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A fully integrated rapid on-chip antibiotic susceptibility test – A case study for Mycobacterium smegmatis

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ABSTRACT

Antibiotic resistance is one of the most pressing scientific and societal issues of our age. There is an urgent need to develop new diagnostic technologies which can quickly determine whether an infection is susceptible or resistant to different treatments so that rational antibiotic prescribing can take place. The main objective of the study was therefore to develop a rapid, simple, cost effective and comprehensive antibiotic susceptibility/resistance test based on rapid nucleic acid profiling. To do so, we integrated a microelectrode sensor within a microfluidic chip that combined bacterial incubation, lysis, and electrochemical detection chambers in a single simple set-up. As a case study, *Mycobacterium smegmatis* was investigated as a surrogate organism for *Mycobacterium tuberculosis*. The novelty of the work lies in developed capability of performing incubation, lysis, fragmentation, and detection process in a comprehensive yet simple lab-on-a-chip device called 'MycoCHIP'. A gold microelectrode 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 Differential Pulse Voltammetry (DPV) and Square-Wave Voltammetry (SWV). The results demonstrated that it was possible to detect bacterial nucleic acid sequences and distinguish antibiotic incubated (Ab-i) cells from non-incubated (Ab-n) cells on MycoCHIP with a label-free molecular detection. The antibiotic susceptibility test showed that through measuring 16SrRNA levels from *M. smegmatis*,sensitivity to antibiotic was apparent after 24 h incubation, with a developed protocol representing a potential approach to determining antibiotic susceptibility more quickly, reliable and economically than current methods.

1. Introduction

The emergence of Antimicrobial Resistance (AMR) is a very worrying phenomenon, which has been identified by influential organizations such as the United Nations and WHO as a major scientific problem in need of urgent, coordinated international action. The emergence of resistant pathogen strains around the world requires not only careful monitoring but the adoption of improved behaviors in terms of antibiotic (AB) selection and consumption [1,2]. There have even been recent and very worrying reports of the emergence of bacterial strains resistant to the ABs of last resort [3,4] and there is obviously a need to develop new drugs. However, the adoption of behaviors which include; reduced antibiotic consumption and judicious prescription of ABs in turn will not only reduce resistance development rates and better

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maintain the efficacy of current drugs, but also result in more sustainable use of antibiotics into the future [5]. Through technological advances which address the problem of AB stewardship it will be possible to achieve substantial reductions in the spread of AMR [5,6].

Mycobacterium is a genus of Actinobacteria, given its own family, the Mycobacteriaceae. The genus includes pathogens known to cause serious diseases in mammals, including tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*). *M. tuberculosis* is a member of the mycobacterium genus and due to the waxy coat surrounding the cell wall does not take up the Gram Stain [7]. It is a well-known and established bacterium which over time has developed a number of strategies and resistance mechanisms which allow it to propagate amongst human populations [8]. It is thought that *M. tuberculosis* latently infects just under one quarter of the entire human population [9].

Improving antimicrobial susceptibility testing is an important component of a coordinated solution to the larger antibiotic resistance problem which also includes searching for new compounds, better infection control measures and effective surveillance of resistant strain spread. Antibiotic susceptibility encompasses a number of traditional techniques which include the broth dilution method (also known as Minimal Inhibitory Concentration (MIC)), the disk diffusion test and the gradient diffusion test [10]. There can often be a very significant lag between taking sample and obtaining a result and in the intervening time clinicians are left with little choice but to speculatively prescribe antibiotics, particularly broad spectrum therapies. The literature clearly shows, and openly discusses, that there is a requirement for new technologies which guide clinicians with antibiotic prescription in a timely way so that the correct drug can be administered quickly [11,12]. Having such a capability will reduce the erroneous prescription of ABs and the resultant spread of drug resistant strains.

Some previous approaches to AMR detection have included piezoelectric detection, thin film biosensors, light scattering nanoparticle assays, electrophysiology, isothermal FISH assays using a fluorescently decorated PNA probe and multiplex PCR [13,14]. It is highly advantageous to deploy such biosensors on a chip. For example, AMR methods previously deployed on-chip include fluorescence imaging, metabolic activity indicators, optical imaging without labeling, and magnetic beads rotation measurement [15]. A particularly attractive approach to detection of AMR genes has been the use of electrochemical sensors, which are well suited to integration onto chips as well as having the advantages of being sensitive, specific, label free, cheaply produced and easy to use. We have already produced electrochemical sensors which detect the DNA signature of methicillin resistance (*mecA* gene) in MRSA [13] in a highly sensitive manner without the requirement to perform a PCR reaction with a result time of < 30 mins [16]. Furthermore, we were involved in the development of a selective and sensitive assay for the identification of bacterial strains based on rRNA sequence. Using the approach, it was possible to sensitively measure and identify *Pseudomonas aeruginosa* against other bacterial strains (*E.Coli* and *MSSA*) with high sensitivity (limit of detection = 10 pM) and in similarly quick times to the MRSA Antibiotic Susceptibility study [17]. Previous research has shown that these kinds of biosensors can be enhanced through the use of microelectrodes, electrodes which have one critical dimension on the scale of microns [18]. Moreover, microelectrodes have been shown to be at least 8 times more sensitive for detecting mycobacterial nucleic acid sequences [19].

Lab-on-a-chip (LOC) systems have been an intense area of study in recent years LOC systems have many advantages such as fast analysis, high efficiency, low concentration of analyte, durability and low cost. Moreover, many functions can be performed on a chip easily and in a short time. Optical experiments can be combined with other analytical methods, and the system can be automated [20,21]. The presence of specialised structures inside the microfluidic chip such as channels, valves, mixers and pumps allows the device accommodate one or more types of fluid and move along channel different channels. If necessary, liquid samples can be held in a part of the chip for a desired time period, mixed together to create a specific reaction, and finally the main products and wastes can be transported out of the device by the outlets [22, 23].

This work offers a new direction by developing an antibiotic susceptibility test, which builds on and combines state of the art advances in molecular probe tests with microfluidics to rapidly profile antibiotic susceptibility. The electrochemical detection methodology was applied by using microelectrodes to determine antibiotic susceptibility by measuring nucleic acid levels in response to incubation conditions. To do so, we have designed a novel PDMS-based microfluidic device with bacterial incubation and lysis chambers, combined with the Au microelectrode set-up in a single device. The first microfluidic chamber serves as bioreactor whereas the microelectrode-integrated microfluidic chamber acts as label-free molecular sensing platform. The integration of both platforms is provided through simple PDMS microchannels and microvalves. Bacteria are incubated with and without antibiotic in separate chambers, lysed, and delivered to the sensing platform where their respective nucleic acid content is measured via the microelectrodes within the microfluidic channels. The device we developed within the scope of the project is a portable chip, not a point-of-care device. However, it has the potential to be a point-of-care device. In the MycoCHIP device, there is no chamber for extraction of bacteria. The proposed compact device offers easy sample control over microbiology and electrochemical experiments in a single device. We believe that the application of microelectrode integration into a microfluidic device as such is the first of its kind in the area of on-chip antibiotic susceptibility research.

