Electrochemical Biosensors – A Nexus for Precision Medicine

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Teaser: Electrochemical biosensors are cheap, rapid, portable and sensitive – ideal for point-of-care drug monitoring.

Abstract

Precision medicine is a field with huge potential for improving patient's quality of life, wherein therapeutic drug monitoring (TDM) can provide actionable insights ~~using the drug concentration in the body to optimise dosing regimen~~. More importantly, incorrect drug dose is a common contributor to medical errors. ~~More importantly, incorrect drug dose is a common contributor to medical errors.~~ However, ~~C~~current TDM practice ~~relies on~~is time-consuming, expensive and requires ~~procedures, performed by~~ specialised technicians ~~with expensive equipment~~. One solution is to employ electrochemical biosensors (ECBs), which are inexpensive, portable and ~~possess~~ highly sensitive~~ity~~. In this review, the potential for ~~electrochemical biosensors~~ECBs as a technology for on-demand ~~TDM~~ drug monitoring is explored, including microneedles, continuous monitoring, synthetic biorecognition elements and multi-material electrodes. The review also highlights emerging strategies to achieve continuous drug monitoring, and concludes by appraising recent developments and providing an outlook for the field.

1. Introduction

Precision medicine is a ~~contemporary~~ modern strategic approach for patient management, which sees patients as individuals rather than part of the wider demographic. It uses a holistic approach to therapy, in which data~~,~~ analysis and technologies are central to decision making. ~~-~~ The transition away from the traditional "one-size-fits-all" approach towards personalised therapy gained increasing traction following the implementation of the 2015 Precision Medicines Initiative in the USA. Indeed, recent years have witnessed a growing interest in employing emerging technologies, such as 3D printing and Artificial Intelligence, to support this goal. For instance, the digital pharmacy era could see the combination of digital prescriptions, 3D printing, and novel point-of-care diagnostic and/or drug monitoring strategies to form a continuous, dynamic system of drug prescribing [1]. When coupled to the Internet-of-Things, a decentralised healthcare system can be realised, improving healthcare access and reducing the~~This decentralised style is key to the future of healthcare, through the reduced~~ burden on medical professionals.

As alluded, a key area of precision medicine is a means of measuring the patient's drug levels to modify therapy accordingly. At present, ~~One key area of precision medicine is~~this is achieved through

therapeutic drug monitoring (TDM). TDM is the clinical practice of measuring the concentration of a therapeutic agent in a patient's blood to optimise the dosing regimen. Typically, a clinical expert would use the measurement from TDM to improve the treatment, and thus clinical outcome, ~~for~~of the patient [2]. Apart from optimising efficacy, TDM is critical in ensuring that serum concentrations are kept under toxic levels, protecting patients from potentially fatal adverse effects.

When it comes to improving clinical outcomes, TDM can play a crucial role. Firstly, TDM can help with narrow therapeutic index drugs. Narrow therapeutic index drugs are those with only a small window of effective dosing between under dosing and adverse toxic effects [3]. For such drugs, TDM can help to ensure the concentration within the body is in the therapeutic window, optimising the Time in Therapeutic Range (TTR) and thus helping to improve patient outcomes. Another benefit of TDM is to help reduce inter-patient variability; exposure to a drug can vary depending on physiological, intrinsic and environmental factors, meaning the resultant response can be highly variable [4]. Currently, bioavailability and consequently treatment response is determined using the drug dose given, which is now known to be inefficient. Inter- and intra-patient variations, such as sex differences [5], expression of metabolic enzymes~~–~~ [6]~~[5]~~, and the time of administration [7]~~[6]~~ have been recognised as critical factors that influence a drug's pharmacokinetics and pharmacodynamics.

In addition, TDM can help to reduce antibiotic-resistant strains of bacteria from forming due to exposure to sub-optimal doses [8], which will also reduce the overall costs of healthcare [9,10]. Indeed, TDM not only offers improvements to patient mortality rates, but also economic benefits. TDM may also be suitable for measuring compliance, assessing the effects of changing dosage regimen, monitoring drug-drug interactions, evaluating the effect of changes in the clinical state of the patient, and looking for signs of toxicity [11]. However, these benefits may only currently be present for certain high-risk groups of patients [10]. Therefore, there is a need for low-cost TDM technologies to allow all patients to reap these benefits. Potential drugs candidates that would benefit from TDM include anticonvulsants, cardiac glycosides, psychotropic medications, mood active drugs, immunosuppressants, antineoplastic and anti-infectives [12].

Current TDM practices rely on serum measurements, in combination with relevant physiological signs such as electrocardiograms, blood pressure, other analyte concentrations, organ function and international normalised ratio [13]. However, the majority of serum measurements require invasive sampling of blood in combination with chromatographic or immunoassay-based techniques, which are time-consuming, expensive, lack standardisation and require a technician to operate [12,14]. As a result, the true potential of TDM is not being realised.

~~Indeed, novel means of measuring and monitoring drug serum concentrations is necessary to optimise patient outcomes.~~ An alternative is the use of sensors to monitor drug concentration in the body. Sensors are devices which detect, measure or respond to a change in a physical property. ~~Sensors~~They are ubiquitous across society, where they are used to automate processes, monitor chemicals in hazardous environments and other dangerous or hard-to-reach areas. In the healthcare sector, sensors are already deployed in hospitals for monitoring the patients' vital signs, including blood pressure, heart rate, body temperature and oxygen saturation (SpO_2). ~~Recently, there is increasing interest in using sensors to optimise other clinical practices, such as therapeutic drug monitoring (TDM). TDM is the clinical practice of measuring the concentration of a therapeutic agent in a patient's blood to optimise the dosing regimen. Typically, a clinical expert would use the measurement from TDM to improve the treatment, and thus clinical outcome, for the patient [1]. TDM may also be suitable for measuring compliance, assessing the effects of changing dosage regimen, monitoring drug-drug interactions, evaluating the effect of changes in the clinical state of the patient, looking for signs of toxicity or a lack of response to therapy, and can also be used if toxic doses and disease states are similar [5]. Potential drugs candidates that would benefit from TDM include anticonvulsants, cardio-active drugscardiac glycosides, respirator-acting drugs, psychotropic medications, mood active drugs, immunosuppressants, antineoplastic and anti-infectives [6].~~

