Biosensors and Bioelectronics 165 (2020) 112348

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: http://www.elsevier.com/locate/bios

A portable papertronic sensing system for rapid, high-throughput, and visual screening of bacterial electrogenicity

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ARTICLE INFO

Keywords:
High-throughput sensing
Extracellular electron transfer
Electrogenicity
Exoelectrogens
Papertronics

ABSTRACT

Electrogenic bacteria or exoelectrogens can transfer electrons to extracellular electron acceptors and thus have a wide range of applications to the ever-emerging fields of bioenergy, bioremediation, and biosensing. Standard state-of-the-art techniques for screening of electrogenic bacteria are inefficient, and often prevent rapid, high-throughput analyses. Herein, we created a simple, rapid, and straightforward papertronic 4- and 16-channel sensing platforms that is connected to a visual readout, allowing the naked eye to evaluate and quantify direct bacterial electrogenic capabilities. Our system integrated multiple 2-electrode sensing units into a signal amplifier circuit connected to light-emitting diode (LED) reporting units. The current generated from electrogenic bacteria in the sensing unit was amplified by the transistor and was transduced into LED illumination. The sensing units incorporated on the paper-based printed circuit boards (PCBs) absorbed bacteria-laden suspensions through capillary action, allowing for a rapid assessment (<2 min) of their electrogenic potential. Two well-known exoelectrogens, *Shewanella oneidensis* MR1 and *Pseudomonas aeruginosa* PA01, and many other mutants of the latter were selected to demonstrate the practicality of the proposed sensor. The effectiveness for on-site and portable measurements was validated by testing solid wastewater samples randomly obtained from the environment. Thus, the system described in this work highlights a novel form of a scalable, high-throughput sensing array for simple and rapid quantification of bacterial electrogenicity.

1. Introduction

Bacterial electrogenicity is a basic and untapped concept behind many potential applications in the emerging fields of wastewater treatment, bioremediation, desalination, sensing, fuel production, and electricity generation (Logan et al., 2019; Lovley, 2012). The electrogenic capability of bacteria is based on extracellular electron transfer (EET), an essential process involving their growth and syntrophic metabolism, where electrons from their aerobic or anaerobic respiratory chains can be re-routed to extracellular terminal electron acceptors (Borole et al., 2011). Most bacteria undergo respiration to convert biochemical energy in the form of carbon skeletons stored in nutrients to adenosine triphosphate (ATP), where this activity involves a set of oxidation/reduction reactions mediated by membrane complexes and cytochromes with electrons being transferred ultimately to a terminal electron acceptor. Most of the terminal electron acceptors for bacterial respiration are soluble substances such as nitrate, oxygen, and sulfate. However, some bacterial species, named exoelectrogens, respire solid-phase electron acceptors such as metal oxides and electrodes to obtain energy in the form of ATP (Torres et al., 2010). These organisms transfer electrons produced via respiration across the cell membrane to external conductive electrodes including graphene, graphite, stainless steel, carbon fiber and paper. Cell surface *c*-type cytochromes, exogenous or endogenous electron shuttles or conductive Type IV pili have been reportedly proposed as EET pathway conduits, which play an important role in determining the electrogenicity of a specific organism or microbial consortium (Torres et al., 2010). Electrogenic bacteria are found in biodegradable environmental waters including readily available wastewater or soiled water in puddles, rivers and streams (Gibbons and Gilbert, 2015; Marsili and Freguia, 2018; Koch and Harnisch, 2016). Such liquid samples normally contain the high energy density in the form of biological and chemical oxidation demand (BOD/COD) that can

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https://doi.org/10.1016/j.bios.2020.112348
Received 28 April 2020; Received in revised form 23 May 2020; Accepted 1 June 2020
Available online 10 June 2020

