Insoluble Type II Collagen as a Novel Scaffold Material for Articular Cartilage Tissue Engineering

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Disclosures

All authors have no conflicts to disclose related to this research project.

Introduction

Articular cartilage is a viscoelastic tissue responsible for facilitating compressive resistance and frictionless movement of articulating, weight-bearing joints. It does so via a combination of proteoglycans intertwined with a spatially organized network of type II collagen [1]. Even though type II collagen is the predominant collagen type in articular cartilage, it currently is not a commonly used material in developing tissue engineering-based strategies for articular cartilage repair. Due to the inherent difficulty in obtaining intact, insoluble type II collagen either by material cost or shortcomings with purification efficiency, type I (or even soluble type II) collagen is instead a more attractive option in articular cartilage tissue engineering [1]. In response to these challenges, we developed a method to obtain intact, insoluble type II collagen. After demonstrating internally in our lab its superiority in *in vitro* chondrocyte redifferentiation over intact, insoluble type I collagen, we share here the feasibility of intact, insoluble type II collagen as a scaffold for articular cartilage repair.

Materials & Methods

Type II Collagen: The method to obtain intact, insoluble type II collagen from articular cartilage was adopted from a previous method with modifications [2]. After processing, samples were digested with proteinase-k for DNA harvesting (Qiagen) and quantification (ABCAM) or digested with pepsin for SDS-PAGE analysis. Residual GAGs were determined via uronic acid analysis by reacting hydrolyzed samples with carbazole, using D-Glucuronic acid as the standard. *Type II Collagen Scaffold Fabrication:* Fabrication of scaffolds from the intact, insoluble type II collagen was adopted from our group's collagen microfiber ink technology as described previously [3]. The intact, insoluble type II collagen was cryomilled then sieved to collect microfiber-sized powder ranging from 25-50µm. A paste was then prepared to a density of 0.15g of collagen microfibers per milliliter of buffer by gradual addition of the microfiber powder while vortexing and then degassing via centrifugation. This paste was incubated for 1hr at room temperature prior to injecting into 96well plates, then frozen to either -20°C at a speed of 0.8°C/min or flash-frozen at -80°C and lyophilized to obtain cylinders with a large (LPS) or small (SPS) pore size (respectively). 3mm tall discs were cut from the cylinders, crosslinked with EDC and NHS, then washed with 0.1M Na_2HPO_4 and purified water prior to lyophilization. *Morphology and Pore Structure:* Vertical cross-sections of crosslinked, lyophilized scaffolds were observed via SEM (Hitachi S-3400) and pore diameters from 3 separate scaffolds were measured with ImageJ. *Chondrocyte Isolation:* Fresh bovine knee joints were obtained from the local abattoir. Full-thickness articular cartilage slices were harvested then minced into approximately $1mm^3$ pieces then digested in growth medium (DMEM, 10% FBS, 1% P/S) supplemented with 0.25% collagenase type II overnight in a 37°C incubator under nutation. The solution was then filtered through a cell strainer and centrifuged to collect the isolated articular chondrocytes. The isolated chondrocytes were washed with sterile PBS, seeded into T75 flasks, and then subcultured to P1. *Cell Culture:* 1×10^6 P1 chondrocytes were injected horizontally into EtO-sterilized LPS and SPS scaffolds and cultured in growth medium and normoxia for 1 week, then switched to chondrogenic medium and 5% O_2 for 28 days. Scaffolds were kept on an orbital shaker at 100RPM throughout the study. *Cell Response to Pore Size:* RNA was harvested from scaffolds (n=6) via TRIzol and relative gene expression to GAPDH of Col1α1, Col2α1, and Aggrecan was measured with RT-PCR. Distribution of GAGs relative to the injection pathway was visualized with Toluidine Blue. Student's t-test was used to determine significance ($p<0.05$).

