BIOL

Controlling Biofilm Formation Through the Use of Conducting Polymers

Ashlyn Young
Biomedical Engineering, University of North Carolina at Chapel Hill

NNIN iREU Site: Centre Microélectronique de Provence, Ecole Nationale Supérieure des Mines de Saint Etienne, France
NNIN iREU Principal Investigators: George Malliaras and Róisín Owens, Department of Bioelectronics, Centre Microélectronique de Provence, Ecole Nationale Supérieure des Mines de Saint Etienne, France
NNIN iREU Mentor: Adel Hama, Department of Bioelectronics, Centre Microélectronique de Provence, Ecole Nationale Supérieure des Mines de Saint Etienne, France
Contact: youngat@live.unc.edu, malliaras@emse.fr, owens@emse.fr, hama@emse.fr

Introduction:

In liquid environments, microorganisms have the tendency to create complex communities on surfaces as a means of survival [1]. These microbial systems consist of a variety of organisms that thrive within a self-assembled matrix, often very resilient to the external environment. This can prove to be a serious nuisance, as biofilm accumulation commonly occurs on marine vehicles, biomedical implants, and industrial pipelines, and can be very difficult to prevent and remove [2, 3]. To combat this issue, biofouling agents have been formulated that resist and prevent unwanted biofilm growth on surfaces. These antifouling agents are commonly a pollution risk to the outside environment, as they leak biocidal agents into the surrounding marine communities or water sources. As a competitive alternative to harmful antifouling agents, the effects of the semiconductive p-doped polymer poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) on biofilm growth has been explored under difference oxidative states [4]. A 96-well microliter plate was fabricated using photolithographic techniques, consisting of gold lines and PEDOT:PSS pixels. A continuous ± 1 V bias was applied to adjacent gold lines, which were connected via salt solution to produce different oxidative states. Prior to biasing, an *Escherichia coli* (*E. coli*) biofilm was produced *in situ* to adhere to the polymer after variable times, ranging from 0 to 22 hours. Preliminary results optically displayed that biofilms adhered better to an oxidized surface after 22 hours of constantly applied bias, with greater surface area coverage on the oxidized polymer in comparison with the reduced.

Methods:

Device Fabrication. Using soft photolithography techniques and AZ positive photoresist, clean rectangular glass slides were patterned through a 10 second UV exposure and development. Surface activation was then achieved with oxygen plasma and a 50 nm layer of chromium, followed by a 150 nm layer of gold were deposited on top of the patterned resist. Lift off was then performed with acetone and isopropanol, revealing a gold patterned device characterized by parallel conductive lines with empty square pixels to allow video microscope imaging. After gold deposition, two methods were available to attain PEDOT:PSS patterning.

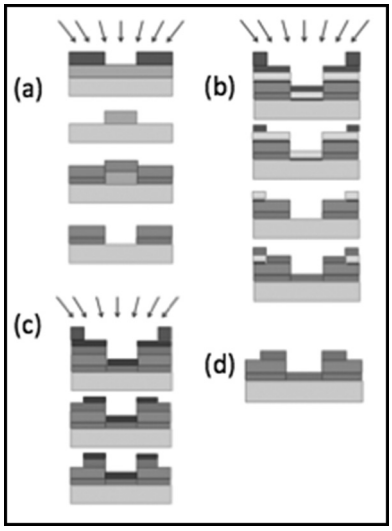

Figure 1: (a) Soft photolithography was used with positive photoresist to create Au, layered on top of Cr, conductive lines. (b) A negative photoresist was used to selectively etch a layer of soap and parylene. PEDOT:PSS polymer was spun on the parylene, which was then peeled off to create the PEDOT pixels. (c) As an alternative to parylene, orthogonal positive photoresist was used on top of PEDOT:PSS to selectively etch the pixel pattern. (d) The final device included lines of Au conductive lines with PEDOT:PSS pixels used as the active areas for E. coli *culture.*

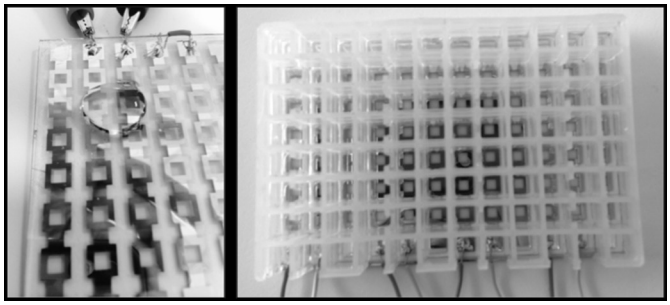

Figure 2: Three NEXTERION® MPX-96 superstructures were used atop the fabricated device. Glued together with PDMS, the top two silicon pieces were cut to allow media flow between two columns, therefore oxidizing one column of pixels and reducing the other when a ± 1 V voltage was applied to the conducting gold lines. Reduced PEDOT:PSS can be identified by a light blue color change. (See full color version on page xxxvi.)

