IN SITU SENSOR ELECTRODE PATTERNING ON URINARY CATHETERS TOWARDS INFECTION PREVENTION

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ABSTRACT

This paper presents a novel *in situ* fabrication strategy for patterning electrodes directly on a urinary catheter surface via an innovative electroplating process. Bacterial biofilms present an enormous problem on catheters, leading to severe catheter-associated urinary tract infections. Our approach enables biofilm monitoring via real-time impedance measurement without interfering with the operation of the catheter and overcoming manufacturing challenges associated with fabricating devices on flexible substrates and subsequently conforming them to complex, curved surfaces vulnerable to biofilm. The fabrication process yields isolated gold electrodes directly adhered to the inner lumen of a urinary catheter. These electrodes maintain their integrity after testing with biofilm formation as determined by impedance spectroscopy. The sensor displayed a significant decrease in impedance of approximately 10% over the 24-hour growth period, as opposed to a slight increase of 19% in control experiment, indicating the potential for this approach as a facile strategy for fabricating *biomonitoring electrodes* for safer use of medical devices that are vulnerable to bacterial colonization and infection.

KEYWORDS

Biofilm, impedance sensors, electroplating, electroless plating, 3D printing

INTRODUCTION

Bacterial Biofilms

Biofilms are the predominant mode of growth for bacteria in a wide range of environments. These films form when bacteria adhere to a hydrated surface and, at a threshold population, encase themselves in a protective extracellular matrix (ECM) which increases their resistance to stressors such as antimicrobial treatments. Bacteria in biofilms require 500-5000x higher doses of antibiotics for removal compared to their planktonic counterparts [1]. Bacterial cells may slough off of mature biofilms and spread throughout the environment. Thus, biofilms can serve as a source of recurring infections in human healthcare, particularly when they form on implanted or inserted medical devices [2]. Urinary catheters are one of the most commonly utilized medical devices which are routinely colonized by bacterial biofilms, leading to catheter-associated urinary tract infections (CAUTIs). These catheters lead to infection at a rate of 5-7% per day of implantation [3]. The guideline for the prevention of CAUTI, provided by the Centers for Disease Control and Prevention (CDC), instructs medical practitioners that catheters should not be replaced in fixed intervals but based on clinical indications [4]. Thus, sensors seamlessly integrated onto catheters have the potential to inform better strategies for CAUTI prevention with the on-demand early detection, ultimately allowing prompt treatment or catheter removal before symptoms begin.

Integrated Device-based Approaches

Wearable and flexible devices have proliferated in recent years due to their potential for providing extensive data for healthcare monitoring on complex surfaces [5],[6]. There are limitations, however, with regard to how these transducers can be fabricated for use in confined geometries. Sensors on medical devices such as catheters, tracheotomy tubes, or prosthetic implants have the potential to provide valuable information for diagnosing infections or evaluating the host tissue response. In particular, urinary catheters can benefit from integrated sensors for monitoring bacterial biofilm growth on their surface. Sensors which have been utilized to monitor biofilms include optical density sensors, surface acoustic wave sensors, and impedance sensors [7]-[9]. The impedance sensor is a relatively straightforward, sensitive, and low-power approach comprising two electrodes with an AC excitation between them. An impedance sensor fabricated on a flexible polymer substrate, rolled, and inserted into a urinary catheter has been utilized to monitor biofilm growth [10]. This insertion process presents a limitation as the rolling/deforming of the thin-film metal electrodes can lead to unwanted fractures. Furthermore, electrode-based transducers fabricated on polymeric substrates do not conform well to more complex surfaces.

In this work, we overcome this potential challenge by incorporating direct plating of the electrodes onto the catheter surface, with 3D-printed templates serving to selectively screen the catheter surface. Our process does not rely on cumbersome fabrication technologies including photolithography and physical vapor deposition. Instead, an aqueous electroless plating process is carried out directly on the inner lumen of the catheter with the 3D-printed mold, and a subsequent electroplating process completes the electrode patterning. We successfully demonstrate electrode fabrication and biofilm sensing directly on the cylindrical polymeric surface of the catheter. Given the multitude of medical devices with similarly complex polymeric surfaces that would benefit from integrated sensors, this technique has the potential to be readily applied for a wide range of applications.

