AN EQUIPMENT-FREE PAPERTRONIC SENSING SYSTEM FOR POINT-OF-CARE MONITORING OF ANTIMICROBIAL SUSCEPTIBILITY

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ABSTRACT

We create a simple, rapid, equipment-free papertronic sensor array that is connected to a visual readout, allowing the naked eye to access antibiotic effectiveness against pathogenic bacteria, *Pseudomonas aeruginosa* PA01. The sensing approach quickly monitors microbial bioelectricity which is based on their metabolic activities and is inversely proportional to the concentration of antibiotics. Each sensing system consists of a two-electrode microbial sensing unit, an interface circuit for sensor signal amplification, and an electronic visual display with a light-emitting diode (LED), which are all mounted onto a paper-based printed circuit board. The bioelectricity in the sensing unit is amplified by the transistor and is transduced into LED illumination when a pre-defined electric current corresponding to a bacterial sample with a certain antibiotic concentration is obtained. Only within an hour, the system generates reliable, discrete visual responses to monitor antibiotic efficacy and provides the right doses for treatment against bacterial infections.

KEYWORDS

Antimicrobial susceptibility testing, papertronics, extracellular electron transfer, microbial fuel cells, high-throughput sensing

INTRODUCTION

Bacterial infections are extremely difficult to be correctly diagnosed and effectively cured because disease can rapidly occur at any places in human body and can be easily spread to other humans and animals [1]. Moreover, a recent study shows that COVID-19 patients have a much greater chance of acquiring secondary bacterial infections, significantly increasing the mortality rate [2]. Effective treatment of the infections relies on the capability to rapidly assess antibiotic effectiveness against the bacterial samples and promote appropriate antibiotic use [3]. The careless and excessive use of antibiotics can lead to a strong natural selection for antimicrobial resistance, posing a more serious threat to public health and the economy [4-7]. Therefore, a rapid and simple antimicrobial susceptibility testing (AST) for anyone, anywhere, and anytime is urgently required to guide effective antibiotic usages and to surveil the antimicrobial resistance rate [8-10]. Although emerging genotypic ASTs are highly sensitive and rapid, the technique must go through complicated central laboratory tests with bulky, expensive, and sophisticated sequencing-based molecular analyzers and prior knowledge of the resistance genes is required. This renders the genotypic ASTs unfit as surveillance or early detection methods for unknown bacterial infections [9, 10]. Typical "gold standard" phenotypic ASTs are based on trained personnel and relatively time-consuming process for culturing and monitoring, practically preventing rapid and early diagnostic analysis of antibiotic resistance [7, 8]. Even our latest advance in paper-based phenotypic ASTs required external readout systems, hampering on-site point-of-care (POC) diagnostic testing [11].

In this work, we allowed rapid POC antibiotic susceptibility testing with an equipment-free all-electrical sensing approach integrated into a low-cost, disposable, and portable papertronic device (Figure 1). Our AST approach monitored extracellular electron transfer (EET) by bacterial cells, parallely and controllably formed on this papertronic sensing array (Figure 2). The transferred electrons are based on microbial metabolic activities and are inversely proportional to the concentration of potential antibiotics, leading to the rapid antimicrobial susceptibility testing (Figure 2).

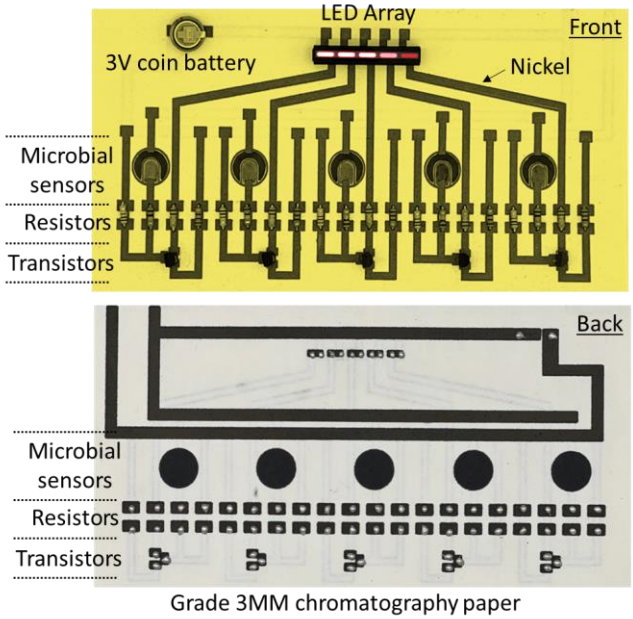

Figure 1: Photo images of the papertronic sensing system for point-of-care monitoring of antimicrobial susceptibility (Front and back views).