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~~When it comes to improving clinical outcomes, TDM can play a crucial role. Firstly, TDM can help with narrow therapeutic index drugs, such as some of those mentioned earlier. Narrow therapeutic index drugs are those with only a small window of effective dosing between under dosing and adverse toxic effects [9]. For such drugs, TDM can help to ensure the concentration within the body is in the therapeutic window, optimising the Time in Therapeutic Range (TTR) and thus helping to improve patient outcomes. Another benefit of TDM is to help reduce inter-patient variability; exposure to a drug can vary depending on physiological, intrinsic and environmental factors, meaning the resultant response can be highly variable [10]. In addition, TDM can help to reduce antibiotic resistant strains of bacteria from forming due to exposure to sub-optimal doses [11], as well as reduce the overall costs of healthcare [12,13]. Indeed, TDM has been shown to be of both economic benefitnot only offers improvements to patient and improve mortality rates in patients, but also economic benefits.~~

However, , these economic benefits may only curently be present for certain high-risk groups of patients [13]. Therefore,, and so there is a need for lower low-cost cost TDM technologies to allow all patients to reap these benefits.

One such technology that could help to realise the full benefits potential of TDM is electrochemical biosensor (ECB). In particular, electrochemical biosensors (ECB) could help to realise the full potential of TDM is electrochemical biosensor (ECB). A biosensor can be defined as a device which measures a biological or chemical reaction by generating a signal which is related to the concentration of the analyte in the reaction [15]. ECBs are biosensors in which the reaction can be measured through changes in electrical properties [16]. They have already been widely adopted in other scientific fields, such as for glucose detection [16,17] [17,18], environmental monitoring [19,20], gas detection [21], pathogen detection [22], and disease diagnosis [22-26]. ECBs are now available commercially [27,28]. However, despite their success in similar fields, ECBs have not yet reached clinical or commercial success for TDM.

In this review, ECBs as a technology for improving the process of TDM is discussed, with particular detail focus on the studies which show the greatest promise for advancing the field and reaching clinical applications. In addition, the future of ECBs is evaluated, including potential roadblocks to translating the technology to clinical settings.

2. Techniques and Background

Electrochemistry relates electrical quantities to chemical processes. A chemical reaction where a set number of electrons are exchanged is called a redox reaction. The redox reaction only occurs at a specific electrochemical potential (measured in volts), which is dependent on the species involved. This quantity is called both voltage and potential.

Electrochemical measurements evaluate the number of redox reactions that occur in a given system. Each redox reaction occurs at a particular electrochemical potential, and involves the transfer of a set number of electrons. For example, Equations (1) and (2) show a redox reaction involving an oxidising agent gaining n electrons and a reducing agent losing m electrons;

$$Ox + n \cdot e^{-} \rightarrow Ox^{n-}, \quad (1)$$

$$Red^{m-} \rightarrow Red + m \cdot e^{-} \quad (2)$$

This reaction will only occur at a specific potential (measured in volts); the potential that this occurs at is dependent on the species involved. If an electrode is close to one of these species and is at that particular potential, it will transfer electrons with the species.

An electrochemistry experiment typically consists of a solution, electrodes, and a potentiostat, as shown in Figure 1A. The electrodes are used to donate or receive electrons from the redox-active species. The rate of this electron transfer gives the current~~, which is what is measured~~ (measured in amperes). The ratio of the potential and current is the impedance, which gives the total resistive effect of the circuit in response to an applied alternating potential.

~~Typically, an electrochemistry experiment consists of a solution, electrodes, and a potentiostat, as shown in Figure 1A. Most commonly~~Generally, three electrodes are used: (1) ~~;~~a working electrode (WE), where the reaction of interest and measurement takes place~~;~~ (2) a reference electrode (RE), ~~which~~ that provides a stable potential against which the potential of the other electrodes can be measured~~;~~ and (3) a counter/auxiliary electrode (CE), which completes the circuit with the WE [27]. The WE is normally made from Platinum, Silver, Gold or a form of Carbon. The choice of electrode material is important as this will affect critical factors including the corrosion resistance, stability, ability to be functionalised, conductivity, cost and biocompatibility [28]~~., all of which are important factors. The CE is often made from the same material, but must have a higher surface area so as not to be the limiting step in the reaction.~~ The CE is normally made from an inert, conducting material. The RE is ~~normally~~ commonly Ag/AgCl, which has a low half-cell potential, low impedance and ~~can easily be manufactured~~ easy manufacturability [29]. Commonly, electrodes require pre-treatment and polishing to remove contaminants and improve the reproducibility.

The solution contains the ~~compound~~ analyte of interest, but must also have additional ions to allow charge to flow between the WE and CE. ~~Finally, the potentiostat is connected to a~~All three electrodes are connected to the potentiostat, ~~and~~which controls the electrical parameters at the WE ~~as well as~~and measures the response. Electrochemical measurements can also be made using Field-Effect Transistor (FET) biosensing, where the conductance across a region between electrodes interacts with the target analyte to give a measurable signal [30,31].

In order to improve the sensitivity (how much the signal changes in response to a change in the analyte concentration), selectivity (how well the sensor detects the analyte instead of interferents), reproducibility (the extent to which different identically manufactured sensors in the same solution give the same concentration of the analyte) and ~~stability~~ reusability (the ability of the sensor to be used multiple times) of ~~a signal~~the system ~~from a reaction~~, the electrodes can be modified with biorecognition elements and nanostructures.

