The Covalently Grafting of Chitosan onto Titanium Surface Stimulate Initial Osteogenesis of Osteoblast

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Statement of Purpose: Titanium (Ti) is considered to be a key implant material in the orthopedic fields. However, the material is often associated with poor osteogenesis, one of the major complications prevailing in orthopedic implants, that often culminates in implant failure.[1] Here, we covalently grafted chitosan onto Ti surface to improve the osteogenic capacity of Ti.

Methods: In this study, Ti foil was cut into samples with a size of 1x1 cm^2. The samples were ultrasonically cleaned, 48% H_2SO_4 treated, and functionalized with chitosan. The experimental groups were H_2SO_4 treated Ti (SA-Ti), the intermediate product, and chitosan treated Ti (SA-CS-Ti), the final product. Untreated Ti (UN-Ti) served as controls. The surface properties of each material were determined by contact angle goniometry and optical profilometry. Osteoblast-like cells, SaOS-2, were used to determine the effect of the materials on their osteogenic capacity. The osteogenic capacity was assessed by measuring cell attachment and calcium deposition.[2]

Results: The extracellular microenvironment plays a key role in controlling cellular behavior. The changes of surface roughness and hydrophobicity of Ti after the treatments were investigated and shown in Table 1. The mean surface roughness value of UN-Ti was 0.19±0.0073 µm, which was approximately tripled in SA-Ti (0.60 ± 0.153 µm) and SA-CS-Ti (0.70±0.053µm). This was in agreement with the optical profilometry images (second row in Table 1) of each surface. Contact angle goniometry results showed that there was a significant increase ($P<0.05$) in hydrophobicity (water contact angle) after the H_2SO_4 treatment (81.58°±9.16) and the subsequent chitosan modification (94.1°±4.32).

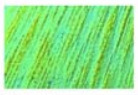
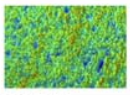
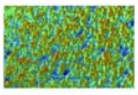
	UN-Ti	SA-Ti	SA-CS-Ti
Average roughness(µm)	0.19±0.007	0.6±0.153*	0.70±0.053*
Optical profilometry images			
Contact Angle (°)	70.3±0.68	81.58±9.16*	94.1±4.32*
Contact angle (camera shots)			

Table 1: Surface Characterization of different titanium samples. (*) denotes significant difference ($p<0.05$) compared with untreated titanium.

Cell attachment, one of the key indicators of osteogenic activities of osteoblasts on implants, was evaluated using scanning electron microscopy (SEM) observation and MTT quantification. As shown in Fig. 2A on all the samples, the level of adherent cells gradually increased with the increase of contact time from 5 min (Fig. 2A, top row), to 120 min (Fig. 2A, middle row) and then to 240 min (Fig. 2A, bottom row). At 120 min and 240 min, the cells attached on SA-Ti and SA-CS-Ti produced more extracellular matrix than the cells attached on UN-Ti. Quantitative result in Fig. 2B confirmed that significantly higher ($P<0.05$) number of cells attached on the SA-Ti and SA-CS-Ti at 120 min and 240 min than on the UN-Ti. Furthermore, after 480 min of cell seeding, significantly higher ($P<0.05$) number of cells was still observed on SA-CS-Ti than UN-Ti (data not shown).

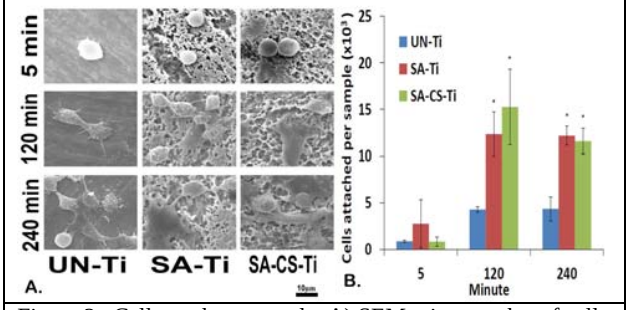


Figure 2. Cell attachment study. A) SEM micrographs of cells attached on UN- Ti, SA-Ti and SA-CS-Ti at 5 min , 120 min and 240 min. B) Quantitative comparison of osteoblast-like cell attachment at different time points. (*) denotes significant difference ($p<0.05$) compared with untreated titanium at respective time points.

Mineralization is a widely used marker to evaluate osteoblast maturation. In the current study, calcium deposition was tested at day 28 and 48 to determine mineralization and compare the osteogenic capacity of each material. Fig. 3 showed that the calcium deposition level on UN-Ti was significantly lower ($P<0.05$) on day 28 than on day 42, while this level was not statistically different on SA-Ti and SA-CS-Ti on Days 28 and 42.

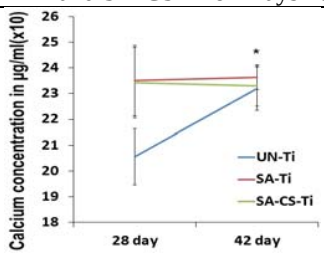


Figure 3: Quantitative representation of calcium deposition on UN-Ti, SA-Ti and SA-CS-Ti on Days 28 and 42 (n=4). (*) denotes significant difference ($p<0.05$) within the UN-Ti groups.

Conclusions: We have demonstrated that Ti surface grafted with chitosan stimulate initial osteogenesis of osteoblast and provided a new insight in future implant design to manage poor osteogenesis.

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[2] Zhang F., et al. Biomaterials. 2008; 29: 4751-9.