Efficient functioning was maintained for 12 h. When testing 50 saliva samples, good correlation coefficients were obtained between the biosensors and a commercial kit [82]. Advances in terms of miniaturization were obtained realizing a microflow-injection system housing a disposable screen-printed biosensor based on the reactivation (in 15 min) of the enzyme cholinesterase (AchE) (inhibited after exposure to organophosphate) using pralidoxime iodide. A 3-fold dilution of saliva samples showed a reproducible response (less than 4% of average relative standard deviation) demonstrating (through its low cost, simplicity and sensitivity) the opportunity to assess subclinical organophosphate exposure [83]. A similar configuration was also used to monitor amylase, in which the sensitive element was based on an amperometric biosensor containing glucose oxidase and peroxidase. This device was characterized by a linear range of 0–190 kU L^{-1}, using a sample volume of 50 μL (injected at a flow rate of 0.79 mL min^{-1}), with no significant degradation of the enzymatic activity during continuous use over a period of 48 h [84].

Glucose and pH are potential targets of great interest for minimally invasive systems. While knowledge of glucose levels could assist improvements in terms of diabetes management, pH is very important in GERD monitoring, which relies otherwise on very invasive esophageal manometry over a period of 24 h. Monitoring of glucose levels in saliva was reported using pyrene-1-boronic acid functionalized carbon nanotubes transistors realized via standard photo-litographic techniques. It was found that with increasing glucose concentration, the drain current was lower because of the increased carrier scattering due to the presence of boronate anions, which decreased the mobility in the transistor. The sensor response was estimated to be 1.3 s, with quite long recovery times (1 h). They showed insensitivity to interferents such as lactose (for levels lower than 1 mM), with a limit of detection of 300 nM, suggesting potential future use as an integrated device able to detect glucose variations in saliva [85]. Similarly, dual-screen printed pH ISE/reference combination electrodes were used to realize a fully operational and disposable pH device. This system was able to work within the pH range 4–8, and the data were highly correlated with results obtained using a bench top pH meter [86].

A more radical strategy to improve wearability of devices employed direct integration of the sensing device within the mouth of a patient. Different biosensors were successfully developed, for example, to detect lactate variations in saliva [87,88] but only Wang et al. [89] proposed a full integration into a mouth guard platform. In this work, three screen printed electrode configuration of a printable Prussian-Blue layer was covered by a poly-orthophenylenediamine layer that entrapped the lactate oxidase (avoiding potential interferences) as working, an Ag/AgCl layer as reference and a Prussian-Blue graphite ink as counter electrode. It was suitable for monitoring lactate levels over the physiological range 0.1–0.5 mM, enabling the estimation of the lactate via a standard addition method [89]. However, the system evaluation was restricted to bench measurements, and no in-mouth real-time measurements were reported.

Another application was devoted to monitoring different bacteria through the use of nanosensors printed as a graphene layer on a water-soluble silk thin film and contacted by gold interdigitated electrodes connected to an inductive coiled antenna (see Fig. 6a). The nanosensors were then transferred onto suitable substrates (such as tooth enamel or tissue) while the water-soluble silk layer was completely dissolved, allowing the attachment of the Au–Graphene nanosensor to the substrate of interest. The graphene layer was functionalized with a dodecapeptide graphene binding peptide, a triglycine linker and the naturally occurring antimicrobial peptide odorranin-HP, working as a biorecognition element. The resulting sensor showed activity towards *Escherichia coli*, *Helicobacter pylori* and *Straphylococcus aureus*. Detection of a single bacterium was claimed, due to the highly sensitive change in the electrical conductivity of the graphene layer, modulated and wireless monitored through an inductively coupled RF reader device. Detection of helicobacter pylori cells within saliva samples with a limit of detection of ~100 cells was also reported [90].

6. Tears fluid monitoring

The opportunity to integrate glucose sensors within contact lenses represents a high impact challenge for diabetes monitoring. It is then fundamental to correlate better blood glucose levels to corresponding concentrations in tears [91], and to do so a better understanding of the mechanisms behind tear secretion is needed. For example the mechanical stimulation of tears induces higher glucose levels [92]. Moreover, it is known that diabetes mellitus can affect the production of tears, their composition, the anterior ocular environment [93] and the structure and function of the tissues that contribute to their production [91]. Therefore the future implementation of sensor technologies within a contact lenses platform may lead to new continuous, noninvasive solutions for monitoring glucose and other important analytes [93,94].

