Electrochemical impedance spectroscopy biosensor platform for evaluation of biofilm

PhD Dissertation Defense

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Microbial biofilms are organized communities of surface-attached microorganisms encased in a self-produced extracellular matrix that pose significant challenges in medicine, the environment, and industry. Biofilms can cause chronic infections, biofouling, and equipment failure, while existing methods for biofilm detection are slow, costly, and labor-intensive. Recently, the use of microfabricated electrochemical impedance spectroscopy (EIS) biosensors has emerged as a promising technique for evaluating biofilm growth in real-time with advantages of small-size, adaptability, low-cost, and high-sensitivity.

In this work, EIS biosensors featuring gold micro-interdigitated electrodes were produced using standard microfabrication techniques. Sensors were integrated into a custom 3D-printable flow cell system, enabling EIS measurements and confocal laser scanning microscopy (CLSM) imaging simultaneously. Green fluorescently labeled *Pseudomonas aeruginosa* PA01, a model biofilm forming bacteria, was introduced into flow chambers and subsequent growth was monitored by EIS, CLSM, and biomass enumeration. Using the system, biofilm growth, dispersal, and the effects of cell-signaling suppression were evaluated. The sensors were also tested in an oil-water emulsion and field-tested on an alpine snow-patch and pond.

Improved stability of EIS measurements was achieved by coating the sensors' counter and reference electrodes with an electrically conductive polymer. Biofilm growth was successfully detected using EIS biosensors at an optimized single-frequency, with average decreases in impedance of ~22% by 24 hours. Likewise, biofilm dispersal via chemical treatments were successfully detected with average increases in impedance of ~14% over the ensuing 12 hours. When cells were exposed to a quorum sensing inhibition agent, impedance did not decrease for 18 hours. Impedance changes due to biofilm growth, dispersal, and effects of quorum sensing inhibition were validated by CLSM images and biofilm enumeration. Similarly, in an oil-water emulsion, the biosensors successfully detected biofilm growth, dispersal, and effects of quorum sensing inhibition. In an alpine field-test,

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samples containing varying concentrations of microbes could be detected using the EIS biosensors.

This work demonstrates that EIS biosensors are a promising tool for real-time monitoring of biofilm dynamics in a variety of aqueous environments. Overall, EIS biosensing holds great potential for in situ and real-time data regarding biofilm colonization that is not possible with existing techniques.