be convertible to electrical energy by the metabolism of specific exoelectrogens in what are commonly termed microbial fuel cells (MFCs). Thus, sensors for on-site characterization of bioelectrogenic capacity derived from the metabolism of environmental wastes are highly desirable and timely. Such sensors will allow for rapid, realistic and practical energy resources from remote and/or resource-limited settings. Furthermore, there is a huge potential for creating customized and engineered exoelectrogens due to current rapid advances in the field of synthetic biology (TerAvest and Ajo-Franklin, 2016). A portable and simple sensing platform for rapid and high-throughput screening of bioelectrogenicity will be revolutionary and accelerate the discovery and characterization of customized and novel exoelectrogens with an in-depth understanding of their electron transfer pathways. Recently, colorimetric, dielectrophoretic, or electrochemiluminescent methods have provided an indirect means for simple characterization of bacterial electrogenic capacity with the potential to scale-up for high-throughput analysis (Yuan et al., 2013; Zhou et al., 2015; Wen et al., 2014; Wang et al., 2019; You et al., 2019). However, these techniques are limited to certain bacterial species having a specific EET mechanism through extracellular *c*-type cytochromes or they could pose issues with low-sensitivity for quantitative color analyses (You et al., 2019). Furthermore, these techniques are equipment- and reagent-intensive, leading to higher costs, greater variations in operating leverage and lack of portability. The most widely employed method is to use an MFC in which the electrogenicity of bacteria is directly characterized by simply measuring voltages through external resistors (Biffinger et al., 2009; Cao et al., 2009). This direct measurement of bacterial EET can provide the most straightforward, accurate and reliable information to screen electrogenic performance. To date, many MFC arrays have been proposed as a high-throughput screening tool, thereby discovering new or improved electrogenic bacterial species (Biffinger et al., 2009; Cao et al., 2009; Hou et al., 2009; Mukherjee et al., 2013). However, the conventional MFC sensing arrays must have a continuous fluidic feeding system, which requires a considerable amount of organic fuels and labor-intensive and time-consuming operation modalities, thus eventually hampering rapid and high-throughput characterization.

Recently, our group pioneered paper-based MFC arrays for high-throughput screening of bacterial electrogenicity without using a continuous fluidic feeding system, simplifying loading of bacterial cultures and device operation (Fraiwan and Choi, 2014; Choi et al., 2015; Gao et al., 2017; Tahernia et al., 2019a). Furthermore, the capillary force of the paper could accelerate bacterial attachment to the electrode, providing rapid power assessment with even a small bacterial inoculum (Gao and Choi, 2018). We successfully characterized the electrogenicity of 95 bacteria species within 30 min from a 96 well microtiter dish model MFC culture system (Tahernia et al., 2020). However, screening of the bacterial electrogenic capacity required an unwieldy data acquisition platform and downstream readout systems. These bulky, cumbersome systems required simultaneously measured voltages from multiple sensors with varying load resistors that process significant amounts of data for polarization curves and power density for each sample. Furthermore, as the number of sensors in the array increases for high-throughput analysis, the signal readout becomes extremely complicated. This involves more external wires, a multi-channel

