Novel Fully Biodegradable Biomimetic Scaffolds for Bone Regeneration and Repair

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Statement of Purpose: Porous structure and biological properties of the scaffolds are two of the most important factors in promoting tissue regeneration. Using proprietary 3D Precision Microfabrication Technology, we are able to fabricate porous biodegradable polymer scaffolds with well controlled porous structures that are optimized for bone and cartilage regeneration. To further render desired biological properties to the porous polymer scaffolds, we are further developing a bio-mimetic coating process to coat the porous scaffolds with ECM by culturing cells (including normal and genetically engineered cells). Living cells and residual DNAs will be removed after the coating process. These biomimetic scaffolds will have a cellular derived ECM enriched with proper growth factors, such as BMPs, to recruit stem cells to the defect sites for repairing. These novel hybrid biomimetic scaffolds are tissue equivalents that can be mass produced in a factory but without the risk of disease transmission and immune response that are associated with the use of donor tissue. They can be terminally sterilized and ready for use.

Methods: Porous PCL scaffolds were engineered using 3D Biotek's Precision Micro-fabrication Technology (Fig. 1). Uniquely, fiber diameter is controlled by nozzle diameter while spacing between fibers is controlled by a motion control system. The struts of each layer are oriented 90ºC relative to the struts of the layer immediate below (Fig. 1B-C). Before use, scaffolds are tissue culture surface treated and γ-radiation sterilized. This study implemented 96-well compatible 3D Insert™-PCL scaffolds, 1.6 mm in diameter (Fig. 1D), with a configuration of 300 µm fiber diameter and 300 µm pore size (PCL3030).

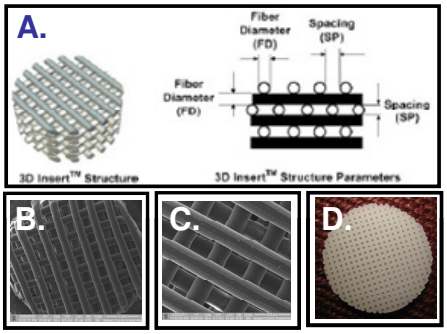

Figure 1. Six-layered structural design of PCL 3030 scaffolds.

Following 3D Biotek's static seeding protocol, human mesenchymal stem cells (hMSCs) and human fibroblastic cells (hFB) were seeded into the porous 3D PCL scaffolds. Cells were seeded at concentrations of $1x10^4$ cells/3D PCL scaffold. hMSC-osteoblastic differentiation was performed according to manufacturer's instructions.

Results: Fibroblastic and osteoblastic cell growth on 3D Insert™-PCL was monitored using an inverted light and fluorescent microscope. Fluorescent images show that osteoblastic cells are viable and grow along PCL fibers (Fig. 2A, C) and within the pores (Fig. 2B). Fluorescent DNA assays performed at weeks 1-4 confirmed increases in fibroblastic cell proliferation and a plateau of osteoblastic proliferation, indicative of their differentiation into osteoblasts (data not shown).

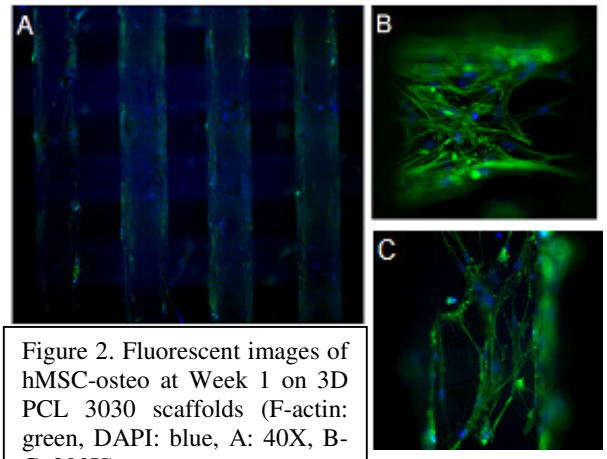

Figure 2. Fluorescent images of hMSC-osteo at Week 1 on 3D PCL 3030 scaffolds (F-actin: green, DAPI: blue, A: 40X, B-C: 200X).

Compared with fibroblastic cells, osteoblastic cells on PCL scaffolds demonstrated increasing and enhanced alkaline phosphatase (ALP) activity at all time points during culture (data not shown). Furthermore, Von Kossa staining (Fig. 3) and calcium deposition assay (data not shown) reveal that 3D Insert™-PCL scaffolds support effective hMSC differentiation into the osteoblastic lineage.

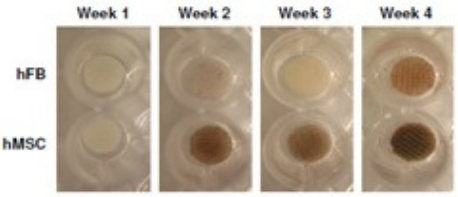

Figure 3. Von Kossa staining of hFB and hMSC-osteoblastic cells.

Conclusions: This study demonstrates the intention of combining 3D Insert™-PCL scaffolds with 3D Biotek's new biomimetic coating process to engineer powerful bioactive scaffolds for bone regeneration and/or repair.

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