Research Article

Microchip-based wearable monitoring system for the optimisation of antimicrobial dosing

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IMSE Research Article

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Antimicrobial resistance poses a great threat to global health. β-lactam is a widely used antibiotic in clinical practice, but overuse of β-lactam can produce β-lactamase in the human body, which hydrolyzes and inactivates the susceptible antibiotcs, resulting in drug resistance. Some antibiotics are secreted in sweat; therefore, sweat sampling provides a great opportunity to reduce unnecessary usage of drugs by optimising antimicrobial dosing, which can help maximize the effectiveness of antibiotics and minimize their toxicity when treating bacterial infections. This article introduces two novel methods to measure the concentration of benzylpenicillin (penicillin G) in sweat: (1) electrochemical detection with the penicillin-binding aptamer as a biological component, which can detect β-lactam concentrations from 1 µM to 200 µM and (2) colorimetry for visual readout using pH indicators to detect pH changes generated by the reaction between β-lactamase and penicillin G. We evaluated and tested the sensitivity of penicillin-binding aptasensor and enzyme-based sensors to penicillin G concentrations. The electrochemical sensor can be used to measure β-lactam concentration in sweat. In the colorimetric sensing method, phenol red, neutral red and bromothymol blue (100 µM) in phosphate-buffered saline (PBS, pH 7.4) served as pH indicators, the colour change resulting from the reaction between penicillin G (100 µM) and β-lactamase (167 mg/ml) can be recognized by the naked eye. For the sweat sampling requirements of the sensors, two microfluidic cells were fabricated using 3D printing technology, the multilayered design allowed for sweat collection and avoided sample contamination. In addition, a hydrophilic tape was pasted on the microchannels to guide the flow of sweat into the chamber by capillary action. The device combined research in the fields of electronics, colorimetric assays, and microfluidics to establish several key design considerations and performance attributes. The combination of sensors and microfluidics provide chances to develop wearable devices to monitor drug levels in a non-invasive, real-time, rapid, and convenient way, which could combat antimicrobial resistance and protect patients from toxic side effects.

1. Introduction

Many of the miracles and developments in medical science can be linked to the discovery and application of antibiotics. However, with the widespread and unabridged use of antimicrobials in clinical practice, antimicrobial resistance (AMR) emerged which not only makes the usage of antibiotics critical, but also the emergence of "super-resistant bacteria" poses a serious threat to global health.[1] Most antibiotics, especially when administered orally, are given on a fixed-dose basis.[2] This strategy risks overdosing or underdosing because the dosage of antibiotics varies according to weight, height, and age of the individual. Although therapeutic drug monitoring (TDM) can be used to maximize the effectiveness of antibiotics while minimizing their toxicity when treating infections[3] based on the relationship between antibiotic concentration and effectiveness,[4] a simple, reliable, rapid, non-invasive, and laboratory-independent TDM technology is still needed. For the assessment of antimicrobial dosing, recent research has focused on biofluids such as interstitial fluid,[5] urine[6], and sweat.[7] Sweat contains a wealth of biomarkers that could provide continuous clinical data for health monitoring, medical diagnosis, and treatment.[8-10] For

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example, monitoring the chloride concentration in sweat has been historically used as the clinical gold standard for cystic fibrosis in infants;[11] the loss of sodium and chloride in sweat reflects the electrolyte balance, which can be used in sports physiology. Compared to blood collection by puncturing the skin, sweat can be collected naturally, continuously, and non-invasively, which provides the opportunity for clinical diagnosis using wearable platforms. Since antibiotics are distributed in biofluids and tissues,[12] drugs like β-lactams[13] as well as rifampicin[14] are excreted through sweat glands. In this context, we conducted studies using biosensors to measure β-lactam concentration in sweat for dose optimisation.

Biosensor consists of a molecular recognition system with a biological component (such as enzyme, antibody, antigen, DNA) and a transducer that transforms bioreceptor output into a readable signal for obtaining useful information like concentration of an analyte in samples. As a bioreceptor, the aptamer is generated from random single-stranded oligonucleotide library through an in vitro technique named systematic evolution of ligands by exponential enrichment (SELEX).[15] After being screened and enriched by SELEX, aptamers can match the sensitivity of antigen-antibody reactions and are easier to synthesize and more stable.[16] Paniel et al.[17] selected aptamers for penicillin, which can work using electrochemical impedance spectroscopy in a wide range of penicillin concentrations (from 0.4 μg/l to 1000 μg/l). Nucleic acid aptamers are widely used in the field of biosensors because of their high specificity and selectivity in binding with a variety of targets.[18] When the aptamer specifically binds to the target, the configuration of the aptamer itself will change accordingly. Based on the configuration changes of the aptamer, many electrochemical sensors have been developed, known as electrochemical aptasensors, which provide the desired operability, low cost, and sensitivity in sample analysis.

