Label-free Molecular Detection of Antibiotic Susceptibility for *Mycobacterium smegmatis* Using a Low Cost Electrode Format

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Abstract

Today, the emergence of antibiotic-resistance in pathogenic bacteria is considered an important problem for society. Excessive consumption of antibiotics, long-term treatments, and inappropriate prescriptions continually increase the severity of the problem. Improving antibiotic stewardship requires improved diagnostic testing and therefore, *in vitro* antibiotic susceptibility testing (AST) is becoming increasingly important. This research details the development of an antibiotic susceptibility test for *Mycobacterium smegmatis* using Streptomycin as an antibiotic. This strain was selected because it is a member of the slow growing *Mycobacterium* genus and serves as a useful surrogate organism for *Mycobacterium tuberculosis*. A commercially available and low-cost screen-printed gold electrode in combination with a specifically developed nucleic acid probe sequence for the 16SrRNA region of the mycobacterial genome were employed to monitor *M. smegmatis* nucleic acid sequences using the techniques of Square-Wave Voltammetry (SWV) and Electrochemical Impedance Spectroscopy (EIS). The results show that it was possible to detect *M. smegmatis* sequences and distinguish antibiotic-treated cells from untreated cells with a label-free molecular detection. As a result, the *in vitro* antibiotic susceptibility test revealed that *M. smegmatis* showed sensitivity to streptomycin after a 24 hour incubation, with the developed protocol representing a potential approach to determining antibiotic susceptibility more quickly and economically than current methods.

Keywords: Antibiotic susceptibility, Electrochemical biosensors, Antimicrobial resistance (AMR), Electrochemical impedance spectroscopy (EIS), Square-wave voltammetry (SWV)

1. Introduction

Antimicrobial resistance (AMR) of pathogenic bacteria has become well-known as an important challenge in recent years [1]. Antibiotic resistance is defined as the ability of bacteria to resist the effects of an antibiotic through use of acquired or evolved resistance genes [2]. The

important events in formation of bacterial resistance includes mitigation of antibiotic activity by enzymatic action, gene mutation, modification of antibiotic targets, and activation of the porin channel [3, 4]. Every year, at least 50,000 people in Europe and USA and hundreds of thousands of people in other regions die from antimicrobial resistant infections [5]. There are important differences in AMR models due to various global problems. Hence, a one-health approach to combat AMR consists of improved sanitation and animal husbandry, better infection control strategies, appropriate stewardship across all sectors, better regulation and policy, the development of new antimicrobial drugs and development of reliable and rapid AMR tests [6].

When investigating a patient sample, traditional phenotypic and genotypic antibiotic susceptibility testing (AST) methods are carried out for screening appropriate antibiotic drugs [7, 8]. For example, disk diffusion on agar plates and micro-dilution are conventional AST methods. Such tests are slow and time-consuming to identify drug-susceptible cells from drug resistance cells [9]. Example test times are 12-72 h and in the case of *Mycobacterium tuberculosis* up to one month with such long wait times leading to the use of the wrong antibiotic or prescription of a broad spectrum drug (which further drives resistance rates). Other technologies such as polymerase chain reaction (PCR) can be applied quickly to identify resistant bacteria, but the technique is expensive to implement, requires complex sample processing and highly trained staff [10]. Thus, new technologies for AST require low complexity of sample processing [6] and simple assay readout.

Today, electrochemical sensors are becoming more commonly employed to determine AST because of their potential for low-cost production and label-free detection [11, 12]. One such electrochemical technique is Electrochemical Impedance Spectroscopy (EIS) which captures changes in phase and amplitude as signal passes through the system. EIS is a method that can be applied to determine DNA hybridization events on electrodes. This technique

recently has been applied to the detection of small molecules and proteins and the label-free detection of nucleic acids [13-16]. Square-Wave Voltammetry (SWV) is another electrochemical measurement technique suitable for sensitive electrochemical measurements, mechanistic study of electrode processes, analytical applications, biologically active substances and many more [17, 18]. SWV negates the effects of capacative charging currents in the electrochemical response and is the most sensitive voltammetric technique currently available.

Screen-printed electrodes (SPE) are often applied as the sensing element in electrochemical biosensors from a range of various other commercially available electrode types. SPEs allow the analysis of low sample volumes with sensitive and selective analysis at low cost. These electrodes have been shown to fulfill the criteria for the reliable detection of antibiotic susceptibility, bacteria and antibiotics [19].

