Tissue Engineering of Nucleus Pulposus using a Novel Cartilage ECM-derived Bioscaffold and Chondrogenic Bone Marrow-derived Mesenchymal Stem Cells

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INTRODUCTION:
Degeneration of the intervertebral disc (IVD) represents a significant musculoskeletal disease burden. Total disc replacement (TDR) is an effective, motion-preserving alternative to treat degenerative disc disease (DDD). However, however, it has many drawbacks and their longevity is unknown. Tissue-engineered nucleus pulposus (NP) is becoming a promising alternative to conventional IVD replacement prostheses.

Ideal scaffold should mimic the structure and function of native tissue and organs, and biologic scaffolds derived from decellularized tissue is the right choice. Cartilage ECM has the similar ECM as nucleus pulposus, and both of them mainly compose of GAG and Type II collagen. In this study, we has investigated cartilage ECM-derived biomaterials for use as scaffolds for nucleus pulposus tissue engineering.

The objectives of the study were to (1) produce and characterize a cartilage ECM-derived scaffold for NP tissue engineering, (2) assess the growth of BMSCs on the novel scaffold in vitro to construct NP-like tissue.

METHODS:
Human cartilage was physically shattered, then decellularized sequentially with hypotonic buffer, TritonX-100, and then was lyophilized and made into a suspension. The scaffold was fabricated by simple freeze drying and cross-linking technique using EDAC and NHS.

Cartilage ECM-derived scaffolds were characterized by SEM, histology (Safranin O, II collagen immunofluorescence and H33258 staining) and cytotoxicity assay. BMSCs were isolated and chondrogenically induced (Safranin O, II collagen immunofluorescence) and then were labeled with the fluorescent dye PKH26. Then cells were seeded on scaffold. The constructs were cultured in vitro. Then harvested tissue was evaluated by histology, immunohistochemistry and Immunofluorescence examination.

RESULTS:
On histology, scaffolds showed most of the ECM components after removal of the cell fragments (Fig.1 A, B, C), and scanning electron microscopy revealed a 3-D interconnected porous structure (Fig.2 B,C,D). Cellular viability assay revealed no cytotoxic effects. In vitro study showed that the novel scaffold could provide a suitable 3-D environment to support the adhesion, proliferation and differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) to NP-like cells in culture with chondrogenic medium after 21 days.

NP-like cells labeled with PKH26 were then grown on scaffolds (Fig.4) and cultured in vitro for 2 week. NP-like tissue formed, with positive staining for Safranin O, toluidine blue and collagen II. Cells in the samples seemed to confirm that they originated from the labeled BMSCs, as confirmed by in vivo fluorescent imaging and immunofluorescence examination (Fig.5,6,7).

DISCUSSION:
This project demonstrates the feasibility of developing a cartilage ECM-derived 3-D interconnected porous scaffold for NP tissue engineering. The novel scaffold retains most of the cartilage ECM components (GAG and Type II collagen) and has good structure and biocompatibility, which makes it a suitable candidate as a supportive structure for NP-like cell repopulation. In vitro study demonstrated that the cartilage ECM-derived scaffold provides adequate 3-D support for the attachment, proliferation and differentiation of BMSCs into NP-like cells.

REFERENCES