2.1. ~~Biorecognition Elements~~Modifications

An assortment ~~variety~~ of biorecognition elements (BRE) exist, and~~. These~~ can be classified as conformation-changing, redox reaction and binding-only molecules. ~~[32]~~

~~Binding-only molecules -~~Figure 1 A) Illustration of a typical 3 electrode setup. B) Example of a conformation changing BRE, where the DNA sequence binds to the analyte, causing it to fold. C) Demonstration of a redox reaction BRE, where the enzyme catalyses a reaction which produces a redox active molecule (the yellow circle). D) Representation of a binding-only BRE, where the antibody binds to the analyte. The images of antibodies, molecules (triangles), enzymes, electrodes, and aptamers used in these figures are from Servier Medical Art licensed under the Creative Commons Attribution 3.0 Unported Licence ~~D. These are the elements, such as molecularly imprinted polymers (MIP) and antibodies, to which the target analyte binds. This binding can be detected, often through the corresponding increased steric blocking of redox active species to the electrode.~~

When ~~C~~c**onformation-changing molecules** ~~-~~(~~-~~Figure 1B)~~,:. The~~ bind~~ing~~ to~~of~~ ~~such a~~ the analyte, ~~species~~ molecule ~~(usually an aptamer) to the target analyte causes~~ the ~~species to change shape~~molecule's structure ~~to~~ changes. ~~These are often used with a redox probe~~These molecules, which are usually aptamers, ~~are often conjugated~~can also be used to conjugate ~~to~~ a redox probe~~, which is;~~ (a compound ~~which~~ that can readily undergo a redox reaction by transferring an electron with the electrode). Attaching a probe to the end of an aptamer means that when it changes shape, the electron transfer ~~coefficient~~ rate~~(ease with which the electrons can move from the probe to the electrode)~~ change~~s. This is reflected by a change in the maximum current amplitude, which can be measured. ~~and monitored.s, and it is this change which is measured at the electrode.~~

Redox reaction molecules ~~-~~(~~-~~Figure 1~~Figure 1B~~C)~~:. Here,~~T ~~the elements~~, such ~~and~~ as enzymes or a redox~~-~~reactive species, cause a redox reaction to occur at a set potential. Th~~is reaction~~ese electrons can be directly measured by the electrodes.

Binding-only molecules (~~-~~Figure 1D)~~: These~~ are ~~the~~ elements, such as antibodies or molecularly imprinted polymers (MIP) ~~and antibodies~~, ~~to~~ which the ~~target~~ analyte binds to. The binding event reduces the access of redox active species to the electrode, thereby hindering electron transfer.~~The binding event reduces the access of the redox active species to the electrode, thereby hindering electron transferThis binding can be detected, often through the corresponding increased steric blocking of redox active species to the electrod.e.~~

The choice of biorecognition element depends on the particular analyte and matrix; in each instance there will be a trade-off between selectivity ~~(how well the sensor detects the analyte instead of interferents)~~, sensitivity ~~(how much the signal changes in response to a change in the analyte concentration)~~, reproducibility ~~(the extent to which different identically manufactured sensors give~~

~~the response to the same concentration of analyte)~~ and reusability ~~(how many times the sensor can be used without losing sensitivity, selectivity or reproducibility)~~ [32,33]~~[33]~~.

As well as ~~using a suitable technique~~BREs, the signal can be improved through modifying the electrode. One such modification is to incorporate a barrier or surface alteration to protect against interfering species, increase the linear range of enzymatic reactions and improve the biocompatibility of the sensor [34]. Other common ~~examples of~~ surface modifications include the addition of metallic, carbonic or conducting polymer nanomaterials, graphene/graphene oxide ~~[35], nanoparticles/ nanostructures [36], multi-walled~~ carbon nanotubes ~~[37]~~, and quantum dots [35]~~[38]~~. Alternative nanostructures, such as electrospun nanofibers, can also be used [36]. Monolayers can be formed on the electrode surface to allow for BRE functionalisation and prevent non-specific adsorption [27].

2.2. Electrochemical Techniques

There are a ~~range~~ selection of ~~different~~ electrochemical techniques that can be used to measure the ~~concentration of a compound~~redox reactions occurring.~~;~~ ~~t~~ These techniques can be categorised into groups; **amperometry**, in which the potential is held constant and the current is measured as a function of time; **voltammetry**, where the current is measured in response to different potentials; **coulometry**, in which the total charge accumulated at the electrode is measured as a function of time; **potentiometry**, where the potential difference between two electrodes is measured; and ~~conductometry, in which the conductivity (ease with which current can pass through) of the solution is measured;~~ **impediometry**, where both the total ~~resistive and reactive components of~~ impedance ~~are the solution are~~of a circuit ~~are~~ is measured in response to a small voltage signal.~~n alternating current [15].~~

~~Some of the most common techniques are;~~Perhaps the most common technique is

Cyclic Voltammetry (CV), where the ~~monitors the response in current to changes in potential. The~~ potential is 'swept' forward and back between ~~2~~ two points and the current response is measured with respect to this potential.~~, either linearly or in a staircase wise manner. It has the advantage that~~While ~~it is quick and simple to perform, however, the detection limit of CV is much lower than~~ that oft ~~other techniques, largely due to background reactions such as double layer charging and redox surface processes~~ [15] ~~[40].~~ In addition,

Square Wave Voltammetry (SWV), and **Differential Pulse Voltammetry (DPV)** are both commonly used voltammetric techniques. These use staircase increases in the potential, but with a square wave or short potential pulse, respectively, superimposed at each 'step'.~~ also monitors the response in current to changes in potential. However, in SWV, the potential is changed in a staircase~~

manner with a square wave superimposed at each 'stair'. Theseis reduces the effects from double-layer charging and so can detect downthe detection limit for to lower concentrations of the analyte is lower. It is also fast, making it good for reversible and quasi-reversible processes but not suitable for slow kinetics [36].[41,42].[41,42].In addition, **Stripping Voltammetry (SV)** can be used. This is a two-step process involving electrolytic deposition followed by stripping of the deposition. The current is measured during the deposition.

- **Differential Pulse Voltammetry (DPV)** is very similar to SWV, except that rather than a square wave being superimposed at each stairstep, it is a short potential pulse. This also has a similar reduction on double-layer charging and non-faradaic effects, but is generally much slower than SWV [36].

One frequently used impediometric technique is **Electrochemical Impedance Spectroscopy (EIS)**, which looks at the effect of on a current from changing the frequency of a small, sinusoidally varying potential. This gives both a resistive and reactive component. This has the advantage that equivalent circuit models can be built from the data, however, this impedance is dependent on the ionic strength of the solution and so is not applicable in samples where this varies greatly. On top of this, because of the time required to sample at a suitable number of frequencies, it can be slow.

