Published in final edited form as:

Electrochem commun. 2017 September ; 82: 1–5. doi:10.1016/j.elecom.2017.07.011.

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

Timothy Miles Rawson¹, Sanjiv Sharma², Pantelis Georgiou³, Alison Holmes^{1,4}, Anthony Cass², and Danny O'Hare⁵

¹National Institute for Health Research Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Imperial College London, Hammersmith Campus, Du Cane Road, London. W12 0NN. United Kingdom

²Department of Chemistry & Institute of Biomedical Engineering, Imperial College London, London, SW7 2AZ, United Kingdom

³Department of Electrical and Electronic Engineering, Imperial College London, South Kensington Campus, London, SW7 2AZ, United Kingdom

⁴Imperial College Healthcare NHS Trust, Hammersmith Hospital, Du Cane Road, Acton, W12 0NN, United Kingdom

⁵Department of Bioengineering, Imperial College London, London, SW7 2AZ, United Kingdom

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 $K_m (\pm 1SD) = 0.0044 \pm 0.0013$ 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 $K_m (\pm 1SD) = 0.0077 \pm 0.0187$ 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.

*Corresponding author: Dr Timothy M Rawson, Health Protection Research Unit in Healthcare Associated Infections & Antimicrobial Resistance, Hammersmith Hospital, Du Cane Road, London.W12 0NN. United Kingdom. timothy.rawson07@imperial.ac.uk, Telephone: 02033132732.

Keywords

Beta-lactam antibiotic monitoring; minimally invasive; continuous monitoring; antibiotic resistance

1.0 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 (DRI's) is at an alarming level, with estimates that over 10 million people will die each year due to DRI's by 2050 [3]. One of the major drivers of DRI's 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 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 mitigate 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.

2.0 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 $>15 \text{ M}\Omega\text{cm}$. Phosphate Buffer Solution (PBS, 0.1M 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 ($\text{IrCl}_4 \cdot \text{H}_2\text{O}$, 0.15 g), aqueous hydrogen peroxide (H_2O_2 30%wt, 1 ml), oxalic acid ($(\text{COOH})_2 \cdot \text{H}_2\text{O}$, 0.5 g), and anhydrous potassium carbonate (K_2CO_3 , 3.9 g), leaving the solution to stabilise for 72 hours 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 ($\text{C}_5\text{H}_8\text{O}_2$, 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 $20 \text{ cm}^3 \text{ s}^{-1}$ and shot volume of 4.4 cm^3 and a cooling time (t_c) of 5 seconds. Each polycarbonate microneedle structure ($25 \times 25 \times 2 \text{ mm}$) comprised of four 4x4 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 seconds for three cycles with an interval of 10 minutes 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 minutes 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 hours 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 - 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. OCP's were recorded over 600 seconds, or until stable potential was reached. Calibration plots were fitted using the Hill equation (1) with K_m values estimated from concentration (M) – potential (E) plots.

$$V = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

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.

3.0 Results and Discussion

3.1 pH calibration

Figure 1a demonstrates the pH calibration results for three independent microneedle array's following AEIROF. pH calibration for iridium oxide between 4.0 and 8.0 demonstrated a median (SD) sub-Nernstian response of 48 ± 11 mV/pH for the microneedle array with $r^2 = 0.929 \pm 0.028$. This was compared to a super-Nernstian response observed (figure not shown) on platinum disc electrodes of ($n = 3$) 64 ± 4 mV/pH ($r^2 = 0.997 \pm 0.001$). Variation occurs in both the apparent $E^{\circ'}$ (pH = 0) value and in slope of calibration, as is commonly found with these devices [46]. The sources of these variations have been discussed in detail by Hitchman [47]. Apparent $E^{\circ'}$ varies with the varying molar ratios of Ir(III) : Ir(IV) within individual AEIROFs and is affected by the chemical irreversibility of the interconversion [47]. Since the molar ratio, the charge storage capacity, and counter ion access will vary with depth and the morphology of the polymeric cross-linked oxyhydroxide film, the precise form of the calibration working curve will depend on underlying substrate properties. Whilst potential scanning can ameliorate this phenomenon for bulk electrodes, the mechanical stability of films on sputtered substrates suffers, and a build-up of relatively non-conductive Ir(III) impairs performance [47,48]. We have nonetheless shown elsewhere [48,49] that reproducibility for any given device is good and that acute sensitivity is stable for days, enabling biologically useful pH measurement to be undertaken.

