Comparative cell behavior on titania nanotubes filled with HAP

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Statement of Purpose:

Optimizing dental and orthopedic implants involves modifying the implant surface chemistry and/or surface topography. Both factors are known to be the most important on the protein adsorption, affecting thus the biological reactions[1]. Nanostructured materials give completely new types of interactions between implant surfaces and cells because the surface area is increased and the surface topography can be modified to resemble native bone tissue[2]. Topography of titanium implants can be tailored by anodization process to produce titania nanotubes. Oh et al.[3] found that TiO_2 nanotubes accelerated significantly the osteoblast adhesion. A common chemical surface modification is to use hydroxyapatite (HAP) as a thin coating deposited with different techniques. It is, however, difficult to make these coatings to remain adherent to Ti due to differences in elastic modulus with subsequent failure at the bone-implant interface or titanium-hydroxyapatite interface[4]. As it is comparably easy to control the nanotube diameter changing the anodization parameters, and to avoid the adhesion drawbacks of HAP coatings, a simple method is proposed here: filling the TiO_2 nanotubes with HAP nanoparticles. In this study, we investigate the fibroblast behavior on TiO_2 nanotubes filled or not with HAP, compared to plain titanium surfaces.

Methods:

Ti foils (0.25 mm, 99.8% purity, Alfa Aesar) were degreased by sonicating in acetone, ethanol, and deionized water (DI) during 10 min each. Samples were then anodized using an electrolyte containing 79.3 vol.% diethylene glycol (DEG), 0.7 vol.% hydrofluoric acid, and 20 vol.% of DI water. Anodization was performed potentiostatically at 60 V for 6 hours using a conventional two-electrode configuration with a platinum foil as a cathode. Following the anodization, the samples were rinsed with DI water and dried in a nitrogen stream, and subsequently annealed in air atmosphere for 10 h at 530 °C. Anodized samples were sonicated during 30 min in ethanol containing HAP nanospheres (< 200 nm, Aldrich), then rinsed with DI water and dried in air. Surface morphology of the TiO_2 nanotubes (TiNT) was characterized using a scanning electron microscope. Human dermal fibroblasts were cultured, expanded, and seeded on TiNT, TiNT+HAP and Ti surfaces at a density of 10^4 cells/cm^2. They were allowed to adhere and to proliferate for 24 h, at 37 °C, in a DMEM medium in a humidified atmosphere of 5% CO_2. Cell viability was determined using an MTT based toxicology assay and cell morphology was observed using scanning electron microscopy. In addition, cells live were viewed under fluorescence microscopy using CMFDA (cytoplasm), DAPI (nucleus), and rhodamine phalloidin (actin) stains to determine cell survival, adherence, morphology.

Results:

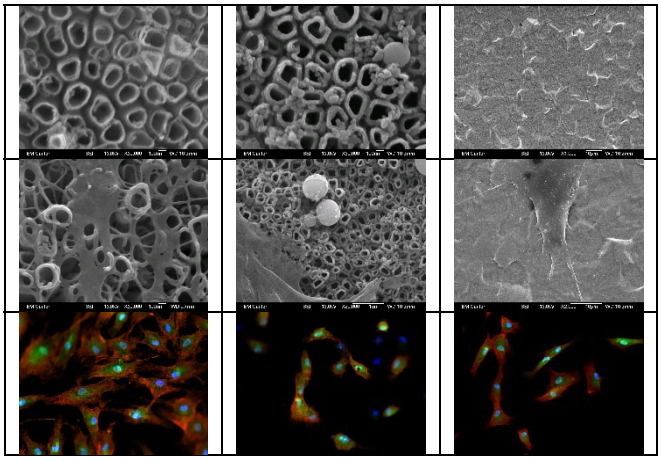

Figure 1. (a-c) SEM showing the TiNT, TiNT+HAP and Ticp; (d-f) SEM of fibroblast cells grown after 24h; (g-i) Representative fluorescence micrographs.

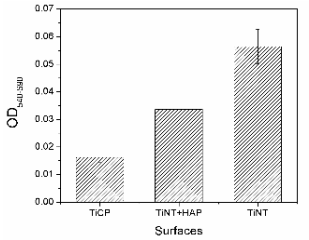

Figure 2. MTT assay data showing the optical density (OD) after 24 h of incubation.

Conclusions:

It is shown that the fibroblasts adhesion/propagation is improved by the topography of the nanotubes. The number of the cells on the TiO_2 nanotubes increases significantly as compared to the cells adhering to the Ticp. This is caused by the significantly increase in surface area on anodized samples. The cells activity on HAP filled nanotubes is lower than on TiNT and higher than Ticp. A possible explanation is that the nanospheres fill the gaps among the nanotubes, suggesting that the surface area has more effect than surface chemistry on cell propagation. Such an array of TiO_2 nanotubes can be useful as a bioactive surface layer with cell adhesion significantly accelerated.

References:

[1] X. Zhu et al., Effects of topography and composition of titanium surface oxides on osteoblast responses, Biomaterials 25 (2004) 4087-103.

[2] P.M. Brett et al., Roughness response genes in osteoblasts, Bone 35 (2004) 124-33.

[3] S. Oh et al., Significantly accelerated osteoblast cell growth on aligned TiO2 nanotubes, J. Biomed. Mater. Res. A 78A (2006) 97-103.

[4] R. Mcpherson, N. Gane, Structural characterisation of plasma sprayed hydroxylapatite coatings, J. Mater. Sci. 6 (1995) 327-34.