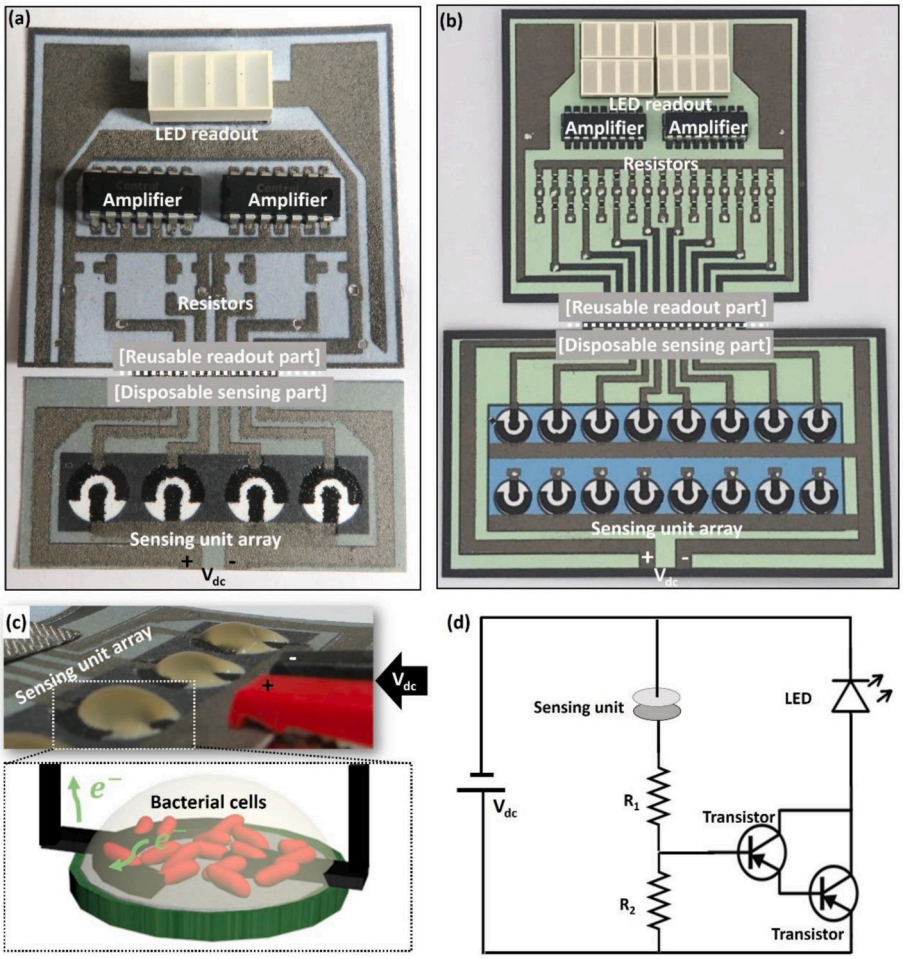

Fig. 1. 4-Channel and 16-channel sensors. (a) A photo of the 4-channel LED sensor, (b) a photo of the 16-channel LED sensor, (c) a photo and schematic illustration of the sensing unit with a bacterial sample, and (d) a schematic circuit diagram of the proposed sensor.

voltmeter with computer processing capacity, such that the sensing system cannot be simplified for portable on-site measurements.

To address the aforementioned issues in this work, we created a simple, portable, equipment-free papertronic sensing platform that achieves facile, rapid, and high-throughput characterization of bacterial bioelectrogenicity (Fig. 1). The 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), upon which all are mounted onto a paper-based printed circuit board (PCB) (Fig. 1a and b). The platform has two components: a disposable sensing array and a reusable readout, so that the bacteria-containing sensing units can be detached from the readout and disposed safely. A current signal generated from a bacterial sample in the two-electrode sensing unit (Fig. 1c) is amplified by field-effect transistors with adjustment of the resistors, sufficient to power the LED for the direct visual readout with the naked eye (Fig. 1d). Very recently, *Lacina* et al. developed a new electrochemical sensor for the transduction of an enzymatic redox signal to a visual readout using a transistor amplifier (Lacina and Skladal, 2015; Lacina et al., 2016). Their system was successfully demonstrated as a glucose sensor by monitoring the presence of H_2O_2 with the naked-eye readout. This technique fosters a "best-fit" solution for the simple, portable, disposable, and equipment-free screening of bacterial electrogenicity when integrated into a paper-based fluidic and electronic array platform. Paper inherently provides the most favorable conditions for easy, rapid, and high-throughput control of a small quantity of microbial suspension. Paper can be an excellent substrate for electronics because of its flexibility, low cost, eco-friendliness, sustainability, and its outstanding mechanical and dielectric properties (Hamedi et al., 2016; Tobjork and Osterbacka, 2011). The sensing and reporting capability could be extended to an array for high-throughput analysis. Each sensing unit could act as a variant resistor with the varying degree of bacterium-specific electrogenic capacity, controlling the opening of transistors and consequently illuminating the LED (Fig. 1d). Therefore, the intensity of a LED, which emits light proportionate to the amount of input electricity, can thus serve as a measure of bacterial electrogenicity. Thus, a 4-channel LED sensor (Fig. 1a) and a higher-throughput 16-channel LED sensor (Fig. 1b) were developed for the first time for the simple visual characterization of bacterial electrogenicity and their effectiveness was validated by testing well-known exoelectrogens and their genetically modified mutant strains. We previously had reported a proof-of-concept prototype of the 4-channel sensor (Tahernia et al., 2019b). Here, this current work demonstrated that our sensor can force a paradigmatic shift for the architectural design of a scalable, high-throughput sensing arrays with exceptional design characteristics and innovative functionalities. Finally, the electrogenic capability of three carbon-rich wastewater samples was characterized to test the future practicality of the sensor for on-site measurements.

2. Experimental

2.1. Materials

Conductive graphite ink (NC1044060) was purchased from Fisher Scientific™. Silver conductive epoxy was obtained from Ted Pella, Inc. Thin-film plastics (1/16-inch acrylic sheets) were purchased from McMaster-Carr. Whatman qualitative filter paper (Grade 1) was obtained from VWR International, LLC. Nickel conductive spray was obtained from MG Chemicals. Other electronic components including quad bipolar transistors (MPQ7093), 4-wide LED arrays (SSB-LX2620IW), and resistors were purchased from Digi-key Electronics. MPQ7093 for signal amplification was comprised of four independent PNP silicon transistors mounted in a 14-pin dual in-line package (DIP) socket.