Enzymes are proteins or RNAs produced by living cells that are highly specific and catalytic to their substrates. Because of enzymes, some chemical reactions can be carried out with high efficiency and specificity even under mild conditions. β-lactamase can hydrolyze and inactivate β-lactam rings, resulting in local pH changes. Gowers et al.[19] fabricated a microneedle-based sensor coated with a pH-sensitive iridium oxide layer to detect such pH changes. Additionally, colorimetric sensors can be used for both quantitative and qualitative analysis to optically indicate the presence of a certain chemical substrate (like hydrogen ions). Therefore, in this work, the pH changes generated by the reaction of antibiotics and β-lactamase were detected by colorimetric assay. The key to colorimetric analysis is to choose the appropriate reaction and control the appropriate reaction conditions. The colorimetric sensing method has the advantage of detecting targets without electrical contacts, leading to flexible design and portable usage; however, such a single-use patch has disadvantages such as lack of continuous monitoring and low measurement accuracy compared to electrochemical sensors.

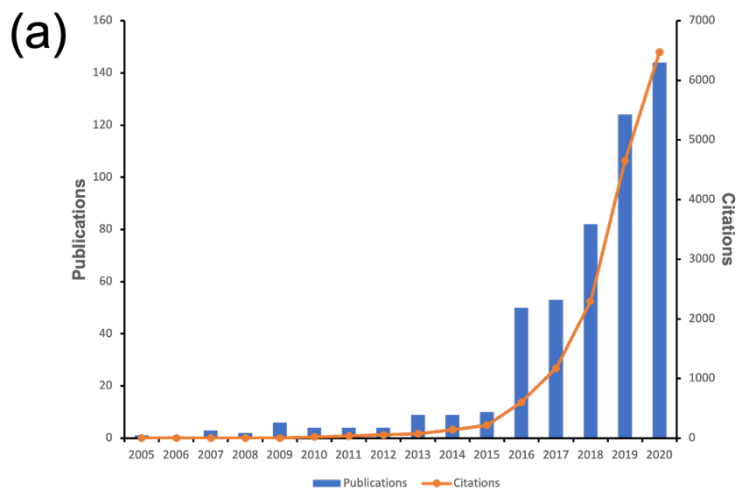
The main aim of the project is to develop wearable sweat sensors to measure β-lactam concentration, so a flexible device is needed for sweat collection. Though there are a couple of issues using sweat as a biofluid including sweat evaporation, contamination, or biodegradation, a microfluidic flow cell can address these problems. Microfluidic chips can be used to capture, store sweat naturally released from the surface of the skin as it can softly seal to the skin in a way that allows sweat glands to pump sweat into microfluidic networks.[20] With the continuous multidisciplinary development of technology in microfluidic materials, fabrication methods and analysis mechanisms, wearable microfluidics for healthcare monitoring have emerged over the past decade.[21] While soft lithography is the mainstream fabrication method for wearable microfluidic sensors as it can provide excellent resolution; the manufacture is limited by the high cost of advanced equipment and the requirement of a cleanroom. The development of 3D printing technology has had a huge impact over microfluidics as it allows people without intense training to design their chips in a cheap and rapid manner.[22]

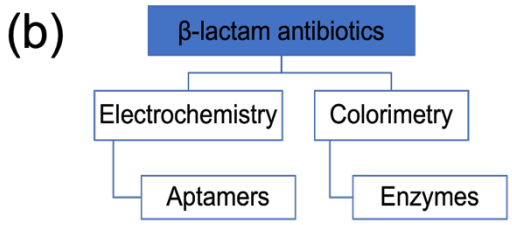
Benzylpenicillin (Penicillin G) is a β-lactam that is clinical mainly used for the treatment of tonsillitis, otitis media, bronchitis, scarlet fever, and other infections. Here, we use two different methods to detect penicillin G in sweat, that is, bonding aptamers to gold nanoparticles (AuNPs) and electrochemically transducing them to obtain electrical signals; β-lactamase is used such that the pH in aliquots in the presence of drugs changes and the colour changes are detected by pH indicators. The most challenging part is the low antibiotic concentration in sweat because of many cellular barriers leading to higher dilution of larger molecules like antibiotics.[23] This research project is extremely significant as optimization of antibiotic doses using TDM technology can have a major impact in combatting AMR.

2. Methodology

2.1. Sweat sensing analysis

Commercial health monitors mainly track physical activities and vital signs like heart rate or movements, which only





(c)

Key words	Number of publications
beta-lactam	22,357
beta-lactam + aptamers	9
beta-lactam + aptamers + sweat	0

(d)

Key words	Number of publications
beta-lactam	22,357
beta-lactam + enzymes	1,800
beta-lactam + enzymes + sweat	0

Figure 1. Literature survey on wearable sweat sensors and the main objectives of the project. (a) Research articles published on wearable sweat sensors according to Web of Science, the numbers of publications and citations have undergone a rapid increase over the past five years. (b) Two main aims of the project, electrochemical sensing using aptamers and colorimetric sensing with β-lactamase. (c) The number of publications by searching beta-lactam, aptamers, and sweat on Web of Science. (d) The results of searching beta-lactam, enzymes, and sweat on Web of Science.

provide limited insights into the ongoings in a human body. Physiological monitoring at molecular levels can help people pursue and lead a healthier lifestyle.[24] As a representative biofluid, sweat contains many biomarkers relevant to human health, such as (1) electrolytes: sodium (Na^+), chloride (Cl^-), potassium (K^+), hydrogen (H^+), and calcium (Ca^{2+}), etc; (2) metabolites: lactate, glucose, urea, and uric acid, etc; (3) xenobiotics: heavy metals (Cu, Hg, Pb) and ethanol, etc. With the advances in artificial intelligence algorithms, electronics, electrochemical biosensors, optical sensors, and microfluidics, wearables sweat sensing has seen tremendous increase (nearly 10-fold) in publications over the past five years (Figure 1a). The biomarkers available for testing in sweat are already well documented,[25, 26] but the clinical worth of a lot of analytes like antibiotics is still underdeveloped. Brasier et al.[27] proved the concept that sweat analysis could be used as a reliable, rapid, non-invasive method to measure antibiotics concentration and may serve as the first TDM method. Figure 1b shows a brief plan for sweat sensing in this project. Electrochemical sensing using aptamers and colorimetric sensing using enzymes based on sweat samples have not been published yet (Figure 1c, d), so our project provides a novel method to measure antibiotic concentration for dose optimization.

