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Nanotube Diameter Influences **MSC Osteogenic Differentiation**

Authors:

David Detwiler, PhD
Sabrina Huang, PhD
Alan Kraft, BSNE
Kreigh Williams, BS

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Introduction

This paper looks at the publication by Oh et al. and the influence of nanofeature size on the differentiation of mesenchymal stem cells (MSCs) down the osteogenic pathway[1]. When mesenchymal stem cells differentiate, they express genes and proteins specific to the cell type they are becoming. When the stem cells become osteoblasts, those cells can build bone. Nanosurfaces are becoming popular in the orthopedic, spine and dental markets for their abilities to direct differentiation of stem cells, recruit vasculature, improve bone-to-implant contact, and speed fixation. Nanofeatures can be difficult to tune to specific size ranges. Acid etching provides a broad range of feature sizes while anodization of titanium creates nanotube structures with tightly controlled feature sizes. This tight control of feature sizes allows for the more specific signal from biological properties of nanofeatures to be distinguishable from the noise. This signal allows multiple cell types to be more specifically directed. The tunability of the titanium nanotube anodization process allows for more precise optimization of biological outcomes.

Methods

Oh et al. anodized titanium foil to create nanotube sizes at 30, 50, 70, and 100 nm. Human MSCs were cultured on control titanium and nanotube samples for collection of messenger ribonucleic acid (mRNA) after 14 days. The cultures on titanium and nanotubes did not use any osteogenic induction chemical additives. A positive control cultured MSCs on tissue culture plates without nanofeatures were used with the addition of chemical osteogenic inducers. Semi-quantitative polymerase chain reaction (PCR) was done to visualize gene expression for alkaline phosphate (ALP), osteocalcin (OCN) and osteopontin (OPN), genetic markers of osteogenic differentiation. The positive control was used to define the expression level when chemicals were used to induce differentiation. The samples used without chemical inducers were shown as a relative expression to the induced control.

Results

The team found that the nanotube size plays a critical role in protein adsorption and distribution that alters how cells attach, changing their shape. Smaller diameter nanotubes had more protein attachment while larger diameter had less protein and wider spacing between proteins. This led small diameter tubes to encourage higher levels of cell attachment and proliferation. Larger diameter tubes led to cell elongation and differentiation down the osteogenic lineage. ALP, OCN and OPN all had dramatic increases between 50 and 70 nm nanotube sizes. ALP and OCN had mRNA expression of 75-80% of the chemically induced positive control. OPN also increased between 50 and 70 nm structures but the increase was much less than the positive control.

