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REVIEW

Blood, sweat, and tears: developing clinically relevant protein biosensors for integrated body fluid analysis

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Biosensors are being developed to provide rapid, quantitative, diagnostic information to clinicians in order to help guide patient treatment, without the need for centralised laboratory assays. The success of glucose monitoring is a key example of where technology innovation has met a clinical need at multiple levels – from the pathology laboratory all the way to the patient's home. However, few other biosensor devices are currently in routine use. Here we review the challenges and opportunities regarding the integration of biosensor techniques into body fluid sampling approaches, with emphasis on the point-of-care setting.

Introduction

Biosensors aim to deliver important diagnostic data into the hands of patients or their treating clinicians in real-time, without the need for centralised laboratory infrastructure. Biosensor technology can be applied in a variety of clinical settings: (a) the emergency situation where urgent diagnostic information will change the course of treatment, *e.g.* acute coronary syndromes[1]; (b) the hospital inpatient setting where immediate results are more desirable even though full pathology laboratory testing may be available, *e.g.* standard blood panel[2]; (c) the outpatient setting where a test result is required to dictate overall management but this has not been attended to by the patient ahead of time, *e.g.* quarterly HbA1c monitoring in diabetic patients[3], or (d) in the patient home for screening or follow-up, *e.g.* glucose monitoring[4]. In addition to these examples, biosensors can be used in low-resource settings without the need for highly trained medical staff, and have the potential to greatly improve patient care[5] in disease outbreaks where complex sample handling is undesirable (*e.g.* the recent West African Ebola epidemic[6, 7]). The endocrinology community has been at the forefront of the clinical adoption of biosensor technology with patient-driven glucose monitoring becoming a mainstay of diabetes care since the 1970's.[4] Indeed, this trend has continued with the widespread adoption of in-office testing of HbA1c (a measure of aggregate glycaemic control over the preceding 3 months),[3, 8] however relatively few assays/methods move beyond the central laboratory.

A biosensor is comprised of three key operations – first the sample collection, followed by assay chemistry, and finally, detection and recording of a quantifiable signal (noting that the chemistry/transduction are often linked). The combined assay/detection technique that has proven most successful in making the transition from the central lab to the point-of-care involves detection of small molecules, *e.g.* glucose, lactate, using enzymatic electrochemical methods. Detecting larger molecules, *e.g.* proteins, lipids, nucleic acids, *etc.*, is a much more challenging problem, mainly due to non-specific adsorption of body fluid components at transducing sensor surfaces, and the general lack of enzyme/analyte pairs for many protein targets. However progress is being made in the development of affinity-based sensors to meet this need, and is reviewed elsewhere[9]. However, progress at the sampling stage lags behind both the assay chemistry and detection methods in terms of research output and perceived importance.[10, 11] Accordingly, the majority of sample collection and processing techniques, for any class of analyte, are still reliant on 20th, and in some cases, 19th century technology (*e.g.* needles and blood tubes[12]). It is thus becoming clear that significant research effort needs to be directed to the development of innovative body fluid sampling strategies that integrate or simplify the downstream operations of the diagnostic testing process.

Looking to the future, it is likely that lower abundance analytes will be of increasing importance to meet the goals of early disease detection, and biosensors should be key tools in this emerging field. Instead of non-specific metabolites and electrolytes (*e.g.* the standard blood panel which includes glucose), these are more likely to be disease-specific proteins, nucleic acids, lipids, or even whole cells, which have been validated in discovery-focused studies.[13-15] In recent times, a range of ultra-sensitive bioassays has been developed to partially address this challenge, often incorporating aspects of nanoparticles and nanotechnology, and mainly using affinity-based interactions between analytes and antibodies, aptamers,

ionophores, or other high-affinity binders, all of which have been thoroughly reviewed elsewhere.[2, 16-19] However, given that the blood volume of a human is on the order of 5 L, and the interstitial fluid volume is ~17 L,[20] the relatively low abundance of these biomarkers leads to an inexorable statistical sampling issue which cannot be solved without addressing the limitations of bulk fluid sampling. As elegantly described by Labuz *et al.*[10] and Mariella *et al.*[11], Poisson statistics dictates that as analyte concentration is reduced, the probability increases that a collected sample of body fluid does not contain any analyte (37% from 1 mL of sample containing a concentration of 1000 molecules/L). Unchecked, this would (or possibly already has, in some circumstances) lead to a stochastic distribution of false negative results, which have nothing to do with the downstream assays chemistry or detector sensitivity – it is simply that the sample volume may not contain the analyte. This could certainly be the case in the emerging areas of ultra-sensitive protein detection (<fg/mL[21]), circulating tumor cells (<50 cells/mL[10]), and microbial sepsis (<100 cfu/mL[22]). In these cases, it is likely that novel sampling approaches will be required in combination with ultra-sensitive detection tools.

The ultimate application of a clinical biosensor is to measure the concentration of a biomarker (or panel thereof), in a real-time, continuous manner directly in body fluids. This would reduce the need for frequent sample collection and potentially open up new approaches in biomarker-guided therapeutic intervention.[23] It is also considered the ultimate goal because if biosensors were capable of real-time sample monitoring, it follows that they could also be applied to simple endpoint testing applications. Some would argue that biosensors, by their very nature, are already capable of real time and continuous sensing (*e.g.* real time binding kinetics, etc.). However, as the majority of applications involve measurement of an analyte in an isolated sample, this definition does not apply to the practice of monitoring analyte concentrations in real time. The benefits of real-time monitoring must be connected with real time sampling to meet this ultimate clinical utility.

The purpose of this review is to identify emerging protein biosensor technologies applied in clinically relevant situations using integrated body fluid sampling strategies. We have deliberately used a broad definition of the term "biosensor" so as to capture emerging technologies. However, we limit our scope generally to bioanalytical methods that currently or potentially combine all three steps of a diagnostic process into an integrated device, requiring minimal sample processing or user input (*e.g.* washing steps), and for which a quantifiable indicator of analyte concentration can be detected, preferably in real time. A focus on *in vitro* bioassays is therefore beyond the scope of this review, and readers are directed to a range of other excellent reviews on related topics throughout this review.

Technical complexities of diagnostic sampling

Body fluids are highly complex mixtures that contain a variable concentration of cells, proteins, macromolecules, metabolites and small molecules. Complex biochemical reactions occur naturally in these fluids (*e.g.* blood clotting), hence it is logical that removal and handling of these fluids by either passive (*e.g.* urine collection) or active (*e.g.* the standard blood draw) methods can alter the composition, resulting in problems prior to the assay even being performed. This issue is commonly referred to as "pre-analytical variability," and even with recent improvements in quality control and standardization in clinical laboratories, it is estimated that over 90% of errors in the diagnostic process are related to this problem.[24] There is already evidence that this problem affects the performance of biosensors exposed to body fluids, even those diluted or otherwise treated to account somewhat for the variation.[10] Taking blood as a case in point, many studies have identified changes in biomarker levels as a function of time to analysis,[25, 26] different collection tubes and associated fittings,[12, 25] and the degree of hemolysis (ruptured red cells leak hemaglobin into serum/plasma which changes colour of the sample leading to inaccurate results in optical assays[12]), which is in turn affected by the sampling method, sampling site, needle gauge, collection flowrate and the size/flow properties of the specific vein involved. Clearly, attempts to address the issue of pre-analytical variability at the sampling stage could pass "savings" on downstream.

Clinical complexities of diagnostic sampling

There are significant practical aspects of sample collection that are rarely discussed in the context of analytical device development. In the clinical setting, poor venous access is a key limitation in the delivery of intravenous therapies, but it can also be problematic for simple sample collection in some patients. Access to a vein for routine blood sampling relies on a trained health care professional to visually identify a reasonable vein, then perform accurate venepuncture and maintain sterility both during and in-between sampling events and tube changes. Factors contributing to difficulty in accessing veins for peripheral cannulation and sample collection include: extremes of patient weight, clinician inexperience, and clinician judgement of poor venous access.[27-29] Other contributors include: extremes of patient age, exposure to cytotoxic drugs (*e.g.* previous chemotherapy), anatomical factors (*e.g.* previous surgical procedures close to sampling site), and prolonged hospital stay requiring the siting of multiple short-term peripheral cannulae. Collection of other fluids can also be highly reliant on clinician skill (*e.g.* lumbar puncture to collect cerebrospinal fluid which also requires patient sedation), and patient's ability to produce a sample in accordance with instruction (*e.g.* urine). Uncontaminated urine can also be difficult to collect in unwell patients or the elderly, confused, incontinent of urine, or who require permanent indwelling catheters. Circumventing these complex and user-dependent

Table 1: Key properties of human body fluids

Body Fluid	Sampling Techniques	pH	Unique proteins (%, in comparison to plasma)	Total Protein Concentration (mg/mL)	Viscosity (mPa.s)
Blood	Needle, lancet	7.35 – 7.45[30]	NA	60 – 80 mg/mL[30]	Serum: 1.52 – 1.54[31] Plasma: 1.58 – 1.60[31] 1.18 – 1.28[32] Blood: 4.69 – 5.2 (92s^{-1})[31] 4.25 – 4.61 (583s^{-1}) [31]
Saliva	Swab	6.2 – 7.4[33]	38,[34] 31[35]	0.2 – 5 mg/mL[36]	2-8 (90s^{-1})[37] 1.5-4 (90s^{-1})[38]
Urine	Passive collection or catheter	4.5 – 8.0[39]	30[40]	<150 mg/day excreted[39] and <0.1mg/mL[41]	0.6-1.2[42]
CSF	Lumbar puncture	7.31 – 7.35[43]	40,[44] 28[45]	1:20 – 1:100[46] (blood plasma)	0.55-0.7 (360-1460s^{-1})[47] 0.7-0.74 (5-100s^{-1})[48]
Tear fluid	Swab, contact lens	6.5 – 7.5[49]	34[50]	6 – 10 mg/mL[50, 51]	1.5 – 3 (20-160s^{-1})[52]
Exhaled breath	Bag, cold trap	7.5 – 7.65[53]	-	1 – 4 mg/mL[54]	-
Sweat	Swab, tattoo	4.0 – 6.8[55]	20[56]	0.1 – 0.7 mg/mL[57]	0.9197
Interstitial fluid (skin)	Tape-strip, iontophoresis, microdialysis, microneedle array	7.2 – 7.4[58]	32[59]	13 – 20 mg/mL[60]	-

collection methods with biosensors could therefore improve access to diagnostic information for significant number of patients.