Table 1 presents the concentrations of β-lactam antibiotics in eccrine sweat after taking standard doses.[13] After calculation, the concentration of penicillin G in sweat is approximately 7.3 μM; therefore, our research aims at detecting β-lactam concentration in the range 1 - 10 μM. The lowest concentration of penicillin that the aptamer can detect is 1.2 nM, and the limit of detection of β-lactamase is 6.8 μM, so β-lactam antibiotics in sweat can theoretically be detected using the method mentioned in this article. After reviewing the literature on sweat composition[28] and artificial sweat sensing using electrochemical sensors, an artificial sweat

Table 1. β-lactam antibiotics concentration in eccrine sweat.

Antibiotics	Location	Concentration (μg/ml)
Ceftazidime	Axilla	28.4
Ceftazidime	Forearm	11
Ceftriaxone	Axilla	8.9
Ceftriaxone	Forearm	2.5
Benzylpenicillin	Axilla	2.6-0.1
Phenoxymethylpenicillin	Axilla	0.4

Table 2. Composition of Artificial Sweat.

Component	Concentration (g/l)
Sodium Chloride	1.81
Potassium Chloride	0.455
Ammonium Hydroxide	0.268
Urea Solution	0.60
Uric Acid	9.92×10^{-3}
Lactic Acid	1.26
D+ Glucose	30.6×10^{-3}
Ascorbic acid	1.76×10^{-3}
L-Glutamic Acid	54.4×10^{-3}

solution was generated with components shown in Table 2. The solution was prepared by adding 500 mL of deionised water (DI water) to a beaker on a mixing platform, then adding electrolyte components, followed by the metabolites and organic acids, finally topped up to 1 L with DI water. This solution was used to verify the feasibility of using aptamers to detect penicillin G in human sweat.

2.2. Depositing penicillin-binding aptamers on gold electrodes

The aptamer 5'-GAGATGTAGAT***GAGGCTCGATC***CGAATGCGTGACGTCATCGGAATACTCGTTTTTACGCCT-3' (Biomers, bold italic indicates docking sequence, 12n) was probed on to the sensor surface as an affinity reagent. It was achieved by coating the surface of the working electrode with a solution containing aptamers of interest to form a self-assembled monolayer (SAM).[9] To avoid any non-specific binding, 6-Mercapto-1-hexanol (MCH) was used to block aptamer-free open surface area.[29] The as attached aptamer on the AuNPs is stable for several months. The detailed process was graphically illustrated in Figure 2a.

Typically, aptamers are bonded to AuNPs,[30] here, we used gold electrodes to test the performance of the aptamer. The bulk gold electrodes (working electrodes) were polished with 1uM, 0.3 uM, 0.05 uM alumina respectively, and ultrasonically cleaned twice for 15 minutes each. Oxygen plasma treatment (Plasma Etch PE-25) was used to clean the surface of electrodes, followed by electrochemical cleaning to bond AuNPs on the sensor surface. 100 µM of DNA was mixed with 20 mM tris(2-carboxyethyl) phosphine hydrochloride (TCEP-HCl) in a 1: 200 ratio by volume and then placed in dark for 1 hour, diluted with phosphate-buffered saline (PBS, pH 7.4) to 200 nM. The gold electrodes were immersed in this solution for 1 hour at room temperature. The electrodes were then washed with DI water, and incubated with 20 mM MCH solution (Figure 3b) overnight, in dark for blocking. After rinsing with DI water, the sensors were ready to detect β-lactam antibiotics.

Chronoamperometry is a simple and widely used electrochemical detection technique. Since the stimulated redox reaction is proportional to the analyte concentration, it works by measuring the current response as a function of time after applying a fixed potential to the working electrode of an electrochemical system. Voltammetry is another electrochemical analysis method based on the relationship between the indicated electrode potential and the current passing through the electrolytic cell, the concentration is determined by the resulting current features. After using