2. Materials and methods

2.1. Design and fabrication of microelectrodes

The microelectrode design consists of two electrodes, namely a 2 mm-wide counter (CE)/ reference electrode (RE) and a 100 μm working electrode (WE), as shown in Fig. 1. The length of the electrodes was 12 mm. Microelectrodes were designed using AutoCAD software. The PET made-photomask was fabricated by Cozum Tanitim (Turkey) and classical photolithography and lift-off process was carried out, Fig. 1a [24], in order to transfer electrode patterns onto the glass slide (25 mm by 75 mm), as depicted in Fig. 1b. A chromium (Cr) layer (approximately 100 nm) was deposited on the glass slide to improve adhesion. 300 nm of Au deposition was carried out via thermal evaporation process on the Cr layer. Gold (Au) was chosen as the material for electrodes due to its ability to allow semi-covalent bonding on its surface in order to

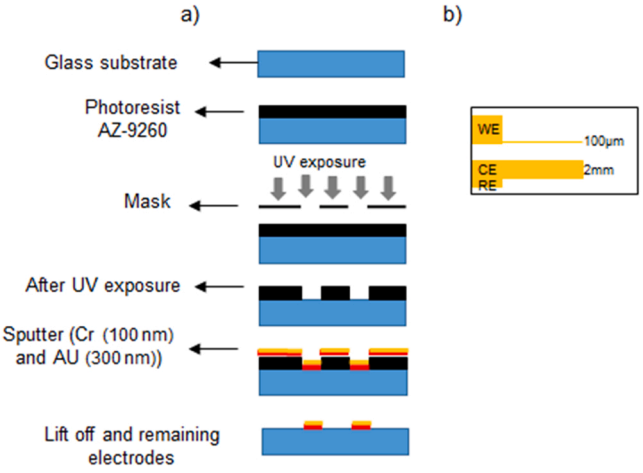

Fig. 1. a) Fabrication process of the electrodes, b) top down schematic of the electrodes.

immobilize bioreceptors, its biocompatibility, favorable electron transfer kinetics with high conductivity, and high stability [25,26]. Cold Solder Silver Epoxy electrically conducting adhesive (M.G. Chemicals, Ontario, Canada) was applied on the electrode terminals in order to create electrical connections between clips and electrodes. The electrodes was kept on a hotplate set to 60 °C for 24 h to cure the epoxy.

2.2. Design and fabrication of the microfluidic chip and microvalve

The integrated MycoCHIP consists of three layers as depicted in Fig. 2; (a) a top incubation layer, (b) a middle microchannel layer, and (c) the bottom microelectrode layer. The microfluidic incubation layer consist of two bioreactors; a bioreactor with antibiotic input (antibiotic susceptibility test section) and the other bioreactor with antibiotic-free input (control section). The left bioreactor has two inlets for receiving a solution-containing antibiotic and bacteria, while the right bioreactor has an inlet for receiving only bacteria. In this step, two samples were prepared and called antibiotic incubated (Ab-i) and nonincubated (Ab-n). Both bioreactors have an outlet to transfer incubated bacteria into the detection platform. The outlets were controlled by a microvalve in order to manipulate the fluid infusion into the microelectrode chamber in a controllable manner. The bioreactor layer was connected to the electrochemical chamber via a middle microchannel layer and microvalves. The width of the bioreactor and electrochemical microchannel was 150 and 300 μm. The microvalve was made by a 3D printer and comprised of a rod and hole with 2 mm and 500 μm diameter. The diameter of the hole in the microvalve was kept at least twice the microchannel width [27].

Polydimethylsiloxane (PDMS) based microfluidic channels were fabricated through conventional optical and soft lithography method [28]. The mask was designed in AutoCAD and printed on PET film (Cozum Tanitum, Turkey). SU8 2050 (Nippon Kayaku, MicroChem, Japan) photoresist was spin-coated onto a 4 in. silicon wafer (Prime CZ-Si, Nanografi, Turkey). Then, a negative replica of the mold was obtained using Sylgard 184 (Dow Europe GmbH) in a mixing ration of 5:1 elastomer:curing agent. To reduce the gas permeability of the PDMS, the amount of elastomer was used at half the standard level [29]. The mixture was poured onto the silicon wafer and cured for 24 h at 50 °C in a hot plate. Inlets and outlets were open using a biopsy punch (1.0 mm with Plunger (Box of 20), Selles Medical, UK). In the following, PET tubes (Harvard Apparatus, USA) were inserted into the inlet in order to connect the microfluidic chip to a syringe pump (New Era, USA).

2.3. Assembly of the MycoCHIP

The middle microchannel layer and bottom glass slide, the microelectrode layer, were bonded together after the plasma treatment using a custom made plasma device. The top bioreactor layer, consisting of the microfluidics, was then connected onto these two integrated layers via plasma bonding as well. Before bonding for each step, the layers were washed with acetone and then dried with nitrogen.

2.4. Electrode functionalisation procedure

All steps of the electrode functionalisation procedure was performed through the inlets on the top bioreactor layer, located on the top of the electrochemical analysis part. Before functionalisation procedure, the microelectrode surfaces were cleaned by injecting of 0.1 M H_2SO_4 solution and using cyclic voltammetry (CV). Then, the electrode surfaces were washed with distilled water (DI) and soluble redox mediator (1 mM ferri/ferrocyanide $[Fe(CN)_6]^{3-/4-}$) was injected onto the microelectrodes to measure CV, differential pulse voltametry (DPV), and electrochemical impedance spectroscopy (EIS) for pre-probe analysis. To create the target-capture surface, 3 μM probe DNA with 15 μM tris(2-carboxyethyl) phosphine (TCEP) in 1 × PBS was first immobilized on the electrode surface for 24 h. We used single-stranded DNA sequences as complementary to 16 S ribosomal RNA and non-complementary sequences, seperatly (Table 1). Pinholes in the DNA self-assembled monolayer (SAM) were filled by next incubating with 3-Mercapto-1-propanol (MCP- Sigma-Aldrich, USA) with 5 mM TCEP in a background of 1 × PBS for 1 h. Afterwards, DPV and SWV were performed to monitor for effectiveness of immobilisation process in post-probe analysis. In the last stage, the post-target analysis was carried out using fragmented oligonucleotide samples, which were delivered to the electrode from the bioreactor. After 1 h incubation, the electrodes surfaces were washed with 5% PBS solution [24] and then SWV and DPV were monitored to realize oligonucleotide hybridization events on microelectrodes through measuring a decrease in the SWV and DPV peak

Table 1
DNA sequences used for the experiments.

Function	Sequence (5'→3')
Complementary Probe	[ThiC6] [SP18] CATGCGACCAGCAGGGTGT
Non-complementary Probe	[ThiC6] [SP18] ACCACAAGACATGCATCCCG

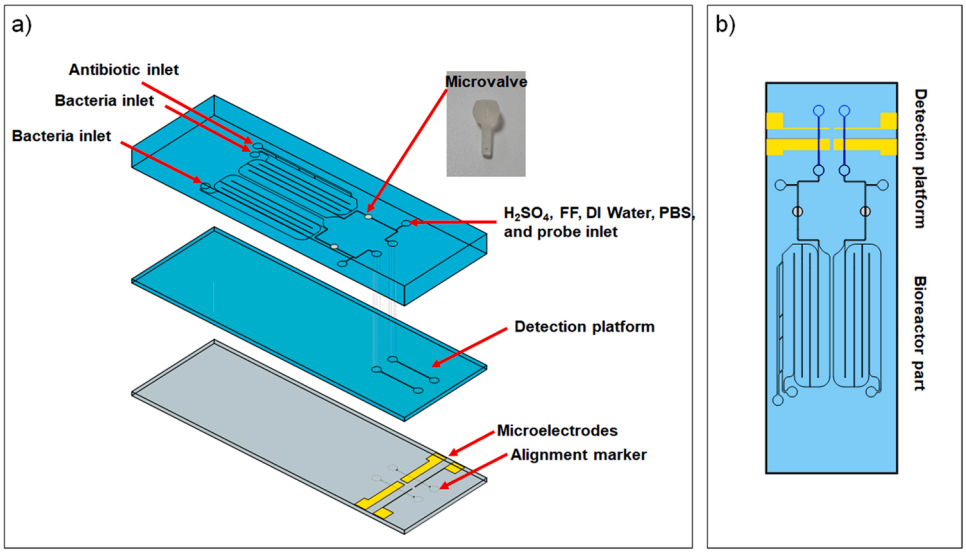

Fig. 2. a) 3D and b) 2D schematic images of the MycoCHIP.

height [16].

