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Antimicrobial particulate silver coatings on stainless steel implants for fracture management

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ABSTRACT

We have used particulate silver coating on stainless steel to prevent in vivo bacterial infection. Stainless steel is commonly used as an implant material for fracture management. The antimicrobial use of silver has been well documented and studied, therefore the novelty of this research is the use of a particulate coating as well as facing the real world challenges of a fracture repair implant. The variable parameters for applying the coating were time of deposition, silver solution concentration, voltage applied, heat treatment temperature between 400 and 500 °C and time. The resultant coating is shown to be non-toxic to human osteoblasts using an MTT assay for proliferation and SEM images for morphology. In vitro silver release studies of various treatments were done using simulated body fluid. The bactericidal effects were tested by challenging the coatings with *Pseudomonas aeruginosa* in a bioreactor and compared against uncoated stainless steel. A 13-fold reduction in bacteria was observed at 24 h and proved to be statistically significant.

1. Introduction

Every year there are an estimated 50 million road traffic accidents [1,2]. The resulting injuries are prone to infection because they can occur in remote locations and often involve an open wound being exposed for extended periods of time [3–5]. By the time the patient is brought to the hospital, infection is often a great concern. Even under the best surgical conditions, infection is still a concern. Since 90% of all road traffic injuries occur in developing nations [6–8], there is often a greater risk of infection as ideal surgical circumstances are not always possible.

Antibiotics use for prevention of infections onto or into orthopedic devices has met with limited success. Limited success has also been seen with non-antibiotic remedies such as chlorohexidine and mupirocin. Historically, amino-glycoside antibiotics were highly effective, as opposed to tetracyclines, cephalosporins and macrolides, common antibiotics prescribed for almost every patient who ever had either an upper respiratory or urinary tract infection. It was believed that such little-used antibiotics may not present a threat towards the development of drug resistance. However, this theory has been proven incorrect. Silver, has been in use for long time especially for topical treatment. Silver is biocidal in the ionic form and, unlike many antibiotics, has at least six different mechanisms of action [9–13]. Silver has the added benefit of being highly toxic to microorganisms with a relatively low toxicity to human tissues [14–17].

Stainless steel has long been a standard material used for fracture management implants due to its mechanical properties, corrosion resistance and low cost. In particular, it is a common material used in cases of fracture repair, such as those caused by road traffic accidents. Therefore, it could be beneficial to apply an antimicrobial coating to stainless steel to address the risk of infection from open wounds and surgical conditions. The antimicrobial agent used was ionic silver.

The objective of this research is to develop a silver coating that will be adherent to the stainless steel implants and offer an antimicrobial surface to prevent infection. This coating should operate within the constraint that the silver release should not be toxic to the surrounding tissue. To accomplish this, it is hypothesized that electrochemical routes can be used to deposit particulate Ag^{+} on stainless steel which can be used on fracture management implants to prevent infection. This approach was chosen because it is cost effective and easy to use. Also, it can be incorporated with current manufacturing processes for a finished product before sterilization. There is a knowledge gap that exists concerning how a particulate coating can be applied. Film based silver coatings have been developed and are currently being used in industry. However, particulate based silver coatings are not yet fully explored. A particulate coating has the advantage that it does not completely cover the surface, which leaves the substrate material exposed and retains the substrate's properties. The challenge is to address how particles can be adhered to the surface such that the coating will remain viable even after implantation.

The novelty of this research is that we address the challenges of the real world. Developing an adhesion mechanism that can be applied after final machining without degrading the product is essential to the

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utility of this method. This research is also novel because we employ a particulate based silver coating as opposed to the more common film based coatings. We have shown that a particulate silver coating can be applied to fracture management devices, survive realistic situational challenges and still release silver above the minimal inhibitory concentration.

To better understand the electro-deposition process that can yield adherent silver to a SS substrate with an appropriate load of Ag^+ ions, a broad range of experiments have been performed. Parameters such as solution concentration of silver nitrate during electrodeposition, deposition current and time, and post deposition heat treatment times and temperatures have been experimented with towards a viable silver coating approach. After the coating's optimization, bacteria cultures were examined to see the antimicrobial effect of the treatment and in vitro Ag^+ release studies were performed. In addition, human osteoblast cell culture was performed to ensure that the coating did not adversely affect human cell attachment and proliferation.

2. Materials and methods

2.1. Sample preparation

FDA approved stainless steel (316L) nails from the Surgical Implant Generation Network (SIGN, WA) designed for the repair of long bone fractures were used in this study. It should be noted that multiple studies were performed during the course of this research and a wide range of process parameters were investigated. Samples were first cleaned with DI water and acetone prior to electrodeposition of silver. Electrodeposition was performed using an aqueous solution of $AgNO_3$ with concentration between 0.001 and 0.5 M with platinum as the anode material. The deposition was carried out by applying a DC current (0.05–0.25 A) between 2 and 7 min at room temperature. SEM images were taken to verify and categorize the deposition of silver. It was observed that the as-deposited coating would not adhere strongly enough to the SS to survive handling, sterilization and surgical procedures. Therefore, post deposition heat treatments were designed and performed. The coated samples were heat treated in a vertical tube furnace in air atmosphere between 300 and 600 °C for 2 to 20 min and allowed to cool naturally at room temperature. Finally, the samples were sprayed with DI water and wiped with a towel to remove any loose silver attached to the surface.

