Recent Developments in Continuous Monitoring Diagnostics with Microneedle Arrays

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Abstract. Compared with therapeutics, diagnostic devices account for a relatively small proportion of healthcare expenditure (less than 10%) and yet timely diagnosis as well as continuous monitoring of molecular markers can have a major impact on disease outcomes. In particular point of care (or near patient) tests can empower individuals to become active participants in the management of their conditions, giving them and their medical support greater insight into both their conditions and their response to treatment. In an extension of the point of care paradigm, continuous monitors of biomarkers and/or therapeutics allow high frequency data to be gathered and patterns of variation to be analyzed in ways that are not possible with infrequent and sporadic testing. The advent of novel materials, fabrication methods and data analysis have opened the way to new devices, assay formats and molecular targets. In this paper we will discuss some aspects of our work in this area with a particular focus on microneedles for continuous, minimally invasive sensing and the use of nucleic acid aptamers in both electrochemical and lateral flow assays.

Keywords: Microneedles, Electrochemical, Aptamers.

1 Microneedle Sensor Arrays

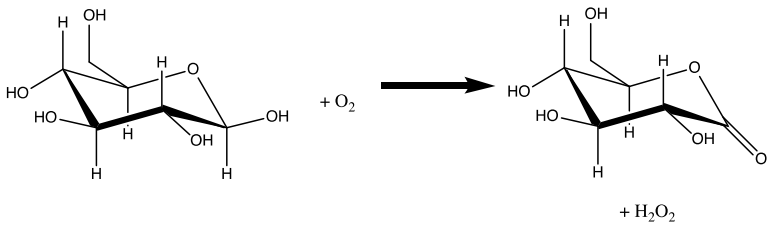
Continuous measurement of physiological parameters such as blood pressure, heart rate, electrophysiological patterns (electrocardiograms, electroencephalograms), and respiration rate are very familiar procedures whether in hospital or, increasingly at home. However, as a general observation this approach has not been applied to the measurement of molecular parameters (biomarker and drugs). The only significant exception is in continuous glucose monitoring (CGM) in Type 1 diabetes and even here only a small proportion of individuals with Type 1 diabetes regularly use CGM devices. There are several well documented reasons for this including cost, discomfort, inconvenience and concerns over reliability. Typically, such CGM is performed with a subcutaneously implanted needle modified to generate a glucose dependent current when an electrical potential is applied. Over the past several years we have taken a different approach to the design and fabrication of continuous sensing devices

that we believe overcome many of the limitation of current implanted sensors and allow a straightforward extension to continuous monitoring of many other molecular species. These microneedle sensor arrays are fabricated by low cost, scalable and flexible methods, reside in the dermal interstitial compartment, less than 1mm below the surface of the skin, whose fluid (interstitial fluid or ISF) is very close in composition to serum. This compartment sits above the capillary bed and nerve endings and so the microneedles cause neither pain nor bleeding upon insertion. The microneedles are shown in Figure 1 and are typically configured as four separate arrays, each array comprising 16 electrically contiguous solid microneedles. This format offers a large sensing area whilst resulting in no adverse tissue response (inflammation or irritation) or collagen capsule formation.

The base structures are made in polycarbonate by injection moulding and then metallized with gold, silver or platinum. Finally, they are functionalized with the relevant sensing chemistries. A complete set of protocols are described in our recent *Methods in Enzymology* chapter [1] and here the use of the devices in continuous amperometric and potentiometric sensing will be described. Both these sensing modalities are well established and have been used with a range of molecular recognition chemistries, in the case of amperometric sensors through oxidation or reduction generating an electrical current and in the case of potentiometric sensors through reactions generating an electrical potential via a local ion flux (most commonly of protons).

1.1 Amperometric Sensing

Many different substrate oxidizing enzymes are known, but one of the earliest and still a widely used example is glucose oxidase from *Aspergillus niger* which catalyses the following reaction:

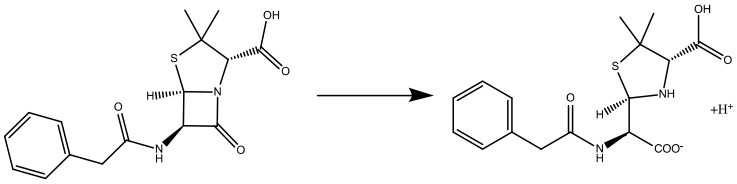


The reaction is characteristic of the class of enzymes that utilize oxygen as the electron acceptor and reduce it to hydrogen peroxide. This product is then reoxidised at a positive potential producing a glucose dependent current. The high turnover number, robustness, low cost and specificity for glucose, when linked to the importance of glucose measurements in the management of diabetes has made it the major application for microneedle sensors of many different designs [2]. In our current design the enzyme is entrapped in a polyphenol coating which provides sufficient mechanical robustness to survive skin insertion. We have recently published data from 24-hour

monitoring of 5 study participants with Type 1 diabetes [3]. In this case the calibration of the sensor was based on a single point at the start of the monitoring period with no further recalibration. Although glucose has been the most intensively studied metabolite in ISF with microneedles, others are both tractable and relevant. Perhaps the example of lactate is a good one. Like glucose there is an accessible lactate oxidase and continuous monitoring of lactate has applications in both sports performance and in the management of sepsis.

