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Biofilm vivacity and destruction on antimicrobial nanosurfaces assayed within a microbial fuel cell

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Abstract

A novel method was developed to assay the antimicrobial capacity of nanostructured surfaces for medical implants in a bicathodic microbial fuel cell. Nano-structured gold surfaces with protruding nanopillars and nanorings were investigated. *Escherichia coli* K12 were used as a model microbe to record electronic effects caused by the interaction with nanosurfaces. The nanostructured gold surfaces enabled power density maxima up to 1910 mW/m^2, indicating fair vivacity, while flat surfaces on the nanoscale provided almost no power 0.35 mW/m^2. The biofilm presence on antimicrobial nanosurfaces was confirmed by the addition of ampicillin and its bactericidal effect resulted in oscillating and declining potentiometric signals. Current density experiments showed that biofilms on antimicrobial nanostructured electrodes caused low currents, indicating that *E.coli* biofilm remained functional before destruction. The bicathodic microbial fuel cell sensor is a novel tool for evaluating antimicrobial effects caused by nanosurfaces and antibiotics.

Key words: Antimicrobial nanosurface; Antibiotic; Microbial fuel cell; Biofilm; *E.coli*

Anti-microbial surface properties of medical implants are crucial for avoiding infections and ensuring patients' rapid recovery after surgery. Currently there are no suitable standardized methods for biofilm detection and quantification, probably due to the complexity of various biofilm structures and compositions, but also because there are not many materials that fulfil easily needed properties. This could however change due to the recent discovery of antimicrobial resistance with insect wings. It was observed that dead cicadas (*Psaltoda claripennis*) degraded in an unexpected pattern. While their body is easily digested by microbes from the environment their tin wings do not degrade. Microscopy showed that the effect was related to a phalanx of nanopillars anchored on the cicadas' wings, which destroyed approaching microbes.[1] The antimicrobial capacity of these nanostructured surfaces is enhanced by a strong hydrophobicity.[2] However, the microbes dead or vital remained on the nanostructured surface. This raised the question as to whether or not such biofilms are functional, and whether there is antimicrobial action, that can be analysed within a microbial fuel cell (MFC). To do so the nanosurfaces need to be made conductive by sputtering them with a conductive material or fabricated entirely from conductive material. Such antimicrobial nanopillars can be synthesized using nanoimprint lithography[3] or a templated electrodeposition technique.[4]

Metallic surfaces are rarely employed with MFCs due to passivation issues that most metals pose as well as, because of the risk of irreversible attachment of biomolecules on metal surfaces and biofouling. An exceptional metal in this respect is gold (Au), which is known to be highly stable when it comes into contact with biological systems. This stability is due to its electrode standard potential of 1.69 V, which makes it inert in microbial environments.[5] Flat gold surfaces in microbial fuel cells were found to be recalcitrant to biofilm adhesion.[6] To circumvent this problem a composite material approach was used to improve biofilm adhesion, with gold sputtered carbon paper clearly enhancing MFC performance. A certain surface roughness seems therefore to be beneficial for biofilm adhesion to gold. To avoid biofilm formation nano roughness need to disrupt and destroy approaching microbes.

In this work *E. coli* served as a model organism to study the antimicrobial activity of potentially antimicrobial nanosurfaces.

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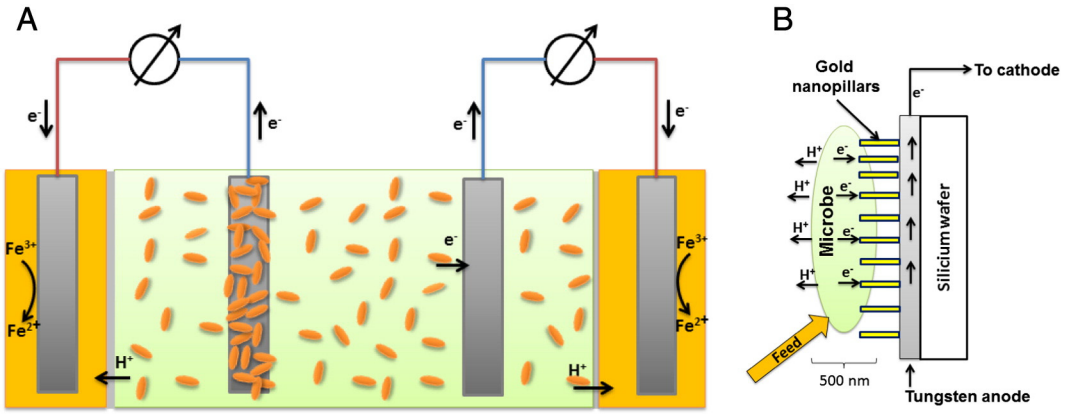

Figure 1. **(A)** Bicathodic microbial fuel cell sensor setup (MFC-1) with a nanostructured gold electrode (right) and reference electrode (left) in the anode and carbon fibre electrodes with 0.5 M hexacyanoferat solution in the cathodes.**(B)** Nanopillars in physical contact with the microbe eventually disrupt the microbe's outer cell membrane causing an alternate electron density flux.