2.5. Susceptibility experiment

Middlebrook 7H9 broth (Sigma-Aldrich, USA) was used to culture the *M. smegmatis* strain ATCC 14468, and supplemented with 2% glycerol (Sigma-Aldrich, USA) and 0.5% Tween (Sigma-Aldrich, USA) in order to eliminate coagulation. The bacteria were kept at 0.5 McFarland (10^8 CFU/ml) using a densitometer device (Biosan, Latvia) and antibiotic concentrations were selected using established MIC values (10 μg/ml). Ab-i sample was prepared by adding 0.1 g streptomycin into 100 ml water. Later, bacteria and antibiotics were injected to the MycoCHIP bioreactor. In the on-chip experiment, the left bioreactor was initially filled with antibiotic and bacteria from inlets with a syringe pump at a flow rate of 3.4 and 0.34 μl/min, respectively. On the other hand, bacteria were injected to the right bioreactor with a syringe pump at a flow rate of 3.4 μl/min (no antibiotic addition step). Inlets and outlets were closed with a stopper when the chips were filled by bacteria and antibiotic, and then were kept in the incubator for 24 h.

In order to reduce gas permeability, the chip was immeresed in a water bath. The incubated samples were next placed on the heater at 90 °C for 10 min to perform thermal lysis and DNA fragmentation. For electrochemical measurement, the valves were immediately opened and the fragmented oligonucleotides were directed onto the microelectrodes via the middle microfluidic layers using the syring pump.

2.6. Electrochemical and UV–vis measurements

CV was implemented by sweeping the potential from − 0.4–0.7 V at 100 mV/s scan rates. The SWV and DPV were scanned out from − 0.3–0.5 V with a 100 mV and from 0.1 to 1.3 V with 100 mV amplitudes, respectively. For EIS, the initial potential was set to 100 mV and a range of frequencies between 100 kHz and 0.1 kHz were employed. A portable potentiostat (PalmSens PS4, Houten, the Netherland) was employed for electrochemical measaurements. The experimental Nyquist plot was fitted using the Randles equivalent circuit to determine the charge transfer resistance (R_{ct}). Moreover, to confirm electrochemical results a NanoDrop™ 2000/2000c spectrophotometer was employed (Thermo Scientific™, USA) for the benchmark measurements of the genomic DNA and RNA concentrations.

2.7. Statistical analysis

Paired sample t-test in SPSS software was applied for statistical analysis. Statistical significance threshold was adjusted at 0.05 ($p < 0.05$). Error bars depict a standard deviation of the mean ($n = 9$).

3. Results and discussion

We developed a label-free electrochemical sensor integrated lab-on-a-chip platform for antibiotic susceptibility testing based on nucleic acid detection. In a typical on-chip molecular antibiotic susceptibility test, bacterial lysis and incubation is implemented outside the chip [30–32]. One critical feature of our approach lies in the fact that MycoCHIP can perform label-free testing of antibiotic resistance of *M. smegmatis* based on the binding of a specific 16SrRNA probe to complementary nucleic acid sequences after bacterial incubation (with and without antibiotic), lysis and oligonucleotide fragmentation are performed in the device. In order to accomplish this task, we combined the capability of PDMS-based microfluidic chips with microelectrodes to detect molecular probes electrochemically. Microfluidic chips in this case are utilized as bioreactors.

3.1. Microfluidic chip development

Plug microvalves were used to connect two platforms via a middle microfluidic layer. Güler et al. [27] have applied plug microvalves for two-layer microfluidic chips with PDMS/PDMS and PDMS/PMMA, and PMMA/PMMA structures. In this study, we were able to control the microchannels in our device by using microvalves for PDMS (Bioreactor)/PDMS (detection platform) layers. Herein, we can direct and control the flow from the bioreactor to the electrodes through the microvalve.

Fig. 3 demonstrates the top view of the microvalve on MycoCHIP. As can be seen in Fig. 3a, the valve is in the open position and the flow clearly moves from the valve towards the detection platform. Red dye has been used to highlight the channels. The width and height of the microchannel here is approximately 150 μm and 50 μm, respectively. In Fig. 3b, when the valve is closed, it can be seen that the liquid flow is prevented. Fig. 3c-f depict the move of the flow step by step from the bioreactor to the electrochemistry microchannel. We used a maximum flow rate of 3.4 μl/min in our studies. However, we increased the flow rate of the pump up to 20 μl/min to examine the tightness of the valve. The valve was resistant to a flow rate of 20 μl/min with a 500 μm hole diameter. During the experiment, no leakage was observed around the microvalve. This therefore means, while the valve is open, the fluid flow at rates used in this study occurs without any issue from the bioreactor to the sensing channel.

3.2. Off chip development of microelectrode measurements of antibiotic susceptibility

In this study, electrochemical analysis techniques were performed because of their novel advantages such as simplicity, low cost, high sensitivity, selectivity, and rapid analysis time [33,34] Conventional methods of AST (disk diffusion and microdilution) require initial isolation of the pathogen from clinical samples, delaying per-preparation up to 18 h and couple of days to perform the assay [35,36]. Electrochemical biosensors are well suited for molecular diagnostics and do not need an isolation process from clinical samples. We previously described an electrochemical biosensor based on specific hybridization of 16 S rRNA oligonucleotide sequence of bacteria for the molecular identification of pathogens [37].

A sensing platform using Au-microelectrodes and capable of performing several electrochemical experiments such as EIS, SWV and DPV was used. EIS is a highly sensitive label-free technique that can measure impedimetirc changes that occur at the electrode/liquid interface [38–40]. In this study, off-chip experiments were first carried out as control measurements to examine the applicability of the microelectrodes - in our case to the detection of specific oligonucleotides from *M. smegmatis*. To do so, 0.5 McFarland bacteria were taken using a densitometer and incubated for 24 h at 37 °C. *M.smegmatis* was first lysed off-chip in the same manner as described above. The bacterial lysate was then used in electrochemical measurements, acting as the source of target molecules for detection. Previously we showed the successful label-free detection of antibiotic susceptibility testing of *M. smegmatis* using commercially available screen-printed electrodes [39]. In a similar fashion, we also reported the detection of nucleic acid sequences of *M. Tuberculosis* using microelectrodes [19]. Here in this work, the electrochemical detection of nucleic acid sequences of *M. smegmatis* was investigated using on chip microelectrodes in combination with the incubation and lysis capabilites on MycoCHIP. Successful cleaning of microelectrode surfaces was proven by recording CVs in 1 mM ferri/ferrocyanide ($Fe(CN)6]^{3-/4-}$) (Fig. 4b) and observing the peak-to-peak separation. The peak-to-peak separation of CV-cleaned microelectrodes was around 97 mV, which is not unlike peak separations observed from gold screen printed electrodes but is somewhat above the ideal value of 58 mV (according to the Nernst equation) [41]. Thus, we concluded this level of peak separation was acceptable for a viable sensor, especially given that more rigorous cleaning methods would likely cause delamination of the thin gold layers which comprised the electrode structures. The surface of the electrodes was then

