Differential response of Staphylococci and osteoblasts to varying titanium surface roughness

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Statement of Purpose: Titanium-based materials have been widely used as dental and orthopaedic implant materials because of their mechanical strength, corrosion resistance, and biocompatibility. However, despite their high rate of success, biomaterials-associated infection has emerged as a leading failure mechanism associated with these and other implant materials. The surface roughness of metallic orthopaedic implants has typically been used to influence osseointegration and spatially control load transfer to the surrounding bone. However, roughened surfaces have been generally thought to enhance the accumulation of infectious bacteria, and there are few or no reports which have addressed how roughness over various length scales affects the different surface interactions with Staphylococci and osteoblasts together. Thus, the present in vitro study aims to evaluate and compare the effects of surface topography on *Staphylococcus epidermidis* (*S. epidermidis*) and human fetal osteoblast (hFOB) behavior on four clinically relevant titanium surfaces [1].

Methods: Ti alloy (ASTM F136) disks were prepared with four different surface conditions: satin, grit-blasted, mirror-polished and plasma-sprayed. These surfaces were characterized by scanning electron microscopy (SEM) and atomic force microscopy. *Staphylococcus epidermidis* (NJ9709, 4×10^5 CFU/cm^2), were seeded onto each Ti disk and then incubated for 24 h. Human fetal osteoblastic cells (hFOB 1.19, ATCC CRL-11372) were cultured in DMEM/FBS for 1, 4, 8 and 16 days. The initial adhesion and subsequent proliferation of *S. epidermidis* and osteoblasts were visualized both by SEM and by three-dimensional reconstruction of image stacks from confocal laser scanning microscopy (CLSM). In addition to morphological studies, osteoblast proliferation and phenotype were studied by measuring cell viability, the alkaline phosphatase activity and calcium expression.

Results: Morphological characterization of titanium substrates shows the four different categories of titanium samples all have distinct surface topographies occurring over different length scales.

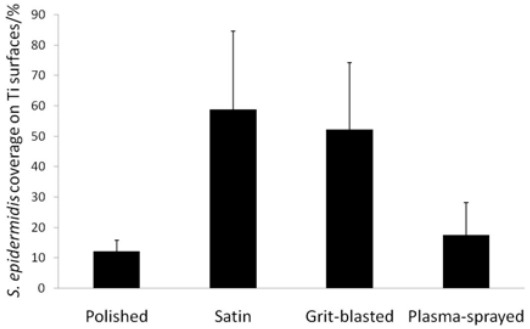

Figure 1. *S. epidermidis* colonization on Polished Ti, Satin Ti, Grit-blasted Ti and Plasma-sprayed Ti

We find that *S. epidermidis* adhesion and growth is substantially higher on the satin and grit-blasted surfaces than on the polished or plasma-sprayed surfaces (Fig. 1). The former are both substantially rougher at length scales comparable to that of bacteria. In contrast, based on imaging and biochemical assays of proliferation, differentiation and matrix formation, we find that

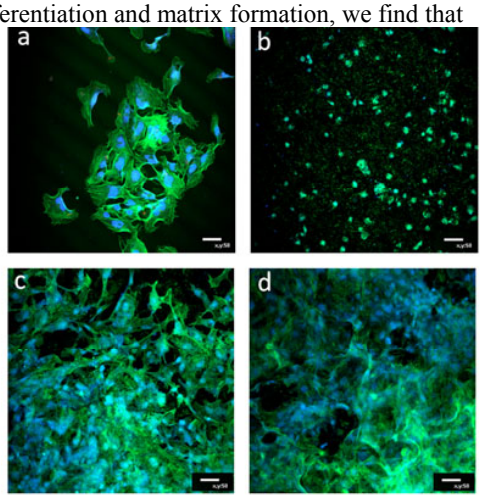

Figure 2. Perpendicular 2D projections of 3D CLSM image stacks of hFOB osteoblast cells cultured for 16 days on: (a) Polished Ti; (b) Satin Ti; (c) Grit-blasted Ti; (d) Plasma-sprayed Ti. Scale bar = 50 μm.

desirable osteoblast adhesion, spreading and differentiation are all promoted by the microscopically smooth and macroscopically rough surfaces provided by a plasma-sprayed surface (Fig. 2). Here the surface topography changes at length scales on the order of tens of microns corresponding roughly to the size of an individual tissue cell.

Conclusions: An important difference between the four Ti surfaces was the length scales characterizing roughness in both the vertical and lateral directions. Scalar roughness parameters are insufficient to adequately characterize elements of surface topography important to the differential attachment by bacteria and by osteoblasts. Foremost among these is the lateral length scale associated with the roughness and, in particular, how this lateral roughness scale compares to the dimensions of bacteria and osteoblasts. Implant surface finishes have historically been varied to control osseointegration and early fixation in different portions of an implant, and, generally speaking, our findings suggest that these varying surface topographies can be further optimized to also minimize bacterial colonization.

References: [1] Wu Y. et al. Biomaterials (2010), doi:10.1016/j.biomaterials.2010.10.001