

# A membrane-free micro-fluidic microbial fuel cell for rapid characterization of exoelectrogenic bacteria

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Received: 28 June 2016 / Accepted: 3 October 2016 / Published online: 11 October 2016  
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**Abstract** A membrane-free micro-fluidic microbial fuel cell ( $\mu$ MFC) has been developed in this work, in which the bacteria-mediated organic fuel oxidation process is physically separated from the proton exchange process that occurs between the laminar co-flows of anolyte and catholyte streams on a micro-fluidic chip. This new strategy aims to shelter exoelectrogenic bacteria in the anode chamber from the potential influence of the agents from the catholyte stream and enable much larger anode surface for bacteria adhesion in order to enhance the electron transfer efficiency. This  $\mu$ MFC reveals considerable difference in the relative open-circuit voltage produced by *Shewanella oneidensis* MR-1 and *Escherichia coli* DH5 $\alpha$ , which can be established and stabilized within 2 h. This platform can be

used for rapid characterization of the exoelectrogenic capability of various microorganisms or the development of a microbe-based electrochemical biosensor.

**Keywords** Microbial fuel cell · Micro-fluidics · Biosensor · Laminar flow · Exoelectrogen

## 1 Introduction

Microbial fuel cell (MFC) is a bioelectrochemical system using microorganisms as catalysts to decompose organic compounds and generate electric current. The electrons produced by bacteria during this process are transferred to the anode and flow through an external circuit to the cathode (Logan et al. 2006; Lovley 2008; Rabaey and Verstraete 2005). These electricity-generating microorganisms are also referred to as exoelectrogens, which have attracted extensive attention in recent years as an important alternative source for green and sustainable energy (Du

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**Electronic supplementary material** The online version of this article (doi:10.1007/s10404-016-1811-5) contains supplementary material, which is available to authorized users.

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et al. 2007; Nor et al. 2015; Rabaey and Verstraete 2005; Wang et al. 2015). Many natural exoelectrogens have been reported, and genetically engineered exoelectrogens are under investigation for more efficient power generation (Kane et al. 2012; Mathuriya 2013; Voeikova et al. 2013; Wu et al. 2013).

As one of the major challenges in this field, it is highly laborious and time-consuming using conventional macro-scale MFCs for screening numerous genetically modified microbial strains to identify models with optimal stability and yield. To characterize these bacterial strains in a rapid fashion, some miniaturized MFCs have been conceived recently (Hou et al. 2010; Qian et al. 2011). A micro-fluidic MFC ( $\mu$ MFC) is usually featured by a large surface-area-to-volume ratio and thus enhanced affiliation between the bacteria and the anode, which can reach stabilized power output within less time while consuming less amount of carbon source compared to conventional MFCs (Wang et al. 2011; Yang et al. 2013). The proton exchange membrane (PEM) is extensively used in MFCs to separate cathode and anode. However, membrane fouling usually occurs in PEM, which compromises the performance of MFCs (Chae et al. 2008; Xu et al. 2012). Utilization of PEM also develops pH gradient between two electrodes, causing additional energy loss (ElMekawy et al. 2013). In addition, the incorporation of membrane in the micro-fluidic fuel cell further increases the manufacturing cost and complexity of the device (Wang and Su 2013). To address these issues, Wang and Su (2013) proposed a membrane-free MFC implementing a novel design that avoided using PEM. Specifically, the PEM-free MFC was achieved by taking advantage of the laminar co-flow generated in a micro-fluidic channel, which considerably improved the overall efficiency of characterizing the bacterial electrogenic activity. The laminar co-flow of the two electrolyte streams allows ionic conduction while reducing the mixing across the two co-flow streams (Ferrigno et al. 2002; Goulet and Kjeang 2014). However, maintaining a laminar co-flow poses a constraint on the width of the channel, limiting the effective contact area between the electrode and the bacteria. Moreover, because the anode is usually located nearby or in parallel with the co-flow region in most existing  $\mu$ MFCs, the constrained co-flow region limits the effective surface area of the anode and hence the electron transfer efficiency.

Herein, we present a  $\mu$ MFC on a chip that can effectively address the above major limitations of the existing membrane-free  $\mu$ MFCs. Specifically, two expanded reaction chambers are designed in the upstream before the catholyte and anolyte converge and form the laminar co-flow. This special design uses the upstream chambers for redox reactions, while maintaining the proton exchange process in the downstream co-flow region. This strategy aims to physically separate the proton exchange process

from the bacteria-mediated oxidation process, so that the exoelectrogenic bacteria in the anode chamber can be sheltered from the influence of potential agents diffused from the catholyte stream. In addition, this strategy enables a much larger anode surface for bacteria adhesion in order to enhance the electron transfer efficiency and lower the system inner resistance (Du et al. 2007). A micro-stopper array is fabricated in the anode chamber to help retain the bacteria and slow down the local medium flow speed, which could provide a more amenable microenvironment for bacterial growth.

