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Volume 14 V2

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Background

Metal implant surfaces were not originally designed to integrate with the body's biology but were there to fill a structural roll to restore orthopedic function of the musculoskeletal system. Over time, stronger and tougher materials such as cobalt chrome, stainless steel, and titanium, were used in implants to prevent device failures on the structural side. The biologic side was not developed until recently with nanoscale hydroxyapatite coatings, submicron roughening techniques, and acid etching, to gain access to more surface area and to achieve a better biologic response[1-4]. What these surfaces lacked was tunability of the nanoscale features that can provide the body with a precise biological signal that interacts with the immune system, vasculature, stem cells and ultimately osteoblasts to make more high-quality bone at a faster rate[5-9].

Anodized titanium nanotubes, such as the nanoVIS Ti™ Surface Technology, provide this precise tunability in nanofeature size control. It has been demonstrated by others that the 60-100 nm range is optimal for mesenchymal stem cell differentiation, osteoblast function, vascular ongrowth, and better bone-to-implant contact[7-10]. Nanovis has commercialized this technology with multiple FDA clearances, 20,000+ implants and produced data to support FDA Nanotechnology Designations. This paper demonstrates earlier differentiation of human osteoblasts on the nanoVIS Ti™ surface compared with the plain titanium surface.

Methods

Grade 5 commercially pure titanium (CP Ti) coupons were anodized to have nanotubes with an average diameter of 70 nm using the Nanovis' patented anodization process. The surfaces of the coupons were examined with an atomic force microscope (AFM) to determine the roughness of the surface at three different scales: 25 × 25 µm, 5 × 5 µm, and 1 × 1 µm. Surface roughness is presented as root mean square (RMS) roughness. Human osteoblasts were cultured on the coupons for 1, 2, 3, and 4 weeks to quantify the presence of alkaline phosphatase (ALP) activity, collagen (Col) production, and calcium (Ca) mineralization. These measurements were performed using an alkaline phosphatase assay kit, Sirius Red staining of collagen, and a calcium assay kit, respectively.

Results

The atomic force microscope was able to measure the surface roughness at three different settings, as listed in Table 1. The anodized nanotube surface (CP-A) exhibited a higher RMS roughness than the control (CP-N) at the 1 × 1 µm scan but showed a similar RMS roughness to the control at the 25 × 25 µm scan. This suggests that the nanotubes influence nano-roughness but not micro-roughness.

Table 1: AFM Scans for anodized nanotube surface (CP-A) and non-treated control surface (CP-N). The asterisk mark (*) indicates significant differences based on a Student's T-test, p-value < 0.05.

AFM Scan	Anodized Nanotubes (CP-A) RMS Roughness (nm)	Non-Treated Control (CP-N) RMS Roughness (nm)
25 x 25 µm	1.6 ± 0.6	1.7 ± 0.4
5 x 5 µm	12.4 ± 0.6	11.3 ± 0.5
1 x 1 µm	25.5 ± 3.6*	14.7 ± 2.1