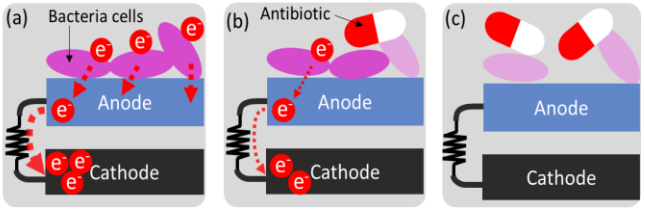

Figure 2: Schematic illustration of our AST technique. The sensing approach quickly monitors microbial bioelectricity which is based on their metabolic activities and is inversely proportional to the concentration of antibiotics.

EXPERIMENTAL PROCEDURE

Device fabrication

To define hydrophilic and hydrophobic regions, the paper substrate (Whatman 3MM Chr paper) was wax-printed by a solid-wax printer (Figure 3) [12, 13]. This is followed by a

heat-treatment at 160°C for 50 seconds. On the patterned hydrophilic regions, the microbial sensing units and the electrical interfaces (i.e. metallic wires) were constructed. The sensing unit has an anode and a cathode separated by a wax-based ion exchange membrane in a horizontal configuration. The anode was engineered to be conductive and hydrophilic so that the aquatic bacterial samples can be readily introduced and their electrogenic activities can be accessed.