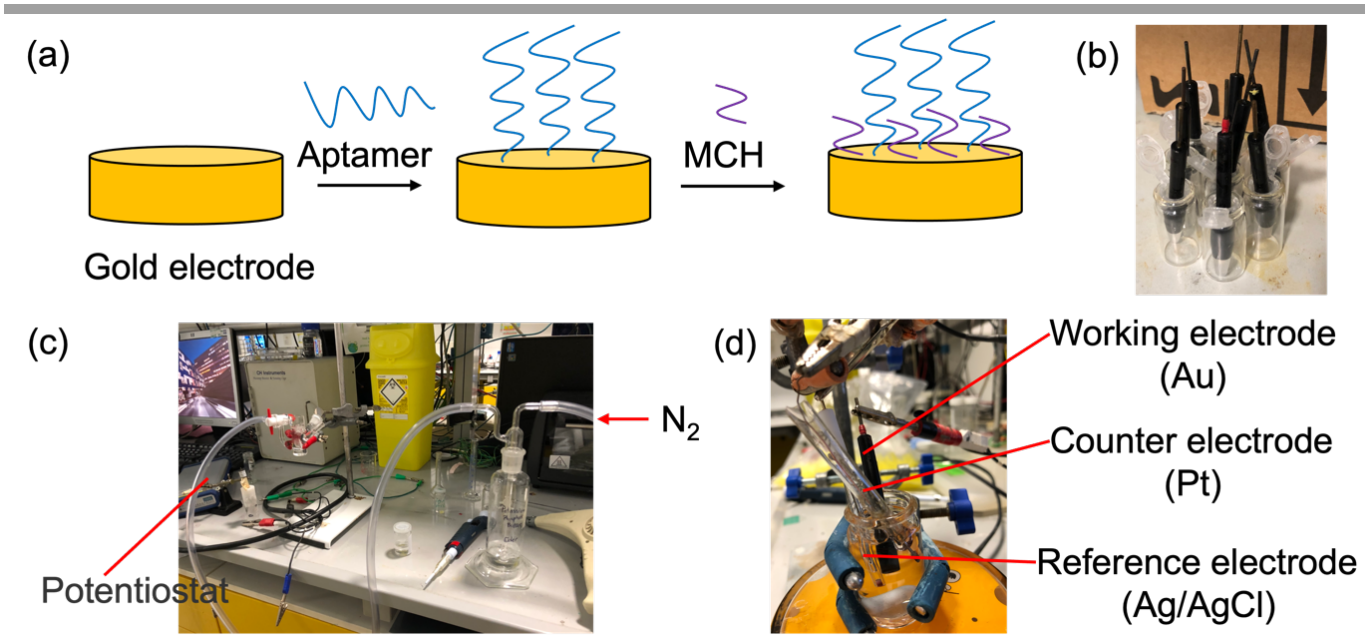


Figure 2. Preparation of penicillin-binding aptamers and experimental images. (a) The sensing mechanism of the aptasensor, aptamers are deposited on the surface of the gold electrode and small molecules are used to block reagents. (b) Experimental image of incubating aptamers with 20 mM 6-Mercapto-1-hexanol (MCH) solution overnight. (c) Image of degassing the PBS solution with hydrogen gas, the potentiostat was connected to the PC. (d) Image of testing artificial sweat with a working electrode (gold), counter electrode (platinum), and reference electrode (silver/silver chloride) to simulate real sensor operating environment.

hydrogen gas (N_2) to remove oxygen gas in the PBS solution for 30 minutes, chronoamperometry and voltammetry were performed using a potentiostat (CompactStat.h, Ivium Technologies) to study the electrochemical characteristics of the sensor (Figure 2c). Then penicillin G was added to the PBS solution to obtain the relationship between electrochemical signal and drug concentration. Since the output signal of PBS solution showed good results, we tested the artificial sweat without a degassing system to verify the feasibility of using this sensor to detect antibiotics in sweat, as shown in Figure 2d, with only three bulk electrodes.

2.3. Forming the colorimetric pH assays

β-lactamase can bind to the β-lactam ring resulting in cleavage of the β-lactam ring and destruction, thus losing its antibacterial activity, and releasing carbon dioxide (CO_2). In this work, phenol red, neutral red, thymol blue, bromothymol blue, and bromocresol purple (50 mM in stock) served as pH indicators. To test the pH ranges of these indicators, pH buffer solutions were prepared by mixing tricine (100 mM) and potassium chloride (KCl, 1 M) with DI water. The pH value of sweat is normally acidic, around 5. As aerobic activity increases and sweating rate increases, the pH of sweat increases towards 7 and above. Therefore, we adjusted the pH values of buffer solutions with sodium hydroxide (NaOH), or hydrogen chloride (HCl) as needed, a Sentron pH probe determined various pH values (i.e., 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0).

The dyes were diluted to the optimal concentration of 0.1 mM, which was adapted from an earlier literature.[31] After incubation of pH indicators (100 µM), penicillin G (100 µM) in PBS solution (pH 7.4) for 40 minutes, β-lactamase (167 mg/ml) was added to the solution. The solution was then incubated for an additional 20 minutes as per a previous report on measuring enzymatic activity by HPLC analysis.[32] Then the solution was fully mixed and showed colour changes. The experiment was conducted again to verify the results.

2.4. Fabrication of the microfluidic device

In literature, microfluidic cells for sweat sampling are often fabricated in multilayers,[33, 34] which was adopted in this work. 3D printing technology can be used to fabricate microfluidic cells with tiny and complicated structures containing multilayers,[21] and is suitable for prototype design. Wearable devices should be high on the comfort scale, so the usage of soft material for designing microfluidic channels is essential such that they can be attached to the skin easily. In this study, a rubber-like material was used to print the microfluidic chip. AutoCAD software (Onshape) was used to design the 3D structures. Microchip for the electrochemical sensor was manufactured by a 3D printer (Figure 4 Standalone, 3D Systems); and for the colorimetric sensor by ProJet MJP 2500, 3D System. The support material is still left on the printer and can be melted away by heat afterwards. In our lab, we use steam and oil baths to remove wax, isopropyl alcohol solution (IPA) to clean precision structures and then wash the microchips with tap water.

