Effect of Bioactive Surface Coating on Protein Adsorption, Bone Cell Differentiation and Immune Response

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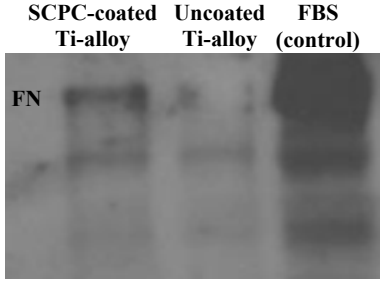
Statement of Purpose: Bioactive ceramic coating promotes bone bonding and fixation of orthopedic implants. Protein adsorption mediates cell-material interaction and so it plays an important role in tissue responses to implant materials. An ideal implant surface should therefore be able to selectively bind attachment proteins, expose their active sites for specific cell adhesion, and trigger an intracellular cascade reaction that leads to bone deposition. The objective of the present study is to coat the surface of medical grade Ti-6Al-4V implant material with a bioactive silica-calcium phosphate nanocomposite (SCPC)[1] and evaluate bone cell responses to this material by RT-PCR. The effect of SCPC coating on fibronectin adsorption is reported. Moreover, the secondary structure of adsorbed proteins was analyzed and co-related to cell function.

Methods: SCPC was coated on Ti-6Al-4V samples by electrophoretic deposition. A voltage of 50 V was applied for 30 sec in a 10 wt% SCPC suspension in ethanol followed by thermal treatment at 800 ˚C/1 hr under argon.[2] Ti-6Al-4V discs with and without SCPC coating were immersed in 1 mL of FBS at 37 ˚C for 4 hr. The adsorbed protein was extracted and analyzed by Western blot and ELISA techniques. A mouse monoclonal antibody was employed for the detection of adsorbed fibronectin. In addition, total protein adsorption was quantified using a Micro BCA assay kit (Thermo Scientific, Rockford, IL). The conformation of the adsorbed protein was analyzed using FTIR in the diffuse reflectance mode. Secondary structures within the amide I functional group, in addition to the ratio of amide I/amide II envelope, were determined. MC3T3-E1 osteoblast-like cells were seeded on the substrates and the expression of osteoblast-associated products was analyzed by measuring levels of mRNA encoding collagen-I, osteonectin, osteopontin, and osteocalcin by RT-PCR. Moreover, the inflammatory response to the substrates was determined by measuring the expression of inflammatory cytokines IL-6, IL-12 and RANK-L. In addition, the ionic composition of the tissue culture medium incubated with the different substrates in presence and absence of cells was also evaluated.

Results: Total protein analysis showed that SCPC-coated substrates adsorbed significantly higher amounts of serum protein than uncoated Ti-alloy ($p<0.05$). Moreover, Western blot and ELISA analyses showed preferential adsorption of fibronectin on SCPC-coated samples (Fig. 1a). FTIR studies of the adsorbed protein showed that the ratio of amide I/amide II functional groups was significantly higher ($p<0.05$) on SCPC-coated than that on the uncoated samples (Fig. 1b). RT-PCR results showed that the cells attached to SCPC-coated substrates expressed higher levels of osteocalcin mRNA compared to that expressed by cells attached to uncoated Ti-alloy. In addition, the release of the inflammatory cytokines IL-6 and RANK-L was significantly lower ($p<0.05$) on SCPC-coated compared to uncoated Ti-alloy implants.



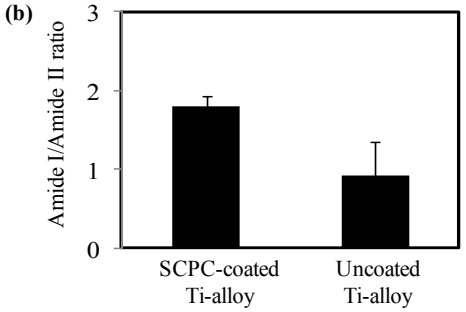


Fig. 1: (a) Western blot showing preferential fibronectin adsorption to the SCPC-coated substrate; (b) FTIR analyses showed that proteins bound to SCPC-coated samples demonstrated significantly higher ratio of amide I/amide II functional groups than the uncoated samples.

Conclusions: The results of the present study show that SCPC-coated substrate selectively adsorbs a significant amount of fibronectin, an attachment protein known to stimulate bone cell adhesion and function. Moreover, SCPC coating induces a favorable protein conformation with high ratio of amide I to amide II. Published studies have shown that higher amide I expression enhances cell adhesion and cellular proliferation leading to enhanced tissue integration.[3] The superior protein adsorption and the bioactive characteristics of the implant coating material synergistically stimulates the rapid expression of osteoblast products associated with de novo bone deposition in the absence of a significant inflammatory response. The minimal production of pro-inflammatory cytokines would be anticipated to limit leukocyte recruitment to the implant site and restrict osteoclast maturation and activation.[4] As such, the use of SCPC-coated Ti-alloy implants has the potential to improve bone-tissue integration and enhance implant longevity.

References:

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