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Flexible substrate sensors for multiplex biomarker monitoring

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Abstract

Wearable healthcare technologies should be non-invasive, robust to daily activity/environments, easy to use and comfortable to wear. Flexible substrate devices for biomarker monitoring can contribute to wearable diagnostic applications. Single target biosensors have extensively been developed for health monitoring applications, however recently multiplex biomarker tests have generated clinical interest. Targeting multiple biomarkers in diagnostic systems (wearable or point of care) offers more focused diagnosis and treatment as changes in a single biomarker can be caused by a series of physiological conditions. This review highlights flexible substrates that have been successfully demonstrated for multiplex biomarker detection with potential for healthcare monitoring.

Introduction

The single target biosensor evolution from laboratory to wearable functionality was demonstrated by the classical glucose oxidase (GOx) biosensor, first described by Clarke in 1962 and developed by Medtronic in 2005 for continuous blood glucose monitoring. Single target sensors have extensively been researched and commercialised for metabolites, antibodies and proteins etc. Bio-fluids such as saliva, tear, sweat and interstitial fluid generate significant research around disease specific biomarkers. Such fluids are naturally secreted by the body and are painlessly sampled unlike invasive blood draw. Saliva incorporates protein biomarkers [1] relevant to local cell activity and biomolecular function. It is used to monitor creatine, fibrinogen, haemoglobin, triglyceride, glucose levels and correlates to blood pressure [2]. Tear fluid contains lipids, electrolytes, metabolites and proteins [3] suitable for disease monitoring. Sweat is extensively used to measure physiological parameters [4,5,6] and incorporates protein biomarkers associated with genetic diseases[7]. While single target assays monitor specific conditions such as diabetes, multiplex protein screening offers improved diagnosis, prognosis and treatment for cancer. Recent reviews [8,9,10] have highlighted wearable technology progress around materials, assays and instrumentation. Flexible substrates offer mechanical properties suitable for wearable devices with Young's moduli compatible with skin applications [11]. Device structure typically consists of flexible layers, including support substrate, active layer and electrical connections. Careful design [12] avoids device failure (cracking and delamination), caused by stretching and bending. Flexible substrate materials include paper, polymer and textiles. All offer biocompatibility and robustness during device fabrication and biomolecule immobilisation. Natural polymers include cellulose, silk, wool and cotton, while synthetic polymers include nylon, polyethylene, polyester and teflon. Wearable biosensors progressed as conductive polymers (polypyrole, polyaniline, polythiophene) improved device integration with flexible substrates, delivering good electrical properties for sensor applications. Incorporating graphene[13,14,15], carbon nanotubes[16,17], metal nanoparticles[18,19] and semiconductor materials into active layers improved electrical and mechanical properties[20] of flexible devices. Issues around fouling and biomolecule interference have been alleviated with biomolecule selective

membranes, immobilisation matrices and antifouling layers [21,22,23]. Such technologies have contributed to advancement in wearable sensors. Electrochemical sensors offer distinct advantages over optical detection in conformal substrates for biomarker detection. For multiplex assays, multiple fluorophores or microarray approaches require complex optics (e.g. sources,lens, filters, sensor arrays) to be integrated on a single rigid substrate to maintain optical alignment. Flexible electrode systems facilitate bending and twisting having minimal signal impact. Microelectrode arrays manufactured on single substrates, facilitate high density multiplex detection. Signal to noise enhancement in optical systems often require long optical pathlengths (e.g. absorption) or high power sources (e.g. fluorescence) to reach clinically relevant biomarker levels. Such approaches can increase system size, power and may require specific thermal management. With electrochemical sensors, performance enhancing approaches include target selective membranes, materials (e.g. carbon nanotubes) enhancing electrical performance which can be incorporated without size or power impact. In this review we highlight applications of flexible substrates to multiplex biomarker monitoring with potential for health monitoring applications.
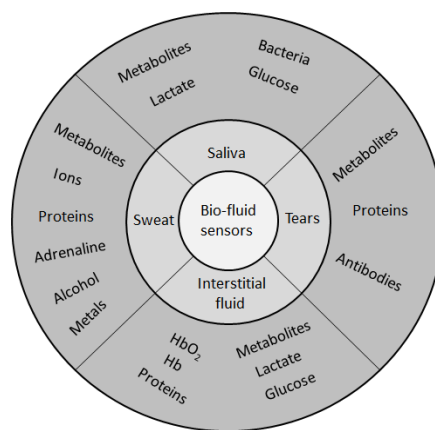
Figure 1: Overview of target biomarkers within saliva, tear, sweat and interstitial fluid (ISF), to be monitored at the eye, skin and mouth locations with potential for multiplex combinations.

Saliva, sweat and tears liquids contain biomarkers (figure.1), which are easily accessed using wearable sensor technology, to diagnose and manage a range of clinical conditions. Significant potential for multiplex monitoring exists, due to biomarker diversity within each sample type. In this review we highlight applications of flexible substrates to multiplex biomarker monitoring with potential for health monitoring applications. The potential for wearable biomarker devices to non-invasively monitor a range of physiological conditions has generated significant interest as outlined in table 1, where clinically relevant biomarker ranges to monitor healthcare conditions are highlighted. For each flexible substrate type, device fabrication, assay implementation and performance is outlined for a range of multiplex applications screening for cancer biomarkers, electrolyte imbalance and proteins etc. This review focuses on electrochemical detection methods as they have progressed more than optical techniques for wearable applications. The approach taken is to review the three main flexible substrate

categories: (i) Paper/paper hybrids, (ii) synthetic polymers and (iii) fabrics as reported in the literature and to highlight multiplex biomarker combinations demonstrated with each substrate type.

Biomarker	Sample type	Health condition	Range	Ref.
Glucose	Saliva,Sweat	Diabetes	0.5mM - 1.6mM	24
Glucose	Tear	Diabetes	0.025-1.475mmol/l	25
Proteins	Sweat & Saliva	Disease screening	ng/ml - pg/ml	26
Electrolytes	Sweat	Dehydration	0-110mM	27
Interleukin 6	Sweat	Inflammation	0.02pg/ml - 20pg/ml	28
Lactate, salts	Saliva	Dehydration	0 - 110mM	29
Zn,Cd,Pb,Cu,Hg	Sweat	Heavy metal poisoning	100 - 300µg/l	30
Potassium	Sweat & Saliva	Hypo & hyper Kalemia	3.6 -5.2 mmol/l	31
Alcohol/Ethanol	Sweat	Intoxication	0 - 36mM	32
Cortisol	Saliva & sweat	Hypertension	7-28 µg/dl	33
Pathogen cells	Sweat/urine	Infectious disease	10 – 100 cells/ml	52

Table 1: Examples of common target biomolecules, sample fluid and clinical concentration ranges used as applications for wearable health monitoring systems.

Paper/hybrid devices

Cellulose is an abundant, natural, low cost polymer (trees, plants, bacteria, algae) extensively used in bioassays [34]. Paper assays generate significant research interest, they're inexpensive, easy to use, flexible, consume low reagent volume and deliver rapid results [35,36,37]. In this section we highlight paper and hybrid/paper devices applied to multiplex biomarker monitoring, in applications including cancer, metabolites and pathogen detection. Microfluidic paper-based analytical devices (mPADs) is a term used for paper and hybrid test devices [38-42].Capillary action makes paper an ideal material for wearable/point of care applications, avoiding the need for pumps, as local surface modification (e.g.wax [43],PDMS [44]) manipulates sample flow to reaction sites. Tests are primarily based on enzyme linked immuno-assays (ELISA), where reagents can be directly adsorbed onto porous paper at reaction sites. Electrochemical detection is extensively used as low cost printing techniques (inkjet) dispense materials forming electrode patterns. Electrochemical detection is extensively used in portable systems due to low power consumption and simple instrumentation, a significant advantage over optical systems.

Glucose, lactose, uric acid

Assays incorporating catalytic reactions, identifying multiple analytes have been demonstrated on paper substrates. A multiplex electrochemical device was demonstrated for glucose, lactose and uric acid detection in human serum samples [45]. Electrodes were screen printed from carbon ink containing Prussian blue for the working electrode (WE) and counter

electrodes (CE), the reference electrode (RE) was Ag/AgCl. Prussian blue (PB) is extensively incorporated as a mediator into electrochemical assays, facilitating electro-potential shift to mitigate against competing biomolecules. Test areas were prepared by spotting 0.3µL of glucose oxidase, lactate oxidase and uricase solutions onto WE areas. Chrono-amperometric detection was used to monitor enzyme reactions within each target zone, at a sampling rate of 10Hz. PB as an electrode mediator, reduced catalytic reaction potentials over the range -0.2mV to 0.2mV (Ag/AgCl) minimising interference from uric and ascorbic acid. Detection was based on reduction of H_2O_2 at 0V. The limit of detection (LODs) were; glucose 0.21mM (range 0-100mM), lactate 0.36mM (range 0-50mM) and uric acid 1.38mM (range 0 – 35mM) in human serum. Direct enzyme immobilisation on WE's is a popular approach to electrochemical sensor implementation. However incorporating 3D structures can improve performance and enhance specificity. A hydrogel-paper hybrid assay demonstrated glucose & protein detection in urine [46,47]. Microfluidic channels were defined by patterning hydrophilic paper with hydrophobic polymer for controlled liquid flow, delivering a low cost approach to multiplex biomarker detection. The paper substrate was soaked in SU8 polymer solution, following UV curing, non-crosslinked polymer was removed in a propylene glycol monomethyl ether acetate (PGMEA) solution. Three dimensional hydrogels enhance reagent immobilisation and assay sensitivity, through increased target capture and optimising enzyme activity [48]. A novel screen-printed microfluidic paper-based analytical device, with all-carbon electrode-enabled electrochemical assay (SP-ACE-EC-µPAD), simultaneously detected glucose and uric acid in urine [49].Carbon ink electrodes were deposited on the substrate using low cost screen printing. Glucose oxidase and uricase were deposited in reservoir locations by spotting 2µL of enzyme solution, followed by air drying (20 minutes).Glucose and uric acid were detected in urine using chrono-amperometry, providing fast and accurate results. Spiked glucose (0.25,0.5,0.75Mm) and uric acid (0.1,0.2,0.3mM) samples (20µL) were evaluated on the device, delivering results within three minutes. This simple detection approach applied 300mV step potential with current monitored over time and was highly suitable for wearable applications.

