IGF-1 Gene-supplemented Type II Collagen Scaffolds for Mesenchymal Stem Cell-driven Chondrogenesis in Vitro

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Introduction: Collagen scaffold mediated non-viral gene delivery has been successfully used to provide a locally sustained therapeutic level of the targeted protein [1]. Undifferentiated adult progenitor cells (mesenchymal stem cells, MSCs) have the capacity to differentiate into committed connective tissue cell lineages including chondrocytes when cultured under certain conditions in vitro. Due to minimal donor site morbidity and nearly unlimited availability, MSCs are undergoing investigation for tissue engineering-based strategies for the treatment of articular cartilage defects. In this context IGF-1 has been identified as a potential factor to stimulate stem cell-based chondrogenesis in vitro [2] and in vivo [3]. The purpose of this study was to evaluate the effects of an IGF-1 gene-supplemented type II collagen scaffold on stem cell-driven chondrogenesis in vitro.

Materials and Methods: Highly porous collagen sheets were fabricated by ramp-freeze-drying and dehydrothermal treatment (DHT) of a porcine type II collagen slurry (Geistlich Biomaterials, Wolhusen, Switzerland). 10μg IGF-1 plasmid DNA per scaffold was complexed to a lipid-mediated transfection reagent (Gene Therapy Systems, Inc., San Diego, CA, USA) and incorporated into 8mm-diam. discs of the collagen scaffolds as follows. A 5μg plasmid load was chemically cross-linking to the scaffolds using an aqueous carbodiimide solution for 30 minutes. After rinsing the scaffolds in PBS they were briefly dried on filter paper and another 5μg of lipid-complexed pIGF-1 was added to each scaffold followed by one hour incubation time. Bone marrow-derived MSCs were isolated from six Spanish goats, subcultured separately to passage two with rhFGF-2 (10ng/ml) supplementation, and seeded onto the collagen sponges (4 million cells). Scaffolds with or without pIGF-1 were cultured for 14 days in serum-free chondrogenic media containing either rhTGF-β1 (100ng/ml) or with additional rhIGF-1 (100ng/ml). At 3 and 14 days, DNA and GAG contents were determined by the Picogreen and dimethylmethylene blue assays, respectively, and the scaffold diameter was measured (n=6). For histochemical analysis, scaffolds were embedded in paraffin, serially sectioned and stained with Safranin-O/Fast green for sulfated glycosaminoglycans (GAG).

Results: All scaffolds showed a comparable amount of cell-mediated contraction during 14 days of culture. After 3 days the number of cells in the scaffolds decreased significantly. This effect was most prominent in IGF-1 gene-supplemented and rhTGF-β1-treated scaffolds in which the number of cells was reduced to about 30% and 45% of the starting number. From 3 to 14 days of culture all groups displayed a significant increase of GAG deposition. Scaffolds supplemented with plasmid IGF-1 exhibited the greatest extracellular accumulation of GAG/per cell after 14 days, with a significant difference when compared to rhTGF-β1 or rhIGF-1 (Figure 1). Histomorphological analysis revealed chondrocyte-like cells in lacunae, surrounded by a glycosaminoglycan rich extracellular matrix (Figure 2E). Histochemical staining with Safranin-O/Fast Green for sulfated GAG was consistent with the biochemical data (Figure 2).

Discussion: Our findings demonstrate the potential of plasmid IGF-1-supplemented type II collagen scaffolds to facilitate chondrogenic differentiation of MSCs and stimulate GAG biosynthesis in vitro. Transient incorporation of plasmid IGF-1 into type II collagen scaffolds may be of use in selected cartilage repair procedures in vivo to stimulate chondrogenesis when MSCs are involved in the reparative process (e.g., when the gene-supplemented scaffolds are seeded with exogenous MSCs or implanted in microfracture-treated sites). Further studies on chondrogenic differentiation using MSCs may serve to identify other factors able to further induce an articular cartilage phenotype using this non-viral scaffold-based strategy.


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