## 2 Materials and methods

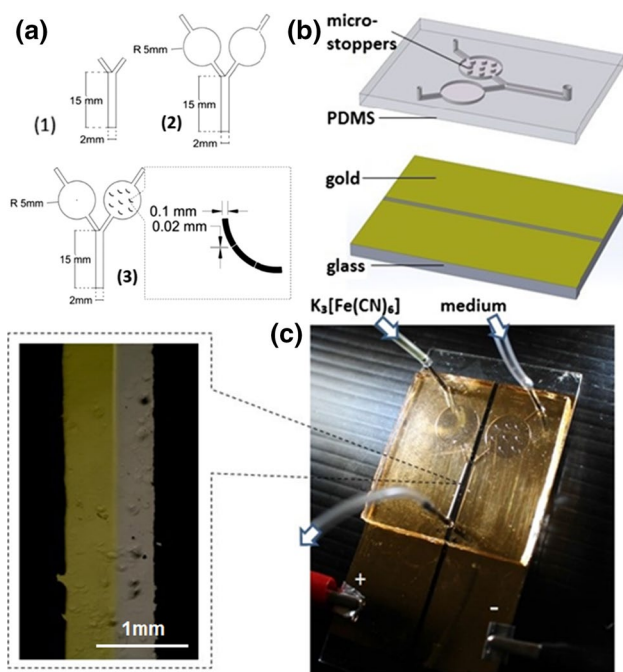
### 2.1 Materials and reagents

The materials and chemical reagents are listed as follows. Polished silicon wafers, SU8 developer and SU8 photoresist were purchased from Microchem, USA. Sylgard 184 silicon elastomer kit was obtained from Dow Corning Inc., USA. Sodium lactate,  $\text{NH}_4\text{Cl}$ ,  $\text{KCl}$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES),  $\text{NaCl}$ ,  $\text{CaCl}_2$ ,  $\text{Na}_2\text{MoO}_4$ ,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{CoCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{ZnCl}_2$ ,  $\text{H}_3\text{BO}_3$ ,  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ ,  $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ ,  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  and riboflavin were obtained from Sigma-Aldrich. Deionized (DI) water was collected from Millipore Synthesis A10 (Molsheim, France). *Shewanella oneidensis* MR-1 (ATCC 700550) was acquired from American Type Culture Collection (Manassas, VA), and *Escherichia coli* DH5 $\alpha$  (DSM 6897) was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) (Braunschweig, Germany).

### 2.2 Chip design and fabrication

Three types of  $\mu$ MFC chips were designed to compare their performance and identify the optimal design (Fig. 1a). The first one has a simple Y-shape. The second is with expanded cathode and anode chambers. The third is with micro-stoppers in the anode chamber. The purpose of including the expanded chambers and stoppers is to maximize the bacteria retention on the anode and increase the surface area of electrode for efficient electron transfer.

A glass substrate (1.5 in.  $\times$  3 in.) was used to pattern the electrodes. A layer of negative photoresist (SU8-3010) was spin-coated on the glass substrate and then exposed to UV irradiation through a film photomask. A 1.0-mm-wide pattern of photoresist (to separate anode and cathode) was formed on the glass substrate after rinsing in SU8 developer. A 200-nm layer of gold was deposited on the glass wafer by sputtering (coaxial magnetron sputter).



**Fig. 1** Design and assembly of membrane-free  $\mu$ MFC. **a** Three types of  $\mu$ MFC chip designs for comparison: (1) Y-shape; (2) expanded cathode and anode chambers without micro-stoppers; and (3) micro-stoppers in the expanded anode chamber. **b** Schematic illustration of the major components of  $\mu$ MFC. **c** The image of an assembled  $\mu$ MFC. The gold electrodes were connected to a digital multimeter for OCV measurement. The enlarged *inset* shows the laminar co-flow in the downstream microchannel after electrolytes are pumped in. The yellow solution on the left is catholyte (color figure online)