Results

Figure 1 shows the SDS-PAGE band of the processed articular cartilage, as well as the average residual DNA and uronic acid content across 3 separate lots of processed articular cartilage. These results demonstrate our method's successful decellularization, majority removal of the proteoglycan content, as well as removal of other components of the raw articular cartilage to obtain mainly intact, insoluble type II collagen. Figure 2 shows that scaffolds featuring either large pores (137 ± 48µm) or small pores (45 ± 12µm) can be fabricated from the same collagen microfiber paste by changing the freezing condition. Figure 3A-B shows that after both 14 and 28 days in CDM, chondrocytes cultured in LPS scaffolds expressed a greater average Col2/Col1 ratio as well as average Aggrecan gene expression. Figure 3C shows horizontal cross-sections of cultured scaffolds stained with Toluidine Blue. Cells depositing GAGs can be observed throughout the LPS scaffolds, whereas the majority reside near the injection pathway in the SPS scaffolds. Overall, Figure 3 demonstrates an average scaffold pore size generally greater than 100µm to be positive for chondrocyte migration, proliferation, and redifferentiation *in vitro*.

Discussion

Collagen is an attractive choice as a biomaterial for tissue engineering due to its capabilities to be resorbed, remodeled by resident cells, and to influence certain favorable cell behavior when compared to synthetic polymers alone. For articular cartilage repair, soluble type II or type I collagen has been commonly used even though the tissue is composed of mainly insoluble type II collagen. We presented here the feasibility of producing and using intact, insoluble type II collagen as a scaffold material for articular cartilage tissue engineering. Our purification process utilizes familiar collagen isolation methods, allowing for straightforward and large-scale procurement of intact, insoluble type II collagen. Via our microfiber ink technology, we can fabricate high-density scaffolds that can translate to higher mechanical properties which is critical for weight bearing tissues. Even at such high collagen density, scaffolds can still be fabricated with pore sizes appropriate for chondrocyte growth and function by controlling the freezing condition. This work we hope will persuade the use of intact, insoluble type II collagen in future developments of articular cartilage repair strategies.

Significance

Material choice is one of the first considerations taken when developing scaffolds for tissue repair. Obtaining type II collagen in its native form and demonstrating its utility as a scaffold material is critical in developing an implant with an appropriate material composition for articular cartilage engineering.

References [1] Wu Z, *et al*. Biomater Biosyst. 2021;4:100030. [2] Steven FS, Thomas H. Biochem J. 1973;135(1):245-247. [3] Caparino K, Li S-T. Poster presented at: *Annual Meeting of the Orthopaedic Research Society*; February 10-14, 2023; Dallas, Tx.

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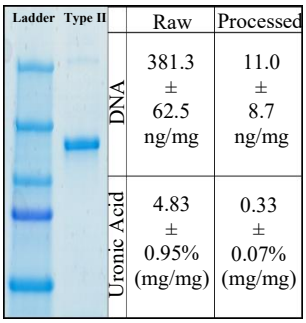

Figure 1: Residuals testing.

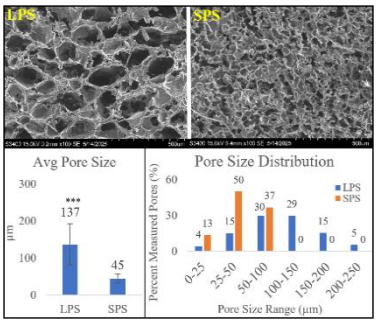

Figure 2: Pore structure & measurement. ***p<0.001

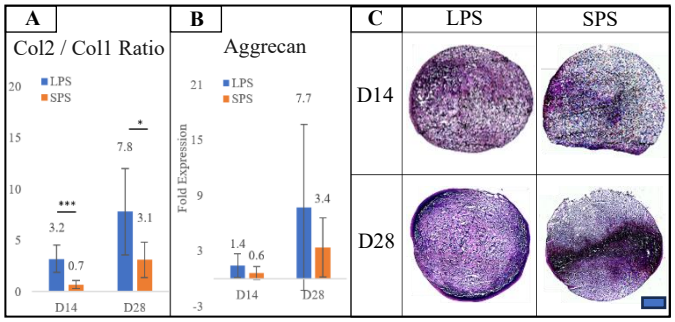

Figure 3: Cell Response. Gene Expression (A,B). Toluidine Blue staining on 10µm cryosections (C). *p<0.05 ; **' p<0.001; Scale bar is 1mm.