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Early detection of *Candida albicans* biofilms at porous electrodes

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ABSTRACT

We describe the development of an electrochemical sensor for early detection of biofilm using *Candida albicans*. The electrochemical sensor used the ability of biofilms to accept electrons from redox mediators relative to the number of metabolically active cells present. Cyclic voltammetry and differential pulse voltammetry techniques were used to monitor the redox reaction of $K_3Fe(CN)_6$ at porous reticulated vitreous carbon (RVC) (238.7 cm^2) working electrodes versus Ag/AgCl reference. A shift in the peak potential occurred after 12 h of film growth, which is attributed to the presence of *C. albicans*. Moreover, the intensity of the ferricyanide reduction peak first increased as *C. albicans* deposited onto the porous electrodes at various growth times. The peak current subsequently decreased at extended periods of growth of 48 h. The reduction in peak current was attributed to the biofilm reaching its maximum growth thickness, which correlated with the maximum number of metabolically active cells. The observed diffusion coefficients for the bare RVC and biofilm-coated electrodes were 2.2×10^{-3} and 7.0×10^{-6} cm^2/s, respectively. The increase in diffusivity from the bare electrode to the biofilm-coated electrode indicated some enhancement of electron transfer mediated by the biofilm to the porous electrode. Verification of the growth of biofilm was achieved using scanning electron microcopy and laser scanning confocal imaging microscopy. Validation with conventional plating techniques confirmed that the correlation ($R^2 = 0.9392$) could be achieved between the electrochemical sensors data and colony-forming units.

Biofilms are complex multispecies communities of microorganisms that adhere to the inert or living surfaces and are generally enclosed in a self-produced polysaccharide matrix known as extracellular polymeric substance (EPS).[1] Biofilms play a key role in the pathogenesis of many bacterial infections as well as in a plethora of medical implants and devices such as hip replacements and catheters. They represent a major problem in the oil industry and computer manufacturing. The high densities of microorganisms in cooling systems or waste pipes have been shown to affect the operational efficiency of those systems to transport water and to adversely affect the performance of those systems through impeding heat transfer or metallic corrosion [1–4]. The ability of biofilms to corrode pipes has been widely reported in the oil, gas, and shipping industries as well as in sewer lines, polluted coastal waters, and medical devices [5–10]. Testing for biofilms uses traditional culturing techniques such as plating and incubation methods [8]. Once the EPS becomes established, the challenge to remove the biofilm requires more dramatic approaches such as shutting down the equipment and scraping the interior of the pipes. Hence, there is a need for rapid detection methods suitable for early detection in order to curb the mounting costs of removing biofilms and plant maintenance.

Traditional microbiology techniques such as plating do not provide an accurate determination of colony-forming units (CFUs) because the number of planktonic bacteria does not directly correlate with the amount of CFUs in a biofilm colony [11–13]. Contemporary methods for detection of biofilms rely on techniques such as scanning electron microcopy (SEM), scanning confocal microscopy, and epifluorescence microscopy. These techniques provide high magnification and image contrasts and are particularly useful for studying the biological contamination of wastewater treatment facilities and the formation of the biofilms on opaque surfaces such as metals, plastics, tissues, and indwelling medical devices. However, these techniques are not suitable for early detection in situ monitoring or for rapid analysis of the presence of biofilms. Moreover, techniques such as SEM generate artifacts and require the sublimation of the excess water, leading to the collapse of the EPS layer and incorrect characterization of the biofilms.

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[1] *Abbreviations used:* EPS, extracellular polymeric substance; CFU, colony-forming unit; SEM, scanning electron microcopy; CV, cyclic voltammetry; DPV, differential pulse voltammetry; RVC, reticulated vitreous carbon; PBS, phosphate-buffered saline; YPD, yeast peptone dextrose; ppi, pores per inch.