Proteins

Colour change detection is an instrument free approach, extensively used with lateral flow assays. Such tests usually identify a single target. In a novel approach to multiplex diagnostics, hydrogel was formed on a paper substrate using an aptamer crosslinker, trapping glucoamylase (GA)[50]. Glucose detection, based on enzymatic oxidation of iodide to iodine, altered test spot colour from clear to brown. For protein detection, spots changed from yellow to blue following tetrabromophenol blue (TBPB) ionisation. The device used a target responsive aptamer crosslinked hydrogel, for selective target recognition. With the target present, the hydrogel collapsed releasing GA into solution, amylose hydrolysed by GA generated glucose as the liquid progressed along the paper. A catalytic GOx reaction along the channel converted glucose to gluconic acid & H_2O_2, resulting in a brown colour change as horse radish peroxidase (HRP) catalysed poly(DAB) from colorless 3,3'-diaminobenzidine (DAB). The resulting colour change length along the test strip correlated to target concentrations. The

flexibility of the hydrogel – aptamer structure facilitated multiple target detection in urine, i.e. glucose (0.7–10.5mM), cocaine (0–100µM) and adenosine detection (0 to 800µM). Adenosine is a cancer biomarker used to monitor disease progression [51]. While colour based detection is fast, results are subjective and may suffer from reduced sensitivity. The authors highlight how distance based colour detection is less subjective compared to spot colour change and performance was comparable to commercially available dipstick tests.

Cell targets

ECL combines electrochemical activity with optical detection by incorporating a chemiluminescent molecule into the ELISA assay. This approach is useful for quantitative detection and gives enhanced sensitivity over purely colour based assays. Conventionally ECL assays are implemented in microwells or on microfluidic devices, however paper based ECL has been demonstrated. A hybrid paper-PDMS device was manufactured for rapid multiplex pathogen detection [52]. The hybrid approach offered a simple, biocompatible, 3D material for reagent storage and immobilisation, without complex surface chemistry, while PDMS microfluidics defined reaction zones. Fluorescent aptamers functionalised with graphene oxide (GO) were deposited by pipette on chromatography paper. The aptamer-GO solution was adsorbed onto paper defining a probe microarray, screening for target pathogens. GO in close proximity to a fluorescence probes caused quenching, thus switching CY3 labelled aptamers to an "off" state. The aptamer became rigid with specific target binding, increasing GO-Cy3 separation, switching "on" fluorescence signal by reducing quenching. The simple test required sample loading followed by incubation (10min), without washing prior to fluorescence detection. This approach offered direct pathogen specific detection without nucleic acid screening techniques (e.g. PCR). *Lactobacillus acidophilus* detection was demonstrated over the range 0 – 300cfu/ml with an estimated LOD of 11cfu/ml. Multiplex pathogen detection was demonstrated for *Staphylococcus aureus* and *Salmonella enterica*, achieving good specificity (figure 2). The detection range for *S.enterica* was 42.2‑675.0 cfu and for *S.aureus* 104‑106 cfu/mL, with LOD of 61.0 cfu/mL (*S.enterica)* & 800.0 cfu/mL (*S.aureus*). Performance was comparable to cell culture and molecular diagnostic approaches.
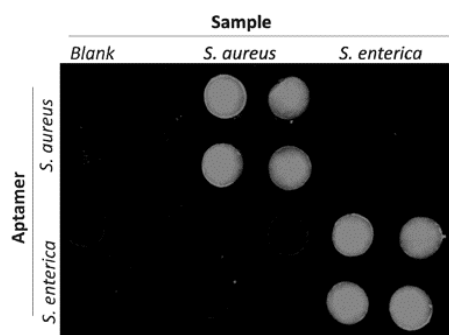
Figure 2: A cross reaction assay incorporating *S.aureus* (10^6 cfu mL^{-1}) and *S.enterica* (1375 cfu mL^{-1}) demonstrate the selectivity of the sensor to multiple pathogen targets for each immobilised aptamer. (Reproduced from [52] with permission from Royal Society of Chemistry).

A novel lab-on-paper based chemiluminescence assay was demonstrated for cancer biomarkers [53]. Reaction chambers were formed by screen printing hydrophobic wax layers into porous paper, with covalent immobilization of capture antibodies using a glutaraldehyde linker molecule. The assay consisted of: (i) immobilising sample specific capture antigens, (ii) addition of HRP-labelled signal antibodies to detection zones, and (iii) injection of luminol-p-iodophenol-H$_2$O$_2$, triggering chemiluminescence. Incubation time was optimised at 210 seconds. Increasing concentrations of three tumour markers were evaluated in PBS buffer, delivering linear ECL responses for each target range; (i) AFP 0.1–35.0 ng/ml, (ii) CA125 0.5–80.0 U/mL and (iii) CEA 0.1–70.0 ng/mL. The LODs of the three targets were within clinically acceptable limits when evaluated with human serum, in agreement with a commercial ECL cancer test. A paper based ECL assay was also implemented screening for four cancer biomarkers (r-fetoprotein (AFP), carcinoma antigen 125(CA125), carcinoma antigen 199(CA199) and carcinoembryonic antigen (CEA)[54]. ECL detection was demonstrated in human serum using TPA (tris-(bipyridine)-ruthenium(II)(Ru(bpy)32þ)-tri-n-propylamine). Eight reaction chambers with feeder channels were defined by wax printing. Eight working electrodes incorporated into the device stimulated ECL during voltage sweeps (0.5V – 1.1V). For manufacture, fluidics were first fabricated followed by electrode screen printing (Carbon WE, Ag/AgCl reference). Capture antibodies (2µl, 20mg/ml) were immobilised on each WE using chitosan coating and GA cross-linking. For target capture, sample was added to each electrode and incubated for thirty minutes. ECL detection was realised by adding TPA (0.01mM) and monitored during voltage sweeps. The test was evaluated with blood serum from high risk cancer patients, measured biomarker concentrations agreed with commercial cancer test results. An origami like paper device was developed to simultaneously screen for four cancer cells (MCF-7,HL-60,K562,CCRF-CEM)[55].Target specific aptamers were immobilised on gold electrodes and porous AuPd nanoparticles labelled with Concanavalin-A acted as probes. The nanoparticles selectively bound to mannose on the captured cell surface. Carbon WEs were screen printed in each capture reservoir with a single Ag/AgCl reference electrode. Au nanoparticles were grown on WE surfaces before aptamer modification, forming an Au-thiol monolayer. Wax channel and chambers were defined for liquid handling. For detection, test solutions (10µl) were delivered to each chamber and incubated for fifteen minutes. After cell capture, the bio-conjugate solution (AuPd@Con-A) was added labelling captured cells. ECL measurements were performed by sweeping voltage (-0.3 V to -1.8V, scan rate 100mV/sec) on each electrode while the monitoring optical signal. A log relationship existed between ECL signals and cell concentration (working range 450 – 10^5 cells/ml) demonstrating potential for low cost, rapid cancer cell screening. The test exhibited good specificity with slight signal increase to non-targeted cancer cells. Test variation was <4% (CV) and devices were viable for up to five weeks. Similar assays were also developed for specific cancerous cell screening [56][57]. Paper stacking was

demonstrated as a novel approach to assay implementation [58]. Paper sheets, pre-incubated with biological reagents were skived into multiple test sheets facilitating mass device manufacture for multiplex applications. The width of a single paper sheet formed each reaction site, with multiple sheets assembled to implement multiplex barcode assays. Test readout was performed using a commercial barcode scanner and simultaneously distinguished positive results for HBV,HCV,HIV,TP over negative samples. Barcode assays were fabricated by gluing white paper, red paper and paper immobilised with capture probes together in a defined manner. Lateral flow delivered target to immobilised capture probes, while AuNPs labels changed paper colour (white to red) indicating a positive test. Devices evaluated with human samples demonstrated reproducible and specific virus detection at clinically relevant levels. An emerging area is paper based molecular diagnostics using amperometric [59] and impedance[60] detection, however multiplex assays using these detection methods on flexible substrates has yet to be demonstrated. A challenge for paper assays is valved fluidic control, a novel paper device (figure.3) incorporated active electromagnetic valving and timed incubation. Active on paper valving was implemented as sample wicked between two electrodes, completing a circuit to activate electromagnetic valves. The device screened for multiple cancer biomarkers and programmed incubation times facilitated specific protein detection. Significant progress has also been made with mPAD multiplex devices, screening for specific biomarker combinations. Catalytic detection is the most popular technique adapted with mPADs assays and aptamers are easily incorporated to enhance selectivity and sensitivity. ECL has enhanced signal benefits over visual colour change assays and electrochemical based techniques offer potential for portable point of care and wearable biomarker detection. Challenges for paper based assays include long term biomolecule activity with refrigeration required to maintain viable assays. Paper assay manufacture is low cost and easy implemented for developing countries, however humidity and temperature variation can impact reproducibility. Capillary flowrates can be modified by change in sample viscosity due to medication, thus internal controls are required for test verification. Wearable paper devices have primarily focused on monitoring interstitial fluid and sweat constituents (e.g. electrolytes, glucose, lactate) using simple assays, while point of care paper applications extend to urine, saliva and blood biomarkers. A future step will be to incorporate complete sample preparation and detection into a single test.
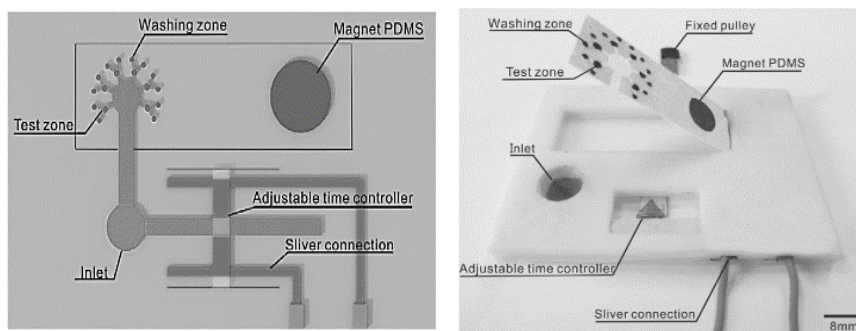
Figure 3: Programmed incubation time and active electromagnetic valving, were implemented on a paper based ECL assay, screening for protein cancer biomarkers alpha-fetoprotein (AFP) and carcinoma antigen 125 (CA-125) in human serum sample (Reprinted from [102] Copyright Sensors & Actuators B).