6.1. Electrochemical sensors for monitoring tear-fluid

Most research aimed at improving glucose monitoring in tears has relied on electrochemical approaches. The first attempts to detect glucose levels in the lacrimal duct fluid were based on a device inserted directly in the canaliculus due to the development of flexible, thick-film, miniaturized electrodes. The working electrode was based on a carbon ink containing glucose oxidase (10,000 U GOx/gm) while the reference was an Ag/AgCl layer, both characterized by etched copper leads connected to insulated wires. An insulating layer was then applied on almost all the electrode area and then rolled in a tubular configuration (0.7 mm) suitable for insertion into polyethylene tubing that functioned as the electrode body. The measurements were carried out at a potential of +0.7 V and the linear range was reported as 20–200 μM, with a detection limit of 8 μM [95]. This configuration did not have the expected degree of uptake in the market, mainly due to its quite invasive nature. However, other research groups followed this trend through an amperometric enzymatic needle type glucose sensor, with improved performance, such as a working range of 1.5–800 μM and minimal interferences from the most common molecules found in tears (e.g. %RE for ascorbic acid = 7.56, uric acid = 11.16 and acetaminophen = 4.85). Glucose levels recorded in tears and blood every 30 min showed intra subject consistency but poorer performances when considering the whole sample population. The development of a general model that would allow correlation of blood and tear glucose concentrations is thus not a straightforward task and requires further investigation [96]. Further improvements were obtained by using a coulometric rather than amperometric approach, thanks to coulometric enzyme based biosensor characterized by a lower limit of detection of 0.38 ± 0.13 μM (dynamic range 10–800 μM). Tear and blood samples were collected every 30 min within 7 h. However, intersubject variability was still manifested when glucose levels in blood were compared with tear fluid in the right and left eyes of the same subjects. A possible solution might involve the precalibration of the system before each measurement using tear and blood samples in order to obtain the exact ratio between blood and tear glucose concentrations [97]. A similar configuration based on

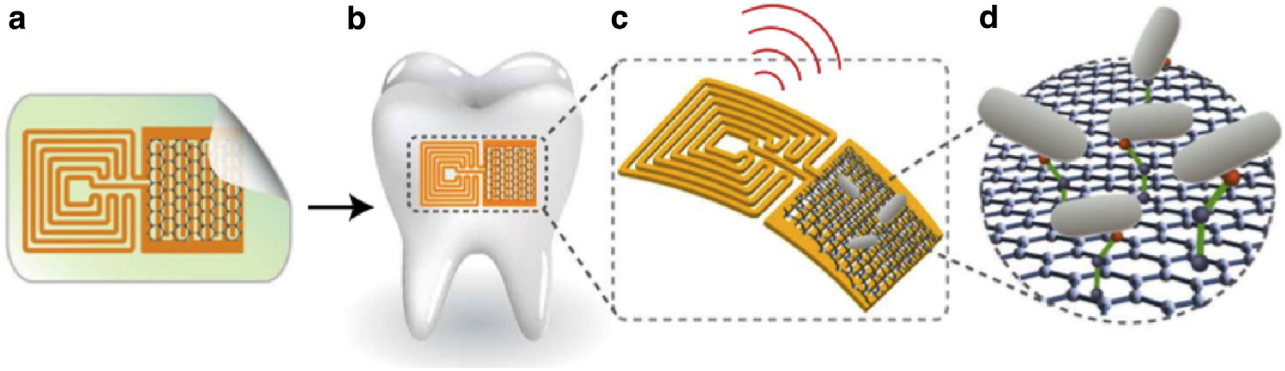

Fig. 6. (a) Tattoo based wireless nanosensor for bacteria monitoring within the mouth. (b) Sensor positioned on a tooth. (c) and (d) Wireless transmission of the signal during analyte interaction with the graphene sensitive sensor layer.
Adapted from Ref. [90].

flexible Teflon tubing integrating a three working-electrode system allowed variations in dopamine, ascorbate and glucose to be tracked in tear fluid. Thanks to its flexibility, this device represents one of the best examples of minimally invasive point of care systems [98].