1.2 Potentiometric Sensing

Classically, potentiometric sensors are based on establishing a potential difference across an ion selective membrane due to differences in concentration between the two compartments separated by the membrane. More recent developments have replaced the two-compartment/membrane configuration by solid-state ion sensing electrodes (ISE's) that offer greater robustness and more straightforward fabrication procedures. Miller and colleagues have demonstrated the combination of a solid-state potassium ISE with hollow microneedles to produce a transdermal potassium sensor [4].
Enzyme linked ISE's have typically linked hydrolytic enzymes to pH sensors and we have recently demonstrated microneedle sensing of penicillin with an iridium oxide (IrOx) pH sensor coated with a hydrogel layer containing entrapped β-lactamase [5].



This device could have particular utility in enabling dynamic dosing of antibiotics based upon continuous monitoring and a feedback loop [6].

2 Beyond Enzyme Sensing-Aptamers

2.1 Affinity Sensing.

Enzymes exhibit the dual properties of molecular recognition and catalysis, transforming the target analyte and in the process generating a detectable product (hydrogen peroxide and protons in the above examples. Affinity reagents simply bind with the requisite specificity and affinity to the target analyte and that process then needs to be coupled to signal generation. There are many possible affinity reagents and signal generation schemes, in respect of compatibility with microneedle sensors we have chosen redox labelled nucleic acid aptamers as the basis for sensing target molecules

that are not amenable to enzymatic sensing. This group comprises protein biomarkers and drug molecules amongst others and offers the opportunity to move the single measurement, point of care paradigm to continuous monitoring.
If the application of microneedle sensor arrays is to be of use here, then the target analytes should be present in the dermal ISF and studies over the past few years have shown that this is indeed the case for both proteins [7] and therapeutic drugs [8].

Nucleic acid aptamers are short single stranded DNA or RNA sequences selected to bind target molecules with good affinity and specificity. The selection typically uses the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) to identify binding sequences in a large (10^{14}) pool of random sequences [9]. Once known the DNA sequence can be synthesized and modified, for example with a redox active group, such that analyte binding brings about a structure switch (Figure 1). This alters the distance between the redox group and the electrode surface and so changes the current flowing [10].

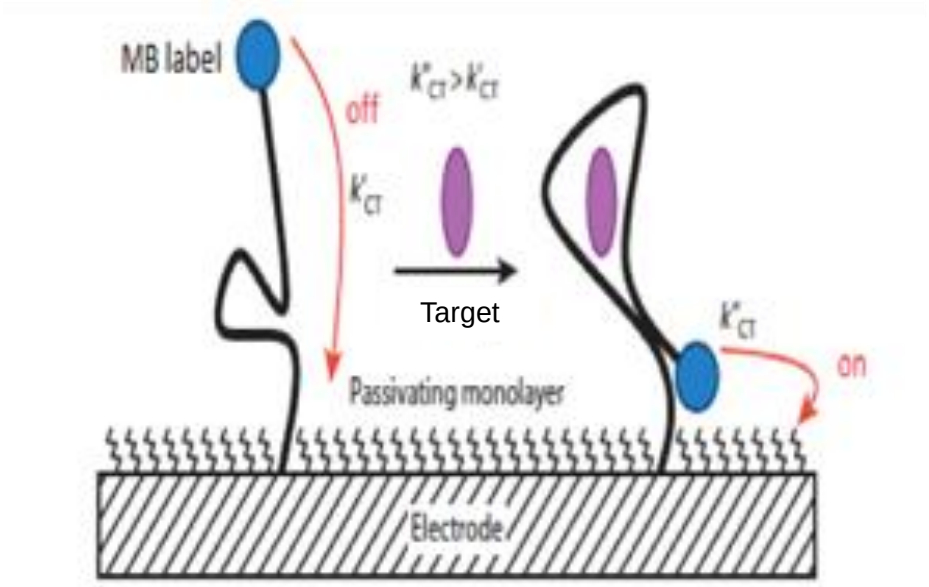


Fig. 1. Electrochemical transduction of structure switching in an aptamer. MB is the redox group methylene blue and k'_{CT} is the charge transfer rate constant

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