

Isolation of S-Phase Osteoblasts: Focal Contact Quantification on Nano-Pitted Substrates

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INTRODUCTION: Cell-substrate interactions have always played a pivotal role in determining the performance and ultimate acceptance of a foreign material placed within an *in vivo* environment. Man-made structures, which can induce cell attachment and maintain differentiation of e.g. osteoblasts, while possessing suitable mechanical properties can be viewed as the paradigm of tissue engineering. Integrin receptors are shown to cluster together at discrete foci, resulting in anchoring complexes, known as focal adhesions (FAs). FA's are linked to many mechanotransductive pathways and hence cell proliferation and differentiation. However, with the viewing of FA's, there are problems associated with cell cycle phase and FA rearrangement. Thus, to eliminate the discrepancies obtained in focal contact number and size, it is favourable to quantify FA formation within a synchronised population of cells. Bromodeoxyuridine (BrdU), a thymidine analogue, was used to label S-phase primary osteoblasts cultured on nano-pitted polycarbonate (PC) after serum starvation and then feeding to induce S-phase in the cell population. Incorporation of BrdU into the nucleus of cells undergoing increased DNA synthesis was subsequently fluorescently labeled at the same time as FA detection.

METHODS: Primary human osteoblasts (HOB) were cultured on the control (flat) and test materials (100 nm diameter, 300 nm centre-centre spacing originally produced by electron beam lithography in orthogonal, near orthogonal, hexagonal and random symmetries) injection moulded in PC. The cells were serum starved for 4 days before adding fresh media to the culture system. Cells were left for 17 hours after feeding before the addition of 10 μ M BrdU, in which they were incubated for 3 hours. Subsequently, DNase treatment and immuno-labelling were used to view S-phase nuclei and FA's. Images were processed using ImageJ.

RESULTS: Osteoblasts were shown to form extensive arrays of focal contacts with the underlying substrate. These were shown to be both of the dot and dash variety, indicating a dynamic system of focal complex formation and maturation. Focal contacts were seen to be increased in osteoblasts cultured on near-orthogonal and random nano-patterns while numbers of cells in S-phase were decreased on flat and orthogonal nano-pitted substrates (Figure 1).

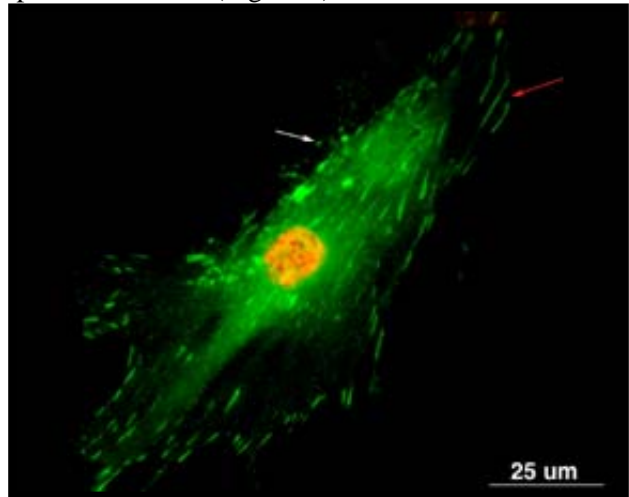


Fig. 1: Typical S-phase Osteoblasts cultured on nano-pitted polycaprolactone. Dot FA's (white arrow) and dash FA's (red arrow) are evident.

DISCUSSION & CONCLUSIONS: Osteoblast focal contact formation is influenced by nano-topography, Highly ordered arrays of nano-pits such as orthogonal and hexagonal patterns result in decreased cellular adhesion and reduced focal contact formation. It is anticipated that the above technique will yield invaluable information as to how specific cells respond and adhere to various substrates.

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