The goal was to investigate whether nano-structured surfaces cause detectable bioelectric signals and thereby enable the detection of antimicrobial nanosurface effectiveness. This was tested through potentiometric, amperometric and polarisation experiments that were performed using a novel microbial fuel cell sensor approach.

Methods

Microbial fuel cell construction

MFC-1: A 500 mL bicathodic microbial fuel cell made from PVC was constructed in house (Figure 1, *A*). It was designed to simultaneously record bioelectric effects on a nanostructured anode and a reference flat anode within the same bioanode cavity.[7]

MFC-2: A 50 mL autoclavable glass MFC (2 × 25 mL half cells) was used to analyse vivacity by polarization and current density experiments. Two identical glass half cells, each with four service channels and a feeding port, were assembled with a clamp holder. Teflon™ disks and rubber joints were added to fix the cation exchange membrane in between the two glass half cells.[8]

Nafion® proton exchange membranes separated anode and cathode compartments for both MFCs. The cathode electrode was made either from carbon fibres or reticulated vitreous carbon (RVC) 20 × 4 × 20 mm^3 (ERG aerospace, USA).

Nanopillar and nanoring fabrication by electrodeposition

Nanopillar electrodes were fabricated by electrodeposition of (Au) into a template of multiple nanopores, which were created by electrochemical anodization of an Al-film.[9] The preparation began by sputtering a 300 nm tungsten layer on a silica wafer. Next vapour was deposited on the W-layer, which consisted of 300 nm of Al-film. The anodization of the top aluminium film was performed in 6% phosphoric acid under 60 V using a platinum mesh counter electrode. During the anodization, the aluminium was oxidized and self-organized vertical nanopores were electrochemically tooled. As illustrated in Figure 2, gold nanopillars were electrodeposited into the nanopores using the tungsten (W) layer as electrode. Subsequently, the aluminium template was dissolved and the golden nanopillar surface was created (Figure 2). The process was performed in a cyanide-free gold electroplating solution (Metalor, ECF 60, 10 g/L) under − 1 mA in a cell of 2.5 cm in diameter. Nanorings (Figure 3, *B*) were obtained by plasma etching the buried tungsten layer before gold electrodeposition.

Electrode wiring

The nanopillar, nanoring and carbon fiber anodes were wired to insulated copper cables using conductive silver epoxy glue (MG Chemicals, Canada) and dried at ambient temperature for four days. A second non-conductive epoxy glue Araldite® (Angst and Pfister, Switzerland) was applied to insulate the cable–electrode connection and other none-structured electrode parts; and dried for 3 h at ambient temperature.

Biofilm growth and stability monitoring using a bicathodic MFC configuration

The 500 ml bicathodic microbial fuel cell (MFC-1) was decontaminated overnight with 1% Korsolex endo-disinfectant solution. The two 10 ml cathodes were equipped with 12 cm^2 carbon fibre electrodes and 0.5 M hexacyanoferrat ($K_3Fe(CN)_6$) and prepared in a 0.1 M phosphate buffer of pH 7. The central anode compartment contained a cultivation medium made of 20 g/l glucose, 5 g/l yeast extract, 8.2 g/l KH_2PO_4 and 10 g/l $NaHCO_3$. *Escherichia coli* K12 ATC 23716 DSM 498 [Deutsche Sammlung für Mikroorganismen, Germany] was precultivated overnight and the 500 mL anode was inoculated with 5 mL of preculture. A nanostructured and a none-structured reference anode were inserted simultaneously into the anodic cultivation, and open circuit potentials were recorded on both sides of the bicathodic MFC for 20 h at 2 min intervals. After 18 h of growth biofilms were treated with 200 μg/mL of Ampicillin, IUPAC name (2S,5R,6R)-6-([(2R)-2-amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, to induce biofilm destruction.