Molecular or genotypic AST is an effective emerging approach that eliminates the spread of deadly infections, the possibility of contamination, long incubation, and tedious bacterial cultures [20]. In this study we applied SPEs to investigate antibiotic susceptibility of *Mycobacterium smegmatis* which is a type of Acid fast bacteria [21]. *M. smegmatis* is a semi-rapidly growing non-pathogenic mycobacterium strain that is often used in molecular biological experiments as model system to study pathogenic *M. tuberculosis* [22]. Streptomycin was chosen as an antibiotic to evaluate AST effects by measuring the levels of specific nucleic acid sequences in reponse to incubation with the drug. Streptomycin is an aminoglycoside antibiotic which applied in the treatment of tuberculosis and sensitive Acid fast bacterial infections. Characterizations of this study were carried out with EIS and SWV techniques as for the reasons described above. The results indicate that the development of a sensitive assay of *M. smegmatis* oligonucleotides is possible with potential for scale up through use of a low cost, commercially available electrode. The simplicity of the repoted approach lies

in the fact that it was possible, using a specific nucleic acid sequence developed for *Mycobacterium* species to measure differential expression levels within two samples, one incubated with streptomycin and one without. This approach offers the prospect of carrying out simple bacterial cultures and then using an SPE to perform readout of the result. The approach is highy suited to use in resource limited settings and demonstration with a well established surrogate organism for TB shows that the approach offers potential to improve antibiotic stewardship for this infection.

2. Materials and Methods

2.1. Target Preparation

A liquid growth medium which is Middlebrook 7H9 broth (Sigma-Aldrich, USA) was applied to the culture of the *M. smeg*matis (ATCC 14468) strain and the bacterial media was supplemented with % 0.5 of Tween (Sigma-Aldrich, USA) and %2 of glycerol (Sigma-Aldrich, USA) in order to prevent coagulation. The cultured bacteria were incubated at 37° C to reach the exponential phase of growth. To do so, 0.5 McFarland propagated bacteria were taken using a Densitometer (Biosan, Latvia) and incubated for 24 h at 37° C. In this step, two samples were prepared and called antibiotic incubated (Ab-i) and non-incubated (Ab-n). Streptomycin (Sigma-Aldrich, USA) was adjusted and added to bacteria according to MIC value (10 µg/ml). To prepare Ab-i sample 0.1 gr streptomycin was solved in 100 ml water. In the following 10 µl streptomycin solvent was added to 1000 µl bacteria. The incubated samples were centrifuged (DAIHAN, South Korea) for 5 min at 5000 rpm to eliminate supernatant. Then, the supernatant was removed and an equal amount of the SDS and Triton X-100 buffer (Sigma-Aldrich, USA) was added to precipitated bacteria. The buffer was prepared by adding 0.045 gr SDS to 14 ml water and then mixed with 1.08 ml Triton X. After slowly shaking bacteria solution for 10 min, bacteria were again centrifuged to remove insoluble cell debris and collect supernatants at

10000 rpm for 10 min. Finally, collected supernatants were ready for fragmentation step which was carried out during electrochemistry testing at 95° C for 5 min [23]. Thereby, the target was prepared from Ab-i and Ab-n samples. The mentioned steps are illustrated in Fig. 1. In this study, supernatants were not purified after the lysis process, whereby there are DNA, RNA, and proteins in the solution of target sample. Therefore, sensors were exposed to DNA/RNA sequences during the EIS experiment.
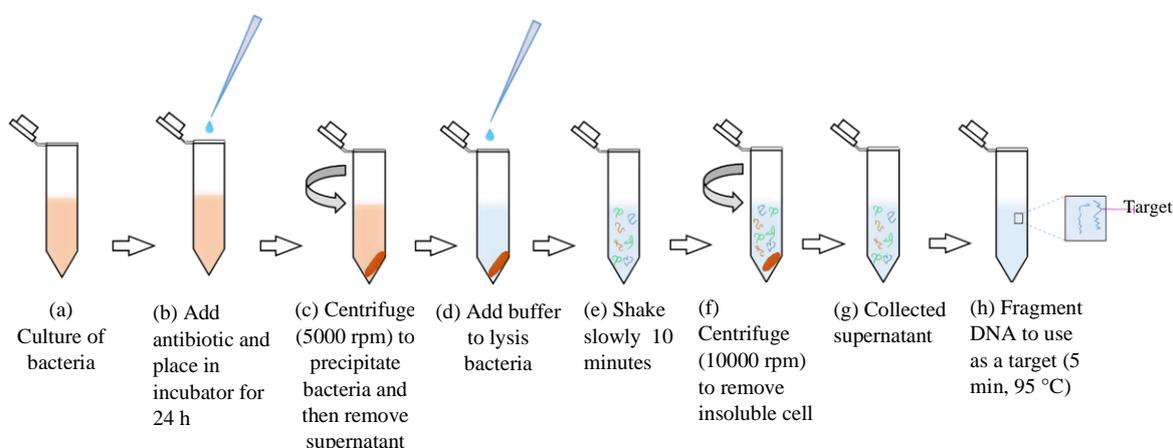