The photoresist was then stripped by acetone. The polydimethylsiloxane (PDMS) micro-fluidic channels were fabricated following soft lithography method. A 3-in. silicon wafer was spin-coated with a negative photoresist (SU8-2075) at 2000 rpm for 120 s to achieve coating thickness of 100  $\mu$ m. The wafer was then pre-baked on a hotplate at 65  $^{\circ}$ C for 5 min and at 95  $^{\circ}$ C for 20 min, followed by UV irradiation through a film photomask containing the pattern of microchannels. After UV exposure, the wafer was baked at 65  $^{\circ}$ C for 5 min and 95  $^{\circ}$ C for 10 min and then immersed in SU-8 developer with gentle shaking for 7 min for complete developing. A mixture of silicon elastomer base and curing agent at a mass ratio of 10:1 was cast onto the SU-8 mold and cured at 70  $^{\circ}$ C for 3 h. Then, the PDMS slab with designed channel structure was sliced and peeled off from the mold. Before assembling the parts, the PDMS chip and gold-patterned glass substrate were rinsed with DI water and autoclaved at 120  $^{\circ}$ C under 10 atm. for 20 min for sterilization. The PDMS chip was manually stacked onto the substrate with the microchannel aligned along the gap between two electrodes (Fig. 1c). A dual-syringe pump (KDS Legato 180) was used to infuse the anolyte and catholyte into the micro-chambers.

## 2.3 Data acquisition

A digital multimeter (3146A ESCORT) was used to measure the open-circuit voltage (OCV) produced by the  $\mu$ MFC. The gold electrodes were connected to the digital multimeter by electrical clips. An aluminum foil was placed between the gold electrode and the electrical clip to ensure close contact for electric conduction. Data were recorded at 2-min interval for totally 1–1.5 h until a stabilized output was achieved. The polarization curves and power curves of the system were measured using linear sweep voltammetry (LSV) at a scan rate of 1  $\text{mVs}^{-1}$  over a range from open-circuit voltage to 0 mV. The LSV data were recorded using an Autolab PGSTAT 302 N potentiostat (Metrohm, the Netherlands) and NOVA 1.9 software. The internal resistance of the  $\mu$ MFCs was calculated based on the polarization curves (Fan et al. 2008). For the linear (ohmic) region of the polarization curves, the relationship between the external voltage ( $E$ ) and the current ( $I$ ) is expressed in the following equation.

$$E = E_b - R_{\text{int}}I \quad (1)$$

where  $E_b$  denotes the intercept of the linear (ohmic) region of the polarization curve with the voltage axis.  $R_{\text{int}}$  represents the internal resistance of the  $\mu$ MFC, which can be determined by linear fitting of the polarization curve.

## 2.4 Cell culture

*Shewanella oneidensis* MR-1 and *E. coli* DH5 $\alpha$  were used as two model bacteria for this proof-of-concept study. The bacteria were cultured in standard Luria broth (LB) media under aerobic conditions at 30  $^{\circ}$ C for MR-1 and at 37  $^{\circ}$ C for DH5 $\alpha$  under shaking speed of 200 rpm overnight, followed by another passage under the same condition for 8 h; 100  $\mu$ L culture solution with an optical density (600 nm) between 0.6 and 0.7 was transferred to room temperature to ensure a consistent concentration throughout the experiments. The minimal medium contained 28 mM  $\text{NH}_4\text{Cl}$ , 1.34 mM KCl, 5 mM  $\text{Na}_2\text{HPO}_4$ , 0.7 mM  $\text{Na}_2\text{SO}_4$ , 1 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 20 mM PIPES, 52 mM NaCl, 0.2 mM  $\text{CaCl}_2$ , 5 mg/L  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.5 mg/L  $\text{Na}_2\text{MoO}_4$ , 0.02 mg/L  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ , 10 mg/L  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ , 3 mg/L  $\text{CoCl}_2 \cdot 4\text{H}_2\text{O}$ , 2 mg/L  $\text{ZnCl}_2$ , 0.2 mg/L  $\text{H}_3\text{BO}_3$ , 1 mg/L  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ , 0.06 mg/L  $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$  and 0.08 mg/L  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$  (Ding et al. 2014; Zachara et al. 1998). The lactate medium was prepared by adding 20 mM sodium lactate as the carbon source and 5 mg/L riboflavin as the electron shuttle mediator to the minimal medium. The fresh anolyte was prepared by mixing lactate medium solution, bacterial culture solution and 2 % LB solution at a volume ratio of 9:1:1. The fresh catholyte contained 50 mM potassium ferricyanide in buffered solution (pH 7.5).