Further pH calibration was performed on an individual platinum disc electrode to document any difference in pH calibration slopes during the sensor fabrication process. pH calibration was performed at 3 individual steps on this electrode; Calibration of the AEIROF alone (step 1), following application of the PEI layer (step 2), and following enzyme fixation (step 3). This can be observed in Figure 1b, which illustrates the response of the electrode to pH calibration between 4.0-8.0 following each step. Whilst there was an observed fall in the apparent $E^{\circ'}$ between these steps, the calibration slopes remained similar, suggesting that response of the AEIROF to pH change is not significantly altered during the sensor fabrication process. This is likely to be secondary to the buffering effect of the PEI and hydrogel layer.

3.2 Beta-lactam calibration

Figure 2 shows the penicillin-G calibration curves and log-concentration plots for the microneedle array sensors calibrated in PBS following fabrication and after 28 days storage. It also shows a comparison of observed results for calibration of fabricated disc electrodes to penicillin-G, amoxicillin, and ceftriaxone. Penicillin-G calibration curves were similar on

both platinum disc electrodes and the microneedle arrays. Using the Hill equation (1), mean K_m ($\pm 1SD$) values were calculated demonstrating $K_m = 0.0044 \pm 0.0013$ M. The relatively large dispersion of K_m values reflects the range of microenvironments of the enzymes. Log(concentration) plots demonstrated a mean ($\pm 1SD$) slope of 29 ± 1.8 mV/decade change with concentration. The response of the microneedle remained similar after 28 days storage at 4°C with a K_m value of $K_m = 0.0062$ M and log(concentration) slope of 32 mV/decade ($r^2 = 0.933$). This suggests that the devices are robust, maintaining a similar response and rate of change in potential to change in concentration and similar enzyme kinetics following prior use and storage.

Following this, calibration with other beta-lactam antibiotics, amoxicillin and ceftriaxone, was performed with the platinum disc electrode to explore the response of the sensor to these antibiotics. The table in Figure 2 provides a comparison of K_m values and slopes for each antibiotic tested. For amoxicillin the K_m was similar to that of penicillin-G ($p=0.37$; 95%CI: -0.010 – 0.005). However, the mean ($\pm 1SD$) slope for the log(concentration) plot was significantly different at 16 ± 1.1 mV/decade change ($p<0.01$; 95%CI: 8.35 – 17.56). For ceftriaxone, only one calibration run was possible. However, the K_m and slope values observed were much lower. In this run $K_m = 0.00011$ M and the log(concentration) slope was 1.5 mV/decade ($r^2 = 0.991$). These findings may be explained by the relatively improved resistance of such antibiotics to hydrolysis by beta-lactamase enzyme when compared to penicillin-G [50,51]. Future work will now compare use of extended spectrum beta-lactamase enzyme (acquired from Sekesui diagnostics) versus the enzyme used within this study to further characterise this response.

3.3 Calibration in artificial interstitial fluid

After demonstration of the potential reproducibility of individual microneedle arrays for detection of penicillin-G, we investigated the effects of artificial interstitial fluid on the accuracy of the sensor. This is an important next step given previous reports of biofouling and reduced sensitivity following the absorption of protein, such as albumin, onto biosurfaces such as iridium oxide based pH sensors [52,53]. This was performed using 2 newly fabricated microneedle array's with testing performed as described above. The mean ($\pm 1SD$) response of the microneedle arrays to penicillin-G calibration in interstitial fluid ($n = 4$) was $K_m = 0.0077 \pm 0.019$ M, with a slope of 34 ± 1.85 mV/decade ($r^2 = 0.966$). There was no significant difference in observed K_m values between PBS and interstitial fluid runs with the microneedles ($p = 0.77$; 95%CI: -0.03 - 0.02). Variation was also minimal in observed K_m values between both microneedle arrays used for the interstitial fluid runs ($p=0.94$; 95%CI: -0.08 – 0.08).

This observation is important for informing future biosensor designs as it allows estimation of the potential working range of the biosensor. As the concentration increases to $[S] > K_m$ the biosensor response begins to move out of the linear range as saturation of the enzyme occurs and the reaction tends towards zero order. By determining the K_m value of the sensor in interstitial fluid, this allows us to ensure that this operating window remains within the linear range required for use in ISF (i.e. when $[S] < K_m$) [54]. Given the likely concentration of free drug in the interstitial fluid is likely to be below 100 to 1000 μ M [44,45] this suggests

that the microneedle biosensors working range will be within an appropriate operating window.

