Electro-deposition of Antimicrobial Silver Coatings on Stainless Steel Implants

Paul DeVasConCellos, Susmita Bose, Amit Bandyopadhyay
W. M. Keck Biomedical Materials Research Laboratory
School of Mechanical and Materials Engineering, Washington State University, Pullman, WA 99164.

Introduction: Stainless steel is commonly used as an implant material for fracture management. In the case of fractures and open wounds, infection is a great concern. Antibiotics are the standard procedure with open wound surgeries, however there is a rise in antibiotic resistant bacteria. This makes silver coatings on stainless steel an excellent option as an antimicrobial agent as cases of silver resistant bacteria are rare. It would also have the benefit of combating bacteria at the site of infection and helping to prevent biofilm formation *in vivo*. 316L stainless steel was used as a substrate material due to its prevalence of industry use. The aim of this study is to optimize a method for applying an antimicrobial coating onto stainless steel.

Methods: Electrodeposition was performed using an aqueous solution of $AgNO_3$ with concentrations between .001-.5 M with platinum as the anode material. The deposition was carried out by applying direct current (DC) for 2-7 minutes at room temperature. SEM images were taken to verify and categorize the deposition of silver. It was observed that as-deposited coating would not adhere strongly enough to SS to survive handling, sterilization and surgical procedures. Therefore, a post deposition heat treatment was optimized and incorporated. The coated samples were heat treated in a vertical tube furnace at 300-700˚ C for 3-12 minutes and allowed to cool at room temperature.

In order to examine how much silver would be released into the body upon implantation, simulated body fluid studies were performed. The SBF was adjusted to a PH of 7.38 and maintained at a temperature of 37˚ C for the duration of the study. Upon completion, the solution was analyzed for Ag^+ content using an atomic absorption spectrometer (AAS).

The effect of Ag^+ coating on human osteoblast cells was studied using an established hFOB cell line. Proliferation was then measured using an MTT assay. After five days, additional samples were also examined using confocal microscopy. Vinculin (Sigma, MO, USA), the specific protein expression relevant to adhesion formation, has been assayed for test samples by fluorescent staining and confocal laser scanning microscopy (CLSM) observation. At three and seven day points, cell morphology was observed using SEM.

Antimicrobial efficacy was tested by challenging the samples with *p. aeruginosa*. This was performed in a bioreactor containing growth media while bubbling air into the reactor. Serial dilutions were carried out and cell counting was performed.

Results: Silver particle deposition was observed to have a size distribution in the range of .5-50 µm. As-deposited adhesion was found to be inadequate and so a post heat treatment was included in the processing. It was found that varying process parameters such as deposition time, voltage, solution concentration, heat treatment time and temperature had an impact on silver release.

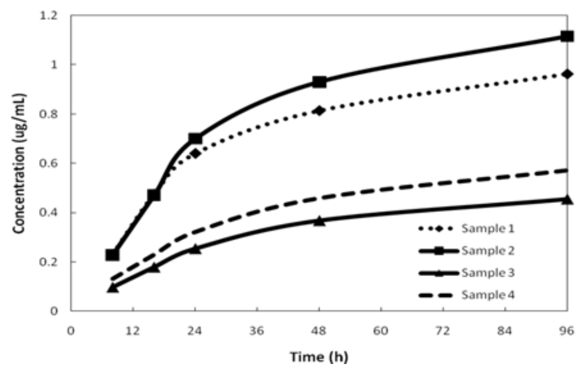

Figure 1. Effects on Ag^+ release rate of varying duration of electrodeposition and heat treatment. Samples 1 and 2 were prepared with deposition times of 1.5 and 3.5 minutes respectively, and identical heat treatments. Samples 3 and 4 were prepared using deposition times of 1.5 and 3.5 minutes respectively, and a more rigorous heat treatment than Samples 1 and 2.

The proliferation study showed that the samples with a silver coating had as many if not more active cells as the control. This can be attributed to the higher surface energy of the samples caused by applying a coating.
Coated samples showed a significant reduction in bacteria when tested. At the 24 hour data point, a 13 fold reduction in colony forming units per cm^2 was observed.

Conclusions: With the rise of antibiotic resistance, silver provides a viable antimicrobial agent that can be applied directly onto implants. This research has expanded on the understanding of applying a coating using silver nitrate and post heat treatment of stainless steel. This coating has been shown to be effective against bacteria while being non-threatening to human tissues. These findings can be useful for optimizing other coating setups and applying similar coatings onto other substrate materials. Our results can be applied to a variety of biomedical devices where infection is a serious concern.