- **Stripping Voltammetry (SV)** is a two-step technique, with electrolytic deposition followed by stripping of the deposition, where the current is measured against potential. The electrolytic deposition concentrates the analyte to give higher sensitivity [34].

Further, one common amperometric technique is **Chronoamperometry**-, which measures the change in current with over time at a fixed potential. For interested readers, furtherMore in-depth information on these techniques and others is available fromin the referenced articles [16,27]Bard and Faulker, and Grieshaber et. al.. It is especially applicable to enzymatic reactions. As a technique, it provides a fast response [15].

3. **Open circuit potential** is the difference(OCP) measures the difference in potential between the WE and RE with no current flowing between them. It is a simple and quick measurement to make, but is prone to noise.

4.3. Recent Work

The clinical success of electrochemical biosensors for detecting glucose has encouraged the use of ECBs for monitoring drug. Research has been undertaken to develop a similar point-of-care technology for TDM. , which Aa n updated list of relevant examples is enumerated in ***Table 1***. In

addition, a selection of the salient developments is discussed in more detail. A long-term aim of ECBs is to employ the technology in clinical environments, and hence studies that provided *in vivo* data were prioritised for this review. A secondary objective is to highlight recent innovations in achieving a continuous TDM. Continuous measurements can provide real-time monitoring of drug concentrations, particularly for drugs wherein their concentration fluctuates as a function of time. Continuous TDM ~~are~~ is valuable for taking proactive measurements to ensure patient safety.

~~4.1.~~3.1. Microneedles-based Electrode Design

Microneedles are devices comprising of micron-sized needles, typically arranged on a small patch. The needles are able to penetrate through the stratum corneum and epidermis~~,~~ but is unable to reach the underlying dermis layer. As pain receptors reside deep inside the dermis, microneedles are painless. In this way, microneedles take the best attributes of transdermal patches and hypodermic needles (i.e. painless and effective transdermal delivery respectively), while overcoming their shortcomings [57]. Extensive research on microneedles have been focused on their potential as novel transdermal drug delivery systems [57]. However, recent research ~~have~~has explored using hypodermic microneedles to sample interstitial fluid (ISF) for ex vivo analysis [58] and solid microneedles for continuous glucose monitoring [59]. Given that ISF has been recognised as a possible medium to provide reliable measurements of drug concentrations, solid microneedle arrays have been explored as novel strategies for continuous therapeutic drug monitoring [60].

~~There are a number of studies of~~Several microneedle-based ECBs ~~which~~ have successfully transitioned beyond *in vitro* experiments~~the laboratory~~ and onto *in vivo* testing. ~~For example, One of these is the measurement of~~ For example, ~~P~~phenoxymethylpenicillin, a common antibiotic, was successfully quantified ~~measured~~ in extra-cellular fluid (ECF) using microneedles and by measuring open-circuit potential. ECF is comprised of both plasma and ISF. Open circuit potential (OCP) measures the difference in potential between the WE and RE with no current flowing between them. ~~to make measurements in the extra-cellular fluid (ECF)~~ (Figure 2A~~C~~) ~~- Figure 2C.~~ The microneedles were made of polycarbonate, layered with g~~G~~old ~~or~~ and s~~S~~ilver for the WE ~~or~~ and RE respectively. The WEs were further layered with ~~I~~iridium ~~O~~oxide (for detecting local pH changes from the hydrogen released in the enzyme reaction), functionalised β-lactamase hydrogel, poly(ethylenimine) ~~-~~and PEG-DE (Figure 2A~~,~~ B) [61]~~, Figure 2A,B~~. A control electrode was used to correct for drift. Patients received five 500 mg doses of ~~P~~phenoxymethylpenicillin every 6 hours, with the final dose taken at the start of the measurements. The open~~-~~-circuit ~~-~~potential was measured between the WE and RE over a 4-hour period, and measurements were compared to that obtained from microdialysis of ECF and high-

performance liquid chromatography-mass spectrometry (HPLC-MS) of blood samples ~~(~~(Figure 2C~~D~~). The microneedle and microdialysis results were seen to give a similar trend for concentration but, as shown in Figure 2D~~E~~, the correlation was just R^2 = 0.15. The pharmacokinetic parameters obtained from the three~~3~~ measurements showed that, ~~to~~with a 95% confidence interval, the maximum concentration (C_{max}) and time to C_{max} (T_{max}) were different between the microneedles and microdialysis. ~~, but~~ However, the area-under-curve measurements did not show statistical difference between all three sampling methods, and T_{max} was not statistically different~~ce~~ between microneedles and free-~~-~~blood. While the results from this study are ~~mixed~~not conclusive, ~~this~~ it nonetheless demonstrates the potential for biosensors to be used in continuous, minimally ~~-~~invasive, real-~~-~~time monitoring [39,61]. This is being taken further by using it in a clinical trial for closed-loop control [62,63].

~~As well as for detecting Phenoxymethylpenicillin~~Additionally, microneedles have ~~also~~ been used to detect methyl paraoxon, an organophosphate used for treatment of glaucoma. The microneedles ~~(Figure 3A)~~ were made from an acrylate-based polymer, with carbon paste electrodes for the WE and CE, and Ag/AgCl modified carbon paste electrodes for the RE. Organophosphorus h~~H~~ydrolase enzyme was immobilised onto the WE. SWV was used for electrochemical detection. After optimisation, the electrodes were able to detect paraoxon over a linear range of 20 to 180 µM, with a limit of detection (LOD) of 4 µM. ~~(Figure 3B).~~ The microneedles were then tested *ex vivo* on mice at 50, 100 and 150 µM, ~~which~~ and gave recoveries of between 84 and 93 %. It was suggested that the lower recoveries is ~~a result of~~due to the more viscous fluids in the skin compared to the ~~solution calibration~~calibration solution. However, this does still demonstrate the potential of microneedles for measurement of paraoxon [56].

