

The Effect Of Cell Support Geometry On Osteogenic Differentiation Of H1 Human Embryonic Stem Cells.

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INTRODUCTION: Differentiation of Human Embryonic Stem Cells (HESC) to the osteogenic lineage could allow repair of bone defects that could otherwise not be remedied. The associated cell delivery devices may have an influence upon the outcome of repair, not only by chemical composition but also the geometry and associated characteristics [1].

METHODS: To test osteogenic differentiation of HESC on a range of scaffold geometries a series of P_(D,L)LA scaffolds were manufactured (Fig 1) and Human Embryonic Stem Cells (HESC) grown and differentiated upon them *in vitro*.

Scaffolds were prepared from the same batch of P_(D,L)LA (60K Mw) and included; supercritical CO₂ scaffolds, salt leached scaffolds (containing; square, round, square interlinked and round interlinked porogens), spherical and irregular, heat sintered (60°C) scaffolds and PLA fleece (from Cellon).

To each of these scaffold types 100,000 H1 HESC were added using an orbital shaker (2 cm radius at 60 rpm; n=6; simultaneously to minimise culture variables). HESC were prepared by culture upon mitomycin-C inactivated MEF feeder layers as undifferentiated cells, "semi-differentiated" in pellet culture and culture expanded in monolayer, before being added to the scaffolds in the presence of ascorbate (10 µM) dexametahasone (10 nM) for 14 days (Differentiation protocol modified from ref [2]).

To estimate cell number, metabolism was measured via the Alamar Blue (AB) assay (as per manufacturers instructions; Serotech Ltd).

To observe the position of cells within the scaffolds Live/Dead (Calcein-AM and EthD1; Molecular probes) was used (as per manufacturers instructions).

To estimate the osteogenic response, activity was measured using conversion of pNPP in an alkaline buffer with comparison to known concentrations of pNP (as modified from ref [3]).

RESULTS: The results suggest that geometry alone can have an influence upon the osteogenic

response, indicated by alkaline phosphatase activities in comparison to cell metabolism. Scaffolds in order of osteogenic permissivity (AP/AB) were - cube pore, irregular sintered particles, spherical pore, sintered spheres, spherical fused pores, CO₂ foamed, fleece and fused cube porogen. The range of scaffolds tested all supported cell growth and differentiation and none appeared to specifically inhibit osteogenesis.

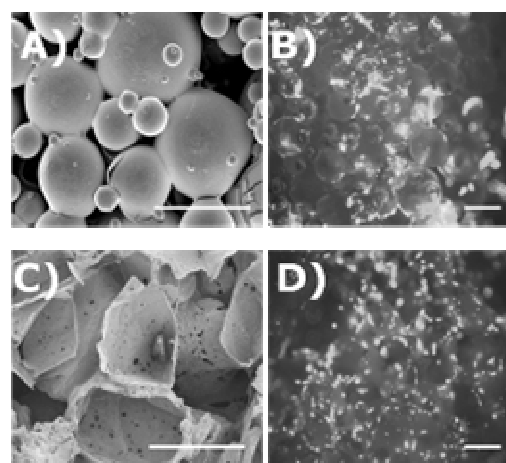


Fig. 1: Examples of two different scaffold geometries of convex-spherical and concave-square (A and C) with HESC location highlighted using live/dead staining (B and D, Bar = 300 µm).

DISCUSSION & CONCLUSIONS: The scaffold range varied in other aspects, such as cell seeding, pore connectivity and sub-micron scaffold surface topography all of which could influence the outcome.

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ACKNOWLEDGEMENTS: Many thanks to Lloyd Hamilton for helpful discussion of polymer characteristics. This work was supported by the B.B.S.R.C.