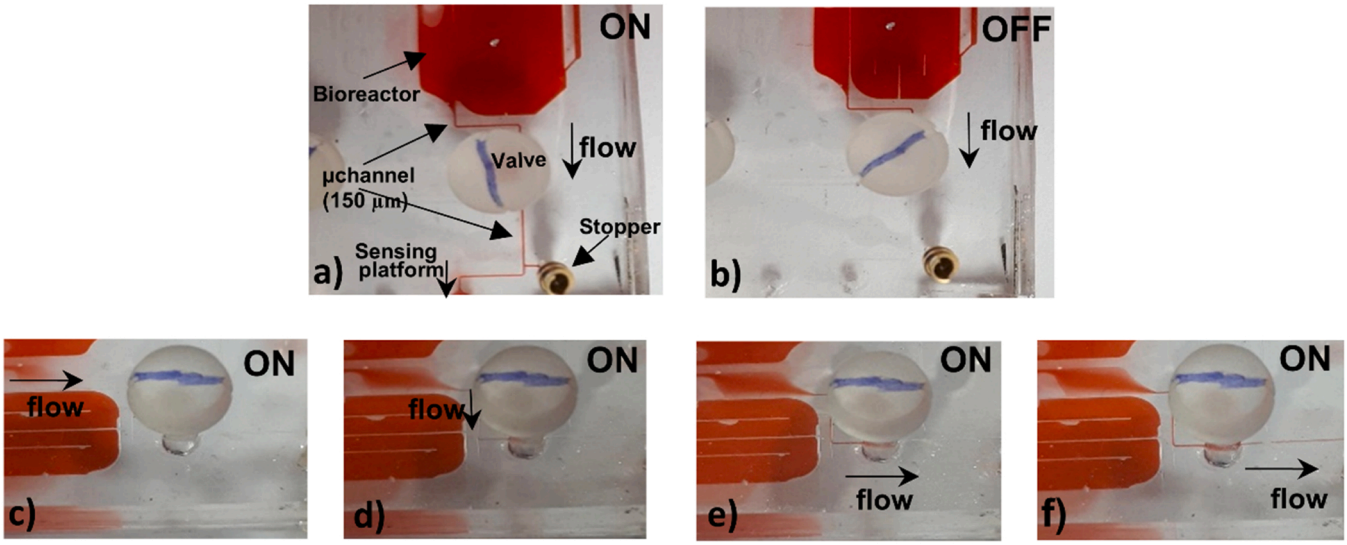

Fig. 3. Top-view photographs of the microvalves integrated onto PDMS/PDMS microchannels. Plug microvalve at a) ON and b) OFF position, c, d, e, and f) image of fluid flowing from bioreactor into microelectrode channel in a controlled manner.

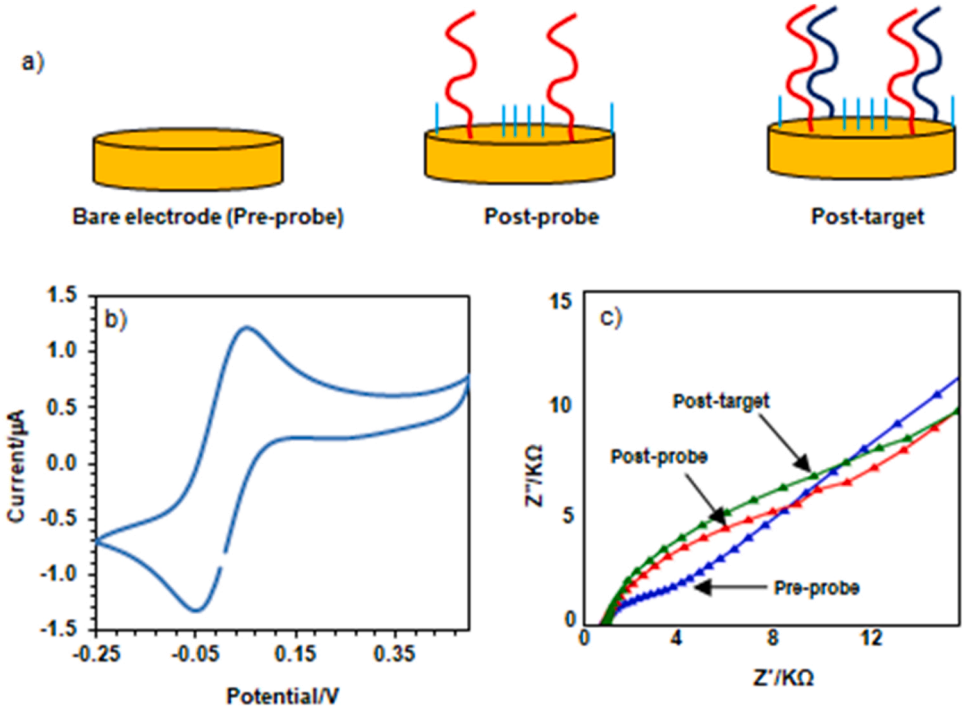

Fig. 4. a) Schematic for the stepwise preparation of working electrode, b) CV voltammogram, c) EIS spectrum.

functionalised with 16srRNA probes specifically designed for capture of nucleic acid sequences from *M. smegmatis*, as depicted in Fig. 4a. The immobilisation method led to a formation of the semi-covalent Au-thiol bond with relatively uniform distribution of the probe on the surface of the electrodes [42]. Successful functionalisation of probe layers was confirmed by a significant increase in the impedance signal. The Nyquist plots obtained from the bare electrode (pre-probe), post-probe functionalization and post-target exposure measurements showed the classic charge transfer semi-circle transitioning into a 45° line. The observed response was characteristic of a typical redox reaction where the R_{ct} increased following immobilization of the probe and hybridization with target molecules, as shown in Fig. 4C. This is a consequence of *M. smegmatis* DNA target hybridising with directly complementary 16 s rRNA probe sequence and therefore the electron transfer at the electrode/liquid interface is reduced. EIS-based detection methodologies have already been reported for *M. tuberculosis* with a DNA aptamer [43] to recognize MPT64 protein and living mycobacteria (*M. bovis BCG* and *M. smegmatis)* by using MGIT™ system [44]. In our method, we were able to perform the detection easily with a simple mechanism for *M. smegmatis* culture and lysis, using microelectrodes, probe immobilization and nucleic acid hybridization. These off chip experiments clearly showed the specific *M.smegmatis* nucleic acid detection could be achieved using the microelectrode sensors when uncoupled from the MycoCHIP device.

3.3. On chip incubation experiments and analysis of nucleic acid levels

Once off-chip testing was completed, we performed the experiments using the full MycoCHIP. First, bacteria were introduced into the right and left bioreactors with and without antibiotic, Ab-i and Ab-n, respectively, until 48 h using an incubator. Once the incubation was considered to be effective, the bacteria was lysed thermally at 50 °C for 20 min. We have already shown the successful thermal lysis conditions of *M. Smegmatis* in a separate study and found that thermal lysis at 50 °C was sufficient [45]. Although lysis of mycobacterium at a temperature above 80 °C is also recommended by many groups, there are similar reports in the literature from off-chip lysis experiments showing lower temperatures and durations can be employed effectively [46–48]. A NanoDrop™ spectrophotometer was applied to measure the genomic DNA and RNA concentrations from incubated samples. Fig. 5 shows the genomic DNA and RNA concentrations in samples collected from the Ab-n and Ab-i *M.smegamtis* cultured for 48 h. Streptomycin was chosen as a test antibiotic, because it can kill bacteria by inhibiting Mycobacterial ribosome protein synthesis [49,50] and is a common first line treatment for TB. As depicted in Fig. 5, genomic DNA and RNA concentrations approximately remained constant for Ab-i experiments for 48 h. While, for Ab-n experiments, genomic DNA and RNA concentrations increased approximately 2 and 4.5-fold in 24 h and 48 h, respectively. Based on the obtained results, it was concluded that *M. smegmatis* was susceptible to the dose of streptomycin antibiotic employed (10 μg/ml).