Comparing different body fluids

Table 1 compares and contrasts key aspects of the body fluids under review. Sample collection methods vary widely across the fluids. They are dominated by bulk fluid sampling approaches that pass complex samples into the downstream assay/detection processes, potentially limiting sensitivity and specificity due to contamination of sensing surfaces with non-specific material. Some methods are more acceptable to some patients (*e.g.* urine preferred to blood; but either would be preferable to lumbar puncture); some methods could be classified as "active" or "passive" (*i.e.* either requiring the patient to actively produce the sample versus passive collection). While most fluids have a physiological pH range similar to that of blood, it is interesting to note that both urine and sweat are quite acidic, and also have quite a variable pH range, which would certainly be expected to affect biosensor readings. While there is significant variation in total protein concentration across the fluids, with blood or plasma the most concentrated fluids, others including saliva, tears, and skin fluid contain a relatively high concentration as well. Encouragingly, all of the fluids possess both a unique proteome (20-40% in comparison with blood plasma) highlighting the need for body fluid-specific assays; yet there is enough overlap with blood in many cases to highlight that there may be situations in which blood sampling is not required to access circulating analytes. It is important to note that the analysis may not be that simple; indeed the data presented in Table 1 does not take into account key complexities in the molecular weight distribution of proteins in each fluid, nor the relative concentration of individual proteins, which can cover 12 orders of magnitude for blood alone.[20, 61] Finally, body fluids all appear to show non-Newtonian, shear-thinning, behaviour as a function of shear rate. Interestingly, some fluids (saliva, blood, plasma) show this behaviour more than others, which could be considered to have constant visco-elastic properties under most testing conditions (*e.g.* urine, sweat, CSF). However, to our knowledge this is an incomplete dataset as the visco-elastic behaviour of these fluids have not all been investigated, thoroughly or otherwise.

Biosensor application with commonly sampled fluids

Blood is the most commonly collected sample for clinical diagnostics, and the blood proteome and the range of clinical tests available are thoroughly reviewed elsewhere.[20, 61] As most cell and tissue excretory products present in the blood, it contains a mixture of classic plasma proteins, secreted proteins, short- and long-range receptor ligands, tissue leakage products, aberrant secretions and foreign proteins, along with metabolites and electrolytes – many of which can be correlated to disease diagnosis, progression ad treatment response. Over 200 proteins are used in clinically approved tests in the USA[61] and the standard blood panel of metabolites and electrolytes (sodium, potassium, chloride, calcium, bicarbonate, glucose, urea and creatinine) is the lab test most frequently requested by clinicians.[7] Lateral flow assays have proven extremely successful in providing a simple and minimally invasive biosensor options for consumers (*e.g.* pregnancy testing), and especially in remote locations (*e.g.* infectious diseases) and have been thoroughly reviewed recently by Yetisen *et. al.*[62] However they are directly reliant on lancets or needles for sample collection, and are unlikely to find application in real-time applications. Electrochemical analysis is also commonly employed here and is well-suited to the detection of low-molecular weight molecules, and is also the basis of most implantable devices, as described thoroughly by Bernhardt *et. al.* [63] (fundamental basis) and Kotanen *et. al.*[64] (applications) in recent reviews. However, this approach requires both (a) an analyte-specific enzyme which reacts with the analyte to

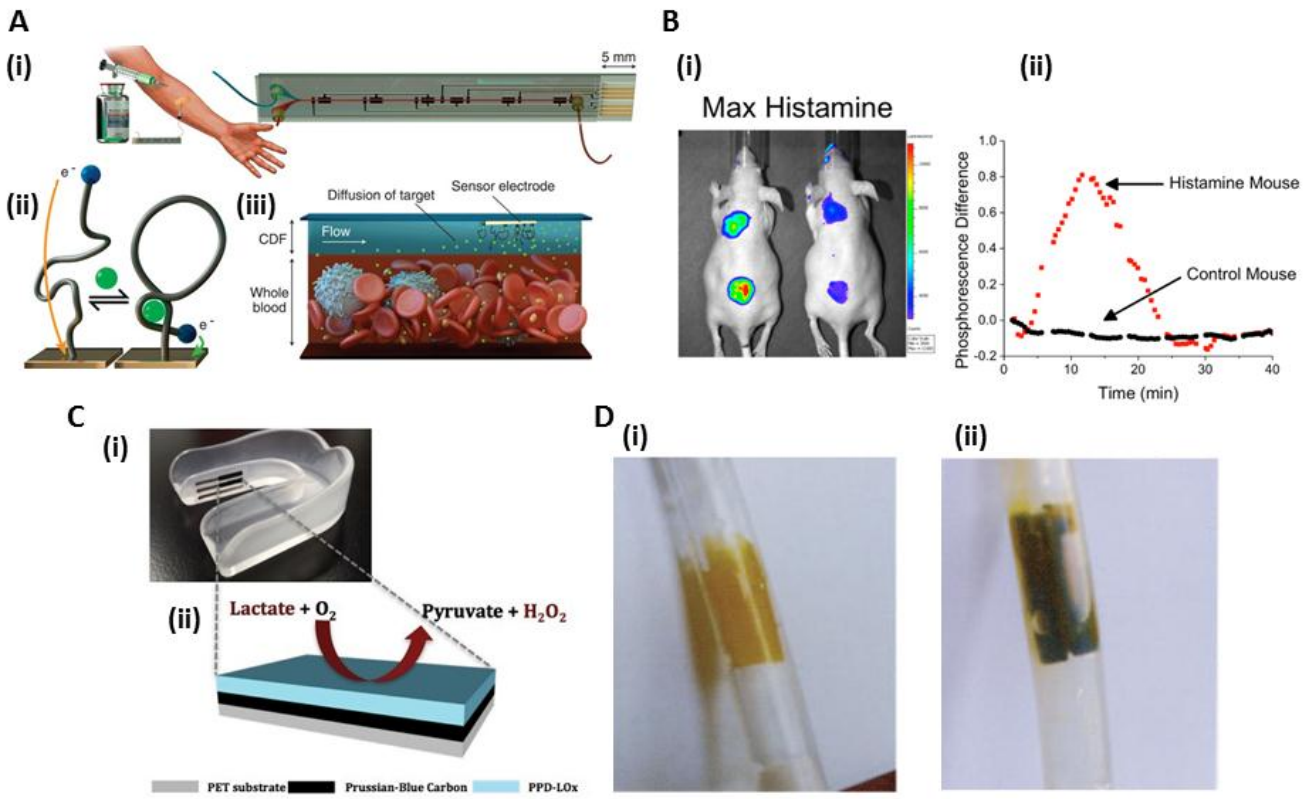

Figure 1: Examples of emerging biosensor technologies for commonly sampled body fluids. (A) "MEDIC" device, which incorporates a microfluidic chamber fed with blood via a catheter (i), detecting doxorubicin in a reversible and real-time manner, using an aptamer-based affinity electrochemical assay, using a "continuous-flow diffusion filter" to limit non-specific fouling of the electrode (iii). (B) optode microparticles that, upon injection in to the subcutaneous tissue of mice (i), can be used to measure the levels of small molecule electrolytes/metabolites in a continuous manner (ii); (C) electrochemical enzymatic sensor (ii) integrated into a mouthguard (i) for continuous monitoring of lactate in saliva. Images for (A-D) adapted with permission from references 65, 67, 75 and 97, respectively.

produce a detectable current at a transducing surface, and (b) a low molecular weight cut-off filter surrounding the device that reduces non-specific interference by allowing only the passage of low molecular weight species to the sensor. However, if the device is to be operated *in vivo*, or if large macromolecules or proteins are the target analytes, then non-specific adsorption of blood proteins interferes significantly with the electrochemical signal. To overcome this limitation, new strategies are being developed for affinity-based electrochemical sensors.[9] Optical approaches are also being developed, for which non-specific adsorption does not necessarily affect the optical detection signal.

In one of the very few examples of an electrochemical assay using an affinity-based approach for real-time sensing, Ferguson *et al.* [65] recently demonstrated real-time detection of doxorubicin, a chemotherapy agent, in a real-time, continuous assay in rats *in vivo* (Figure 1A). The "MEDIC" device comprises a catheter inserted into the patient that diverts blood (~0.75 mL/hr) into a microfluidic device containing an electrochemical, aptamer-based sensor. Upon specific drug binding, the aptamer probe undergoes a reversible conformational change that modulates electron transfer between the terminally bound methylene blue redox reporter and the electrode. Importantly, the aptamer also showed rapid kinetics (k_{on} ~3 $\mu M^{-1}min^{-1}$ and k_{off} ~ 1.35 $\mu M^{-1}min^{-1}$) such that the doxorubicin concentration in the blood could be monitored stably over ~4 hours. A crucial aspect of the design is the inclusion of a "continuous-flow diffusion filter", in which a buffer stream flowing across the sensor is combined with the blood flow, in a laminar regime, such that only the molecules with large enough diffusion constants (*e.g.* small molecule drugs) are able to diffuse from the blood into the buffer in sufficient time to be detected by the sensor. This filter serves the same purpose as the polymeric matrices employed in traditional electrochemical devices, with the same limitation

that developing assays for larger protein analytes could be problematic. Using a custom-designed algorithm based on the charge-transfer kinetics to reduce sensor drift, the MEDIC device is capable of stable, continuous, quantitative monitoring of doxorubicin in human blood for at least 4 hours.

An interesting alternative to the routine blood panel analysis has been developed in Clark's group (reviewed here[3]), involving the *in vivo* analysis of analyte-specific fluoroescence in a real-time and continuous format (Figure 1B). These "optodes" (named based on their conceptual similarity to ion-selective electrodes) consist of plasticised microparticles that are loaded with analyte-specific ionophores and a pH-sensitive fluorescent dye. In the absence of analyte, the ionophore is protonated, but upon selective binding of the analyte, the dye deprotonates to maintain the charge balance in the particle, resulting in a concentration-dependent change in optical properties. This approach has been used to measure common blood panel analytes, both *in vitro*[66] but also in a real time, continuous manner. Clark's group have demonstrated that following injection of the particles into the subcutaneous tissue, various small molecules and electrolytes (including histamine,[67] sodium,[68] glucose[69]) can be measured in real-time by whole body fluorescence imaging, and most recently via photo-accoustic imaging.[70] This approach is extremely promising, however again a key challenge is to move beyond the standard blood panel for real-time, continuous monitoring of proteins and other macromolecules. Furthermore, optical detection methods that are practical in clinical environments are yet to emerge.