Polymer substrates

A diverse range of flexible synthetic polymer materials are available for sensor applications including polyimide, polyethersulfone, polyetheretherketone, poly(ethylene napthalate), polycarbonate and polyethylene terephthalate (PETE). Such materials are compatible with mass manufacture methods for thin flexible substrates (e.g. spin coating, extrusion, injection moulding). They are compatible with processing steps to deposit electroactive layers (organic or inorganic) for device fabrication (electrode, FET, nanowire) and biochemistry protocols immobilising capture biomolecules (antibodies, DNA) or enzymes, for target capture/recognition. Electroactive materials incorporated into flexible sensors include; conductive polymers (polypyrole, polyaniline, polythiophene), semiconductor materials (ZnO, In_2O_3, graphene) and nanowire/nanoparticle materials (carbon nanotubes). Microelectronic deposition techniques (CVD, evaporation) are extensively used to realise pure films defining devices and electrical contacts. Approaches where prepolymer liquids incorporate nanowire/nanoparticle materials have been implemented by electrospinning, micro-contact printing, spin-coating etc. Such approaches produce robust devices amenable to substrate bending, twisting and stretching.

Glucose, Electrolytes, lactate

A flexible substrate for multi-target (glucose,lactate ,Na^+,K^+) sweat screening was demonstrated for exercise monitoring [61]. Sensors were fabricated on flexible PET substrates conforming to skin contour. Gold electrodes were deposited on the substrates using a lift off process. Enzymes (GOx,LOx) immobilised on the substrate using a polysaccharide chitosan membrane facilitated amperometric measurement. Ion selective electrodes (ISEs) incorporating PEDOT:PSS as an ion to electrode transducer, facilitated selective Na^+ and K^+ detection. The reference electrode (Ag/AgCl) was coated with a polyvinylbutyral (PVB) membrane containing carbon nanotubes enhancing electrical properties. Such coatings facilitate long term measurements and minimise signal drift. Incorporating Prussian blue dye, shifted reduction potentials to 0V removing the need for additional sensor power, an important consideration for wearable devices. The ion sensors demonstrated detection over physiologically relevant ranges (K^+ 10 -160mM & Na^+ 1-32mM). Temperature variation was found to have significant impact on enzyme activity and thermal compensation addressed overestimation in glucose and lactate measurements. The system was evaluated during moderate activity on an exercise bike. After perspiration onset, decrease in measured glucose and lactate levels was observed, however lactate levels stabilised as expected with continuous steady exertion. Na^+ increased and K^+ decreased as perspiration commenced and stabilised as exercise continued. When applied to

different body locations (forehead, wrist) the system recorded different trends due to varied sweat volumes and skin characteristics. During high intensity exercise, measured trends varied across four volunteers for Na^+ and K^+. During intense exercise, observed glucose trends were well correlated across subjects, however lactate levels varied between subjects. Highlighting how large scale population testing is required to establish biomarker relevance. A fully integrated autonomous platform (figure 4) [62], was developed to quantify Na^+, Cl^- and glucose in sweat sample for cystic fibrosis (CF) and diabetes monitoring. Iontophoresis electrodes coated with pilocarpine loaded hydrogel were incorporated to locally stimulate sweat glands in sedentary patients. The measurement consisted of two phases; (i) application of a local current to generate sample and (ii) parameter measurement using ion selective electrodes.
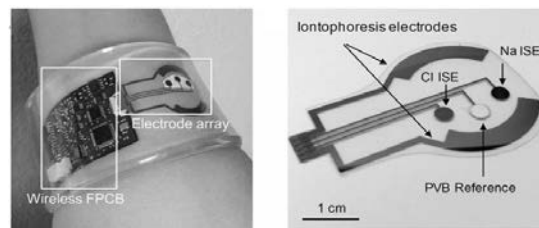

Figure 4: The flexible patch type system incorporating electrodes and electronics for wireless Na^+, Cl^- and glucose monitoring in sweat sample. (Reprinted from [62] Copyright PNAS 2017).

Ag electrodes were deposited on a PET substrate facilitating mechanical flexibility for direct skin contact. An Ag/AgCl electrode for Cl^- detection was deposited by modifying an Ag electrode with a $FeCl_3$ solution. The Na^+ detection electrode was realised by depositing a Na^+ selective layer upon an Ag electrode. PVB coatings saturated with chlorine ions was applied to formulate reference electrodes. For CF diagnostics the system was evaluated by measuring sweat chloride concentration. Levels >60mM indicated increased probability of disease. Measurements were made on three CF patients and six healthy volunteers, the average Na^+ and Cl^- for CF sufferers were 82.3mM and 95.7mM respectively, while average healthy readings were 26.7mM and 21.2mM. For glucose measurements a correlation between blood and sweat levels was demonstrated on a group of fasting and post fasting volunteers. A skin mounted micro-analytical flow system was demonstrated [63] to monitor sweat lactate and glucose levels during light indoor cycling sessions. An optimised microfluidic sweat collection device was designed to deliver sweat sample collected from the skin to measurement electrodes. This was the first time a microfluidic interface was optimised for sample collection on a skin wearable sensor. The device structure consisted of two PDMS layers, one incorporating the electrode substrate and the second defining fluidic channels. Gold electrodes (WE, CE) were modified with PB, GOx and LOx for detection. The device also incorporated an Ag/AgCl reference electrode. When evaluated with spiked artificial sweat sample, flowing at 200ml/min, standard deviations of 1.2% & 1.6% were recorded for lactate (12mM) and glucose (10mM). The linear operating ranges for lactate was 4-20mM and glucose 2-10mM. Detection sensitivity was 29.6μM/μA. On body tests were undertaken with two healthy subjects over twenty minutes of moderate

indoor cycling. Different signals were obtained compared to in-vivo tests due to temperature, pH and flowrate variation. Similar measured metabolite trends were previously reported [61,64,65]. Flexible conformal biosensor arrays were manufactured using printed ultrathin metal oxide semiconductor technology for pH and glucose detection [66]. Arrays were manufactured from indium oxide films (3.5nm) with low impurity concentration. The metal oxide films were robust to stress/strain caused by substrate bending, twisting and stretching. The surface was easily modified for biomolecule immobilisation and the sensor demonstrated both pH and glucose detection for diabetes and wound monitoring. Flexible field effect transistor (FET) devices were formed on ultrathin polyimide films (2μm), the active layer (In_2O_3) was spin coated then annealed at 250°C. Interdigitated electrode arrays (IDEs) were patterned on the metal oxide film defining metal contacts. Sensor thin films were released from support substrates by delamination in water, facilitating stretching and unfolding. Protonation of surface hydroxyl groups and primary amines of aminopropyltriethoxysilane (APTES) occurred on the metal oxide surface, as pH decreased from pH5.5 – pH9. For glucose detection, GOx immobilised on FET surfaces produced hydrogen peroxide from glucose oxidation. Measured currents were recorded over a clinically relevant range (100μM - 400μM) and devices were suitable for simultaneous on-skin glucose and pH measurements. Flexible organic electrochemical transistors (OECT) formed on a flexible substrate demonstrated simultaneous detection of uric acid and glucose [67]. The device was formed by deposition of electroactive PEDOT:PSS layers on a flexible PET substrate (50μm) with platinum contact electrodes. Flexible devices could be attached to conformal surfaces and were robust to repeat bending cycles (1000 times).The device was sensitive to H_2O_2 formed during enzymatic reactions. To overcome sensitivity of the platinum gate electrodes to interfering molecules (e.g. dopamine, glucose, UA, and ascorbic acid), electrodes were modified with a bilayer consisting of graphite flakes and nafion, then encapsulated with a conductive polymer (polyaniline-PANI). After surface modification positively charged molecules (e.g. dopamine) were repelled from the surface (Nafion repelled UA and AA), while larger molecules (e.g. glucose) could not penetrate the PANI nanometer sized pores. Gate modification had minimal impact, as H_2O_2 penetrated the bilayer delivering good detection (LOD 10^{-9}). Uricase (UOx) was immobilized on PANI using Graphene Oxide (GO) and oxidation of Uric acid also generated H_2O_2. A physiologically relevant response range was recorded (100 x 10^{-9}M - 500 x 10^{-6}M) for uric acid. The device detected UA in saliva at 173±20 × 10^{-6} M and saliva glucose in a healthy individual (103±10 × 10^{-6}M). The bilayer blocking effect was relevant only to potentiometric sensors as fields associated with amperometric detection negated bilayer charge blocking at the device surface. Blocking layer efficiency is an important parameter along with electrode poisoning, requiring consideration in catalytic sensor methods. A flexible multi-sensor patch was fabricated to simultaneously monitor sodium, pH and lactate sweat during exercise sessions [68]. The patch adhered to skin on the lower back where a flexible microneedle array and microfluidic channel collected sweat sample for electrochemical measurement. The innovative sampling mechanism continuously drew sample across the sensors for real-time detection. The overall patch thickness was 180μm facilitating comfortable long term application. For Na+ detection, a PVC ion selective membrane was drop cast onto Pt/PEDOT electrode gasket, the reference electrode was prepared by doping

the polymeric membrane with a lipophilic salt. For lactate detection the reference electrode was treated with potassium dichromate. For pH detection Iridium oxide was electrochemically deposited on electrodes by chemical oxidation. Lactate oxidase drop cast onto electrodes coated with SPEES/PES for selective lactate detection. Sensor signals were wirelessly transmitted to a mobile phone for data analysis. The system was evaluated on six male volunteers during exercise sessions. Temperature recalibration was applied to the sensors compensating for modified enzyme activity. Saliva samples were taken during exercise to determine cortisol concentration using a commercial immunoassay. Measured sodium, lactate and pH levels were in agreement with previous reported studies and in line with clinical levels for exercise sessions and were repeatable with CV's of 6% (Na), 7% (pH) and 9% (lactate). The sensors were robust to interfering sweat constituents e.g. uric acid, ascorbic acid and glucose. A disposable patch incorporating microfluidics and assay reagent implemented a passive test screening for Cl, Zn and Na [69]. The device was composed of three layers; (i) adhesive, (ii) microfluidic and (iii) light shield. Microfluidic channels, valves and chambers realised a passive pump mechanism delivering sweat sample from skin to assay reservoirs. Fluorescent probes reported target concentrations, with signals read using a smartphone via miniaturised optical fluorescence system. The fluidic system (figure 5) contained check valves to facilitate timed delivery of sweat sample to each reaction chamber once a threshold liquid pressure was achieved. The light shield layer protected the probes during skin application for sweat sampling and assay reaction. For Na and Zn detection, there was a direct relationship between intensity and target concentration correlating to physiologically relevant ranges (Na 20–60 mM, Zn 1.4–27μM). An inverse fluorescent signal was correlated to increasing Cl sweat content over the physiological range 20–60 mM.
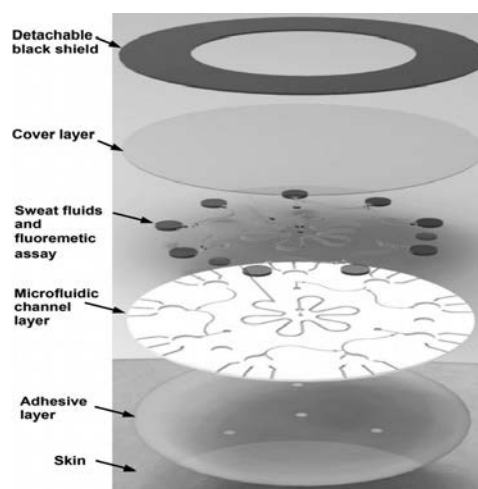
Figure 5: A multilayer patch incorporating adhesive, microfluidics and assay reagents was demonstrated for detection of Cl, Na and Zn over clinically relevant ranges. (Reproduced from [69] with permission from Royal Society of Chemistry).

