Osteoblast Response and Osseointegration of Direct Metal Laser Sintered Titanium Implants

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Statement of Purpose: Dental implants are placed in over 500,000 people each year in the United States, most of which are successful [1]. However, dental implant failure rates increase dramatically for the elderly, patients with diabetes, a history of smoking, or are otherwise compromised [2]. Titanium is a commonly used material in dental implants, with good biocompatibility and mechanical strength. It has been shown that titanium implant surfaces modified with combined micro and nano surface roughness increase osteoblast differentiation, which can lead to improved implant osseointegration [3]. However, traditional manufacturing methods produce surfaces that require a series of additional treatments to induce roughness. 3D additive manufacturing methods such as laser sintering have increased efficiency and decreased waste, while maintaining good spatial resolution. The purpose of this study was to analyze effects of a 3D laser sintered titanium alloy implant surface with combined micro-/nano-roughness *in vitro* and in a novel *in vivo* model.

Methods: Disks 15mm in diameter and 1mm in height and screws 3.7mm in diameter and 8mm in height were manufactured using direct metal laser sintering. Titanium alloy (Ti6Al4V) particles 24-45 μm in diameter were sintered with a ytterbium laser spot size of 0.1mm at 1054nm, continuous power of 200W and scanning rate of 7m/s. Surfaces were characterized for topography with scanning electron microscopy (SEM), chemistry with x-ray photoelectron spectroscopy (XPS) and energy dispersive x-ray spectroscopy (EDX), roughness with laser confocal microscopy, and wettability with sessile drop contact angle. Machined disks (M), laser sintered machined disks (M-LST), laser sintered disks blasted with $CaPO_4$ (LST) and laser sintered disks blasted with $CaPO_4$ particles and acid etched (A-LST) were used for *in vitro* studies. MG63 osteoblast-like cells were cultured at 20,000 cells per surface and assayed for DNA, alkaline phosphatase (ALP) specific activity, osteocalcin (OCN), bone morphogenetic protein 2 (BMP2), vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF2), integrins α2 and β1 (ITGA2, ITGB1). OCN, OPG and BMP2 were also analyzed from normal human osteoblast (NHOST) cultures. For *in vivo* studies, 8 male New Zealand white rabbits 4±0.25kg in weight were implanted with a laser sintered implant in the left femur and a control implant in the contralateral bone. Micro-computed tomography (micro-CT) was used to analyze bone-to-implant contact after 6 weeks of implantation.

Results: After production, SEM revealed LST surfaces had a rough microtopography, while A-LST surfaces also included a fine nanotopography at high magnification. Quantitative roughness (R_a) values of 3.37±0.62μm and peak-to-valley height (S_z) of 62.74±9.81μm were obtained for LST disks. LST surfaces were also hydrophilic, exhibiting contact angle of 34±7 degrees. XPS analysis showed that titanium (87.8±0.5%), aluminum (8.3±0.7%) and vanadium (3.9±0.2%) were the three most dominant elements, although calcium and phosphorous were also revealed through EDX analysis of A-LST surfaces due to the surface treatment. For MG63 cells, DNA was elevated on all surfaces compared to the M group. ALP activity was increased on LST and A-LST compared to both M and M-LST. OCN increased on all groups with increasing surface roughness. BMP2 and ITGA increased on all surfaces compared to M, and increased on LST and A-LST compared to M-LST. VEGF increased on LST and A-LST compared to the M group only. FGF2 increased on LST and A-LST compared to both M and M-LST. ITB1 increased on all surfaces compared to M, and A-LST compared to M-LST. Expression of OCN, OPG and BMP2 by NHOST cells increased in a roughness-dependent manner on M, M-LST, LST and A-LST surfaces. Micro-CT analysis of bone-to-implant contact of implants retrieved after 6 weeks of implantation revealed no significant differences between control and experimental implant groups in the superior cortical, inferior cortical or trabecular bone, although differences may have been obscured by the implant shadowing.

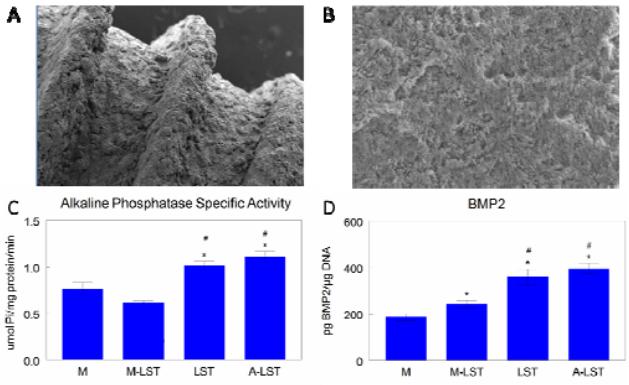

Figure 1. LST implant (A); high magnification of A-LST surfaces showing nano-roughness (B); MG63 expression of ALP activity (C); and BMP2 protein (D)

Conclusions: 3D laser sintering is a method of producing titanium alloy surfaces and implants that are inherently hydrophilic and can be modified for varying surface roughness. Surfaces with combined micro-/nano-roughness enhanced osteoblast response and have potential for increased osseointegration *in vivo*.

References:

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