Effect of Titanium Nanotopography on Mesenchymal Stem Cell Fate

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Statement of Purpose: Nanotechnology is a powerful tool to modulate the osteoblast responses to titanium (Ti) implant surfaces, which directly affect the process of osseointegration. Our group has observed the positive effect of a nanostructured Ti surface (Ti-nano) on the osteoblast phenotype expression in primary osteogenic cultures.[1] It has also been shown that nanostructured Ti surfaces themselves affect osteoblast phenotype expression of mesenchymal stem cells (MSCs) and the participation of integrins in the osteoblast differentiation of cells grown on biomaterials.[2,3] Based on these findings, here we investigated whether Ti-nano could potentiate the MSCs differentiation toward an osteoblast lineage in the absence of osteoinductive factors, and the possible involvement of integrins in this process.

Methods: Discs of commercially pure grade 2 Ti, with 12 mm in diameter and 1.5 mm thick, were polished using 320 and 600 grit silicon carbide. Samples were treated with a blend of 10 N H_2SO_4 and 30% aqueous H_2O_2 (1:1 v/v) for 4 hours at room temperature to produce the surface nanotopography. Ti-nano and untreated (Ti-control) Ti discs were rinsed with deionized H_2O, autoclaved and air-dried. Bone marrow was obtained from femora of 5-week male Wistar rats (120-150 g) under the regulation of the Committee of Ethics in Animal Research of the University of Sao Paulo and plated into culture flasks containing growth media i.e., alpha-minimum essential medium (α-MEM – Invitrogen-Life Technologies, Grand Island, NY) supplemented with 10% fetal calf serum (Gibco-Life Technologies), 50 μg/ml gentamycin (Gibco-Life Technologies) and 0.3 μg/ml fungisone (Gibco-Life Technologies). MSCs were selected by adherence to polystyrene and expanded in the same media until subconfluence. First passaged cells were cultured in 24-well culture plates on Ti-nano and Ti-control discs at a cell density of 2×10^4 cells/disc in growth media, for 10 days. Cultures were kept at 37ºC in a humidified atmosphere of 5% CO_2 and 95% air; the medium was changed 3 times a week. SYBR Green-based quantitative real-time PCR was carried out to evaluate the gene expression of runt-related transcription factor 2 (Runx2), a master regulator of osteoblast differentiation and α1, α5 and β1 integrins. The relative gene expression was normalized to GAPDH. The assays were carried out in triplicates (n=3) and data of Ti-nano were compared with Ti-control by Mann-Withney U-test with the level of significance set at $p\leq0.05$.

Results: At the nanoscale, Ti-control showed a surface with grooves while Ti-nano exhibited a network of nanopits (Figure 1). A higher gene expression of Runx2 ($p=0.001$), α1 integrin ($p=0.001$) and β1 integrin ($p=0.001$) was noticed in MSCs grown on Ti-nano while α5 integrin gene expression was lower ($p=0.001$) in MSCs cultured on Ti-nano compared with Ti-control (Figure 2).

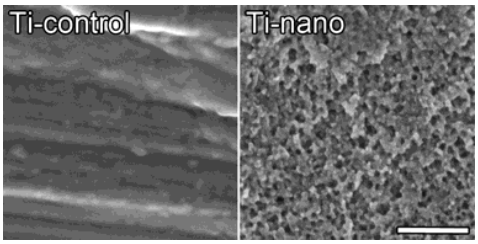

Figure 1. High resolution scanning electron micrographs of Ti-control and Ti-nano surfaces. Ti-control presents a surface with grooves while Ti-nano exhibits a network of nanopits. Scale bar = 200 nm.

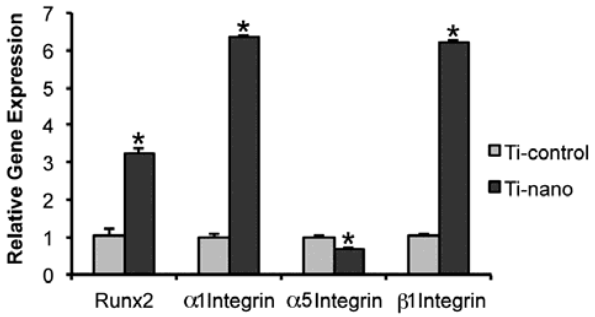

Figure 2. Gene expression of Runx2, α1, α5 and β1 integrins of MSCs cultured on Ti-control and Ti-nano surfaces in a non-osteogenic milieu for 10 days. The data are presented as mean ± standard deviation (n=3).
* indicates statistically significant difference ($p\leq0.05$).

Conclusions: We have shown that the Ti-nano surface drives the MSCs differentiation toward the osteoblast lineage by itself as indicated by the higher Runx2 gene expression. Furthermore, our results suggest that the integrin signaling pathway is, at least in part, involved in this osteogenic effect, since we noticed higher expression of α1 and β1 integrins in MSCs cultured on Ti-nano compared with Ti-control.

References

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