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Towards a minimally invasive device for beta-lactam monitoring in humans

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ABSTRACT

Antimicrobial resistance is a leading patient safety issue. There is a need to develop novel mechanisms for monitoring and subsequently improving the precision of how we use antibiotics. A surface modified microneedle array was developed for monitoring beta-lactam antibiotic levels in human interstitial fluid. The sensor was fabricated by anodically electrodepositing iridium oxide (AEIROF) onto a platinum surface on the microneedle followed by fixation of beta-lactamase enzyme within a hydrogel. Calibration of the sensor was performed to penicillin-G in buffer solution (PBS) and artificial interstitial fluid (ISF). Further calibration of a platinum disc electrode was undertaken using amoxicillin and ceftriaxone. Open-circuit potentials were performed and data analysed using the Hill equation and log(concentration [M]) plots. The microneedle sensor demonstrated high reproducibility between penicillin-G runs in PBS with mean K_m (± 1SD) = 0.0044 ± 0.0013 M and mean slope function of log(concentration plots) 29 ± 1.80 mV/decade (r^2 = 0.933). Response was reproducible after 28 days storage at 4 °C. In artificial ISF, the sensors response was K_m (± 1SD) = 0.0077 ± 0.0187 M and a slope function of 34 ± 1.85 mv/decade (r^2 = 0.995). Our results suggest that microneedle array based beta-lactam sensing may be a future application of this AEIROF based enzymatic sensor.

1. Introduction

The concept of organisms developing resistance to beta-lactams is well understood and commonly involves mutations in the genetic sequence coding penicillin binding proteins [1,2]. The global threat to patient safety and modern medicine from drug resistant infections (DRIs) is at an alarming level, with estimates that over 10 million people will die each year due to DRIs by 2050 [3]. One of the major drivers of DRIs is the inappropriate use of antimicrobial agents [4]. Whilst much emphasis has been placed upon prudent prescribing and antibiotics, a major area that still requires intervention is in optimising the dosing of antibiotics to ensure that the correct amount is given to maximise bacterial killing, whilst avoiding the harmful consequences of therapy such as DRI and toxicity. Recently, this problem has been highlighted by reports that up to 75% of critically ill patients in intensive care may not be receiving appropriate doses of beta-lactam antibiotics [5] leading to a growing consensus that antibiotic dosing must be provided on an individualised basis [6]. Current therapeutic drug monitoring (TDM) strategies typically rely on single time point plasma blood samples that require transporting and analysis, which is rarely commercially available for clinical practice [7]. There is an urgent need to develop novel, minimally invasive techniques for TDM that will allow real-time assessment of antimicrobial concentrations that are not constrained by current processes to allow maximum impact from precision dosing interventions.

Microneedle technology was first demonstrated as a suitable mechanism for monitoring and drug delivery over 20 years ago [8]. Since then technology has progressed rapidly with data supporting the use of this microneedle sensor technology for monitoring glucose and lactate concentrations in humans [9–12]. The microneedle works by penetrating the stratum corneum layer of the skin accessing the interstitial fluid, whilst avoiding the nerve fibres and blood vessels that are found within the dermis, thus offering a minimally invasive method for drug or metabolite monitoring.

Electrochemical sensors for antimicrobials in the environment, agriculture, and humans have been demonstrated for a wide range of

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agents used in human medicine [13–38]. However, attempts to translate these into mechanisms for real-time monitoring of drug concentrations in humans currently require invasive vascular catheter insertion or extraction of interstitial fluid using classical microdialysis [32,39]. These have their limitations with vascular based devices only being acceptable in very specific situations in clinical practice such as critical care or at the time of surgery and pose their own risks to the patient, including thrombosis [32]. Such invasive devices would not be acceptable in the vast majority of individuals who receive antimicrobial therapy in settings outside of critical care. Furthermore, microdialysis techniques require transfer of small volumes of interstitial fluid, which not only presents technical challenges but also leads to delays that militate against their application in real-time control [39]. Electrochemical sensors for antimicrobial sensing are largely based on aptamer, antibody linked, and enzyme sensors [32,40,41]. These have demonstrated high sensitivity for monitoring of antimicrobials. Enzymatic penicillin-G sensors are some of the oldest reported antimicrobial sensors reported in the literature [41].

Here, we report of a sensor that exploits an iridium oxide pH sensing layer to detect changes in pH arising from beta-lactamase hydrolysis of the analytical target. A range of beta-lactams are investigated and testing in physiological relevant media is undertaken as an initial step towards minimally invasive monitoring of beta-lactam antibiotics.

2. Method

2.1. Reagents and equipment

All agents were purchased from Sigma Aldrich (UK) unless otherwise stated. All rinsing and aqueous solution preparation was undertaken using deionised water with a resistivity of > 15 MΩcm. Phosphate Buffer Solution (PBS, 0.1 M phosphate, pH 7.4 at 25 °C) was used unless otherwise stated. Iridium oxide plating solution (100 ml) was prepared as described by Yamanaka [42] using iridium chloride hydrate ($IrCl_4{\cdot}H_2O$, 0.15 g), aqueous hydrogen peroxide (H_2O_2·30 wt%, 1 ml), oxalic acid ($(COOH)_2{\cdot}H_2O$, 0.5 g), and anhydrous potassium carbonate (K_2CO_3, 3.9 g), leaving the solution to stabilise for 72 h before use [42].