Alcohol, glucose

A flexible sensor was developed to simultaneously monitor alcohol and glucose with low sample volume (1 - 3μL), this combination facilitated a wearable system to monitor correlation between alcohol consumption and diabetes [70]. Flexible

porous polyimide membranes were used as a substrate to fabricate Zinc oxide electrodes. The porous polymer membrane wicked sweat away from the skin to the sensor surface. Selective biomolecule transport through the porous membrane enhanced signal by minimising interference from ions and lipids. The sensor was evaluated on synthetic sweat spiked with glucose and ethanol over clinically relevant ranges, with impedance measured over the frequency range 50 – 500Hz. Variation in impedance due to electron charge transfer and electrical double layer modulation, modified electrode capacitance as target biomolecule concentration varied. Changes in imaginary impedance correlated to ethanol concentration over the range 0.01mg/dl to 200mg/dl. Sweat pH variation (pH 4 to pH 8) had an impact on ethanol estimates. The sensor also demonstrated selectivity against interfering biomolecules (e.g. uric acid, glucose, lactate, creatine etc.). For combinatorial experiments, glucose measurements were made at 100Hz and the performance of glucose and alcohol measurements were comparable to commercial devices for glucose (Accu-Chek®) and alcohol (BACtrac®) and within acceptable limits of error.

Heartrate, lactate

Combining electrophysiological and biochemical measurements on a single patch [71] augmented heartrate monitoring with lactate measurements, offering a more comprehensive fitness monitor compared to electrophysiological measurements alone. The system was composed of a three electrode lactate sensor and a bipolar electrocardiogram sensor fabricated on a flexible substrate for skin adhesion. The device was fabricated by screen printing electrodes on a flexible polyester sheet. Portable instrumentation incorporated a potentiostat and an electrocardiogram with Bluetooth telemetry. The hybrid device was tested on three subjects during cycling exercise sessions. The patch size (7cm x 2cm) was set by electrocardiogram electrode separation. Placing the patch on the chest region was optimal for heartrate monitoring and also generated sufficient sweat sample for lactate measurement. The lactate electrodes were fabricated between the heartrate electrodes. Prussian blue ink was used to print the working electrodes, which were highly sensitive to hydrogen peroxide produced by enzymatic lactate oxidation. The Ag/AgCl reference electrode was also screen printed. The sensors were separated by an ecoflex hydrophobic layer preventing signal distortion through multiple electrical pathways thus minimising crosstalk between sensors. Results from continuous cycling highlighted heartrate measurements between 60bpm (resting) and 120bpm (full exertion), at exercise commencement no variation in lactate was measured on the lactate sensor due to lack of perspiration, however with sweat onset the LOx sensor shows signal increase correlating to heartrate and exertion. Dilution of sweat lactate content after a period of time due to profuse sweating was also detected by the sensor. During exercise cooldown the heartrate returned to normal and the lactate readings reduced as expected under normal clinical conditions.

Heavy metals

Metal detection is well established in electrochemistry [72] and has been applied to environmental [73], agriculture [74] and health [75] applications. Portable amperometric technology is suitability for system miniaturisation [76]. Metal screening in wearable health devices has emerged as an area of interest as metal deficiency (e.g. Cu,Zn) causes disease [77,78], while metal build-up has negative health implications. Screening sweat for heavy metals using electrochemical techniques [79, 80], has been adapted to wearable technologies [81]. Heavy metal detection (Zn,Cd,Pb,Cu,Hg) in body fluids, was demonstrated using square wave anodic stripping voltammetry on Au and Bi electrodes [82]. A five electrode microarray was evaporated and patterned (lift-off) on a PET substrate, then coated with a Nafion protective layer [83]. A PDMS sample reservoir (20-30μl) was sealed on the sensor substrate for sweat sample retention, providing a stable sensor liquid measurement interface. Highlighting how microfluidic designs can be incorporated with flexible sensor substrates to enhance biomarker detection.

Proteins & DNA

Single target immunoassays have limited diagnostic value as many biomarkers (e.g. proteins) are associated with a number of conditions (e.g. cancers), thus there's significant interest in implementing multiplex tests to improve diagnosis accuracy. Gold working electrode microarrays were deposited on flexible polyimide (PI) substrates for cytokine detection using impedance measurements [84]. The same group fabricated Poly(pyrrole) (PPy) microwires (PPYμWs) on flexible substrates including PETE, cyclic olefin copolymer (COC), PEN and PI using microcontact printing (μCP) [85]. The flexible sensors demonstrated multiplex cytokine detection by impedance spectroscopy. PPy is a good conductive polymer for electrical biosensors due to its electrical conductivity, environmental stability, biocompatibility and easy synthesis [86]. Human interleukin-10 antibodies were chemically immobilised on PPyμWs using gluteraldehyde (GA) crosslinker. Impedance spectroscopy was used to detect rhIL-10 biomarkers over the range 1–50 pg/mL, with sensitivity 0.026(pg/mL). This group also devised a PDMS μCP process defining PPy nanowires on flexible thermoplastic surfaces (PETE, PEEK), by covalent bonding. Carbodiimide crosslinker chemistry attached specific monoclonal antibodies (anti-human IL-6) to diazonium functionalised nanowires, defining impedance sensors for IL-6 detection. The LOD was 0.013pg/mL with a linear operating range 1 – 50pg/mL and demonstrated potential for multiplex cancer screening using IL-6 antibody biomarkers. Target specific aptamers were combined with Nanographene Oxide (NGO) sensor arrays to selectively screen for nine cancer related proteins using fluorescence detection [87] at nanomolar target concentrations. Multiple protein targets were also detected using aptamer/NGO substrates with NGO modifying polymer substrate elasticity [88]. NGO also demonstrated superior limits of detection over graphene-oxide due to increased oxygenated reactive sites delivering enhanced affinity for a wide range of protein biomarkers. OECT devices were also demonstrated for label free DNA detection [89], where the device was fabricated on a PET substrate and integrated within a PDMS microfluidic channel. The transistor active layer was PEDOT:PSS with Au electrodes. The device electrical characteristics (I_{DS}, V_G) showed similar values before and during bending. The

electrical transfer curve shifted positively after DNA hybridisation and demonstrated sensitivity down to 10pM target oligonucleotide with enhanced hybridisation conditions. While multiplex target detection was not demonstrated the potential microarray application could be realised.

Cell Targets

Label free electrochemical biomolecule detection has generated significant interest in recent years, removing reporter molecules can significantly reduce assay cost and complexity. Organic electrochemical transistors have been demonstrated for detection of sialic acid screening for cancer cells [90]. Such approaches exploit the concept of field effective transistors, detection is realised by monitoring source to drain electrical characteristics as gate voltage is modified with biomolecule interaction. In a highly efficient approach to cancer cell detection, sialic acid was directly monitor at a PABA modified GC electrode by exploiting direct binding between SA and phenylboronic acid. The transistor active layer was based on PEDOT:PSS with screen printed carbon source and drain electrodes. The OECT device showed a response to free SA test solution and HeLa cells with relative standard deviation of 6.3% (SA) and 12.9% (HeLa) respectively. The device has demonstrated potential to distinguish between different cell types (figure 6).
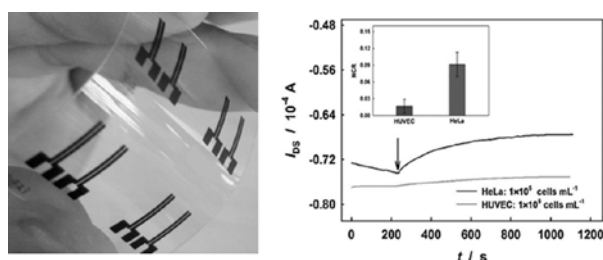
Figure 6: OECT devices were incorporated into a flexible transparent substrate (left) to screen for sialic acid. The potential to differentiate between different cancer (HeLa) and normal (HUVEC) cell types was demonstrated (right). (Reprinted from [90] Copyright Sensors & Actuators B).

Fabric substrates

Textile based sensors are a growing research area, most clothing materials are hydrophilic, biocompatible and naturally conform to the body profile. Approaches to achieve woven fabric sensors include :(i) modified fibres/threads, defining conductive paths in cloth or (ii) electrodes printed onto finished garments using flexible print/deposition approaches. Unlike paper and polymer substrates requiring multiple detection and fluid handling layers, fibres are selectively woven into the fabric, defining sensor and fluidic functionality in a single layer manufacture process. Textile manufacture incorporates a range of low cost high throughput techniques [91] (embroidery, weaving, braiding, coating & printing) compatible with

multiplex biomarker screening devices. Fabrics incorporating measurement technologies are referred to as smart fabric sensors (SFSs) [91]. Stretchable SFSs can be realised by using elastic fibres/threads [92], greatly enhancing suitability for wearable applications.