Saliva has a long history of use in clinical diagnostics due to the ease of sample collection (swab or passive drool) and the wide variety of both host biomarkers and those associated with infection. A key issue with saliva, as shown in Table 1, is the extreme range of fluid viscosity, which is a key challenge for device engineering. While there has been significant overlap with blood serum observed in terms of proteomics, the concentration of protein in saliva is significantly lower (~30%) and there are additional dynamic changes relating to diet and fluid intake[36]. Nasopharyngeal fluid is a related sample that can also be collected from the nasal passages for specific pathogen detection, and is currently routinely collected for respiratory virus DNA via PCR, often for a multiplex panel of 6-8 common viruses.[71] There is often a lengthy waiting period between sample collection and the attainment of final results, an issue that has been problematic during influenza epidemics.[72, 73] (*e.g.* H1N1) Development of sensitive protein biosensors may help to rapidly identify the disease-causing pathogen in a timely fashion in some cases. Biosensors have been applied to detect a range of analytes including small molecules (lactate[74, 75], cortisol[76, 77], biogenic amines[78]), proteins and organisms (salivary alpha-amylase[79-84], CA15-3[85], influenza virus[86], mutans streptococci[87, 88]). These studies generally used optical immunoassay approaches to detect those proteins for which no enzyme partner was apparent, or enzymatic electrochemical assays (in the case of amylase). Interestingly, Aluoch *et al.*[84] developed an electrochemical immunoassay biosensor for salivary amylase which compared favourably to a sensitive ELISA, however it has not yet been tested in real fluids. However, in nearly all of these cases, saliva was collected via the "passive drool" method and often processed (*e.g.* by dilution, buffer exchange, *etc.*) prior to analysis, hence limiting the potential for real-time sample analysis.

A mouthguard sampling device recently developed by Kim *et. al.*[74] is a novel collection device with the potential to convert the current trend of passive, end-point saliva sampling into a minimally-invasive continuous monitoring system (Figure 1C).[74] Since salivary lactate concentrations correspond well to blood lactate levels, the former is of interest in fitness monitoring. The mouthguard consists of a polyethylene terephthalate (PET) substrate coated with a printable Prussian-Blue (PB) transducer, and overlaid with lactate-oxidase enzyme entrapped in poly-orthophenylenediamine (PPD). The PB transducer detects the hydrogen peroxide products of the oxidase reaction, while the PPD acts to protect the biosensor surface and prevent fouling. In buffered media, the sensor could detect lactate in saliva over the physiological range, with a detection limit of ~0.1 mM. Addition of physiological levels of other electroactive species (ascorbic acid and uric acid) had negligible effect on the lactate detection at 0.5 mM, suggesting the PPD layer provided adequate protection. In human saliva samples, the device measured background lactate levels at ~0.01 mM, which is in the normal range for unstimulated saliva, with a linear response to 0.5 mM. In continuous operation mode, the device was tested every 10 minutes over a 2-hour period, without significant loss of function. Future work will focus on miniaturization of circuits, and detailed toxicology and biocompatibility analysis.

Urine is a commonly collected sample for clinical and non-clinical testing, especially due to the ease of collection, usually without the need for invasive procedures. Invasive sampling is occasionally required in infants where a suprapubic aspirate is performed for collection of a sterile sample, or the incontinent elderly where an 'in-out' catheter must be inserted and then withdrawn from the urinary bladder. Lateral flow assays have also been designed for endpoint analysis of a range of analytes including pregnancy hormones, glucose, bilirubin, ketones and drugs of abuse.[62] Indeed, these devices are far better suited to urine than blood, because the latter requires lancets or needles to provide the sample to be analysed. However, urine samples require active production of the sample by the patient, which can then only be used for endpoint analysis. Furthermore, as demonstrated in Table 1, only analyte amount can be quantified for urine analysis, as the volume produced by different people at different times renders concentration readings effectively meaningless. Urine biosensors applied to human sampling have typically focussed on enzymatic small molecule analysis, that may be indicative of renal tract pathology (oxalate[89], glucose[90-92], uric acid[93-95]), with more complex systems emerging to

detect proteins. In one case the authors reported detection of bladder cancer marker NMP22[96] in clinical samples using an electrochemical affinity-based biosensor, although samples needed to be diluted 1:10 in buffer for successful quantitative detection. Samples are usually collected in a suitable vessel for endpoint analysis, followed by processing via buffer dilution and/or pH neutralization, and solids removal. However, for continuous analyte detection, the best example is the development of smart catheter devices that respond to the presence of infectious agents.

Integration of biosensors into in-dwelling urinary catheters can be used to provide early warning of infection. The key advantage of such systems is that a real-time and continuous indication can be provided, without relying on active participation by the patient, which can identify signs of infection days before catheter lines become encrusted and blocked. While these devices might not be considered to fit the traditional definition of a biosensor, their clinical application is aligned. For example, Stickler *et al* have developed a sensor which can be placed inside a catheter bag which changes colour in response to pH changes. The pH change is usually related to the presence of pathogens in the urine, and could be used as an early indicator of line infection prior to catheter blockage (Figure 1D). The sensor consisted of a pH-sensitive dye (Bromothymol Blue – BTB) embedded in a cellulose acetate matrix. Infections caused by *P. mirabilis* and other urease-positive microorganisms causes increase in pH of urine, and the sensor changes colour ~12 hours following infection, in a model system.[97] This compared to ~55 hrs for blockage of catheter due to encrustation, which is usually the clinical endpoint reached prior to replacement which may require emergency referral. The sensor was then tested in a clinical trial to assess performance in comparison to blockage time.[98] The sensor only changed colour in response to *P. mirabilis* infection (15 patients), and did not change colour in samples from patients where the infection was not identified (5 patients). Importantly, in agreement with the earlier study, the sensor was able to detect infection up to 12 days prior to catheter blocking, so that catheter replacement could be performed long before emergency referrals were necessary. Recently an improved design was reported that overcame previous manufacturing limitations that prevented scale-up. The new material was based on a PDMS substrate, and the sensing reagents could be incorporated into the 2-part curing system.[99] The newer device showed similar performance to the original in clinical trials[100], and further work is planned to further reduce the time between sensor colour change and catheter blockage, to reduce the number of replacements required for any given patient.

Cerebro-spinal fluid is an excellent example of an important clinical sample that must be collected to rule out potentially life-threatening conditions, for which there is no viable biosensor. Sampling is painful for patients, and currently requires the presence of highly practiced medical professionals, whereby a needle is inserted into the space between L3 and L5 lumbar vertebrae to withdraw fluid. CSF is a clear, colourless fluid which in health, has a lower cell count and significantly lower protein concentration than blood (Table 1). The most common reason to sample this fluid is to establish the presence of central nervous system (CNS) pathology (e.g. infection, malignancy, autoimmune disease), and is also sometimes used in the acute setting to rule out meningitis. Recent studies have also shown detection of amyloid-products in the CSF of patients with dementia and related conditions,[101] and given that at least 20% of the CSF proteome is unique when compared to blood (Table 1), more CSF-specific biosensors are likely to emerge if convenient sampling approached are developed. To date, no viable alternative to LP-sampling has been developed (outside the setting where the patient has an extra ventricular drain inserted, often for continuous monitoring of CSF pressures and removal of excess fluid as a therapeutic approach). Hence new non-invasive (or less-invasive) techniques for body fluid analysis of CSF and cranial fluids could, at the very least, reduce the pain and discomfort for patients, but could also facilitate the development of novel tests for CNS-related diseases. While this field is in its infancy, readers are directed to a recent ACS virtual issue ("Chemistry and the BRAIN initiative) that highlights recent progress and future directions.[102]

Exhaled breath is of particular interest in the analysis of breath volatile organic compounds (VOCs),[103, 104] which can be related to a range of respiratory conditions (*e.g.* asthma, smoking-related illnesses, cystic fibrosis, *etc.*) and other diseases. A key example is fractional exhaled nitric oxide (FENO) which is significantly increased in the breath of asthma patients and others with lung inflammation.[105] Protein-containing material can also be isolated using a cold-trap system to condense the gas[103] however analysis of the breath condensate is technically challenging.[106] To date, the predominant protein species in this fluid are type I and II cytokeratins (originating from the lung), along with inflammatory cytokines.[107, 108] Traditionally, analysis of exhaled breath is carried out using gas chromatography and mass spectrometry, both of which currently are limited to centralised laboratories. In recent years, there has been more interest in the use of biosensors, which could potentially offer a quick and inexpensive way for detection of breath analytes. End-tidal carbon dioxide monitoring is used routinely in hospitals (both in intensive care units and in surgery) to measure the carbon dioxide concentration in the breath of intubated patients,[109] using a simple optical approach. A number of studies describe the development of electrochemical arrays for single or multiplexed analyte detection ("electronic noses"),[104, 110-115] yet as there are no widely accepted standardised methodology for sample collection and analysis[116], development and use of breath testing for the purpose of disease diagnostic has been limiting.[103] The availability of simple devices for collection of exhaled breath and condensate (*e.g.* the RTube™ - a nebulizer that non-invasively captures expired breath condensate under