~~4.2.~~3.2. Closed-loop for Monitoring and Dosing

Unsurprisingly, continuous monitoring is meaningless without prompt corrective action. The time-consuming process of manual dose calculation will invariably lead to delays in corrections to the drug serum concentrations. This will reduce the mean TTR, although still higher than without TDM entirely. Therefore, to maximise the TTR and optimise drug treatment, several research groups have developed closed-loop drug monitoring and delivery systems. These are capable of delivering feedback doses of drug calculated using a pre-programmed mathematical algorithm in response to the measured drug serum concentrations.

One key example is a biosensor developed for the *in vivo* measurement of ~~V~~vancomycin, a glycopeptide antibiotic. Vancomycin is a good target for therapeutic drug monitoring. ~~because i~~It has a ~~small~~ narrow therapeutic window ~~[11]~~, high inter-patient~~individual~~ variability, and trough plasma

concentrations of ~~troughs in the plasma concentration~~ <10 mg/L can give rise to bacteria strains with characteristics similar to that of vancomycin-intermediate *S. aureus* strains [8]. ~~resistant-like characteristics.~~

The sensor used was built from ~~3~~ three thin wires. The WE was a gold wire~~,~~ ~~with the WE being gold~~ functionalised with a methylene blue redox reporter attached to a structure-switching aptamer (~~–~~ Figure 3~~4~~A). The sensor was tested *in vivo* by inserting the sensor and an infusion line into the jugular veins of a live rat. Using SWV, the sensor produced ~~this showed~~ high precision measurements of patient-specific pharmacokinetic parameters, ~~with~~ and measurements of ~~key~~ elimination time constants fitted with a precision of ~~fitted to~~ better than 20% (error at 95% confidence interval of fitted trace). The sensor outputs were then used to drive a controller for the infusion rate (Figure ~~4~~3B). This ~~which~~ achieved the target plasma concentration rapidly and stably for more than 5 hours (~~–~~ Figure ~~4~~3C), despite changes in drug elimination rate (~~,~~ Figure ~~4~~3D)~~.,~~ ~~demonstrating~~ This example highlighted the effectiveness of ECBs for closed-loop delivery and continuous therapeutic drug monitoring [38].

In another study, continuous *in vivo* drug monitoring and closed-loop feedback was achieved using ~~Another study also managed to achieve *in vivo* continuous monitoring and closed-loop dosing; here,~~ an electrochemical detector ~~was~~ combined with a microfluidic device. Like the aforementioned example, a gold ~~working electrode~~WE~~The WE was gold~~ functionalised with a methylene blue redox reporter attached to a structure-switching aptamer was used ~~(– Figure 5A)~~. A catheter was connected to the microfluidic device via a silicone tubing. This permitted ~~Tubing was used to connect a catheter to the microfluidic device, allowing for~~ a continuous flow of blood from the animal to mix with a buffer solution and flow to the electrodes ~~(– Figure 5B)~~. This was used to measure ~~D~~doxorubicin; a chemotherapeutic drug with high ~~variability, clinically meaningful differences in pharmacokinetics between individuals~~ inter- and ~~even~~ intra-~~individual~~ patient variations in pharmacokinetic parameters over the course of a treatment [64]. The readings from the sensor ~~was~~ were deployed to control the drug dosing ~~of~~ in live, conscious rabbits. A concentration within 20% of the set point therapeutic concentration was maintained, and specific dosing profiles were achieved ~~(Figure 5D)~~. This outperformed the clinical standard for dosing, based on body surface area ~~(, shown in Figure 5C)~~. Drug-drug interactions were measured with co-administration of cisplatin, a drug ~~(which is~~ known to extend the half-life of ~~D~~doxorubicin.~~);~~ T~~t~~he sensor-controller achieved time-in-range of 97% compared to 34% for traditional dosing ~~(Figure 5E, F)~~. When the system was tested in anaesthetised rats, and a mathematical model of the control system achieved similar results to ~~that~~those with rabbits ~~(Figure 5G, H)~~, despite differences in weight, blood volume and clearance time [65].

~~4.3.~~3.3. Molecular Imprinted Polymers

Other investigations have used molecular imprinted polymers (MIPs) instead of aptamers or enzymes as the biorecognition element~~; one of the more advanced studies measured the chemotherapeutic immunosuppressant cyclophosphamide~~. MIPs are synthetic polymers with a predetermined selectivity for a given analyte, and can enrich the electrode with high selectivity and specificity. Compared to traditional enzyme/antibody based biorecognition layers, MIPs are inexpensive, simple to prepare, exhibit good stability and are less susceptible to environmental factors. Furthermore, the production of MIPs does not involve animals [66].

Huang et al. (2017) quantified cyclophosphamide by us~~ed~~ing a gold WE modified with n~~N~~itrogen and s~~S~~ulfur co-doped activated graphene and an MIP layer~~—~~ (Figure ~~6~~4A). The measurements were made by immersing the sensor in the analyte-containing solution for 10 mins, then washing with distilled water and performing CV in ferri/ferrocyanide solution. Binding of cyclophosamide to the MIP gave increased blocking for the ferri/ferrocyanide molecule, reducing the rate of reduction and so changing the peak current. ~~increased impedance for the ferricyanide molecule to reducing at the electrode, and so a change in the peak current.~~ ~~From~~ T~~t~~his achieved~~,~~ an LOD ~~limit of detection~~ of 3.4 pM and linear range of detection of 8 pM – 800 nM ~~were measured~~(~~,~~ Figure ~~6~~4B). This also performed well in spiked rabbit blood samples. When a rabbit was dosed at 12 mg/kg, results obtained from ~~it was found~~ the biosensor ~~gave~~ was found to be similar ~~results~~ to that from HPLC (~~,~~ Figure ~~6~~4C). Interestingly, the sensor was found to possess ~~, but was able to detect to lower concentrations~~greater sensitivity than HPLC (~~, as shown in~~ Figure ~~6~~4D). However, sample pre-treatment (dilution in methanol and centrifugation in this case) is necessary, ~~the sample pre-treatment steps of diluting in methanol and centrifuging show~~ suggesting that MIPs are not as developed as aptameric biosensors [43].

