**Why is Titanium Biocompatible?**

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**Introduction:** Titanium (Ti) is the material of choice for orthopedic applications because of its known biocompatibility. In order to enhance osteogenic properties of the Ti implants, it is necessary to understand the origin of this osteogenic property. This paper addresses the origin of Ti biocompatibility through theoretical modeling, the precise determination of Ti surface chemistry by X-ray photoelectron spectroscopy (XPS), and the study of fibronectin (FN) adsorption as a function of Ti (near) surface properties by Enzyme-linked immunoabsorbent assay (ELISA).

An enhancement of protein adsorption encourages cell adhesion and growth, thus, rapid bone growth and osteointegration at the implant surface. The adsorption of proteins such as FN and vitronectin is a key factor in cell adhesion and bone formation at an implant surface.[1] The adhesive proteins contain the cell receptor-binding RGD amino acid sequence motif and mediate the cell adhesion to the surface of the implant (Figure 1). For cells, adhesive proteins play the role of an anchor on the implant surface that binds the protein binding site to the cell receptors (such as integrin). The biocompatibility and bio-integration of the implanted surface depends very strongly on the initial immobilization and possible structural changes of mediating proteins at the implant surface.[2]

Materials and Methods: Stainless steel and CoCr implants are not considered an ideal choice for cementless implants. On the other hand, Ti is biocompatible and encourages osteoblast ingrowth. It is important to know why Ti has good biointegration properties. We applied XPS to analyze the near surface chemistry of several Ti specimens. XPS experiments indicated the presence of native Ti oxides that include a sub-oxide component with a stoichiometry of approximately TiO. We applied ELISA to determine FN adsorption as a function of surface chemistry and morphology. In these experiments, we used a chicken polyclonal anti-FN antibody and a peroxidase conjugated rabbit anti-chicken antibody as first and second antibodies, respectively. Tetramethylbenzidine was used as a staining agent. After 3 hours of incubation time, the adsorption of FN to the surface of TiO2 and Ti (orthopedic grade and nanostructured) was determined by the application of a Microplate Reader at a wavelength of 655 nm.

Results: High resolution XPS spectra of the Ti 2p region obtained for stoichiometric TiO2 film fabricated by ion beam assisted deposition (IBAD) and native Ti-oxides. Note there are only two peaks in the IBAD TiO2 spectrum (corresponding to photoelectrons emitted from the split 2p level at 458.4 eV and at 464.2 eV), signifying a pure TiO2 composition. In contrast, the spectrum of the native Ti-oxide shows additional peaks beside the major TiO2 peaks. The binding energies of these additional peaks indicate the presence of Ti (not oxidized, BE=453.3 eV and 460 eV) and Ti suboxides with binding energies between those characteristic for Ti and for TiO2, respectively. The presence of Ti peaks indicates the native oxide is so thin (probably a few nm only) that it does not fully mask the underlying metal.

We have carried out comparative ELISA experiments to study an adsorption of FN onto the elemental Ti and stoichiometric TiO2 deposited onto glass substrates by IBAD at room temperature in ultrahigh vacuum chamber. Figure 3 clearly shows that more FN was adsorbed on the TiO2 (anatase phase) as compared to the Ti and CoCr. These results are consistent with those we observed for mesenchymal stromal cells (OMA-AD) adhesion and growth of the same materials.

Discussion: We have shown that an insulating oxide surface layer such as TiO2 or ZrO2 plays an important role in enhancement of protein adsorption to an implant surface [2]. Comparing Figures 2 and 3 we can clearly see that the increase of FN adsorption is due to the improvement of the surface oxide quality at the surfaces with similar topology. The ability of TiO2 to trap charges on its surface (due to surface defects) strengthens non-specific interactions, i.e. electrostatic and van der Waals, between the adhesion protein and the oxide surface layer compared to un-oxidized surfaces. Such localized charges cannot be trapped on the pure metallic surfaces. Transition from macrostructure to nanostructure increases the density of surface features (hillock edges and vertices, steps etc.) further improves the protein adsorption.

Significance: This work indicates that the biocompatibility of Ti metal is due to the presence of thin native substoichiometric titanium oxide layer which enhances the adsorption of mediating proteins on the surface which strengthens non-specific protein-surface interactions. Improving the surface oxide quality, i.e. fabricating stoichiometric oxides as well as nanoengineering the surface topology that matches the dimensions of adhesive proteins, is crucial for increase of protein adsorption and, as a result, the biocompatibility of Ti implant materials (See Fig. 3).