3.4 Limitations & future work

There were several limitation within this study. We have not investigated the ability of the sensor to monitor dynamic concentration changes. This will be investigated through the development of a dynamic system to simulate the observed human pharmacokinetic variations in ISF. Furthermore, the role of enzyme inhibitors, such as clavulanic acid or tazobactam, which are often combined with penicillin antibiotics, on the enzyme reaction rate were not explored. This will form of future research that will inform refinement of the sensor device before testing on human patients receiving beta-lactam antibiotics.

4.0 Conclusion

The hydrolysis reaction that occurs when beta-lactamase acts upon penicillin provides a mechanism for sensing through the application of pH sensitive sensors, as demonstrated with AEROFs in this study. We have demonstrated that the sensitivity of the sensor and level of detection is within the range expected in human patients ISF at steady state. To our knowledge this is the first report of such a sensor being developed on a microneedle array, which may provide a mechanism of direct sampling from the ISF. This has the added benefit that it facilitates direct interstitial fluid sensing in a minimally invasive fashion, whilst not relying on microdialysis. If successful, this sensor may lead to development of closed-loop control systems, based on continuous monitoring of beta-lactam concentrations.

References

- [1]. Kong K-F, Schnepfer L, Mathee K. Beta-lactam Antibiotics: From Antibiosis to Resistance and Bacteriology. APMIS. 2010; 118:1–36. DOI: 10.1111/j.1600-0463.2009.02563.x [PubMed: 20041868]
- [2]. Sime FB, Roberts MS, Peake SL, Lipman J, Roberts JA. Does beta-lactam pharmacokinetic variability in critically ill patients justify therapeutic drug monitoring? A systematic review. Ann Intensive Care. 2012; 2:35.doi: 10.1186/2110-5820-2-35 [PubMed: 22839761]
- [3]. O'Neill, J. Tackling Drug-Resistant Infections Globally: an Overview of Our Work the Review on Antimicrobial Resistance. 2016. <https://amr-review.org/>
- [4]. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJV. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet. 2016; 387:176–187. DOI: 10.1016/S0140-6736(15)00473-0 [PubMed: 26603922]
- [5]. Roberts JA, Ulldemolins M, Roberts MS, McWhinney B, Ungerer J, Paterson DL, Lipman J. Therapeutic drug monitoring of beta-lactams in critically ill patients: Proof of concept. Int J Antimicrob Agents. 2010; 36:332–339. DOI: 10.1016/j.ijantimicag.2010.06.008 [PubMed: 20685085]
- [6]. Roberts JA, Abdul-Aziz MH, Lipman J, Mouton JW, Vinks AA, Felton TW, Hope WW, Farkas A, Neely MN, Schentag JJ, Drusano G, et al. Individualised antibiotic dosing for patients who are critically ill: Challenges and potential solutions. Lancet Infect Dis. 2014; 14:498–509. DOI: 10.1016/S1473-3099(14)70036-2 [PubMed: 24768475]
- [7]. Roberts JA, Norris R, Paterson DL, Martin JH. Therapeutic drug monitoring of antimicrobials. Br J Clin Pharmacol. 2012; 73:27–36. DOI: 10.1111/j.1365-2125.2011.04080.x [PubMed: 21831196]

