Rosette Nanotube (RNT) and Nanophase Hydroxyapatite (HA) Composites for Orthopedic Applications

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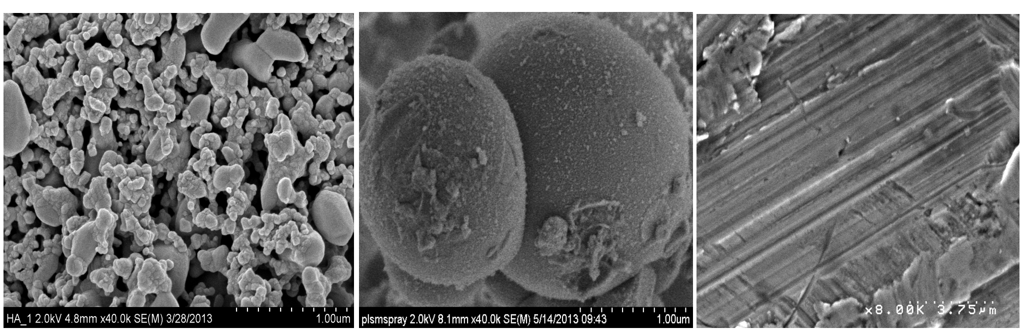
Orthopedic implants fail due to various reasons such as extensive bacterial colonization, poor osseointegration, extensive inflammation and osteolysis due to wear debris. In order to reduce orthopedic implant failures, the main objective of this study was to create a biomimetic bone tissue engineering material based on the self assembling properties of rosette nanotubes (RNTs), the osteoconductive properties of nanophase hydroxyapatite (HA), the bacterial inhibition properties of the MAM7 peptide[1], the quorum sensing inhibitor RNAIII peptide[2], and the anti-cancer properties of the PCK3145 peptide[3] as well as curcumin[4].

Nanophase hydroxyapatite was synthesized by a wet chemical synthesis process using Ca $(NO_3)_2 4H_2O$, KH_2PO_4,distilled water, ammonia and acetone mixing while stirring for 1 hour and aging for 24 hours followed by filtering using Whatman's grade:1 filter paper followed by sintering at 900 °C (Figure 1). To determine its antibacterial and osseointegrative properties, HA was then coated onto Ti-6,4 samples cut into 1cm x 1cm squares by electrophoretic deposition. HA were tested for cell growth and viability using human osteoblasts(ATCC-C12720) and performing MTT adhesion and proliferation assays. Bacterial adhesion and proliferation assays were performed using a *Staphylococcus aureus(*ATCC® 25923)strain of bacteria. Samples conventionally plasma sprayed with micron sized HA were obtained from the firm HIMED and used for comparison. Results demonstrated for the first time that nanophase HA possessed significantly greater wettability properties (Figure 2) and decreased bacterial colonization while increasing osteoblast adhesion and proliferation compared to micron sized conventionally used HA (Figures 3 and 4).[5]



(a) (b) (c)

Figure 1: SEM image of the EPD coated nanophase HA on Ti-6Al-4V. The surfaces showed enhanced nano-features which provided a conducive surface for osteoblast adhesion. (b) SEM image of the conventional plasma sprayed micron sized HA on Ti-6Al-4V which shows reduced nano features and clumping of the HA powder due to the high heat involved in the process. (c)SEM image of plain Ti.

(a) (b) (c)

Figure 2: (a) Contact angle for EPD coated nanophase HA on Ti-6Al-4V: $< 6^0$ (Complete wetting), (b) Contact angle for plasma sprayed micron sized HA on Ti-6Al-4V: 48.79 ^{0}and (c) Contact angle for plain Ti-6Al-4V: 65.28^0.The total spreading of the water droplet on the surface of the EPD coated sample depicts increased hydrophilicity, making it favorable for cell attachment and growth. The hydrophobic nature of the plasma coated sample might make it an optimal surface for bacterial attachment.