## 2.5 Bacterial fixation and imaging

After operation for about 1.5 h, the  $\mu$ MFC was disassembled and rinsed with minimal medium. The adherent bacteria were fixed in 2 % glutaraldehyde solution overnight at 4 °C and then dehydrated by sequentially transferring through 50, 70, 90 and 100 % ethanol for 10 min in each solution and dried at 50 °C in a thermostatic oven for 2 h. Before imaging, the fixed samples were sputter-coated with platinum. Images were taken using a field emission scanning electron microscope (JSM-6700F). The bacteria adhered inside the PDMS anode chamber were imaged with an inverted microscope (Olympus IX71).

## 2.6 Statistical analysis

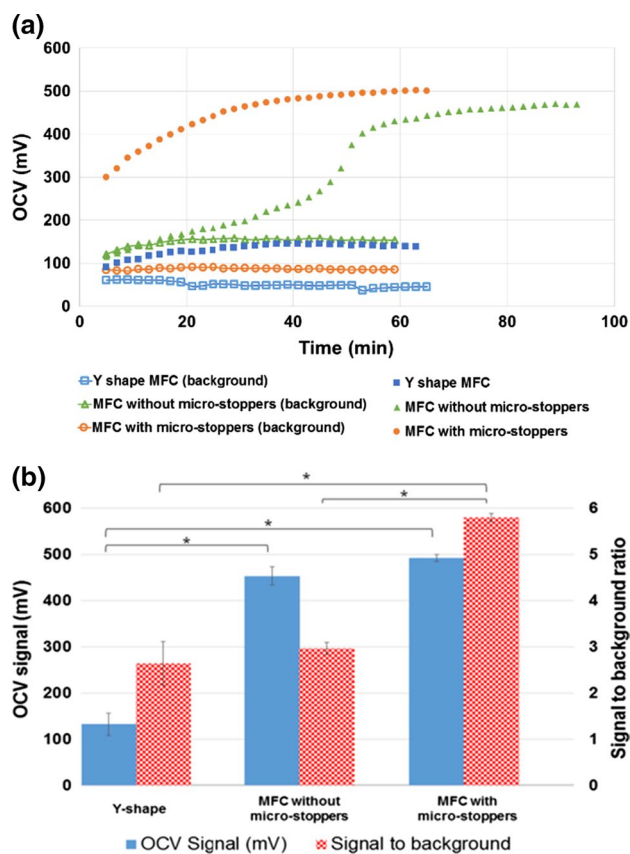
One-way analysis of variance (ANOVA) and Turkey honestly significant difference (HSD) test were used to statistically analyze the significant difference of OCV and signal-to-background ratio among three designs. Student's *t* test was used to analyze the significant difference of the OCV output between MR-1 and DH5 $\alpha$ . The difference between data were considered statistically significant if the *p* value was <0.05.

## 3 Results and discussion

Rather than designing an MFC for power generation, this study was focused on the optimization of a  $\mu$ MFC for characterizing the exoelectrogenic capability of bacteria. Specifically, the performance of the  $\mu$ MFC was evaluated with two different bacterial models, namely *S. oneidensis* MR-1 and *E. coli* DH5 $\alpha$ . The OCV of the  $\mu$ MFC was used as a simple and effective indicator to evaluate the exoelectrogenic capability of the particular bacterial strains (Logan et al. 2006).

The system was set up by infusing anolyte and catholyte into the  $\mu$ MFC at flow rate of 60  $\mu$ L/min. The flow rate usually affects the laminar co-flow in a microchannel and the mixing rate of small molecules across the two-stream boundary (Choban et al. 2004). Under this applied flow rate, the Reynolds number (*Re*) in the co-flow region was <10. Therefore, a laminar co-flow was maintained to minimize the mixing of oxidation agent and other species from the catholyte stream with the anolyte stream. In the meantime, proton exchange was allowed across these two streams.

The background signals in Fig. 2a were the OCVs measured for the bacteria-free chips with three different designs as negative control. These background voltages were generated due to different redox potentials between the medium in anode chamber and the buffer in cathode



**Fig. 2** Characterization of  $\mu$ MFCs. **a** Background OCVs and OCVs produced by MR-1 in three chip designs. **b** Average stabilized OCVs produced by MR-1 and the signal-to-background ratios. The data are shown as mean  $\pm$  SD ( $n = 3$ ). \**p* value <0.05 (Turkey HSD test) indicates significant difference between two groups

