Interactions of *Escherichia coli* HM22 Biofilm with Electrochemically Active Commercially Pure Titanium Surface

Jin Guo, Jeremy L. Gilbert

Department of Biomedical and Chemical Engineering, Syracuse Biomaterials Institute, Syracuse University, Syracuse, NY 13244

Introduction: Bacterial infection is a major concern in orthopedic implants that may lead to implant failure and revision[1]. The resistance of bacterial biofilms to antibiotics increases the difficulties of fighting against infections[1]. It has been reported that reduction electrochemistry at metallic biomaterial surfaces can negatively affect mammalian cell viability[2]. A systematic study of reduction electrochemistry effects on bacterial biofilm behavior would provide a clear view of the therapeutic benefits of electrochemical treatment of infection. The goal of this study is to understand the effect of voltage and solution on cell viability of *E. coli* HM22 cultured on electrochemically active commercially pure Titanium (cpTi) surfaces.

Materials & Methods: CpTi discs were wet-polished to 600 grit, sonicated and sterilized in 70% ethyl alcohol for 24 h. The discs were placed into a custom-made electrochemical cell culture chamber with electrical contacts to cpTi disc as a working electrode, a graphite counter and a chlorided silver wire reference electrode. *E. coli* HM22 was first cultured overnight in 25 ml LB medium supplemented with 25 μl DPA at 37°C with shaking at 200 rpm. 1 ml overnight cell culture was plated on cpTi surface and kept at open circuit potential (OCP) at 37°C for 30 minutes. The surfaces were then gently rinsed with saline solution (0.9 % w/v NaCl) 3 times. Fresh medium (LB or Saline solution) were then added to immerse the counter and reference electrodes which were connected to a potentiostat. The Ti sample was potentiostatically held at -1000 mV at 37°C for 24 h compared to control samples held at OCP. The current of each chamber was measured for 24 h in 28 s intervals and average current densities over 24 h were calculated. Electrochemistry Impedance Spectroscopy (EIS) was then performed. To assess cell viability, a LIVE/DEAD® bacterial viability kit was used according to manufacturer's instructions. Cells were imaged with an inverted microscope (Zeiss Axiovert 40 CFL). Cell area was analyzed using ImageJ software. 2-way ANOVA was performed with α=0.05 (n=3 for all groups, 5 spots randomly selected from each sample). The sample surfaces were gently rinsed in phosphate buffered saline (PBS) and fixed with 4% formaldehyde solution. The samples surfaces were then dehydrated in gradient ethanol, sputtered with gold and assessed by scanning electron microscope (SEM) (JEOL 5600).

Results & Discussions:

After 24-hour voltage treatment at -1 V, the average current density (5 μA/cm^2) measured with bacterial cells in LB medium was lower than current density (40 μA/cm^2) of control groups without cells in LB medium. In Figure 1, the voltage treatment decreased the impedance of the Titanium interface compared to control samples held at OCP in the presence of bacterial cells. Titanium surfaces in LB medium had lower impedance value than surfaces in saline solution at -1V. The presence of bacterial cells slightly changed the impedance in both LB medium and saline solution at -1V.

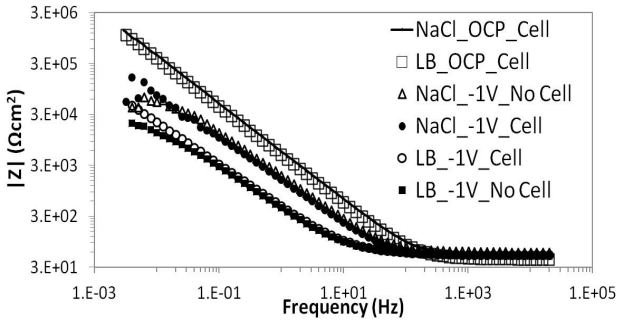

Figure 1. The impedance of the cpTi- Biofilm interface in saline solution and LB medium, with and without bacteria, at -1 V and OCP

Figure 2 shows the cell viability fraction of different conditions at 24 h. The cell viability was determined by area fraction ratio of viable cells over all the cells. The voltage treatment in saline solution negatively affected *E. coli* cell viability compared to control groups held at OCP ($P<0.01$). The effect of voltage treatment in LB medium was not significant on cell viability. The presence of voltage treatment and limited nutrients in saline solution adversely impacted the cell viability on the cpTi samples, compared to OCP and LB medium (2-way ANOVA, $P<0.01$). The mechanism of this killing effect may be due to the toxic substances produced as a result of electrolysis or decreased bacterial activity[1].

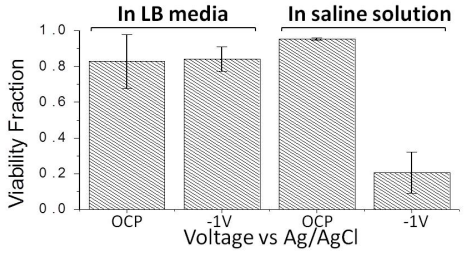

Figure 2. Cell viability fraction. Note the large loss of viability at -1V in saline solution and little loss in LB medium at -1 V compared to controls at OCP.

Conclusions: This study demonstrates that the viability of *E. coli* HM 22 cells cultured on titanium surface is affected by voltage treatment and electrolyte solution contents. In particular, voltage treatment in saline solution negatively affects the cell viability. On the other hand, the electrochemical impedance of the titanium-oxide-bacteria interface is dependent on the contents of the electrolyte solutions and the presence of voltage. These results could ultimately be used to design new devices with a capability to actively control bacterial infections and inflammation on the implant surface. Further work will be done to investigate the effects of voltage and time on the cell viability, cell morphology and biofilm structure of bacteria cells cultured on electrochemically active metallic surface.

References: (1) J. W. Costerton, et al., Science 1999;284: 1318-1322 (2) Ehrensberger et al., JBMR-A 2010;93A:1500-1509.