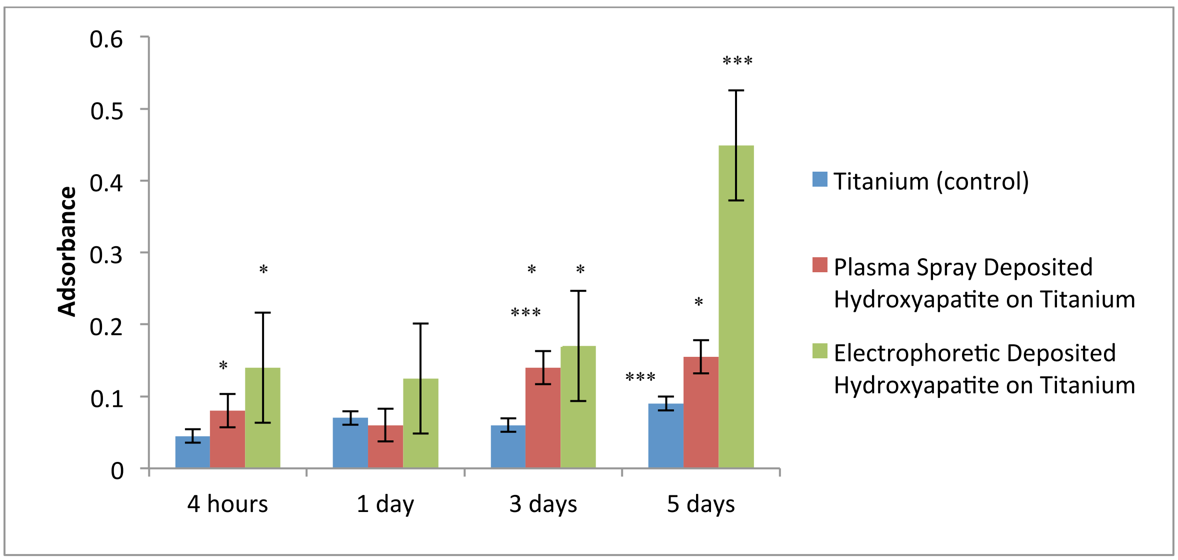


Figure 3: Increased Osteoblast Density on Nanophase Hydroxyapatite After5 Days of Culture. Data = mean+/-SEM; N = 3; * $p < 0.01$ compared to Ti (control) at the same time period; ** $p < 0.01$ compared to plasma sprayed deposited hydroxyapatite on Ti at the same time period; *** $p < 0.01$ compared to the previous time period on the same substrate. There was 6 and 3 times more osteoblasts on nanophase hydroxyapatite compared to Ti (control) and plasma spray deposited hydroxyapatite on Ti, respectively.

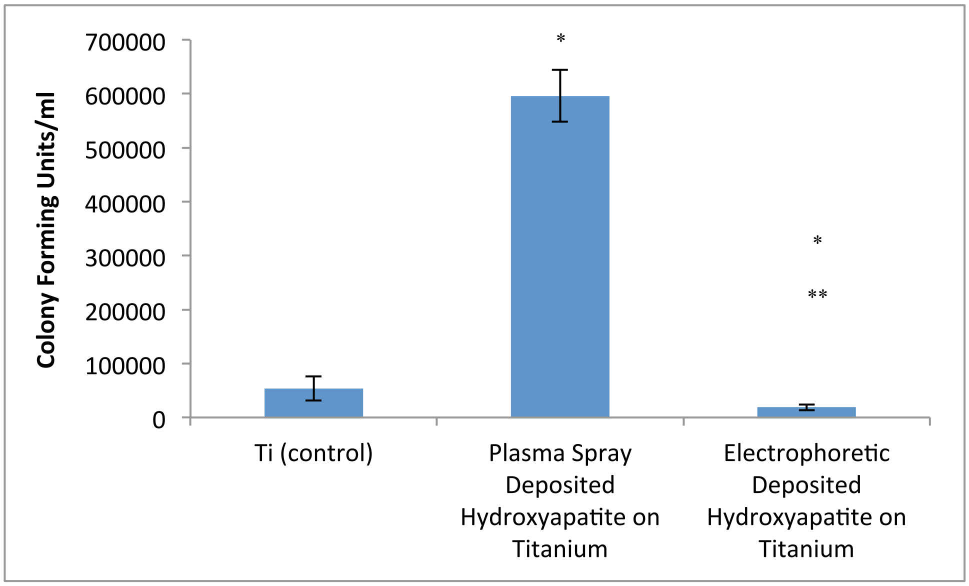


Figure 4: Significantly Decreased Bacteria Density on Nanophase Hydroxyapatite after 18 Hours of Culture. Data = mean+/- SEM; N = 3; * $p < 0.01$ compared to Ti (control); ** $p < 0.01$ compared to conventional plasma sprayed deposited hydroxyapatite on Ti. There was 2.9 and 31.7 times less bacteria on nanophase hydroxyapatite compared to Ti (control) and conventional plasma spray deposited hydroxyapatite on Ti, respectively.

Rosette nanotubes (RNTs) with lysine side chains were synthesized using general synthetic strategy. They were then functionalized with the desired peptides by covalent grafting. The functionalized RNTs were dissolved in dH_2O to achieve a 0.1 mg/ml stock solution. The stock solution was diluted with dH_2O to obtain a 0.001 mg/ml solution of the same. 250 mg of nanophase HA was mixed with 5ml 70% ethanol and sonicated for 20 minutes to obtain a homogeneous solution. 5 ml of 0.001 mg/ml of the initial solution was added to the homogenous nanophase HA solution. The material was characterized using H NMR, C NMR spectra, high resolution ESI-MS and elemental analysis. Cell growth and viability was tested using osteoblasts and osteosarcoma cells to perform MTT adhesion and proliferation assays. Bacterial adhesion and proliferation was tested using *S.aureus* and *S.epidermidis* strains of bacteria. Animal studies were then conducted.

Future studies will further develop an ideal biomaterial that offers improved osseointegration, decreased chances of bone cancer formation and reduces bacterial infection.

References

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