- [8]. Henry S, McAllister DV, Allen MG, Prausnitz MR. Microfabricated microneedles: a novel approach to transdermal drug delivery. *J Pharm Sci.* 1998; 87:922–5. DOI: 10.1021/js980042+ [PubMed: 9687334]
- [9]. Sharma S, Saeed A, Johnson C, Gadegaard N, Cass AE. Rapid, low cost prototyping of transdermal devices for personal healthcare monitoring, *Sens. Bio-Sensing Res.* 2013; 15(1):101–115. DOI: 10.1089/dia.2012.0188
- [10]. El-Laboudi A, Oliver NS, Cass A, Johnston D. Use of Microneedle Array Devices for Continuous Glucose Monitoring: A Review. *Diabetes Technol Ther.* 2013; 15:101–115. DOI: 10.1089/dia.2012.0188 [PubMed: 23234256]
- [11]. Moniz ARB, Michelakis K, Trzebinski J, Sharma S, Johnston DG, Oliver N, Cass A. Minimally invasive enzyme microprobes: an alternative approach for continuous glucose monitoring. *J Diabetes Sci Technol.* 2012; 6:479–80. DOI: 10.1177/193229681200600239 [PubMed: 22538163]
- [12]. Trzebinski J, Sharma S, Moniz Radomska-Botelho A, Michelakis K, Zhang Y, Cass AEG. Microfluidic device to investigate factors affecting performance in biosensors designed for transdermal applications. *Lab Chip.* 2012; 12:348. doi: 10.1039/c1lc20885c [PubMed: 22130554]
- [13]. Caras SD, Janata J. pH-based enzyme potentiometric sensors. Part 3. Penicillin-sensitive field effect transistor. *Anal Chem.* 1985; 57:1924–1925. [PubMed: 4037346]
- [14]. Anzai J, Hashimoto J, Osa T, Matsuo T. Penicillin Sensors Based on an Ion-Sensitive Coated with Stearic Acid Langmuir-Blodgett Field Effect Membrane Transistor. *Anal Sci.* 1988; 4:247–250.
- [15]. Yerien TD, Christian GD, Ruzicka J. Flow injection analysis as a diagnostic technique for development and testing of chemical sensors. *Anal Chim Acta.* 1988; 204:7–28. DOI: 10.1016/S0003-2670(00)86342-4
- [16]. Gao X, Zhen R, Zhang Y, Grimes CA. Detecting Penicillin in Milk with a Wireless Magnetoelastic Biosensor. *Sens Lett.* 2009; 7:6–10. DOI: 10.1166/sl.2009.1002
- [17]. Lee SR, Rahman MM, Sawada K, Ishida M. Fabrication of a highly sensitive penicillin sensor based on charge transfer techniques. *Biosens Bioelectron.* 2009; 24:1877–1882. DOI: 10.1016/j.bios.2008.09.008 [PubMed: 18977651]
- [18]. Wang H, Wang Y, Liu S, Yu J, Guo Y, Xu Y, Huang J. Signal-on electrochemical detection of antibiotics at zeptomole level based on target-aptamer binding triggered multiple recycling amplification. *Biosens Bioelectron.* 2016; 80:471–476. DOI: 10.1016/j.bios.2016.02.014 [PubMed: 26878484]
- [19]. Daprà J, Lauridsen LH, Nielsen AT, Rozlosnik N. Comparative study on aptamers as recognition elements for antibiotics in a label-free all-polymer biosensor. *Biosens Bioelectron.* 2013; 43:315–320. DOI: 10.1016/j.bios.2012.12.058 [PubMed: 23353009]
- [20]. Pikkemaat MG. Microbial screening methods for detection of antibiotic residues in slaughter animals. *Anal Bioanal Chem.* 2009; 395:893–905. DOI: 10.1007/s00216-009-2841-6 [PubMed: 19484227]
- [21]. Huet AC, Fodey T, Haughey SA, Weigel S, Elliott C, Delahaut P. Advances in biosensor-based analysis for antimicrobial residues in foods. *TrAC - Trends Anal Chem.* 2010; 29:1281–1294. DOI: 10.1016/j.trac.2010.07.017
- [22]. Willander M, Khun K, Ibupoto ZH. Metal oxide nanosensors using polymeric membranes, enzymes and antibody receptors as ion and molecular recognition elements. *Sensors (Switzerland).* 2014; 14:8605–8632. DOI: 10.3390/s140508605
- [23]. Ismail F, Adejolu SB. Galvanostatic entrapment of penicillinase into polytyramine films and its utilization for the potentiometric determination of penicillin. *Sensors.* 2010; 10:2851–2868. DOI: 10.3390/s100402851 [PubMed: 22319276]
- [24]. Bi X, Hartono D, Yang KL. Real-time liquid crystal pH sensor for monitoring enzymatic activities of penicillinase. *Adv Funct Mater.* 2009; 19:3760–3765. DOI: 10.1002/adfm.200900823
- [25]. Gonçalves LM, Callera WFa, Sotomayor MDPT, Bueno PR. Penicillinase-based amperometric biosensor for penicillin G. *Electrochem Commun.* 2014; 38:131–133. DOI: 10.1016/j.elecom.2013.11.022