2.2. Preparation of paper-based PCBs and sensing units

PCBs are a critical part of the paper-based sensing platform and provide support for integrated circuits and electrical connections between the electric components and their sensing units. With the exception of the bacterial sensing units and the metallic wire regions, the entire paper was asymmetrically wax-printed by a solid-wax printer (Xerox Phaser8570dn), followed by heat-treatment (150° for 30 s) to allow complete penetration of the wax throughout the paper (Fig. 2). This hydrophobic wax pattern provided the paper substrate a dielectric insulating property as a PCB for preventing electrical shorts between metallic wires. Furthermore, the reverse side of the sensing unit was wax-printed and a hydrophilic region in the unit was defined by controlling the penetration depth of the melted wax to contain the sample in the sensing units without leaking through the bottom of the paper (Gao and Choi, 2017). The sensing units harboring the bacterial samples were constructed with two graphite ink electrodes by screen-printing (Fig. 2a & b). The PCB wire designs and thin-film plastic stencils for metal deposition were prepared by using AutoCAD software. The plastic stencils were micromachined by laser cutting (Universal Laser System VLS 3.5). Then, nickel was sprayed on the patterned hydrophilic regions of the paper through the stencils. The conductive nickel ink in liquid penetrated through the hydrophilic regions pre-designed with hydrophobic wax barriers, forming metallic wires through drying processes. All electrical components were mounted through functional vertical interconnect accesses (vias) patterned on the multiple paper PCB layers and connected to metallic wires with the conductive silver paste (Fig. 2c). The 4-channel sensor required 2 PCB layers while the 16-channel device used 4 PCB layers to form a complete and functional system. Finally, the reusable readout circuit component was connected to the sensing unit array though the stainless-steel mesh connector. The sensing unit array can be easily attached or detached as a disposable part while the readout continuously serves as a reusable part.

2.3. Bacterial inoculum and environmental wastewater samples

To demonstrate our papertronic sensing platform as a screening tool for bacterial electrogenicity, two well-known exoelectrogens, *Shewanella oneidensis* MR1 and *Pseudomonas aeruginosa* PA01, and mutants of *P. aeruginosa* were selected as the test species. Previously, our reports showed that the genetic modification of naturally occurring exoelectrogens significantly affects the electrogenicity of *P. aeruginosa* mutants (Fraiwan and Choi, 2014; Choi et al., 2015; Gao et al., 2017; Tahernia et al., 2019a, 2020; Gao and Choi, 2018). In our current work, the effectiveness of the papertronic sensing system was validated as a simple but powerful platform to evaluate the electrogenicity of such strains. Furthermore, three carbon-rich wastewater samples randomly obtained from three different environmental sites in Binghamton, NY were tested. Were our portable sensing system able to rapidly screen the bioelectrogenic properties of bacteria metabolizing such environmental wastes, a realistic and practical energy resources can be quickly explored, especially in resource-limited developing countries. The 4-channel LED sensor evaluated three test sets; #1 - *bdlA*, MR1, *lasR rhlR*, and *fliC*, #2 – MR1, PA01, *pmpR*, and *bdlA*, and #3 – tap water, wastewater1, wastewater2, and wastewater3. The 16-channel LED sensor evaluated a test set of PA01 isogenic mutants, *wapR, pslpel, PA0962, pilY1, moaA1, gor, lasl rhll, norCB, pmbA, mvfR, relA, pilT nirS, acnC,* and *pilT* while Luria-Broth (LB) (a carbon-rich medium) was used as the negative control. The mutants were genetically engineered by using classical allelic exchange or transposon mutagenesis methods (Kulasekara et al., 2015). The detailed information of those genetically engineered mutants and the rationale are described in our previous reports (Choi et al., 2015; Gao et al., 2017; Tahernia et al., 2019a, 2020; Gao and Choi, 2018). All bacterial samples were prepared in LB media (1 w/v% tryptone, 0.5 w/v% yeast extract and 0.5 w/v% NaCl) with an OD_{600} (optical density at 600 nm) of 2.5 which saturated the sensor unit

