Developments in Micro and Nanoscale Sensors for Biomedical Sensing

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ABSTRACT

The widespread use of point of care testing in biomedical and clinical applications is a major aim of the electrochemical field. A large number of groups are working on lab-on-a-chip systems or sensor arrays which are underpinned by electrochemical detection methodologies. Miniaturised transducers have the potential to be adopted in such systems for diagnosis of a range of diseases in both clinical and non-clinical settings. In this review we will present the current trends and state of the art for a selection of miniaturised sensing elements (microelectrodes, nanoelectrodes and field-effect transistors) and provide an impression of current technologies, their associated

performance characteristics and also considering the major barriers to adoption and how they might be surmounted in future so these technologies can fulfil their early promise.

Keywords: Microelectrodes, Nanoelectrodes, Field-effect transistors, Biomedical-Sensing, Fabrication, Biosensors

1. INTRODUCTION

Miniaturised sensor systems have an important role to play in both the future development of medical devices and biomedical research. The ability to sample in real time at the point of care or through implantation will be vital to the effective diagnosis, monitoring of treatment response and measurement and prognostication for long term chronic medical conditions. In biomedical research, miniature systems have shown great promise in measuring cellular behaviour, monitoring metabolism, and developing new treatments (e.g. bioelectronic medicine). With this in mind, in this review we have chosen to focus on the transducing element of such systems, namely the sensor. The review examines recent developments for three types of sensor suitable for incorporation into microsystems (microelectrodes, nanoelectrodes and field-effect transistors (FETs)) with particular emphasis on biomedical applications, and makes an assessment of the state of progress for these technologies. Figure 1 presents an overview of the various technologies and application areas this review covers.

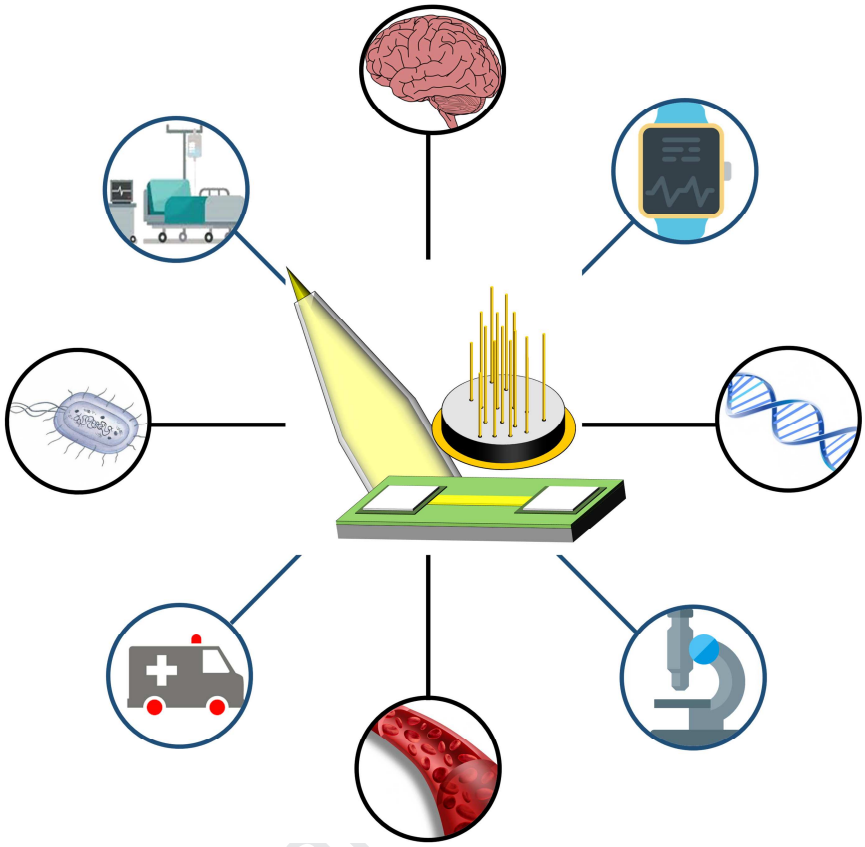

Figure 1. Overview of the technologies and application areas covered in this review utilising micro and nanoscale sensors for biomedical sensing.