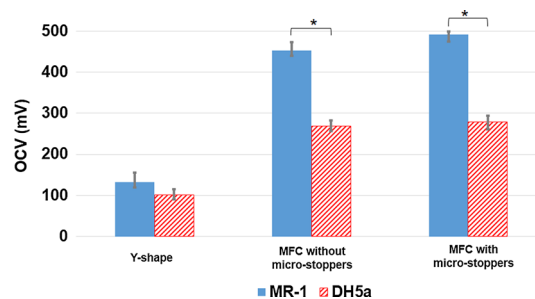
chamber (Logan et al. 2006), which needed to be considered to accurately evaluate the exoelectrogenic capability of the bacteria. The different background OCVs measured in three designs were due to the different  $\mu$ MFC configurations, including the surface area of electrodes and the fluid velocity distribution inside the chambers, which influenced the resultant redox potential. The experimental results indicated that the Y-shape  $\mu$ MFC with the smallest electrode area exhibited consistently the lowest background at 50 mV. For designs with expanded reaction chambers, the background OCV increased considerably to 155 mV, while the one with micro-stoppers in anode chamber reduced the background to a moderate level of 87 mV. This notable difference was due to the unique micro-stopper design. Although the micro-stoppers did not change the surface area of the electrode significantly, they increased the flow resistance and decreased the flow velocity in the anode chamber as shown by numerical simulation (Fig. S1, Supporting Information). As reported in the literature on laminar flow-based micro fuel cells (Choban et al. 2004; Sprague et al. 2009), the change of anolyte flow patterns



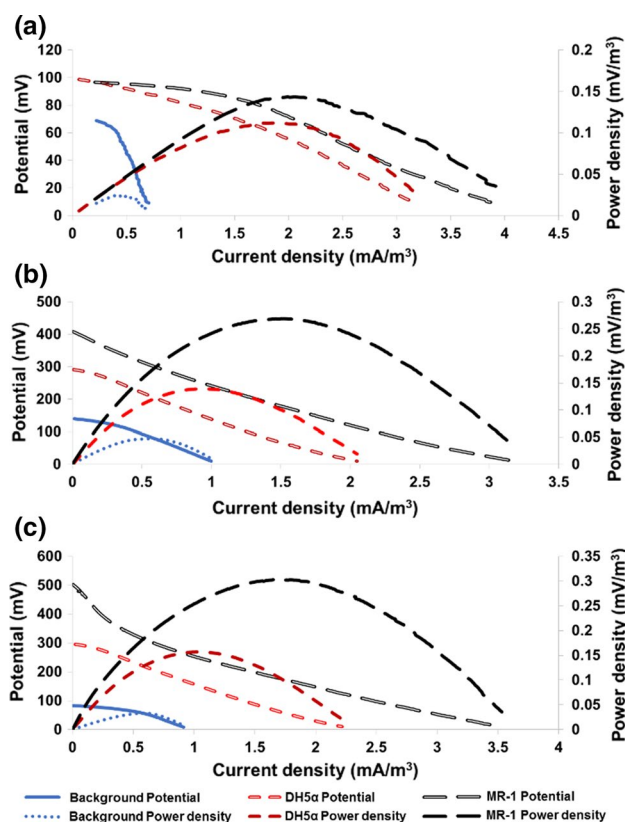
could affect the  $\mu$ MFC internal resistance to proton transport and hence the OCVs.

The laminar co-flow was developed immediately after the catholyte and bacteria-laden anolyte were introduced into the  $\mu$ MFC (Fig. 1c, inset). The measured OCV quickly adjusted and increased to a stabilized output (Fig. 2a). The time required to reach a stabilized output ranged from about 10 to 90 min, depending on the specific chip design and bacterial species. The  $\mu$ MFC with expanded reaction chambers typically required a longer time for output stabilization. In contrast, it usually takes a few days for conventional MFCs to reach stabilized output (Fan et al. 2012; Yu et al. 2011), while a few hours for many existing miniaturized MFCs (Qian et al. 2011). In addition, it was obvious that three different  $\mu$ MFC designs produced different levels of output OCV after stabilization (Fig. 2b). The two  $\mu$ MFCs with expanded reaction chambers (with and without micro-stoppers) generated significantly higher output OCV (at  $492 \pm 7.55$  and  $453 \pm 19.97$  mV, respectively) compared to the Y-shape design that had a final output of only  $132 \pm 23.33$  mV ( $p$  value  $< 0.05$ ), which implied that larger electrode surface area in the  $\mu$ MFC played an important role to enhance the total output OCV. Meanwhile, the  $\mu$ MFC with micro-stoppers produced higher OCV than the design without micro-stoppers, indicating that micro-stoppers enhanced the bacteria-electrode interaction and thereby increased the output OCV signal. On the other hand, the background OCV also increased considerably due to the enlarged electrode surface (Fig. 2a). Because the background OCV was not contributed by the bacteria, we measured the signal-to-background ratio (Fig. 2b) to evaluate the contribution solely by the bacteria, which showed significant difference ( $p$  value  $< 0.05$ ) between the  $\mu$ MFC with ( $5.79 \pm 0.09$ ) and without ( $2.96 \pm 0.13$ ) micro-stoppers. For the Y-shape  $\mu$ MFC, however, the output OCV was considerably lower than the designs with expanded chambers (Fig. 2a, b) because it had much smaller surface area of the electrodes. Although the Y-shape  $\mu$ MFC produced the lowest background OCV, the considerably lower output OCV caused the lowest signal-to-background ratio. Therefore,  $\mu$ MFC with micro-stoppers produced the highest signal-to-background ratio among three different designs due to the unique micro-stopper feature, which reduced the background OCV while increasing the output OCV and thereby enhanced the overall sensitivity for exoelectrogenic bacteria screening.

