Fabrication of nanoscale titania coating and their osteoblast responses

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Statement of Purpose: The success of titanium (Ti) implants is due to osseointegration or the direct contact of the implant surface and bone without a fibrous connective tissue interface, where the surface Ti is a very thin layer of amorphous titania (TiO_2).[1] Recently, in vitro cellular models have shown that osteoblast proliferation, differentiation and mineralization were significantly greater on sintered nanoscale crystal TiO_2 bulk ceramics than on conventional microscale formulations of the same ceramics.[2] It was hypothesized in this study that nanoscale crystal TiO_2 coating on Ti implant enhances osteoblast activity and there exists an optimal nanoscale crystal size. In order to test the hypothesis, nanoscale crystalline TiO_2 coatings were deposited on Ti disks by radiofrequency magnetron sputtering followed by heat treatment at various temperatures. Osteoblast precursor cells were used to evaluate cell responses to nanoscale coating.

Methods: Commercially pure Ti disks with 15 mm diameter and 2 mm thickness were polished using 600 grit paper, degreased and passiviated (ASTM F86-91) and used as controls. TiO_2 coatings were deposited at 1.0~1.5 mbar and a power of 300 W at a coating rate of 170 nm per hour using a CMS-18 radiofrequency magnetron sputtering system (Kurt J Lesker Company, PA). After deposition, samples were left either as-deposited or heat-treated at 400°C and 600°C for 1 hour in air. Samples were sterilized by polyethane oxide. Samples were characterized using water contact angle, surface roughness, scanning electron microscope (SEM), atomic force microscope (AFM), and x ray diffraction (XRD). Human embryonic palatal mesenchymal cells (HEPM), an osteoblast precursor cell line, was used to evaluate cell attachment, proliferation, and differentiation on nanoscale coatings compared to Ti disks. A subtractive cell attachment was measured using coulter counter. dsDNA was quantified via PicoGreen assay, total protein production was measured using a BCA assay, alkaline phosphatase was measured by conversion p-nitrophenyl phosphate to p-nitrophenol via a colormetric endpoint assay. Data were statistically compared using the ANOVA, with the Student Newman-Keuls procedure as the post hoc test for the evaluation of significant differences at the $P < 0.05$ level.

Results / Discussion:

SEM observation indicated homogenous coatings of nanoscale crystals (Figure 1). The average crystal sizes for as-deposited TiO_2 coating, 400°C heat-treated TiO_2 coating and 600°C heat-treated TiO_2 coating were observed to be approximately 20nm, 40nm and 80 nm, respectively. Hexagonal crystals of 10 micron were observed on Ti control. Fracture cross-section morphology of deposited and heat-treated TiO_2 coatings indicated a dense and columnar structure. Thin film XRD analysis on the as-sputtered coatings indicated the presence of amorphous, whereas the rutile and a trace amount of anatase titania were observed on heat-treated coatings. Water contact angles and surface roughness of different surfaces are shown in Table 1. The as-sputtered TiO_2 exhibited significantly lower surface roughness compared to the other three groups. No differences in surface roughness were observed between the Ti controls, 400°C and 600°C heat treated TiO_2 coatings. The as-sputtered TiO_2 exhibited significantly greater water contact angle compared to the other three groups. The heat treated TiO_2 coatings exhibited significantly lower water contact angle compared to the Ti controls, suggesting that the as-sputtered TiO_2 reduced the hydrophilicity, but heat treated TiO_2 enhanced the hydrophilicity compared to the Ti controls.

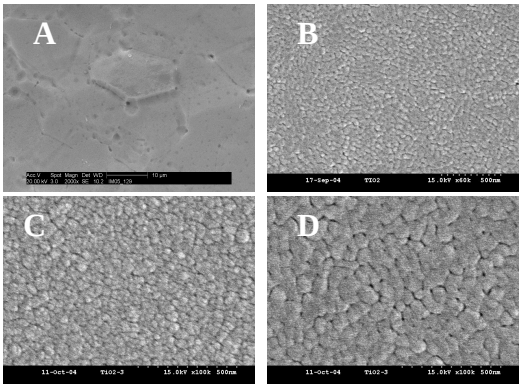

Fig.1 Representative surface morphology. A) Ti; B) As-deposited TiO_2; C) 400°C heat-treated TiO_2; and D) 600°C heat-treated TiO_2. bar = 500nm

Table 1 Surface roughness and contact angle of different surfaces

	Surface roughness (μm)	Water contact angle (°)
Ti control	0.37 ± 0.03	37.4 ± 5.4
as-sputtered TiO_2	0.25 ± 0.02	70.3 ± 0.82
400C heat treated TiO_2	0.45 ± 0.11	28.2 ± 2.78
600C heat treated TiO_2	0.44 ± 0.03	26.1 ± 2.80

Nanoscale TiO_2 significantly enhanced cell adhesion compared to microscale Ti control. The concentration of dsDNA was observed to increase over time. A significant higher alkaline phosphatase on 400C heat-treated TiO_2 coating was observed at day 6 ($p<0.05$) when compared to other groups.

Conclusions:

It was concluded that nanoscale crystal TiO_2 coatings enhanced osteoblast adhesion and differentiation compared to the Ti controls.

References:

1. Schmale GA, et al, J Arthroplasty 2000; 15(6): 718-729. 2. Kasemo B. J Prosthet Dent 1983; 49: 832-837 3. Gutwein LG, et al. Biomaterials 2004; 25: 4175-4183.

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