Another approach is to integrate electrochemical sensors within contact lenses. This solution dates back to 2007 when an amperometric biosensor was realized on a flexible polymeric substrate through Soft-MEMS layer by layer techniques. It was based on GOx immobilized on a polypropylene gas permeable membrane placed on a film-type oxygen electrode located on a Pt working electrode. The sensor was able to cover the relevant normal range of glucose in tear fluid (0.025–1.475 mmol L^{-1}) while located on the pupil of a rabbit and kept in position with fixing tape. Upon obtaining a stable baseline signal, changes in the signal were used to monitor the effect of oral administration of glucose. Blood glucose concentration was monitored simultaneously, and this was characterized by an almost immediate response after bolus injection. In contrast, equivalent event tracked in tears were delayed by 10–20 min [99]. The same sensor was employed to measure kinetics of tear secretion by glucose instillation (20 μL) within the eye (5 mmol L^{-1}, 10 mmol L^{-1}). The current was stable at 0.5 μA and then increased after glucose instillation up to values of 0.61 μA (5 mmol L^{-1}) and 0.73 μA (10 mmol L^{-1}) after 45 s [100]. While this approach is exciting, the low biocompatibility of the employed materials was a significant issue [99].

This problem was overcome integrating the GOx within a polytetrafluoroethylene membrane, using the phospholipid based polymer 2-methacryloxyloxyethyl phosphorylcholine (HPC) copolymerised with 2-ethylexylmethacrylate (EHMA:PMEH). The same design was implemented with the only difference being the use of a PDMS substrate. The flexible, wearable electrode worked within the range 0.05–1.00 mmol L^{-1} (the reported range of glucose in tears is 0.14–0.23 mmol L^{-1}), with an average response time of 41.6 s and sensitivity of 0.95 μA mM^{-1} [101]. The glucose basal concentration was extrapolated from the calibration curve obtained before the positioning of the sensor within the eye (0.11 mM) [102]. The real-time response was also monitored after glucose addition within the eye, which was characterized by an increase in the current signal, immediately followed by a dropping back towards the baseline levels, indicating dilution due to secretion of new tear fluid corresponding to 29.6% ± 8.42 min^{-1} [103]. Inflammation effects were absent within the trials but the fact that the system employed physical wire inter-connects to the potentiostat was a drawback [102].

These difficulties were sorted through the development of a wearable, wireless contact lens based on a PET substrate integrating an amperometric concentric three-electrode design (Fig. 7a). The GOx solution was immobilized in a titania sol–gel membrane covered by a Nafion layer. The pads for the potentiostat connection were placed at the border of the contact lens, to allow unobstructed vision for the wearer (Fig. 7b) [104]. This design was improved using a differential sensing arrangement based on an activated glucose oxidase (working electrode) and de-activated glucose oxidase ('control' electrode) employed in differential mode. They were integrated with a printed Au antenna and readout/telecommunication circuitry realized via microelectromechanical techniques. Tests showed that the differential approach enabled the signal component arising from the main interferent species at the concentrations normally found within tears (ascorbic acid 50 μM, lactate 10 mM and urea 10 mM) to be subtracted from the activated glucose oxidase electrode signal. The combined system was characterized by a linear range of 0–2 mM. However, while a stable signal was obtained for ca. 12 h (97.4% of initial activity), performance deteriorated over time, and by day 4 it had declined to 54% of the initial signal. The effect of protein fouling was significant, causing a decrease to 49.9% of the signal recorded. Nevertheless, these results are promising, and they suggest that a use model based on daily replacement may be possible. Because of its reasonable stability, and its wearable and wireless nature, this system could potentially represent a significant first step towards the realization of a smart contact lens [105] that could revolutionize glucose management in diabetic patients.

