

Patterns of Genomic Regulation In Mesenchymal Stem Cells Cultured on Osteogenic Nanotopographies.

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INTRODUCTION: A key tenet in orthopaedic implant design is differentiation of the native mesenchymal stem cells in to mature, bone producing, osteoblasts.

Previously, we have published immunohistological evidence of primary human mesenchymal stem cells producing increased levels of osteocalcin (OCN) and osteopontin (OPN) in response to a variety of nanotopographies when cultured in basal media alone^{1, 2}. OCN and OPN are osteoblast specific matrix proteins and thus, our results allude to the nanotopographies having osteogenic properties.

In order to expand on our understanding of the genomic process of osteogenic differentiation on the nanotopographies, two further selection processes have been used. Firstly, stem cell response to a selection of nanomaterials was analysed with 1.7k gene arrays. The most interesting of these were subsequently analysed with both 19k gene arrays and also 101 gene osteospecific arrays and compared to stem cells treated with dexamethasone (dex).

METHODS: Stro-1 selected mesenchymal stem cells were cultured for 21 days on materials fabricated by photolithography and polymer demixing for 1.7k arrays and then both 14 and 28 days for 19k arrays. Cells treated with dex were also cultured for 14 and 28 days. Both the 1.7k and 19k arrays were from the Ontario Microarray Centre and contained general, well-characterised expressed sequence tags. Also, 101 gene osteospecific oligo arrays from Superarray Bioscience were used to detect early osteoblast differentiation at 14 days.

The topographies were all produced by embossing in polymethylmethacrylate (PMMA) via Ni intermediaries fabricated by sputter coating and electroplating of the master topographies. Flat PMMA was used as a control.

Cluster analysis and iterative group analysis were performed with the Ontario arrays. For the osteospecific arrays, numbers of gene hits for each gene were counted.

RESULTS & DISCUSSION: Cluster analysis revealed large distinct areas of similar differential expression of genes on the nanotopographies compared to planar control. These results infer that as the cells differentiate large numbers of similar genes are, for the most part, turned on (it is noted that smaller clusters of down-regulations were observed). Critically, up-regulations

tended to include clusters involved in matrix regulation and cell signalling. Down-regulations were seen in areas such as proliferation. We demonstrate for the first time, using these osteo-specific arrays that the nanotopographies exhibit a similar effect to dex with significant implications for materials/cell science.

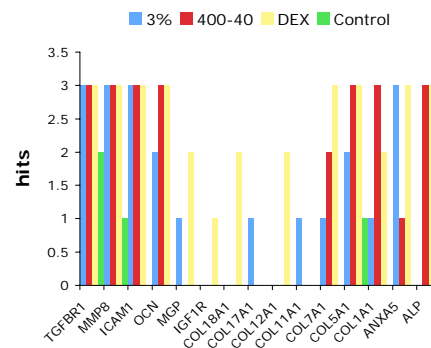


Fig. 2: Number of hits for osteospecific genes (just a small selection).

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REFERENCES:

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2. Dalby, M. J et al. *Biomaterials* **2006**, 27, 2980-7.