~~MIPs as mentioned can be modified to obtain the desired property.~~ Stoi~~a~~a~~i~~n et al~~.~~. (2020) recently explored MIP for detecting ~~A~~azithromycin (AZI), where they demonstrated a simple approach ~~to~~ for synthesising MIPs~~.~~ ~~[67].~~ Following mechanical polishing of the glassy carbon electrode, the electrodes were placed in a container with the reagents needed for synthesising the MIP. These reagents include ~~, which included~~ a crosslinker, the functional monomer, and the analyte of interest, AZI. Subsequently, a voltage was applied to the system, where the MIP were synthesised via electropolymerisation. This synthesis procedure is indeed rapid, wherein the process took less than five minutes. Moreover, the electropolymerisation is obtained with the same instrument used for electrochemical sensing; the benefit of which is that both laboratory footprint and capital cost are minimised. Excess reagents were rinsed under acidic conditions~~, which~~ to release~~d~~ AZI molecules, leaving behind an imprinted site for selective re-binding. This facile approach to producing a highly selective biorecognition layer will indeed expedite research [67].~~.~~

~~4.4.~~3.4. Multi-Material Biorecognition Layer

In addition to design consideration, the choice of material is also an important factor for electrode design. Aptamers in particular are becoming popular~~,~~; ~~and~~ as evidenced by **Table 1**, these have been applied to different active pharmaceutical ingredients. Interestingly, innovative approaches ~~are~~ include combining aptamers with other synthetic materials, primarily carbon allotropes. Qin et al. (2016) produced a novel label-free method for detecting kanamycin [41]. The study combined aptamers with a porous platinum-copper alloy and a functionalised graphene. The alloy served to immobilise the aptamers onto the electrode surface through the -NH_2 group of the aptamer, ~~,~~ ~~W~~whereas~~,~~ the functionalised graphene served to facilitate conductivity. The resultant combination of aptamer-alloy-graphene yielded an electrode with both a lower detection limit and a wider linear response range. ~~The~~ ~~R~~recent studies indicate there is a potential for a multi-material combining aptamers with other materials. Interestingly, the use of multi-materials is actively being pursued by the three-dimensional (3D) printing field. One can envisage~~s~~ a streamline electrode functionalisation approach using 3D printing.

El-Weiki et al. (2020) also employed a multi-material approach to achieving an ultrasensitive electrode for detecting oxaliplatin, where [60]~~. For their study, which was validated~~ validation was done using~~in~~ both human serum and urine samples~~,~~. ~~t~~The electrode was constructed using reduced graphene oxide~~,~~ and multi-walled carbon nanotubes ~~that were~~ loaded with both gold and platinum nanoparticles. Interference studies in the presence of other platinum-based chemotherapeutic agents revealed that the voltagram for oxaliplatin was unchanged~~.~~ [53].

It is worth remarking that the use of aptamers can yield low-cost electrodes. Whilst care should be taken when studies claim to be lost cost [68]~~,~~ ~~–~~ ~~T~~this will be a welcome addition to healthcare institutes given the economic climate faced by many institutes across society. An example of this was demonstrated by Roushani and Shahdost-fard (2019), who constructed an electrode using aptamers, gold nanoparticles and quantum dots [46]. The authors noted that the composited approach produced a synergistic effect, and recorded a LOD of 33.33 aM for ibuprofen. A value in the atto-molar range is unprecedented for ibuprofen when compared to analytical techniques~~,~~ such as HPLC, colorimetric and other electrochemical biosensor studies, as noted in the study. It is also worth echoing the authors' claim that, despite the use of multiple materials, ~~that~~ the fabrication process is both a facile and green approach to producing an ultrasensitive electrode. Interestingly, the study relied on quantum dots, which are a recent material discovery~~.~~, ~~and~~ This is illustrative of how emerging technologies in an allied discipline, such as material science and engineering, could advance ECB innovation.

Ilkhani et al. (2016) combined Ssurface-Eenhanced Rraman Sspectroscopy (SERS) with electrochemical biosensors for both screening chemotherapeutic drugs and their effect on DNA samples. [62]. Whereas most studies in the field focus solely on drug detection, the authors investigated the feasibility of simultaneously monitoring drug and pharmacological activity. Incidentally, the electrode surface comprised of carbon allotropes, and metallic nanoparticles. Since SERS is a non-destructive technique, it also allows for continuous measurements without affecting the bioanalytes [52].

4.5.3.5. Summary of Recent Developments

The examples detailed in this section demonstrate the recent progress made in TDM using ECB. More specifically, it concentrates on the modifications performed onto the electrode to overcome challenges, such as low sensitivity or poor stability. Disclosed herein are examples of novel methodologies that combined multiple materials to collectively improve electrode performance. In addition, innovative designs are used to achieve continuous drug monitoring. The recent developments highlights the potential for achieving a closed-loop system for precision medicine. In addition to TDM, Ultimately, the beneficiaries of the study, in addition to TDM, will ultimately include environmental monitoring, anti-counterfeiting, and food quality. In addition, the simplicity of ECB could replace complex standard laboratory analytical techniques that are complexcomplex, such as HPLC. These characterisation techniques also tend to be. HPLC is a delicate characterisation technique that isand consequently susceptible to machine downtime. Therefore, ECBs could affordprovide reliability and considerable time-savings that will accelerate research.

5.4. Future Outlook and Perspectives

ECBs have a number of strengths and benefits for their use. With increased further research and study, EBCs are poised to become an important tool in the management of disease and therapy. However, there are also a number ofseveral challenges that need to be overcome and criterion met.

5.1.4.1. Advantages of ECBs

ECBs possess an assortment of advantages making them particularly applicable to TDM. Firstly, their they possess fast measurement and analysis times, and do not along with no necessity forobviating incubationrequire incubation in most cases. Hence, means a reading can be taken more rapidly than other biosensingsimilar techniques, such as chemiluminescence [69]. This is especially important for the continuous monitoring of drugs with fast clearance times of the drug; the response time must be sufficiently quick to resolve these timescales [65]. In addition to this, tThe capability

ability to perform continuous monitoring ~~means~~ enables these biosensors to be ~~can then be~~ used in closed-loop monitoring, where the measurement is used to adjust the dosing regimen, giving better control over the serum concentration and patient outcomes.

