Enhanced Nutrient Transport Improves Depth-Dependent Properties of a Tri-layered HA Construct With Zonal Co-culture of Chondrocytes and MSCs

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Introduction

Biomimetic design in cartilage tissue engineering is a challenge given the complexity of the native tissue. Further, while chondrocytes (CHs) are one source for tissue engineering, they are limited in number and so mesenchymal stem cells (MSCs) are considered a promising alternative, especially when co-cultured with CH. In this context, CHs can enhance the initial efficiency of MSC chondrogenesis, as well as limit hypertrophic changes in some instances.[1,2] Moreover, a number of studies have shown that zonal CHs seeded in layered hydrogels can produce depth-dependent inhomogeneity, suggesting that these zonal CHs maintain their identity in 3D culture.[3] Recently, we showed that the zonal CH populations retained their native production levels and influenced MSC fate decisions in hyaluronic acid (HA) hydrogel co-cultures,[4,5] and created a tri-layered HA construct with mixed zonal CHs and MSC subpopulations to mimic the depth-dependent properties of cartilage.[6] One limitation of that study was that matrix deposition in the core region of the construct was limited, due to poor nutrient transport. To improve nutrient supply, recent studies have used dynamic loading, media perfusion, microbubbles and macro-channels.[7] Here, we used a porous hollow fiber (combined with cotton thread) as a transport pathway, and investigated the effects of this modification on the maturation of the tri-layered construct and on its zonal integration when used to fill a cartilage defect.

Methods

(Study 1) MSCs (P3) and zonal articular CHs were isolated from juvenile bovine knees. Full-thickness cartilage was excised from the femoral condyle and divided into three layers to obtain CHs from the superficial (SCH; top 100 μm), middle (MCH; top half of remaining cartilage), and deep (DCH) zone (bottom half of remaining cartilage). CHs were isolated by collagenase digestion and expanded through passage 4. MSC-only or mixed cell populations (MSC:CH ratio = 4:1) were encapsulated at 60×106 cells/mL in 1% w/v HA hydrogel (Lifecore Biomedical).[8] Tri-layered constructs were created by exposing the first layer (DCH-MSC) of the cell-HA solution to UV light for 2 minutes, followed by polymerization of the second layer (MCH-MSC) for 4 minutes, and finally adding the third layer (SCH-MSC) and completing polymerization via UV for another 6 minutes (Figure 1A).To improve nutrient transport, a porous hollow fiber (HF; ID=700μm, pore size=0.1μm) (alone or with a cotton thread passed through it) was inserted through the core along the axial direction on day 3 (Figure 1B-C).[9]

(Study 2) To investigate the effects of zonal chondrocytes on integration with native cartilage, tri-layered constructs were used to fill a defect (ID: 5 mm) by polymerizing the layers in situ or by placing the construct into the defect after in vitro polymerization (Figure 1J-M), with zone-to-zone matching (Figure 1L). Constructs (Ø4.8 × 3.5 mm) were cultured in a defined medium containing 10ng/mLTGF-β3, with media changed thrice weekly, and constructs were turned regularly to improve growth through the depth. Cell viability, distribution and proliferation of mixed populations of MSCs (blue), superficial (red), middle (purple), and deep (green) zone chondrocytes were followed using CellTracker (Molecular Probes). Bulk properties were assessed via unconfined compression, and local properties[10] were determined using a custom microscope compression device and texture correlation.[11] Glycosaminoglycan (GAG) and hydroxyproline contents were determined. Paraffin or cryo-sections (8μm) were stained with Alcian Blue for proteoglycans (PG). Significance was determined by two-way ANOVA with Tukey's post hoc tests ($p<0.05$).

Results

MSC and CH were viable over the 16 week culture period, with each cell population well mixed within its appropriate layer (Figure 1D-F). Constructs with HF and HF/thread maintained initial construct dimensions without swelling, and cells in the core were more viable (data not shown). Bulk properties and GAG content in all groups increased with time, reaching ~600 kPa and 4%WW at 16 weeks (Figure 1G-H), levels comparable to native cartilage (dashed line). Cells in the core region without HF enlarged (became