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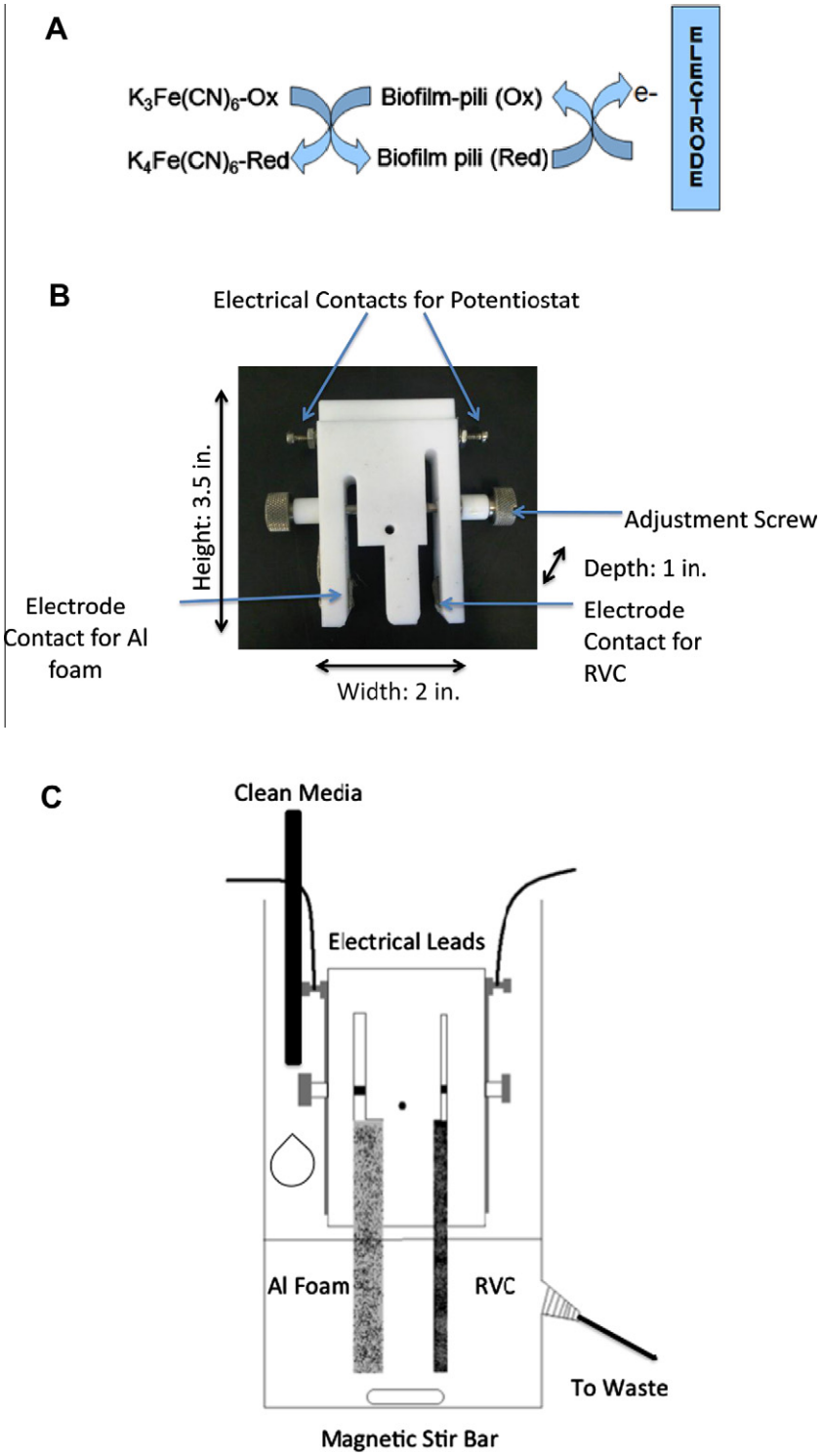

Fig.1. (A) Sensor concept and electron exchange between conducting pili in biofilms and solution-bound redox mediators. Distal or axial transfer of electrons occurs between the biofilm redox proteins (via conductive filaments) and electrode. The biofilm pili facilitate super exchange and enhanced conductivity while circumventing mechanical detachment from the cell surface. (B) Sensor platform designs and placements of the porous electrodes. (C) Schematic of the complete electrochemical sensor biofilm reactor setup.

Electrochemical techniques allow for sensitive detection and fast data processing [9]. Various techniques such as cyclic voltammetry (CV), impedance spectroscopy, rotating disk electrode, and differential pulse voltammetry (DPV) have been used to detect various species of biofilms [9,13–16]. The goal of the current work was to develop a real-time in situ sensor platform that can be used for various species of biofilms. As with most biofilms, there are three phases involved in the development of fungal biofilms: early phase (0–11 h), intermediate phase (12–30 h), and maturation phase (38–72 h) [17,18]. These are characterized by (i) adhesion of yeast cells to the surface of the device, (ii) formation of a matrix in which there is dimorphic switching from yeast to fungal form or as it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated, and (iii) an increase in the