Electrochemically Generated Hydrogen Peroxide for the Prevention of Biofilm Formation

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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]. These antimicrobial effects are thought to be related to the faradaic modifications (i.e. alkaline pH and generation of reactive oxygen species such as hydrogen peroxide (H_2O_2)) in the microenvironment adjacent to the stimulated metal. The CVCES magnitudes used in previous studies resulted in water reduction being the dominant cathodic half-cell reaction. However, recent unpublished work in our lab shows that peak H_2O_2 production occurs at lower CVCES magnitudes (-700mV vs. Ag/AgCl for Ti and -600mV vs. Ag/AgCl for stainless steel(SS)) where oxygen reduction is the dominant cathodic half-cell reaction. The goal of this work is to determine the extent to which H_2O_2 contributes to the antimicrobial activity of low magnitude CVCES applied to Ti and 316L SS. *Staphylococcus aureus (S. aureus)* is a common orthopedic pathogen and is a catalase positive bacteria, so it has a mechanism to resist the antimicrobial effects of H_2O_2 to some degree. In order to confirm that any reduction in the bacterial viability observed in this study is due to H_2O_2, a catalase deficient *S. aureus* mutant strain was used as a comparison.

Methods: The bacterial strains used in this work were a wild type *S. aureus* (Newman) and a catalase deficient mutant of the Newman strain (ΔKatA). A 24-well plate assay and an electrochemical treatment assay were used in this work. For the 24-well plate assay, Ti (Grade 4, McMaster Carr) or 316L SS (McMaster Carr) disks (4mm x 11mm dia) were placed in the bottom of individual wells and incubated for 24 hours with 1 mL of fresh bacterial inoculum (~10^3 colony forming units (CFU) per mL of tryptic soy broth with 0.25% glucose (TSBG)). Each inoculum was chemically modified to achieve H_2O_2 concentrations of 0 µM, 5 µM, 15 µM, 25 µM, 50 µM, and 100 µM. For the electrochemical treatment experiments, rectangular Ti and 316L stainless steel samples with a surface area of 4.36 cm^2 were incubated with bacterial inoculum (same as plate studies) in a custom agar test chamber that utilized a standard 3-electrode potentiostatic configuration to apply CVCES to the metal samples (-700mV vs. Ag/AgCl for Ti and -600mV vs. Ag/AgCl for 316L SS) for 24 hours as previously described [1,3]. Antimicrobial effects were quantified at the end of the tests by reductions in planktonic and coupon-associated bacterial CFU enumerated by the spread plate method.

Results: The results of the 24-well plate assay (Fig.1A-C) showed significant reductions planktonic and coupon associated CFU for both bacterial strains at H_2O_2 concentrations over 15 µM. The ΔKatA strain was found to be more susceptible to H_2O_2 than the Newman strain. The electrochemical assay for Ti substrates (Fig.1D-E)

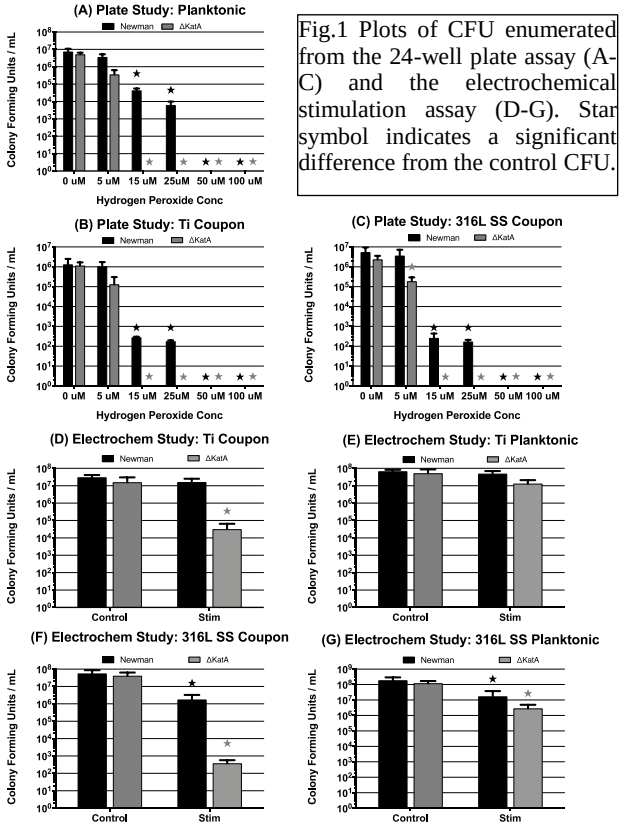

Fig.1 Plots of CFU enumerated from the 24-well plate assay (A-C) and the electrochemical stimulation assay (D-G). Star symbol indicates a significant difference from the control CFU.

showed the ΔKatA coupon associated CFU were significantly reduced by the -700 mV CVCES, while the planktonic CFU were not impacted. The viability of the Newman stain was not reduced by CVCES. This suggests CVCES of -700mV can affect bacteria close to the stimulated Ti surface, and bacteria that express catalase are able to withstand the H_2O_2 that is generated by the CVCES. The electrochemical assay for 316L SS substrates (Fig.1F-G) showed significant reductions in the planktonic and coupon associated CFU for both strains when -600 mV CVCES was applied to 316L SS. The planktonic bacteria had a 1-log reduction for the Newman strain, and a 2-log reduction for the ΔKatA strain. The coupon associated bacteria for the Newman strain were reduced by ~1 order of magnitude, while the ΔKatA strain had an approximately 5-log reduction. The greater susceptibility of the ΔKatA strain observed in these studies indicates that antimicrobial mechanism of low magnitude CVCES is likely associated with the generation of H_2O_2. Studies are ongoing to evaluate further optimization of low magnitude CVCES for Ti and 316L SS implants, including the role of combined antibiotic and low magnitude CVCES.

References: 1) Ehrensberger,M et al. Biomaterials, 2015. 41(0)97-105. **2)**Nodzo,S et al. Clin ortho rel res, 2016. 474(7): 1668-753. **3)** Canty, M et al. mSphere, 4(3), e00178-19, 2019. **4)**Schneider S Bioelectrochemistry. 2018;121:84-94. **5)** Wang H AMB Express. 2017;7:204. **6)** Park B, J Bacteriol. 2008;190:2275-8.