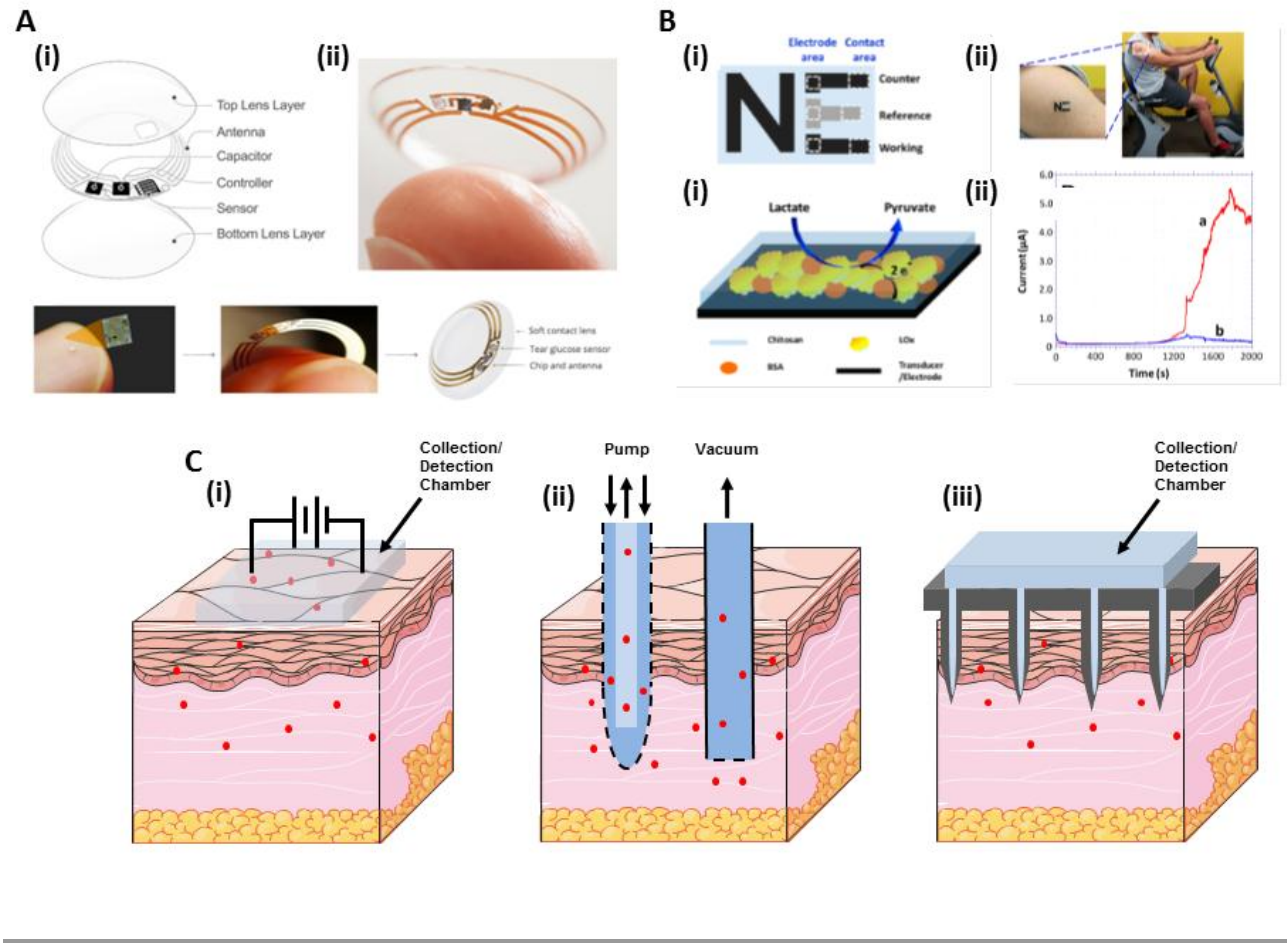

Figure 2: Examples of emerging biosensor technologies for emerging body fluids. (A) A contact lens glucose sensor showing the sensor construction (i) and real-size comparison (ii). (B) Sweat tattoo sensor (i) designed for enzymatic electrochemical lactate detection (ii), with the sensor shows applied to skin (iii), and the real-time readout on an exercising human (iv). (C) Schematic of emerging skin sampling devices, based on iontophoresis (i), microdialysis (ii), and microneedle array (iii). Images (A) and (B) adapted with permission from references 123 and 131, respectively

normal breathing) may speed up device development, and several groups appear to be integrating sensors into these devices.[117, 118]

Biosensor application with emerging body fluids

Analysis of **tear fluid** is a relatively new concept, and to date glucose is the only analyte targeted for detection. The concentration of glucose in tears has been shown to be highly correlated to blood glucose with a lag time of ~10 minutes making tear glucose sensors a worthwhile alternative to finger pricking for repetitive or continuous monitoring. Tears are also a promising fluid for protein detection, given that the appreciable protein concentration and unique protein content (Table 1). However, to date no published studies are available on biosensing in this context. One of the challenges when sampling tear fluid for a quantitative readout, is that any irritation can cause an increase in tear production leading to a reduction in biomarker concentration.[119] There are several potential solutions which have been explored such as minimally invasive capillary collection at the corner of the eye[120] to calibration with a continuous monitoring device such as an electrode embedded contact lens[121]. Many groups have turned to contact lenses (Figure 2A) because a significant amount of research has already been carried out on the fabrication, biocompatibility and fouling mechanisms on these surfaces,[122] as discussed in a recent review.[123] The substrates chosen for biosensor construction (excluding electrodes) are almost exclusively polymeric in nature due to the biocompatibility and fouling properties that can be produced. Although some early work was performed on disposable fluorophore-doped contact lenses,[124, 125] electrochemical detection has since become the favoured method of quantification, [120, 126, 127] due to the ease of integration with continuous and wireless readouts. [121, 128] The optimisation of enzyme and electrodes for glucose detection in tear fluids is an active area of research. [129]

Sweat is an acidic, electrolyte-rich fluid whose production is induced by exercise and results in secretion of metabolites including lactate, glucose and uric acid.[55] However, in terms of biosensor systems in development, efforts have focussed on the electrochemical detection of the metabolites lactate, glucose and uric acid, because the protein content is extremely low (Table 1). Sample collection methods include simple swabbing of the skin, or fluid collection with a microsyringe, however these methods are yet to be integrated with sensors. The Macroduct™ system uses iontophoresis in the presence of pilocarpine to induce and then collect sweat fluid, which has been used for clinical sodium chloride analysis for cystic fibrosis diagnostics, and also in proteomic studies of sweat.[56, 130] Sample collection tools that can be applied for continuous analysis, or those that do not rely on active sweat production could result in very useful biosensors, due to the non-invasive nature of analysis. However the key limitation is that patients cannot easily control their sweat production for sampling/analysis, and it is also affected significantly by environmental factors including temperature and humidity.

An example of a continuous sweat "tattoo" biosensor was developed by Jia *et. al.*[131] for measurement of exercise-induced lactate (Figure 2B). The device consists of a screen-printed electrode on a flexible substrate, with lactate oxidase immobilised onto the working electrode with multi-walled carbon nanotubes acting as the transducer surface, and tetrathiafulvalene (TTF) added to enhance low-voltage electrocatalytic conversion of lactate. Testing carried out *in vitro*, with the sensor attached to both rigid and flexible substrates, showed that the amperometric response was stable to repeated mechanical bending, was unaffected by the presence of physiological concentrations of other metabolites (*e.g.* creatinine, ascorbic acid, glucose, uric acid), with a linear response rate for lactate of 1 – 20 mM (typical physiological levels up to 25 mM). Epidermal testing was also performed over ~30-minute period of exercise, with excellent agreement to laboratory testing. Colorimetric analysis of sweat pH and metal ions has also been demonstrated in sweat *in* situ by Huang, *et. al.*[132] Such devices could be extremely useful in a range of non-invasive applications, especially if advances are made that facilitate sensitive protein detection (as discussed with regards to blood). Furthermore, this method is limited in that sufficient electrolyte fluid (sweat) must be in contact with the sensor for the amperometric signal generation; hence it currently relies on active sweating.

While the composition and origin of **skin interstitial fluid** (ISF) remain difficult to define [133], its diagnostic potential arises from its ease of access, high degree of vascularisation [134], and passage of blood biomarkers into the ISF under hydrostatic and osmotic pressure. In particular, skin capillary vessels readily exchange fluid and small molecules with the ISF, whilst having a lower permeability towards macromolecules, such as proteins [135]. Thus, much of the focus on skin sampling to date has been on using ISF as a proxy for blood sampling of small molecules, such as glucose [136, 137], lactate [138], cortisol [139], and urea [140]. While analysis of the skin ISF proteome for biosensing applications has been largely overlooked, several studies suggests that macromolecular biomarkers originating from blood may also be readily accessed from the ISF, along with unique skin-specific proteins.[59, 141] The lack of interest in protein-based skin biosensors to date has been partly due to the challenges associated with developing affinity based biosensors (as discussed for other fluids), and partly due to a lack of convenient approaches developed to sample skin fluid. A number of local skin diseases such as, eczema, psoriasis, cancer and skin based infections present opportunities for diagnosis by altering skin chemistry (pH) and other biomarkers, as recently covered in a review by Paliwal *et al.* [142]. Furthermore, the skin's role in preferentially accumulating some disease markers originating from other sites was also noted, such as amyloid B from Alzheimer's disease and biomarkers of cardiovascular disease risk. A range of bulk fluid/tissue sampling approaches have thus been developed, which include tape stripping, suction blisters and biopsies. Others including iontophoresis, microdialysis and microneedles have been integrated with biosensors and tested in pre/clinical models. One issue that has received little attention is the damage caused to the skin using these approaches, which may in turn affect the levels of target analytes.

In reverse iontophoresis (RI) an electrical current is applied to the skin surface to extract charged, and by inducing fluid flow, uncharged molecules for subsequent analyte detection (Figure 2C(i)). RI has been demonstrated for the sampling of a range of small analytes including phenylalanine [143, 144] urea [140] and glucose [136, 145]. The best known example of an RI extraction system with an incorporated biosensor (in this case electrochemical) is the GlucoWatch Biographer [136]. This device was approved in humans and commercially available for continuous glucose measurement, providing reasonable prediction of blood glucose levels. The device, however, was eventually withdrawn due to a high false positive rate [146]. Although RI is not biomarker selective, molecules migrate to different extents according to their charge and size, which can provide selective purification of the sample during extraction [147]. This typically limits the extraction of proteins, which has the benefit of reducing fouling to electrochemical sensors, and in the case of glucose measurement, results in migration of electroactive interfering molecules to the non-sensing electrode compartment. Recently, the Wang group has developed a proof of concept wearable "tattoo" device that incorporates an RI system with electrochemical glucose detection, [148] using a low-potential Prussian-Blue transducer that potentially allows for more selective and sensitive analysis. Following successful *in vitro* characterization of the specific electrochemical response of the sensor to glucose, the device was trialled on human volunteers by detecting an increase in glucose levels following a meal.