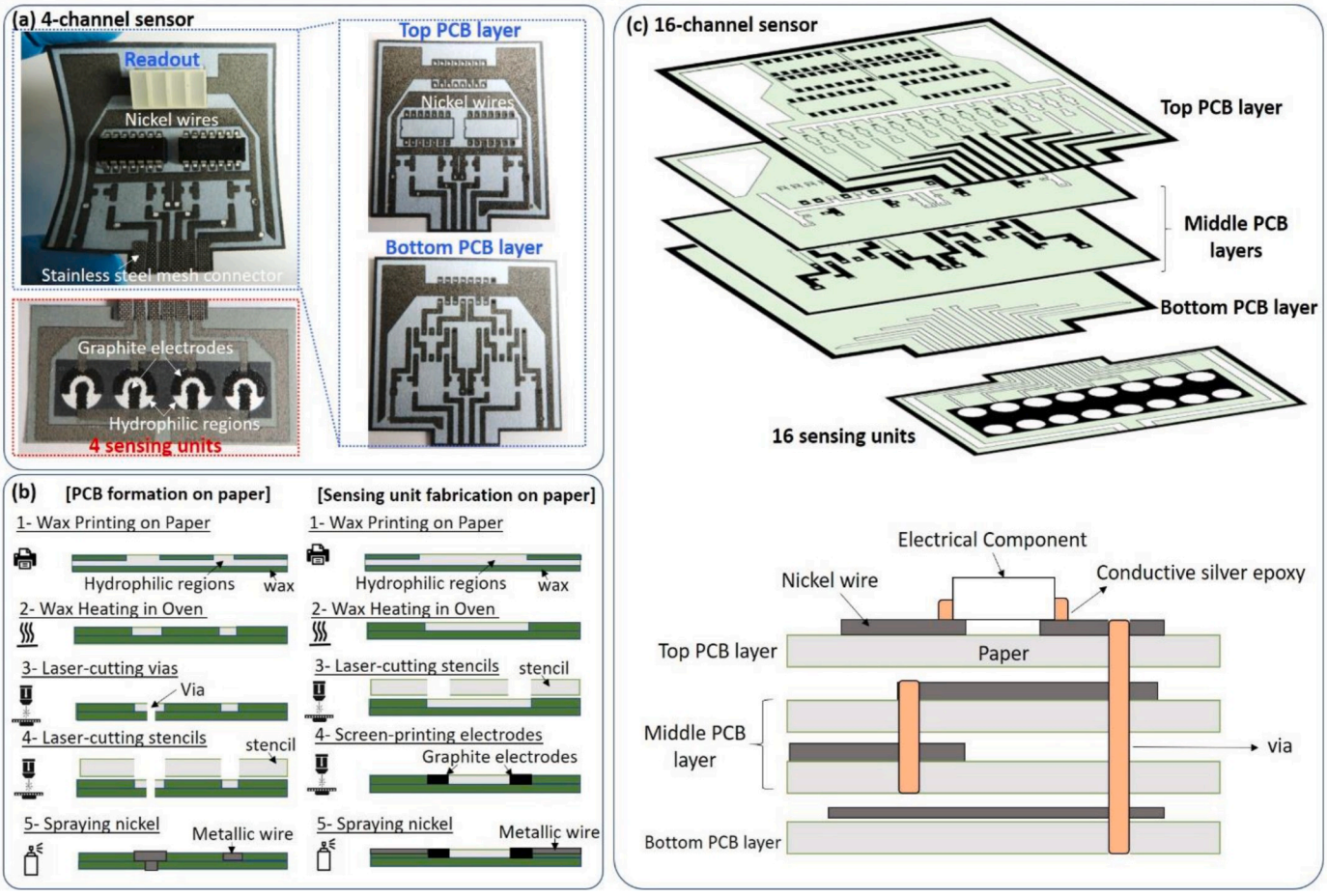

Fig. 2. Device configuration and fabrication process. (a) 4-channel LED sensor displaying sensing and reporting units constructed on the multiple paper PCB layers, (b) fabrication processes for papertronic PCBs and sensing units, and (c) 16-channel LED sensor showng individual PCB layers and its cross section.

(Tahernia et al., 2019a).

2.4. Circuit configuration

The sensing unit array was connected to the readout LED array through two transistors. The bacterial electrogenic capability was characterized by the transistor amplifier with the two-electrode sensing unit sequentially connected to their bases and the LED behind the amplifiers. With the input voltage (V_{dc}, 6V), a redox potential can be applied to the bacterial cells, generating a current and thus reducing the resistance of the unit. By adjusting the value of two resistors (R_1 and R_2), a certain level of electrogenicity of bacterial sample in the sensing unit can be monitored by opening the transistors because of the reduced resistance and thus lighting the LED.