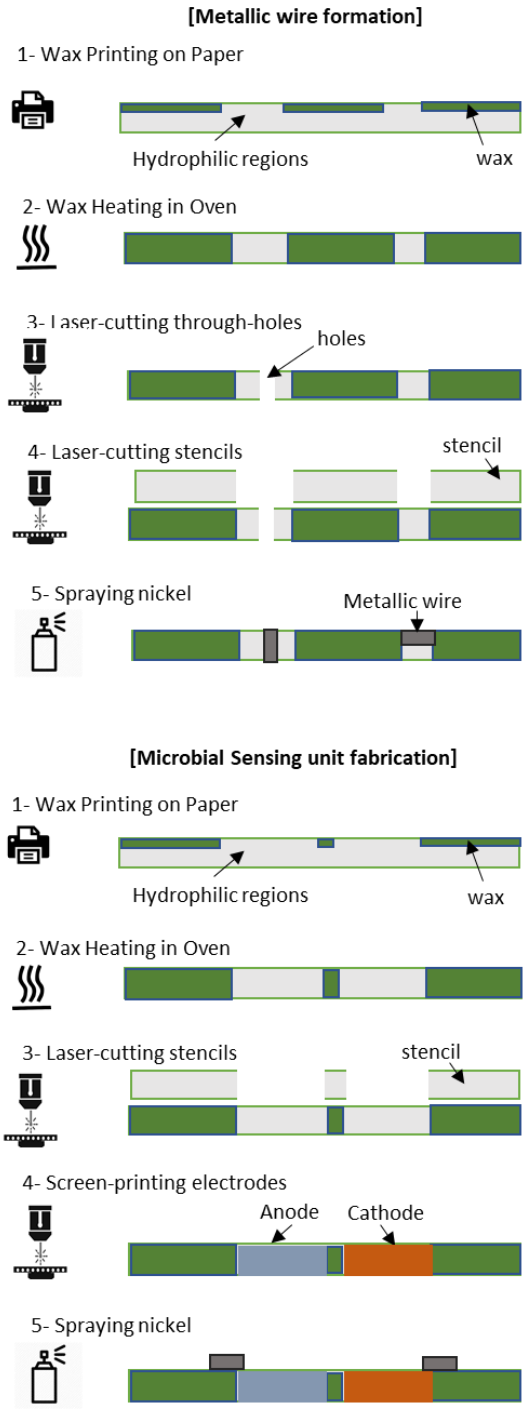

Figure 3: Fabrication process

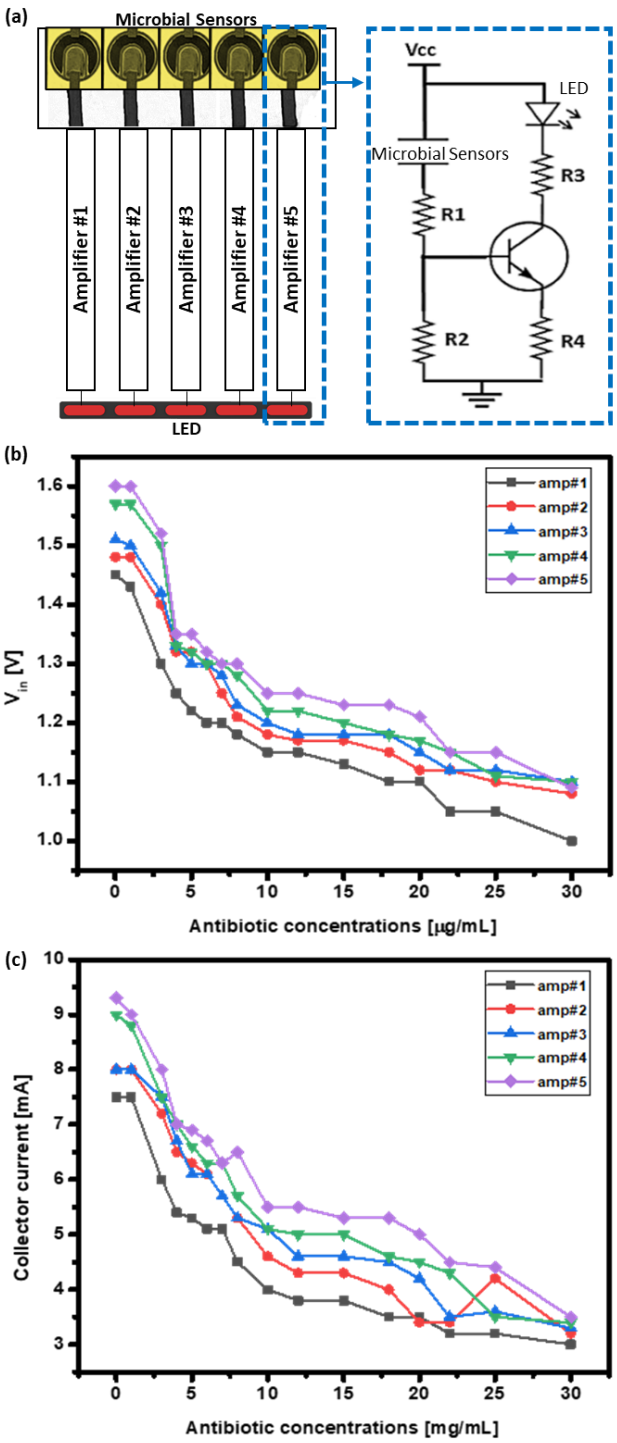

Figure 4: (a) Schematic illustration of the electric circuit for five antibiotic concentrations in the bacterial sample, (b) transistor voltage inputs, and (c) their collector currents in response to varying antibiotic levels.

A mixture of poly (3,4-ethylene dioxythiophene): poly (styrene sulfonate) (PEDOT: PSS) and dimethyl sulfoxide (DMSO) was introduced into the anodic region, followed by addition of 3-glycidoxypropy-trimethoxysilane. The cathode was prepared with Ag_2O catalysts. When the bacterial cells perform the metabolic activities, electrons and protons are produced where the electrons

