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Accelerated Human Osteoblast Differentiation on CP Titanium

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Volume 14

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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].

Nanotubes anodized on titanium, 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 the data to support FDA Nanotechnology Designations. Data presented here demonstrates the added biological signal that the nanoroughness of nanoVIS Ti™ Surface Technology at 70 nm provides to human osteoblasts to encourage early differentiation.

Methods

Sample coupons of commercially pure titanium, Grade 5, (CP Ti) were anodized to have nanotubes that were on average 70 nm in diameter with 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: 25x25 microns, 5x5 microns, and 1x1 micron. Roughness is presented as Root Mean Square Roughness (RMS). Human osteoblasts were cultured on the surface of 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.

Table 1: AFM Scans for Nanotube Anodized (CP-A) and Control (CP-N) samples. 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

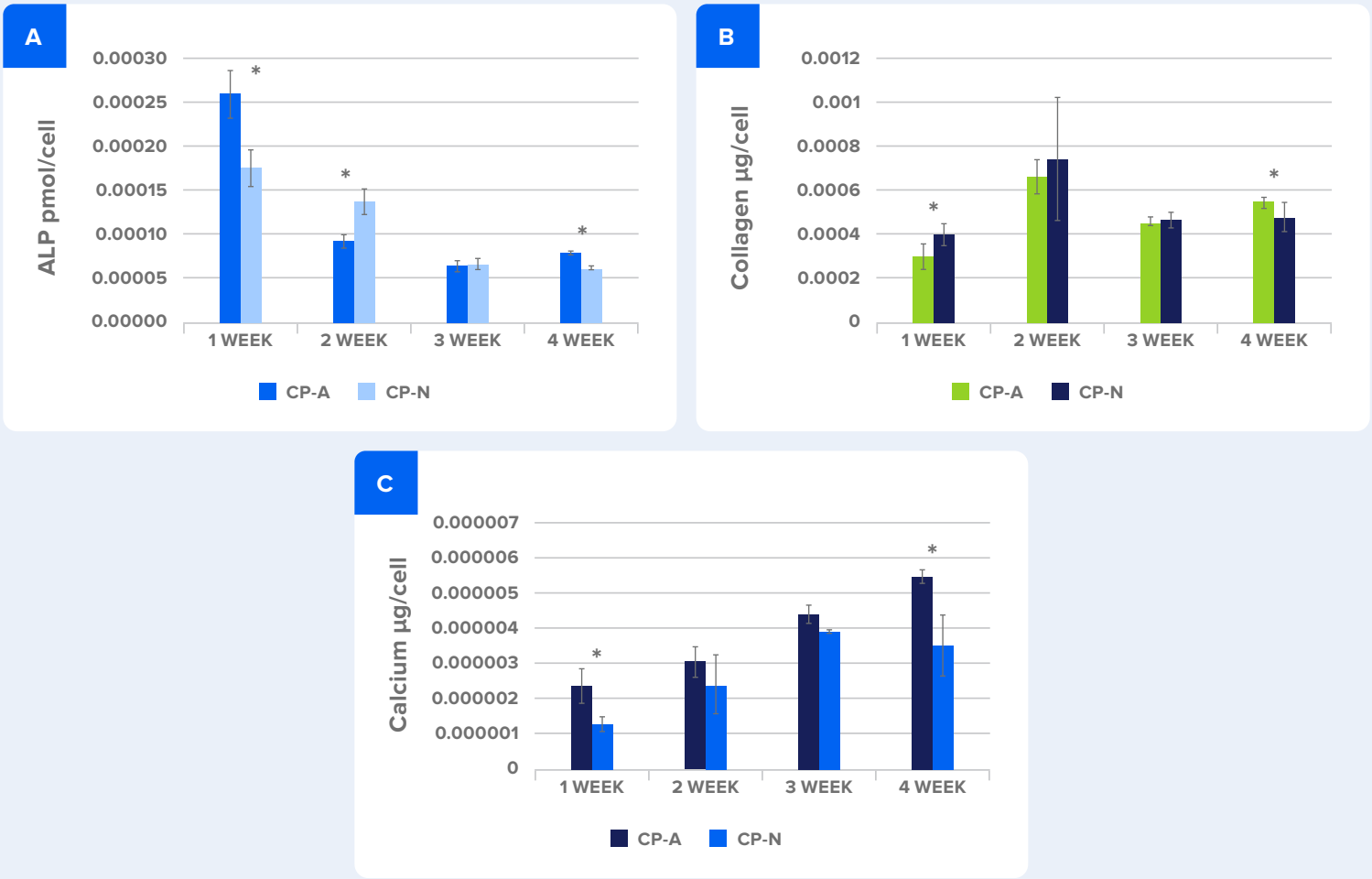

Figure 1 - A) Alkaline phosphatase activity, B) Intracellular collagen production, C) Calcium deposition from human osteoblast cultures at 1, 2, 3, and 4 weeks of culture. All results are presented on a per cell basis. CP-A = anodized nanotubes CP Ti; CP-N control surface non-treated CP Ti. The asterisk mark (*) Indicates significant differences based on a Student's T-test, p-value<0.05.

Results

The atomic force microscope was able to measure the surface roughness at three different settings, Table 1. The nanotube surface exhibits a higher RMS than the control at the 1 × 1 µm scan but is similar to the control at the 25 × 25 µm scan. This suggests that the nanotubes influence nano-roughness but not micro-roughness. Alkaline phosphatase activity, Figure 1A, was significantly higher on anodized samples (CP-A) after the first week and then dropped off. Control samples (CP-N) were also the highest after the first week and maintained most of that expression after the second week before dropping off. Intracellular collagen production, Figure 1B, was indistinguishable between nanotube and control surfaces at all time points. Both surfaces had the highest collagen production after 2 weeks of culture. Calcium deposition, Figure 1C, into the extracellular matrix produced by osteoblasts increased over time for both surfaces. Anodized nanotube surfaces showed significantly higher calcium deposition after 1- and 4-weeks of cell culture.

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. At the submicron scale, the roughness of the anodized nanotubes becomes apparent and is significantly different than the control surface. The nanoscale roughness is what provides the signal and accounts for the differing biological response that cells have when in contact with the nanosurface.

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

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 provides an additional biological signal, driving stem cell and osteoblast differentiation, reducing pro-inflammatory signals, and encouraging rapid vascular on growth that results in better bone implant contact to enable patients to have an implant with high stability over the long term[5,7,8,10].

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