2. DEVELOPMENTS IN MICROELECTRODE SENSORS

2.1 Microelectrode Sensors

A microelectrode can be defined as an electrode with one critical dimension on the scale of microns. This dimension can be e.g. diameter, edge length or band width. In practice, this dimension is usually ~25 µm and is around the point where the critical dimension is smaller than the diffusion layer. The electroanalytical advantages of microelectrodes are well known and include: reduced *iR* drop (allowing measurements in poorly conducting media), reduced RC constant (allows measurement of fast processes), improved signal to noise (enhanced Faradaic

over non-Faradaic current ratio), simplified analytical expressions for data interpretation and relative insensitivity to convection (ability to measure in a flow). These analytical advantages mean that microelectrodes have found widespread application. To date, in biomedical sensing, microelectrodes have been most widely adopted in the field of neuroscience where microfabricated microelectrodes, microelectrode arrays (MEAs) and glass-pulled carbon fiber electrodes have been widely used for *in vitro* experiments, detection of neurotransmitters and for implantable studies. New applications of microelectrodes are being found so as well as neuroscience, this section will cover a range of areas where studies showing the biomedical application of microelectrodes have been recently published.

2.2 Fabrication

A key factor in producing either single electrodes or arrays is fabrication, with advances in this area constantly permitting more facile and reliable sensor production. Improvements in fabrication lead to more robust devices which can sample for longer, give more precise measurements and of special interest to this topic dampen the inflammatory response associated with implantation through use of e.g. inert or biocompatible materials. Recently, it has been shown that a Hafnium oxide layer produced by atomic layer deposition (ALD) when deployed in a bilayer with parylene-C is a suitable insulator for producing well defined planar microelectrodes [1**]. The significance of this result is that conventional insulation materials such as CVD films of silicon dioxide or nitride can contain “pinholes” which can act as points of weakness and provide additional electrode area above that defined by the photomask. In addition, the thickness of common insulation layers means that mass transport to a recessed electrode can deviate significantly from the expected behaviour. The HfO_2 film produced by ALD was 10-60 nm thick and showed good passivation of the electrode, low leakage currents

and reproduction of signal across time, implying high stability of the passivation material. The layout of the glass chip used in this study is shown in Figure 2. Other notable advances in fabrication include, production of a silver bi-band microelectrode which has two 25 µm band working electrodes which can be fabricated easily, is easy to sterilise and which can be used for a range of clinically relevant measurements [2] and an outline of a combined soft-lithographic and electrochemical method for production of nano-structured Pt microelectrodes for a range of biomedical applications [3].