Microdialysis (MD) employs a semi-permeable probe inserted into the dermis or subcutaneous tissue, enabling partially selective sampling of proteins and small molecules based on the membrane molecular weight cutoff (MWCO) of the probe (Figure 2C(ii)) [149-151]. The implanted MD probe is perfused with an isotonic liquid that collects molecules below the MWCO of the membrane through diffusion, which can then be collected and analysed. Since the pioneering work of Jansson *et al.* [149] and Anderson *et al.* [150] in the late 1980's MD has been extensively used for the measurement of small molecules [152-154], whilst some high molecular weight molecules have also been detected, including cytokines (IL-6 ~ 29kDa) [154], albumin [155], and high molecular weight dextrans (*in vitro* only, up to 150 kDa) [156]. Sampling of large molecules is somewhat limited, however, due to the loss perfusate from probes with very large effective pores, reducing sample recovery [155]. To date the application of MD has been limited to scenarios where invasiveness is far outweighed by benefit of early detection of complications arising during surgery and intensive care.[153, 157] MD has, however, been demonstrated for continuous glucose measurement in self-monitoring glucose devices [158-160], such as the GlucoDay [158], with good correlation to blood glucose levels. While well suited to continuous monitoring and generally excluding fouling proteins from electrochemical sensors [161], MD inherently involves a significant lag time due to the slow pumping rates required allow equilibration of analyte [155] and probes are prone to long term fouling and degradation [162, 163].

Microneedles (MNs) and MN arrays consist of hollow projections typically hundreds of microns to a few millimetres long, with an inner channel diameter less than 100 µm (Figure 2C(iii)) [164, 165]. These MNs/MN arrays penetrate through the outer epidermal layers of the skin to provide direct access to ISF and blood with reduced invasiveness, making them suitable for repeated or real time monitoring. Without the molecular weight cutoff issues of MD probes, MNs/MN arrays offer the potential for real time sampling of small *and* large molecules at the ISF concentration. In principle this includes pharmacokinetics, metabolites (glucose, lactate, glutamate) [138, 166], ions (Na^+, K^+ and pH) [138, 167], cytokines, proteins (infectious disease, cardiovascular disease) and RNA/DNA. Furthermore, microfabrication technology used for MN fabrication is compatible with miniaturised fluid handling and electrochemical sensor fabrication meaning MNs can easily be integrated with backside compartments for processing and analyte recognition/transduction. In pioneering work, Zimmermann *et al.* [168] demonstrated the first MN array for ISF glucose measurement consisting of 8 × 8 hollow MNs integrated with a flow through sensor which extracted ISF by capillary force and was shown to detect glucose in human skin *in vivo*. The channels, however, did not continue to passively extract ISF once filled at a sufficient rate for real time glucose measurement suggesting more complex systems with active extraction (such as pumps) may be required for continuous monitoring. A similar concept was demonstrated by Mukerjee *et al.* in human skin [169], however, glucose was detected qualitatively with a glucose test strip, rather than with a sensor. In a series of publications the Narayan and Wang groups have developed hollow MN arrays integrated with solid carbon fibre, carbon paste or Pt electrodes within the MN channels themselves. Using these MN array electrodes they have employed electrochemical detection schemes for hydrogen peroxide [170], lactate [138, 170], glucose and glutamate [166] detection *in vitro* with the ultimate aim of developing a wearable sensor. Significantly they have also demonstrated multiplexed detection of pH, glucose and lactate *in vitro* using a single MN array. To date, however, this promising approach has not been demonstrated *in vivo* in human skin.

In our own group, solid MNs arrays (i.e. microprojection arrays or MPAs) have been also been developed to sample protein biomarkers from the skin, including IgG [171, 172], dengue NS1 protein [173]and malaria *pf*HRP2 [174]. To our knowledge this is the first demonstration of MNs or MPAs to sample skin proteins either selectively or non-selectively. The surface of these MPAs were modified with biorecognition probes that selectively capture circulating proteins from skin ISF/blood, which has been demonstrated to be highly selective for the target protein. Thus, the collected sample represents only a molecular fraction and avoids fluid handling and processing. A wearable version of this design has also been demonstrated to increase the total amount of protein captured *in vivo* for up to 6 h [171], which may have application to accumulate low concentration or rare analytes over extended periods that are not otherwise detectable in small fluid volumes [175]. Although this approach achieves selective sampling of proteins from ISF/blood, at this early stage analyte is detected with *in vitro* assays upon MPA removal from skin and is not integrated with a biosensor, though future designs aim to incorporate this with an external biosensor cartridge.

Emerging trends and future opportunities

There are some interesting trends identified in this review, particularly when it comes to the challenge of detecting proteins and other macromolecules in body fluids *in vivo* or, without significant sample processing, *in vitro.* We suggest that consideration of the issues and concepts in the following discussion could open up new research areas and possibly lead to innovative solutions to key challenges in this field.

The concept of "selective sampling" approaches is emerging to avoid the processing of bulk samples, of which the majority is irrelevant to the outcome of the test. This approach is not necessarily all that new, as it is the basis of how glucose monitoring and related electrochemical devices are able to operate in complex fluids, namely via encapsulation of the device in a polymeric matrix to limit mass transport of large molecules to the sensor. However, new methods are emerging; the most promising of which may be the direct enrichment of a target analyte at a surface whilst in contact with a body fluid *in vivo*, or at least without treating an extracted sample. In our

group, we have used this approach to develop microneedle surfaces with anti-fouling polymers and affinity probes, in order to selectively extract protein analytes from the skin ISF. Several of the methods highlighted here use a similar approach – Clark's group directly inject their nanosensors thus avoiding sampling; Ferguson's study effectively "diverted" a small but continuously flowing blood sample into an analyte-selective microfluidic channel; and Wang's group have moved the sensor directly into the body fluid (saliva, sweat, or skin) for selective monitoring of small moelcules. Indeed, the utility of the selective sampling approach across a wide variety of body fluids and biosensor platforms suggests that it could be applied across a range of methodologies, regardless of the detection techniques employed.

While the examples raised in this review are predominantly *in vivo* examples, there is no reason why selective sampling approaches could not also be integrated with emerging *in vitro* diagnostic devices. An excellent example is the case of microfluidics technologies, for which a significant device footprint is required for bulk sample processing prior to biomarker isolation and detection.[10, 11] The rapid expansion of microfluidic technologies has opened up a plethora of new opportunities in diagnostics, [176, 177] however bulk sampling with needle or lancet devices remains the predominant sample collection approach. Integration of microfluidic approaches with selective body fluid sampling could not only remove the need for sample processing operations on these devices, but could also help to address the challenge of rare event analysis. In the case of circulating tumor cell analysis, there is already a significant number of microfluidic devices available to isolate these cells from blood samples;[178, 179] if they could be used to isolate these very rare cells from the entire blood volume of a patient, in a minimally invasive manner, this could significantly improve the clinical utility of these devices. Ferguson's study shows that microfluidic systems can indeed be integrated into body fluid sampling for real-time and continuous monitoring approaches, and we hope to see more demonstrations of this in the future, for a range of different classes of biomarkers.

We suggest that a number of relatively commonly collected, or easily collected, body fluids have been under-utilised in clinical biosensor development. Each fluid has its own list of technical and clinical challenges in terms of utility, this broadening the range of fluids sampled may provide clinicians with more diagnostic options. While blood, saliva, urine, and to a lesser degree subcutaneous tissue (mainly for implantable glucose sensors) have been widely used, the prospect of using relatively protein-rich fluid, with unique proteome sub-sets, is certainly intriguing. Furthermore, the comparison between body fluids that are related by physiology (*e.g.* blood, skin ISF, subcutaneous tissue, sweat) could also yield new insights into biosensor development and disease investigations.

Finally, the real-time and/or continuous approach is also becoming popular. A common definition of a biosensor includes the real-time/continuous attribute. However, endpoint analysis, is usually the goal for *in vitro* clinical sample analysis, for which real-time and continuous measurements have are usually not relevant, unless dynamic information (*e.g.* activity/affinity or related measurements) is specifically required. As biosensors become better integrated with body fluid sampling, we expect that real-time analysis will open up avenues into biomarker-directed therapies, with dynamic information collected over time, from many parts of the body. Certainly, we look forward to a future in which biosensor-based approaches may indeed begin to tackle the immense challenges in detecting low abundance analytes in complex fluids in real-time, including ultra-low protein analytes, circulating tumor cells, and microbial sepsis.

Conclusions

In conclusion, there is significant potential for the integration of biosensors into clinical practice. However, in order to achieve their full potential, we suggest that better integration between body fluid sampling and the biosensor itself is required. A key technical hurdle across all body fluids is the jump from using enzymatic methods tailored to small molecule analysis, through to approaches in which macromolecular proteins and other analytes can be also be detected in real-time, with high specificity and selectivity. Furthermore, there are significant opportunities for technology developers to develop new methods to non-invasively analyse body fluids for which there are currently very few acceptable approaches available, if any.