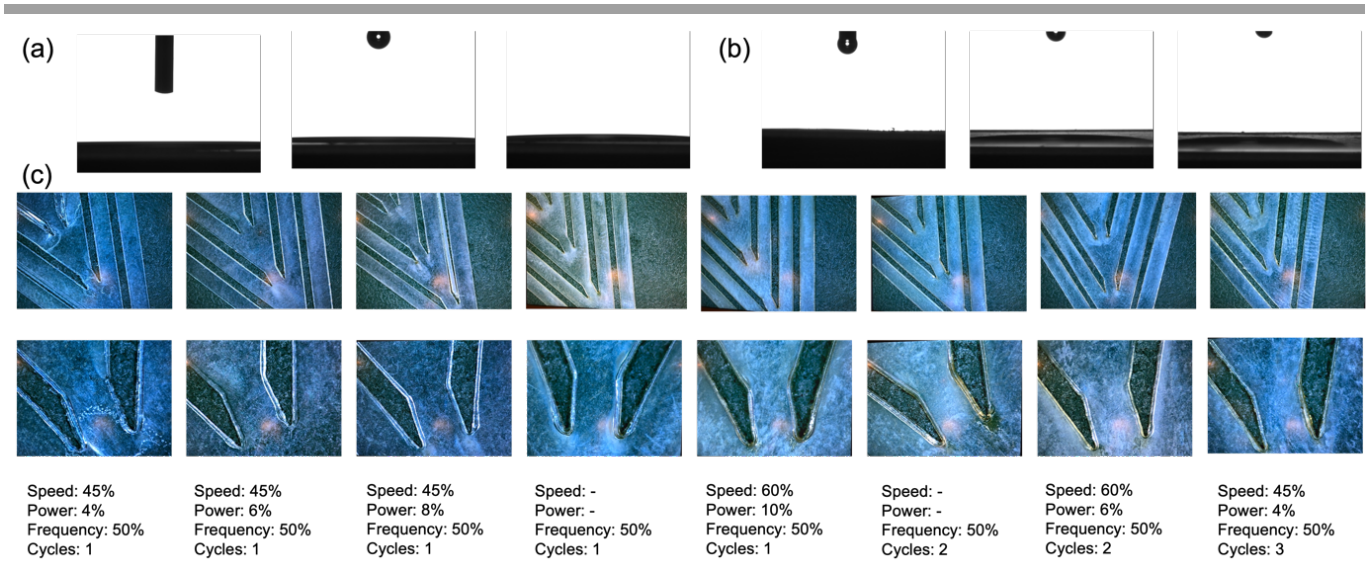


Figure 3. Investigation on hydrophilic tapes. (a) The DI water contact angles of the hydrophilic tape. (b) The artificial sweat contact angles of the hydrophilic tape. (c) Photos of the hydrophilic tape after laser cutting under a microscope, the laser cutter was set at a different speed, power, frequency, and cycles each time to test the optimum settings for the hydrophilic tape.

The microchannels are sealed by hydrophilic tape (ARfow 93049, Adhesives Research) so that once the fluid is collected from the adhesive layer, it can automatically wick into the chamber by capillary action.[35] We tried to test the contact angle of the hydrophilic tape with water (Figure 3a) and artificial sweat (Figure 3b), but could not measure the contact angles as the liquid drop immediately spread with angles less than 10 degrees for both DI water and artificial sweat liquid probes. This confirmed that the hydrophilic tape has a highly hydrophilic pressure-sensitive adhesive. Next, we cut the hydrophilic tape with a laser cutter (VersaLASER VLS3.50) using the settings as 45% speed, 8% power, 50% frequency, and 1 cycle (Figure 3c). Afterwards, the hydrophilic tape was stuck on the microchannel to guide fluid flow.

3. Results and discussion

3.1. Electrochemical sensing

The aptamer used in this work was from Paniel et al,[17] they used SELEX to select the sequence of DNA and tested penicillin G in milk. Here, we deposited the aptamer (200 nM) on gold electrodes and transduced it electrochemically. Figure 4a presents the voltammetry of penicillin G in DI water, although there are some noises in the results, the plot shows a good linear relationship (Figure 4b). This confirms that the aptasensor can be used to measure penicillin. Then we tested artificial sweat and removed the degassing system to better simulate the real sensor working environment. It showed good, curved data in figure 4c with voltammetry at 10 mV/s from -0.4 V to 0 V with equilibration time of 15 s. Next, we