Fig. 1. Schematic for the preparation of target from cultured bacteria.

2.2. Electrode Pretreatment

Prior to measurement, all electrodes were cleaned by immersion in ethanol solution to obtain a clean and uniform surface. The clean electrode improves Self-Assembled Monolayer (SAM) formation. The electrodes were placed in a bath Sonicator (ISOLAB Laborgeräte GmbH, Germany) for 10 minutes at room temperature in order to clean process. In addition to ethanol, the cleaning process was compared with another method to determine the best probe immobilization. Electrodes were soaked in %10 H_2SO_4 solution for 30 min without CV cleaning.

2.3. Detection Method

After the cleaning process, the electrodes were thoroughly washed using DI water (10 seconds). The soluble redox mediator (1 mM Ferri/Ferrocyanide (Fe $[CN]_6^{3-/4-}$)) was placed on the electrodes, then EIS and SWV were performed at room temperature to obtain pre-probe results. Fig. 2 shows the stepwise preparation of the working electrode. In order to form the SAM, the probe solution was prepared using 3 μM probe DNA with 15 μM tris(2-carboxyethyl) phosphine (TCEP) in 1 x PBS and backfilling solution was made by mixing 1 mM 3-Mercapto-1-propanol (MCP-95%, Sigma-Aldrich, USA) with 5 mM TCEP in a background of 1 x PBS [24]. Then, the probe and backfilling solutions were added onto a working part of the electrode surface and incubated for 24 h and 1 h, respectively. Following each incubation, electrodes were washed with DI water for 10 s. Consequently, electrodes were functionalized with single stranded DNA (5'-[ThiC6][SP18]CATGCGACCAGCAGGGTGT-3'), with a sequence complementary to 16S ribosomal RNA [25]. Moreover, a non-complementary probe sequence (5' [ThiC6][SP18] ACCACAAGACATGCATCCCG 3') was used to assess specificity. After the post-probe process, EIS and SWV were measured to effectiveness of immobilization process, through measuring an increase in charge transfer ressitance (R_{ct}) or decrease in SWV peak height, as the probe inhibits electron transfer at the electrode surface. Finally, the post-target incubation was done using fragmented oligonucleotides which were put on the electrode for 1 hour at room temperature. The electrodes were then washed in %5 PBS solution for 20 s [24]. EIS and SWV were applied to find oligonucleotide hybridization events on electrodes through a futher increase in R_{ct} or decrease in SWV peak height [23, 26].
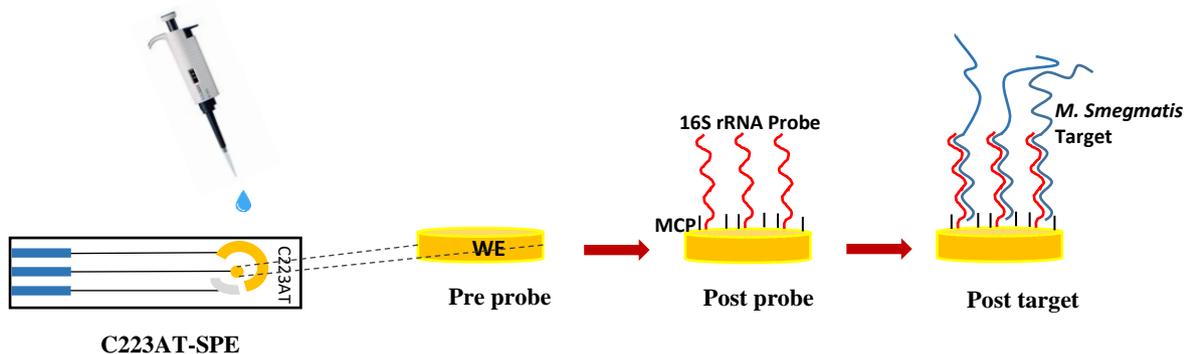
Fig. 2. Schematic for the stepwise preparation of working electrode.

