In Vivo Hyaline Cartilage Regeneration Without Cell Transplantation

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Statement of Purpose: Articular cartilage does not mount an effective healing response to injury, resulting in progressive joint degeneration and disability. Clinical treatments with cell or osteochondral plug transplantation require tissue harvest and an open joint surgery; however, they do not reliably regenerate a durable weight-bearing surface. We are pursuing a novel strategy to engineer cartilage healing based on recruitment of endogenous mesenchymal stem cells (MSC) to the defect site. The synovial membrane has been shown to be a rich source of local endogenous MSC (sMSC) with superior potential for cartilage repair [1,2]. Our approach is based on signaling molecule release from a biomaterial scaffold to induce sMSC chemotaxis to, and proliferation and differentiation within, the cartilage defect. Our objective was to test cartilage regeneration by endogenous sMSC recruitment to a defect in an animal model.

Methods: A porous, elastic chitosan-gelatin scaffold (Fig. 1) served as the basis for signaling molecule delivery. Heparin was bound to the amino groups of the scaffolds to bind and protect signaling molecules for in vivo delivery. The heparin binding reaction was catalyzed by 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). In the treatment group, scaffolds were loaded with 1 μg each of HGF, IGF-1, IGF-BP2, FGF-basic, and TGFB-1. Control scaffolds were loaded with 1 μg IGF-1 only. The scaffolds were press fit into osteochondral defects of 3 mm diameter and 2 mm depth in the patellar groove of male New Zealand white rabbits (5 per group) at age 3 months. The distal femurs were collected at 6 and 12 weeksk post surgery for macroscopic and histologic evaluation.

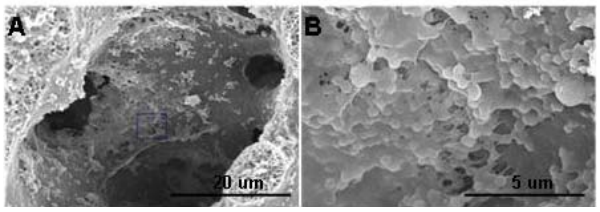

Figure 1. Elastic chitosan-gelatin scaffold with A) an interconnected macroscopic pore structure and B) gelatin beading on the pore surfaces.

Results: Extensive cartilage and subchondral bone regeneration had occurred in the treatment groups by 6 and 12 weeks post surgery (Fig. 2). In the regenerated tissue, vibrant staining (red) by Safranin O demonstrated extensive production of glycosaminoglycans, positive immunohistochemical staining (brown) demonstrated an abundance of type 2 collagen, and negative immunohistochemical staining (no brown) demonstrated a lack of type 1 collagen. Collectively, this staining pattern demonstrated that the regenerated tissue was hyaline cartilage. Extensive regeneration of subchondral bone was also present. The regenerated cartilage was more cellular than the surrounding cartilage, indicating that the tissue was not yet mature. In the control groups, very little tissue regeneration occurred. The scaffolds were not well-infiltrated, and the tissue that formed at the surface of the defect was primarily fibrous tissue, with some subchondral bone formation. Results for the 6 and 12 week groups were similar.

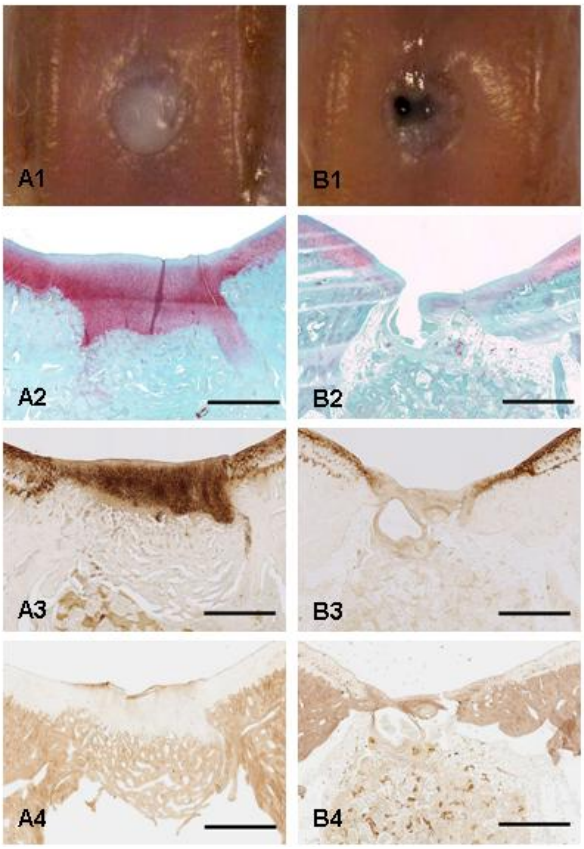

Figure 2. Representative 12 week treatment (column A) and control (column B): macroscopic images (row 1) and Safranin O fast green (row 2), type 2 collagen (row 3), and type 1 collagen (row 4) histology. Scale bar is 1 mm. Column B is the most extensive regeneration by a control.

Conclusions: Our approach to cartilage injury is novel because it focuses on engineering a healing response in situ. Our results demonstrate proof-of-concept for hyaline cartilage regeneration by endogenous MSC recruitment. Ongoing efforts are focused on sequential, rather than all-at-once, signaling molecule delivery. This research lays the groundwork for programming a clinically effective healing response without the need for cell transplantation.

References: 1) Sakaguchi Y. Arthritis Rheum. 2005;52:2521-2529. 2) Vanden Berg-Foels WS. BMES Oct. 2009;OP9-3-13D.

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