Class B beta-lactamase from *Bacillus cereus* 569/H9 was purchased from Merck Millipore with a mixture of beta-lactamase I & II. For enzyme immobilisation 5% aqueous polyethylenimine (PEI) was used. The following three solutions were also prepared: (i) 5 ml 0.1 M phosphate buffer (pH 7.4) with 25 mg/ml beta-lactamase; (ii) 5 ml of 0.1 M phosphate buffer (pH 7.4) with 50 mg/ml bovine serum albumin; and (iii) glutaraldehyde solution ($C_5H_8O_2$, 2.5%). Several different approaches were initially tested and validated on standard disc electrode devices.

For calibration of the sensor, penicillin-G, amoxicillin, and ceftriaxone were obtained from Sigma-Aldrich and stock solutions of each beta-lactam prepared in PBS or artificial interstitial fluid (described below) for dilution.

The base microneedle array was fabricated as previously described by Sharma and colleagues [9]. Bare microneedle arrays were then sputtered with chromium (15 nm)/platinum (50 nm) to obtain the working electrodes. One of the microneedle arrays was sputtered with Ag (150 nm), which was modified to an Ag/AgCl reference electrode by treating with a saturated solution of $FeCl_3$ [9]. Cyclic voltammetry, iridium oxide deposition, and open circuit potentials (OCP) was performed using CHI 650a potentiostat. pH calibration curves were recorded with a Mettler Toledo SevenEasy pH meter. 5 mm diameter platinum disc electrodes, purchased from Alvatek Ltd., were also used where stated.

2.2. Microneedle arrays

The microneedles array structures described here were fabricated in a three-stage process. The solid work designs were transferred to a FANUC ROBOCUT α-OiC (Series 180is-WB) machine for wire erosion. It was set to make three milling passes over the copper-tungsten (Cu-W) (Erodex, UK) block to create master electrodes for spark erosion. The Cu-W master was then used for spark erosion (JOEMARS EDM AZ50DR) of an aluminium block (Erodex, UK) in to obtain the metal inlay. This metal inlay was used for the injection moulding of polycarbonate pellets. The polycarbonate pellets were dried at 110 °C for 24 hours under vacuum prior to use before injection moulding process at T_m = 270 °C (PC melt temperature), T_w = 80 °C (tool temperature) at injection speed of 20 $cm^3\,s^{-1}$ and shot volume of 4.4 cm^3 and a cooling time (t_c) of 5 s. Each polycarbonate microneedle structure (25 × 25 × 2 mm) comprised of four 4 × 4 microneedle arrays. Fabrication of microneedle array structures have been described in detail before [9,43].

2.3. Microneedle array electrochemical sensor preparation

The microneedle arrays were prepared by rinsing the surface with ethanol. Iridium oxide was then deposited on the platinum at a constant potential of 0.95 V for 300 s for three cycles with an interval of 10 min between cycles. pH calibration of anodically electrodeposited iridium oxide films (AEIROFs) was performed in PBS with OCP recorded for the pH range 4.0–8.0.

Polyethylenimine was layered onto the AEIROFs for mechanical stability. Beta-lactamase was then immobilised onto the electrode surface by depositing beta-lactamase and BSA solution with 2.5% glutaraldehyde solution, which was left for 90 min and then rinsed. Finally, another layer of beta-lactamase solution alone was deposited onto the outer membrane, and after drying a final layer of PEI was added. Sensors were stored for at least 24 h at 4 °C before use. 5 mm platinum disc electrodes were modified using the same method.

2.4. Beta-lactam antibiotic calibration

A stock solution of beta-lactam was prepared in PBS. OCP were recorded for increasing concentrations of beta-lactam from 50 to 5000 μM, based on reports of similar concentrations of beta-lactam detected in patients subdermal interstitial fluid [44,45]. This was achieved by adding the concentrated stock solution to PBS under gentle stirring. OCPs were recorded over 600 s, or until stable potential was reached. Calibration plots were fitted using the Hill Eq. (1) with Km values estimated from concentration (M) – potential (E) plots.

$$V = \frac{V_{max}\,[S]}{K_m + [S]} \tag{1}$$

where V is the velocity of the enzyme reaction ($M\,s^{-1}$), V_{max} is the maximum velocity of the reaction ($M\,s^{-1}$), [S] is the substrate concentration (M), and K_m is the half maximal concentration constant for the reaction.

The slope of log(concentration [M]) – potential [E] plots of the data was also investigated to allow comparison of the linear response of the sensors.

After calibration, the sensor was then stored at 4 °C for 28 days and the calibration repeated to assess response over time.

2.5. Artificial interstitial fluid preparation and calibration

Artificial interstitial fluid was prepared by mixing; standard physiological solution (0.9% NaCl), 11 g/l total protein made up of bovine serum albumin and human alpha-globulins (cohn factor IV-1) in a ratio of 60:40, and 5 mM dextrose. Proclin 150 (6 mg/l) was added as a preservative. Penicillin-G calibration was then performed and OCP recorded using the methodology described in Section 2.4. This was performed using two microneedle arrays fabricated using the same methodology described above.