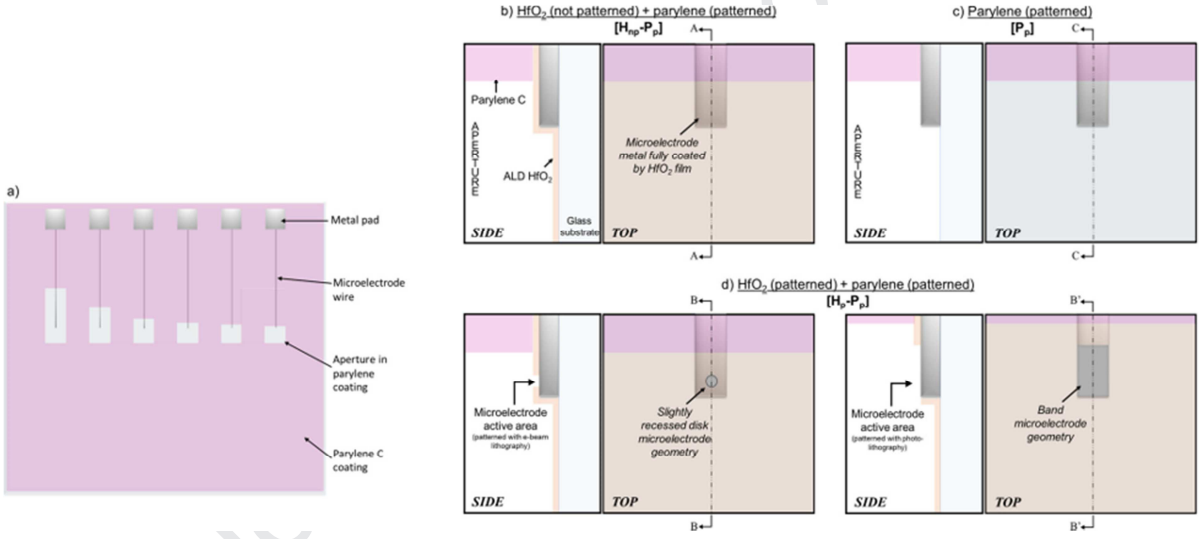

Figure 2. (a) Layout of the glass chip patterned with six devices. Parylene coating with various aperture sizes. (b-d) Side and top: close-up views of the region around the microelectrode active area with various insulator coatings. Not to scale. Reprinted from [1], Copyright 2019, with permission from American Chemical Society.

2.3 Nucleic Acid and Protein Detection using Microelectrodes

Recent studies have emerged showing the functionalisation protocols necessary to modify microelectrodes alongside interpretation of the sensor response for short chain alkane thiols [4], DNA [5] and antibody molecules [6]. For nucleic acid detection, studies have shown detection of antimicrobial resistance genes using an RPA assay in combination with electrophoresis [7],

graphene modified MEAs for DNA detection [8] and a polypyrrole modified MEA for detection of hypermethylation of DNA in cancer diagnosis [9]. Within the area of immunosensing, interdigitated microelectrodes (IDEs) have been employed commonly, often with the use of a capacitive measurement. Example studies for immunosensing in clinically important areas include detection of the prostate cancer biomarker PSA using a 1D [10**] and 3D hydrogel modified IDE array [11], amyloid as a marker for dementia and neurodegenerative disease [12, 13] and HIV detection via an electrochemical steric hindrance assay utilising microelectrodes [14].

2.4 Neuroelectrochemistry

This is an area of research where microelectrode measurements have been in regular use for decades. As a result, microelectrodes are well established, especially the carbon fibre microelectrode, particularly in combination with fast scan cyclic voltammetry (FSCV). This topic is well covered elsewhere so studies of note in other areas include: enhanced detection of dopamine via amperometry using a carbon fibre electrode bearing small islands of electroplated gold [15], glutamate detection amongst brain organoids using self-organised nanostructured microelectrodes [16], the use of platinum-iridium films to improve behaviour of chronically implanted electrodes [17], polypyrrole grafted gold nanoparticle modified microelectrodes for enhanced signal recording from U87MG cells [18] and finally a key study on the role of inflammation on the functionality of microelectrodes [19**].

2.5 Cellular Behaviour and Metabolism

Microelectrodes and in particular MEAs are becoming increasingly popular for measurements of cells, cells in culture, cellular communication and metabolism. Examples of complete culture systems include: measurement of anti-oxidant compounds from NIH-3T3 cells

in a combined culture plate with MEA [20], and MEA system for osteogenic differentiation of mesenchymal stem cells under electrical stimulation [21]. Notable studies detailing the measurements and/or detection of cellular analytes and metabolic products include: important studies detailing modified microelectrodes for enzyme free detection of glucose via a nanostructured gold film on the microelectrode surface [22] and detection of alanine and glucose using a silver-copper composite [23], the detection of dihydroxybenzoic acid isomers using a carbon fibre microelectrode modified with gold nanoparticles themselves sensitised with carbon nanotubes [24], the detection of compounds important in taste via an MEA functionalised with cardiomyocytes [25**] and Pt nanoparticle modified carbon fibre microelectrodes for detection of the important oxidative metabolite hydrogen peroxide [26]. Finally, the use of the "impacts" or "collisions" principle where current pulses are recorded on a microelectrode; for platelet detection in an electrochemical flow cytometry assay [27] and for bacterial detection assays which can discriminate *E. coli* vs *Stenotrophomonas maltophilia* (which has promise for quickly identifying the correct antibiotic to use in treatment) [28] and measurement of collisions of live bacteria in a real time label-free manner using a poised microelectrode [29].