3. Results and discussion

3.1. A 2-electrode sensing configuration

Typically, a reliable and reproducible evaluation of bacterial electrogenicity can be conducted in a 3-electrode MFC configuration with working, counter and reference electrodes (Babauta et al., 2013). By setting the working electrode to a different electrical potential from that of the reference electrode, we constructed an activation energy barrier that must be overcome for the bacteria to trigger the electron transfer process. However, the 3-electrode MFC configuration requires the use of a bulky reference electrode, which can pose a problem for miniaturization and integration (Shinwari et al., 2010). Furthermore, the 3-electrode configuration is difficult to expand into an array format because it is challenging to independently control the potential of each electrode in parallel (Sun and Crooks, 2005). A 2-electrode electrochemical configuration is required to simplify the readout circuit and interconnection between the sensing electrochemical units and the LED reporting units. It has also been reported that 2-electrode enzymatic analytical cells without the reference electrode can be successfully demonstrated by improving their redox reactions (Lee et al., 2018). The redox electroactive site of the enzymes is embedded deeply within their active sites and, as such, reduces the efficiency of electron transfer from catalytic enzymes to electrodes. Creating effective EET pathways, which can be well characterized even in a 2-electrode configuration, can make bacterial electrogenic activity much more efficient in lighting the LED (Osman et al., 2010, 2011). Our 2-electrode sensing array will make it possible to create an inexpensive, scalable, time-saving, high-performance and user-friendly platform that facilitates studies of fundamental bacterial electrogenicity.

3.2. A 4-channel sensing array

A 6.2 cm × 8.6 cm 4-channel LED sensing platform was developed on the paper PCB by integrating four 2-electrode sensing units, eight resistors (4 sets of R_1 and R_2), two transistor amplifier chips (MPQ7093, Darlington transistor configuration), and a 4-array LED. (Fig. S1 & Fig. 3). Each sensing unit functioned as an active resistor with the bacterial samples. The readout circuit contained an adjustable voltage divider including the sensing unit, and two resistors R_1 and R_2. When a sensing unit was filled with the LB media in the absence of bacterial cells