Electrolytes, pH

Fabric sensors are especially suited to skin applications monitoring sweat or interstitial fluid parameters (e.g. electrolytes). Commercial cotton yarn, dyed with carbon nanotube ink defined an ion-selective membrane for target specific measurement [93]. The electrodes were woven into a band aid type material (figure. 7), with a commercial miniature reference electrode incorporated into the structure measuring pH, potassium and nitrates in liquid sample. Optimising carbon nanotube ink concentrations achieved stable electrode dye for cotton based applications.
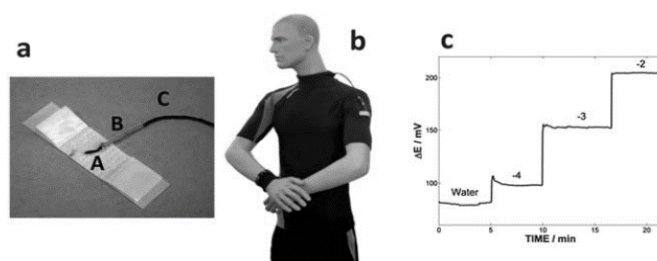
Figure 7: Band aid plaster material incorporating ion selective membranes, designed to measure pH, potassium and nitrates. (Reprinted from [93] with permission from Royal Society of Chemistry.)

Impedance measurements were made using these band aid devices and yarn based electrodes were found to be stable over extended time (one month), with structure bending/stretching having minimal impact on performance. Selectivity and sensitivity of the yarn electrodes were similar to conventional planar electrodes. The device incorporated a cellulose interface between electrodes, which rapidly soaked sweat, eliminating direct skin contact. Device manufacture was convenient and suitable for low resource countries, due its low manufacture cost and disposable nature. Silk is also an interesting material for fabric based electrochemical sensors, individual threads were coated with conductive inks and woven into flexible fabric electrodes [94]. Silk threads were coated with reagents and electrode materials before being incorporated into fabric patches realising sensor arrays. This approach was preferred over conventional screen printing to reduce reagent waste, while incorporating hydrophilic and hydrophobic threads into the sensor design controlled fluid flow and miniaturised sensor footprint. An organic electrochemical transistor formulated on a cotton fibre [95] simultaneously measured adrenaline and NaCl in sweat sample by oxidation at the gate electrode, forming adrenochrome. Metal wire was used as gate electrodes controlling charge on a PEDOT:PSS conduction channel formulated on a cotton fibre. When switched on, cations flowed from the electrolyte solution to the conductive channel reducing current flow between drain and source, the

current amplitude was proportional to sweat adrenaline concentration. The sensor response was measured over the range 10nM to 10mM, changing the gate electrode to silver facilitated salt content measurement.

Glucose, lactate, hemoglobin

Electrochemical detection was used to detect glucose (chrono-amperometry) and hemoglobin (digital pulse voltammetry) [94]. This approach incorporated different reagents on electrodes, in a fashion not previously demonstrated with textile devices, also enhancing mechanical strength for incorporation into wearable devices. The glucose sensor consisted of carbon ink coated onto silk fibre (counter electrode), with thread coated in a carbon ink/potassium ferricyanide mix for the WE, followed by glucose oxidase deposition. An Ag/AgCl thread coating was used for the reference electrode. Identical materials and manufacture technique was used for the hemoglobin sensor, with a carbon RE replacing Ag/AgCl and WEs left uncoated. For multiplex detection uncoated carbon RE's were incorporated into a four electrode design with common RE and CE's for multiplex detection. Each sensor was 2cm × 1.2 cm × 0.1 cm. The multiplex sensor was evaluated with lysed blood sample of known glucose and haemoglobin content. For glucose detection 5µl of blood was delivered to the sensor, a 0.5V fixed voltage was applied between WE and RE with current measured over time. Glucose was oxidised forming gluconic acid, while potassium ferricyanide reduction produced the measured current. The sensors showed good sensitivity over a clinically relevant range (80mg/dL to 600 mg/dL) with <5% CV. For hemoglobin detection, red blood cell lysis released target into solution. Carbon WE's were used to detect oxyhemoglobin by reduction at -0.42V. Using digital peak voltammetry (DPV), peak size at -0.42V increased with increasing oxyhemoglobin over the clinically relevant range 2.3g/dL to 14g/dL. Electrochemical sensors were embroidered into fabric, to monitor glucose and lactate in whole blood [96]. Conductive thread was woven to define complex electrode designs using a commercial embroidery machine. Electrochemical detection using complex electrode design patterns was not previously demonstrated with weaved fabric. Carbon-ink coated thread defined the working and counter electrodes, while Ag/AgCl ink was used for the reference electrode. Passive enzyme adsorption (e.g. GOx or LOx) was undertaken on the working electrode for selective detection. Resistivity of the Ag/AgCl and carbon coated threads were in line with literature, to reduce oxidation resistance the Ag/AgCl was flux coated. The assays were stable, reproducible and target specific in liquids containing glucose, lactate and uric acid. Blood spiked with glucose and lactate were measured over the range 0-40mM, demonstrating suitability for clinical measurements. The sensor performance was stable against mechanical deformation e.g. bending, stretching and twisting. An array of graphene FET devices (figure.8) suitable for multiplex detection was fabricated on a silk substrate [97]. A thin silk film substrate (10µm) was prepared from silk fibres. Glucose oxidase (GOx) was incorporated into silk solutions prior to drying achieving GOx (1%) loaded substrates. Graphene grown on a nicol (Ni) substrate was transferred to pure silk films using a PDMS stamp technique. Gold electrodes were deposited by low temperature evaporation (40°C) and patterned to realise source and drain electrodes on graphene channels. The GOx silk substrate was placed on the silk-graphene layer and adhered using water. The FET gate was then

deposited and patterned on the Silk-GOx layer. The glucose - GOx reactions modulated FET conductance, resulting in increased source drain current with increased glucose concentration. With V_g = 0V and V_{ds}=100mV, the measured current (I_{ds}) demonstrated a linear response over glucose concentration 0.1–10 mM (LOD =0.1mM), with average sensor sensitivity 2.5 A/mM. Sensor response time was < 10sec, with up to ten sensors deposited per sensor patch, facilitating multiplex detection. The device was selective with minimal impact from uric acid (10mM), bovine serum albumin (BSA) or ascorbic acid (10mM).
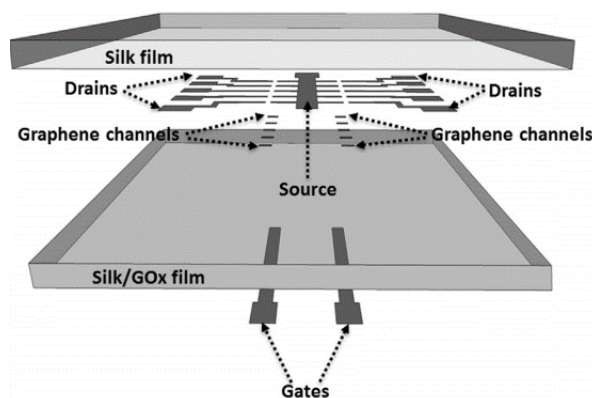
Figure 8: Outline of the incorporation graphene FET device array onto a silk substrate for spatial multiplex detection of glucose in a wearable system. (Reprinted from [97] Copyright Sensors & Actuators B).

Flexible fabric sensors have been extensively deployed for electrophysiological [98] and blood pressure [99] measurement and a recent review [100] on flexible strain sensors highlighted polymers and functional nanomaterials emerging in resistive/capacitive devices. They also highlighted how semiconductor materials significantly improve piezo-resistivity over metals in flexible sensor applications, a similar trend is evident with flexible biomarker devices. However for biomarker monitoring additional challenges exist around surface fouling, sweat and humidity. Sampling appropriate volumes of target biomolecule is also challenge in wearable devices and may require fluidic reservoir storage or pre-concentration prior to detection.

Organic electrochemical transistors (OECTs) were fabricated by coating nylon fibres with multilayers of Cr/Au/PEDOT:PSS [101]. The stretchable fibres were incorporated into fabric using a commercial weaving system, without any impact on performance. Bending the fibre had a small change in resistance (26 to 51Ω cm^{-1}) compared to a fibre coated only with Cr/Au (39 - 401Ω cm^{-1}). To define the gate electrode Pt was deposited on the nylon fibre using a Ti adhesion layer. Detection of glucose (30 – 100 x 10^{-9} M), uric acid and dopamine were demonstrated with the device. For glucose detection GOx was deposited on the gate electrode with a modified blocking polymer layer. For UA detection the gate electrode was modified with Nafion-graphine/polyaniline /uricase–graphene oxide multilayers and detection limits were similar to a planar device (30 x 10^{-9}M) with a linear response up to 300 x 10^{-6}M. The gate was modified with graphene flakes for dopamine detection

with a limit of detection 10×10^{-9}M. To demonstrate the practical application the OECT sensors were woven into diapers and used to determine glucose levels in spiked artificial urine.

Concluding remarks and future perspectives

In recent years significant growth has occurred with consumer devices, monitoring physical activity (hearth-rate, motion etc.) for the fitness and wellbeing markets. Flexible substrate sensors based on piezoresistance, piezocapacitance and piezoelectric technologies have been commercialised where product design deliver robust, easy to use and comfortable devices. To date, less progress has been made in commercialisation of wearable biomarker monitoring devices (e.g. glucose, Na^+ etc.). Research and commercial devices have extensively demonstrated single target biomarker detection, however there's increased activity around multiplex detection due to more representative diagnostics. New biomarkers emerging from genomic/proteomic research offers opportunities for real-time health monitoring in accessible biofluids (e.g. saliva, sweat, tear fluid). Flexible substrates facilitate on body wearable diagnostics enhancing non-invasive health monitoring. Materials progress has advanced wearable sensors through: (i) biocompatible polymers realising flexible substrates for integrated electrodes/electronics, (ii) conductive nanomaterials defining flexible electrodes, (iii) target selective interface layers (ionogels,hydrogels) addressing biofouling and enhancing biomarker selectivity. Integration of graphene, semiconductor and nanowire materials with conductive polymers have delivered significant progress in flexible sensor technology by enhancing electrical and mechanical performance. Microfluidics incorporated into flexible sensor devices improve liquid volume sampling [63] and deliver active device metering/valving [102].

