Enhanced Osteoblast Functions on Nano Rough Micron Patterned Titanium

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Statement of Purpose: Research has demonstrated increased osteoblast (bone-forming cells) functions on nanotextured titanium surfaces compared to their conventional counterparts due to the fact that the constituent components in bone (collagen and hydroxyapatite) are nanostructured. However, to better create an implant that is more biomimetic of natural bone, surface orientation also needs to be accounted for since the collagen fibrils are spontaneously aligned. Therefore, this research focused on examining osteoblast functions on nano rough micron patterned titanium surfaces created using electron beam evaporation. In addition, this study was interested in determining if the width of these aligned dimensions was a significant factor in controlling osteoblast response. Results of this study provided in vitro and in vivo evidence that the presence of nano features on titanium surfaces as created by electron beam evaporation improved osteoblast functions. In addition, osteoblast behavior varied based on the dimensions of these aligned features indicating there are optimal conditions needed to produce an environment that best supports osteoblasts growth.

Methods: To create nano rough micron patterned substrates, copper grids containing parallel features were attached to the surface and electron beam evaporation was performed. This technique involves electron beam bombardment to deposit a material, in this case titanium, onto another material, also titanium here. As the beam rotates and hits the surface of the source material, heating and vaporization occur. The vapor flow then condenses onto the substrate surface located at the top of the vacuum chamber. In this study, Ti was deposited onto the Ti substrates at a rate of 3.5 Å/s and at a thickness of 500 nm. Three types of patterned substrates were examined: 1) a nano rough region of 80 µm/micron rough region of 45 µm, 2) a nano rough region of 48 µm/micron rough region of 35 µm, and 3) a nano rough region of 22 µm/micron rough region of 30 µm. Complete nano rough substrates and standard, unmodified commercially pure titanium were also studied. Surface characterization of these surfaces included SEM, AFM, and XPS, all of which were completed to indicate that the surfaces contained both nanotextured and aligned features. Cellular proliferation studies were completed in addition to standard calcium, alkaline phosphatase, and collagen assays. Finally, in vivo data was completed by inserting electron beam modified and unmodified titanium screws in the femur of rats for up to 28 days.

Results: Surface characterization of these patterned substrates indicated a linear configuration that contained alternating regions of nano rough and micron rough features. XPS revealed that there was no chemical difference in the surface oxide layer of the nano rough and micron rough regions indicating that topography alone played a significant role in osteoblast behavior.
Proliferation studies were completed to determine overall osteoblast responses on these surfaces. These studies revealed there was an increase in total osteoblast density on these patterned substrates compared to conventional, unpatterned titanium substrates. In addition, there was a difference observed in total osteoblast density among the varying patterned substrates. More specifically, the patterned substrates with the larger nano rough region (80:45 µm) contained the greatest osteoblast density. In addition to overall osteoblast density, the osteoblast density per region was also examined. It was noted that more osteoblasts grew on the nano rough region than on the micron rough region and that among the various patterned substrates, those with the largest nano rough region (80:45 µm) contained the most osteoblasts.
Initial calcium, alkaline phosphatase, and collagen deposition studies revealed a similar trend as that of the proliferation studies in that osteoblast differentiation increased on the patterned substrates compared to the conventional, unmodified titainum.
Lastly, in vivo studies revealed there was bone ingrowth along the nano rough screws compared to the conventional, unmodified screws (Figure 1).

Conventional Screws   Nano Rough Screws

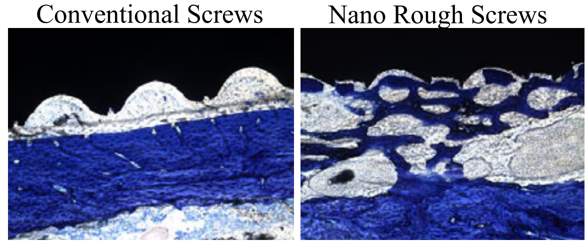

Figure 1. In vivo data of screws implanted into the femur of rats for 28 days. The conventional screws represented on the left showed no bone ingrowth whereas the nano rough pins clearly showed the presence of new bone ingrowth (represented by the blue toluene blue stain)

Conclusions: This study provided evidence that creating surfaces more biomimetic of natural bone, including both orientation and size, results in improved osteoblasts behavior and growth. More specifically, improved osteoblast function on linearly aligned nano rough micron patterned substrates was observed. This study also revealed that pattern dimensions have an effect on the long term functions of osteoblasts. Finally, this study revealed through in vivo data that the nano rough surface created using electron beam evaporation clearly promotes bone ingrowth. Therefore, the results from this study provide evidence that this technique should be further researched as a means to improve orthopedic implant longevity and efficacy.

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