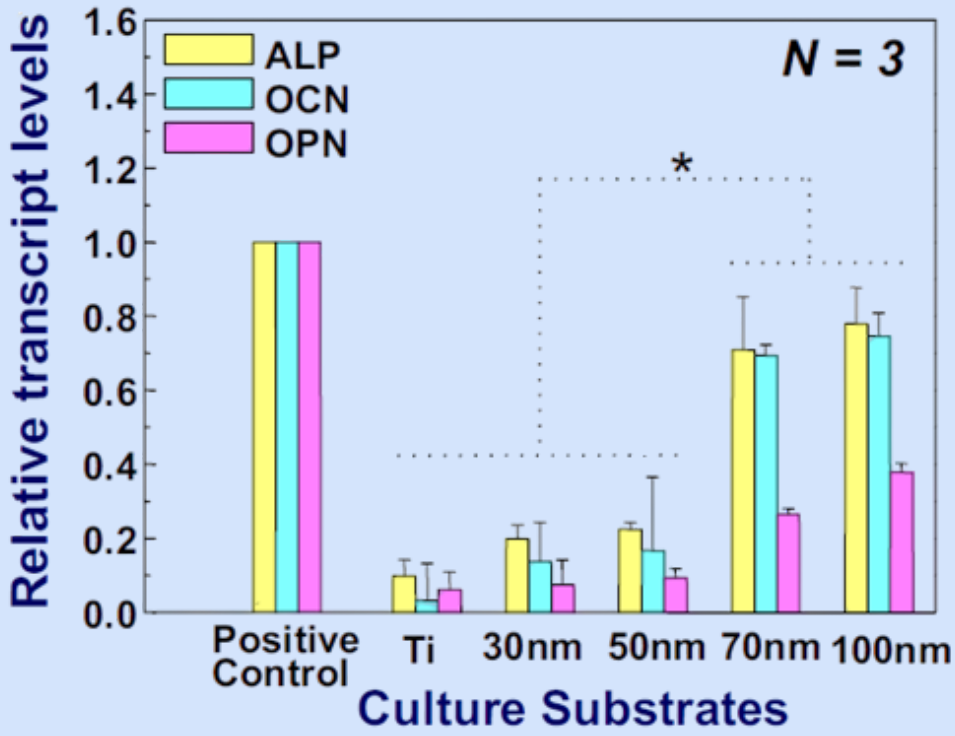

Figure 1: Quantitative PCR analysis for ALP, OCN, and OPN. The plastic cell culture plate with osteogenic-inducing media was used as a positive control for osteogenic differentiation. Asterisk mark (*) represents significant differences between Ti, 30- and 50-nm nanotubes vs. 70- and 100-nm nanotubes for ALP, OCN, and OPN gene expressions ($P < 0.01$). This figure was extracted from Oh et al.

Discussion

Oh et al. showed that no chemical inducers are needed to differentiate MSCs. They showed that the size of the nanotube has a dramatic ability to induce MSC differentiation with the size of the nanofeature alone. They also demonstrated that the nanosurface provides a signal that allows the transition from minimal differentiation of MSCs to a strong signal for differentiation to occur across a narrow size range (Fig. 1.). This can be thought of as tuning a radio dial. If the output is between 90 and 100 on the radio, all the signals in that range come out as static noise. Maybe there is a signal that can overcome the noise, but it might be combined with another signal instructing cells to do opposing things, proliferate or differentiate. But, if the radio can be tuned to a specific “station”, the cells can receive the desired signal clearly. Acid etched surfaces provide a large surface area with a broad range of surface features. Nanotubes provide a large surface area but can be tuned to a narrow size range to provide that clear desired signal. Oh et al. also showed that cells are making a choice between adhesion and differentiation based on the nanotube adhesion of proteins and the spacing of those proteins (Fig. 2). Nanofeatures that maximize protein adsorption maximize cell adhesion while nanofeatures that create more space between protein cause cell elongation and differentiation. The host bone has lots of activated MSCs around the implant. The implant doesn’t need to allow more cells to grow, the implant needs to be engineered to encourage differentiation of the available resources to make more bone around the implant.

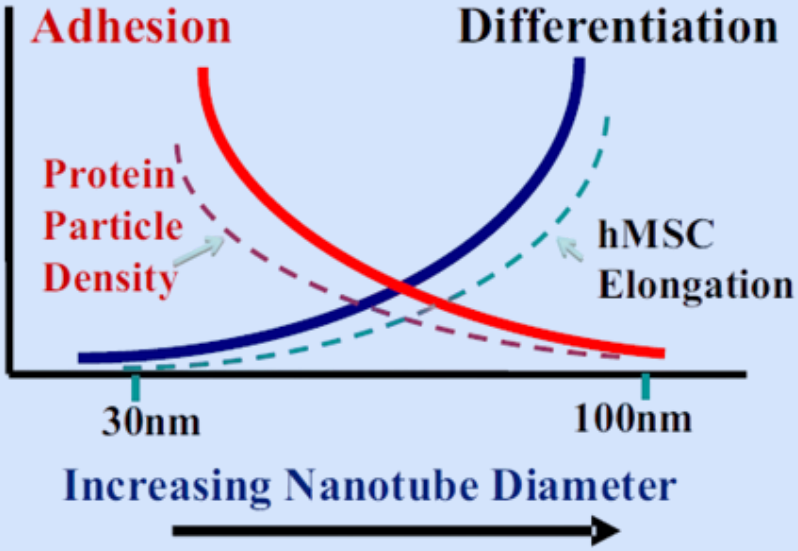

Figure 2: Schematic illustration of the overall trends of nano cue effects on hMSC fate and morphology after a 24-h culture. The change in hMSC cell adhesion and growth without differentiation (solid red line) has the same trend as protein particle density (broken red line), whereas that of differentiation (solid blue line) has the same trend as hMSC elongation (broken blue line). This figure was extracted from Oh et al.

Conclusion

Nanofeatures increase surface area and protein binding as a general feature. This publication demonstrates that more surface area is not always better. In this case, precision control over nanotube size that spreads out protein attachment sites for cells encourages elongated cell shapes that promote differentiation of the MSCs. Acid etched surfaces provide a nanosurface with a wide range of nanofeatures that are better than flat titanium but don't provide a clear signal for cell differentiation. Anodized titanium nanotube surfaces provide a tunable nanofeature size that can be optimized for bone cell differentiation. An implant that can provide the body with the signal to make bone on its own surface has a better chance of integrating into bone, creating a more stable and durable interface for the patient.

References

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