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1. T. E. Caragher, B. B. Fernandez, F. L. Jacobs and L. A. Barr, *Journal of Emergency Medicine*, 2002, **22**, 1-7.
2. T. T. Ruckh and H. A. Clark, *Analytical Chemistry*, 2014, **86**, 1314-1323.
3. Cagliero E, Levina DV and N. DM, *Diabetes Care*, 1999, **22**, 1785-1789.
4. S. E. Clarke and J. R. Foster, *British Journal of Biomedical Science*, 2012, **69**, 83-93.
5. D. Mabey, R. W. Peeling, A. Ustianowski and M. D. Perkins, *Nature Reviews Microbiology*, 2004, **2**, 231-240.
6. L. Simonsen, A. Kane, J. Lloyd, M. Zaffran and M. Kane, *Bulletin of the World Health Organization*, 1999, **77**, 789-800.
7. http://loinc.org/downloads/usage/orders.
8. C. D. Miller, C. S. Barnes, L. S. Phillips, D. C. Ziemer, D. Gallina, C. B. Cook, S. D. Maryman and I. M. El-Kebbi, *Diabetes Care*, 2003, **26**, 1158-1163.
9. A. Barfidokht and J. J. Gooding, *Electroanalysis*, 2014, **26**, 1182-1196.
10. J. M. Labuz and S. Takayama, *Lab on a Chip*, 2014.
11. R. Mariella, *Biomedical Microdevices*, 2008, **10**, 777-784.
12. R. A. R. Bowen, G. L. Hortin, G. Csako, O. H. Otanez and A. T. Remaley, in *Clinical Biochemistry*, Pergamon-Elsevier, Oxford, 2010, vol. 43, pp. 4-25.
13. R. Aebersold, L. Anderson, R. Caprioli, B. Druker, L. Hartwell and R. Smith, *Journal of Proteome Research*, 2005, **4**, 1104-1109.
14. R. Etzioni, N. Urban, S. Ramsey, M. McIntosh, S. Schwartz, B. Reid, J. Radich, G. Anderson and L. Hartwell, *Nature Reviews Cancer*, 2003, **3**, 243-252.
15. L. Hartwell, D. Mankoff, A. Paulovich, S. Ramsey and E. Swisher, *Nature Biotechnology*, 2006, **24**, 905-908.
16. C. J. Feng, S. Dai and L. Wang, *Biosensors & Bioelectronics*, 2014, **59**, 64-74.
17. P. J. Conroy, S. Hearty, P. Leonard and R. J. O'Kennedy, *Seminars in Cell & Developmental Biology*, 2009, **20**, 10-26.
18. P. D. Skottrup, *Analytical Biochemistry*, 2010, **406**, 1-7.
19. P. D. Howes, R. Chandrawati and M. M. Stevens, *Science*, 2014, **346**, 53-+.
20. N. L. Anderson and N. G. Anderson, *Mol. Cell. Proteomics*, 2002, **1**, 845-867.
21. D. M. Rissin, C. W. Kan, T. G. Campbell, S. C. Howes, D. R. Fournier, L. Song, T. Piech, P. P. Patel, L. Chang, A. J. Rivnak, E. P. Ferrell, J. D. Randall, G. K. Provuncher, D. R. Walt and D. C. Duffy, *Nature Biotechnology*, 2010, **28**, 595-599.
22. P. Yagupsky and F. S. Nolte, *Clinical Microbiology Reviews*, 1990, **3**, 269-279.
23. M. L. Rogers and M. G. Boutelle, *Annual Review of Analytical Chemistry, Vol 6*, 2013, **6**, 427-453.
24. G. Lippi, G. C. Guidi, C. Mattiuzzi and M. Plebani, *Clinical Chemistry and Laboratory Medicine*, 2006, **44**, 358-365.
25. S. A. Randall, M. J. McKay and M. P. Molloy, *Proteomics*, 2010, **10**, 2050-2056.
26. J. Z. Yi, Z. X. Liu, C. A. Gelfand and D. Craft, *Serum/Plasma Proteomics: Methods and Protocols*, 2011, **728**, 161-178.
27. Sebbane M, P. Claret, Lefebvre S, Mercier G, Rubenovitch J, Jreige R, Eledjam JJ and de la Coussaye JE, *Journal of Emergency Medicine*, 2013, **44**, 299-305.
28. P. Juvin, A. Blarel, F. Bruno and J. M. Desmonts, *Anesthesia and Analgesia*, 2003, **96**, 1218-1218.
29. A. F. Jacobson and E. H. Winslow, *Heart & Lung*, 2005, **34**, 345-359.
30. C. A. Burtis, E. R. Ashwood and D. E. Burns, *Tietz textbook of clinical chemistry and molecular diagnostics*, Elsevier Saundeers, St Louis, Missouri, USA, 2006.
31. M. Gudmundsson and A. Bjelle, *Angiology*, 1993, **44**, 384-391.
32. O. Cakmak, C. Elbuken, E. Ermek, A. Mostafazadeh, I. Baris, B. E. Alaca, I. H. Kavakli and H. Urey, *Methods*, 2013, **63**, 225-232.
33. D. J. Aframian, T. Davidowitz and R. Benoliel, *Oral Diseases*, 2006, **12**, 420-423.
34. J. A. Loo, W. Yan, P. Ramachandran and D. T. Wong, *Journal of Dental Research*, 2010, **89**, 1016-1023.
35. W. H. Yan, R. Apweiler, B. M. Balgley, P. Boontheung, J. L. Bundy, B. J. Cargile, S. Cole, X. P. 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. Oiao, N. L. Rhodus, J. R. Sevinsky, D. States, J. L. Stephenson, S. Than, J. R. Yates, W. X. Yu, H. W. Xie, Y. M. Xie, G. S. Omenn, J. A. Loo and D. T. Wong, *Proteomics Clinical Applications*, 2009, **3**, 116-134.
36. B. L. Schulz, J. Cooper-White and C. K. Punyadeera, *Critical Reviews in Biotechnology*, 2013, **33**, 246-259.
37. P. J. F. Rantonen and J. H. Meurman, *Acta Odontologica Scandinavica*, 1998, **56**, 210-214.
38. M. S. Park, J. W. Chung, Y. K. Kim, S. C. Chung and H. S. Kho, *Oral Diseases*, 2007, **13**, 181-186.
39. V. Thongboonkerd, S. Mungdee and W. Chiangjong, *Journal of Proteome Research*, 2009, **8**, 3206-3211.
40. L. L. Jia, L. Zhang, C. Shao, E. L. Song, W. Sun, M. X. Li and Y. H. Gao, *Plos One*, 2009, **4**.
41. J. Adachi, C. Kumar, Y. L. Zhang, J. V. Olsen and M. Mann, *Genome Biology*, 2006, **7**.
42. B. A. Inman, W. Etienne, R. Rubin, R. A. Owusu, T. R. Oliveira, D. B. Rodriques, P. F. Maccarini, P. R. Stauffer, A. Mashal and M. W. Dewhirst, *International Journal of Hyperthermia*, 2013, **29**, 206-210.
43. B. K. Siesjo, *Kidney International*, 1972, **1**, 360-&.
44. A. Zougman, B. Pilch, A. Podtelejnikov, M. Kiehntopf, C. Schnabel, C. Kurnar and M. Mann, *Journal of Proteome Research*, 2008, **7**, 386-399.
45. S. E. Schutzer, T. Liu, B. H. Natelson, T. E. Angel, A. A. Schepmoes, S. O. Purvine, K. K. Hixson, M. S. Lipton, D. G. Camp, P. K. Coyle, R. D. Smith and J. Bergquist, *Plos One*, 2010, **5**.
46. F. Abdi, J. F. Quinn, J. Jankovic, M. McIntosh, J. B. Leverenz, E. Peskind, R. Nixon, J. Nutt, K. Chung, C. Zabetian, A. Samii, M. Lin, S. Hattan, C. Pan, Y. Wang, J. Jin, D. Zhu, G. J. Li, Y. Liu, D. Waichunas, T. J. Montine and J. Zhang, *Journal of Alzheimers Disease*, 2006, **9**, 293-348.
47. I. G. Bloomfield, I. H. Johnston and L. E. Bilston, *Pediatric Neurosurgery*, 1998, **28**, 246-251.