2.2. Silver release rate

In order to simulate how much silver would be released into the body upon implantation, a simulated body fluid (SBF) was prepared keeping the ionic concentration the same as blood plasma according to the method presented by Kokubo et al. [18,19]. The SBF was adjusted to a pH of 7.38 and maintained at a temperature of 37 °C for the duration of the study. Each sample was placed in a sterile vial with 10 mL of SBF. At each time point the solution was replaced with fresh SBF. Upon completion, the solution was analyzed for Ag^+ content using an atomic absorption spectrophotometer (AAS). The measurements were completed using a Shimadzu AA-6800 Atomic Absorption Spectrophotometer (Shimadzu, Kyoto, Japan). The samples were tested in "Flame Mode" using air and acetylene (C_2H_2) fuel, and data collection was carried out using Shimadzu WizAArd software. To calibrate the machine, standards of 0.5, 2.5 and 5 μg/mL were created with ultra-pure water by diluting known concentration samples (High-Purity Standards, Charleston, SC, USA). During testing, a pre-spray time of 30 s and an integration time of 10 s were used.

2.3. Antimicrobial activity

To test how effective the coating would be against bacteria that are a common threat in surgical situations, silver coated SS samples were challenged with *Pseudomonas aeruginosa*. Samples were ultrasonically cleaned and rinsed with DI water and acetone before coating. They were coated with an electrodeposition technique and heat treated as outlined above. A stock of *P. aeruginosa* was incubated for 24 h in growth media prior to injecting it into the prepared bioreactor. The bioreactor used was a 1 L beaker that has a custom airtight lid which holds columns that extend down into the beaker. These inert columns hold the samples with set-screws such that they can all be subject to similar, laminar flow. The bioreactor setup can be seen in Fig. 1. All components used were sterilized using an autoclave prior to use. Once the samples were fixed onto the columns and growth media was added, cell stock was injected in the amount of 10% by volume of the growth media in the bioreactor. The media was kept in motion by using a cross stir rod on a stir plate. Air was also slowly bubbled into the media and was allowed to escape via a filtered outlet. After 24 h, the samples were removed from the bioreactor, scraped and rinsed into test tubes using a buffer solution. The solution was then mixed using a homogenizer to break up cell clusters and ensure uniformity. Next a serial dilution was carried out,

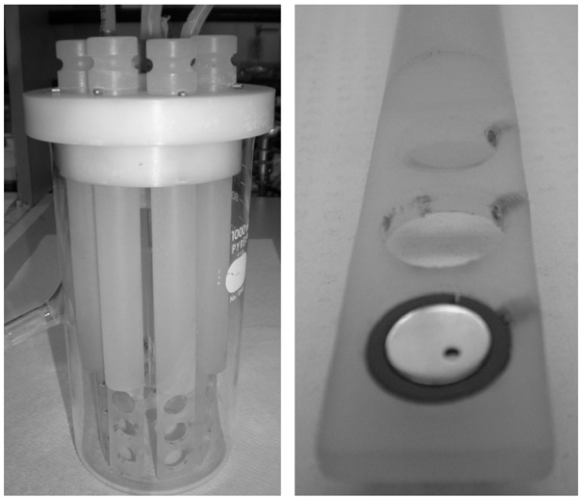

Fig. 1. Bioreactor setup. Multiple samples or controls can be tested by placing disk samples in the circular cutouts. They are held in place with set screws. This setup allows for air supply and outgoing tubes. Once samples were in place and growth media was added, the bioreactor was sealed and bacteria were injected at the top. Smaller diameter samples required an adapter cuff seen in dark gray (right).

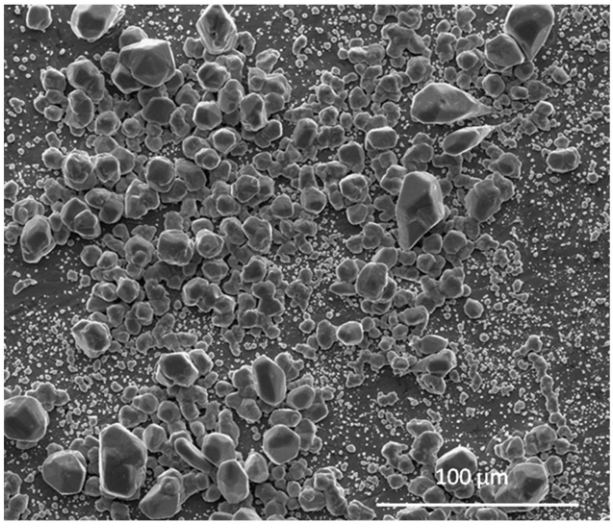

Fig. 2. SEM images of electro-deposited silver particles. There is a bi-modal distribution of particles. While the majority of the smaller particles are less than one micron in size, large particles are mostly between 10 and 20 μm.