Challenges still exist e.g. (i) analyte leaching from interface layers, (ii) long term stability for bio-recognition molecules and (iii) stable interface potentials between sample and sensor interfaces. Enzyme free glucose detection has emerged as a key research topic, enabled by enhanced electro-catalytic nanostructure properties [103,104]. These remove surface immobilisation and ameliorate issues around enzyme stability and lifetime. However device reproducibility is an issue with up to 50% signal variation reported across non-enzyme nanoparticle sensors [105]. Non-linear response/sensitivity and interference from electroactive species is also reported [105]. Enzyme free electrochemical glucose detection has yet to impact flexible biomarker systems and enzyme mediated detection is still widely implemented. For future wearable flexible biomarker monitoring system, sampling will present challenges; (i) minimum sample volumes required to reliably detect target concentrations, and (ii) sample/biomarker replenishment rates. Current wearable electrochemical devices have to overcome lifetime and stability issues. Biofouling and electrode poisoning (H_2O_2) impact long term stability limiting measurements to <24hours, acceptable for short term disposable devices (e.g. contact lens for glucose monitoring) but limiting long term biomarker monitoring (e.g. days). A challenge for wearable potentiometric devices is avoiding interface equilibrium between reference electrode material and the sample, resulting in signal drift and limited sensor lifetime [106].

Flexible substrate multiplex biomarker system are a natural fit for wearable health monitoring applications. They can provide rapid, reliable information for the end user, facilitating decision making on medication, performance or lifestyle. In future developments, flexible polymers with physical characteristics (Young's modulus) similar to skin will advance comfort and robustness of skin worn devices. For non-invasive monitoring, reliability and clinical performance [107] are key aspects for device performance. Blood diagnostics [108-110] are the benchmark against which wearable systems are evaluated and lower target concentration, lag times and sample variation are challenges to be addressed. Progress in wearable sensor technology has been significant, flexible substrates has been facilitated by new nanomaterials, polymers and novel fabrication technologies. To date, diabetes monitoring has been the commercial and technology benchmark [111,112,113], with glucose and lactate extensively used to demonstrate proof of concept in research devices [114-119]. Emerging biomarkers from genome and proteome research are generating clinical interest as biomarkers linked to specific diseases. Currently many commercial diagnostic assays monitor single biomarkers (e.g. glucose), but the possibility to monitor multiple biomarkers promises more efficient health monitoring where multiple parameters inform clinical diagnosis. The potential for non-invasive wearable biomedical sensors as alternatives to invasive monitoring is significant and the explosion in physical activity and lifestyle monitoring devices illustrate how issues around biofouling, calibration and signal interference can be addressed.

Acknowledgements

References

[1] W.Apweiler, B.Balgley, P.Boontheung, J.Bundy, B.Cargile ,S.Cole,X.Fang, M.Gonzalez-Begne, T.Griffin, F.Hagen, S.Hu, L.Wolinsky, C.Lee, D.Malamud, J.Melvin ,R.Menon, M.Mueller, R.Qiao, N.Rhodus, J.Sevinsky, D.States, J.Stephenson, S.Than, J.Yates, W.Yu ,X. Xie, Y.Xie, G.Omenn, J.Loo,D. Wong. Systematic comparison of the human saliva and plasma proteomes. Proteomics Clinical applications. 3 (2009) 116-134.

[2] M.Soukup, I.Biesiada, A.Henderson, B. Idowu, D. Rodeback, L. Ridpath, E. G. Bridges, A.M. Nazar, K.G. Bridges. Salivary uric acid as a noninvasive biomarker of metabolic syndrome. Diabetol Metab Syndr. 4 (2012) 14.

[3] R.Semba, J.Enghild, V.Venkatraman, T.Dyrlund, J.E. Van Eyk. The Human Eye Proteome Project: Perspectives on an emerging proteome. PROTEOMICS (2013) 2500–2511.

[4] C.Huang, M.Chen, L.Huang, I.Mao. Uric acid and urea in human sweat. Chin. J. Physiol. 45 (2002) 109–115.

[5] W.Latzka, S.Montain. Water and electrolyte requirements for exercise. Clin. Sports Med. 18 (1999) 513–524.

[6] S.Shirreffs, L.Aragon-Vargas, M.Chamorro, R.Maughan, L.Serratosa, J.Zachwieja. The sweating response of elite professional soccer players to training in the heat. Int. J. Sports Med. 26 (2005) 90–95.

[7] W.Zeng, L.Shu, Q.Li, S.Chen, F.Wang, X.Tao. Fiber based wearable electronics: a review of materials, fabrication, devices, and applications. Adv. Mater. 26 (2014) 5310−36.

[8]S.Choi,H.Lee,R.Ghaffari,T.Hyeon,D.HKim. Recent Advances in Flexible and Stretchable Bio-Electronic Devices Integrated with Nanomaterials. Adv. Mater. 2016, DOI: 10.1002/ adma.201504150.

[9]M. Stoppa, A.Chiolerio. Wearable electronics and smart textiles: a critical review. Sensors 14 (2014) 11957−92.

[10] M.Raiszadeh, M.Ross, P.Russo. Proteomic analysis of eccrine sweat: implications for the discovery of schizophrenia biomarker proteins. Journal of Proteome Research 11 (2012) 2127–2139.

[11] S. Park, J. Ahn, X. Feng, S. Wang, Y. Huang, J.A. Rogers. Theoretical and Experimental Studies of Bending of Inorganic Electronic Materials on Plastic Substrates. Adv. Funct. Mater. 2008, 18, 2673–2684.

[12] F. H. Silver, J. W. Freeman and D. DeVore, Skin Res. Technol., 2001, 7, 18–23.

[13] L.Peng, Z.,L. Jingjing, C.Hongyan ,S.Yan,Y. Nailiang. Air-Stable Black Phosphorus Devices for Ion Sensing. ACS Appl. Mater. Interfaces. 7 (44) (2015) 24396–24402.

[14] Y. Bo, H. Yang, Y. Hu, T. Yao, S. Huang. A novel electrochemical DNA biosensor based on graphene and polyaniline nanowires. Electrochimica Acta. 56 (2011) 2676–2681.

[15] X.Kang, J.Wang, H. Wu, I.A. Aksay, J. Liu, Y. Lin. Glucose Oxidase–graphene–chitosan modified electrode for direct electrochemistry and glucose sensing. Biosensors and Bioelectronics 25 (2009) 901–905.

[16] S.Kumar Vashist, D. Zheng, K. Al-Rubeaan, J.H.T. Luong, F.S. Sheu. Advances in carbon nanotube based electrochemical sensors for bioanalytical applications.Biotechnol Adv. Mar-Apr;29(2) (2011) 169-88.

[17] C.B. Jacobs, M.J. Peairs, B.J Venton. Review: Carbon nanotube based electrochemical sensors for biomolecules. Analytica Chimica Acta 662 (2010) 105–127.

[18] K. Saha, S.S. Agasti, C. Kim, X. Li, V.M. Rotello. Gold Nanoparticles in Chemical and Biological Sensing. Chem. Rev. 2012, 112, 2739–2779.

[19] X. Luo, A. Morrin, A. J. Killard, M.R. Smyth. Application of Nanoparticles in Electrochemical Sensors and Biosensors. Electroanalysis. DOI:10.1002/elan.200503415.

[20] S.R. Forrest. The path to ubiquitous and low-cost organic electronic appliances on plastic. Nature, Vol. 428, 29 April 2004.

[21] Y.Liu, L.Shi, M.Wang,Z. Li, H. Liu, J. A novel room temperature ionic liquid sol-gel matrix for amperometric biosensor application. Green Chem. 7 (2005) 655- 658.

[22] N.Nishi, H.Murakami, Y.Yasui, T.Kakiuchi. Use of Highly Hydrophobic Ionic Liquids for Ion-selective Electrodes of the Liquid Membrane Type. Anal. Sci. 24 (2008) 1315-1320.

[23] B.Penga, J.Zhub, X.Liua, Y.Qina. Potentiometric response of ion-selective membranes with ionic liquids as ion-exchanger and plasticizer. Sens. Actuators B. 133 (2008) 308–314.

[24] G. Reach. Continuous Glucose Monitoring and Diabetes Health Outcomes: A Critical Appraisal. Diabetes Technology & Therapeutics. April 2008, Vol. 10, No. 2: 69-80.

[25] S.Iguchi, H.T.Saito. A flexible and wearable biosensor for tear glucose measurement.Biomed Microdevices (2007) 9: 603.

[26] W.Yan, R.Apweiler , B.M.Balgley, P.Boontheung, J.L.Bundy, B.J.Cargile ,S.Cole, X.Fang, M. Gonzalez-Begne, T.J.Griffin, F.Hagen, S.Hu, L.E.Wolinsky, C.S.Lee, D.Malamud, J.E.Melvin , R. Menon, M.Mueller, R.Qiao, N.L.Rhodus, J.R.Sevinsky, D.States, J.L.Stephenson, S.Than, J.R.Yates, W.Yu ,X. Xie, Y.Xie, G.S.Omenn,J.A Loo, D.T. Wong. Systematic comparison of the human saliva and plasma proteomes. Proteomics Clinical applications. 2009, 3, 116-134.

[27]Bergeron,M.F. (2003) Heat cramps: fluid and electrolyte challenges during tennis in the heat. J. Sci. Med. Sport 6, 19–27.

[28] L.S.Selva-Kumar, X.Wang, J.Hagen, R.Naik, I. Papautsky, J.Heikenfeld. Label free nano-aptasensor for interleukin-6 in protein-dilute biofluids such as sweat. Anal. Methods, 2016, 8, 3440 – 3444.

[29] W.A. Latzka, S.J. Montain. Water and electrolyte requirements for exercise. Clin. Sports Med., 18 (1999), pp. 513–524.

[30] C.G.Fraga. Relavance, essentiality and toxicity of trace elements in human health. Mol. Aspects. Med. 2005, 26, 235.

[31]S.R. Newmark, R.G. Dluhy. Hyperkalemia and hypokalemia. JAMA 231, 631–633 (1975).

[32] Gamella, M. (2014) A novel non-invasive electrochemical biosensing device for in situ determination of the alcohol content in blood by monitoring ethanol in sweat. Anal. Chim. Acta 806, 1–7.

[33] T. Umeda. Use of saliva for monitoring unbound free cortisol levels in serum. Clin. Chim. Acta 110, 245–253 (1981).

[34] M. Sher, R. Zhuang,U. Demirci, W. Asghar. Paper-based analytical devices for clinical diagnosis: recent advances in the fabrication techniques and sensing mechanisms. Expert Rev Mol Diagn. 2017 Apr; 17(4): 351–366.