2.4. Electrochemical measurements

Commercially available Gold (Au) Screen Printed Electrode (SPE) were purchased from Metrohm, Herisau, Switzerland. The SPE applied in this study was C223AT (high-temperature cure), which contains a three-electrode system with a silver reference and gold working and counter electrodes. The structure of the SPE is demonstrated in Fig. 2. All measurements were performed using a portable potentiostat (PalmSens PS4, Houten, Netherland) three times.

CV was carried out by sweeping the potential from 0.5 to −0.25 V at 100 mV/s scan rates. The SWV scan was implemented from −0.3 to 0.5V with a 100 mV amplitude. The EIS response was measured at frequencies from 100 kHz and 0.1 Hz and the generated Nyquist and Bode impedance plots were fitted by the Randles equivalent circuit in order to determine the charge transfer resistance (R_{ct}).

3. Results and Discussion

3.1. Detection by Electrochemical Impedance Spectroscopy

EIS and SWV are sensitive and label-free methods that are frequently used to determine the changes in the electrical properties at the sensor-sample interface [27]. In this study, a low-

cost electrode was chosen to observe antibiotic susceptibility testing using these techniques. We worked with six SPEs to perform six experiments; three electrodes were assigned to Ab-i experiments and another three electrodes applied to Ab-n experiments. All of the six experiments showed similar electrochemical impedance response curves. Fig. 3 displays one such experiment with the EIS response for a 1 mM potassium ferro and ferrocyanide 1 x PBS solution on the working electrode with the Randles equivalent circuit used for data fitting. The EIS response shows a semi-circle transitioing into a 45⁰ line which is typical of a charge transfer reaction followed by diffusional behavior from the redox couple in solution. Of particular importance to these experiments was the semi-circular portion of the plot which was used to extract the R_{ct}, which is indicative of electron transfer rates at the sensor surface. It is clear that the sensors detect the presence of probe and target on the working electrodes since the charge transfer resistance increased through successive incubations. The green curve is the signal obtained from the SPE prior to probe immobilisation (measured after ethanol cleaning) where the surface is at its cleanest and most unhindered and which gives rise to the fastest electron transfer rates and thereby a negligible semi-circle in the observed response. Immobilization of thiol- probes by self-assembly on working electrodes is frequently used in DNA biosensors and fabrication of electrochemical systems [28]. Binding of thiol groups to the gold surface (working electrode) is a result of strong interaction (chemisorption) between the sulfur and gold atoms [29]. The attachment process resulting from formation of the DNA probe layer caused a large impedance signal increase represented by the red curve. The EIS response for post-probe confirmed that probes were thoroughly immobilized on the working electrode. Hybridization of oligomeric DNA can be detected based on electrical frequency-dependent response without using of expensive equipment or mediators and labelling methods [30]. As shown in Fig. 2, the 16s rRNA probe was examined with a *M. smegmatis* DNA target directly complementary to the probe sequence. EIS measurement of post-target (blue curves)

