

Controlled Surface Functionalisation of Orthopaedic Titanium Implants: In-vitro Osteogenic Bioactivity.

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INTRODUCTION: Controlled surface topography may induce favourable cell reactions upon implantation and, at a later stage, increase bone-implant contact (BIC) thus leading to greater stability¹. The latter is especially important in areas of low bone density. Variations of surface topography produced using the current methods produce extremely randomised surface patterns in terms of shape, spacing and distribution. This may negate potentially controllable, reproducible and possibly predictable post-implantation responses of cells contacting the implant surface.

We report on the in-vitro bioactivity of surface topography produced using a novel patented method for orthopaedic implant structuring. This method produces micro-nano surface patterns in an easy to apply, industrial scale, setting.

METHODS: *Sample preparation* Samples were produced from grade 316L stainless steel alloy. The patterned surface consisted of 50 and 30 µm diameter hemispheres and 20 µm distance between adjacent hemisphere circumferences (samples codes 50_20 and 30_20).

Cell culture Human bone marrow stromal cells (HBMC's) cultivated in α-MEM (Invitrogen, supplemented 10% FCS, 1% PSN, 50 µM Ascorbate-2-phosphate, 10 nM Dexametasone, 2 mM β-Glycero phosphate and 50 µM Vitamin D3. Cultures were maintained in a humidified atmosphere, at 37° C and 5% CO₂. Cells were seeded at ~ 2.5 x 10³ cells/ cm².

Immunocytochemistry At various time points, the cell actin microfilaments labelled using Phalloidin (Alexa Fluor 488, 1:40, Invitrogen). Cells were also labelled using α-vinculin mouse monoclonal IgG (clone HVIN-1, 1:300, Sigma) followed by secondary goat α-mouse IgG (Alexa Fluor 546, 1:100, Molecular Probes).

Polymerase chain reaction At day 14 in culture, cells were lysed and total RNA purified using Quiagen RNeasy kit (Quiagen). The RNA was reverse transcribed into cDNA using iScript cDNA Synthesis kit (BioRad) and osteoblastic gene regulation was assessed using a BioRad iCycler.

RESULTS: The cell-substrate interaction was mediated by the formation and clustering of thick patches of focal adhesions related to the

hemisphere structure (Figure 1A). This particular pattern was not seen on non-structured controls surfaces. The seeded cells also exhibited morphology associated with 3 dimensional matrices (Figure 1B).

PCR experiments revealed an enhanced osteogenic differentiation in cells seeded on the structured surfaces compared to cell culture control surfaces (results not shown). The genes (ALP, Osteocalcin) investigated are directly related to formation and maintenance of a mineralised bone matrix.

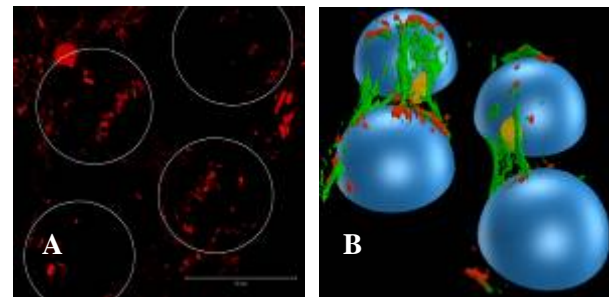


Fig1. Confocal laser microscope images of HBMC cells seeded on 50_20 structured 316L surfaces at day 7 in-culture. The labelled cellular components are actin (green), vinculin (red) and nuclei (yellow). A: Focal adhesion formation and an outline of the hemisphere bases. B: 3D reconstruction of confocal laser microscope image stack showing cells attaching to the hemispheres.

DISCUSSION & CONCLUSIONS: Defined, positive hemispheric, exhibited significant bioactive properties in influencing osteogenic differentiation. Further studies are currently underway to investigate cell migration, proliferation and expression of phenotype as a function of this particular surface topography.

REFERENCES: ¹ A. Bruinink, J.P. Kaiser, C. Meyer (2005) *Adv Eng Mater.* **7**, 411-8. ,

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