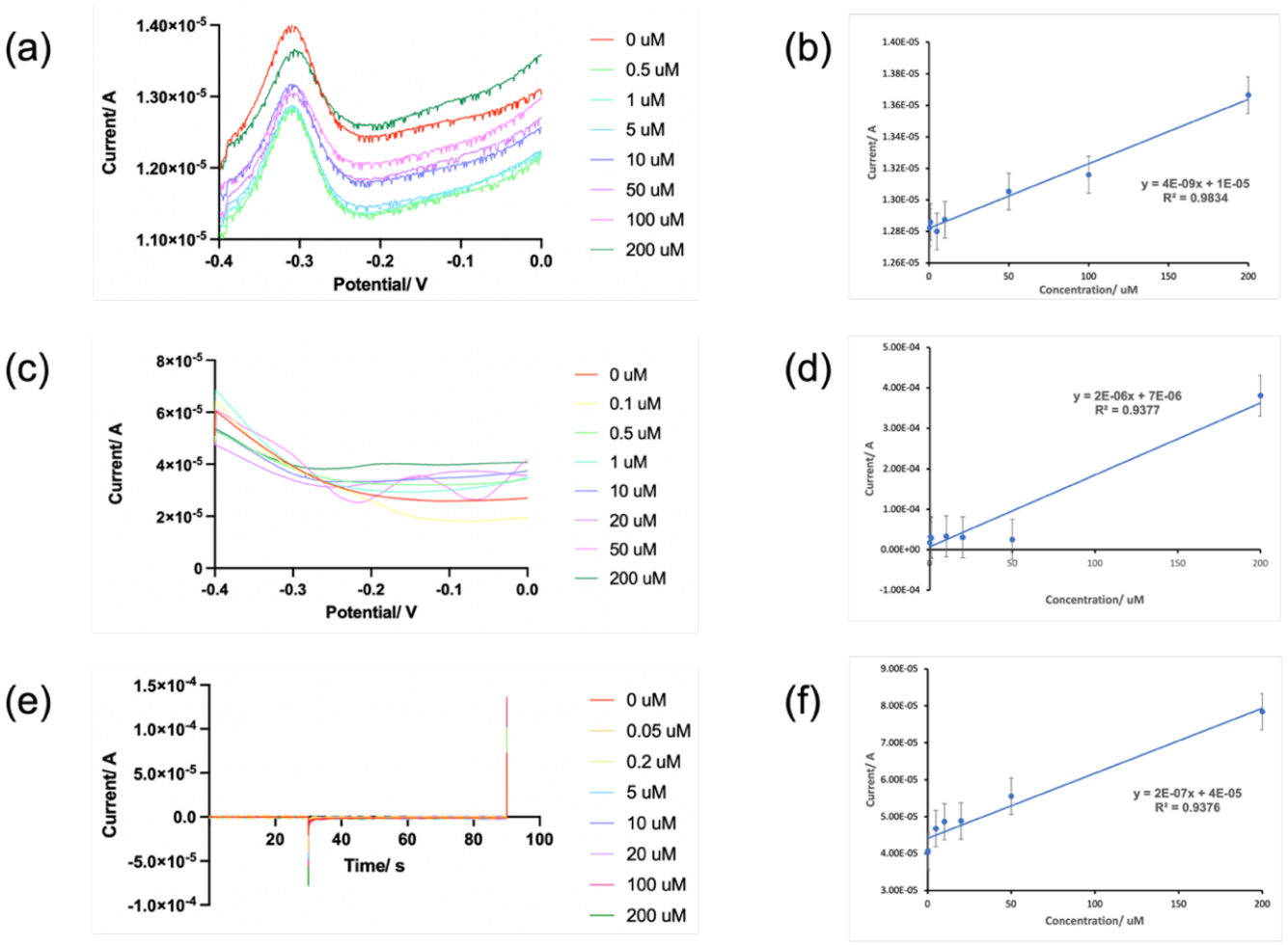


Figure 4. Voltammetry of different concentrations of penicillin G in (a) PBS solution and (c) artificial sweat at 10 mV/s from -0.4 V to 0 V with equilibration time of 15 s. (e) Chronoamperometry of penicillin G in artificial sweat. (b, d, f) Plots of electrochemical data.

analysed the chronoamperometry of penicillin G in artificial sweat (Figure 4 e) to compare the results. All electrochemical data showed linear relationships in these tests (Figure 4b, d, f), confirming that the aptamer is suitable for detecting penicillin in sweat.

3.2. Colorimetric sensing

The pH of sweat is typically 5-7. β-lactamase can react with penicillin at pH 4.5-8.0, so it is suitable for sweat sensing. The results of the tests with five pH indications, namely phenol red, neutral red, thymol blue, bromothymol blue, and bromocresol purple (100 µM) and pH buffers (pH from 5.0 to 8.0 at increments of 0.5) to obtain the working pH ranges of these dyes have been shown in Figure 5a. Figure 5b presents the mechanism of the reaction between penicillin G and β-lactamase, which releases carbon dioxide into the solution thus reducing the pH value of the aliquot. Table 3 summarises the working pH ranges of dyes, since the optimum pH for β-lactamase was 7.4, we selected dyes showing colour changes between 7 and 7.5. Therefore, phenol red (PR), neutral red (NR), and bromothymol blue (BB) were used for this study. PR, NR, and BB (all 100 µM) were incubated with penicillin G (100 µM) in PBS solution (pH 7.4) for 40 minutes, then β-lactamase (167 mg/ml) was added into the left tube in each colorimetric group and allowed to sit for 20 minutes, the colour changes have been shown in Figure 5c. All three dye groups had slight colour changes with (left) and without (right) β-lactamase and the colour change of the NR was the most obvious. Then the experiments were repeated, and we observed similar results. The minimum concentration of penicillin for β-lactamase detection is 6.8 µM in PBS based on published work, which is below that of sweat (7.3 µM). Future work should obtain a linear relationship between RGB value and concentration by changing the concentration of antibiotics. Also, the concentration of β-lactamase should be varied to optimise the chemical reaction.

