Nanostructured composite coatings to reduce bacterial infection on titanium orthopedic implants

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Introduction: A critical first step in the propagation of infection in implants is bacterial adhesion onto a material's surface. Hence, an important strategy in the prevention of infection is the reduction of bacterial adhesion, which must be accomplished without sacrificing the bone growth properties of the implant.[1] This work proposes new types of implant coating materials incorporating nanophase hydroxyapatite, selenium and cerium oxide particles that can help achieve reduced bacterial adhesion via the combined effect of nanotopography and their very own chemical composition. Selenium (Se) is an antioxidant essential trace element for human health that can remove free radicals *in vitro*. Nanoscale Se is found to possess excellent bioavailability, low toxicity, and contribute to a wide spectrum of health-promoting as well as disease prevention and treatment activities. Cerium oxide nanoparticles are amongst the most widely used rare earth compounds and they have enormous potential as antioxidant and radioprotective agents for cancer applications [2]. Hence, this study focuses on combining the use of these 2 nanoparticles with conventional hydroxyapatite to coat titanium surfaces via electrophoretic deposition to offer increased bacterial resistance and improved bone growth without the use of any externally delivered antibiotics.

Materials and Methods: Nanoscale hydroxyapatite, cerium oxide and selenium particles were synthesized using previously-established wet chemistry synthesis procedures.[1,2]. The nanoparticles were deposited onto 1-cm squares of titanium sheet metal (Alfa Aesar) after the surface was cleaned by sonication using ethanol and acetone. The coated surfaces were then exposed to different strains of bacteria namely *Staphylococcus aureus* (ATCC® 29740™) and *Pseudomonas aeruginosa* (ATCC® 39324™). Tryptic soy broth (0.03% TSB) and agar plates (Sigma-Aldrich) were used as the media The cell suspension was diluted to achieve an optical density of 0.52 – 0.54 at a wavelength of 562 nm corresponding to a cell density of 10^9 cells/ml. After sterlization with 70% ethanol for 20 minutes, a concentration of, 10^6 cells/ml were seeded onto each sample and allowed to incubate for 24 hours. Afer incubation, the samples were rinsed and sonicated in PBS to remove the bacteria from the substrate. This solution was then diluted to various degrees and plated on the TSB/Agar plates to grow for another 18 hours before the colonies formed were counted. All experiments were conducted in triplicate and differences between means were determined using analysis of variance followed by Student's t-tests.

Results and discussion: Composite coatings were successfully achieved by the process of electrophoretic deposition and verified by SEM (Figure 1).

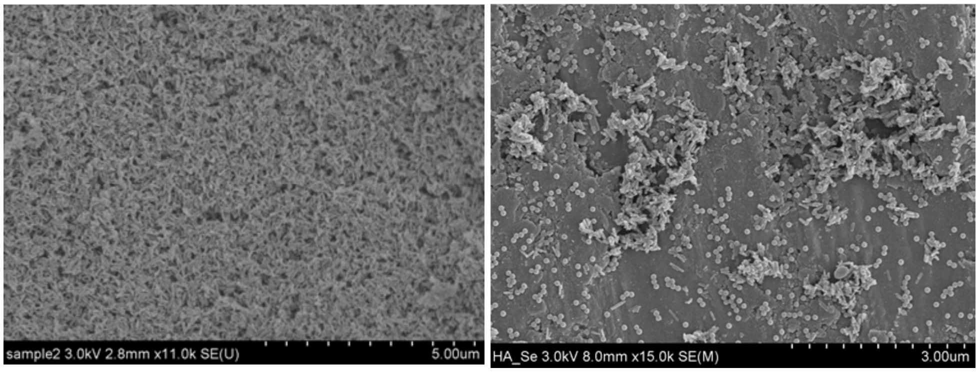

Figure 1: SEM images of nanophase HA and Ceria composite coating and nanophase HA and selenium composite coatngs.

The coatings were found to show a nanoscale topgraphy that would potentially alter the surface energy and roughness of the implant. Preliminary data also indicated reduction in bacterial activity for both the strains tested on the coated samples, Figure 2, where it is seen that the composite HA/Se coatings showed 5 times more reduction in bacterial activity as compared to plain Ti..

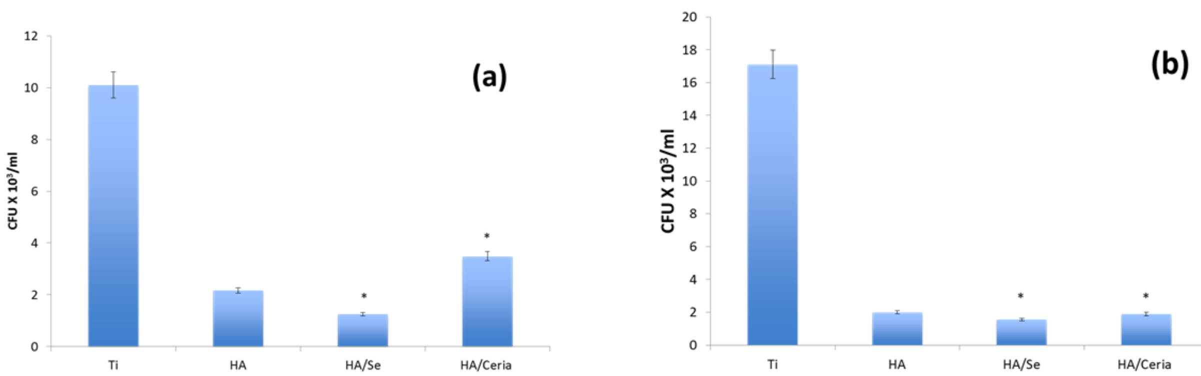

Figure 2: (a)*P. aeruginosa* and (b)*S.aureus* adhesion on coated samples after 18 hours of incubation. *$p<0.05$ as compared to plain Ti.

This reduction in bacterial activity is tentatively attributed to the changes in the surface morphology of the implant brought about by the nanostructured coatings and the chemical properties of the selenium and cerium oxide nanoparticles which is to be confirmed by further experiments and observations.

Conclusion: Collectively, results demonstrated promise for the continued development of composite nanophase hydroxyapatite, cerium oxide and selenium EPD coated titanium implants to improve device performance

Acknowledgement: The authors would like to thank Northeastern University for funding.

References:

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