3.4. On chip development of microelectrode measurements of antibiotic susceptibility

Afterwards, susceptibility testing was investigated on-chip following three steps: bacterial incubation, fragmentation while the microvalve was on and sensing while the microvalve was off. Incubated and fragmented Ab-i and Ab-n samples were sent to the electrochemistry chamber via the middle microfluidic layer after the valve was opened. Pre-electrochemistry experiments were conducted while the valve was off, in parallel to the on-chip microbiology experiments involving the bioreactor. The cleaning performance of the electrode was examined by calculating the potential difference between the two peaks obtained from the CV graph. According to Nernst equation, the ideal value is 58 mV [51] and this value was calculated as 62 mV for on-chip measurements. The calculated value indicates that the cleaning was successfully performed and it is interesting to note that cleaning was more successful when performed on chip in the full MycoCHIP format.

The amount of target hybridisation on the electrode surface was monitored by measuring DPV and SWV responses from the electrodes. We tried to investigate the electrochemical analyses firstly using the EIS technique using MycoCHIP, as in the off-chip experiments. However, noise dominated the EIS reponse,presumably originating by the use of microchannels in combination with the microelectrodes. Since MycoCHIP was designed for in the field use, it was concluded not to continue with EIS and employ pulsed voltametric techniques since it would be impractiacal to employ shielding in the field. Horney et al. [52] previously reported impedance measurements using a 30 μm working electrode in a microfluidic channel [52], however interestingly impedance measurement with a 100 μm working electrode in our case was not successful. This may be due to the different size of the microelectrodes, the small size of the electrodes increases the sensitivity of the measurement [53]. There may possibly be a set of conditions where detection with EIS on the microfluidic integrated microelectrodes is possible, however for clarity in this study we did not continue with EIS detection, instead applied SWV and DPV for further analysis. SWV and DPV techniques have some advantages over other commonly used methods including EIS, as they are more suitable for the application of the point-of-care, where generating AC frequencies and automated analysis of the response would be more complicated and expensive to deploy in the sophisticated device instrumentation [19].

The label-free detection mechanism of the electrochemical assay technique using these approaches is based on the change of interfacial electron transfer kinetics of oxidation and reduction of ferri-ferro upon probe–oligonucleotides binding events. Fig. 6 demonstrates experimental responses and signal changes (target-to-probe ratio) of SWV and DPV analyses. The reason for the high error bars in the results of this study can be mentioned as lack of bacteria purification and non-optimality of the used channels and electrodes dimensions. Figs. 6b and 6d show the experimental responses of SWV and DPV pre-probe, post-probe functionalisation and post-target exposure. Figs. 6c and 6e display SWV and DPV signal change, where blue and orange columns are complementary and non-complementary (NC) probes, respectively. The Ab-i sample showed lower signal change than antibiotic Ab-n sample in SWV analysis, brought about by differential 16SrRNA expression and differences in total nucleic acid content when compared to bacteria not incubated with antibiotic. However, statistical significant could not be established (The T-Test demonstrates that the p-value is higher than 0.05). The amount of oligonucleotides captured on the immobilised electrode surface is often proportional to the oligonucleotides in the solution [54]. In addition, DPV analyses also support the SWV results. SWV displays both a higher signal change and a lower variation than DPV likely because SWV is more sensitive, a finding consistent with previous research [19]. The MycoCHIP demonstrates specific and sensitive electrochemical-based assay for *M. smegmatis* based on the use of a specific Mycobacterial 16SrRNA probe sequence. We also have previously reported antibiotic susceptibility test with EIS and SWV techniques for *M. smegmatis* using screen printed electrode [39]. Though SWV signals obtained using the MycoCHIP showed higher signal change and variation in comparison with off chip experiments performed using

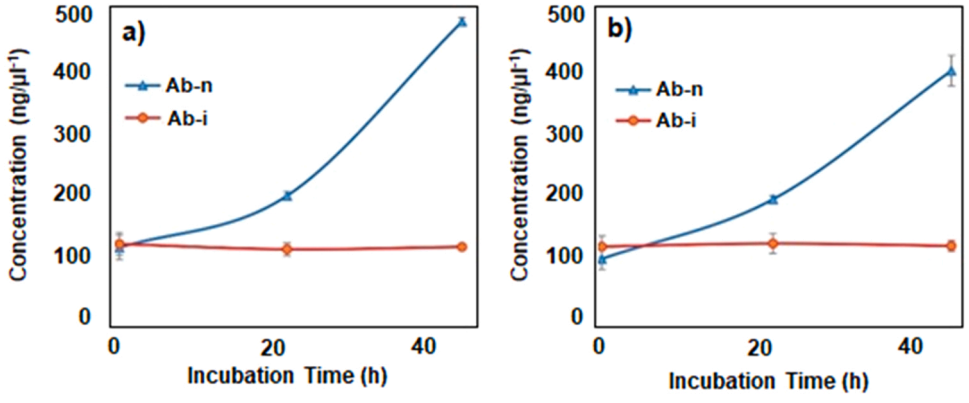

Fig. 5. NanoDrop results tested over 48 h. a) DNA and b) RNA concentrations at the beginning, after 24 and 48 h.