Based on these observations, the  $\mu$ MFC with expanded reaction chambers and micro-stoppers exhibited the highest efficiency among three designs with regard to OCV generated by the exoelectrogens. We further measured and compared the raw OCVs produced by DH5 $\alpha$  (Fig. S2, Supporting Information) and MR-1 (Fig. 2), respectively, using three different designs. The average OCVs produced



**Fig. 3** Comparison of the stabilized OCVs produced by MR-1 and DH5 $\alpha$ . The data are shown as mean  $\pm$  SD. \* $p$  value  $< 0.05$  (Student's  $t$  test) indicates significant difference between two groups



**Fig. 4** Polarization curves obtained from three different designs using linear sweep voltammetry (LSV) methods at a scan rate of 1 mV/s. **a** Y-shape MFC; **b**  $\mu$ MFC without micro-stoppers; and **c**  $\mu$ MFC with micro-stoppers

by MR-1 and DH5 $\alpha$  in three designs are shown in Fig. 3. According to the statistical analysis, it was evident that there was no significant difference between the OCV produced by two bacterial strains in the Y-shape design, while the OCV produced by MR-1 was significantly greater than that by DH5 $\alpha$  in the other two designs ( $p$  value  $< 0.01$ ). For the Y-shape  $\mu$ MFC, MR-1 generated 30 % higher OCV than DH5 $\alpha$ . Meanwhile, the OCV produced by MR-1 was

**Table 1** Current and power generation details of different designs

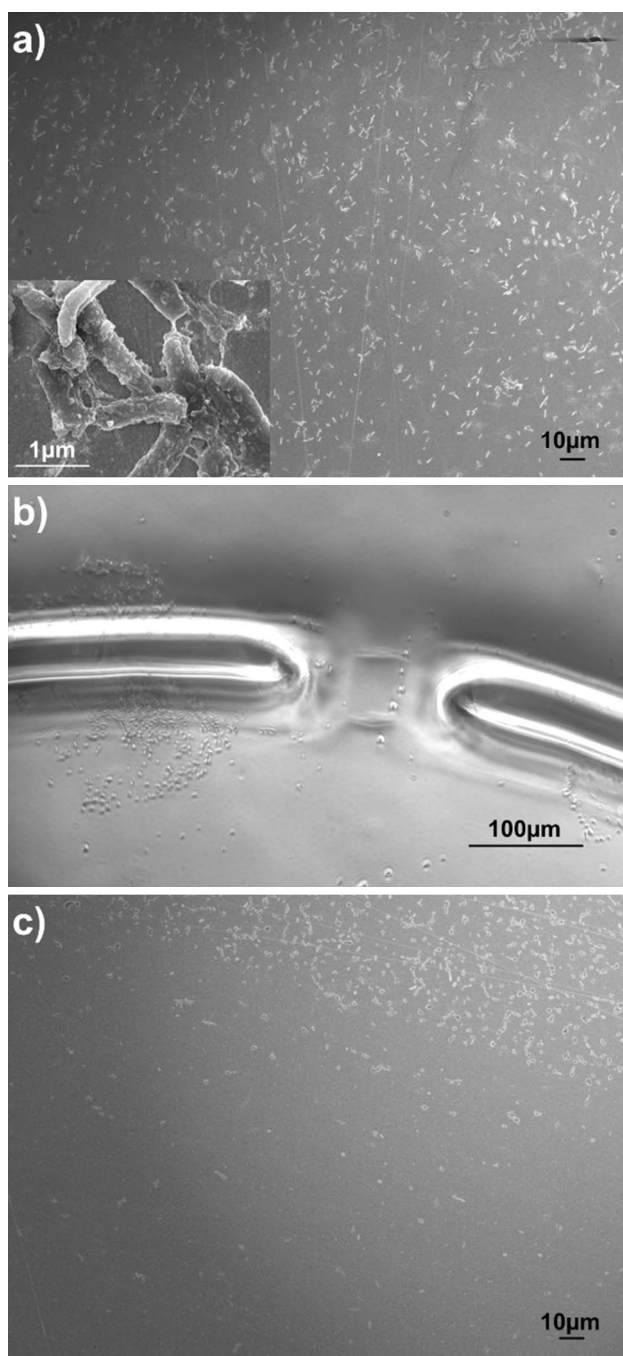
	Y-shape MFC		MFC without micro-stoppers		MFC with micro-stoppers	
	DH5 $\alpha$	MR-1	DH5 $\alpha$	MR-1	DH5 $\alpha$	MR-1
Anode chamber volume ( $\times 10^{-9}$ m <sup>3</sup> )	2.00	2.00	98.5	98.5	98.5	98.5
Projected anode area ( $\times 10^{-5}$ m <sup>2</sup> )	1.25	1.25	9.10	9.10	9.10	9.10
Maximum power (nW)	1.399	1.796	12.625	24.439	14.287	27.579
Maximum power density (volumetric) (W/m <sup>3</sup> )	0.699	0.898	0.128	0.248	0.145	0.280
Maximum power density (by area) (mW/m <sup>2</sup> )	0.112	0.144	0.139	0.269	0.157	0.303
Calculated internal resistance (M $\Omega$ )	2.91	2.58	1.76	1.28	1.65	1.13