showed a significant change in spectra. The results displayed the high sensitivity of the system. Therefore, immobilization and hybridization were successfully performed for all six of these initial experiments.
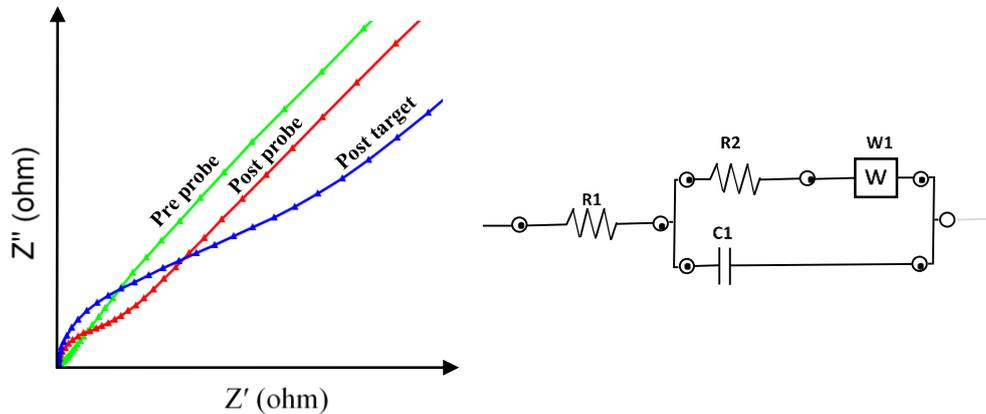

Fig. 3. The experiment spectrums of EIS with the Randles equivalent circuit used for data fitting. The electrodes were incubated for 1 h with the target which prepared from 0.5 McFarland bacteria suspension.

For measuring EIS of DNA probe immobilization, probe concentration and electrode cleaning are very important in probe immobilization process onto electrode surface. Standard cleaning protocols for gold electrodes usually involve performing cyclic voltammetry in dilute sulphuric acid [31, 32]. However this protocol is time consuming and usually only capable of cleaning one electrode at a time. In order to decrease the time and increase the throughput of the detection, both ultrasonication in ethanol and immersion in sulphuric acid was investigated as alternatives. Electrodes were either immersed in %100 ethanol and sonicated at 25 °C for 10 minutes or soaked in %10 sulphuric acid for 30 minutes. Fig 4 shows CVs of the ferri/ferro redox reaction after these treatments. The CVs show a typical response of a solution-based redox reaction with well-defined peaks. This cleaning effect is further demonstrated by comparing R_{ct} values after cleaning, which are presented in Table 1. The low and repeatable R_{ct} values give confidence that both of these procedures are capable of producing clean

electrodes. As it yielded lower R_{ct} values, the sonication in ethanol was chosen for the rest of the measurements performed in this paper. This could indicate that the majority of contamination on the surface were compounds soluble in ethanol, for example organic molecules or gold oxide species. Moreover, immobilization studies were performed by increasing the concentration of the probe solution by 3 times, which was 9 µM probe DNA with 15 µM TCEP in 1 x PBS, since it is known that 7×10^{-5} - 3×10^{-4} probes/nm^2 is optimum hybridization surface densities [33]. After the oligonucleotide probes were incubated for 24 h, a high increase was observed in R_{ct} values for all 6 experiments. Numerically, the probe count was enhanced on the electrode surface due to the more concentrated immobilisation solution. Increasing the probe density resulted in higher sensitivity in the antibiotic susceptibility test with these electrochemical sensors. The number of bacteria in the sample incubated with streptomycin was significantly lower than for the control sample (antibiotic-untreated sample) after 24 h incubation and this was observable in the electrochemical response of the more densely formed sensing layer.

Table 1
EIS results of experiments after cleaning under a) H_2SO_4 without CV cleaning (Electrodes soaked to H_2SO_4 solution for 30 min.) and b) ethanol.

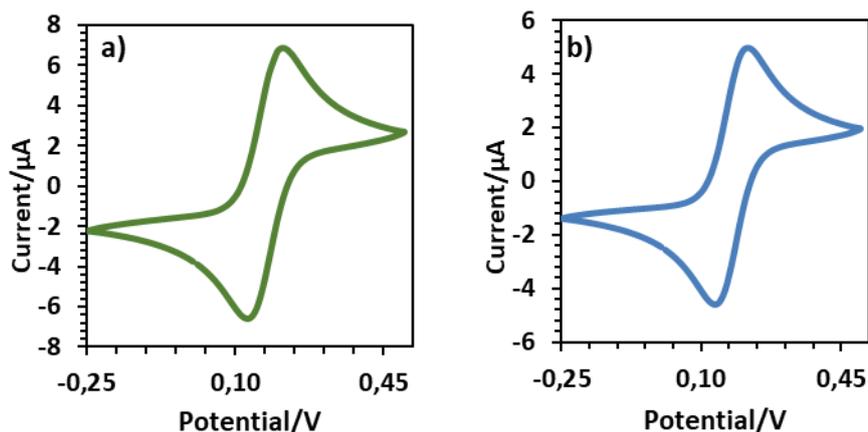|		Rct (Ω)									
		1. E	2. E	3. E	4. E	5. E	6. E	7. E	8. E	9. E	10. E
a)	Pre probe	303	343	297	423	377	243	288	284	296	300
	Post probe	341	347	307	450	381	257	R	R	R	R
	Post target	341	R	R	R	R	R	-	-	-	-
b)	Pre probe	137	127	120	129	141	130	132	155	122	134
	Post probe	156	129	133	R	R	138	139	R	157	R
	Post target	198	R	R	-	-	R	R	-	R	-

