

Three-Dimensional Inverse Opal Scaffolds for Culture and Differentiation of Human Mesenchymal Stem Cells

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INTRODUCTION: Strategies to promote cartilage tissue regeneration involves a combination of multipotent mesenchymal stem cells (MSCs), appropriate stimuli (in vivo or in vitro) and three-dimensional (3D) scaffolds. Effective scaffold systems and methods to induce a stable articular chondrocyte phenotype from MSCs are lacking. We have developed novel 3D inverse opal scaffolds consisting of either chitosan or PLGA interconnected nanofibrous matrices containing a highly uniform pore structure. The potential use of these scaffolds to promote MSC viability and differentiation is currently being explored.

METHODS: Scaffold Synthesis- A simple fluidic device was used to generate monodisperse gelatin microspheres as a template. Following sonication and heat treatment, the resulting gelatin lattice pellet was infiltrated with PLGA solution followed by freeze-drying. Washing and a second freeze-drying step was carried out to remove the gelatin microspheres. Scaffolds were stored in ethanol until further use. See Choi, *et al*¹ for more experimental details related to chitosan scaffold production. Fig.1 shows an SEM of a PLGA scaffold. **MSC Isolation and Culture-** Human MSCs were obtained from iliac crest-derived bone marrow aspirates by standard filtration and density centrifugation procedures². Resulting bone marrow-derived MSCs (**BM-MSCs**) were cultured in low glucose DMEM containing serum and FGF-2 (10ng/ml). **Scaffold/Cell Cultures and Analyses-** Scaffolds were washed with PBS and immersed in a BM-MSC or a chondrocyte cell line (TC28) suspension (approx. 1×10^5 cells / ml / scaffold) with gentle mixing for 2h. Scaffolds were then cultured in 24 well plates in regular growth medium containing serum and FGF-2. Cell viability and proliferation were tested over time using a LIVE/DEAD assay kit (Invitrogen) and MTT assay, respectively.

RESULTS: Fig. 1A shows a magnified SEM view of the top surface of a generated PLGA inverse opal scaffold. Note the uniform pore

structure. Fig 1B shows the presence of human BM-MSCs within a PLGA scaffold after 21 days in regular growth medium. Note that the majority of cells are viable and well distributed throughout the matrix. Similar results were noted for the chondrocyte cell line, TC28 (not shown). Over the culture period in vitro, there was no sign of significant scaffold degradation.

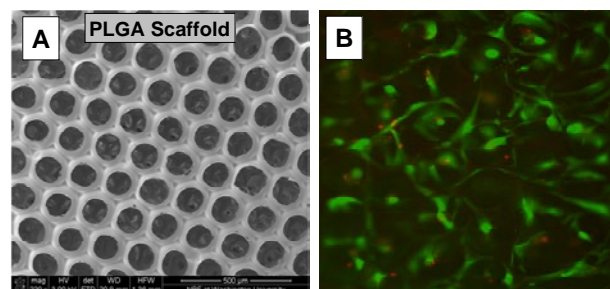


Fig. 1: A. Magnified SEM view of the top surface of a PLGA inverse opal scaffold. B. Fluorescent image of human BM-MSCs within a PLGA scaffold after 21 days in culture. Green = live cells; Red = dead cells.

DISCUSSION: In addition to successful culture and proliferation of a pre-osteoblastic cell line within 3D inverse opal chitosan scaffolds¹, we now show growth and viability of human BM-MSCs and chondrocytes in similar PLGA-based scaffolds over time in vitro. The PLGA scaffolds are more robust than chitosan scaffolds and may better serve the purposes of cartilage tissue engineering. We are currently investigating the differentiation potential of BM-MSCs toward a chondrogenic lineage within these scaffold systems.

REFERENCES: ¹ Choi *et al*, Adv. Mater. 2009, 21:1-5. ² Yoo *et al*, JBJS.1998, 80A: 1745-1757. ³ Barry *et al*, Exp Cell Res. 2001, 268 : 189-200.

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