The same configuration was also examined for monitoring lactate levels, using a smaller working electrode (area 0.19 mm^2) based on lactate oxidase immobilized in a film based on bovine serum albumin (BSA) covalently and cross-linked glutaraldehyde, and a 'control' electrode (same composition as the working electrode but without the enzyme). These electrodes were once again used in a differential configuration to enable common mode signal components arising from interferents to be automatically compensated to some extent. The entire system was covered with a medical grade polyurethane layer to reduce enzyme leaching, and a final Nafion outer layer. The sensors had a linear range 0–1 mM (limit of detection 50 μM, ca. 20 times below the minimum concentration of lactate in tears), and a response time of 25 s. Moreover, while the response was not linear within the range 1–5 mM, there was sufficient resolution and accuracy to enable this part of the analytical signal to be employed for clinical measurements. The system was reported to function satisfactorily for 24 h [106].

6.2. Optical sensors for tears monitoring

As described in Section 6.1, commercial contact lenses represent a good substrate for immobilizing transduction elements. Because of its high affinity for different types of sugars, fluorophore labelled

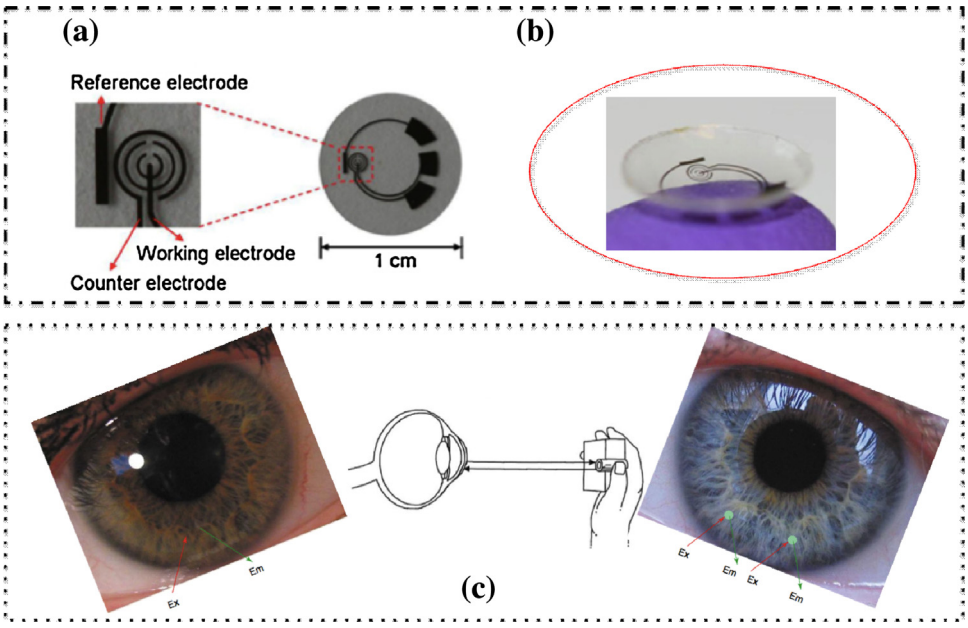

Fig. 7. Examples of devices used to monitor glucose in tears. (a) and (b) Integration of a wireless electrochemical sensor within a contact lens (adapted from Ref. [104]). (c) Glucose sensitive contact lenses doped with a boronic acid glucose receptor together with the schematic of a possible glucose monitoring device for measuring the emission intensity of the system (adapted from Ref. [109]).

boronic acid derivatives (BAFs) represent one of the most studied optically responsive materials for glucose monitoring because of their ability to chelate different monosaccharides. Research specifically related to their application within commercial contact lenses has been mainly carried out by Badugu et al. [107] commencing around 2004 [108]. This group examined a number of derivatives in order to identify the ones with particularly high glucose binding affinity [109]. The dynamic range of the water-soluble BAFs was found to be reduced when solution experiments were compared to equivalent measurements with BAF immobilized within the contact lenses [108]. The best performance was obtained using 6-methylquindinium, used as the fluorescent indicator along with the ortho isomeric form of N-(boronobenzyl)-6-methoxyquinolinium bromide (*o*-BMOQBA). It was found that the pK_a of the probe was also reduced (which is an advantage because of the slightly acidic environment of the contact lens), and the boronate diester formed after the sugar complexation was stabilized. The addition of glucose and its subsequent binding to the boronic acid receptor caused a decrease in the fluorescence intensity (for example, the decrease is ca. 13% for glucose concentrations lower than 1 mM) [108]. The response range was reported as 50–500 μM [110]. Initially, this new fluorescent boronic acid probe was photo-physically characterized versus control probes that did not contain the boronic acid functional group. Subsequently, the contact lenses were functionalized with the sensitive compound and subjected to leaching, interference, pH and shelf-life stability tests [107]. It was found that the modified lenses changed colour in response to changes in glucose concentration, with a response time of 10 min [109]. A significant disadvantage of this approach is the need to excite the fluorophore by passing light into the eye in order to collect the emission spectra, as shown in Fig. 7c [107].