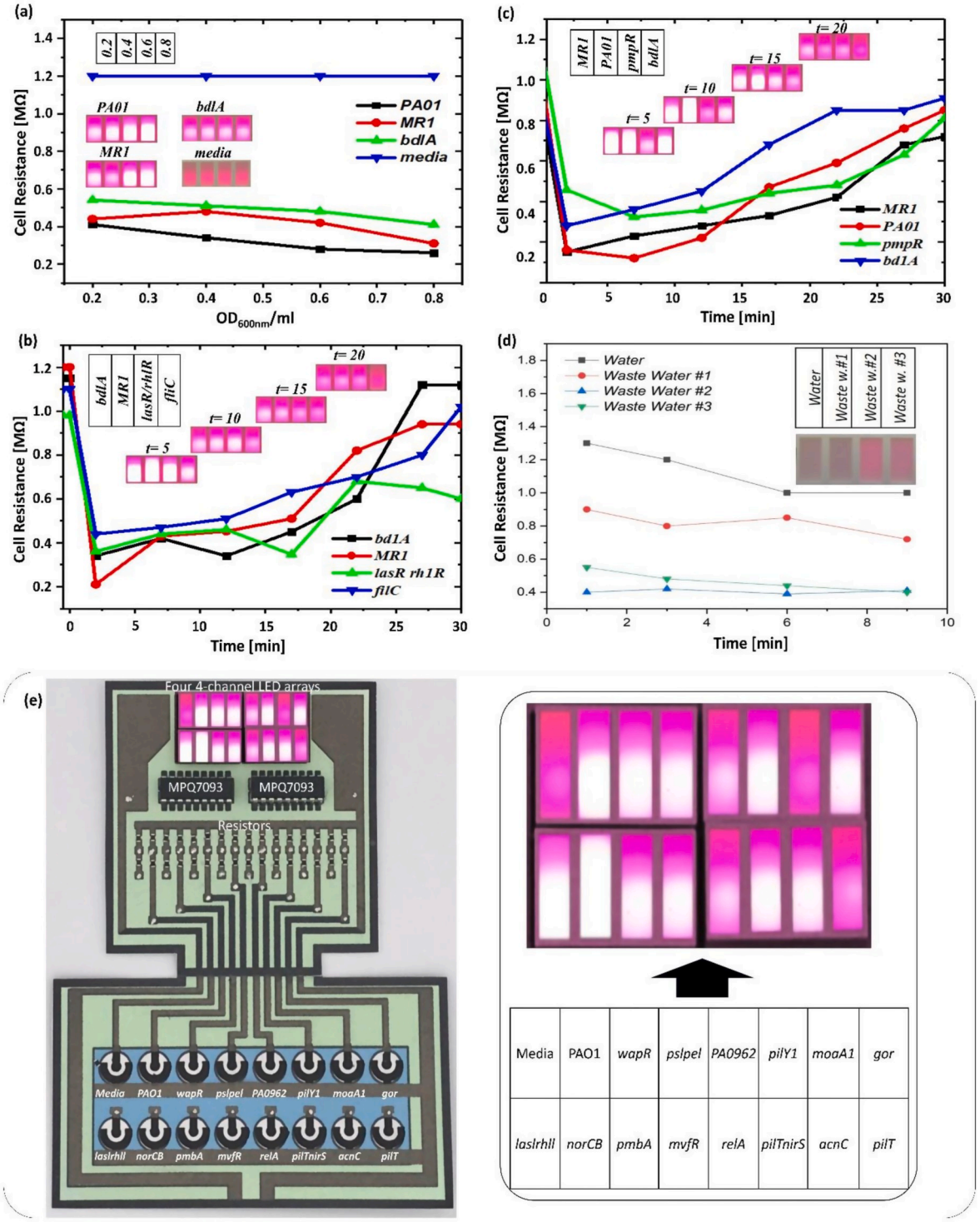

Fig. 3. The 4- and 16-channel LED sensors with LED illumination. Resistance values of the sensing units and corresponding LED responses of (a) different types of bacteria according to their concentration, and (b) & (c) different types of bacteria as a function of time. (d) Resistance values of the sensing units and the LED response at 1 min Of different wastewater samples. Wastewater samples #2 and #3 demonstrated weak electrogenicity while sample #1 and the tap water control was incapable of powering the LED. (e) LED optical outputs of the 16-channel sensor with 15 bacterial strains and media as a negative control.

and the V_{dc} was applied, the value of two resistors (R_1: 200 kΩ and R_2: 1MΩ) were chosen selectively to provide a minimum current (i.e., background current) to open the transistors and consequently activate the LED with minimum illumination (red color) (Figs. S2 and 3a). The resistor R_1 regulated the voltage across the sensing unit so that the current was divided into the resistor R_2 and the base of the Darlington device (two-transistor amplifier). The overall current gain of this two-transistor amplifier is the product of the two individual gains of the transistors multiplied together, and very high gain values are obtained compared with a single transistor circuit. The resistor R_2 was selected for the transistor to be properly biased for a suitable operating point. The intrinsic capillary force of the hydrophilic sensing unit allowed for rapid adsorption of the bacterial liquid sample (~40 μL) and promoted immediate bacterial attachment to the electrode. With the applied voltage

(V_{dc}), bacteria in the sensing unit generated current, reducing the resistor value of the unit and thus increasing the current through R_1 above the level of the background current. Because the increased current was greater than the threshold voltage of the transistors, the LED emissions became brighter (white color) than those with the background current. Therefore, the electrogenicity of bacterial samples (compared to the negative control, LB media) could be characterized by the increased light intensity emitted by the LED.

Fig. 3a shows the resistance values of the sensing units and the corresponding LED responses with the different bacterial types and their varying titers. The control with LB media in the absence of bacterial cells produced a much higher resistance value (1.2MΩ) than the other bacterial samples and a minimum red color illumination. With the increasing concentration of two wild-type exoelectrogens, *S. oneidensis MR1* and *P. aeruginosa* PA01, the unit cell resistance decreased with the increasing LED light intensity. The *bdlA* mutant produced brighter LED illumination than the negative control, indicating some electrogenic capability but much lower than the two wild-type exoelectrogens. This result is in good agreement with our previous work (Gao et al., 2017), indicating an inability of this strain to disperse and re-sculpt a mature, electrogenic biofilm.

