Live Cell Imaging of Bacterial Biofilm Disruption by Cathodic Voltage-Controlled Electrical Stimulation

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Statement of Purpose: Previous studies have shown that cathodic voltage-controlled electrical stimulation (CVCES) of titanium (Ti) implants is an effective and broad-spectrum antimicrobial treatment for implant associated infections[1-3]. The purpose of the study was to utilize a custom, electrochemically controlled live cell microscopy system to directly visualize in real time how CVCES disrupts methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms from Ti substrates.

Methods: A clinical isolate of MRSA (strain NRS70) was used in all experiments. Commercially pure Ti (cpTi) coupons (give dimensions) were mirror polished, sterilized with UV light, and incubated overnight with freshly inoculated bacterial cultures (containing ~10^7 colony forming units (CFU) per ml in tryptic soy broth medium supplemented with 0.25% glucose (TSBG)) to allow for biofilm formation on the coupons. Subsequently, the biofilm coated samples were mounted into a saline-filled custom microscopy chamber that utilized a standard 3-electrode potentiostatic configuration to apply CVCES (-1.5V, -1.8V, or -2.2V vs Ag/AgCl) via a potentiostat (Interface 1000, Gamry Instruments) to the samples for 1 hour. A graphite rod counter electrode and an Ag/AgCl reference electrode were electrically connected via an agar bridge. Utilizing reflected light differential interference contrast microscope, we captured live cell videos of the bacteria biofilm being exposed to CVCES. All samples were observed at open circuit potential for 5 minutes prior to stimulation. Following the test period, the coupon and surrounding saline were extracted for enumeration of planktonic and coupon-associated CFU by dilution plating. A minimum of 3 independent samples were conducted for each test condition. The reported CFU were compared across CVCES magnitudes by a 1-way ANOVA followed by a Tukey post-hoc test where appropriate.

Results: Figure 1 presents still frame images captured 5 mins prior to CVCES and then after 2, 10, and 60 mins of CVCES treatment. It was observed that CVCES disrupted the MRSA biofilm in a time-dependent and magnitude-dependent manner. Biofilm disruption occurred within 2 mins for -2.2V, ~10 mins for -1.8V, and ~60 mins for -1.5V. Figure 2 reports the MRSA bacterial burden following CVCES treatments and indicates that application of -2.2V for 1 hour significantly reduced both the coupon and planktonic CFU by over 3-logs as compared to all other test conditions.

Conclusions: An electrochemically controlled live cell microscopy system has been developed and used to show that CVCES displays time-dependent and magnitude-dependent antimicrobial effects and disruption of MRSA biofilms on cpTi surfaces.

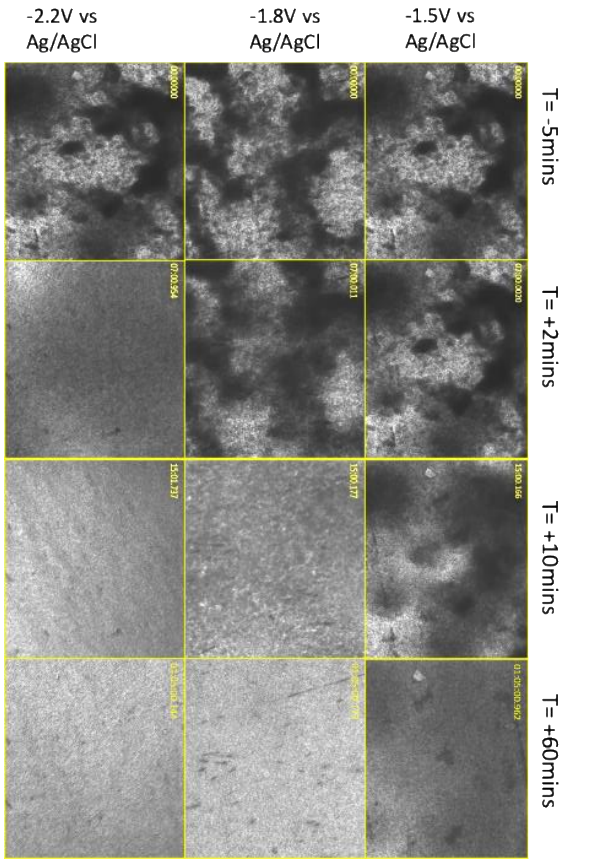

Figure 1: Summary of still frame images taken from live cell imaging videos showing MRSA biofilm disruption during CVCES treatments (-2.2V, -1.8V, and -1.5V)

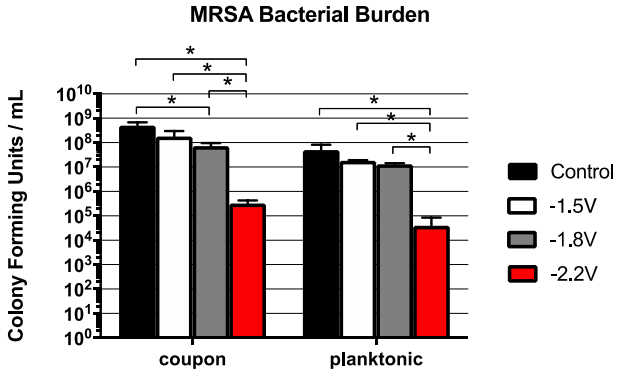

Figure 2: Plot of coupon and planktonic CFU enumerated following CVCES treatments at -2.2V, -1.8V, and -1.5V.

References: 1. Ehrensberger, M et al. Biomaterials. 2015;41(0):97-105. **2.** Nodzo, S et al. Clin Orthop Rel Res. 2016;474(7): 1668-753. **3.** Canty, M et al. mSphere. 2019;4(3):e00178-19.