3. DEVELOPMENTS IN NANOSCALE SENSORS

3.1 The Benefits of Nanoelectrodes

As microelectrodes shrink in size, their sensing properties become further enhanced. Capacitive charging becomes quicker, enabling them to measure even faster chemical processes, and signal to noise ratio improves through reduced *iR* drop and more efficient mass transport [30]. Their small size can also result in pronounced migration effects and electrode kinetics, as opposed to the purely diffusional mass transport observed in microelectrodes [31]. Their rapid response times and reduced dimensions mean nanoelectrodes also enjoy very high spatial and

temporal resolution, making nanoelectrodes extremely convenient for probing biological systems and processes.

3.2 Biosensors that employ Nanoparticles

These benefits have led many authors to functionalise macroelectrodes with nanoparticles to imbue the larger, and easier to produce, electrodes with improved sensing properties. An example of this is where Nunna et al. decorate gold interdigitated microelectrodes with a thiourea monolayer, to which gold nanoparticles are bound, before being functionalised with CA-125 capture antibodies [32]. The gold nanoparticles provided a higher sensing signal when detecting CA – 125 antigens, explained by the increased surface area. An interested reader is pointed to the many reviews on the subject of nanoparticles for electrochemical biosensing [33, 34]. Other popular strategies along these lines include the use of carbon nanotubes and graphene. Silva et al. bound carbon nanotubes to a glassy carbon electrode and tethered it with the lectin cratylia mollis, which enabled specific and sensitive detection of the fetuin glycoprotein, a prostate cancer biomarker. The sensor detected down to 0.017 μg/mL using square wave voltammetry and also successfully measured glycoprotein in patient samples [35].

3.3 Traditional Nanoelectrodes

Although nanoparticles and nanostructuring have the benefits of cost effectiveness and ease of use; producing controllable and reproducible films is challenging, especially in a scalable manner [36]. Biosensors based on nanosized electrodes themselves are somewhat less common in literature, their use mostly hampered by the cost and complexity of producing them. One of the more common methods has been the use of a nanoporous template material, through which metal structures such as nanowires can be electroplated [37, 38]. This method has been successfully used many times to create ensembles of nanoelectrodes or nanoneedles for

biosensing. For the creation of more precise and controllable nanoelectrode arrays, micro and nanofabrication methods are generally required (for further reading, see a review published in *Current Opinions* recently on nanoelectrode arrays and ensembles for biosensing [39]). Popular methods include electron beam lithography and, more recently, nanoimprint lithography [37, 40, 41]. For example, Delle et al. used nanoimprint lithography to produce batches of nano interdigitated electrodes and utilised them to detect complementary DNA through hybridisation with a single stranded DNA probe layer on the electrode surface [42]. Nanoelectrodes have also found their way into full lab on a chip systems, with Triroj et al. reporting a microfluidic chip with an integrated nanoelectrode array, for detecting prostate-specific antigen (PSA) [43**]. The antigens were labelled with a GOx enzyme and provided an electrochemical signal at the electrode surface, which decreased as the GOx was displaced by the target PSA in solution. A novel "woodpile" type nanoelectrode array, using CeO2 to detect glucose was developed by Zhou et al. and is shown in Figure 3 [44]. The sensors demonstrate a sensitivity of 42.8 μA mM–1 cm–2 and a linear range from 20 μM to 2.5 mM, which is competitive in literature while benefiting from comparatively simple fabrication. Microlithography has also been used to create precise arrays of nanoband electrodes [45] and were employed by Piper et al. to demonstrate electrochemical growth of a hydrogel layer, which decreased biofouling when performing DNA – DNA hybridisation sensing [46].

