Electrospinning of collagen type II-nanofibers for potential cartilage repair
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Introduction: Matrix-based autologous cell transplantation marks a promising approach for the treatment of osteo-/chondral lesions, although none of the clinically used biomaterials reflects both biochemical composition and ultrastructural aspects of articular cartilage. Collagen type II is the major extracellular matrix protein of hyaline cartilage and organized as a triple helical homotrimer of three alpha-1 chains, which form fibrils with a diameter of 50-400 nm. Recent findings have shown that electrospun three-dimensional nanofibrous structures that exhibit morphological similarities to extracellular provide a biomimetic environment for seeded cells (1,2). Human bone-marrow derived stem cells (MSCs) are capable of extensive self-renewal and can undergo chondrogenic differentiation (3). Hence, the use of MSCs for matrix-based articular cartilage repair strategies addresses shortcomings of autologous chondrocytes, such as limited availability and donor-side morbidity. In this study, we investigated the fabrication of a collagen type II-based nanofibrous scaffold via electrospinning for the potential use in cartilage repair. Various collagen type II concentrations produced in two different solvents were used to fabricate nanofibrous scaffolds and physico-structural characterization was performed to evaluate the properties of the scaffolds. For cell culture, biocompatibility of the obtained scaffolds was assessed using a cell proliferation assay.

Materials and Methods: Collagen type II was isolated from articular cartilage of knee joints of 6-8 month old calves, which are mechanically disrupted and pre-treated with 0.15 M NaOH for 12 hours. Afterwards, the remaining solids were rinsed with ddH2O, digested with pepsin in acetic acid (pH 2.8) for 24 hours and the remaining solids were discarded. The solubilized collagen type II was precipitated with NaCl (0.9 M), centrifuged (20,000 x g at 4°C, 1h) and dissolved in 0.5 M acetic acid, this step was repeated 3 times. Collagen type II was extensively dialysed against 0.5 M acetic acid, lyophilized and stored at -80°C. Purity of the isolated collagen was verified by 5% SDS-PAGE gel. Electrospun scaffolds were fabricated using different solvents, hexafluoropropanol (HFP) or 50% acetic acid, with varying concentrations of collagen type II. Morphological characteristics of the different scaffolds were assessed by scanning electron microscopy (SEM) and fibre diameter and pore size were determined. Optimized nanofiber scaffolds were subject to chemical cross-linking using 10% hexamethylene-diisocyanate in iso-propanol to prevent rapid biodegradation. Cross-linked scaffolds (8mm diameter, 1mm thick) spun from HFP (10% collagen II) and acetic acid (40% collagen II) were seeded with 40,000 MSCs and cultured for 21 days in basal medium (10%FBS, 1% Pen./Strep.). Cell proliferation was assayed using the Cell-Titer 96™ Aqueous One Solution Cell Proliferation Assay. In addition, cell mediated contraction was analyzed by direct measurement of the scaffold diameter over time.

Results: Collagen type II was successfully isolated and purified and no contaminating proteins were detected by electrophoresis. Collagen type II could be electrospun in a concentration ranging from 7.5 to 17.5% when using HFP as a solvent, from 35 to 45% when using 50% acetic acid, respectively. In general, nanofibers produced using HFP showed a larger diameter (400-3000 nm) when compared to fibers spun from acetic acid (150-650nm), which also appeared more homogeneous. In addition, fiber size increased with higher collagen type II concentration for each solvent (Fig. 1). Cross-linking of the scaffolds was necessary to stabilize and prevent the structure from rapid biodegradation; utilization of hexamethylene-diisocyanate cross-linking did not alter the morphology of the nanofibers. MTS assay revealed an increase in cell number during the three-week culture period for both types of scaffolds - 2.1-fold increase was seen on the scaffolds obtained from acetic acid, and a 1.41-fold increase seen when seeding MSCs on HFP derived nanofibers (Fig.2A). The nanofiber constructs exhibited a stable shape over 21 days in culture and uncontrolled contraction did not occur. After 21 days, cell-mediated contraction was 23% in the acetic acid derived scaffold and 12% when using HFP as the solvent (Fig.2B).

Discussion: We have successfully electrospun collagen type II using a weak acid solvent to produce collagen type II-based nanofibrous scaffolds. Our results show that fiber sizes are dependent on fabrication parameters. By using cross-linking reagents, the collagen type II nanofibers are structurally stable during the culture period, and support continuous cell proliferation. Taken together, these findings suggest that promising electrospun collagen type II nanofibers have biologically favorable structure and properties and represent tissue engineered scaffolds applicable for cartilage repair.


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