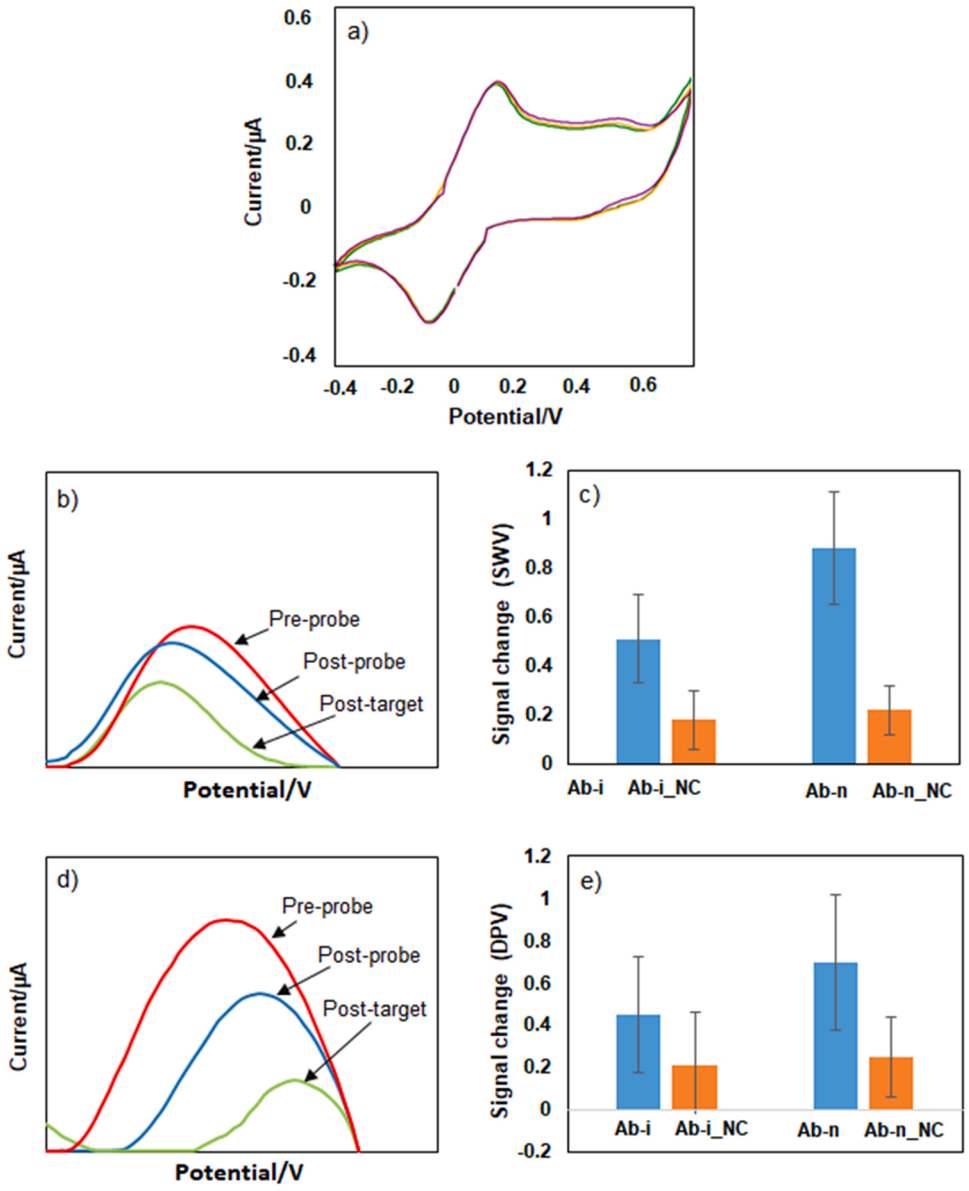

Fig. 6. Graphs showing SWV and DPV results. a) CV voltammogram, c and e) The experiment responses of SWV and DPV measurements, b) and d) average SWV and DPV signal changes after exposure to target using fully complemtanry probes and non-specific probes (used as control measurements). Error bars represent standard error of the mean (n = 9), $p > 0.05$. (The pre-probe, post-probe, and post-target were chosen among 9 experiments performed to better illustrate).

screen-printed electrodes. High single change is probably owing to the use of microelectrodes and high variation could be due to presence of microchannels and microelectrodes dimension. Both enhanced sensitivity and higher variation in measurements have been previously observed when using microelectrodes for DNA detection in this manner.

In this study we used 10^8 CFU/ml as initial concentration to detect and determine the susceptibility and resistance. In the literature, detection limit of *M. tuberculosis* was obtained as 10^4 CFU/ml with electrical impedance spectroscopy in suspensions using interdigitated microelectrode [55]. Therefore, we consider that MycoCHIP device can perform AST experiments up to 10^4 CFU/ml level, in the event that optimization of the chip is further considered.

Finally, we believe that our results are consistent with the above electrochemical and UV-Vis off-chip control experiments results. Here as a proof-of-concept, it is possible to incubate a bacterial sample, fragment and then quickly determine the susceptibility of *M. smegmatis* to streptomycin within 24 h. *M. tuberculosis* divides almost every 18 h on average and *M. smegmatis* divides every 3–5 h [56]. Jeon et al. [57] already reported on chip AST with LCR Meter device for E. coli samples [57]. They proved AST in 150 min based on their evaluated bacterila levels. Safavieh et al. [58] developed an on-chip biosensor for rapid (<90 min), real-time and label-free bacteria isolation from conventional blood and antibiotic susceptibility tests [58]. The target bacteria in their study are captured with screen-printed electrodes using antibodies and the electrical response is monitored in the presence and absence of antibiotics after an hour of incubation. Moreover, Yang et al. [59] applied a lock-in amplifier to evaluate AST in microfluidic chip [59]. They reported the electrical resistance of the microchannels by measuring the number of bacteria in the microchannels. In the literature, there are various methods to apply on chip AST. For example, Ma et al. [60] reported an on-chip AST following conventional agar dilution methods to detect *Campylobacter* bacteria [60]. Dong et al. [61] utilized a microfluidic chip system to personalize antibiotic treatment of urinary tract infections by using metabolic activity indicators (ATP bioluminescence assay) [61]. We designed a comprehensive microfluidic chip in this study. All operations are carried out within the chip, so it leads to a less

laborious assay which still represents a significant time saving and leaves less opportunity for contamination.

4. Conclusion

In this study, the development of an on-chip label-free molecular-based antibiotic susceptibility test for the compariatively slow-growing *M. smegmatis* (doubling time 3–4 h), MycoCHIP, was presented. MycoCHIP consists of a bioreactor for bacterial incubation, lysis and a microelectrode functionalised with nucleic acid sequences specific for the bacteria of interest. The connection between the two platforms were provided through a microvalve and a middle microfluidic layer. The valves developed on the chip made the opening and closing operations of the microchannels easier between the incubation chambers and detection platform with high quality. Hybridization of genetic sequences and detection thereafter were successfully completed. Electrochemistry measurements proved that the rapid, qualitative analysis of such slow-growing bacteria was possible. The target binding and measurement processes took ~80 min. We then went on to show that *M. smegmatis* was susceptible to the streptomycin antibiotic as also confirmed by UV–vis spectroscopy and electrochemical methods. In short, MycoCHIP proves to be capable of performing antibiotic susceptibility testing of *M. smegmatis* on a single chip within 24 h. We are confident that the concept of the MycoCHIP detection scheme can be easily applied to other bacterial strains and further functionalised for multiplexed detection such as drug and multi-drug resistant TB detection in low resource settings.