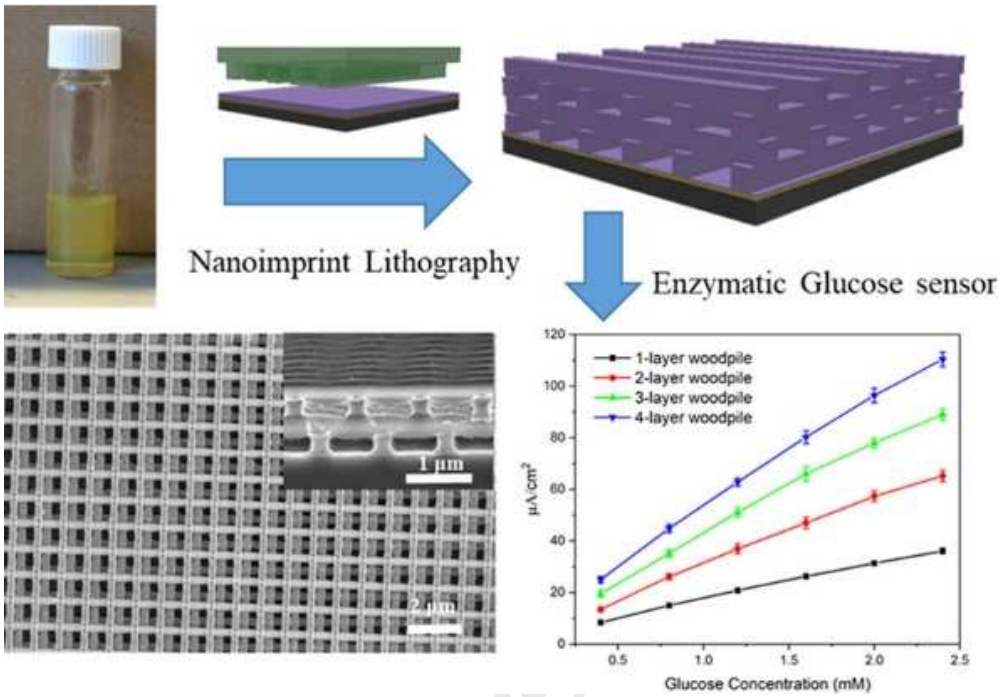

Figure 3. The stacked 'woodpile' nanoelectrode glucose sensor presented in [44]. Parts of the nanoimprint lithography steps are shown, along with a schematic and SEM of the finished device. Its performance as a glucose sensor is shown as a function of how many layers the structure incorporates. Reprinted from [44], Copyright 2019, with permission from American Chemical Society.

3.4 Fundamental Behaviour of Nanoelectrode Biosensors

A remaining challenge in the application of nanoelectrode biosensors which employ biorecognition films, is the fundamental behaviour of those films on nanoscale electrodes. Pittino et al. present a numerical simulation of the positon and orientation of single and double stranded nucleic acid films on nanoelectrode arrays, for capacitive biosensors [47]. They found that sensitivity is greater near the edge of the electrode, which correlates with the improved sensing properties of micro and nanoscale/functionalised electrodes. Veselinovic et al. investigated the formation of DNA biorecognition layers on various nanostructured electrodes and its subsequent effect on biosensor performance [48**].

4. FIELD-EFFECT TRANSISTORS AND RELATED ARCHITECTURES FOR BIOMEDICAL SENSING

4.1 Field-Effect Transistor Biosensors

Field-effect transistors (FETs) are increasingly gaining prominence as biosensors due to several key advantages including their scalability, low-cost, and ease of integration with simple electronics for measurement and readout. FETs can act as amplifiers within electronic circuits and typically consist of three terminals; the gate, source and drain. A change in gate voltage (which can be induced by a biorecognition element, DNA attachment, ion presence etc.) causes a change in the current flowing between the source and drain. Many FET architectures exist; the optimum in each case being commonly linked to the choice of semiconductor. To date, crystalline silicon (Si) remains the most common semiconductor material, largely due to its well established fabrication. However, organic semiconductors are increasingly finding use in devices to produce organic field-effect transistors (OFETs) [49] and organic electrochemical transistors (OECTs) [50]. Such OFET/OECT architectures can meet the demands of emerging biosensor applications by providing improved biocompatibility, low processing temperatures, reduced cost, low-voltage operation, high transconductance, ease of production and compatibility with flexible/stretchable substrates.

4.2 Silicon-based Transistors

A commercially available depletion-mode nMOS transistor has previously been used in conjunction with a gold sensing pad connected via an extended gate for the detection of PNA:DNA hybridisation [51]. The device is low-cost and easy to setup, providing clear changes in potential of ~ 70 mV with a 1 µM DNA target, which increases as a function of nucleic acid concentration. The extended gate architecture has also been used to develop a low-cost FET

sensor for the detection of mutations in the tumour protein 53 gene (TP53) [52]. In addition, extended gate sensors have been used for pH sensing [53] and to control the potential response to small biomolecules [54].