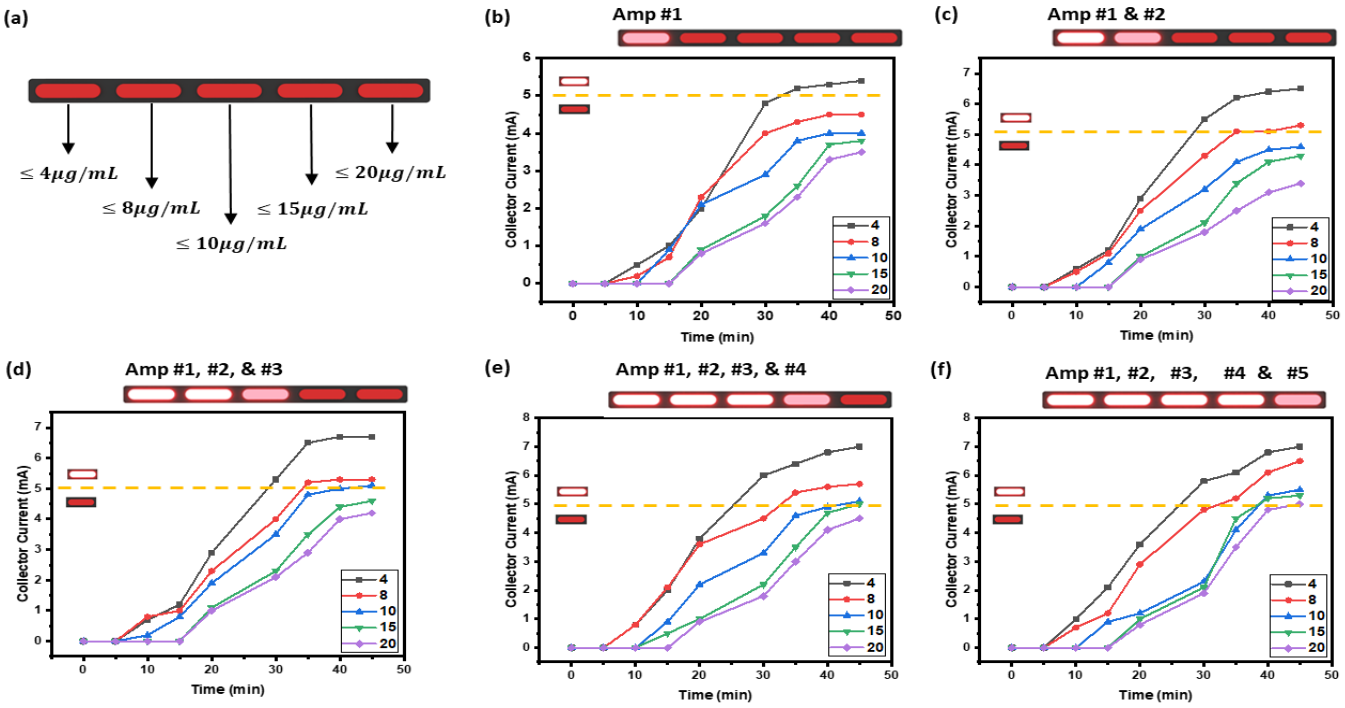

Figure 5: (a) Optical outputs of the AST sensing system (each LED indicator in the array corresponds to a certain antibiotic level in the bacteria sample). (b-f) LED responses and the current outputs of the sample with a different antibiotic level.

move to the cathode through an external load and the protons travel to the cathode through the ion exchange membrane. All electrical components were inserted into plated through holes on the patterned paper and connected to the metallic nickel wires with the conductive silver paste (Figure 3).

Bacterial sample preparation

Pseudomonas aeruginosa PA01 strain was selected as the test species because it is a well-known pathogen that can cause infections in humans and considered as electricity-producing bacteria. The bacterial sample was prepared in Luria Broth (LB) medium with an optical density at 600 nm (OD_{600}) of 1.0.

Antibiotic solution preparation

Gentamicin (GEN) was selected as antibiotics because it is highly effective against *P. aeruginosa* by inhibiting protein synthesis and affecting the integrity of the cell wall [14]. 4 μg/mL, 8 μg/mL, 10 μg/mL, 15 μg/mL and 20μg/mL GEN in sterile LB were prepared. Then, 5uL of the prepared antibiotic samples were dropped on the anodic reservoirs and pre-dried at room temperature for 5h.

