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Nanotube Diameter Influences **MSC Osteogenic Differentiation**

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Background

This paper provides a general overview of the study by Oh et al., which investigated the influence of nanotube diameter on the osteogenic differentiation of mesenchymal stem cells (MSCs)[1]. Osteogenic differentiation is regulated by a combination of physical, biochemical and mechanical signals from the surrounding environment[2-6]. Typical chemical inducers such as dexamethasone can provide a stronger signal than the nano-featured surfaces. By eliminating chemical inducers from the media, this paper demonstrated that MSCs can differentiate into osteoblasts solely in response to physical signals provided by nano-scale surface features.

Methods

Titanium foils were anodized to create nanotube sizes at 30, 50, 70, and 100 nm. Human MSCs were cultured on control titanium and nanotube samples for ribonucleic acid (mRNA) collection 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), which are 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

Smaller diameter nanotubes had more protein attachment while larger diameter had less protein and wider spacing between proteins. These small diameter tubes with high surface area encouraged high levels of cell attachment and proliferation, whereas large diameter tubes led to cell elongation and differentiation down the osteogenic lineage. Quantitative PCR analysis demonstrated that the expression of ALP, OCN and OPN increased dramatically on nanotubes with diameters between 50 and 70 nm, as shown in Figure 1. Within the nanotube diameter range of 70 and 100 nm, 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. As nanotube diameter increased, MSC's phenotype transits from adhesion to differentiation, as shown in Figure 2. The results showed that the nanotube size plays a critical role in protein adsorption and distribution that alters how cells attach, proliferate, and differentiate.

Discussion

Oh et al. demonstrated that chemical inducers are not required for osteogenic differentiation of MSCs. Instead, the diameter of the titanium nanotubes alone provided sufficient physical signals to induce MSC differentiation toward the osteogenic lineage. They also demonstrated that the nanosurface enables the transition from MSCs adhesion, with minimal differentiation, to strong osteogenic differentiation over a narrow range of nanotube diameter. This concept can be thought of as tuning a radio dial. A signal may be present and overcome the noise, but it may also be competing with another signal instructing cells to perform opposing functions, such as proliferation or differentiation. If the radio can be tuned to a specific "station", the cells can receive the desired signal clearly.

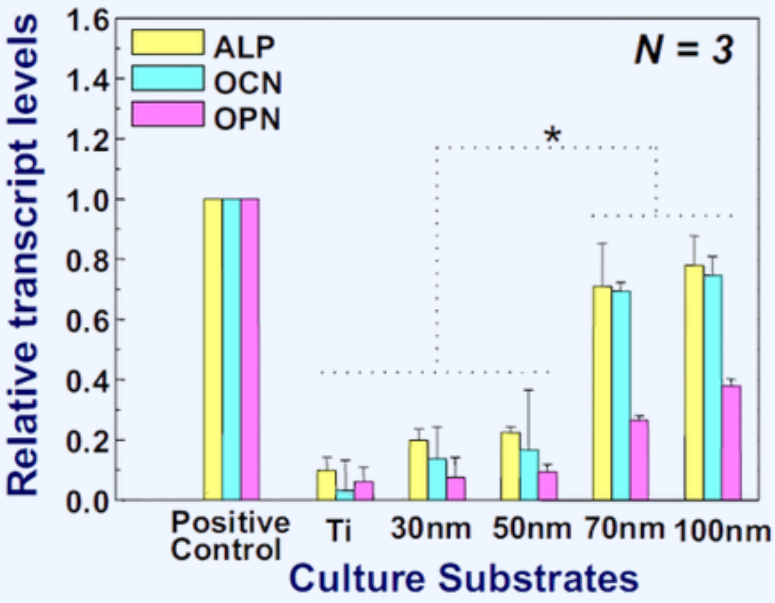

Figure 1: Quantitative PCR analysis for ALP, OCN, and OPN. The plastic cell culture plate with osteogenic-inducing media was used as a positive control for osteogenic differentiation. Asterisk mark (*) represents significant differences between Ti, 30- and 50-nm nanotubes vs. 70- and 100-nm nanotubes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

Conventional surface treatments, such as acid etching, provide a broad range of feature sizes, while titanium anodization creates uniform nanotube structures with tightly controlled diameters. This tight control of nanotube diameters provides more specific mechanotransduction signal (mechanical stimulus that alters gene expression) that directs osteoblast differentiation. The tunability of the titanium nanotube diameters also allows for more precise optimization of biological outcomes.

The paper demonstrated that a specific range of engineered titanium nanotube diameters can promote the osteogenic differentiation of MSCs without the need for osteogenic inducing media. Nanofeatures that maximize protein adsorption maximize cell adhesion, while nanofeatures that create greater spacing between adsorbed proteins promote cell elongation and differentiation. When an orthopedic implant is placed, the surrounding host bone contains many activated MSCs. Rather than promoting MSC proliferation on the host bone, implant surfaces should be engineered to encourage differentiation of these available cells and support new bone formation around the implant.

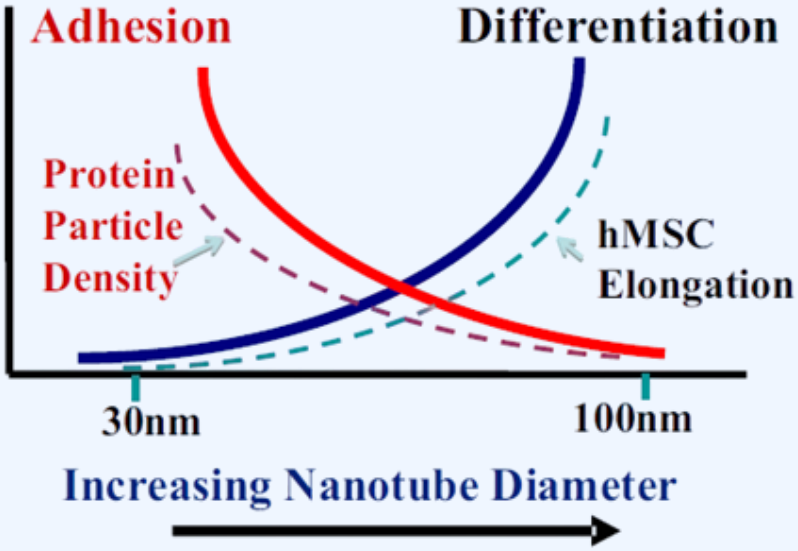

Figure 2: Schematic illustration of the overall trends of nano cue effects on hMSC fate and morphology after a 24-h culture. The change in hMSC cell adhesion and growth without differentiation (solid red line) has the same trend as protein particle density (broken red line), whereas that of differentiation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

Conclusion

Nanofeatured surfaces generally increase surface area and protein binding. However, this paper demonstrated that more surface area is not always better. Precise control over nanotube diameter provided appropriately spaced protein attachment sites that promoted cell elongation and MSCs differentiation. Acid etched surfaces provide nanosurface with a wide range of nanofeatures that perform better than flat titanium but do not provide a clear signal for cell differentiation. Anodized titanium nanotube surfaces provide tunable nanotube diameters that can be optimized to promote bone cell differentiation. An implant that provides the body with a signal to make bone on its surface has a greater chance of integrating with bone, creating a more stable and durable interface for the patient.

References

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