4.3 Carbon Nanotube FETs

Carbon nanotube FETs (CN-FETs) make use of either a single or an array of carbon nanotubes as the channel in the FET architecture offering improved performance such as higher electron mobility and lower threshold voltage than traditional Si-based FETs. A suspended CNT-based FET (Figure 4) was developed for the ultrasensitive label-free detection of DNA. Through separation of the CNT and the substrate, a LoD as low as 10 aM was achieved [55**].

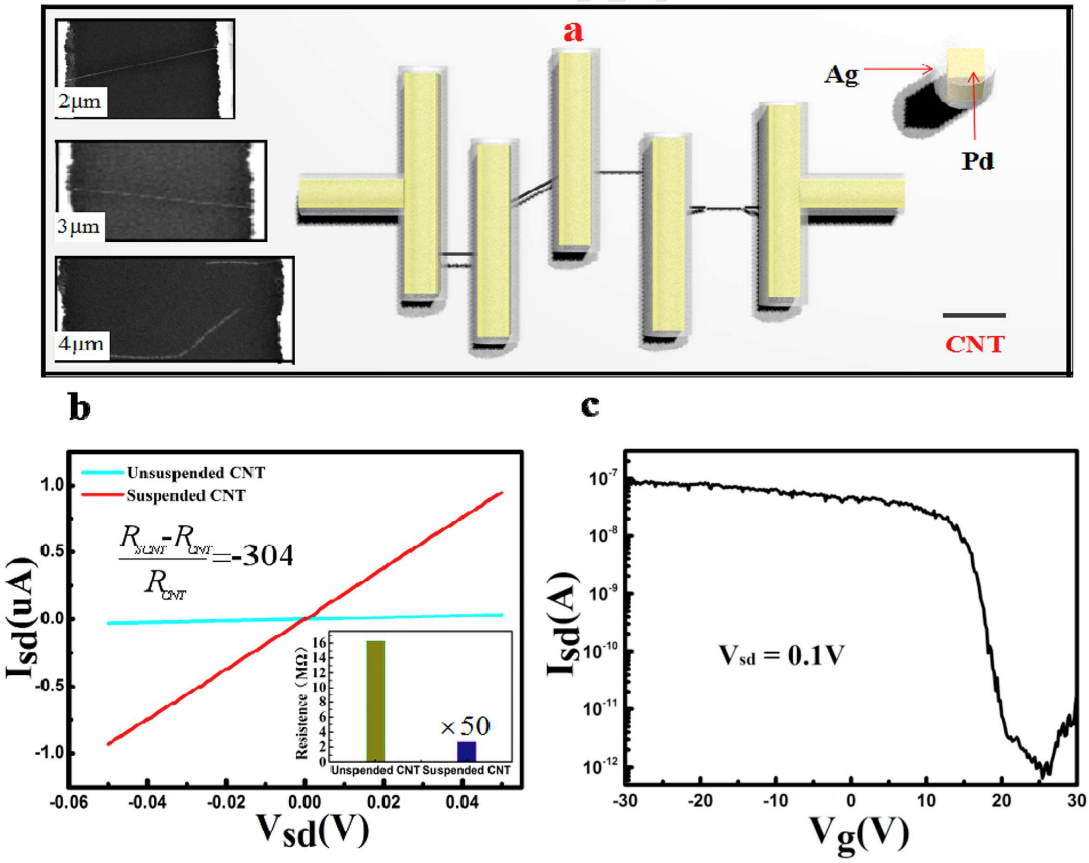

Figure 4. (a) Schematic of a suspended and unsuspended CNT. The inset on the L.H.S shows partially enlarged images of a Suspended CNT FET (SNCT FET) with channel spacing of 2, 3 and 4 µm's. (b) Comparison of the conductivity between a suspended CNT with an unsuspended CNT. In the inset, x 50 represents that the resistance of the unsuspended CNT amplified 50 times. (c) $I_{sd} - V_g$ curve of the SCNT FET. Reprinted from [55], Copyright 2019, with permission from Elsevier.

4.4 Graphene

Graphene is a recently discovered allotrope of carbon consisting of a single layer of atoms in a 2-D lattice structure which displays special properties such as high strength, near transparency and the ability to conduct heat and electricity very efficiently [56]. A graphene FET (GFET) immunosensor was recently developed to sensitively detect Human Chorionic Gonadotropin (hCG), a key biomarker of certain cancers [57*]. The sensor works on the principle of antigen-antibody interaction, and the limit of detection has been established as < 1 pg/mL. Single-stranded DNA detection has also been achieved using a magnetic GFET with a detection limit of 1 pM [58].