- [26]. Müntze GM, Baur B, Schäfer W, Sasse A, Howgate J, Röth K, Eickhoff M. Quantitative analysis of immobilized penicillinase using enzyme-modified AlGa_N/Ga_N field-effect transistors. *Biosens Bioelectron.* 2015; 64:605–610. DOI: 10.1016/j.bios.2014.09.062 [PubMed: 25314619]
- [27]. Healey BG, Walt DR. Improved fiber-optic chemical sensor for penicillin. *Anal Chem.* 1995; 67:4471–4476. DOI: 10.1021/ac00120a007 [PubMed: 8633784]
- [28]. Siqueira JR, Abouzar MH, Poghosian A, Zucolotto V, Oliveira ON, Schöning MJ. Penicillin biosensor based on a capacitive field-effect structure functionalized with a dendrimer/carbon nanotube multilayer. *Biosens Bioelectron.* 2009; 25:497–501. DOI: 10.1016/j.bios.2009.07.007 [PubMed: 19651505]
- [29]. Cháfer-Pericás C, Maquieira Á, Puchades R. Fast screening methods to detect antibiotic residues in food samples. *TrAC - Trends Anal Chem.* 2010; 29:1038–1049. DOI: 10.1016/j.trac.2010.06.004
- [30]. Rowe AA, Miller EA, Plaxco KW. Reagentless Measurement of Aminoglycoside Antibiotics in Blood Serum via an Electrochemical, Ribonucleic Acid Aptamer-Based Biosensor. *Anal Chem.* 2010; 82:7090–7095. DOI: 10.1021/ac101491d [PubMed: 20687587]
- [31]. Wu X, Kuang H, Hao C, Xing C, Wang L, Xu C. Paper supported immunosensor for detection of antibiotics. *Biosens Bioelectron.* 2012; 33:309–312. DOI: 10.1016/j.bios.2012.01.017 [PubMed: 22317834]
- [32]. Ferguson BS, Hoggarth DA, Maliniak D, Ploense K, White RJ, Woodward N, Hsieh K, Bonham AJ, Eisenstein M, Kippin TE, Plaxco KW, et al. Real-Time, Aptamer-Based Tracking of Circulating Therapeutic Agents in Living Animals. *Sci Transl Med.* 2013; 5:213ra165–213ra165. DOI: 10.1126/scitranslmed.3007095
- [33]. Schoukroun-Barnes LR, Wagan S, White RJ. Enhancing the analytical performance of electrochemical RNA aptamer-based sensors for sensitive detection of aminoglycoside antibiotics. *Anal Chem.* 2014; 86:1131–1137. DOI: 10.1021/ac4029054 [PubMed: 24377296]
- [34]. Han S, Li B, Song Z, Pan S, Zhang Z, Yao H, Zhu S, Xu G. A kanamycin sensor based on an electrosynthesized molecularly imprinted poly-o-phenylenediamine film on a single-walled carbon nanohorn modified glassy carbon electrode. *Analyst.* 2017; :218–223. DOI: 10.1039/c6an02338j
- [35]. Zhang X, Zhang Y-C, Zhang J-W. A highly selective electrochemical sensor for chloramphenicol based on three-dimensional reduced graphene oxide architectures. *Talanta.* 2016; 161:567–573. DOI: 10.1016/j.talanta.2016.09.013 [PubMed: 27769449]
- [36]. Jakubec P, Urbanová V, Med íková Z, Zbo il R. Advanced Sensing of Antibiotics with Magnetic Gold Nanocomposite: Electrochemical Detection of Chloramphenicol. *Chemistry.* 2016; 22:14279–84. DOI: 10.1002/chem.201602434 [PubMed: 27529758]
- [37]. Govindasamy M, Chen SM, Mani V, Devasenathipathy R, Umamaheswari R, Joseph Santhanaraj K, Sathiyam A. Molybdenum disulfide nanosheets coated multiwalled carbon nanotubes composite for highly sensitive determination of chloramphenicol in food samples milk, honey and powdered milk. *J Colloid Interface Sci.* 2017; 485:129–136. DOI: 10.1016/j.jcis.2016.09.029 [PubMed: 27662024]
- [38]. Karthik R, Govindasamy M, Chen SM, Mani V, Lou BS, Devasenathipathy R, Hou YS, Elangovan A. Green synthesized gold nanoparticles decorated graphene oxide for sensitive determination of chloramphenicol in milk, powdered milk, honey and eye drops. *J Colloid Interface Sci.* 2016; 475:46–56. DOI: 10.1016/j.jcis.2016.04.044 [PubMed: 27153217]
- [39]. Ranamukhaarachchi SA, Padeste C, Dübner M, Häfeli UO, Stoeber B, Cadarso VJ. Integrated hollow microneedle-optofluidic biosensor for therapeutic drug monitoring in sub-nanoliter volumes. *Sci Rep.* 2016; 6doi: 10.1038/srep29075
- [40]. Hayat A, Marty JL. Aptamer based electrochemical sensors for emerging environmental pollutants. *Front Chem.* 2014; 2:41.doi: 10.3389/fchem.2014.00041 [PubMed: 25019067]
- [41]. Gorchkov DV, Soldatkin AP, Maupas H, Martelet C, Jaffrezic-Renault N. Correlation between the electrical charge properties of polymeric membranes and the characteristics of ion field effect transistors or penicillinase based enzymatic field effect transistors. *Anal Chim Acta.* 1996; 331:217–223. DOI: 10.1016/0003-2670(96)00185-7