Circuit configuration

The microbial sensing units were directly connected to the interface circuit for sensor signal amplification, and the electronic visual display with the LEDs (Figure 4) [15, 16]. The bacterial electricity generation was amplified by the transistor interface circuit and assessed by turning the LED array on and off. The interface circuit included five Darlington transistors with different values of R1 and R2 resistors. R1 and R2 resistors were carefully selected to generate a signal specific to an antibiotic concentration in the bacterial sample. R3 and R4 were chosen to generate a transistor input voltage and to turn the LED on and off.

RESULTS AND DISCUSSION

This work is part of a global effort to enable an innovative, rapid, easy-to-use, and high-throughput AST platform for pathogenic bacteria and thus provide effective antibiotic treatment guidelines while minimizing the unnecessary use of antibiotics [7, 8]. Genotypic and phenotypic AST methods have been dramatically advanced to determine the antibiotic susceptibility of bacterial cells [9, 10]. However, antibiotic susceptibility depends on laborious and time-consuming procedures [17-19]. The bacterial cells are exposed to antibiotics for a relatively long time, and then they are dislodged for microscopic examination of viability. This available standard protocol and susceptibility testing is limited to a few bacterial strains, which is only accessible to the ASTM standard subscribers (www.astm.org). The proposed work of this project is unique. No other group has proposed an AST platform that can provide real-time and high-throughput all-electrical monitoring capability with rapid and easy-to-control incubation time of bacteria with pre-defined antibiotics. This technique will be advanced as the proposed EET-based AST approach enables an entirely new technique in guiding antibiotic treatment for bacterial infections. This will also spur the development of additional theory and understanding of bacterial EET-based sensing, antibiotics distribution through bacterial cells, and antibiotic resistance development.

Our papertronic AST sensor array enabled simple visual monitoring of bacterial metabolic activities against different antibiotic concentrations. Our system integrated five 2-electrode sensing units into signal amplifier circuits connected to LED reporting units (Figure 1). The bioelectricity metabolically produced from the bacterial sample with a different antibiotic level

was amplified by the transistor, which triggered a LED when a pre-defined current level was obtained (Figure 4). The resistors (R1 & R2) were adjusted so that each LED indicator in the array corresponded to a certain antibiotic level in the bacteria concentration with OD_{600} of 1.0 within the 5μL of the sensing reservoir (LED#1: ≤ 4μg/mL, LED#2: ≤ 8μg/mL, LED#3: ≤ 10 μg/mL, LED#4: ≤ 15 μg/mL, and LED#5: ≤ 20 μg/mL) (Figure 5). For example, if all the LEDs light up, the sample has the lowest antibiotic concertation (≤ 4μg/mL). The LED #1 corresponded to 4μg/mL concentration which is the typical minimum inhibitory concentration of antibiotic gentamicin toward *P. aeruginosa* PA01. If the bacteria become resistant to the pre-defined antibiotic level, the current will be decreased to stop the illumination of the designated LEDs.

CONCLUSION

Antibiotic resistance (AMR) has increased worldwide in the 21st century, developing more infections that can be untreatable. Rapid antimicrobial sustainability testing (AST) has become increasingly important as drug-resistant infections become harder to treat. In this work, we reported a rapid AST sensor array by using the direct conversion of the amplified bacterial electrogenic signal to discrete visual perception. The proposed sensor array allowed the simple, easy-to-use, low-cost, high-throughput, sensitive AST by using a paper-based electronics and a amplification circuit with a LED indicator array. Our device can be a practical POC tool that provides immediately actionable healthcare information at a reduced cost, revolutionizing public healthcare in developed and developing countries. It is also expected that research into pathogens for emulating and elucidating multiple, correlated key parameters in forming multiple bacterial strains, thereby enabling a versatile platform for fundamental studies of antibiotic resistance development.

ACKNOWLEDGEMENTS

This work was supported mainly by the National Science Foundation (CBET #2100757, ECCS #2020486, and ECCS #1920979), and partially by the Office of Naval Research (#N00014-21-1-2412).

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