Table 3. Working pH ranges of pH indicators

Dye	pH range
Phenol Red	5-8
Neutral Red	6.5-8
Thymol Blue	7.5-8
Bromothymol Blue	6.5-8
Bromocresol Purple	5-6.5

3.3. Soft epidermal microfluidic device for sweat collection

The soft microfluidic device uses a combination of capillarity and natural pressures associated with perspiration (~ 70 KPa)[36] to capture and route sweat. Layer-by-layer assembly is a simple, versatile, cost-effective surface modification technique, widely used to deposit multifunctional thin films at nanoscale.[37] The collection site for sweat needs to be such

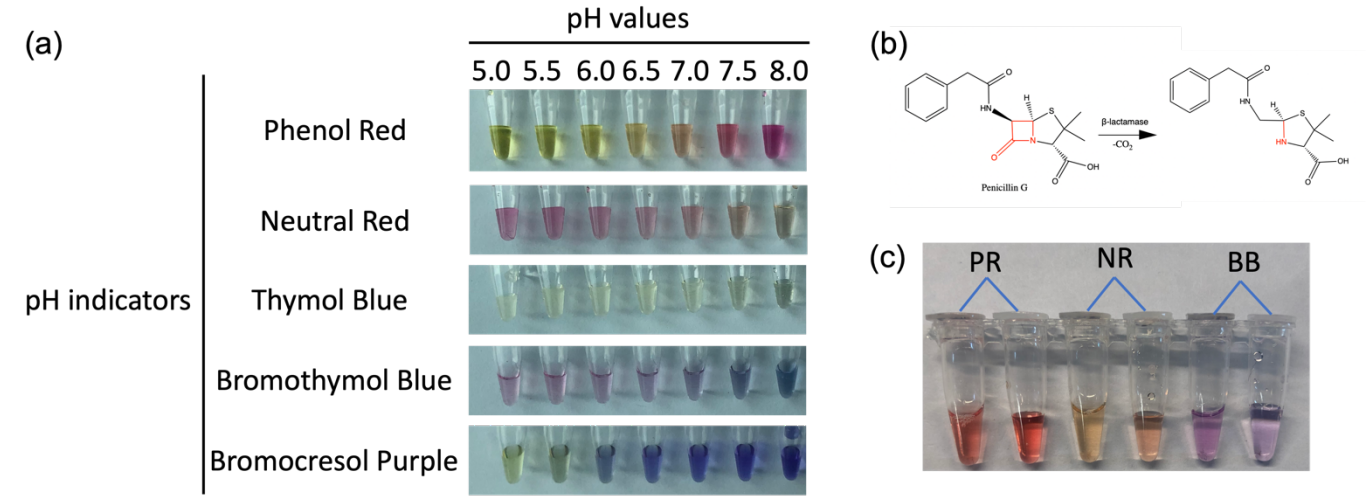


Figure 5. Colorimetric sensing results and the reaction mechanism of β-lactamase. (a) Screening of five pH indicators phenol red, neutral red, thymol blue, bromothymol blue, and bromocresol purple with pH buffers (pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0). (b) Reaction mechanism of β-lactamase cleaving the β-lactam ring (highlighting in red). (c) Screening of PR, NR, and BB with β-lactamase in the left of each dye group.

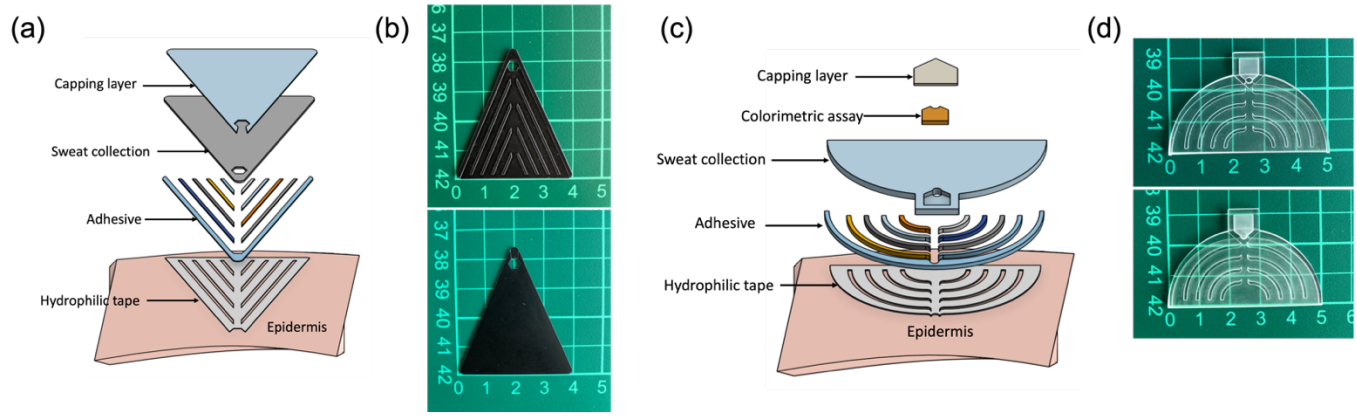


Figure 6. Schematic illustration and optical images of two epidermal microfluidic sweat devices. (a) Exploded view of the device highlighting various functional layers for electrochemical sensing. (b) Optical images of the device for electrochemical sensing. (c) Exploded view of the device highlighting various functional layers for colorimetric sensing. (d) Optical images of the device for colorimetric sensing.