The gold-patterned device was surface activated with oxygen plasma and spin coated at 650 rpm with PEDOT:PSS and soft baked for 60 seconds. Orthogonal negative resist was then spin coated on top of the PEDOT layer, exposed, and developed to protect square pixels of PEDOT:PSS. The unprotected PEDOT:PSS was etched through in the plasma machine, and the final photoresist was removed to reveal square pixels of PEDOT:PSS. An alternative method included a parylene peel off, though orthogonal resist was preferred. A

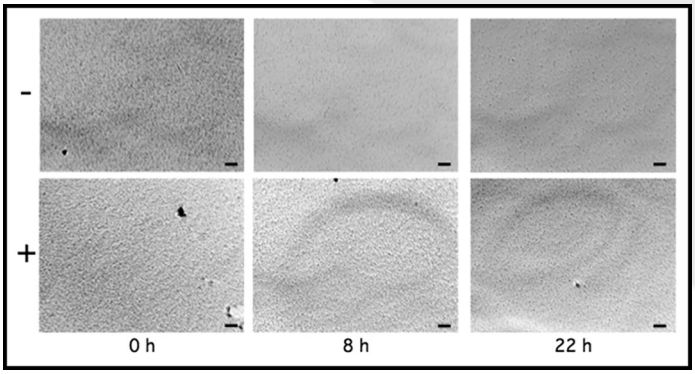

Figure 3: Biofilm growth was assessed under three different adhesion time conditions (22 hours, 8 hours, and 0 hours), and two different oxidative states (oxidized and reduced). Scale bars are 50 µm.

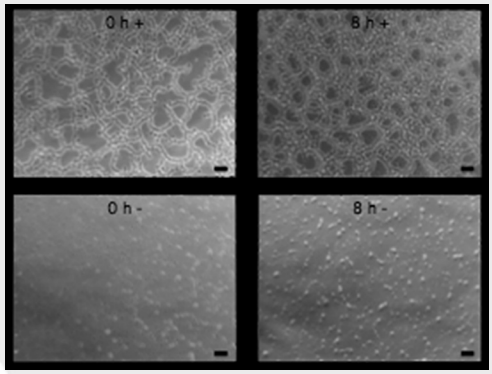

Figure 4: Biofilm was imaged with fluorescent Syto 9/ Propidium Iodide live/dead dye prior to DI water washing. Red indicates dead bacteria, while green indicates living bacteria. Scale bars, 50 µm. (Full color, page xxxvi.)

NEXTERION® MPX-96 superstructure was cut with a scalpel to allow the connection of adjacent rows with media during experimentation. The cut grid was then glued with PDMS to an in-tact grid to isolate bacteria growth on the well plate while still allowing oxidation/reduction to occur on the PEDOT:PSS. The finished device was heated to 100°C on a hotplate and the two-level grid was glued on, exposing PEDOT:PSS pixels, while preventing leakage.

Bacteria Culture. *E. coli* colonies were isolated on an agar plate and stored in the freezer for future use. A colony was selected from the agar plate for each experiment and cultured in 30 mL of LB media for 8 hr. Following three days of subculture in M63% media, 400 μL of the *E. coli* solution was pipetted into four wells in three rows of the device (reduced, oxidized, and control) and left to grow in a humidified 30°C videomicroscope incubator for 0, 8, and 22 hr.

Experimentation. After a biofilm was formed for the determined time condition, 22 hours of ± 1 V bias were applied to adjacent rows in the videomicroscope. A time-lapse video was recorded for the 22 hour condition to observe biofilm formation under bias. Following the bias, the device was removed from the videomicroscope, supernatant fluid was removed, and each well washed twice with deionized water. Cells were then imaged in the videomicroscope. Viability of the bacteria was assessed with Syto 9/Propidium Iodide live/ dead fluorescent dye post experimentation.

Results and Conclusions:

In conclusion, we found that conductive polymer oxidation affected the ability of bacteria to adhere and form biofilm, with the oxidized material displaying more biofilm growth. Through altering the oxidative state of the PEDOT:PSS, we were able to achieve a degree of control over biofilm growth. Reduced biofilm displayed a much lessened biofilm accumulation when qualitatively examined by microscopy. The preliminary images can be better quantified through surface area coverage calculations.

In a time-lapse video collected during bias, it was noted that at 0 hour, oxidized polymer displayed aggregation accumulation of bacteria in early development, a phenomenon very characteristic of biofilm growth. Additionally, this aggregation was not observed as clearly in the reduced polymer. This was solely noted in the 0 hour condition due to the high quantity of bacteria in the samples incubated for longer times prior to bias, making changes in biofilm layer more difficult to view. Fluorescent imaging was used to determine the bacteria viability after bias and was observed under two conditions.

One condition included washing with de-ionized (DI) water; the other only included supernatant fluid removal. The justification for washing with DI water was to avoid the crystallization of media when dried. When washed with DI water prior to fluorescent imaging, it was observed that the bacteria adhered to the glass were all dead, with the only living cells in the supernatant. When avoiding the DI water step, the adhered cells were not fluorescent, possibly due to a protecting layer of fluid and cellular components formed by the biofilm. Despite the bacteria no longer appearing alive, a biofilm was existent on the oxidated polymer, while the reduced polymer did not display such. These observations facilitate the preliminary conclusion that reduced PEDOT:PSS acts as an antifouling agent for the *E. coli* bacterial species.

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