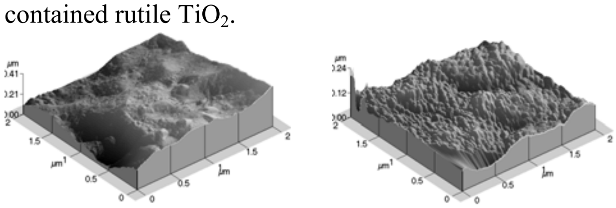
Controlling Osteoblast Function on Nano Rough Micron Patterned Titanium

Sabrina Puckett, Thomas J Webster.
Division of Engineering, Brown University, 182 Hope Street, Providence, RI 02912.

Statement of Purpose: Research to date has yet to evaluate the effect that organized nanosurface features can have on the interaction of osteoblasts with material surfaces. To determine if surface orientation of features can mediate osteoblast adhesion and morphology, this study investigated osteoblast function on patterned titanium substrates containing alternating regions of micron rough and nano rough surfaces prepared by novel electron beam evaporation techniques. The goal behind this concept is to mimic not only the dimension of constituent components in bone, including collagen and hydroxyapaptite, but also their parallel alignment. This study was also interested in determining whether or not the size of the patterned regions had an effect on osteoblast behavior and alignment. Results of this study provided evidence that aligned features on the surface of titanium improved early osteoblast functions (morphology and adhesion) promising for their long term functions, criteria necessary to improve orthopedic implant efficacy.

Methods: Micron rough samples, nano rough samples, and nano rough micron patterned samples were used throughout this study. The micron rough samples came straight from the factory and required no preparation, except for cleaning. The nano rough and patterned substrates were created using electron beam evaporation. The patterned substrates possessed a linear configuration of alternating micron rough and nano rough features by attaching copper grids onto the Ti substrate before being placed into the electron beam evaporator. The nano rough substrates contained no grid and resulted in the entire surface being altered by electron beam evaporation. The surfaces were characterized by SEM, AFM, and XRD and cell adhesion studies were completed on all the above substrates mentioned above.

Results: The topographical differences among the varying substrates used in this study were demonstrated by SEM and AFM (Figure 1). More specifically, the patterened substrates contained modified regions that clearly displayed nanoscale features and unmodified regions that maintained micron rough features. These surfaces created by this deposition process possessed not only a different topography but also a different crystallinity when compared to the conventional surfaces that have not been altered by deposition. More specifically, the nano rough regions of the patterned substrates contained anatase TiO_2 whereas the micron rough regions lacked the presence of anatase TiO_2 and contained rutile TiO_2.



(a) Micron Rough Region (b) Nano Rough Region

Figure 1. AFM images representing the topographical differences of the unmodified, micron rough region (a) and the modified, nano rough region (b) on the patterned Ti substrates.

This study provided evidence that creating Ti surfaces with linearly aligned patterns has an effect on osteoblasts. First, there was an increase in total osteoblast adhesion on the entire nano rough Ti surfaces. Second, this study further provided evidence that creating aligned patterns of alternating nano rough and micron rough regions resulted in the initial alignment of osteoblasts on these surfaces.

Third, this study provided evidence of greater osteoblast adhesion on the modified nano rough region of these patterned Ti substrates. Directed osteoblast adhesion on the nano rough Ti regions of the patterned substrates may be explained by the fact that they contained greater particle boundaries at the surface due to smaller particle size. The fact that osteoblasts were adhering more to the modified, nano rough regions indicates that they favor surfaces that are more like that of natural bone (nano rough), and as a result, better conditions are being derived to create optimal interactions between the device and its surroundings as well as with proteins[1,2].

Lastly, this study was interested in determining if early functions of osteoblasts were affected by the size of the aligned patterns. Results suggested that there is some optimal pattern dimension of nano features to which osteoblasts prefer to function. In other words, a threshold exists in which osteoblasts do not attach and spread properly. More specifically, data from this study revealed that decreasing the width of the nano rough region on these patterned substrates results in decreased osteoblast adhesion on nano rough regions.

Conclusions: The initial conclusions from this study indicated that creating patterns on Ti substrates has an effect on the way osteoblast respond. More specifically, evidence of improved osteoblast adhesion and morphology on Ti substrates with linearly aligned nano rough features was observed. Furthermore, the dimensions of these patterns can affect the alignment and adhesion of osteoblasts on these surfaces resulting in their overall survival and growth. Thus, this study demonstrates that the formation of patterns containing micron and nano features as prepared by electron beam evaporation on titanium should be further studied to increase the longevity of orthopedic implants. This technique provides a way to mimic the size and orientation of collagen and hydroxyapatite in long bones thus explaining the initial results in the study.

References: (1)TJ Webster, C Ergun, RH Doremus, et al, “Specific proteins mediate enhanced osteoblase adhesion on nanophase ceramics,” *J Biomed Mater Res*, vol. 51, 2000, pp. 475-83. (2) Y Yang, R Glover, JL Ong, “Fibronectin adsorption on titanium surfaces and its effect on osteoblast precursor cell attachment,” *Col Surf B: Biointerfs*, vol. 30, 2003, pp. 291-97.