4.5 Silicon Nanowire FETs

Silicon nanowire FETs (SiNW-FETs) are increasing attracting attention due to their high sensitivity, specificity, label-free nature and real-time detection attributes [59]. Recently, a SiNW-FET has been developed to detect α-Fucosidase for Hepatocellular Carcinoma diagnosis [60].

4.6 Organic Electrochemical Transistors

OECTs are commonly based on the conducting polymer PEDOT:PSS, and since PEDOT:PSS is in direct contact with the electrolyte, OECTs can sensitively convert biochemical signals into electrical ones, enabling a wide variety of applications for chemical and biological

detection including DNA [61], lactate detection [62], cell activity [63], cardiac potential [64] and for wearable/textile sensors [65]. Of particular note, OECTs have been used for the detection of the human influenza virus using sialyllactose-functionalised transistors based on PEDOT:PSS [66]. Additionally, OECTs have been integrated with immune-affinity membranes for the label-free detection of interleukin-6 (IL-6) at physiological concentrations via antibody-antigen binding at the gate electrode [67*]. The membrane acts to increase the IL-6 concentration at the sensing electrode, thereby improving device sensitivity (LoD ~ 100 pM). Furthermore, a stable and selective wearable cortisol sensor featuring an OECT integrated with a synthetic and biomimetic polymeric membrane has been developed based on the transistor's ability to tolerate mechanical bending and stretching tests providing a useful wearable sweat sensor [68].

5. CONCLUSIONS

Microelectrodes offer a number of advantages for bioanalytical sensing applications and have to date been commonly employed in neuroscience measurements both *in vitro* and *in vivo.* Increasingly the analytical advantages of these sensors are being utilised for detection of biomarkers (DNA, RNA, proteins etc.). The nature of the microelectrode response and the different conditions required for effective surface functionalisation compared to e.g. macroelectrodes are becoming increasingly understood with the use of arrays and IDEs in both Faradaic and non-Faradaic measurements setups proving popular. Whether a single sensing modality employing microelectrodes will go on to dominate is unclear however in the coming years the emergence of optimised sensing protocols and achievable limits of detection will emerge.

Although the benefits of nanoscale electrodes for biosensing are well known, the fundamental reasons for this, and their behaviours, are not as established. In addition, current fabrication methods for controllable nanoelectrodes are prohibitively expensive for many groups and likely the reason, not only for this lack of fundamental agreement but for the dearth of nanoelectrode biosensors in literature. This, however, is contrasted by the vast number of sensors functionalised with nanomaterials, which showcase the remarkable sensitivity enhancements attainable when working at the nanoscale and has become a commonplace strategy for electrochemical biosensors. Although, the drawback with these sensors again comes down to reproducible and controllable manufacturing. As fabrication processes become more advanced and decrease in cost, it is likely that nanoelectrode will become even more widely used.

Field-effect transistor-based architectures are increasing finding use within the field of biosensing due to many key advantages such as their low-cost, scability, ease of integration with readout electronics and their ability to be mass manufactured, notably on conformable substrates such as ultra-lightweight plastic. Looking ahead, there is still not a consensus about the best FET structure to use and it appears to be rather application specific. What is clear, is that FETs will continue to be used in many fields of biomedical research for many years to come, with organic materials in particular playing a key role in the development of novel and previously inconceivable devices.

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AUTHOR CONTRIBUTIONS

Stuart Hannah: Conceptualization, Writing – original draft, Writing – review and editing. **Ewen Blair:** Conceptualization, Writing – original draft, Writing – Review and Editing. **Damion Corrigan**: Conceptualization, Supervision, Writing – original draft, Writing – Review and Editing.

ADDITIONAL INFORMATION

Competing Interests – The authors declare no competing interests**.**

- Overview of current trends and state of the art in micro and nanoscale biosensors
- Microelectrodes, nanoelectrodes and field-effect transistor systems discussed
- Reviews performance characteristics of sensors and considers barriers to adoption

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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