E.: Experiment
R: Reduction

Fig. 4. Cyclic voltammograms measured in preprobe step for a) H_2SO_4 without CV cleaning (Electrodes soaked into H_2SO_4 solution for 30 min.) and b) Ethanol.

Table 2 represents the pre-probe, post-probe and post-target results for six electrodes, three electrodes were fused for Ab-i experiments and three electrodes for Ab-n experiments. The R_{ct} values of pre probe and post-probe were 126 ± 30 and 788 ± 201 Ω, respectively. Thus, a high concentration of 16S rRNA probe resulted in a higher EIS response in the presence of target DNA and the largest signal change (target-to-probe ratio). We investigated the R_{ct} signal change to determine susceptibility differens in *M. smegma*tis for Ab-i and Ab-n samples as shown in Fig. 5. Following extraction of nucleic acids from Ab-i and Ab-n samples and subsequent incubation on the *Mycobacterial* 16SrRNA probe functionalised electrodes, the R_{ct} signal change of Ab-i and Ab-n samples increased by 2.60 ± 0.08 and 3.63 ± 0.94 times, respectively. The results indicate that the antibiotic free sample gave a larger change in signal. In other words, bacterial growth was seen in the untreated sample following 24 h of incubation. While, the sample containing *M.smegmatis* cells exposed to antibiotic gave lower signal changes in the EIS response. Hence, *M. smegmatis* gave a differential response in terms of the EIS signal when incubated with and without antibiotic. In the following, the performance of both samples was compared with a non-complementary probe to confirm sensor specificity. The experiment results show that non-complementary (nc) probe is not able to hybridize

conserved region of M. smegmatis (Ab-n-nc and Ab-i-nc), as shown in Fig. 5. Therefore, The

EIS results with complimentary probe confirm sensor specificity of the experiments.

Table 2

Electrochemical impedance spectroscopy (EIS) for every experiment.

	Rct (Ω)					
	Ab-n			Ab-i		
	1. E.	2. E.	3. E.	1. E.	2. E.	3. E.
Pre-probe	146	141	132	135	66	134
Post-probe	676	473	900	791	1066	821
Post-target	2143	2226	2575	2138	2745	2089

E.: Experiment

Streptomycin is one of the oldest antibiotics used against *Mycobacteria*. After many

years of use, rates of resistance have risen and its potency has decreased gradually. Nowadays,

with resistance of current anti-mycobacterium drugs, the use of streptomycin has led to a

renewed interest. Streptomycin acts by inhibiting protein synthesis of *Mycobacterial* ribosome

[34] and leads to bacterial death [35]. As seen in this study, bacteria incubated with

streptomycin showed lower singal change in EIS, brought about differential 16SrRNA

expression and differences in total nucleic acid content than for non-incubated bacteria. This

then means the development of a specific and sensitive EIS-based assay for *M. smegmatis*

based on the use of a specific *Mycobacterial* 16SrRNA probe sequence. Recently, limited

studies have been published on the detection of *Mycobacteri*a using EIS-based techniques. For

examples, Sypabekova *et al.*, reported EIS-based detection of *M. tuberculosis* secreted protein