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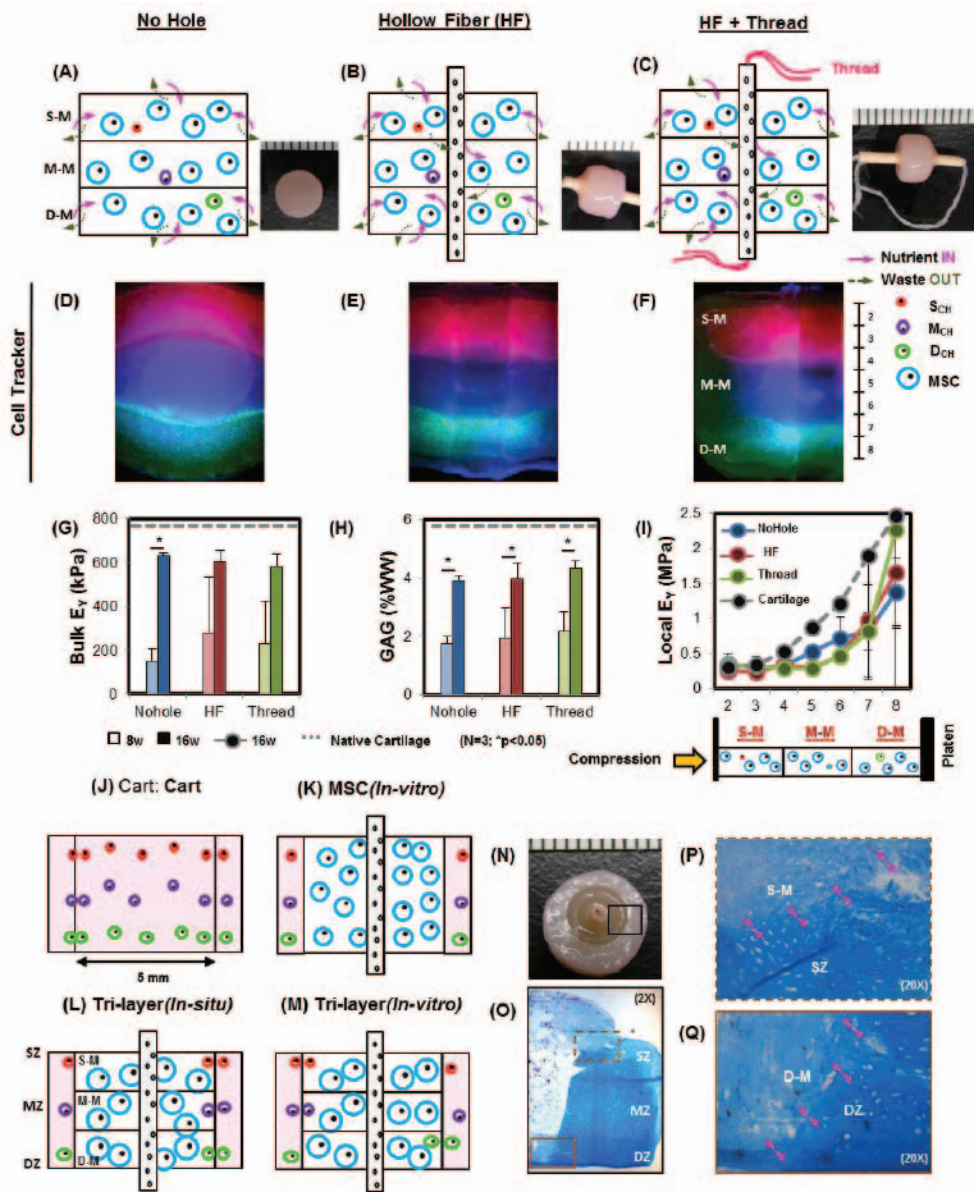

Figure 1. [Study 1] Schematics and macroscopic images (at 16 weeks) or tri-layered construct (A) No hole, (B) Hollow fiber (HF), (C) HF with thread (HF + thread). (D-F) Visualization of superficial zone CHs (red), MSCs (blue), deep zone CHs (green) at 16 weeks (cell mixture, CH:MSC = 1:4). (G) Bulk modulus (kPa), where lighter bars indicate 8 weeks and darker bars indicate 16 weeks. (H) GAG (%WW) content. (I) Local modulus (MPa) in Nohole (blue), HF (red), HF + thread(green), and native cartilage (gray), where region 8 is the deep zone (n=3 *p<0.05). [Study 2] Zone-to-zone cartilage integration schematics: (J) Cartilage/cartilage (control), (K) cartilage/MSC (in vitro), (L) cartilage:Tri-layer (in situ), (M) cartilage:Tri-layer (in vitro). (N) Macroscopic image of cartilage:Tri-layer group (in vitro), (O-Q) Alcian blue staining (pint arrows indicate interface between repair tissue and host cartilage).

hypertrophic), while those with a HF maintained a normal cell morphology (data not shown). The local modulus of the tri-layered constructs showed depth-dependent properties (~0.3MPa in the 'surface' region and ~1.4-2.3 MPa in the deep region, Figure 1I). The local modulus of the construct with HF/thread (green) in the deep region (DCH-MSC) nearly matched native levels. While the overall depth dependence mirrored native tissue, properties in the central regions were still lower than that of the native tissue. When these constructs were used to fill a cartilage defect, zonally-matched integration began from the outer surfaces (Figure 1N-Q).

Discussion

In this study, we fabricated a tri-layered HA construct with zonal co-culture of CH and MSCs in each layer to mimic the depth-dependent properties of articular cartilage. Given the thickness of the scaffold, we included a porous HF (coupled with thread) to improve nutrient transport into the core of the construct. Although the cells in the core region of the construct with HF showed robust PG production, there was neither a positive nor adverse effect on bulk properties, which are mainly governed by peripheral matrix properties. However, the HF did ensure that the tri-layered construct retained a

distinct cellular organization (without swelling) and produced constructs with near-native depth-dependent properties. The ratio of local modulus of the D-M:S-M layer in the tri-layered construct with HF/thread, HF, and control constructs was 6.8, 6.5 and 4, respectively (whereas the ratio for native cartilage was 8.1; DZ:SZ). With the tri-layered construct, we further investigated the effect of zone-to-zone cartilage integration, where zonal chondrocytes in each layer of the construct were matched to the host tissue. Filling cartilage defects in a zonally consistent manner might allow for better interaction between engineered construct and host tissue, though longer term studies are required to validate this finding. Taken together, our results demonstrate that a layer-by-layer fabrication scheme, including co-culture of zone-specific articular CHs and MSCs, can reproduce the depth dependent characteristics and mechanical properties of native cartilage. Such a tri-layered construct may provide critical advantages for focal cartilage repair. Ongoing studies will determine how individual cells within each layer communicate with one another and with adjacent layers, and scale this technology to produce constructs of anatomic relevance for cartilage repair applications.

Significance

Biomimetic design in cartilage tissue engineering is challenging. A tri-layered construct comprised of zonal CHs co-cultured with MSCs generated depth-dependent properties that mirrored native tissue by 16 weeks, particularly when the central nutrient supply was increased via a porous HF (w/ thread). This tri-layered engineered cartilage with zonal CHs/MSCs co-culture holds promise for the repair of focal defects.

Acknowledgments

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