48. F. Yetkin, U. Kayabas, Y. Ersoy, Y. Bayindir, S. A. Toplu and I. Tek, *Southern Medical Journal*, 2010, **103**, 892-895.
49. M. S. Norn, *Acta Ophthalmologica*, 1988, **66**, 485-489.
50. L. Zhou, S. Z. Zhao, S. K. Koh, L. Y. Chen, C. Vaz, V. Tanavde, X. R. Li and R. W. Beuerman, *Journal of Proteomics*, 2012, **75**, 3877-3885.
51. K. B. Green-Church, K. K. Nichols, N. M. Kleinholz, L. W. Zhang and J. J. Nichols, *Molecular Vision*, 2008, **14**, 456-470.
52. J. M. Tiffany, *International Ophthalmology*, 1991, **15**, 371-376.
53. K. Kostikas, G. Papatheodorou, K. Ganas, K. Psathakis, P. Panagou and S. Loukides, *American Journal of Respiratory and Critical Care Medicine*, 2002, **165**, 1364-1370.
54. D. H. Conrad, J. Goyette and P. S. Thomas, *Journal of General Internal Medicine*, 2008, **23**, 78-84.
55. K. Mitsubayashi, M. Suzuki, E. Tamiya and I. Karube, *Analytica Chimica Acta*, 1994, **289**, 27-34.
56. M. M. Raiszadeh, M. M. Ross, P. S. Russo, M. A. Schaepper, W. D. Zhou, J. H. Deng, D. Ng, A. Dickson, C. Dickson, M. Strom, C. Osorio, T. Soeprono, J. D. Wulfkuhle, E. F. Petricoin, L. A. Liotta and W. M. Kirsch, *Journal of Proteome Research*, 2012, **11**, 2127-2139.
57. T. C. Boysen, S. Yanagawa, F. Sato and K. Sato, *Journal of Applied Physiology*, 1984, **56**, 1302-1307.
58. M. Gilanyi and A. G. B. Kovach, *American Journal of Physiology*, 1991, **261**, H627-H631.
59. J. Kool, L. Reubsaet, F. Wesseldijk, R. T. Maravilha, M. W. Pinkse, C. S. D'Santos, J. J. van Hilten, F. J. Zijlstra and A. J. R. Heck, *Proteomics*, 2007, **7**, 3638-3650.
60. A. C. Muller, F. P. Breitwieser, H. Fischer, C. Schuster, O. Brandt, J. Colinge, G. Superti-Furga, G. Stingl, A. Elbe-Burger and K. L. Bennett, *J Proteome Res*, 2012, **11**, 3715-3727.
61. N. L. Anderson, *Clinical Chemistry*, 2010, **56**, 177-185.
62. A. K. Yetisen, M. S. Akram and C. R. Lowe, *Lab on a Chip*, 2013, **13**, 2210-2251.
63. P. V. Bernhardt, *Australian Journal of Chemistry*, 2006, **59**, 233-256.
64. C. N. Kotanen, F. G. Moussy, S. Carrara and A. Guiseppi-Elie, *Biosensors & Bioelectronics*, 2012, **35**, 14-26.
65. B. S. Ferguson, D. A. Hoggarth, D. Maliniak, K. Ploense, R. J. White, N. Woodward, K. Hsieh, A. J. Bonham, M. Eisenstein, T. E. Kippin, K. W. Plaxco and H. T. Soh, *Science Translational Medicine*, 2013, **5**, 213ra165.
66. B. Awqatty, S. Samaddar, K. J. Cash, H. A. Clark and J. M. Dubach, *Analyst*, 2014, **139**, 5230-5238.
67. K. J. Cash and H. A. Clark, *Sensors*, 2012, **12**, 11922-11932.
68. J. M. Dubach, E. Lim, N. Zhang, K. P. Francis and H. Clark, *Integrative Biology*, 2011, **3**, 142-148.
69. K. Billingsley, M. K. Balaconis, J. M. Dubach, N. Zhang, E. Lim, K. P. Francis and H. A. Clark, *Analytical Chemistry*, 2010, **82**, 3707-3713.
70. K. J. Cash, C. Li, J. Xia, L. V. Wang and H. A. Clark, *ACS Nano*, 2015.
71. J. Deng, Z. Ma, W. Huang, C. Li, H. Wang, Y. Zheng, R. Zhou and Y.-W. Tang, *Virologica Sinica*, 2013, **28**, 97-102.
72. J. M. Katz, K. Hancock and X. Xu, *Expert Review of Anti-Infective Therapy*, 2011, **9**, 669-683.
73. M. Pappaioanou and M. Gramer, *Ilar Journal*, 2010, **51**, 268-280.
74. J. Kim, G. Valdes-Ramirez, A. J. Bandodkar, W. Z. Jia, A. G. Martinez, J. Ramirez, P. Mercier and J. Wang, *Analyst*, 2014, **139**, 1632-1636.
75. J. B. Claver, M. C. V. Miron and L. F. Capitan-Vallvey, *Analyst*, 2009, **134**, 1423-1432.
76. S. Lee, J. H. Lee, M. Kim, J. Kim, M. J. Song, H. I. Jung and W. Lee, *Applied Physics Letters*, 2013, **103**, 4.
77. R. C. Stevens, S. D. Soelberg, S. Near and C. E. Furlong, *Analytical Chemistry*, 2008, **80**, 6747-6751.
78. S. Piermarini, G. Volpe, R. Federico, D. Moscone and G. Palleschi, *Analytical Letters*, 2010, **43**, 1310-1316.
79. C. S. Zou, M. Zhou, G. M. Xie, P. Luo, X. L. Xiong, H. J. Xu and J. Zheng, *Chinese Journal of Analytical Chemistry*, 2008, **36**, 1217-1220.
80. M. Yamaguchi, M. Kanemaru, T. Kanemori and Y. Mizuno, *Biosensors & Bioelectronics*, 2003, **18**, 835-840.
81. V. Shetty, C. Zigler, T. F. Robles, D. Elashoff and M. Yamaguchi, *Psychoneuroendocrinology*, 2011, **36**, 193-199.
82. T. F. Robles, R. Sharma, L. Harrell, D. A. Elashoff, M. Yamaguchi and V. Shetty, *American Journal of Human Biology*, 2013, **25**, 719-724.
83. M. Mahosenaho, F. Caprio, L. Micheli, A. M. Sesay, G. Palleschi and V. Virtanen, *Microchimica Acta*, 2010, **170**, 243-249.
84. A. O. Aluoch, O. A. Sadik and G. Bedi, *Analytical Biochemistry*, 2005, **340**, 136-144.
85. Y. H. Liang, C. C. Chang, C. C. Chen, Y. Chu-Su and C. W. Lin, *Clinical Biochemistry*, 2012, **45**, 1689-1693.
86. T. Endo, S. Ozawa, N. Okuda, Y. Yanagida, S. Tanaka and T. Hatsuzawa, *Sensors and Actuators B-Chemical*, 2010, **148**, 269-276.
87. A. Kishen, M. S. John, C. S. Lim and A. Asundi, *Biosensors & Bioelectronics*, 2003, **18**, 1371-1378.
88. A. Kishen, M. S. John, C. S. Lim and A. Asundi, in *Saratov Fall Meeting 2002: Optical Technologies in Biophysics and Medicine Iv*, ed. V. V. Tuchin, 2002, vol. 5068, pp. 194-201.
89. S. Milardovic, I. Kerekovic and M. Nodilo, *Talanta*, 2008, **77**, 222-228.
90. X. J. Gao, W. Y. Yang, P. F. Pang, S. T. Liao, Q. Y. Cai, K. F. Zeng and C. A. Grimes, *Sensors and Actuators B-Chemical*, 2007, **128**, 161-167.
91. K. B. Male and J. H. T. Luong, *Applied Biochemistry and Biotechnology*, 1992, **37**, 243-254.
92. P. G. Yu and D. Zhou, *Analytica Chimica Acta*, 1995, **300**, 91-97.
93. E. Akyilmaz, M. K. Sezginturk and E. Dinckaya, *Talanta*, 2003, **61**, 73-79.
94. J. Ballesta-Claver, I. F. D. Ortega, M. C. Valencia-Miron and L. F. Capitan-Vallvey, *Analytica Chimica Acta*, 2011, **702**, 254-261.
95. P. Kanyong, R. M. Pemberton, S. K. Jackson and J. P. Hart, *Analytical Biochemistry*, 2012, **428**, 39-43.
96. N. Gan, L. Y. Wang, W. M. Xu, T. H. Li and Q. L. Jiang, *Chinese Journal of Analytical Chemistry*, 2007, **35**, 1553-1558.
97. D. J. Stickler, S. M. Jones, G. O. Adusei and M. G. Waters, *Journal of Clinical Microbiology*, 2006, **44**, 1540-1542.
98. D. J. Stickler, S. M. Jones, G. O. Adusei, M. G. Waters, J. Cloete, S. Mathur and R. C. L. Feneley, *Bju International*, 2006, **98**, 1244-1249.

99. S. Malic, M. G. J. Waters, L. Basil, D. J. Stickler and D. W. Williams, *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, 2012, **100B**, 133-137.
100. A. Long, J. Edwards, R. Thompson, D. A. Lewis and A. G. Timoney, *Bju International*, 2014, **114**, 278-285.
101. N. A. Verwey, R. Veerhuis, H. A. M. Twaalfhoven, D. Wouters, J. J. M. Hoozemans, Y. J. M. Bollen, J. Killestein, M. Bibl, J. Wiltfang, C. E. Hack, P. Scheltens and M. A. Blankenstein, *Journal of Immunological Methods*, 2009, **348**, 57-66.
102. A. M. Andrews and P. S. Weiss, *Acs Nano*, 2012, **6**, 8463-8464.
103. G. M. Mutlu, K. W. Garey, R. A. Robbins, L. H. Danziger and I. Rubinstein, *American Journal of Respiratory and Critical Care Medicine*, 2001, **164**, 731-737.
104. R. F. Machado, D. Laskowski, O. Deffenderfer, T. Burch, S. Zheng, P. J. Mazzone, T. Mekhail, C. Jennings, J. K. Stoller, J. Pyle, J. Duncan, R. A. Dweik and S. C. Erzurum, *American Journal of Respiratory and Critical Care Medicine*, 2005, **171**, 1286-1291.
105. K. Alving, E. Weitzberg and J. M. Lundberg, *European Respiratory Journal*, 1993, **6**, 1368-1370.
106. V. S. Kurova, E. C. Anaev, A. S. Kononikhin, K. Y. Fedorchenko, I. A. Popov, T. L. Kalupov, D. O. Bratanov, E. N. Nikolaev and S. D. Varfolomeev, *Clinical Chemistry and Laboratory Medicine*, 2009, **47**, 706-712.
107. M. Fumagalli, F. Ferrari, M. Luisetti, J. Stolk, P. S. Hiemstra, D. Capuano, S. Viglio, L. Fregonese, I. Cerveri, F. Corana, C. Tinelli and P. Iadarola, *International Journal of Molecular Sciences*, 2012, **13**, 13894-13910.
108. K. Bloemen, J. Hooyberghs, K. Desager, E. Witters and G. Schoeters, *Proteomics Clinical Applications*, 2009, **3**, 498-504.
109. R. E. St John, *Critical care nurse*, 2003, **23**, 83-88.
110. S. Sethi, R. Nanda and T. Chakraborty, *Clinical Microbiology Reviews*, 2013, **26**, 462-475.
111. U. Tisch, I. Schlesinger, R. Ionescu, M. Nassar, N. Axelrod, D. Robertman, Y. Tessler, F. Azar, A. Marmur, J. Aharon-Peretz and H. Haick, *Nanomedicine*, 2013, **8**, 43-56.
112. D. Guo, D. Zhang, L. Zhang and G. Lu, *Sensors and Actuators B-Chemical*, 2012, **173**, 106-113.
113. J. Luo, J. Luo, L. Wang, X. Shi, J. Yin, E. Crew, S. Lu, L. M. Lesperance and C.-J. Zhong, *Sensors and Actuators B-Chemical*, 2012, **161**, 845-854.
114. J. Luo, J. Luo, L. Wang, X. Shi, J. Yin, E. Crew, S. Lu, L. M. Lesperance and C.-J. Zhong, *Sensors and Actuators B: Chemical*, 2011, **161**, 845.
115. K. Mitsubayashi, H. Matsunaga, G. Nishio, S. Toda and Y. Nakanishi, *Biosensors & bioelectronics*, 2005, **20**, 1573-1579.
116. M. Phillips, *Analytical Biochemistry*, 1997, **247**, 272-278.
117. N. Strand, A. Bhushan, M. Schivo, N. J. Kenyon and C. E. Davis, *Sensors and Actuators B-Chemical*, 2010, **143**, 516-523.
118. C. E. Davis, M. J. Bogan, S. Sankaran, M. A. Molina, B. R. Loyola, W. X. Zhao, W. H. Benner, M. Schivo, G. R. Farquar, N. J. Kenyon and M. Frank, *Ieee Sensors Journal*, 2010, **10**, 114-122.
119. H. Kudo, T. Arakawa and K. Mitsubayashi, *IEEJ Transactions on Sensors and Micromachines*, 2012, **132**, 451-454.
120. Q. Yan, B. Peng, G. Su, B. E. Cohan, T. C. Major and M. E. Meyerhoff, *Analytical chemistry*, 2011, **83**, 8341-8346.
121. S. Iguchi, H. Kudo, T. Saito, M. Ogawa, H. Saito, K. Otsuka, A. Funakubo and K. Mitsubayashi, *Biomedical Microdevices*, 2007, **9**, 603-609.
122. S. McArthur, 0803869 Ph.D., University of New South Wales (Australia), 2001.
123. N. M. Farandos, A. K. Yetisen, M. J. Monteiro, C. R. Lowe and S. H. Yun, *Advanced Healthcare Materials*, 2014.
124. R. Badugu, J. Lakowicz and C. Geddes, *Journal of Fluorescence*, 2003, **13**, 371-374.
125. V. L. Alexeev, S. Das, D. N. Finegold and S. A. Asher, *Clinical Chemistry*, 2004, **50**, 2353-2360.
126. D. K. Bishop, J. T. La Belle, S. R. Vossler, D. R. Patel and C. B. Cook, *Journal of diabetes science and technology*, 2010, **4**, 299-306.
127. M. X. Chu, M. Mochizuki, K. Mitsubayashi, K. Miyajima, D. Takahashi, T. Arakawa, K. Sano, S.-i. Sawada, H. Kudo, Y. Iwasaki and K. Akiyoshi, *Talanta*, 2011, **83**, 960-965.
128. H. Yao, 3563104 Ph.D., University of Washington, 2013.
129. J. T. La Belle, D. R. Patel, C. B. Cook, E. Engelschall, K. Lan, P. Shah, N. Saez, S. Maxwell, T. Adamson, M. Abou-Eid and K. McAferty, *Journal of Diabetes Science and Technology* 2014, **8**, 109-116.
130. K. B. Hammond, N. L. Turcios and L. E. Gibson, *Journal of Pediatrics*, 1994, **124**, 255-260.
131. W. Z. Jia, A. J. Bandodkar, G. Valdes-Ramirez, J. R. Windmiller, Z. J. Yang, J. Ramirez, G. Chan and J. Wang, *Analytical Chemistry*, 2013, **85**, 6553-6560.
132. X. Huang, Y. H. Liu, K. L. Chen, W. J. Shin, C. J. Lu, G. W. Kong, D. Patnaik, S. H. Lee, J. F. Cortes and J. A. Rogers, *Small*, 2014, **10**, 3083-3090.
133. H. Wiig and M. A. Swartz, *Interstitial Fluid and Lymph Formation and Transport: Physiological Regulation and Roles in Inflammation and Cancer*, 2012.
134. I. M. Braverman, *Microcirculation-London*, 1997, **4**, 329-340.
135. C. C. Michel, *Cardiovasc Res*, 1996, **32**, 644-653.
136. M. J. Tierney, J. A. Tamada, R. O. Potts, L. Jovanovic, S. Garg and T. Cygnus Res, *Biosensors & Bioelectronics*, 2001, **16**, 621-629.
137. N. S. Oliver, C. Toumazou, A. E. G. Cass and D. G. Johnston, *Diabetic Medicine*, 2009, **26**, 197-210.
138. P. R. Miller, S. A. Skoog, T. L. Edwards, D. M. Lopez, D. R. Wheeler, D. C. Arango, X. Xiao, S. M. Brozik, J. Wang, R. Polsky and R. J. Narayan, *Talanta*, 2012, **88**, 739-742.
139. A. Kaushik, A. Vasudev, S. K. Arya, S. K. Pasha and S. Bhansali, *Biosensors and Bioelectronics*, 2014, **53**, 499-512.
140. V. Wascotte, E. Rozet, A. Salvaterra, P. Hubert, M. Jadoul, R. H. Guy and V. Preat, *Eur J Pharm Biopharm*, 2008, **69**, 1077-1082.
141. C. M. Huang, C. A. Elmets, K. R. van Kampen, T. S. DeSilva, S. Barnes, H. Kim and D. C. C. Tang, *Mass Spectrometry Reviews*, 2005, **24**, 647-660.