67 % and 76 % higher than the OCV produced by DH5 $\alpha$  in the  $\mu$ MFC with expanded chambers, without and with micro-stoppers, respectively. There are many prior studies that have investigated either DH5 $\alpha$  (Qiao et al. 2007) or MR-1 (Qian et al. 2011) as the catalysts in various MFC devices. However, to the best of our knowledge, no prior study has characterized and compared the electrogenic capability of these two bacterial strains on the same platform. In the present study, MR-1 and DH5 $\alpha$  exhibited different exoelectrogenic capabilities under the same condition. These results suggested that this  $\mu$ MFC could be used as an efficient tool for rapid analysis of different exoelectrogenic microorganisms.

The polarization curves and power output obtained for different designs and bacterial strains are shown in Fig. 4. It was observed that MR-1 outperformed DH5 $\alpha$  in all three designs in terms of potential and power density. Typically, the power density initially increased to a maximum with the increase in current and then gradually decreased with further increase in current. Table 1 summarizes the major data of different  $\mu$ MFC designs and bacterial strains derived from the polarization curves (Fig. 4). The maximum power density by MR-1 was always greater than that by DH5 $\alpha$ , which was consistent with the above results related to OCV. The maximum power density by area produced by both DH5 $\alpha$  and MR-1 was highest on the  $\mu$ MFC with expanded chambers and micro-stoppers. However, for maximum power density by volume, the Y-shape  $\mu$ MFC exhibited the highest density among three designs, which was due to the fact that the Y-shape  $\mu$ MFC has the highest surface-area-to-volume ratio compared to the other two designs. The maximum power output of the  $\mu$ MFCs with expanded chambers using MR-1 was about 0.3 mW/m<sup>2</sup> (Table 1), which was much lower than that (1.5–6.25 mW/m<sup>2</sup>) by other similar devices reported in the literature (Qian et al. 2009, 2011). In terms of internal resistance, the Y-shape  $\mu$ MFC showed the greatest resistance of 2.91 and 2.58 M $\Omega$  for DH5 $\alpha$  and MR-1, respectively, while the  $\mu$ MFC with micro-stoppers showed the lowest internal resistance of 1.65 M $\Omega$  for DH5 $\alpha$  and 1.13 M $\Omega$  for MR-1. Generally, high internal resistance was expected for  $\mu$ MFCs due to the small contact area