MPT64 using an interdigitated electrode [36]. The authors applied a special receptor, a single

stranded DNA aptamer, which specifically recognizes MPT64 protein. Kargupta *et al.*, recently

reported culture-based detection of living mycobacteria (*M. bovis BCG and M. smegmatis*)

using impedance measurements at multiple frequencies [37]. They claimed the detection

method is faster than current commercial systems. Our study was carried out using SPE as a

sensor platform, which is a very inexpensive electrode. In addition, we not only detected *M.*

smegmatis but also determined the sensitivity to the antibiotic. Therefore, the combination of

the low cost SPE with a simple linear nucleic probe sequence demonstrated a potential approach to determining antibiotic susceptibility.
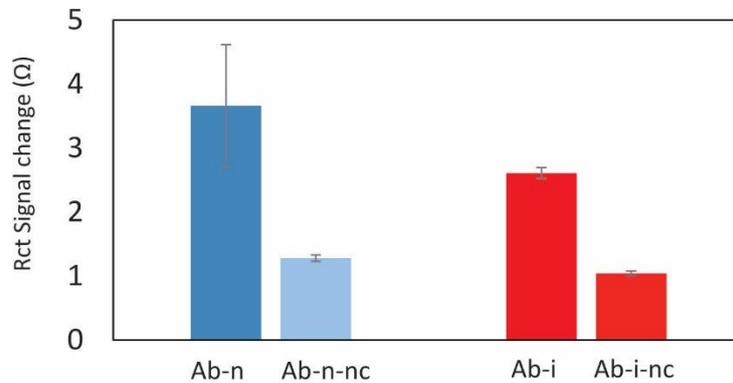

Fig. 5. EIS Signal change after exposure to target for complementary and non-complementary probes.

3.2. Detection by Square-Wave Voltammetry (SWV)

SWV technique has also been applied to characterize the performance of electrochemical biosensors [38]. Here in our study, SWV was examined by six electrodes separately, of which three electrodes were exposed to Ab-n antibiotic and three electrodes Ab-i. As can be seen in Fig. 6a, an example from six experiments were selected to display the SWV response because SWV profiles of these samples have almost the same shape. The highest peak represented by the blue curve was measured before the probe immobilization. Since the electrodes were clean at first, the SWV curve in the pre-probe measurement gives a high peak curent. Then, owing to the immobilization of DNA probe, the peak significantly decreases after the electrodes were incubated with the probe (red curve). With the addition of target, the peak decreases further (green curve). These results are consistent with the EIS results presented earlier. At this stage, it can be said that hybridization took place between the 16SrRNA probe sequence and the *M. smegmatis* sequences. In order to evaluate the sensitivity, SWV signal changes were examined as shown in Fig. 6b. SWV signals changed by 0.59 ± 0.01 and $0.57 \pm$

0.01 for the Ab-i and Ab-n samples, respectively. The results show that when antibiotics were added into media, a higher change was observed in the signal. Thus, our biosensor identified bacteria were sensitive to streptomycin. In line with the EIS results, the SWV results confirm that both probe immobilization and target hybridization have been successfully completed. Demonstration of the potential to perform this antibiotic susceptibility measurement using a voltammetric technique such as SWV means that a simple, low cost circuit and detection platform can be used to perform the antibiotic susceptibility in the field.
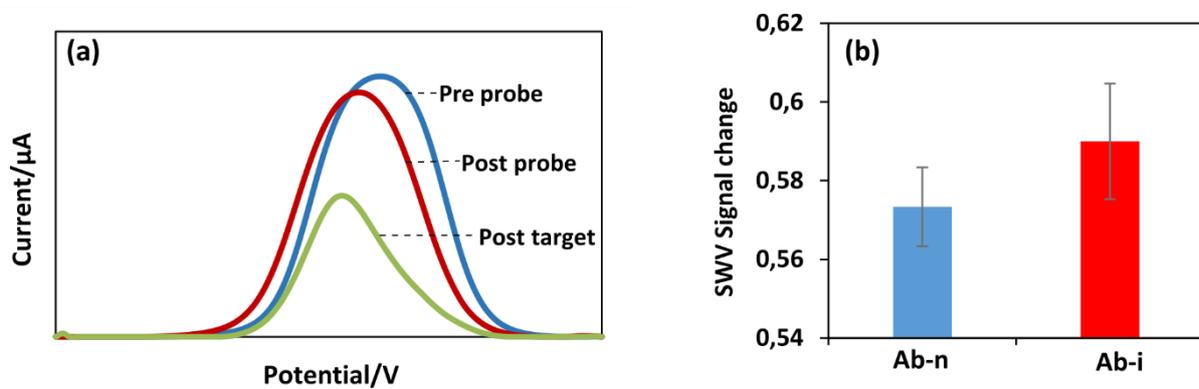

Fig. 6. Graphs showing SWV results. a) The experiment spectrums of SWV, b) Average SWV signal change after exposure to target.

