Pore Diameter Size Affects Osteoblastic Cell Responses to Porous Titanium Surfaces

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Statement of Purpose: Titanium (Ti) implants have been produced in dense forms and some limitations such as interfacial instability with host tissues and lack of biological anchorage for tissue ingrowths may be associated with such design.[1] In this context, porous implants could be an alternative to provide biological anchorage for the bone through the ingrowths of mineralized tissue into the pores. In a previous work, we demonstrated that porous Ti has a positive effect on the culture growth and the cell cycle activity, resulting in a delay in the expression of the osteoblastic phenotype.[2] Furthermore, there is evidence that pore diameter size has influence on osteoblastic cells cultured on porous biomaterials.[3] Considering this, the present study was designed to evaluate cell proliferation and gene expression of the osteoblastic markers, runt-related transcription factor 2 (Runx2), bone sialoprotein (BSP), and osteopontin (OPN), in cultures of human alveolar bone-derived cells grown on porous Ti with three different pore diameter size means and on dense Ti, during the development of osteoblastic phenotype.

Methods: Porous Ti was prepared with three different pore diameter size means, 312 μm (Ti 312), 130 μm (Ti130), and 62 μm (Ti62) by powder metallurgy.[4] All samples, porous and dense Ti, were machined to obtain discs with 10 mm diameter and 2 mm thick. Human alveolar bone fragments were obtained from one healthy donor, using the research protocols approved by the Committee of Ethics in Research. Osteoblastic cells were obtained from these fragments by enzymatic digestion and cultured in α-MEM (Gibco, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (Gibco), 50 μg/ml gentamicin (Gibco), 0.3 μg/ml fungizone (Gibco), 10^{-7} M dexamethazone (Sigma, St, Louis, MO, USA), 5 μg/ml ascorbic acid (Gibco), and 7 mM β-glycerophosphate (Sigma). Subconfluent cells in primary culture were harvested and subcultured in 24-well culture plates on porous and dense Ti discs at a cell density of 2×10^4 cells/sample. During all the culture period, cells were incubated at 37ºC in a humidified atmosphere of 5% CO_2 and 95% air; the medium was changed every 3 or 4 days. Cell proliferation was evaluated at days 3, 7, and 10 by 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyl tetrazolium bromide (MTT - Sigma) assay and data were expressed as absorbance. At days 10, 14, and 17, gene expression of Runx2, BSP, and OPN were evaluated by real-time reverse transcriptase-polymerase chain reaction (real-time PCR), using the SybrGreen system (Applied Biosystems, Warrington, UK). The relative level of gene expression was calculated in reference to β–Gus expression and normalized by the gene expression of cells subcultured on polystyrene, using the cycle threshold method. MTT assay was performed in quintuplicate (n=5) and real-time PCR experiments were carried out in triplicate (n=3). Comparisons were done using the non-parametric Kruskal Wallis test, for independent samples, followed by Fisher test (level of significance: 5%).

Results: Cell proliferation was affected by Ti surfaces as follows: at day 3, dense Ti<Ti130=Ti62<Ti312 and at days 7 and 10: dense Ti<Ti130<Ti62<Ti312.
Runx2 gene expression was affected by Ti surfaces as follows: at day 10: Ti312<Ti130<Ti62<dense Ti and at days 14 and 17: Ti312=Ti130<Ti62<dense Ti. BSP gene expression was affected by Ti surfaces as follows: at day 10 Ti312<Ti130=Ti62< dense Ti, at day 14 Ti312=Ti130<Ti62=dense Ti, and at day 17 Ti312<Ti130<Ti62<dense Ti. OPN gene expression was affected by Ti surfaces at days 10, 14, and 17 as follows: Ti312<Ti130<Ti62<dense Ti. Data are presented in Table 1.

Table 1. Cell proliferation (MTT) and gene expression of Runx2, BSP, OPN in osteoblastic cells cultured on dense and porous Ti surfaces for periods of up to 17 days

	Day	Dense Ti	Ti312	Ti130	Ti62
MTT	3	0.1±0.01	0.2±0.03	0.2±0.01	0.2±0.01
	7	0.2±0.04	0.8±0.09	0.5±0.05	0.4±0.02
	10	0.3±0.02	1.0±0.08	0.7±0.06	0.5±0.03
Runx2	10	1.1±0.08	0.3±0.01	0.6±0.03	0.9±0.10
	14	2.5±0.25	0.7±0.04	0.7±0.02	1.6±0.05
	17	2.2±0.19	0.5±0.11	0.3±0.11	0.7±0.03
BSP	10	0.9±0.10	0.1±0.01	0.1±0.02	0.1±0.04
	14	1.9±0.38	0.2±0.03	0.3±0.03	1.4±0.08
	17	2.9±0.20	0.01±0.0	0.01±0.0	0.8±0.30
OPN	10	9.5±0.6	3.2±1.04	4.7±0.43	5.9±1.23
	14	47.3±2.2	2.4±0.10	3.2±0.21	5.8±1.92
	17	18.9±1.0	0.1±0.02	0.1±0.00	0.2±0.03

Data represent mean ± standard deviation (n=5 for MTT and n=3 for Runx2, BSP and OPN gene expression).

Conclusions: Irrespective of the pore diameter size, porous Ti surfaces favor cell proliferation and induce a delay in the development of the osteoblastic phenotype compared to dense Ti. In addition, Ti62 exhibits lower proliferation rate and higher expression of osteoblastic markers compared to Ti130 and Ti312. In this way, porous Ti with pore diameter size on the order of 60 μm should be considered an advantageous approach for the development of desirable implant surfaces.

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