MATERIALS & METHODS

Electrode Fabrication

The gold impedance sensor electrodes are fabricated

directly on a 22 Fr elastomeric Foley catheter (Figure 1A) using the process depicted in Figure 1B – (i) The catheter is initially coated in a 25 µm-thick layer of parylene-C to reduce the mechanical mismatch between the plated metal and the silicone polymer substrate and improve adhesion. Cracks appear in the metal without this initial parylene-C layer, resulting in a significant loss of conductivity. (ii) The samples are then cleaned using acetone, methanol, and isopropanol, to be prepared for the following Ni electroless plating step. Oxygen plasma treatment (200 W, 1 min) introduces reactive carboxylic groups to the surface of the parylene-C to improve the adhesion of the electrodes. Immediately after oxygen plasma treatment, the catheters are immersed in 0.026 M stannous chloride solution in 1:1 methanol and water, with 0.07 M trichloroacetic acid, for 45 minutes. This sensitization step bonds tin ions to the surface. After rinsing with methanol, a 3D-printed mold is inserted into the catheter, screening portions of the inner lumen from the electroless plating solutions and producing separate electrodes. 3D printing dramatically simplifies the electrode patterning compared to techniques like photolithography, particularly in hard to reach areas like the catheter lumen. The mold is printed using a FormLabs Form 2 SLA 3D printer with photopolymer resin. After the mold has been added, the catheter is filled with a 10 mM sodium tetrachloropalladate solution for 5 hours, which replaces the tin on the surface with palladium. Palladium serves as a nucleation site for the formation of the nickel seed layer for the following gold electroplating step. Immersion for 45 seconds in a nickel electroless plating bath generates a thin nickel layer (<50 nm) on the catheter inner lumen. (iii) The 3D-printed mold is removed for the Au electroplating. The device is immersed in TSG-250 commercial electroplating solution and cycled from 0 to -0.5 V for 100 cycles at a scan rate of 25mV/s. (iv) The resulting electrodes are then coated with a 1-µm layer of parylene-C to prevent ions from leaching into the environment. SEM and EDS analysis are used to examine the cross section of the fabricated device.

Catheter Flow System

As depicted in Figure 2, the catheter sections with plated electrodes are interfaced with tygon tubing to form a flow system for introducing fresh Luria broth (LB) growth media and bacterial cells. Electrical connections are made using 24-gauge wire clipped to each of the two electrodes. The electrical connections and tubing are sealed using epoxy. A peristaltic pump is used to drive flow at 7 ml/h through the system from an LB media reservoir through the catheter with the sensor to a waste reservoir. The media and waste reservoirs are sterilized in an autoclave at 121 °C. The catheter and tubing are sterilized by flushing with ethanol and UV light exposure for an hour. The entire system is assembled in a sterile biosafety cabinet.

Biofilm Impedance Sensing

Escherichia coli K12 W3110 are cultured overnight in a 5 ml culture tube in an incubator shaker at 250 rpm and 37 °C. The bacterial culture is diluted to an OD_{600} of 0.25 and added to the system via syringe. The bacteria are allowed to attach for 2 hours to the sensor surface under a no-flow condition. Then, LB media is flowed for 24 hours as the biofilm grows. No bacterial cells are added in the control samples. Impedance spectra are gathered using a CHI electrochemical workstation from 10-10^6 Hz at a 50 mV amplitude. These spectra are gathered before the introduction of bacteria with LB media only, at the beginning of the 24-hour biofilm growth period, at the end of the biofilm growth period, and after the growth period after cleaning with ethanol. This last measurement with LB examines the electrode integrity. In addition, the impedance is monitored in real-time at 100 Hz throughout the growth period.

