Mineralization from Marrow Cells On Al_2O_3 Grit Blasted Ti6A14V Disks Is Greater Than SiO_2 Grit Blasted Disks

Hippensteel, E A; Vass, S A
DePuy Orthopaedics, Inc., Warsaw, IN

Statement of Purpose: Morphology and composition of the surfaces of implantable devices can directly influence the behavior of osteoblasts and in turn, expression of essential bone forming proteins. Grit blasting is a common procedure used to promote biological attachment of orthopaedic implants. At a certain pressure, particles of varying size and composition can be directed at an implant surface to create a roughened surface. Because of the pressures involved, particles of the grit blast material may become embedded in the implant, adding to the topography of the surface. The objective of this study was to investigate the effect of alumina oxide (Al_2O_3) and silicon dioxide (SiO_2) grit blasted titanium surfaces on mineralization from rat bone marrow cells. This study is aimed to determine the relevance of two methods used on orthopaedic devices on the formation of bone-like mineral *in vitro*.

Methods: Ti6A14V disks (25.4mm diameter, 3mm thick) were blasted using Al_2O_3 (Al) and SiO_2 (Si) media according to current manufacturing specifications. The grit blasted disks were characterized using a surface roughness tester (Mitutoyo) and scanning electron microscopy (SEM) linked with energy-dispersive spectroscopy (EDS). Al and Si disks were gamma sterilized and placed together in one Petri dish for each time point in order to assure the cellular response was due to the substrate texture and composition and not a difference in cell density. Both Al and Si grit blasted disks without cells were incubated in media alone to determine if mineral formation on the surfaces was cell-dependent. For bone marrow cell preparation, cells from the femora of Wistar rats were prepared according to established protocols and 20mL of the cell suspension added to each dish. Cultures were stored in a humidified incubator at 37°C and 5% CO_2 and the culture medium refreshed every 2 days. Cultures were maintained for 10, 12, 14, 16 and 18 days before fixation and staining. Von Kossa samples were stained and imaged with digital photography. Samples for surface characterization were fixed in 0.1M sodium cacodylate buffer (pH 7.4) containing 3% gluteraldehyde, serially dehydrated and dried at room temperature. After drying, samples were analyzed using diffuse reflectance Fourier transform infra-red spectroscopy (FT-IR), thin-film x-ray diffractometry (XRD), and carbon coated prior to SEM-EDS.

Results / Discussion: The roughness (Ra) of the Al grit blasted disks was 5.09±0.50 µm and Si was 3.00±0.46 µm ($p<0.001$). SEM showed similar morphologies for both grit blast materials (Fig 1). There were macro- and micro-sized ridges formed from the removal of and plastic deformation of the Ti substrate. EDS showed the presence of Ti, Al, and V for the Al grit blasted samples (Fig 1a), present in the Ti substrate. EDS for the Si disks (Fig 1b) showed Si, Na, and Ca in addition to Ti, Al and V. The control disks incubated in media alone showed no evidence of Ca or P deposits and, therefore, all mineralization occurring in this experiment was cell-mediated.

For the bone marrow cells, Von Kossa staining indicated a significant difference in the onset and degree of mineralization between the two substrate types. There were mineralized nodules across the entire surface of the Al grit blasted disks by 10 days in culture (Fig 2a). There were areas that stained pink on the SiO_2 disks (Fig 2b), but not stained black, indicating the lack of calcium and phosphate mineral on the disk at day 10. FT-IR, XRD, and EDS also confirmed the presence of CaP on the Al disks, but not on the Si disks. CaP mineral began to form on the Si disks by 18 days in culture. SEM morphological examination revealed that at earlier time points (around 10 days), cells on the Al grit blasted surface were found in clusters on the surface of the disk. Cracks in the nodules (due to high vacuum SEM) revealed mineralized collagen fibers at the lower layers of the nodules. Cells on the Si grit blasted surface were more flattened in shape and created a monolayer across the surface of the disk. At later time points (around 16-18 days), cells on the Si disks began to form CaP nodules. Studies are underway to evaluate the biochemical aspects of this study.

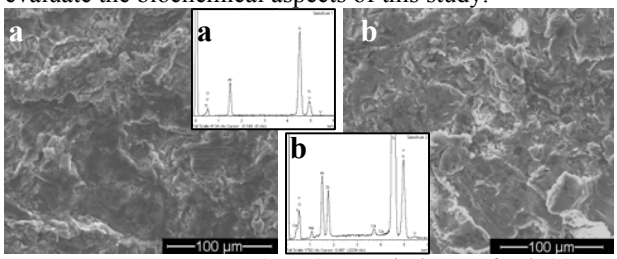

Figure 1: SEM-EDS (500x) morphology of Al (a) and Si (b) grit blasted disks prior to cell culture.

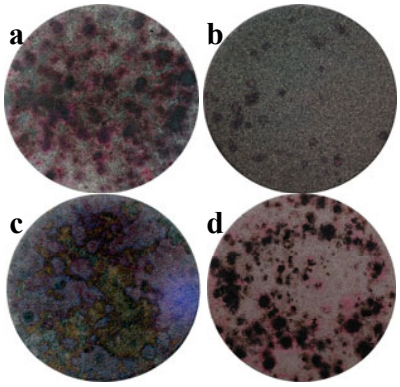

Figure 2: Von Kossa staining of Al (a) and Si (b) samples with (black) areas of mineralization after 10 days and Al (c) and Si (d) after 18 days.

Conclusions: The alumina oxide grit blasted Ti substrate is capable of enhancing better bone mineral-like formation on the surface of the disks. This may be attributed to a combination of surface roughness and resulting surface composition. Earlier mineralization may enable faster bone fixation of orthopaedic devices.