- [42]. Yamanaka K. The electrochemical behavior of anodically electrodeposited iridium oxide films and the reliability of transmittance variable cells. *Jpn J Appl Phys.* 1991; 30:1285–1289. DOI: 10.1143/JJAP.30.1285
- [43]. Cass AEG, Sharma S. Microneedle Enzyme Sensor Arrays for Continuous In Vivo Monitoring. 2017; :413–427. DOI: 10.1016/bs.mie.2017.02.002
- [44]. Roberts JA, Roberts MS, Robertson TA, Dalley AJ, Lipman J. Piperacillin penetration into tissue of critically ill patients with sepsis--bolus versus continuous administration? *Crit Care Med.* 2009; 37:926–933. DOI: 10.1097/CCM.0b013e3181968e44 [PubMed: 19237898]
- [45]. Roberts JA, Udy AA, Jarrett P, Wallis SC, Hope WW, Sharma R, Kirkpatrick CMJ, Kruger PS, Roberts MS, Lipman J. Plasma and target-site subcutaneous tissue population pharmacokinetics and dosing simulations of cefazolin in post-trauma critically ill patients. *J Antimicrob Chemother.* 2014; 70:1495–1502. DOI: 10.1093/jac/dku564
- [46]. Elsen HA, Monson CF, Majda M. Effects of Electrodeposition Conditions and Protocol on the Properties of Iridium Oxide pH Sensor Electrodes. *J Electrochem Soc.* 2009; 156:F1–F6. DOI: 10.1149/1.3001924
- [47]. Hitchman M, Ramanathan S. Thermally grown iridium oxide electrodes for pH sensing in aqueous environments at 0 and 95 C. *Anal Chim Acta.* 1992; 263:53–61. DOI: 10.1016/0003-2670(92)85425-6
- [48]. Ng SR, O'Hare D. An iridium oxide microelectrode for monitoring acute local pH changes of endothelial cells. *Analyst.* 2015; 140:4224–4231. DOI: 10.1039/C5AN00377F [PubMed: 25919982]
- [49]. Bitziou E, O'Hare D, Patel BA. Simultaneous Detection of pH Changes and Histamine Release from Oxyntic Glands in Isolated Stomach. *Anal Chem.* 2008; 80:8733–8740. DOI: 10.1021/ac801413b [PubMed: 18947199]
- [50]. Greenwood, D. *Antimicrobial Chemotherapy*. Fourth Edi. Oxford University Press; Oxford: 2000.
- [51]. Kocsis, B, Szabó, D. Antibiotic resistance mechanisms in Enterobacteriaceae Microbial pathogens and strategies for combating them: Science, technology and education. Mendez-Vilas, A, editor. Formatex; 2013. <http://www.formatex.info/microbiology4/vol1/251-257.pdf>
- [52]. O'Hare, D. *Biosensors and Sensor Systems Body Sensor Networks*. Yang, G, editor. Springer; 2014.
- [53]. Trouillon R, Combs Z, Patel BA, O'Hare D. Comparative study of the effect of various electrode membranes on biofouling and electrochemical measurements. *Electrochem Commun.* 2009; 11:1409–1413. DOI: 10.1016/j.elecom.2009.05.018
- [54]. Bartlett, PN, Toh, CS, Calvo, EJ, Flexer, V. *Modelling Biosensor Responses Bioelectrochemistry*. John Wiley & Sons, Ltd; Chichester, UK: 2008. 267–325.

Figure 1. Anodically deposited iridium oxide film pH calibration

1a. Comparison on iridium oxide pH calibration on separate microneedle devices

1b. Comparison on iridium oxide pH calibration on different stages of biosensor fabrication

Figure 2. Calibration of microneedle biosensors

2a. Calibration curve comparison for microneedle runs with penicillin-G fitted with the Hill equation

2b. Log(concentration) plots of data from penicillin-G calibration

2c. Comparison of results for platinum disc electrode based beta-lactam sensor calibrated against penicillin-G, amoxicillin, and ceftriaxone.