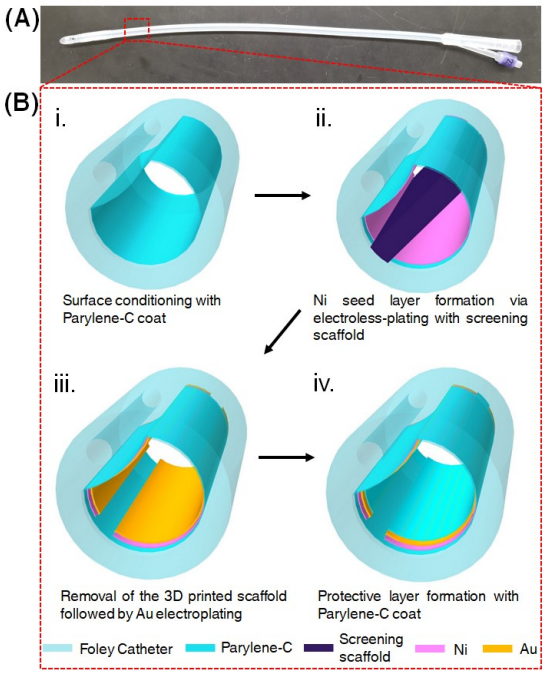

Figure 1: (A) Foley catheter and the (B) process flow depicting the in situ sensor electrode patterning on the inner lumen of a urinary catheter for biofilm detection.

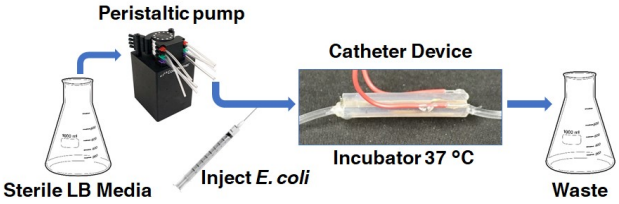

Figure 2: Experimental setup comprised of the catheter device interfaced with media and waste reservoirs via tygon tubing for delivering fresh media during biofilm growth experiments.

RESULTS & DISCUSSION

In Situ Electrode Fabrication

The sensor electrodes patterned on the inner lumen of the catheter are shown in the cross-section image of the catheter section in Figure 3A, demonstrating the viability of this approach for patterning electrodes for sensing on a catheter. The pattern used in this case was a rectangular mold with rounded edges (Figure 3B) to screen the inner catheter lumen for selective electroless plating dark portion of the mold is plated Ni after electroless plating). More complex electrodes with larger sensor interfaces could be produced using more complex 3D printed molds, such as interdigitated patterns. Figure 3A i-iii shows cross-section SEM images from the electrode-patterned catheter with corresponding EDS scans. The silicone, metal, and the two

parylene-C layers are shown distinctly on the surface. EDS analysis confirms each of these layers by means of their signature elements, gold at 2.120 keV (electrodes), chlorine at 2.622keV (parylene-C), and silicon at 1.749keV (silicone catheter). The key difference between the screened (Figure 3Ai) and the exposed (Figure 3Aii) areas is the lack of a metal layer (Figure 3C, inset). The well-defined electrode pattern edge (Figure 3Aiii) clearly indicates the success in use of the 3D printed screening mold for patterning of in situ electroless plating on curved confined surfaces.