142. S. Paliwal, B. H. Hwang, K. Y. Tsai and S. Mitragotri, *European Journal of Pharmaceutical Sciences*, 2013, **50**, 546-556.
143. V. Merino, A. Lopez, D. Hochstrasser and R. H. Guy, *J Control Release*, 1999, **61**, 65-69.
144. N. Longo, S. K. Li, G. Yan, R. P. Kochambilli, K. Papangkorn, D. Berglund, A. H. Ghanem, C. L. Ashurst, S. L. Ernst, M. Pasquali and W. I. Higuchi, *Journal of Inherited Metabolic Disease*, 2007, **30**, 910-915.
145. G. Rao, P. Glikfeld and R. H. Guy, *Pharmaceutical Research*, 1993, **10**, 1751-1755.
146. Diabetes Research in Children Network (DirecNet) Study Group, *Diabetes Care*, 2004, **27**, 722-726.
147. A. Sieg and V. Wascotte, *Journal of Drug Targeting*, 2009, **17**, 690-700.
148. A. J. Bandodkar, W. Jia, C. Yardımcı, X. Wang, J. Ramirez and J. Wang, *Analytical Chemistry*, 2014, **87**, 394-398.
149. P. A. Jansson, J. Fowelin, U. Smith and P. Lonnroth, *American Physiological Society*, 1988, **255**, E281-E220.
150. C. Anderson, T. Andersson and M. Molander, *Acta Derm Venereol*, 1991, **71**, 389-393.
151. U. Ungerstedt, *Acta Anaesthesiologica Scandinavica*, 1997, **41**, 123.
152. S. Klaus, K. Staubach, W. Eichler, J. Gliemroth, M. Heringlake, P. Schmucker and L. Bahlmann, *Annals of Clinical Biochemistry*, 2003, **40**, 289-291.
153. S. Klaus, M. Heringlake and L. Bahlmann, *Critical Care*, 2004, **8**, 363-368.
154. F. Sjogren, C. Svensson and C. Anderson, *Br J Dermatol*, 2002, **146**, 375-382.
155. P. J. Fellows, *Journal of vascular research*, 2003, **40**, 304.
156. R. J. Schutte, S. A. Oshodi and W. M. Reichert, *Analytical Chemistry*, 2004, **76**, 6058-6063.
157. H. Stjernstrom, T. Karlsson, U. Ungerstedt and L. Hillered, *Intensive Care Med*, 1993, **19**, 423-428.
158. I. M. Wentholt, M. A. Vollebregt, A. A. Hart, J. B. Hoekstra and J. H. DeVries, *Diabetes Care*, 2005, **28**, 2871-2876.
159. T. Kubiak, B. Worle, B. Kuhr, I. Nied, G. Glasner, N. Hermanns, B. Kulzer and T. Haak, *Diabetes Technol Ther*, 2006, **8**, 570-575.
160. P. Rossetti, F. Porcellati, C. G. Fanelli and G. B. Bolli, *Diabetes Technol Ther*, 2006, **8**, 326-337.
161. J. W. Mo and W. Smart, *Frontiers in Bioscience*, 2004, **9**, 3384-3391.
162. N. Wisniewski and M. Reichert, *Colloids and Surfaces B-Biointerfaces*, 2000, **18**, 197-219.
163. N. Wisniewski, B. Klitzman, B. Miller and W. M. Reichert, *J Biomed Mater Res*, 2001, **57**, 513-521.
164. M. R. Prausnitz, *Advanced Drug Delivery Reviews*, 2004, **56**, 581-587.
165. R. F. Donnelly, K. Mooney, E. Caffarel-Salvador, B. M. Torrisi, E. Eltayib and J. C. McElnay, *Ther Drug Monit*, 2014, **36**, 10-17.
166. J. R. Windmiller, G. Valdés-Ramírez, N. Zhou, M. Zhou, P. R. Miller, C. Jin, S. M. Brozik, R. Polsky, E. Katz, R. Narayan and J. Wang, *Electroanalysis*, 2011, **23**, 2302-2309.
167. H. Gardeniers, R. Luttge, E. J. W. Berenschot, M. J. de Boer, S. Y. Yeshurun, M. Hefetz, R. van't Oever and A. van den Berg, *Journal of Microelectromechanical Systems*, 2003, **12**, 855-862.
168. S. Zimmermann, D. Fienbork, B. Stoeber, A. W. Flounders and D. Liepmann, A microneedle-based glucose monitor: fabricated on a wafer-level using in-device enzyme immobilization, 2003.
169. E. Mukerjee, S. D. Collins, R. R. Isseroff and R. L. Smith, *Sensors and Actuators a-Physical*, 2004, **114**, 267-275.
170. J. R. Windmiller, N. Zhou, M.-C. Chuang, G. Valdes-Ramirez, P. Santhosh, P. R. Miller, R. Narayan and J. Wang, *Analyst*, 2011, **136**, 1846-1851.
171. J. W. Coffey, S. R. Corrie and M. A. F. Kendall, *Biomaterials*, 2013, **34**, 9572-9583.
172. S. R. Corrie, G. J. P. Fernando, M. L. Crichton, M. E. G. Brunck, C. D. Anderson and M. A. F. Kendall, *Lab on a Chip*, 2010, **10**, 2655-2658.
173. D. A. Muller, S. R. Corrie, J. Coffey, P. R. Young and M. A. Kendall, *Analytical Chemistry*, 2012, **84**, 3262-3268.
174. K. T. Lee, D. A. Muller, J. W. Coffey, K. J. Robinson, J. S. McCarthy, M. A. F. Kendall and S. R. Corrie, *Analytical Chemistry*, 2014, **86**, 10474-10483.
175. J. M. Labuz and S. Takayama, *Lab on a Chip*, 2014, **14**, 3165-3171.
176. C. D. Chin, T. Laksanasopin, Y. K. Cheung, D. Steinmiller, V. Linder, H. Parsa, J. Wang, H. Moore, R. Rouse, G. Umviligihozo, E. Karita, L. Mwambarangwe, S. L. Braunstein, J. van de Wijgert, R. Sahabo, J. E. Justman, W. El-Sadr and S. K. Sia, *Nat Med*, 2011, **17**, 1015-1019.
177. C. D. Chin, V. Linder and S. K. Sia, *Lab on a Chip*, 2012, **12**, 2118-2134.
178. K.-A. Hyun and H.-I. Jung, *Lab on a Chip*, 2014, **14**, 45-56.
179. Y. Dong, A. M. Skelley, K. D. Merdek, K. M. Sprott, C. Jiang, W. E. Pierceall, J. Lin, M. Stocum, W. P. Carney and D. A. Smirnov, *Journal of Molecular Diagnostics*, 2013, **15**, 149-157.