CRediT authorship contribution statement

Hamed Ghorbanpoor: Conceptualization, Methodology, Supervision, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Araz Norouz Dizaji:** Investigation, Methodology, Investigation, Writing – review & editing, **Iremnur Akcakoca:** Investigation, Writing. **Ewen O.Blair:** Investigation, Methodology, Writing – review & editing. **Yasin Ozturk:** Investigation, **Paul Hoskisson**: Investigation. **Tanil Kocagoz:** Investigation, **Huseyin Avcı:** Conceptualization, Methodology, Supervision, Investigation. **Damion K Corrigan:** Conceptualization, Methodology, Supervision, Investigation, Writing – review & editing. **Fatma Dogan Guzel:** Conceptualization, Methodology, Supervision, Investigation, Writing – review & editing, Visualization, Resources, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] C.L.J.P. Ventola, therapeutics, The antibiotic resistance crisis: part 1: Causes and threats, P&T 40 (2015).
[2] A. Norouz Dizaji, D. Ding, T. Kutsal, M. Turk, D. Kong, E.J.J. o Piskin, B.S. Polymer Edition, In vivo imaging/detection of MRSA bacterial infections in mice using fluorescence labelled polymeric nanoparticles carrying vancomycin as the targeting agent, J. Biomater. Sci. Polym. Ed. 31 (2020) 293–309.
[3] P. McGann, E. Snesrud, R. Maybank, B. Corey, A.C. Ong, R. Clifford, M. Hinkle, T. Whitman, E. Lesho, K.E. Schaecher, Escherichia coli Harboring mcr-1 and blaCTX-M on a Novel IncF plasmid: First report of mcr-1 in the United States, Antimicrob. Agents Chemother. 60 (2016) 4420–4421.
[4] J.M. Blair, M.A. Webber, A.J. Baylay, D.O. Ogbolu, L.J.J.N. Piddock, Molecular mechanisms of antibiotic resistance, Nat. Rev. Microbiol. 13 (2015) 42–51.
[5] C.-R. Lee, I.H. Cho, B.C. Jeong, S.H.J.I. j o e r Lee, health, p, Strategies to minimize antibiotic resistance, Int. J. Environ. Res. Public Health 10 (2013) 4274–4305.
[6] Khan, Z.A., Siddiqui, M.F., Park, S.J. D., Current and emerging methods of antibiotic susceptibility testing. 9, 49, 2019.
[7] J. Fonseca, G. Knight, T.D. McHugh, The complex evolution of antibiotic resistance in Mycobacterium tuberculosis, Int. J. Infect. Dis. 32 (2015) 94–100.
[8] T. Smith, K.A. Wolff, L. Nguyen, Molecular Biology of Drug Resistance in Mycobacterium Tuberculosis, Springer, 2012, pp. 53–80.
[9] R.M. Houben, P.J. Dodd, The global burden of latent tuberculosis infection: A re-estimation using mathematical modelling, PLoS Med. 13 (2016), e1002152.
[10] L.B. Reller, M. Weinstein, J.H. Jorgensen, M.J.J.C. i d Ferraro, Antimicrobial susceptibility testing: A review of general principles and contemporary practices, Clin. Infect. Dis. 49 (2009) 1749–1755.
[11] J. Choi, Y.-G. Jung, J. Kim, S. Kim, Y. Jung, H. Na, S.J.L. o a C. Kwon, Rapid antibiotic susceptibility testing by tracking single cell growth in a microfluidic agarose channel system, Lab. Chip 13 (2013) 280–287.
[12] Y. Tang, L. Zhen, J. Liu, J.J.A. Wu, Rapid antibiotic susceptibility testing in a microfluidic pH sensor, Anal Chem 85 (2013) 2787–2794.
[13] D.K. Corrigan, H. Schulze, G. Henihan, I. Ciani, G. Giraud, J.G. Terry, A.J. Walton, R. Pethig, P. Ghazal, J.J.B. Crain, Impedimetric detection of single-stranded PCR products derived from methicillin resistant Staphylococcus aureus (MRSA) isolates, Biosens. Bioelectron. 34 (2012) 178–184.
[14] F.D. Guzel, F. Citak, Development of an On-Chip antibiotic permeability assay with single molecule detection capability, IEEE transactions on nanobioscience 17 (2018) 155–160.
[15] S. Kim, F. Masum, J.S.J.B.J. Jeon, Recent developments of chip-based phenotypic antibiotic susceptibility testing, BioChip J. 13 (2019) 43–52.
[16] D. Corrigan, H. Schulze, G. Henihan, A. Hardie, I. Ciani, G. Giraud, J. Terry, A. Walton, R. Pethig, P.J.A. Ghazal, Development of a PCR-free electrochemical point of care test for clinical detection of methicillin resistant Staphylococcus aureus (MRSA), Analyst 138 (2013) 6997–7005.
[17] G. Henihan, H. Schulze, D.K. Corrigan, G. Giraud, J.G. Terry, A. Hardie, C. J. Campbell, A.J. Walton, J. Crain, R.J.B. Pethig, Bioelectronics, Label-and amplification-free electrochemical detection of bacterial ribosomal, RNA 81 (2016) 487–494.
[18] H.L. Woodvine, J.G. Terry, A.J. Walton, A.R.J.A. Mount, The development and characterisation of square microfabricated electrode systems, Analyst 135 (2010) 1058–1065.
[19] Blair, E.O., Hannah, S., Vezza, V., Avcı, H., Kocagoz, T., Hoskisson, P.A., Güzel, F. D., Corrigan, D.K. J.S., Reports, A., Biologically modified microelectrode sensors provide enhanced sensitivity for detection of nucleic acid sequences from Mycobacterium tuberculosis. 100008, 2020.
[20] G.M.J.N. Whitesides, The origins and the future of microfluidics, Orig. Future Microfluid. 442 (2006) 368–373.
[21] L.R. Volpatti, A.K. Yetisen, Commercialization of microfluidic devices, Commer. Microfluid. Devices 32 (2014) 347–350.
[22] J. Melin, S.R. Quake, Microfluidic large-scale integration: the evolution of design rules for biological automation, Annu. Rev. Biophys. Biomol. Struct. 36 (2007) 213–231.
[23] D.B. Weibel, W.R. DiLuzio, G.M. Whitesides, Microfabrication meets microbiology, Nat. Rev. Microbiol. 5 (2007) 209–218.
[24] K.T. Tran, T.D. Nguyen, Lithography-based methods to manufacture biomaterials at small scales, J. Sci. Adv. Mater. Devices 2 (2017) 1–14.
[25] R. Riahi, S.A.M. Shaegh, M. Ghaderi, Y.S. Zhang, S.R. Shin, J. Aleman, S. Massa, D. Kim, M.R. Dokmeci, A.. Khademhosseini, Automated microfluidic platform of bead-based electrochemical immunosensor integrated with bioreactor for continual monitoring of cell secreted biomarkers, Sci. Rep. 6 (2016) 1–14.
[26] S.R. Shin, Y.S. Zhang, D.-J. Kim, A. Manbohi, H. Avci, A. Silvestri, J. Aleman, N. Hu, T. Kilic, W. Keung, Aptamer-based microfluidic electrochemical biosensor for monitoring cell-secreted trace cardiac biomarkers, Anal. Chem. 88 (2016) 10019–10027.
[27] M.T. Guler, P. Beyazkilic, C.J.S. Elbuken, A versatile plug microvalve for microfluidic applications, Sens. Actuators A Phys. 265 (2017) 224–230.
[28] F.D. Güzel, B.J.M. Miles, N. Letters, Development of in-flow label-free single molecule sensors using planar solid-state nanopore integrated microfluidic devices, Micro Nano Lett. 13 (2018) 1352–1357.
[29] A. Lamberti, S. Marasso, M.J.R.A. Cocuzza, PDMS membranes with tunable gas permeability for microfluidic applications, RSC Adv. 4 (2014) 61415–61419.
[30] Ö. Baltekin, A. Boucharin, E. Tano, D.I. Andersson, J. Elf, Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 9170–9175.
[31] C.M. Kim, E.S. Song, H.J. Jang, H.-J. Kim, S. Lee, J.H. Shin, S.J. Kim, S.H. Jeong, J. Jeong, K. Koh, Development and evaluation of oligonucleotide chip based on the 16S–23S rRNA gene spacer region for detection of pathogenic microorganisms associated with sepsis, J. Clin. Microbiol. 48 (2010) 1578–1583.
[32] Z. Li, R. Ju, S. Sekine, D. Zhang, S. Zhuang, Y.J.L. Yamaguchi, All-in-one microfluidic device for on-site diagnosis of pathogens based on an integrated continuous flow PCR and electrophoresis biochip, Lab Chip 19 (2019) 2663–2668.