In this study, SPSS software was performed for analyzing data and running statistical tests. The paired sample T-Test demonstrates that the p-value for EIS and SWV results are 0.1 and 0.25, respectively. Both of the p-values are higher than 0.05 (> 0.05). Thus, there is no statistically significant difference between Ab-n and Ab-i experiments.

EIS and SWV results show a relatively new and emerging approach to electrochemical AST studies. Generally, electrochemical sensors were applied as detection and diagnostic methods [14, 39-42]. In this study, Ab-n and Ab-i samples were interrogated by electrochemical methods was employed as a benchmark technique. Considering the results of EIS and SWV measurements, it is possible to distinguish between antibiotic and non-antibiotic

incubated *M.smegmatis* samples. These results were obtained following sample incubation times of one hour so substantial opportunity for optimization exists,by for example, reducing the incubation time. The increased probe density on the electrode surface brought about by use of a more concentrated immobilsaton solution may have resulted in faster hybridization in the Ab-n solution (which had more nucleic acid present). In addition, this method is thought to contribute to the determination of the viability of the cells.

There are a number of methods to determine cell viability. Although MTT assay is most commonly used as a cell viability assay, there are different assay methods such as classes of colorimetric tetrazolium reagents, resazurin reduction and protease substrates generating a fluorescent signal, the luminogenic ATP assay, and a novel real-time assay to monitor live cells for days in culture [43]. These methods have advantages and disadvantages for evaluation of cell viability [44]. Besides these, we believe that this electrochemical method has great potential in determining cell viability because it also indicates how many viable cells are remaining at the end of the experiment. Therefore we believe that our results show the capability of EIS and SWV for measuring oligonucleotides and can be used as a proxy for cell viability and going on for that give an accurate impression of antibiotic efficacy, leading to technologies capable of giving simple, low cost susceptibility results leading to rational prescription of drugs and enhanced antibiotic stewardship.

4. Conclusion

The cleaning method employed and probe concentration chosen had a critical impact on producing an accurate and reproducible electrochemical AST. The ethanol cleaned electrodes enabled effective SAM formation, while being faster and allowing a higher throughput than more traditional methods such as repetitive CV cleaning. In addition, probe concentration had a critical importance in these electrochemical AST studies by producing a

stable and reproducible SAM which gave consistent changes upon target hybridisation. When the probe concentration was increased from 3 to 9 μM, a higher R_{ct} value was measured as expected. We then went on to show that *M. smegmatis* was susceptible to the streptomycin antibiotic as confirmed by electrochemical methods. Following apre-culture we were then able to extrct the nucleic acid pool from *M.smegmatis* cultures and without the requirement to amplify the target DNA by PCR or using traditional phenotypic methods measure the antibiotic effect of streptomycin though use of 16SrRNA and global nuleic acid. This work points the way to a rapid, simple, low cost methodology for assessing antibiotic susceptibility and providing rational drug prescriptions in the field or in resource limited settings.

Declaration of competing interest

No potential conflict of interest was reported by the authors.

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Table 1 EIS results of experiments after cleaning under a) H_2SO_4 without CV cleaning (Electrodes soaked to H_2SO_4 solution for 30 min.) and b) ethanol.

		Rct (Ω)									
		1. E	2. E	3. E	4. E	5. E	6. E	7. E	8. E	9. E	10. E
a)	Pre probe	303	343	297	423	377	243	288	284	296	300
	Post probe	341	347	307	450	381	257	R	R	R	R
	Post target	341	R	R	R	R	R	-	-	-	-
b)	Pre probe	137	127	120	129	141	130	132	155	122	134
	Post probe	156	129	133	R	R	138	139	R	157	R
	Post target	198	R	R	-	-	R	R	-	R	-

E.: Experiment

R: Reduction

Table 2 Electrochemical impedance spectroscopy (EIS) for every experiment.

	Rct (Ω)					
	Ab-n			Ab-i		
	1. E.	2. E.	3. E.	1. E.	2. E.	3. E.
Pre-probe	146	141	132	135	66	134
Post-probe	676	473	900	791	1066	821
Post-target	2143	2226	2575	2138	2745	2089

E.: Experiment