With a wide~~The~~ variety of electrode materials, biorecognition elements, modifications and the potential to be combined with additive manufacturing, ~~mean~~ ECBs have the ~~capability potential~~ capacity to be highly personalised and customisable [70]. This supports their use in clinical settings, with one potentiostat unit able to measure multiple different drugs ~~and~~ for various disease states by using different electrodes, designed for each situation. In addition, adjusting the size and shape of the electrode would allow for healthcare to move away from the 'one-size-fits-all' approach and instead be modelled on the patient, conceivably improving compatibility. Further, the combination of TDM and additive manufacturing would allow for complex electrode design to further increase the capability of the sensors [71].

Finally, the reduced costs of these devices, and potential to be incorporated into existing and commonplace infrastructure (such as smartphones, laptops, electronic tablets) [66] allow these to be portable and carried out by the patients [72]. This will, ~~empowering~~ empower ~~the~~ patients to take control of their own healthcare and reducing the burden on ~~the healthcare professional~~healthcare systems. A price evaluation is warranted for low-cost fabrication claims. Other biosensing modalities, such as calorimetry, have also been reported to be low-cost, and a comparison will help researchers and clinicians gauge the economic viability of implementing ECB into their work.

~~5.2.~~4.2. Obstacles to Commercialisation

Despite these advantages, ECBs still have ~~a number of~~several issues preventing them from being used widely. One of the key disadvantages is the error in the measurement; the current standard acceptable relative standard deviation is 20 % [73]. When considering a narrow therapeutic index drug and the potentially adverse effect from slight overdoses, these accuracies are inadequate. In addition to this, the interference from the matrix and selectivity against structural analogues and other redox species needs to be adequately corrected ~~for~~ to reduce this source of systematic error. Often, ~~regularly,~~ these effects are not sufficiently accounted for or recorded.

There is also a need for these sensors to be more robust. Physiological parameters such as pH and buffer strength can vary within the same biological fluid [74,75], and this needs to be offset ~~accounted for~~ [76]. Electrochemical responses can also be influenced by environmental factors, notably temperature and humidity [77]. In particular, aptamer-target binding affinity can be impacted by changes in temperature, potentially resulting in variable performance in different climates and settings [78]. Additionally~~In addition to this~~, in considering implantable biosensors for continuous

monitoring and closed-loop drug delivery, the foreign-body response may need to be ~~overcome~~ mitigated to prevent non-specific adhesion [79]. There are also concerns over sample evaporation, normalisation of the sample volume, and location dependent expression [80,81].

Each biorecognition element also suffers from their ~~individual~~individual ~~has~~ drawbacks. MIPs do not currently perform adequately in real biological fluids, and further work is needed to ~~go~~ transition from laboratory to mass manufacturing settings [82]. ~~, whereas e~~ Enzymes are not sufficiently stable over time and are not suitable for harsh conditions [83], and may be inhibited by charged ions [84]. Selecting a suitable aptamer is a very expensive process, and the aptamer discovered may only be selective under specific conditions, which include temperature and media [79]. In addition, research into antibodies for detection has been questioned for reproducibility and specificity [85,86].

Nanomaterials and structures also suffer from setbacks, such as aggregation, not being cost-effective, batch-to-batch variability, complex production processes, concerns about nanotoxicity and a lack of understanding of structure, composition and reactivity [25,87-89]. Using additive manufacturing also presents setbacks, such as potential drug-polymer interactions [90].

~~5.3.~~4.3. Future Outlook

As the field of ECBs moves forward~~s~~, there is a necessity for the technology and innovation in the laboratory to move forward into clinical and commercial applications. However, as with many fields, current research often involves increased complexity and not applicability, making the designs and advancements achieved in laboratory settings unsuitable for further testing [91]. Future research should focus on improving the robustness of the systems, helping to overcome the issues currently faced by ECBs and thus improve translation and outcome of patients.

There is huge potential for these devices to be incorporated with other technologies. One of the most likely applications of ECBs is with wearable devices, with~~here the~~ rapid response times being particularly important here [92]. Furthermore, these wearable devices could be personalised to the individual to improve measurement accuracy. Wearable devices are being explored in the context of various different scientific fields, for example, for Na^+ detection using a temporary-tattoo sensor [93], or in a bandage using potentiometry to measure pH in the range 5.5 – 8, for monitoring the evolution of the wound [94].

In addition ~~to this~~, the data generated from the ECBs are compatible with AI and cloud technologies, where mass data is key. If the sensors are able to connect ~~remotely~~ to and transmit data via a cloud server, the relevant information can be passed onto a clinician remotely. This allows~~,~~ ~~allowing~~ them to make a judgement about the state of the patient without requiring face-to-face

meetings, ~~allowing~~ effectively enabling the patient to be cared for from their home. This is also useful for remote settings, where seeing a clinical expert may not be possible otherwise. Indeed, with the use of closed-loop monitoring, where AI algorithms (and, in the future, machine learning networks) are introduced, there may well no longer be a need for a clinician to monitor the patient as regularly, freeing the clinician up for other work.

Biosensors can also be used for multiplex detection, where the same platform is used to measure multiple drugs simultaneously. This would be ideal for patients prescribed multiple medications, and in particular for determining drug interactions. Multiplexing has already been demonstrated for simultaneously monitoring various species, such as glucose, lactate, Na^+ and K^+ in sweat in order to assess the condition of the user [95], or the detection of ascorbic acid, uric acid and dopamine [96]. However, multiplexing will require ~~more~~ further research, such as into discovering new redox probes [79].

A novel redox probe, ~~O~~oracet ~~B~~blue, has been shown to be effective for use in human plasma, where it was used with a capture probe, graphene oxide and gold nanorods for the detection of the micro RNA miR-155 in human plasma without sample preparation (although 60 minutes incubation periods were required) with an LOD of 0.6 fM [97].

BREs can also be combined. One example of this was the combination of a MIP with an aptamer for the detection of ~~P~~prostate s~~S~~pecific a~~A~~ntigen; the combination of the BREs was able to enhance the sensitivity of the aptamer by a factor of 3, whilst retaining good selectivity and biofouling resistance [98]. Another illustration of possible improvements from combining BREs ~~as~~is the use of enzymes within an aptamer sensor. Here, m~~M~~ucin 1 p~~P~~rotein (MUC1) was detected using an aptamer to bind to MUC1, and ~~HRP~~h~~H~~orseradish p~~P~~eroxidase enzyme to produce a redox active species. This was able to detect MUC1 down to an LOD of 2.2 nM [99].