A different technique based on the use of optical near infrared spectroscopy and new glucose responsive materials based on polymer crystalline colloidal arrays was also applied to detect glucose in tears. The materials were based on poly(styrene-co-acrylamide-co-3-acryloamido)phenyl boronic acid embedded within a slightly positive hydrogel of poly(acrylamide-co-2-(dimethylamino)ethylacetate). This material showed spectral responses in the near infrared region (around 1722 nm) when binding glucose, which were detected with a UV-VIS-NIR spectrophotometer. The limit of detection was reported as 6.1 μg dL^{-1}, with almost no response to the main interferents such as lactate, albumin, and ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}) (maximum relative error of ±3.3%). The effective temperature range was 15–43 °C, and the response time 22.1 s at a glucose concentration of 7.5 mg dL^{-1}. If integrated within a contact lens, this polycrystalline material may provide a route to non-invasive, continuous optical monitoring of glucose in tears [111]. In the future, photonic crystals capable of glucose-selective light diffraction behaviour should also be considered. When this effect coincides with the visible region, colour changes occur without the need for a dye label, and this effect could be used to determine the glucose concentration in the surrounding fluid through the use of a colour chart or a simple optical sensor. A significant disadvantage is the pH dependence of the binding behaviour of boronic acid receptors [112]. Therefore, significant attention is now focused on materials such as hydrogels based on (3-acrylamidopropyl)trimethylammonium chloride (ATMA) co-polymerised with 2-acrylamidophenylboronic acid (2-APB). These are reported to be sensitive to glucose within the pH range 6.5–7.8, with negligible interference from the biological medium [113].

7. Conclusions and emerging trends

Real-time monitoring of biochemical analytes within biological fluids requires research that crosses disciplines, spanning from electrical engineering and physics to materials science, chemistry and biology. In order to become commercially available for continuous health monitoring, wearable chemical and bio-chemical sensors must overcome significant challenges related to wearability, comfort, device and material biocompatibility, efficient energy usage, acceptable analytical performance and a practical usage model. Despite the wave of optimism that manifested related to implantation of biosensors within the body some 30 years ago [114], in reality, sensor technology is still far from delivering on this early promise of long-term use. Perhaps the most significant obstacle is biofouling, as it rapidly and profoundly affects sensor sensitivity and durability by altering the sensor-body fluid interface, leading to sensor malfunction [115]. Since the sensor

is implanted, the body perceives it as a foreign object and, as a result, biological material builds up on the sensitive sensing surface. In contrast, implants that do not possess these very sensitive surfaces are used successfully in very large numbers. For example, cardiac pacemakers are implanted in 600,000 people per year [116] as part of a standard procedure. However, advances in biosensor performance continue to be reported, such as a glucose sensor (based on an enzyme-immobilized and amperometric detection) that has been implanted fully subcutaneously for extended periods (1–2 years) in pigs. Accurate glucose measurements were obtained over this duration after an initial stabilization period (2–3 weeks after implantation) [117]. In contrast, needle-type glucose sensors implanted percutaneously cause infections and require frequent sensor calibration due to response drift [118]. Understanding and controlling in vivo surface effects constitutes one of the greatest challenges for biosensor research. Strategies investigated include improved enzyme stabilization through entrapment in conductive polymer matrices [119] and ionic liquid membranes [120], coating of the sensor with biocompatible polymers [121], and the use of drug-release strategies for enhanced tissue integration [122–125].