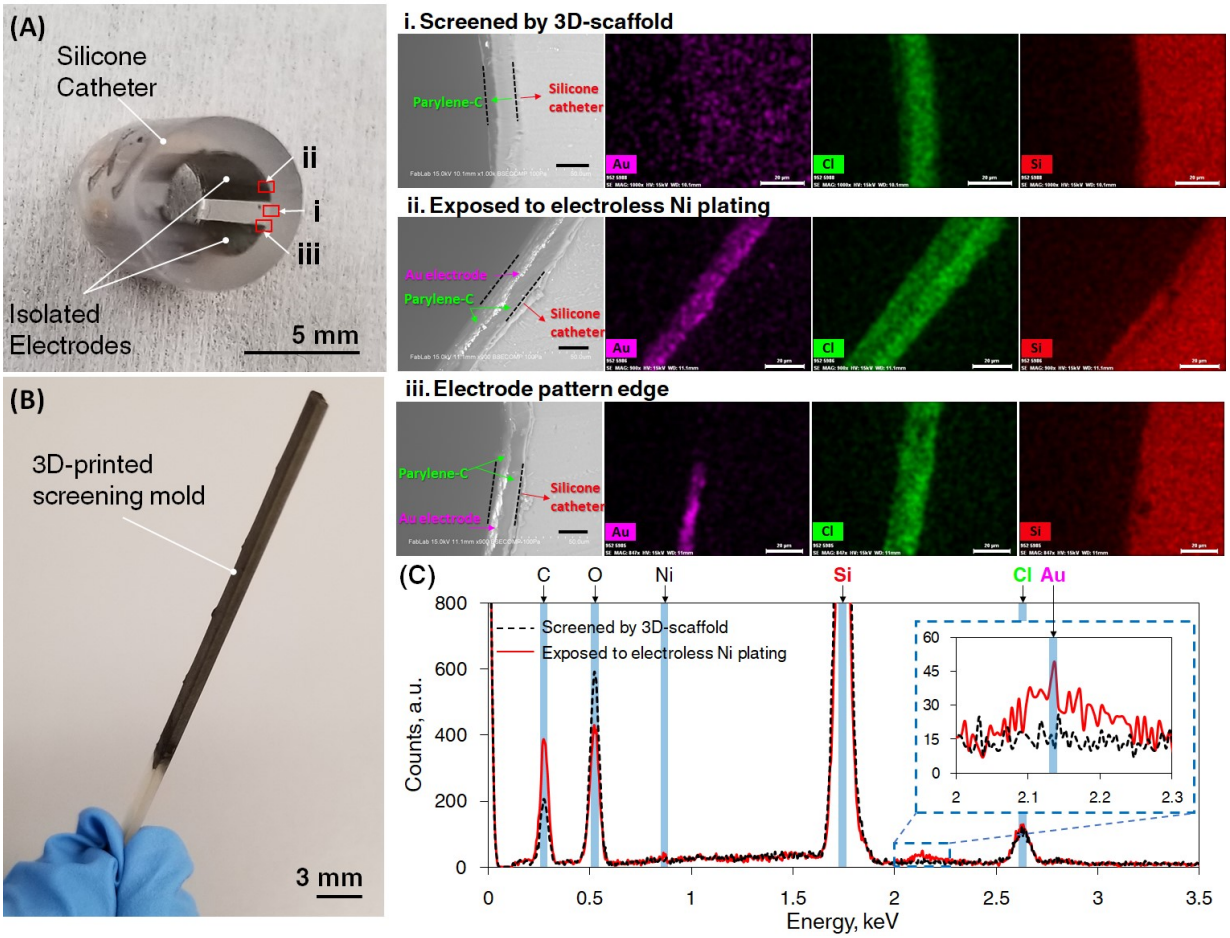

Figure 3: (A) Cross-sectional optical image of the in-situ electrode patterned Foley-catheter with (i-iii) SEM characterization of layer formation with EDS scans from Au, Cl, and Si which are the signature elements for electrodes, parylene-C, and the catheter, respectively (scale bar: 20 µm unless specified). (B) Optical image of the 3D-printed mold for selective screening of electroless plating process, which appears black due to the Ni layer. (C) A comparison EDS spectrum acquired from catheter sections screened by the screening mold (black dotted) and exposed to Ni electroless plating (red), confirming selective pattering of gold electrodes.

Sensor Electrode Characterization

Impedance spectroscopy at the beginning and after 24-hours of biofilm growth displayed a frequency-dependent decrease in impedance associated with biofilm growth (Figure 4A). This is consistent with previous reports indicating that the capacitive component of the impedance changes with biofilm formation due to the accumulation of charged proteins and metabolites [10]. In contrast, control samples without any biofilm showed a frequency-independent increase in impedance (Figure 4B). We suggest that this is related to electrode degradation, potentially caused by the dissolution of metal ions due to the applied voltage in an electrolyte media. This is further evidenced by impedance spectroscopy of the sensor electrodes which had been used to detect biofilm before the cells were added and after cleaning with ethanol. The electrodes displayed a frequency-independent increase in impedance when measuring the impedance in LB media after the electrodes had been cleaned of biofilm (Figure 4C). A similar trend is seen in the control electrodes before and after the growth period (Figure 4D). The increase in impedance after the removal of biofilm with ethanol reinforces the role of biofilm in the decrease in impedance seen with the sensor. Overall, this suggests that this is a viable approach for sensing biofilm on urinary catheters.