between electrolytes and electrodes. The internal resistance of our device (1 ~ 3 M $\Omega$ ) was much higher than those of other existing  $\mu$ MFCs (13–30 k $\Omega$ , Qian et al. 2009, 2011). This was due to the relatively short operation time of the proposed  $\mu$ MFCs (~1.5 h) compared to other  $\mu$ MFCs. Hence, the biofilm coverage on the anode surface was still low (Fig. 5a), which caused relatively higher resistance of the anode. Moreover, the distance between two electrodes in our current devices was 1.0 mm, which was much greater than that in other  $\mu$ MFCs using PEM membrane (~300  $\mu$ m) (Qian et al. 2009, 2011) and hence increased the distance or internal resistance for proton transport. In fact, the lower power density as discussed above was attributed to the higher internal resistance in our device. Further improvement could be achieved by decreasing the gap size between two electrodes, which could shorten the proton transport distance and reduce the internal resistance. Nevertheless, this study demonstrated that the  $\mu$ MFC with expanded chambers and micro-stoppers was effective to improve the sensitivity compared to the previously reported Y-shape  $\mu$ MFC.

To verify the above findings, we also measured the OCVs produced by these two bacterial strains in a regular macroscale dual-chamber MFC (Fig. S3a, Supporting Information), while the culture conditions were maintained exactly same as in  $\mu$ MFCs. The results (Fig. S3b, Supporting Information) showed that both bacterial strains produced higher stabilized OCV (780 mV by MR-1, 454 mV by DH5 $\alpha$ ) in the macro-MFCs as compared to  $\mu$ MFCs (492 mV by MR-1, 280 mV by DH5 $\alpha$ , Fig. 3), which was expected because the macro-MFC was featured by much greater contact area between the electrode and the bacteria, as well as a large proton exchange membrane. Meanwhile, MR-1 outperformed DH5 $\alpha$  in terms of stabilized OCV in the macro-MFCs, which was consistent with the results obtained in  $\mu$ MFC. However, for both bacterial strains, it typically took several days for the macro-MFC to reach stabilized output (4 days for MR-1, 8 days for DH5 $\alpha$ , Fig. S3b), whereas  $\mu$ MFC only took <2 h (Fig. 2a and Fig. S2). Considering the application for rapid screening the exoelectrogenic capability of various microorganisms or as a



**Fig. 5** **a** SEM image of MR-1 adhered on the gold anode of the  $\mu$ MFCs with micro-stoppers. *Inset* MR-1 forming aggregates on the gold substrate. **b** Optical contrast image of MR-1 bacteria retained near a micro-stopper. **c** SEM image of MR-1 adhered on the gold anode of the  $\mu$ MFC without micro-stoppers

microbe-based electrochemical biosensor, the proposed  $\mu$ MFC was obviously more advantageous with rapid analysis, much less reagent consumption and good sensitivity.

After analysis of the bacterial exoelectrogenic capability, the  $\mu$ MFCs with expanded reaction chambers were

disassembled and the bacteria were fixed for visualization. The gold electrode and PDMS anode chamber were examined with a scanning electron microscope and a light microscope. Figure 5a shows the remaining MR-1 bacteria adhered on the gold electrode of the  $\mu$ MFCs with micro-stoppers. A closer examination (Fig. 5a enlarged inset) revealed that some bacterial aggregates started forming biofilm, which could enhance the exoelectrogenic activity of MR-1. The bacterial accumulation was also observed near the PDMS micro-stoppers (Fig. 5b), indicating that the stoppers had facilitated retaining the bacteria inside the anode chamber. In contrast, the MR-1 bacteria adhered very sparsely on the anode of the  $\mu$ MFC without micro-stoppers (Fig. 5c). Particularly, there was almost no bacteria adherent at the center of the chamber where the flow velocity reached the maximum (Fig. S1a, Supporting Information).

## 4 Conclusion

In summary, we have fabricated and compared three different designs of membrane-free micro-fluidic MFC as an efficient tool for rapid characterization of different exoelectrogenic microorganisms on a chip. It has been demonstrated that the  $\mu$ MFC with expanded cathode and anode reaction chambers and micro-patterned stoppers can enhance the bacteria–electrode affiliation and improve the electrogenic output considerably. Comparing the relative open-circuit voltages produced by MR-1 and DH5 $\alpha$ , this device is able to effectively distinguish two bacterial strains with different exoelectrogenic capabilities, which can be further confirmed by the polarization and power curves. We believe that such devices could be used for more extensive applications in exoelectrogenic microbe-related environmental sensing and screening.

**Acknowledgments** This study was supported by a Sustainable Earth Office (SEO) Award at Nanyang Technological University (M408120000) funded to Y.K. and C.B. The PhD scholarship from the Interdisciplinary Graduate School at Nanyang Technological University is gratefully acknowledged by T.C.D.

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