Novel BREs and alternative sensing mechanisms are ~~also~~ being studied in different scientific contexts. One such example is the use of t~~T~~hymine for the detection of m~~M~~ercury; t~~T~~hymine modified g~~G~~old nanoparticles/reduced graphene oxide nanocomposites were used. In presence of Hg^{2+}, t~~T~~hymine-Hg^{2+}-t~~T~~hymine complexes would form, and the Hg^{2+} could be reduced. Using this, m~~M~~ercury could be sensitively detected with a LOD of 1.5 pg/ml, whilst remaining selective against other metal ions.

Advances in ECBs will also allow for these to become a tool for research, rather than the element under investigation. This has already been realised in other fields, such as wearable biomarker detection, where uric acid was found to be higher in sweat and serum for patients with gout [100]. With increased uptake and validation of ECBs in TDM, it is easy to imagine them being used to

investigate drug-drug interactions, or better understand the conditions which lead to inter-individual pharmacokinetics.

~~1.~~5. Conclusion

Electrochemical biosensors can potentially see the transition of healthcare management towards precision medicine, and accordingly, towards improving patient's quality of life. The technology affords short analysis times, low-~~-~~costs and a variety of modifications and techniques, which make ECBs a key technology in TDM. In this review, the different parameters involved in ECBs were summarised, which can be exploited to improve sensing quality. The review prioritised recent studies that provided *in vivo* results to illustrate the progression made in translating the technology to clinical settings. Recent developments have shown that surface modifications can address traditional electrode drawbacks, and offer improved stability and reproducibility. Moreover, engineering microneedle-based electrodes hold the potential for continuous drug monitoring, and thereby providing ~~live~~ real-time readings. Innovations in allied fields, such as new materials and fabrication technologies, are likely to feedback and help fully realise the potential of ECBs.

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Figures:

Figure 1 A) Illustration of a typical 3 electrode setup. **B)** ~~Demonstration of a redox reaction BRE, where the enzyme catalyses a reaction which produces a redox active molecule (the yellow circle). C)~~ Example of a conformation changing BRE, where the DNA sequence binds to the analyte, causing it to fold. **C)** Demonstration of a redox reaction BRE, where the enzyme catalyses a reaction which produces a redox active molecule (the yellow circle). **D)** Representation of a binding-only BRE, where the antibody binds to the analyte. ~~Images adapted from~~The images of antibodies, molecules (triangles), enzymes, electrodes, and aptamers used in these figures are from Servier Medical Art licensed under the Creative Commons Attribution 3.0 Unported Licence [101].

Figure 2 A) Photograph of the microneedles. **B)** Illustration of the microneedles *in vivo*. ~~**B)** Image depicting the different layers of the microneedles, with the polycarbonate base, Chrome, insulating lacquer, Gold, Iridium Oxide, β-lactamase hydrogel and Poly(ethylenimine). **C)** Photograph of the microneeldes~~microneedles~~.~~ **C~~D~~)** Concentration over time graph, showing how 3 sets of microneedles give similar readings to microdialysis. The crosses are all microneedle data converted to concentrations using smoothing and then first order polynomial equations; the purple, grey and red crosses correspond to different microneedles and had correlation coefficients of R^2 = 0.47, 0.43 and 0.62, respectively. Blue pluses are the ECF microdialysis results, and the green pluses are the unbound serum concentration measurements. **D~~E~~)** Plot of microneedle vs microdialysis estimated concentration, showing a correlation of R^2 = 0.15, with an LOD of 0.17 µ~~m~~g/mL and an LOQ of 0.55 µg/ml~~-~~. ~~Figure Adapted from [44].~~Reprinted from [39] under the terms of the Creative Commons CC-BY 4.0 License.

~~**Figure 3 A) i)** The microneedles, with a scale bar (10 mm) with **ii)** the hollow and **iii)** carbon-paste packed microneedles. **B)** The correlation of the peak current vs methyl paraoxon concentration, demonstrating the highly linear correlation. Figure adapted from [61].~~

Figure ~~3~~4 A) Image of the conformation-changing vancomycin aptamer, which was used for **B)** feedback closed-loop drug delivery loop, where the measurement of vancomycin concentration from the sensor was used to control the flow rate on the sensor responsive infuser. **C)** The concentration against time plot for the closed-loop delivery system. **D)** The elimination time constant of vancomycin over this closed-loop delivery. The elimination time constant changed by a factor of roughly 3. ~~Figure adapted from [43].~~Reprinted with permission from [38]. Copyright © 2019 American Chemical Society.

~~**Figure 5 A)** The closed-loop delivery system for Doxorubicin delivery in the rabbit. **B)** An diagram of the microfluidic chip and conformation-changing aptamer used for these measurements. **C)** An example of tradition infusion of Doxorubicin; here the concentration was in the target range just 12 % of the time. **D)** Closed loop infusion for the same rabbit, showing an improved 96 % of the time the concentration was within the desired range. **E)** A concentration against time graph showing how the drug cisplatin can affect Doxorubicin pharmacokinetics. **F)** Measurements from the same rabbit, showing how closed-loop delivery was able to account for these changes in pharmacokinetics to maintain a steady concentration. **G)** The desired and measured concentration against time graphs for a rat, using the model developed with the rabbits. **H)** A close-up of the initial time delay for the closed-loop delivery with rats. Figure adapted from [54].~~

Figure ~~4~~6A) Schematic for the preparation steps used to produce the cyclophosphamide MIP biosensor. **B)** Correlation graph for how the change in current is affected by changes in cyclophosphamide concentration. **C)** Comparison of HPLC and the MIP biosensor for detecting cyclophosphamide in a dosed rabbit, with **D)** showing an enlarged image of the later measurements that the HPLC method was unable to detect. ~~Figure adapted from [48].~~Reprinted with permission from [43]. Copyright © 2016 Elsevier B.V.

Tables:

Table 1 *A selection of studies using ECBs for the detection of drugs*