The Effect of Varying the Architecture of Scaffolds on Mesenchymal Stem Cell Osteogenesis and Chondrogenesis

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Introduction: Articular cartilage has a limited intrinsic ability to heal. For this reason, orthopedic management of these lesions remains a persistent problem for the orthopedist and patient. This has prompted the development of numerous procedures in an attempt to treat these lesions and halt or slow the progression to diffuse arthritic changes. Tissue engineering may eliminate many of the problems associated with the current surgical options. Current tissue engineering methods are aimed at filling the cartilage defects with cells or scaffolds alone, or in combination with one another to promote cartilage regeneration. It appears that the absence of cells leads to a poor quality reparative tissue. Chondrocytes, although widely studied, either alone or in combination with a scaffold, have failed to restore a normal articular surface and the hyaline cartilage formed early on seems to deteriorate with time. Therefore, improved tissue engineering methods are still needed.

At the forefront of investigation is the use of stem cells for tissue engineering therapies because of their ability to differentiate into various cells types and thus, promote the regeneration or repair of the diseased or damaged tissue of interest. Mesenchymal stem cells (MSCs) are multipotent cells that are capable of differentiating along several lineage pathways. MSCs in combination with biomaterials of varying architectures as scaffolds to direct differentiation has yet to be investigated. We propose to investigate MSCs in combination with fibrous scaffolds as a potential tissue engineering therapy for bone and cartilage regeneration. We evaluated two commonly used polymeric compositions in the field of tissue engineering, poly lactic acid (PLA) and poly (D,L-lactide-co-glycolide 75/25 (PLGA), at the nanometer (SF) and micrometer (LF) fiber diameters for MSC attachment, morphology, growth and differentiation along osteogenic and chondrogenic lineages.

Materials and methods:

Fabrication of Tissue Engineering Scaffold: PLA and PLGA were made in to non woven mats of two distinctly different fiber diameters, nanometer (SF) and micrometer (LF) using the electrospinning[1] technique. The polymer concentration and the needle diameter were varied in the electrospinning process to fabricate LF and SF (Table 1). LF and SF were made into 1mm thick non-woven mats and were sterilized prior to cell seeding. They were characterized for fiber diameter and cell morphology using Scanning Electron Microscopy (SEM). Porosity and pore size distribution were analyzed by mercury intrusion porosimetry (MIP) and thermal analysis was performed with a Differential Scanning Calorimeter (DSC).

Cell proliferation: MSC that were isolated from whole bone marrow and subcultured were seeded on to LF and SF. Tissue culture polystyrene plate (TCP) was used as the control. Cell proliferation was assessed using Vybrant MTT Cell Proliferation Assay Kit (Molecular Probes).

Osteogenic and chondrogenic differentiation: MSCs on LF and SF scaffolds were cultured in osteogenic inductive media and compared to MSCs in standard growth media. Mineralization and alkaline phosphatase activity was measured at days 4, 7, 11, and 14. Chondrogenic potential of MSCs on LF and SF was compared to cell pellet (CP) culture. MSCs were seeded at a density of 10^5 cells/cm^2 onto scaffolds and 0.25×10^6 cells were used to make each cell pellet. They were maintained in chondrogenic induction medium supplemented with TGF-β3 (Cambrex BioScience Inc.,). Control group was maintained in standard growth medium. Sulphated glycosaminoglycan (GAG) and type II collagen content on LF, SF and CP were assessed with Blyscan Assay (Biocolor Ltd.) and Arthrogen-CIA Capture ELISA Kit (Chondrex, Inc.) respectively.

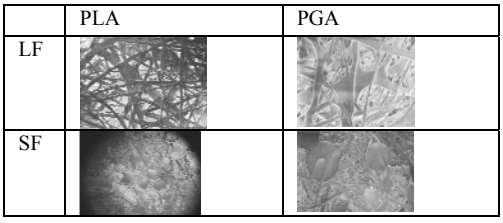
Results and Discussion: The result of image analysis is summarized in Table 1.

Table 1: Process variables and resultant fiber diameter.

	Solution *w/w*	Needle	PLA *(μm)*	PGA *(μm)*
LF	10	13	17 ± 7.6	16 ± 7.6
SF	5	20	0.4 ± 0.92	0.5 ± 0.88

The morphology of the cells on LF, SF is shown in Figure 2. The cells were flat and spread out on LF, but appeared to be rounded on SF.

Figure 2: SEM images of PLLA and PLGA.



The MIP results showed that the microfiber and the nanofiber scaffolds of PLLA had a porosity of 39% and 47% respectively. DSC results showed that the electrospinning process does not alter the characteristic property of the polymer even when processed at a high voltage. The results of the MTT assay showed good growth characteristics on both SF and LF. No significant differences in MSC proliferation were detected between them. However, striking results were detected in cell morphology showing that cells adhere with rounded morphology on SF whereas they appeared flat on LF. The results of the osteogenic differentiation studies demonstrated the highest levels of mineralization occurred on both LF and SF scaffolds as compared to TCP. The rounded morphology of the MSCs on SF might prove beneficial in MSC chondrogenic differentiation ultimately leading to cartilage formation. The chondrogenic differentiation data currently suggests that SF scaffolds support Type II collagen synthesis.

References: 1.Hohman MM.Physics of Fluids 2001; 13: 2201–2220.