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Effect of electrode sub-micron surface feature size on current generation of *Shewanella oneidensis* in microbial fuel cells

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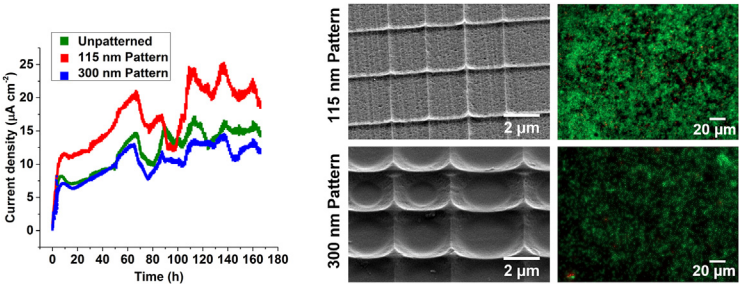
HIGHLIGHTS

- Effect of the feature size was delineated from the effect of increased surface area.
- Effect of the feature size on current production is independent of the surface area.
- The electrode with 115 nm features produces 40% higher areal current density.
- Optimally sized surface features significantly improve bacteria attachment density.
- Majority of the current is produced by electrode-associated biofilm.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial fuel cells (MFCs) are envisioned to serve as compact and sustainable sources of energy; however, low current and power density have hindered their widespread use. Introduction of 3D micro/nanostructures on the MFC anode is known to improve its performance by increasing the surface area available for bacteria attachment; however, the role of the feature size remains poorly understood. To delineate the role of feature size from the ensuing surface area increase, nanostructures with feature heights of 115 nm and 300 nm, both at a height to width aspect ratio of 0.3, are fabricated in a grid pattern on glassy carbon electrodes (GCEs). Areal current densities and bacteria attachment densities of the patterned and unpatterned GCEs are compared using *Shewanella oneidensis* Δ*bfe* in a three-electrode bioreactor. The 115 nm features elicit a remarkable 40% increase in current density and a 78% increase in bacterial attachment density, whereas the GCE with 300 nm pattern does not exhibit significant change in current density or bacterial attachment density. The current density dependency on feature size is maintained over the entire 160 h experiment. Thus, optimally sized surface features have a substantial effect on current production that is independent of their effect on surface area.

1. Introduction

Microbial fuel cells (MFCs) use exoelectrogenic bacteria (e.g. *Shewanella oneidensis* and *Geobacter sulfurreducens*) as catalysts to generate electricity from organic and inorganic substrates. MFCs

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are envisioned to have applications in wastewater treatment, environmental sensing, bioremediation, hydrogen production, and miniature vehicle powering [1–5]. While power density of MFCs has increased by several orders of magnitude over the past decade, an improvement of another one to two orders of magnitude is needed to enable commercial consideration [6]. In this regard, one of the major areas of focus has been the anode, since the extracellular electron transport occurs between the anode biofilm and the anode surface. Anodes of 3D structured materials (e.g. fiber felt [7], non-woven carbon fiber [8], stainless steel foam [9]) have been shown to significantly enhance the performance of MFCs. Modification of the electrode surface by addition of nanomaterials, including nanoparticles [10–12], carbon nanotubes [13–16], carbon nanostructures [17], and graphene [18–20], has also been shown to improve the power generation. These modifications typically changed the surface chemistry (e.g. surface energy, functional groups, or charge) and the surface topography (e.g. feature shape and feature size) making it challenging to identify the specific factors that contribute most to the improved performance. Most studies have attributed the positive effect of the surface micro/nanostructures solely to the increased available surface area for bacterial attachment. However, it is now known that certain topographical feature sizes reduce bacterial attachment density despite the increase in actual surface area [21–25]. Furthermore, it is shown that single cell morphology [26–28], and expression of certain genes [29] are regulated by the size of the surface features. In this work, to investigate the effect of feature size on current production of *S. oneidensis* in MFCs, we utilized our previously developed Spun-wrapped aligned nanofiber (SWAN) lithography technique [30] to texturize electrodes with well-defined and precisely controlled topographical features and disambiguated the effect of topographical feature size from the ensuing effect of surface area increase on bacterial attachment and current production.

