nanoVIS Ti Surface Technology™ Increases Mineralization of Extracellular Matrix Produced by Osteoblasts and Mesenchymal Stem Cells

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Introduction

Dental implants, spinal fusion cages, pedicle screws and joint replacement implants all have a common need: to integrate quickly into bone1-3. To better achieve these goals, implant manufacturers have designed new surface technologies that aim to promote more efficient bone growth around the implants. These technologies could lead to better clinical outcomes with faster stabilization, stronger fixation, and lower risk of complications such as migration or subsidence.

The surface of a device, particularly at the nanoscale, directs protein and cell attachment to drive stem cell differentiation4-6. The nanoscale surface can be designed to enhance the ability to attract proteins from the blood and direct cell differentiation toward the osteoblastic lineage7-11. Mesenchymal stem cells (MSCs) are present in the bone ready to respond to injury and repair needs. Once activated, MSCs proliferate and differentiate into pre-osteoblasts given the appropriate signals.

Osteoblasts are the cells that actively lay down new bone during remodeling and healing processes. Vascular on growth is followed by deposition of the extracellular matrix that will then become mineralized into woven bone. The mineralization assay presented here demonstrates that the surface nanostructure can have a positive biological effect on matrix production and mineralization in vitro12,13.

This white paper compared the mineralization results of osteoblasts and mesenchymal stem cells on four different surfaces after 21 days. The four surfaces were (a) Control Ti64 ELI, (b) Nanorough acid etched, (c) Control CaP, and (d) nanoVis Ti Surface Technology™, as shown in Figure 1. The purpose of this white paper is to summarize the data that Nanovis has submitted to the FDA to demonstrate improved in vitro mineralization of the nanoVIS Ti Surface Technology™.

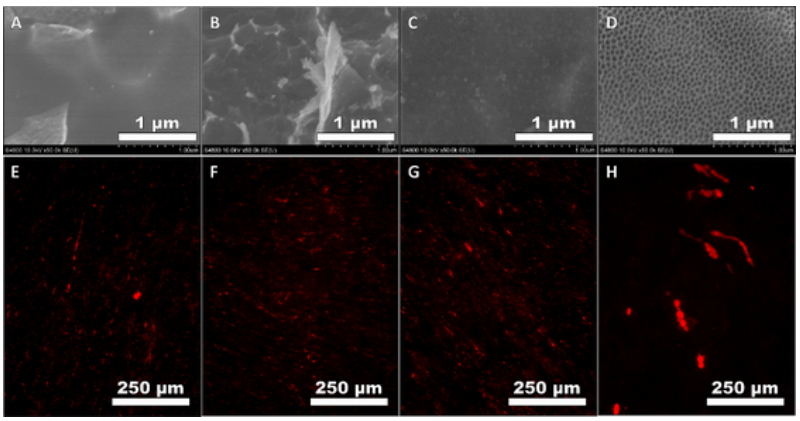

Figure 1 –SEM images of surfaces (A, B,C, D) and Alizarin Red staining of osteoblast differentiation cultures after 21 Days (E, F, G, H); (A, E) Control Ti64 ELI, (B, F) Nanorough acid etched, (C, G) Control CaP, (D, H) nanoVIS Ti Surface Technology™.

Methods

For calcium mineralization, primary human osteoblasts and MSCs were cultured on each surface for 21 days. At day 21, cells were fixed and stained with Alizarin red, which binds to calcium. The samples were then imaged with a fluorescent microscope and subsequently processed to extract and quantify total calcium content with photo spectroscopy. The total calcium value was then analyzed with 2-way ANOVA to determine statistical significance.

Results

The fluorescent imaging for Alizarin Red staining shows that calcium mineralization was present on every surface, however they were significantly larger on the nanoVIS Ti Surface Technology™ (Figure 1H). The quantified extracts show that nanoVIS Ti surface had the highest level of mineralization for all test groups in Figure 2. There was no statistically significant difference between any of the other test groups. The mineralized nodules are approximately 5-10x larger on the nanoVIS Ti Surface Technology™.

Conclusion

The results of these studies show that the nanoVIS Ti Surface Technology™ is beneficial for increasing calcium mineralization of extracellular matrix in vitro. Mineralization is an important part of bone formation, and surfaces that promote mineralization should be beneficial for creating stronger new bones in a healing environment. The data presented in this white paper was reviewed by the FDA, who awarded the technology with a "nanotechnology designation" and the label language "increases and accelerates calcified extracellular matrix production in vitro". NanoVIS Ti Surface Technology™ shows incredible promise for use in spine, dental and orthopedic applications.

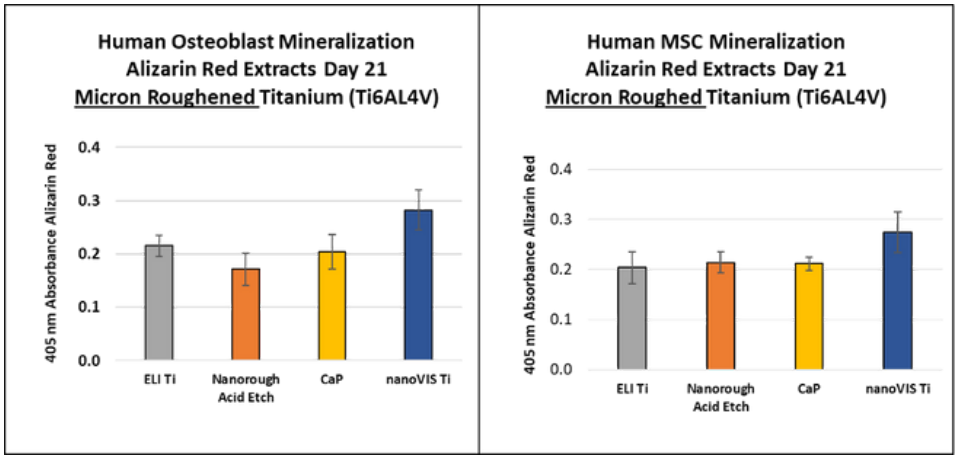

Figure 2 - 21 Day Mineralization assay by Alizarin Red staining and extraction with human osteoblasts and human mesenchymal stem cells on micron roughened titanium alloy (Ti6Al4V).

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