[33] A.N. Dizaji, Z. Ali, H. Ghorbanpoor, Y. Ozturk, I. Akcakoca, H. Avci, F.D. Guzel, Electrochemical-based ''antibiotsensor'' for the whole-cell detection of the vancomycin-susceptible bacteria, Talanta 234 (2021), 122695.
[34] F.D. GUZEL, I. Akcakoca, H. Ghorbanpoor, A.N. Dizaji, Y. ÖZTÜRK, E. Blair, T. KOCAGOZ, D. Corrigan, A. Hüseyin, Highly sensitive label-free electrochemical detection of heat shock protein with low-cost screen-printed electrodes, Eskişeh. Tech. Univ. J. Sci. Technol. A Appl. Sci. Eng. 22 (2021) 344–352.
[35] K.E. Mach, R. Mohan, E.J. Baron, M.-C. Shih, V. Gau, P.K. Wong, J.C. Liao, A biosensor platform for rapid antimicrobial susceptibility testing directly from clinical samples, J. Urol. 185 (2011) 148–153.
[36] M. Rawat, M. Uppal, G. Newton, M. Steffek, R.C. Fahey, Y. Av-Gay, Targeted mutagenesis of the Mycobacterium smegmatis mca gene, encoding a mycothiol-dependent detoxification protein, J. Bacteriol. 186 (2004) 6050–6058.
[37] D. Corrigan, H. Schulze, G. Henihan, A. Hardie, I. Ciani, G. Giraud, J. Terry, A. Walton, R. Pethig, P. Ghazal, Development of a PCR-free electrochemical point of care test for clinical detection of methicillin resistant Staphylococcus aureus (MRSA), Analyst 138 (2013) 6997–7005.
[38] S.O. Akbulut, H. Ghorbanpoor, B.Ö. İpteç, A. Butterworth, G. Avcıoğlu, L. D. Kozacı, G. Topateş, D.K. Corrigan, H. Avcı, F.D. Güzel, Impedance testing of porous Si3N4 scaffolds for skeletal implant applications, SN Appl. Sci. 2 (2020) 823.
[39] F.D. Güzel, H. Ghorbanpoor, A.N. Dizaji, I. Akcakoca, Y. Ozturk, T. Kocagoz, D. K. Corrigan, H. Avci, Label-free molecular detection of antibiotic susceptibility for Mycobacterium smegmatis using a low cost electrode format, Biotechnol. Appl. Biochem. 68 (2020) 1159–1166, https://doi.org/10.1002/bab.2037.
[40] J. Kaur, H. Ghorbanpoor, Y. Öztürk, Ö. Kaygusuz, H. Avcı, C. Darcan, L. Trabzon, F. D.J.I.N. Güzel, Extending the depth of human plasma proteome coverage using simple fractionation techniques, J. Proteome Res. 20 (2021) 1261–1279.
[41] A.J. Bard, L.R.J.E.M. Faulkner, Fundam. Appl. 2 (2001) 580–632.
[42] S.B. Nimse, K. Song, M.D. Sonawane, D.R. Sayyed, T.J.S. Kim, Immobilization techniques for microarray: challenges and applications, Sensors 14 (2014) 22208–22229.
[43] I. Strauss, G. Valle, F. Artoni, E. D'Anna, G. Granata, R. Di Iorio, D. Guiraud, T. Stieglitz, P.M. Rossini, S. Raspopovic, Characterization of multi-channel intraneural stimulation in transradial amputees, Sci. Rep. 9 (2019) 1–11.
[44] R. Kargupta, S. Puttaswamy, A.J. Lee, T.E. Butler, Z. Li, S. Chakraborty, S. Sengupta, Rapid culture-based detection of living mycobacteria using microchannel electrical impedance spectroscopy (m-EIS), Biol. Res. 50 (2017) 1–11.
[45] A.N. Dizaji, Y. Ozturk, H. Ghorbanpoor, A. Cetak, I. Akcakoca, T. Kocagoz, H. Avci, D. Corrigan, F.D. Guzel, Investigation of the effect of channel structure and flow rate on On-Chip bacterial lysis, IEEE Trans. NanoBioscience 20 (2021) 86–91.
[46] P. Shetty, D. Ghosh, D. Paul, Thermal lysis and isothermal amplification of Mycobacterium tuberculosis H37Rv in one tube, J. Microbiol. Methods. 143 (2017) 1–5.
[47] W.J. Valente, E. Pienaar, A. Fast, A. Fluitt, S.E. Whitney, R.J. Fenton, R.G. Barletta, O. Chacon, H.J. Viljoen, Kinetic study of in vitro lysis of mycobacterium smegmatis, a kinetic study of in vitro lysis of mycobacterium smegmatis, Chem. Eng. Sci. 64 (2009) 1944–1952.
[48] M. Miyata, A.C.B. Santos, N.H. Mendes, E.A. Cunha, F.A.F Melo, C.Q.F. Leite, Assessment of the quality of dna extracted by two techniques from Mycobacterium tuberculosis for fast molecular identification and genotyping, Braz. J. Microbiol. 42 (2011) 774–777.
[49] P. Ruiz, F. Rodriguez-Cano, F. Zerolo, M. Casal, Investigation of the in vitro activity of streptomycin against Mycobacterium tuberculosis, Microb. Drug Resist. 8 (2002) 147–149.
[50] L. Bryan, Bacterial resistance and susceptibility to chemotherapeutic agents, CUP Arch. (1982).
[51] A. Butterworth, E. Blues, P. Williamson, M. Cardona, L. Gray, D.K.J.B. Corrigan, SAM composition and electrode roughness affect performance of a DNA biosensor for antibiotic resistance, Biosensors 9 (2019) 22.
[52] M.-C. Horny, M. Lazerges, J.-M. Siaugue, A. Pallandre, D. Rose, F. Bedioui, C. Deslouis, A.-M. Haghiri-Gosnet, J. Gamby, Electrochemical DNA biosensors based on long-range electron transfer: investigating the efficiency of a fluidic channel microelectrode compared to an ultramicroelectrode in a two-electrode setup, Lab Chip 16 (2016) 4373–4381.
[53] P.Q. Li, A. Piper, I. Schmueser, A.R. Mount, D.K.J.A. Corrigan, Impedimetric measurement of DNA–DNA hybridisation using microelectrodes with different radii for detection of methicillin resistant Staphylococcus aureus (MRSA), Analyst 142 (2017) 1946–1952.
[54] S.R. Shin, T. Kilic, Y.S. Zhang, H. Avci, N. Hu, D. Kim, C. Branco, J. Aleman, S. Massa, A.J.A.S. Silvestri, Label-free and regenerative electrochemical microfluidic biosensors for continual monitoring of cell secretomes, Adv. Sci. 4 (2017), 1600522.
[55] A. Pourmir, A. Bahrmand, S. Ettefagh Far, A. Hadizadeh Tasbiti, S. Yari, Rapid diagnosis of mycobacterium tuberculosis with electrical impedance spectroscopy in suspensions using interdigitated microelectrode, J. Anal. Chem. 71 (2016) 676–684.
[56] A. Verma, A.K. Sampla, J.S. Tyagi, Mycobacterium tuberculosis rrn promoters: differential usage and growth rate-dependent control, J. Bacteriol. 181 (1999) 4326–4333.
[57] H. Jeon, A. Khan, Z. Barakat, S. Park, Label-free electrochemical microfluidic chip for the antimicrobial susceptibility testing, Antibiotics 9 (2020) 348.
[58] M. Safavieh, H.J. Pandya, M. Venkataraman, P. Thirumalaraju, M. K. Kanakasabapathy, A. Singh, D. Prabhakar, M.K. Chug, H. Shafiee, Rapid real-time antimicrobial susceptibility testing with electrical sensing on plastic microchips with printed electrodes, ACS Appl. Mater. Interfaces 9 (2017) 12832–12840.
[59] Y. Yang, K. Gupta, K.L. Ekinci, All-electrical monitoring of bacterial antibiotic susceptibility in a microfluidic device, PNAS 117 (2020) 10639–10644.
[60] L. Ma, M. Petersen, X.J.A. Lu, Identification and antimicrobial susceptibility testing of campylobacter using a microfluidic lab-on-a-chip device, Appl. Environ. Microbiol. 86 (2020).
[61] T. Dong, X. Zhao, Rapid identification and susceptibility testing of uropathogenic microbes via immunosorbent ATP-bioluminescence assay on a microfluidic simulator for antibiotic therapy, Anal. Chem. 87 (2015) 2410–2418.

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