Fig. 3b and c shows the varying resistance and the corresponding LED response of the bacterial species, *S. oneidensis MR1*, *P. aeruginosa* PA01, *bdlA*, *lasR/rhlR*, *pmpR*, and *fliC* as a function of time (#1 - *bdlA*, MR1, *lasR rhlR*, and *fliC* and #2 – MR1, PA01, *pmpR*, and *bdlA*). With the introduction of the samples, the resistance of the units significantly decreased with the brightest LED illumination because of the intrinsic advantage of paper showing instant current generation from the bacterial cells (Gao and Choi, 2018). However, the resistance gradually increased with time while the LED intensity decreased accordingly. This was due to decreased bacterial electrogenic activity as nutrients became depleted within the limited volume of the unit. *S. oneidensis MR1* and *P. aeruginosa PA01* demonstrated the highest electrogenicity, followed by *bldA* and *lasR/rhlR*. The *fliC* and *pmpR* mutant yielded the lowest electrogenicity. The *lasR/rhlR* and *pmpR* mutants lack the quorum sensing regulators while a *fliC* mutant is nonmotile and forms very poor biofilms (Gao et al., 2017).

To demonstrate rapid, on-site detection capability of the sensor, three environmental wastewater samples were evaluated as feedstocks for our MFCs (Fig. 3d). A local environmental sample may house many electrogenic bacteria, which can serve as a potential energy resource in remote and resource-constrained settings (Fraiwan et al., 2016). Interestingly, wastewater samples #2 and #3 randomly obtained from our environment successfully demonstrated weak electrogenicity while sample #1 and the tap water control was incapable of powering the LED.

3.3. A 16-channel sensing array

The 4-channel sensing array was readily extended to an 8.5 cm × 15 cm 16-channel sensing system by simply adding more components to the basic 4-channel sensing system configuration. This involved 16 sensing units, four 4-wide LED arrays, two amplifier chips, and 32 resistors (Fig. S3 & Fig. 3e). All circuit and sensing operating principles are the same as the 4-channel sensor. Fifteen bacterial samples were tested and compared to the control with LB media: PA01, *wapR, pslpel, PA0962, pilY1, moaA1, gor, lasl rhll, norCB, pmbA, mvfR, relA, pilT nirS, canC,* and *pilT*. LED responses were observed within 2 min after sample introduction. The *moaA1* and *pilT* mutants displayed similar LED intensity to the negative control, indicating negligible electrogenicity. It should be noted that the *norCB* and *lasl rhll* mutants exhibit greater electrogenicity than that of the wild-type bacteria, indicating that the genetic engineering of microbial metabolic pathways can improve the electrogenic activity of the bacteria. The *wapR, pslpel, PA0962, pilY1, gor, pmbA, mvfR, relA, pilT nirS,* and *acnC* mutants demonstrated weaker electrogenicity than that of the wild-type bacteria.

4. Conclusion

In this work, we developed a simple, portable, equipment-free papertronic sensing platform for rapid, and high-throughput screening of bacterial bioelectrogenicity. The platform revolutionarily included sensing and reporting LED units in an array deposited on disposable and inexpensive paper substrates. The system provided a straightforward assessment of bacterial electrogenic capabilities by direct observation with the naked eye. The intrinsic capillary force of the paper allowed for the rapid introduction of the liquid sample suspension, leading to an instantaneous assessment of bacterial electrogenicity from a small amount of liquid. The simple 2-electrode sensing and readout circuit configuration facilitated a scale-up to a 4-channel or 16-channel sensing array platform. Bioelectrogenicity of 15 bacterial strains were simultaneously characterized. Furthermore, the portability and point-of-care analytic capability of the system were validated by on-site testing of environmental samples potentially including exoelectrogens. Although further improvements in sensitivity are still needed, our innovative papertronic sensing system will be useful for the imminent practical implementation of bacterial electrogenicity evaluation.

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.

CRediT authorship contribution statement

Mehdi Tahernia: Investigation, Methodology, Data curation, Writing - original draft. **Maedeh Mohammadifar:** Investigation, Formal analysis, Writing - review & editing. **Daniel J. Hassett:** Investigation, Formal analysis, Funding acquisition, Writing - review & editing. **Seokheun Choi:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing.

Acknowledgments

This work was supported by the National Science Foundation (ECCS #1703394 & #1920979), Office of Naval Research (#N00014-81-1-2422), Integrated Electronics Engineering Center (IEEC), the Analytical and Diagnostics Laboratory (ADL) at SUNY-Binghamton for providing the fabrication facilities and the SUNY Binghamton Research Foundation (SE-TAE). Other support was a sub-contract from the National Science Foundation (CBET #1605482) to D.J.H.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2020.112348.

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