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INFLUENCE OF A NANOSTRUCTURED TITANIUM SURFACE ON CULTURED HUMAN OSTEOBLASTIC CELLS

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INTRODUCTION: Surface modifications have been applied to titanium (Ti) in an attempt to accelerate/enhance contact osteogenesis. Chemical deoxidation and controlled reoxidation using H_2SO_4/H_2O_2 results in the formation of a unique surface nanotopography that affects early events in osteogenic cell cultures derived from rat calvaria [1,2]. The aim of this study was to evaluate the effect of the nanostructured Ti surfaces on human osteoblastic (hOB) cells.

METHODS: Machined, commercially-pure grade II Ti discs, 12 mm in diameter and 2 mm thick, were chemically treated with H_2SO_4/H_2O_2 for 2 h [1]. Osteoblastic cells were obtained by enzymatic digestion of human alveolar bone and cultured in standard osteogenic condition until subconfluence. First passage cells were cultured ($2x10^4$ cells/well) on nanostructured and machined Ti discs in 24-well culture plates. For cell attachment assay, adherent cells were enzymatically released at 24 hours and counted. Data were expressed as percentage of initial cell number. For proliferation, cells were cultured for 1 and 7 days, enzymatically released and counted. Data were expressed as doubling time in hours [3]. At day 7, cell viability was evaluated using trypan blue and expressed as percentage of viable cells. At days 1, 3, and 7, cell morphology was evaluated with phalloidin and DAPI; alkaline phosphatase (ALP) labeling was performed using a primary mouse anti-human ALP antibody (B4-78). Data were compared by Mann-Whitney test.

RESULTS: Chemical treatment resulted in a nanopitted surface (compare Fig. 1A and 1D). There were no statistically significant differences between nanostructured and machined Ti in terms of cell attachment (p=0.70), proliferation (p=0.28), and viability (p=0.83) (Table 1) of passaged hOB. At day 1, cells were spread and exhibited an elongated polygonal shape on machined Ti whereas on nanostructured Ti they were mostly stellate-shaped (Fig. 1B and 1E). At day 7 both Ti surfaces exhibited areas of cell multilayering and cells were predominantly elongated. ALP positive cells were observed at all time points with a similar labeling pattern on both Ti surfaces (Fig. 1C and 1F).

Table 1. Cell attachment, proliferation, and viability (Mean±SD, n=3) of hOB cells cultured on nanostructured and machined Ti.

	Nanostructured	Machined
Attachment	34.7±6.4	40.3±10.5
Doubling time	50.4±2.7	48.1±2.1
Viability	92.1±2.8	93.1±1

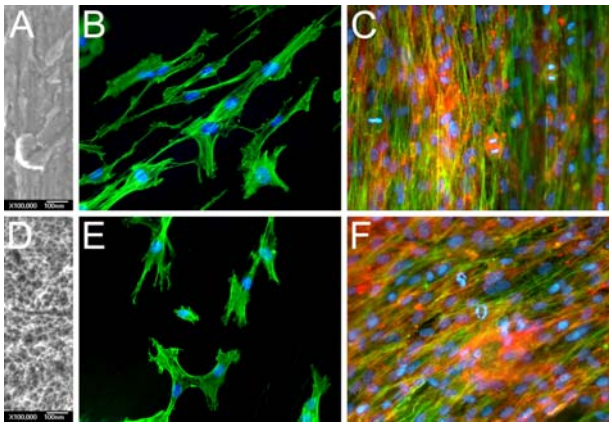

Fig. 1. (A-C) machined, (D-F) nanostructured Ti. (A,D) FE-SEM. (B,E) Cell morphology at day 1; (C,F) ALP labelling (red) at day 7, X40.

DISCUSSION & CONCLUSIONS: Results from these initial studies indicate that nanostructured Ti induces no significant differences in cell attachment, proliferation and viability of passaged hOB when compared to machined Ti. Nanotopography may, therefore, affect other key parameters of in vitro osteogenesis such as the observed influence on cell shape or possibly cell differentiation.

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