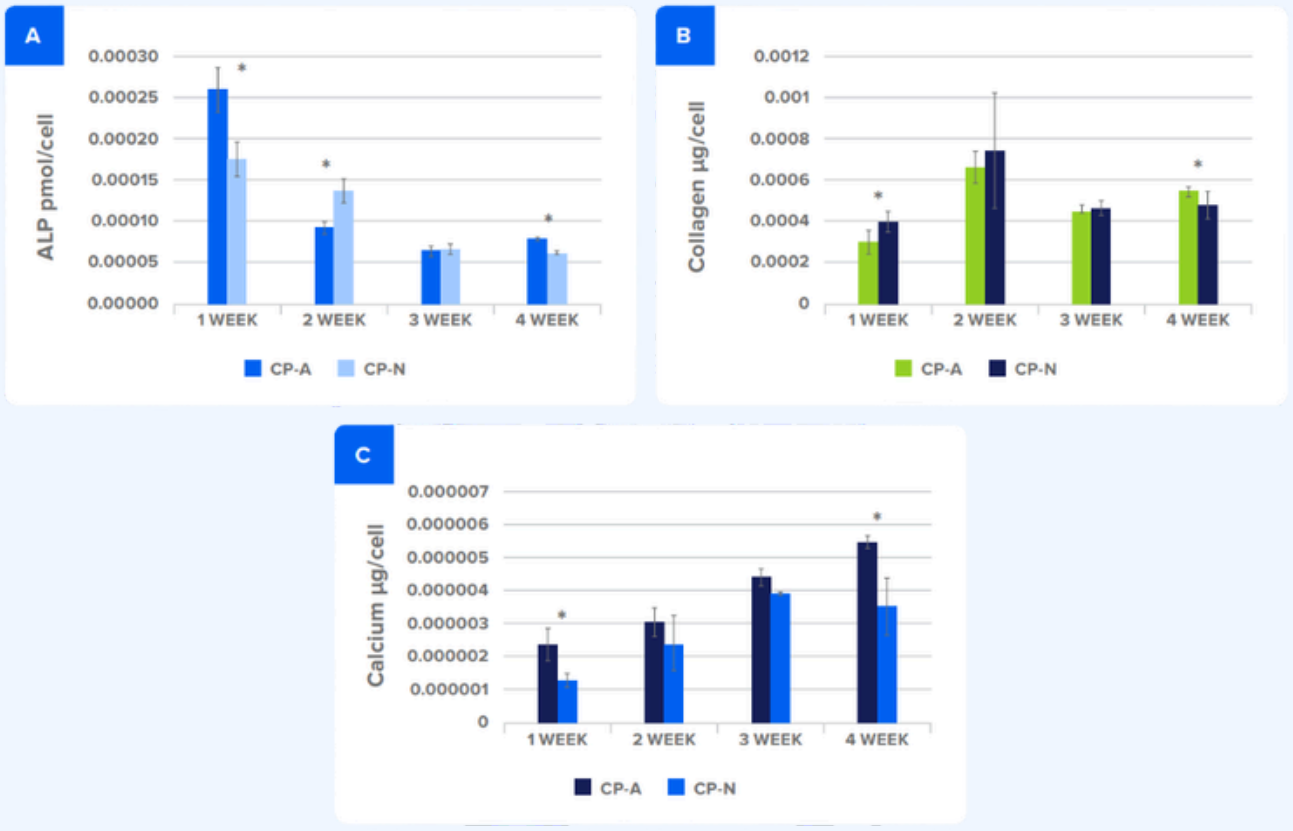

Figure 1: A) Alkaline phosphatase activity, B) intracellular collagen production, C) calcium deposition from human osteoblast cultures at 1, 2, 3, and 4 weeks of culture. All results are presented on a per cell basis, with each measured value normalized to the corresponding number of cells. CP-A: anodized nanotube surface; CP-N: non-treated control surface. The asterisk mark (*) Indicates significant differences based on a Student's T-test, p-value<0.05.

Discussion

The scale of things matters. Micron roughness matters to things that are of similar size, like cells and tissues. But cells interact with surfaces through proteins, which are considerably smaller than cells, a few nanometers to tens of nanometers in size. For that size, nanofeatures size, shape, and distribution become very important cues to allow the implant surface to signal the cells[1-5,8]. The roughness of the nanotube surface can only be appreciated if the probe of the microscope is interrogating the surface below a micron. Table 1 shows that, at the submicron scale of 1 x 1 µm scan, the roughness of the anodized nanotubes became apparent and was significantly higher than the control surface. It is the nanoscale roughness that provides the signals responsible for the different biological responses when cells interact with the nanosurface.

Alkaline phosphatase (ALP) expression is an indication of the change of state of a cell[11]. In this study, higher expression was noted on the nanotube surface when cells underwent differentiation from proliferating pre-osteoblasts to differentiated osteoblasts responsible for matrix production[8,11]. The 70 nm nanotube surface showed increased early ALP expression after 1 week, indicating a rapid and unified differentiation of the osteoblast cultures from a pre-osteoblastic state to a differentiated state, as shown in Figure 1A. Intracellular collagen production was similar on the control and nanotube surfaces with a peak after 2-weeks of culture, as shown in Figure 1B. This does not mean that there was more or less extracellular matrix, but rather that the rate of cellular collagen production was similar. Calcium deposition in the extracellular matrix produced by osteoblasts was significantly higher on the nanotube surface than on the control surface after 1-, and 4-weeks of culture, as shown in Figure 1C. The 70 nm nanotube surface demonstrated an ability to accumulate more calcium in the matrix after 1 week and over time. These results indicate earlier differentiation and greater function of osteoblasts on the 70 nm nanotube surface compared with the control titanium surface.

Conclusion

The nanoVIS Ti™ surface that is commercially available from Nanovis can provide a permanent nano-scale roughness on the surface of a titanium implant. This nano-scale roughness resulted in statistically significant increases in ALP expression and calcium deposition compared with the control surface after 1 week of culture, indicating earlier osteoblast differentiation and greater osteoblast function. The nanoVIS Ti™ surface can accelerate human osteoblast differentiation on commercially pure titanium.

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