Temporal Impedance Monitoring

The *in situ* patterned electrodes were further utilized for continuous, real-time monitoring of biofilm formation. This is an essential element of more effective biofilm infection management, allowing a continuous readout of the state of the device surface. 100 Hz was selected for biofilm monitoring as it is in the middle of the frequency range which displayed an impedance decrease (Figure 4A). The impedance was recorded continuously for samples with and without biofilm over 24 hours (Figure 5). The samples with biofilm showed a sharp decrease over the

first 7 hours, followed by a plateau of relatively little change over the next 17 hours. In total, the impedance decreased approximately 10% over the 24-hour period. This is consistent with the growth dynamics of bacterial biofilm, with a rapid growth phase followed by a stable mature biofilm phase. In contrast, the control sample without any bacterial cells showed an increase in impedance of approximately 20%. This increase was relatively steady throughout the 24-hour period. We suggest that this is due to the electrode degradation.

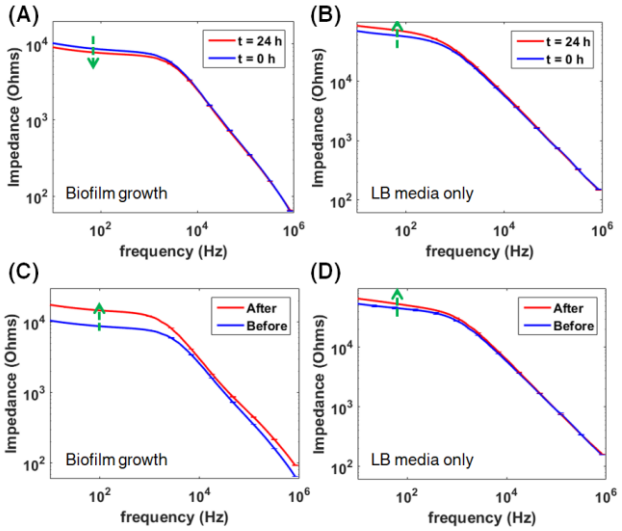

Figure 4: 50 mV impedance spectra with in situ *fabricated electrodes. (A-B) At the beginning of the biofilm growth period (blue) and at the end of 24 hours of biofilm growth (red). Samples with biofilm (A) showed a decrease in impedance, whereas control samples (B) without bacterial cells showed an increase in impedance (N=3 measurements). (C-D) In LB media only before and after use in biofilm sensing experiments for (C) biofilm sensing and (D) control samples (N=3 measurements).*

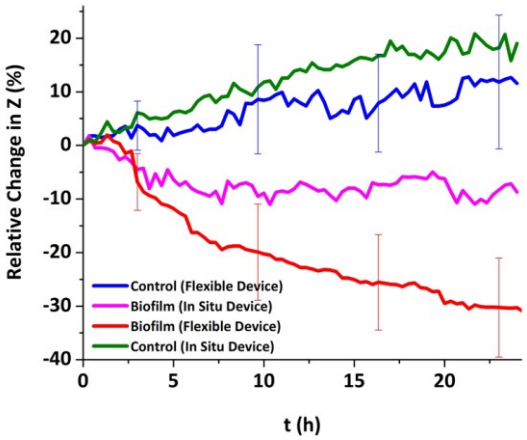

Figure 5: Impedance transients at 100 Hz showing real-time biofilm sensing results. The green and blue lines show the increasing impedance of control samples for the in situ *fabricated device and flexible device, respectively. The magenta and red lines show the decreasing impedance for the biofilm samples of the* in situ *fabricated device and flexible device, respectively.*

These results were also compared to continuous biofilm impedance sensing results using a flexible impedance sensor conformed on a urinary catheter. This device was fabricated monolithically using photolithography, but then must be rolled and inserted into the catheter [10]. Both the control and biofilm sensing samples displayed similar behavior to the *in situ* fabricated electrodes. Each of the biofilm samples showed a significant decrease in impedance, but the *in situ* fabricated electrodes saturated faster. This is not considered a problem as the initial onset of biofilm formation is most important for infection detection. The larger overall decrease with the flexible device is attributed to the larger electrode interface – this device was comprised of interdigitated electrodes.

CONCLUSION

We have introduced an innovative *in situ* fabrication strategy for producing sensor electrodes on inaccessible medical device surfaces, particularly on urinary catheters. The sensors show similar performance to externally-fabricated biofilm impedance sensors, without a complicating integration step. This sensor system has the potential to serve as an integral tool for biofilm infection management. This technique can be applied to additional medical device surfaces that are vulnerable to biofilm colonization, particularly polymeric surfaces with complex geometries. Furthermore, introducing more complex mold designs to increase the electrode interface can improve the performance of these devices.

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