Microstructured Ti-6Al-4V Surface Improves Osseointegration: an in Vitro and in Vivo Study

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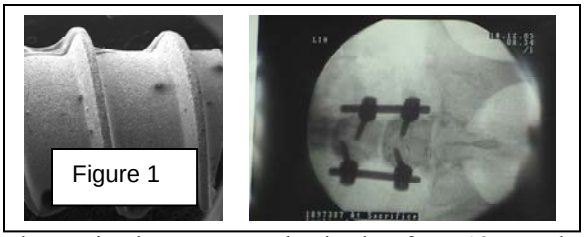
Statement of Purpose: Cell and tissue responses are dependent on biomaterial surface properties, including roughness, chemistry and wettability. Previous studies established that compared to smooth surfaces, microstructured titanium surfaces enhance osteoblast differentiation in vitro [1] and increase the quantity and quality of bone formation in vivo [2]. Similar to Ti, Ti-6Al-4V alloy is also widely used in prosthetics implants because of its good biocompatibility and mechanical property. We and others previously found that osteoblasts are sensitive to rough Ti-6Al-4V (Ti-alloy) surfaces [3]. However, whether the in vitro response of osteoblasts to Ti-alloy substrates with irregular microtopographies prepared by grit blasting is correlated with improved osteogenesis in vivo is unknown. The objective of this study was to test the hypothesis that increased production of factors associated with osteogenesis by osteoblasts in vitro will be positively correlated with increased bone to implant contact (%BIC) in vivo. To do this, we systematically assessed the effects of micron scale structured Ti-6Al-4V surfaces on cell and tissue responses by comparing in vitro results with the osseointegration of pedicle screws in sheep spine.

Methods: For the in vitro study, Ti-alloy disks were prepared by Impliant, Inc. to fit into 24-well plates (ø15x1 mm). Surfaces were either machined to produce 0.2 μm smooth surfaces, or blasted with a calcium phosphate medium (CaP), resulting in 2, 3 or 3.34 μm roughness. Surface morphology and chemistry were characterized by scanning electron microscopy (SEM), X-ray photon spectroscopy (XPS) and energy dispersive analysis of X-rays (EDAX). MG63 human osteoblast-like cells were cultured on the disks as well as on tissue cultured treated polystyrene (plastic) until cells were confluent on plastic. Cell morphology, cell number, alkaline phosphatase specific activity (ALP), and levels of osteocalcin (OCN), prostaglandin E_2 (PGE_2), transforming growth factor-β1 (TGF-β1) and osteoprotegerin (OPG) in the conditioned media, were analyzed. Data are presented as means ± SEM (n=6 independent cultures per variable).

For the in vivo study Ti-alloy pedicle screws (ø5x25 mm) were implanted into L4 and L5 vertebra of 15 2-year-old female Assaf sheep (Figure 1). Fusion rods were connected vertically to attain proper fixation and load bearing. Each sheep received either 4 smooth (Ra=0.2 μm) or 4 rough (Ra=3 μm) screws. Prior to use, screw surfaces were characterized by SEM, XPS and EDAX.



The animals were euthanized after 12 weeks of implantation. Osseointegration was evaluated by histomorphometry and removal torque measurement.

Results: XPS and EDAX confirmed that disk surfaces contained Ti, Al and V. Si and C were also present, but amounts were reduced on the CaP blasted substrates. MG63 cells on smooth surfaces were aligned and elongated. On rough surfaces, the cells were more rounded with cytoplasmic extensions into the pits of grit blasted surfaces. Cell numbers were lower on Ti compared to plastic, and the effect was more profound on rough surfaces. ALP was also reduced in a similar manner. OCN was increased in a surface dependent manner, with highest OCN on roughest surfaces (Figure 2a). Local factors were also regulated by surface microstructure with higher levels of PGE_2 (Figure 2b), TGF-β1 (both active and latent forms) and OPG on rough surfaces.

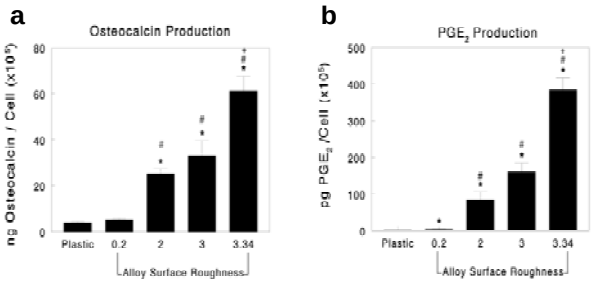


Figure 2

The average age and weight of sheep in the smooth and rough implants groups were the same. Thirteen of the 16 smooth implants and 10 of 14 rough implants supported osseointegration (Figure 3); only these implants were analyzed by histomorphometry (Table I). %BIC was increased on the rough implants. There were no differences in expected BIC (EBIC%) or bone volume (BV%). The required force to remove screws from the bone was more than two times greater for rough implants than for smooth implants.

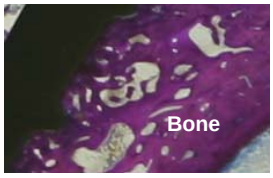


Table I	Smooth	Rough
BIC%	66.25±4.78	80.88±3.97*
EBIC%	83.67±1.74	81.35±2.96
BV%	78.07±1.47	79.45±1.60
Removal Torque(N·m)	2.28±0.32	5.29±0.41 *

Conclusions: The in vitro results indicate that microstructured Ti-6Al-4V surfaces produced by CaP blasting promote a differentiated osteoblast phenotype. These cells release increased levels of factors associated with bone formation and inhibition of osteoclast activity. This study also demonstrates the osteogenic effect of the microstructured Ti-6Al-4V surfaces using a clinically relevant in vivo analysis of pedicle screw osseointegration in sheep spine. Moreover, the study shows a positive correlation of the in vivo outcomes with the results of in vitro assays using MG63 cells as the experimental model. The results indicate that rough Ti-6Al-4V implants support greater and better osseointegration.

References: (1) Boyan et al., Eur Cell Mater 2003 24:22-7; (2) Li et al., J Biomed Mater Res 2002 60:325-32; (3) Lincks et al., Biomaterials 1998 19:2219-32

Acknowledgements: Impliant, Inc.