How voltage and wear debris from Ti-6Al-4V interact to affect cell viability during in-vitro fretting corrosion

Hui, T; Gilbert, JL.
Syracuse University, Syracuse, NY.
gilbert@syr.edu

Statement of Purpose: Fretting corrosion (or mechanically assisted corrosion) is one of the principal mechanisms of degradation of modular designs of the orthopedic implants. Ti-based, Cobalt-based and iron-based materials, which owe their resistance to corrosion to the presence of oxide film, are widely used in orthopedic industry. However, the essence of fretting is to abrade the oxide film, which then, by electrochemical processes, reforms. Two clinical consequences are the release of particles and ions as corrosion-by products and the negative excursion of the voltage of the implant which, in turn, leads to more deleterious result of the underlying metal and the biological system[1]. Though there are many articles investigating the effects of wear particles and ions on the biological system, and the mechanisms of fretting corrosion; there are few studies on the effects of fretting corrosion on cells in-vitro. The goal of this work is to develop an in-vitro fretting corrosion cell culture test system ant to investigate how voltage and wear debris interact to affect cell viability.

System Description: In order to replicate the fretting motion in vivo, a fretting-crevice corrosion pin-on-disk system was developed as shown in Fig.1 left. The fretting motion is provided by a piezo electric actuator (Piezo-Jenna Systems, Germany). A piezo amplifier (Piezo-Jenna Systems, Germany) and a function generator (EZ Digital Co., ltd, USA) are used to control the piezo actuator to get the desired motion (90 um, 1 Hz). Cells are plated on top of the Ti-6Al-4V sample inside of the petri-dish chamber that is glued on top of the metal sample using polymethylmethacrylate (PMMA), as shown in Fig.1 right. The electrochemical signal is acquired by a 3-electrode potentiostat (EG&G Princeton Applied Research, USA) and the data acquisition is achieved by CorrWare software and LabView 2012.

Figure 1

Methods: The fretting motion is introduced by the above mentioned system. The load for all experiments is fixed at 2 N. All metal disks are made of Ti-6Al-4V and the cone-shaped pins are Borosilicate glass. The exposed metal surface area for cells to attach is 0.178 cm^2. The cell used in this study is the MC3T3-E1 subclone 4 (ATCC, VA) pre-osteoblasts. Two groups of experiments were performed, (all trials lasted for 14 hrs): 1. Fretting and monitoring the open circuit potential vs. Ag/AgCl. In this group of experiments, the potential was controlled below the threshold potential[2] (-400 mv vs Ag/AgCl) for MC3T3 cells. 2. Fretting while potentiostatically holding the voltage above the threshold potential and monitoring the current. The fretting motion of group 2 was the same as in group 1.In the positive control, cells were plated on the sample and cultivated for 14hrs, whereas in the negative control group, the sample potential was potentiostatically held at -1000 mv. Live and Dead cell assay (L3224, Invitrogen, Oregon) was performed after each experiment and the percentage of the alive cells were calculated using imageJ; then the samples were fixed using 4% formaldehyde and were properly prepared for SEM imaging. All tests were performed for 3 trials and the statistical analysis were performed using student t-test with confidence level of 95%.

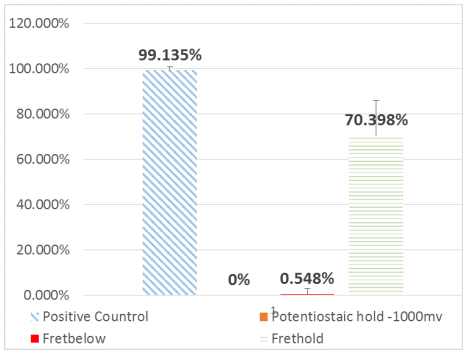

Figure 2

Results: The results from live and dead assay and SEM image are shown in Fig. 3. And Fig. 2 shows, 99.6% of cell in fretting under the threshold potential were dead, and balled up. Only 30% of cells were dead when fretting while the potential was held at -300 mv. From the images we can see even though the cells were surrounded by wear particles, most of them were still alive. The cells in the positive control group were well spread out and the viability was 99% while cells in negative control were 100% dead.

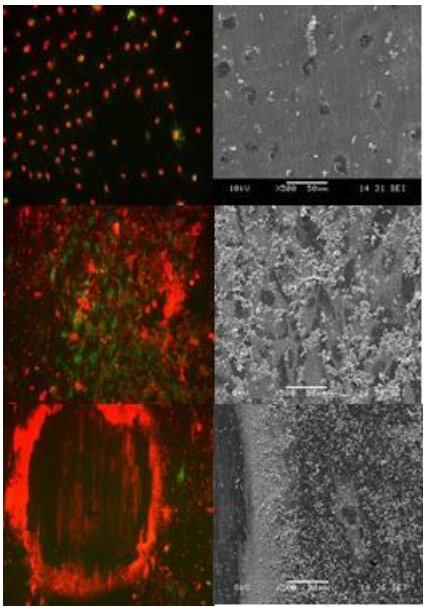

Figure 3

Conclusions: The results of this study show that both voltage excursion and wear particles have effects on cell viability during fretting corrosion. The amount of wear particles in the system in two groups of experiments were the same since the fretting motion and time duration were the same; the fact that cells are more viable in the second group of experiment indicating that cells are more sensitive to the voltage changes than the wear particles surrounding them.

References: (1) Jacobs JJ, et al., JBJS 80.A (2) (1998): 268-82., 2) Sivan S. et al., J BMR- B 101.8 (2013): 1489-497. **Acknowledgements:** Depuy Synthes