REAL-TIME EVALUATION OF EFFECTIVENESS OF ANTIMICROBIAL COATINGS WITH SURFACE PLASMON RESONANCE IMAGING

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ABSTRACT

In this study, Surface Plasmon Resonance imaging (SPRi) was used to evaluate the effectiveness of antimicrobial coatings for preventing initial bacterial attachment and subsequent biofilm formation. SPRi is capable of monitoring of biological interactions in real time on a large (1 cm$^2$) sensor surface. Here, we monitored the growth and biofilm formation of Pseudomonas aeruginosa (CFP PA01) on gold, bovine serum albumin (BSA), casein, and antibiotic surfaces. Patterning of multiple coatings on a single sensor surface and simultaneous exposure to flowing solution containing bacteria allowed a direct, quantitative comparison of the effectiveness of the coatings.

KEYWORDS: Antimicrobial Coatings, Bacteria, Biofilm, Surface Plasmon Resonance imaging

INTRODUCTION

Antimicrobial surface coatings are evaluated typically by determining the amount of bacterial cells that attach to them over the course of several hours or days. The evaluation is done either by staining the biofilm on the surface at the end of the experiment followed by visual inspection or by removing all of the cells from the surface at the end of the experiment and counting them [1]. These approaches do not provide any information about the dynamics of the attachment process and require additional processing steps after the biofilm formation is complete.

SPRi is a relatively inexpensive, label-free technique, which provides the ability to real-time monitor biological interactions that take place within 200 nm of a gold surface [2, 3]. We recently reported that it is possible to use this technique to obtain images of bacterial biofilm formation in static solutions [4]. Here, we investigate the use of SPRi for evaluating coatings for the prevention of bacterial cell attachment when they are exposed to a continuous flow. In this set of experiments, the sensor surface was coated with two different coatings (BSA and casein or BSA and penicillin) at the same time, and the middle part of the surface was left uncoated as bare gold. Figure 1 shows a schematic of the experimental setup. Casein and BSA are both commonly used proteins in microfluidics for preventing non-specific adsorption of proteins onto channel surfaces [4]. Penicillin with streptomycin is a well-known antibiotic combination for treatment of a large number of bacterial species.

![Figure 1: A schematic of the gold-coated prism with two different coatings on the surface and a PDMS chamber for loading bacteria. The fresh media enters the chamber via the inlet tube and the waste media exits the chamber through the outlet tube. In this system, the middle section was left uncoated in order to collect data of bacterial growth on bare gold, and this data was used to normalize the light intensity change on the coatings due to biofilm formation.](image)

The SPRi system provides images of the 1 cm$^2$ area on the surface with sub-10 micrometer resolution [5, 6]. As bacteria attach to the surface, the system is able to detect the attachment site due to a change in refractive index that is caused by biomass displacing aqueous medium. Every three seconds, the system subtracts the current image from the initial image at the beginning of the experiment to generate a
difference image. In the difference images, the bright spots indicate bacterial attachment, and higher brightness refers to a higher concentration of biomass on the surface, which can be quantified to determine the extent of surface coverage.

EXPERIMENTAL

Each experiment was performed by pipetting two different surface coatings directly onto separate regions of a single gold sensor surface and allowing them to dry for 2 hours prior to exposing them to bacterial solution. A custom-made polydimethylsiloxane (PDMS) chamber was pressure sealed around the patterned area. In the first set of experiments, the surface of the sensor was coated with BSA on the left and casein on the right, the middle part was left as bare gold. In the second set of experiments, the left side of the sensor was coated with BSA and the right side was coated with a penicillin/streptomycin solution. The antibiotic mixture was prepared with 10,000 units/mL penicillin and 10,000 µg/mL of streptomycin.

*Pseudomonas aeruginosa* (CFP PA01) was cultured overnight at 37 °C in 6 mL of Lysogeny Broth (LB) growth medium to allow them to reach stationary phase. The solution was diluted 10 to 1 in fresh LB and 200 µL was placed in the PDMS chamber above the patterned sensor surface. The whole system then was placed into the Horiba SPRi-Lab+ device. Fresh sterile LB was then flowed through the chamber and past the sensor surface for 24 hours at 10 µL/min.

RESULTS AND DISCUSSION

Difference images were evaluated at 6-hour intervals. The images showed the smallest increase in brightness for the sections coated with casein compared to sections of the surface coated with other materials, indicating that casein was the most effective at preventing bacterial attachment and further biofilm formation. The area coated with penicillin/streptomycin had significantly less biomass attachment than the bare gold surface during the first 6 hours of the experiment, but then had the highest brightness of all the coatings after 24 hours. This suggests the penicillin/streptomycin were initially effective, but that simply coating a surface with antibiotics does not effectively prevent bacterial attachment for extended amounts of time.

To quantify the results, the average brightness on each coated section was determined with Adobe Photoshop CS5 and the results were normalized based on the value for the uncoated section (bare gold) after 24 hours, which had the highest contrast change. The graphs in Figure 2 show the average normalized brightness of 0.17, 0.80, and 0.84 for casein, BSA, and penicillin/streptomycin coatings after 24 hours, respectively. These values are directly proportional to the amount of biomass located on the surface of each of the coatings compared to the gold surface. Inspection of the surfaces with microscopy techniques after removal from the SPRi instrument qualitatively confirmed the results.

CONCLUSION

In this study, SPR imaging was used, for the first time, to evaluate the effectiveness of common microfluidic antifouling and antimicrobial coatings in preventing the attachment of *P. aeruginosa*. The real-time imaging capabilities of this technique allow the attachment process to be monitored during continuous flow, which simulates real microfluidic situations. Since this is a label-free surface analysis technique, it can be used with any microbe of interest and in complex opaque samples, such as oil shale and whole blood.

Bare gold, BSA, casein, and penicillin/streptomycin solution were compared quantitatively by analyzing the brightness of the pixels in the patterned areas using Adobe Photoshop. The results indicate that casein was the most effective antibacterial coating and that it can potentially be used in microfluidic channels to prevent non-specific bacterial adhesion.

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REFERENCES


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