[35] J.P. Comer. Semi quantitative specific test paper for glucose in urine. Anal Chem 1956;28:1748e50.

[36] A.W. Martinez, S.T. Phillips, M.J. Butte, G.M. Whitesides. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. Angew Chem Int Ed 2007;46:1318e20.

[37] A.W.Martinez, S.T. Phillips, B.J. Wiley, M. Gupta, G.M. Whitesides. Flash: a rapid method for prototyping paper-based microfluidic devices. Lab Chip 2008;8: 2146e50.

[38] R. Mukhopadhyay. Cheap, handheld colorimeter to read paper-based diagnostic devices. Anal Chem 2009;81:8659.

[39] A.W. Martinez, S.T. Phillips, G.M. Whitesides. Diagnostics for the developing world: microfluidic paper-based analytical devices. Anal Chem 2010;82:3e10.

[40] S.K. Sia, L.J.Kricka. Lab on paper. Lab Chip 2008;8:1988e91.

[41] G.M. Whitesides. What comes next? Lab Chip 2011;11:191e3.

[42] R. Mukhopadhyay . Medical diagnostics with paper and camera phones. Anal Chem 2008;80:3949.

[43] Y. Lu ,W. Shi, L. Jiang, J. Qin, B. Lin. Rapid prototyping of paper-based microfluidics with wax for low-cost, portable bioassay. Electrophoresis 2009;30: 1497e500.

[44] S.K. Tang, G.M. Whitesides. Optofluid.: Fundam.,DevicesAppl.(2010)7–31.

[45] W. Dungchai, O. Chailapakul, C.S. Henry. Electrochemical Detection for Paper-Based Microfluidics. Anal. Chem. 2009, 81, 5821–5826.

[46] A.W. Martinez, S.T. Phillips, M. J. Butte, G.M. Whitesides. Patterned Paper as a Platform for Inexpensive, Low-Volume, Portable Bioassays. Angew. Chem. Int. Ed. 2007, 46, 1318 –1320.

[47] A.W. Martinez, S.T. Phillips, E. Carrilho, S.W. Thomas, H. Sindi, G.M. Whitesides. Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis Anal. Chem. 2008, 80, 3699–3707.

[48] F. Kivlehan, M. Paolucci, D. Brennan, I. Ragoussis, P. Galvin. Three-dimensional hydrogel structures as optical sensor arrays, for the detection of specific DNA sequences. Analytical biochemistry 421 (1), 1-8.

[49] Y. Yao, C. Zhang. A novel screen-printed microfluidic paper-based electrochemical device for detection of glucose and uric acid in urine. Biomed Microdevices (2016) 18:92.

[50] X. Wei, T. Tian, S. Jia, Z. Zhu, Y. Ma, J. Sun, Z. Lin, C. Yang. Microfluidic Distance Readout Sweet Hydrogel Integrated Paper. Based Analytical Device (µDiSH-PAD) for Visual Quantitative Point of-Care Testing. Anal. Chem. 2016, 88, 2345–2352.

[51] Y.F. Zheng, G.W. Xu, D.Y. Liu, J.H. Xiong, P.D. Zhang, C. Zhang, Q. Yang, S. Lv. Study of urinary nucleosides as biological marker in cancer patients analyzed by micellar electrokinetic capillary chromatography. Electrophoresis, 23 (2002), pp. 4104-4109.

[52] P. Zuo, X. Li, D. Dominguez, B. Yeb. A PDMS/paper/glass hybrid microfluidic biochip integrated with aptamer-functionalized graphene oxide nano-biosensors for one-step multiplexed pathogen detection. Lab Chip, 2013, 13, 3921–3928.

[53] S. Wang, L. Ge, X. Song, J. Yu, S. Ge, J. Huang, F. Zeng. Paper-based chemiluminescence ELISA: Lab-on-paper based on chitosan modified paper device and wax-screen-printing. Biosensors and Bioelectronics 31 (2012) 212–218.

[54] L. Ge, J. Yan, X. Song, M. Yan, S. Ge, J. Yu. Three-dimensional paper-based electrochemiluminescence immunodevice for multiplexed measurement of biomarkers and point-of-care testing. Biomaterials 33 (2012) 1024e1031.

[55] L. Wu, C. Ma, L. Ge, Q. Kong, M. Yan, S. Ge, J. Yu. Paper-based electrochemiluminescence origami cyto-device for multiple cancer cells detection using porous AuPd alloy as catalytically promoted nanolabels. Biosensors and Bioelectronics63(2015)450–457.

[56] M. Su, L. Ge, S.Ge, N.Li, J.Yu, M.Yan, J.Huang. Paper based electrochemical cyto device for sensitive detection of cancer cells. Anal.Chim. Acta847 (2014) 1–9.

[57] M. Su, L. Ge, Q. Kong, X. Zheng, S. Ge, N. Li, J. Yu, M.Yan. Polymer Based Devices. Biosens.Bioelectron. 63 (2015)232–239.

[58] M. Yang, W Zhang, J. Yang, B. Hu, F. Cao, W. Zheng, Y. Chen, X. Jiang. Skiving stacked sheets of paper into test paper for rapid and multiplexed assay. Sci. Adv. 2017;3:eaao4862.

[59] J. Cunningham, N. Brenes, R. Crooks Paper Electrochemical Device for Detection of DNA and Thrombin by Target-Induced Conformational Switching. Anal. Chem. 2014, 86, 6166–6170.

[60] P. Ihalainen, F. Pettersson, M. Pesonen, T. Viitala, A. Määttänen, R. Österbacka, J. Peltonen. An impedimetric study of DNA hybridization on paper-supported inkjet-printed gold electrodes. Nanotechnology 25 (2014) 094009 (11pp).

[61] W. Gao, S. Emaminejad, H. Nyein, S. Challa, K. Chen, A. Peck, H. Fahad, H. Ota, H.Shiraki,D. Kiriya, D. Lien, G.Brooks. R. Davis, A. Javey. Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. 28 January 2016, Vol. 529 Nature.

[62] S. Emaminejada, W. Gao, E. Wub, Z.A. Davies, H. Nyein, S. Challaa, S.P. Ryan, H. Fahad, K. Chen, Z. Shahpar, S. Talebia, C. Millaf, A. Javey, R.W. Davies. Autonomous sweat extraction and analysis applied to Cystic Fibrosis and glucose monitoring using a fully integrated wearable platform. PNAS 114 (2017) 4625-4360

[63] A. Martín, J. Kim, J.F.Kurniawan, J.R.Sempionatto,J.R.Moreto,G.Tang,A.S.Campbell,A.Shin,M.Lee,X.Liu,J.Wang.Epidermal Microfluidic Electrochemical Detection System: Sweat Sampling and Metabolite Detection. ACS Sens. 2 (2017) 1860−1868.

[64] M.Parrilla, J. Ferr, T.Guinovart, F. Andrade. Wearable Potentiometric Sensors Based on Commercial Carbon Fibres for Monitoring Sodium in Sweat. Electroanalysis. 28 (2016) 1267 – 1275.

[65] G. Matzeu, C. O Quigley, E. McNamara, C. Zuliani, C. Fay, T. Glennon, D. Diamond. An integrated sensing and wireless communications platform for sensing sodium in sweat. Anal. Methods. 8 (2016) 64–71.

[66] Y. Rim, S. Bae, H. Chen, J. L. Yang, J. Kim, A.M. Andrews, P. S. Weiss, Y. Yang, H. Tseng. Printable Ultrathin Metal Oxide Semiconductor-Based Conformal Biosensors. ACS Nano VOL. 9 NO. 12 12174–12181 2015.

[67] C. Liao, C. Mak, M. Zhang, H. W. Chan, F. Yan. Flexible Organic Electrochemical Transistors for Highly Selective Enzyme Biosensors and Used for Saliva Testing. *Adv. Mater.* 2015, *27*, 676–681.

[68] S. Anastasova, B. Crewther , P. Bembnowicz , V. Curto , H. Ip, Bruno Rosa , Guang-Zhong Yang. A wearable multisensing patch for continuous sweat sensing. Biosensors and Bioelectronics 93 (2017) 139-145.

[69] Y.Sekine, S. Kim, Y. Zhang, A. Bandodkar, S. Xu, J. Choi, M. Irie, T. Ray, P. Kohli, N.Kozai, T. Sugita, Y. Wu, K. Lee, K. Lee, R. Ghaffarid, J.A. Rogers. A fluorometric skin-interfaced microfluidic device and smartphone imaging module for in situ quantitative analysis of sweat chemistry. Lab Chip, 2018, Advance Article, DOI 10.1039/C8LC00530C.

[70] A.Bhide, S.Muthukumar, A.Saini, S.Prasad. Simultaneous lancet-free monitoring of alcohol and glucose from low-volumes of perspired human sweat. Scientific Reports | (2018) 8:6507 | DOI:10.1038/s41598-018-24543-4.

[71] S.Imani, A.Bandodkar, A.Vinu Mohan, R. Kumar, S. Yu , J.Wang, P. Mercier. A wearable chemical-electrophysiological hybrid biosensing system for realtime health and fitness monitoring. Nature Communications 7:11650, DOI:10.1038/ncomms1165

[72] B. Bansoda, T. Kumarb, R. Thakurc, S. Ranac, I. Singh. A review on various electrochemical techniques for heavy metal ions detection with different sensing platforms. Biosensors and Bioelectronics 94 (2017) 443–455.

[73] G. March, T. Nguyen, B.Piro. Modified Electrodes Used for Electrochemical Detection of Metal Ions in Environmental Analysis. Biosensors 2015, 5, 241-275; doi:10.3390/bios5020241.

[74] G. Zhao,Y. Sheng, H. Wang, G. Liu. A Portable Electrochemical Detection System based on Graphene/Ionic Liquid Modified Screen-printed Electrode for the Detection of Cadmium in Soil by Square Wave Anodic Stripping Voltammetry.Int. J. Electrochem. Sci., 11 (2016) 54 – 64.

[75] W. Yantasee, Y. Lin, K. Hongsirikarn, G.E. Fryxell, R. Addleman , C. Timchalk. Electrochemical sensors for the detection of lead and other toxic heavy metals: the next generation of personal exposure biomonitors. Environ Health Perspect. 2007 Dec;115(12):1683-90.