that there is reasonable sweat gland density and can offer a flat space to mount a wearable device; therefore, we chose back as collection site. From Taylor et al.,[38] the back sweat rate is reported to be 1.21 $mg \cdot cm^{-2} \cdot min^{-1}$ during exercise, assuming that sweat and water have the same density (1 µL=1 mg), so the back sweat generation rate is 1.21 $\mu l \cdot cm^{-2} \cdot min^{-1}$ during exercise. To design microfluidic chips that can collect enough sweat samples within 15 minutes, the base surface area of the microfluidic device for electrochemical sensing is 653.44 mm^2, for that particular case, the total fill time is 13.87 minutes (during exercise).

The microfluidic devices were composed of multiple layers including: (1) a capping layer, (2) a microfluidic channel layer, and (3) an adhesive layer, both devices had microchannels in the adhesive layer for guiding fluid flow. Figure 6a presents an exploded view of the chip design for electrochemical sensing, the triangle shape and tiny microchannels in the adhesive layer (thickness 0.25 mm) helped the fluid to flow directly into the chamber in the sweat collection layer (thickness 0.5 mm). The capping layer (thickness 0.25 mm) can be directed to the electrochemical sensor and with an outlet to avoid contamination. The total thickness of the device was 1 mm, and the total capacity was 110.60 µL (Figure 6b), which could collect enough amount of sweat for the sensor.[27] The chip for the colorimetric sensing utilised a similar design principle but had a special reservoir (thickness 1 mm) to contain colorimetric assay reagents for pH detection (Figure 6c). After the fabrication of microfluidic chips (Figure 6b, d), hydrophilic tapes were stuck on the channel to guide the flow of sweat.

It is difficult to attach the microfluidic device on the skin to see how much fluid can be collected; to solve this issue, we designed two testing chips to mimic the sweat generation process.[39] The diameter of the outlet holes was 0.5 mm. The thickness of joint parts was increased from outside to make the structure firm and microchannels were added to guide fluid flow (Figure 7a, b). In Figure 7d, e, we designed a curved tube so that fluid can be injected from the top. After fabrication using ProJet technology, these chips can work like human skin to simulate the eccrine sweat production process (Figure 7c, f). The testing microfluidic cartridge was attached to the microfluidic chip (Figure 6b) using a pressure activated double sided tape (3M 9084), then fluid was injected through control syringe to test the fluid flow to mimic human skin. The attached syringe was tuned to obtain different fluidic flow rates. The test results showed that testing sample (water) could flow continuously from the outlet and collected by microfluidic device to carry it to sensor area either for colorimetric or electrochemical sensing.

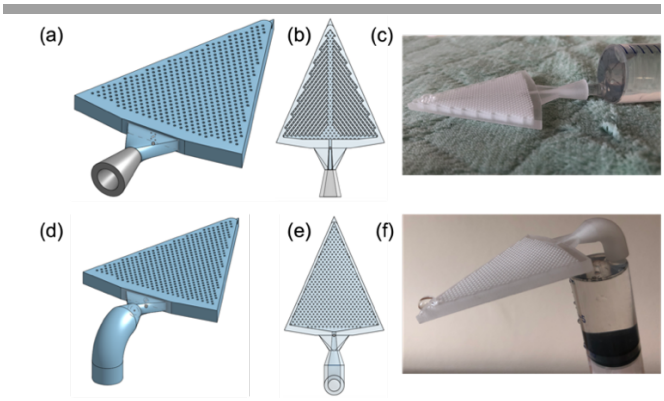


Figure 7. Images of the design and performance of the testing chips. (a, d) Shaded view and (b, e) transparent view of the chips to simulate the sweat generation process. (c, f) Optical images of liquid being injected into the chip.

4. Conclusion

This article addressed methods of aptamer-based electrochemical sensing and enzyme-based colorimetric sensing to quantify antibiotics from different concentration of spiked samples in sweat. The electrochemical data showed that the gold electrode has good stability and high sensitivity to penicillin G. The obtained results are the proof of concept to demonstrate that penicillin-binding aptamers can be used to electrochemically transduce signal for the determination of penicillin G concentrations between 1 and 200 µM. Additionally, phenol red, neutral red, and bromothymol blue can detect the pH changes caused by the reaction of β-lactamase with penicillin G (100 µM) by the naked eye. Additionally, two multi-layered completely bio-compatible microfluidic chips were designed, fabricated and packaged using MJP-3D printing technology for sweat collection. To control the direction of fluid flow, highly hydrophilic pressure-sensitive tapes were used inside the channels to wick sweat onto the sensor. These microfluidic chips could be attached to any part of the skin to provide a flexible platform for sweat sensing. In the future, to obtain a true wearable drug concentration measurement, microfluidic chips should be packaged with electrochemical sensors or colorimetric assays. Further consideration should be the testing of robustness of the device by testing on volunteers during normal physical activity with no human-subject risk.

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