Developing Antimicrobial Dental Implant Surfaces through Silver Nanoparticle Deposition

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Introduction:

Titanium is a highly biocompatible material, which makes it an ideal biomaterial for dental implant placement. While these implants have generally proven successful, peri-implantitis (dental implant infections) may be as high as 14%.[1] Early implant failures especially may arise due to infections resulting from contaminated surgical placement or impaired healing, while plaque-induced peri-implantitis is more typically observed beyond one year of placement. With chronic infections, the patient may have to take large doses of antibiotics to address the infection or even have the implant surgically replaced if the antibiotic treatment fails to clear up the infection. Several studies have reported on the potential for select metals and their respective ions to act as anti-microbial agents.[1] This has prompted surface modification strategies leading to Ag enrichment of relevant implant surfaces in order to modulate bacterial response.[2] The objective of this study was to evaluate the antimicrobial potential of an electrochemical method to deposit silver nanoparticles (AgNPs) on titanium surfaces. It was hypothesized that reductions in bacterial attachment and growth could be achieved without impacting the inherent biocompatibility of the titanium substrate.

Materials and Methods:

0.254 cm thick Ti foil (99.5%, annealed, Alfa Aesar) was cut into discs with diameter of 1.11 cm. Prior to AgNP deposition, all discs were cleaned by sequential sonication (5 min) in acetone, ethanol and lastly nanopure water (18.2 MΩ-cm), followed by air drying. AgNP deposition onto the Ti surfaces was achieved using a 3-electrode chemical cell design and a potentiostatic double-pulse electrodeposition technique involving both nucleation and growth phases (pulses).[3] Here, 1 sec and 30 sec nucleation phases were assessed for a fixed growth phase (120 sec) and electrolyte concentration (10^{-3} M Silver Nitrate, 99.5%, Alfa Aesar). All discs were subjected to a final ethanol rinse and sonication prior to use in cell culture assays. AgNP coverage on the discs was evaluated by scanning electron microscopy (SEM) at high magnification (60,000X).

A 1 x 10^5 Colony Forming Unit (CFU) concentration of *S. aureus* in a 20µL aliquot of either PBS, for 3-hour attachment studies, or Mueller Hinton (MH) broth, for 24-hour growth studies, was centrally placed on the treated titanium discs and incubated in a moist environment at 37°C. The CFU concentration was determined by verified optical density measurements, and untreated titanium discs served as controls. For the 3-hour attachment studies, the discs were passively rinsed in PBS and then the attached CFU's were removed by vortexing and collected. For the 24-hour growth studies, both the passive and vortexed rinses were collected in order to assess cell growth in suspension and on the disc surface, respectively. These collected fractions were serially diluted and then a 20 µL aliquot was re-seeded onto MH agar plates and incubated at 37°C for 24 hours; the number of CFUs were determined by visual inspection. A standard MTT assay was used to assess the potential cytotoxic effects of the deposited silver nanoparticles using NIH 3T3 mouse fibroblasts. One-way ANOVA was used to assess significance ($p<0.05$) between experimental conditions.

Results:

Overall, greater surface coverage and a more distinct bi-modal AgNP size distribution were observed with the longer (30 sec) nucleation pulse time, as shown in Figure 1.

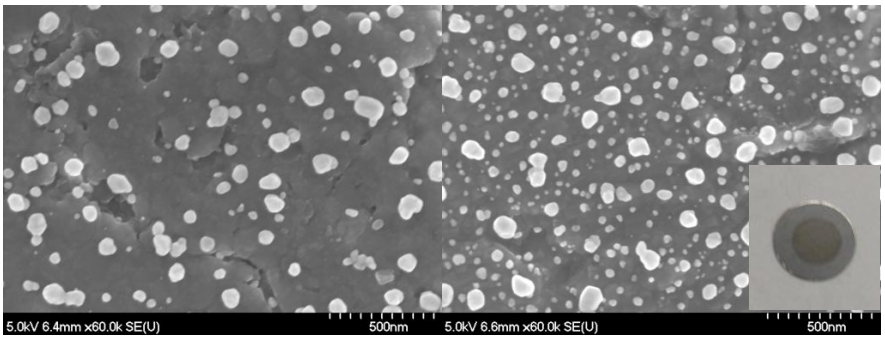

Figure 1: Representative SEM surface images of 1 sec (left) and 30 sec (right) discs; a well-defined central region of AgNP deposition on the discs was obtained (inset).

The results of four repeated microbial assays showed no discernable impact on bacterial attachment. While there was a trend to reduced surface growth of *S. aureus* at 24 hours, no statistically significant differences were noted. However, AgNP deposition did significantly reduce the growth of suspended (unattached or weakly attached) cells. Results for one of these runs is shown in Table 1.

Table 1: Colony Forming Unit (CFU) Counts following culturing of *S. aureus* (n=5)

Sample Condition	**Attachment ($x10^3$)**	**Growth**	
		Surface ($x10^3$)	**Suspended ($x10^6$)**
Control (Ti only)	35.1 ± 23.6	35.3 ± 36.0	3.41 ± 1.03[a]
1 sec Nucleation	46.1 ± 13.6	14.2 ± 12.5	0.03 ± 0.02[b]
30 sec Nucleation	44.2 ± 9.8	15.1 ± 7.8	0.07 ± 0.02[b]

Cytotoxicity assays revealed lower cell activity overall for all disks compared to the positive control (culture plate). However, there was no statistically significant difference in cell response between the experimental control (Ti only) and the AgNP-coated discs.

Discussion:

Assessment of this system was limited in part by a microbiological assay protocol that proved technically challenging due to the small volumes required in order to control surface area coverage of the cell suspension, leading to relatively high intra-group variability. Modifications to the protocol to allow for larger volumes together with an increase in replicates should allow for a more definitive evaluation of the AgNPs on cell attachment and surface growth. Nevertheless, these studies demonstrated that AgNPs clearly have the capacity to significantly reduce growth of *S. aureus* in proximity to the disc surface, presumably through an Ag ion release; efforts are underway to correlate ion release with observed bacterial cell response. It is worth noting that this approach is not necessarily optimized with respect to balancing antimicrobial response with the inherent cytotoxicity of Ag on eukaryotic cells, and further AgNP enrichment may be possible. Future studies will also need to look at the impact on osseointegration potential of surface, while also teasing out the influence of physical modifications to the surface resulting from the electrolytic deposition process.

References

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