[76] X. Xuan, F. Hossain, J. Park. A Fully Integrated and Miniaturized Heavy-metal-detection Sensor Based on Micro-patterned Reduced Graphene Oxide. Scientific Reports | 6:33125 | DOI: 10.1038/srep33125

[77] C.G.Fraga. Relavance, essentiality and toxicity of trace elements in human health. Mol. Aspects. Med. 26 (2005) 235.

[78] M.Schaefer, M.Schellenberg, U.Merle, K.H.Weiss, W.Wilson. Protein expression, copper excretion and sweat production in sweat glands of Wilsons disease patients and controls. BMC Gastroenterol. 8 (2008) 29-31.

[79] A. Crew, D. Cowell, J.P. Hart. Development of an anodic stripping voltammetric assay, using a disposable mercury free screen printed carbon electrode for determination of Zinc in human sweat. Talanta. 75 (2008) 1221-1226.

[80] A.P.De Souza, A.S.Lima, M.O Salles, A.N.Nascimento, M.Bertotti. The use of a gold disc microelectrode for the determination of copper in human sweat. Talanta. 83 (2010) 167-170.

[81] J.Kim, W.R.de Araujo, I.A. Samek, A.J. Bandodkar, W. Jia, B. Brunetti, T. Paixao, J. Wang. Wearable temporary tattoo sensor for real time trace metal monitoring in human sweat. Electrochemical Communications. 51 (2015) 41-45.

[82] W. Goa, H.Y Nyein, Z. Shahpar, H.M Fahad, K. Chen, S. Emaminejad, Y.Goa, L. Tai, H. Ota, E.Wu, J. Bullock, Y. Zeng, D. Lein, A. Javey. Heavy Metal Monitoring of bodily fluids. ACS Sensors 1 (2016) 866-874.

[83] A. Koh, D. Kang, Y. Xue, S. Lee, R. Pielak, J. Kim, T. Hwang, S. Min, A. Banks, P. Bastien, M. Manco, L Wang, K. Ammann, K. Jang, P. Won, S. Han, R. Ghaffari, U. Paik, M. Slepian, G. Balooch, Y. Huang, J.A. Rogers. A Soft, Wearable Microfluidic Device for the Capture, Storage, and Colorimetric Sensing of Sweat. Sci Transl Med. 2016 November 23; 8(366)

[84] A. Baraket, M. Lee, N. Zine, M. Sigaud, N. Yaakoubi, M. Trivella, M. Zabala, J. Bausells, N. Jaffrezic-Renault, A. Errachid. Diazonium modified gold microelectrodes onto polyimide substrates for impedimetric cytokine detection with an integrated Ag/AgCl reference electrode. Sensors and Actuators B 189 (2013) 165– 172.

[85] A.Garcia-Cruz, M. Lee, N. Zine, M. Sigaud, J. Bausells, A. Errachid. Poly(pyrrole) microwires fabrication process on flexible thermoplastic polymers: Application as a biosensing material. Sensors and Actuators B 221 (2015) 940–950.

[86] V. Kamakoti, A. Selvam, N. Shanmugam, S. Muthukum, S. Prasad. Flexible Molybdenum Electrodes towards Designing Affinity Based Protein Biosensors. Biosensors 2016, 6, 36.

[87] A Graphene-Based Sensor Array for High-Precision and Adaptive Target Identification with Ensemble Aptamers. J. Am. Chem. Soc. 2012, 134, 13843−13849.

[88] S. Chou, M. De, J. Luo, M. Rotello, J. Huang, V. Dravid. Nanoscale Graphene Oxide (nGO) as Artificial Receptors: Implications for Biomolecular Interactions and Sensing. J. Am. Chem. Soc. 2012, 134, 16725−16733.

[89] P.Lin, X.Luo, I.Hsing, F.Yan. Organic Electrochemical Transistors Integrated in Flexible Microfluidic Systems and Used for Label-Free DNA Sensing. Adv. Mater. 2011, 23, 4035–4040.

[90] X.Guo, J.Liu, F. Liu, F. She, Q. Zheng, H.Tang, M. Ma, S. Yao. Label-free and sensitive sialic acid biosensor based on organic electrochemical transistors. Sensors and Actuators B 240 (2017) 1075–1082.

[91] L. Castano, A.Flatau Smart fabric sensors and e-textile technologies: a review. Smart Mater. Struct. (2014) 23, 053001 (pp27).

[92] Ohmatex-Smart Textile Technology. Available from: www.oh matex.dk (Accessed 21/2/18).

[93] T.Guinovart, M.Parilla, G.A.Crespo, F.X Rius, F.J Andrade. Potentiometric sensors using cotton yarns, carbon nanotubes and polymeric membranes. Analyst. 138 (2013) 5208 - 5215.

[94] T. Choudhary, G. Rajamanickam, D. Dendukuri. Woven electrochemical fabric based test sensors: A new class of multiplexed electrochemical sensors. Lab Chip. 15 (2015) 2064-2072.

[95] N. Coppede, G. Tarabella, M. Villani, D. Calestani, S. Lannotta, A. Zappettini. Human stress monitoring through an organic cotton-fiber biosensor. J. Mater.Chem B. 2 (2014) 5620.

[96] X. Liu, P.B Lillehoj. Embroidered electrochemical sensors for biomolecule detection. Lab Chip 16 (2016) 2093 – 2098.

[97] Xueqiu You,James Jungho Pak. Graphene-based field effect transistor enzymatic glucose biosensor using silk protein for enzyme immobilization and device substrate. Sensors and Actuators B 202 (2014) 1357–1365.

[98] H. Chou, A. Nguyen, A. Chortos, J. To, C. Lu, J. Mei, T. Kurosawa, W.Bae, J.Tok, Z. Bao. A chameleon inspired stretchable electronic skin with interactive colour changing controlled by tactile sensing. Nat. Commun. 6 (2015) 8011.

[99] S. Lee, A. Reuveny, Reeder, J. Lee, H. Jin, Q. Liu, T. Yokota, T. Sekitani, T. Isoyama, Y. Abe, Z. Suo, T. Someya. A transparent bending-insensitive pressure sensor. Nat. Nanotechnol. 11 (2016) 472.

[100] M. Amjadi , K. Kyung , I. Park, M. Sitti. Stretchable, Skin-Mountable, and Wearable Strain Sensors and Their Potential Applications: A Review. Adv. Funct. Mater. 26 (2016) 1678–1698.

[101] A. Yang, Y. Li, C. Yang, Y. Fu, N. Wang, L. Li, F. Yan. Fabric Organic Electrochemical Transistors for Biosensors. Adv. Mater. 2018, 30, 1800051.

[102] J. Wang, W. Li, L. Ban, W. Du, X. Feng, B. Liu. A paper-based device with an adjustable time controller for the rapid determination of tumour biomarkers. Sensors and Actuators B 254 (2018) 855–862.

[103] Q. Baloach, A. Tahira, A. Begum Mallah, M. Ishaq Abro, S.Uddin, M.Willander, Z.Ibupoto. A Robust, Enzyme-Free Glucose Sensor Based on Lysine-Assisted CuO Nanostructures. Sensors 16, 1878 (2016) doi:10.3390/s16111878.

[104] Y.Bai, W.Yang, Y.Sun, C.Sun. Enzyme-free glucose sensor based on a three-dimensional gold film electrode. Sensors and Actuators B 134 (2008) 471–476.

[105] C. Bell, A. Nammari, P. Uttamchandani, A. Rai, P.Shahand, A. Moore. Flexible electronics-compatible non-enzymatic glucose sensing via transparent CuO nanowire networks on PET films. Nanotechnology 28 (2017) 245502 (11pp)

[106] D. Choi, J. Kim, G.R. Cutting, P. Searson. Wearable Potentiometric Chloride Sweat Sensor: The Critical Role of the Salt Bridge.Anal. Chem. 88 (2016) 12241–12247.

[107] A.P.F Turner. Biosensors: sense and sensibility. Chemistry Society Reviews. 42 (2013) 3184 – 3196.

[108] M. de Planell-Saguer, M. Celina Rodicio. Analytical aspects of microRNA in diagnostics: A review .Analytica Chimica Acta 699 (2011) 134–152.

[109] S.Dijkstra, P.F.A. Mulders, J.A. Schalken. Clinical use of novel urine and blood based prostate cancer biomarkers: A review .Clinical Biochemistry 47 (2014) 889–896.

[110] T. Monbailliu, J. Goossens, S. Hachimi-Idrissi. Blood protein biomarkers as diagnostic tool for ischemic stroke: a systematic review.Biomark Med. 2017 May;11(6):503-512.

[111] https://www.medtronic-diabetes.ie/minimed-system/continuous-glucose-monitoring (accessed 27/2/18)

[112] https://freestylediabetes.ie/ (accessed 27/2/18)

[113] http://www.gluco-wise.com/ (accessed 27/2/18)

[114] H. Lee, T. Choi, Y. Lee, H. Cho, R. Ghaffari, L. Wang, H. Choi, T. Chung, N. Lu, T. Hyeon, S. Choi, D. Kim. A graphene-based electrochemical device with thermoresponsive microneedles for diabetes monitoring and therapy. Nature Nanotechnology volume11, pages 566–572 (2016)

[115] W. Jia. Electrochemical tattoo biosensors for real-time noninvasive lactate monitoring in human perspiration. Anal. Chem. 85, (2013) 6553–6560.

[116] J.Kim, G.Valdés-Ramírez, A.J.Bandodkar, W.Jia, A.G.Martinez, J.Ramírez, P.Mercier, J.Wang. Non-invasive mouthguard biosensor for continuous salivary monitoring of metabolites. Analyst. 139 (2014) 1632.

[117] R.Malon, K.Y.Chua, D.H.Wicaksono, E.Corcoles. Cotton fabric based electrochemical device for lactate measurement in saliva. Analyst 139 (2014) 3009.

[118] S. Liakat, K.A. Bors, L. Xu, C.M. Woods, J.Doyle, C.F.Gmachl. Noninvasive *in vivo* glucose sensing on human subjects using mid-infrared light. Biomedical Optics Express. 5 (2014) 2397-2404.

[119] N.Ozana, Y.Beiderman, V.Mico, M.Sanz, X.Garcia, A.Arnand, J.Baharam, Y.Epstein, Z.Zalevsky. Improved noncontact optical sensor for detection of glucose concentration and indication of dehydration level. Biomedical optics express. 5 (2014) 1926-1940.