On the other hand, wearable sensors placed in close contact with the body are easily accessible and do not suffer to the same extent from biofouling. Perhaps even more importantly, they are amenable to short term use models (hours, days compared to many years for implants). Despite this, as already discussed in the previous sections, the number of effective wearable biochemical sensing devices reported in the literature is limited. Enhancing comfort while minimizing size and maximizing efficiency will increase the likelihood of their adoption by users. Recent advances in materials science are starting to revolutionize this area thanks to flexible and stretchable fibres based on carbon nanotubes (CNTs) and graphene. At the same time, organic electronics is also demonstrating beneficial impact on wearable sensors [69]. Other flexible and stretchable electrolytes based on ionic liquids and polyionic liquids may also have a role to play in the development of wearable electro-chemical and biochemical sensors [120,126,127]. Ionic liquids have shown potential as effective solvent media for many enzymatic reactions, and under certain conditions, several have demonstrated enhanced biocatalyst activity, thermal stability, and reusability [126,128].

Moreover, suitable power sources (including power scavenging approaches) must be considered for practical applications of wearable devices. For example, portable, flexible batteries [129–131] or energy harvesting approaches can be integrated into the wearable platforms and used to power wearable devices.

Other components like antennas, fabricated by printing or etching metal patterns on rigid substrates, can be made flexible and stretchable, using new electronic materials and/or new device configurations. Recently, they have been realized using CNT sheets and polymer substrates [132] or silver nanowires embedded in an elastomeric substrate [133]. These antennas are thus well suited for applications like wireless strain sensing and could be implemented in smart textiles.

The newly developed electronic "tattoo" technology probably constitutes the state-of-the-art in wearable sensors and has been demonstrated for epidermal pH monitoring [73], real-time lactate sensing in human perspiration [75], and detection of bacteria in saliva [90]. These devices are easily fabricated using well established printing technologies and have the advantage of being non-invasive, and mechanically stable when directly applied on the skin [134] or teeth [90]. Although at present there are only few examples demonstrated, the potential of this technology is tremendous [135].

Recent advances in analytical chemistry, materials science, microfluidics and electric engineering are indeed deeply influencing technological approaches to full integrate biochemical sensors into wearable devices. Physical sensors directly embedded into garments are already present on the market, often as the result of close interactions between universities and international companies. Checklight by Reebok, LifeShirt by Vivonoetics, and other "smart" garments (including socks, t-shirts, and bras) by Sensoria are just few examples of commercially available items. Similar collaborations are now advancing research activities aimed at the development of low-cost wearable biochemical sensors. Several small companies (like OrSense) and spin offs (such as Electrozym) that exploit low-cost technologies to realize non-invasive tools for biochemical sensing have recently made some impact in the global market. Simultaneously, larger companies are increasingly interested in the potential applications of wearable biochemical sensors. For example, Google recently filed a patent [136] and announced a project to further develop the electrochemical glucose sensors integrated into contact lenses [137], probably with the idea of eventually linking these sensors with the Google Glass instrumented spectacle platform. This has the advantage of enabling the sensorised contact lens to be inductively powered from the Google Glass platform due to the short distance involved, which greatly simplifies the lens requirements, as no integrated power source is required. Simultaneously, rumours abound on the possible integration of a glucose monitoring function integrated into the forthcoming Apple i-Watch [138], which will further integrate into the Apple 'HealthKit' health information software environment [139]. However, the most likely scenario will be an informatics platform level integration of biochemical information generated with existing technologies, rather than full integration of biochemical sensors into the iWatch platform, unless this can be achieved with a use model based on a replaceable sensing module (maximum use perhaps up to 1 week, more likely daily), as envisaged with the contact lens platform described above.

These are just a few examples of "a new wave" of innovation in the design and fabrication of devices for non-invasive monitoring of biological fluids that has just started, and involves exciting collaborations between university research teams, clinical groups, community health service providers, small companies emerging with specific technology offerings, and huge global corporations keen to leverage their existing technologies into new market offerings. It is clear that wearable biochemical sensors will be a central part of this offerings, which will provide patients and clinicians with extensive information for optimal management of a range of chronic conditions, enabling home-based therapeutic strategies and healthcare provision, which will generate substantial improvements in terms of quality and life expectations of people.

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