2. Experimental methods

2.1. Electrode assembly and surface nanostructure fabrication

Glassy carbon (GC) was chosen as the electrode material due to its high electrochemical activity, biocompatibility, and surface smoothness after polishing. Electrodes were assembled as shown in Fig. 1(a). Before assembly, the back face and edges of the GC chip (10 mm × 10 mm, and 1 mm thick, Hochtemperatur-Werkstoffe GmbH, Germany) were roughened using a 320 grit sandpaper, followed by sonication in deionized water, flushing with dry air and then further drying on a hot plate at 180 °C. A wire was soldered to a copper plate (6 mm × 6 mm), which was then adhered to the back face of the GC piece by conductive epoxy. The back of this assembly was covered by a biocompatible and autoclavable epoxy (Master Bond, Hackensack, NJ), leaving only the front face of the GC chip exposed to the bacteria solution in the experiment. After curing at room temperature overnight, the assembled glassy carbon electrode (GCE) was further cured at 70 °C for another 3 h. The exposed surface of the GCE was polished successively by 1 μm, 0.3 μm, and 0.05 μm alumina slurry to produce a smooth surface finish. Smooth electrodes serve as the control (unpatterned) electrodes and also as the base for fabricating the nanopatterned electrodes. Nanostructures in a grid pattern were fabricated on the unpatterned GCEs using SWAN lithography [30]. First, 7 wt% and 14 wt% polystyrene (PS, molecular weight of 2000 kg mol^{-1}) in xylene solutions were used to deposit 170 nm (for fabrication of the small features) and 370 nm (for fabrication of the large features) fiber masks on the GCE surfaces, using the non-electrospinning spinneret based tunable engineered parameter (STEP) technique [31]. To fuse fibers to the substrate, the fiber-masks were then exposed to tetrahydrofuran vapor for 40 min (170 nm fiber) or 60 min (370 nm fiber). The mask covered electrodes were etched at 2.0 V (vs. Ag/AgCl) in 0.1 M NaOH solution for 5 min (small feature) and 10 min (large feature), respectively. After etching, the masking fibers were gently wiped off and the electrodes were further treated in piranha solution (3:1 H_2SO_4 and H_2O_2) for 40 min to remove the PS fiber residue. The patterned surface nanostructures were characterized by scanning electron microscopy (SEM) and atomic force microscopy (AFM). In order to confirm that the surface activity of the electrodes remained unchanged after mechanical polishing and electrochemical etching, cyclic voltammetry (CV) at a scan rate of 100 mV s^{-1} in a solution of 1 M KNO_3 and 1 mM ferricyanide was conducted (Fig. S2).

2.2. Bacteria culture and bioreactor setup

S. oneidensis Δ*bfe* [32] was streaked from −80 °C frozen stock on a 1.5% Lysogeny broth (LB) agar plate (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 10 g l^{-1} NaCl, and 15 g l^{-1} agar) and cultured at 30 °C for 24 h. A single colony was isolated from the plate and inoculated into a 125 ml flask containing 10 ml LB media and cultured at 30 °C and 150 rpm. Bacteria were harvested after 5 h, centrifuged at 1700 g for 10 min and resuspended in minimal medium (MM) twice, then transferred to a 1000 ml flask containing 100 ml MM with 10 μM flavin mononucleotide (FMN, Sigma-Aldrich, St. Louis, MO), and cultured at 30 °C and 150 rpm. Bacteria were harvested at $OD_{600} = 1$ (Fig. S1), centrifuged, and resuspended in fresh MM supplemented with 10 μM FMN and then introduced into the bioreactor. The MM contains 0.46 g l^{-1} of NH_4Cl, 0.225 g l^{-1} of K_2HPO_4, 0.225 g l^{-1} of KH_2PO_4, 0.117 g l^{-1} of $MgSO_4 \cdot 7H_2O$, 0.225 g $l^{-1}$of $(NH_4)_2SO_4$, 10 ml of a mineral mix (containing 1.5 g l^{-1} of nitrilotriacetic acid, 0.2 g l^{-1} of $FeCl_2 \cdot 4H_2O$, 0.1 g l^{-1} of $MgCl_2 \cdot 6H_2O$, 0.02 g l^{-1} of sodium tungstate, 0.1 g l^{-1} of $MnCl_2 \cdot 4H_2O$, 0.1 g l^{-1} of $CoCl_2 \cdot 6H_2O$, 1 g l^{-1} of $CaCl_2 \cdot 2H_2O$, 0.05 g l^{-1} of $ZnCl_2$, 0.002 g l^{-1} of $CuCl_2 \cdot 2H_2O$, 0.005 g l^{-1} of H_3BO_3, 0.01 g l^{-1} of sodium molybdate, 1 g l^{-1} of NaCl,

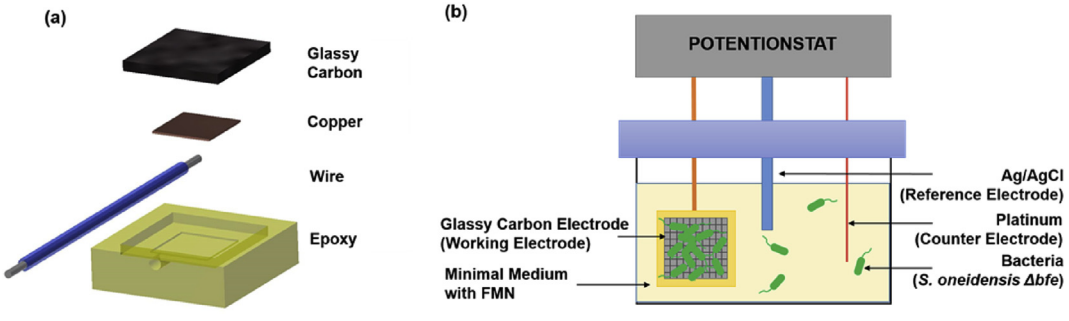

Fig. 1. **(a)** Exploded view of the glassy carbon electrode (GCE) assembly. (**b**) Schematic of the bioreactor setup used for electrochemical performance characterization.