Hydrogels with Gradient Niche Cues Enhance Cartilage Regeneration with Zonal Organization

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Statement of Purpose: Cartilage tissue is characterized with zonal organizations, with increasing stiffness from superficial to deep zone. However, most scaffolds developed to date are homogeneous in nature, and fail to recapitulate the zonal tissue structures and functions. Furthermore, how gradient niche cues such as matrix stiffness influence cartilage development remains largely unknown. The goal of this investigation is to mimic cartilage zonal organization via engineering hydrogels with mechanical gradient cues, and to examine the effects of mechanical gradient cues on modulating cartilage matrix formation by chondrocytes in 3D.

Methods: Hydrogels were constructed using 8-arm poly(ethylene glycol) – norbornene (MW 10K), linear PEG dithiol (MW 1.5K) and methylcrylated chondroitin sulfate. Primary (passage 0) bovine neonatal chondrocytes were mixed with hydrogel precursor solution before loaded to a gradient generator to produce gradient hydrogel solution. Chondroitin sulfate methacrylate (CS-MA) was added to the hydrogel network to better mimic the function of native cartilage extracellular matrix (ECM), and enables enzymatic degradation and matrix turnover by cell-secreted chondroitinase. To visualize the formation of biochemical gradient, FITC-conjugated peptide was used as a model biochemical cue. Cell-containing hydrogels was cultured in chondrogenic medium for up to 21 days to examine the effects of mechanical gradient cues on cartilage formation. To examine the effects of block mechanosensing on cartilage formation, samples were cultured with or without blebbitatin. Outcomes were analyzed using biochemical assays, gene expression, and histology.

Results: Mechanical testing confirmed formation of hydrogels with mechanical gradient cues (Fig. 1A). Using FITC-conjugated peptide, we also confirmed our ability to form biochemical gradients (Fig. 1B). Increasing hydrogel stiffness led to a dose-dependent increase in cartilage gene expression (Sox9, aggrecan and Collagen II), decrease in cell proliferation (Fig.2A), and increase in cartilage matrix deposition by biochemical assays (hydroxyproline and sGAG) (data not shown). Blebbistatin largely abolished the stiffness-dependent increase in cartilage gene expression and matrix formation (Fig. 2A). Immunostaining confirmed that the trend of resulting cartilage zonal organization mimics the superficial to deep zones of native articular cartilage (Fig. 2B). Our results highlight the importance of mechanosensing in cartilage development. Such gradient hydrogels may be broadly useful for elucidating the effects of mechanosensing on influencing other cell fates and tissue development using reduced materials and time.

Conclusions: Here, we report a facile method to fabricate 3D gradient hydrogels with continuous mechanical or biochemical gradient cues that mimic native articular cartilage zonal structures. Our platform supports rapid formation of gradient hydrogels of tissue scale (i.e. cm) that allow homogeneous cell encapsulation in 3D. When encapsulated in 3D hydrogels with mechanical gradient, chondrocytes show stiffness-dependent cellular responses that correspond to specific zones of articular cartilage. We anticipate that this method of forming 3D gradient hydrogels with biomimetic niches cues may be broadly useful for engineering other tissues with heterogeneous zonal organization or reconstructing tissue interfaces such as osteochondral defects, bone-ligament interface and bone-muscle interface etc.

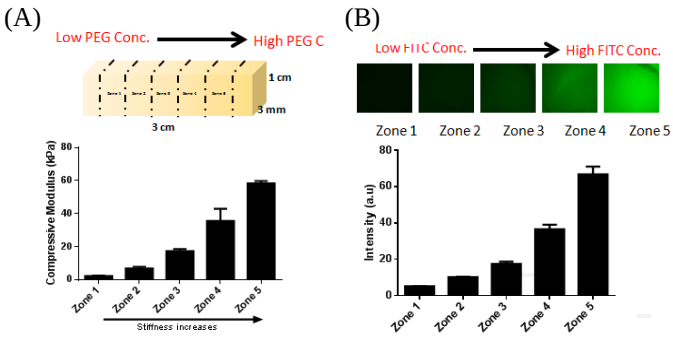

Figure 1. (A) Mechanical testing confirmed the formation of hydrogels with stiffness gradient. (B) Formation of biochemical niche gradient as shown by FITC-conjugated peptide.

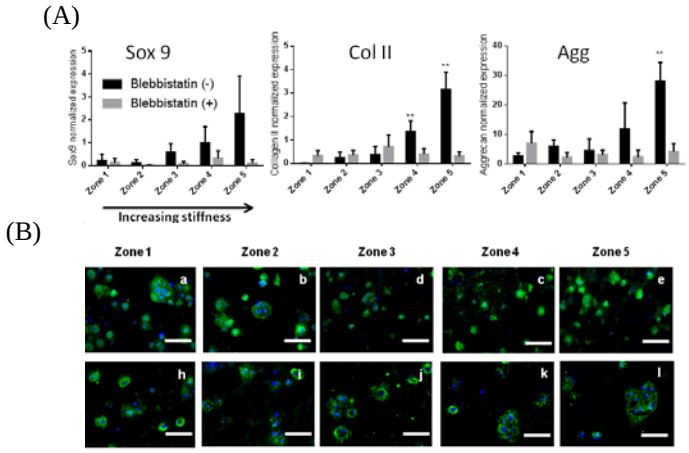

Figure 2. (A) Hydrogels with mechanical gradient cues led to stiffness-dependent cartilage marker gene expressions in 3D. (B) Gradient hydrogels promotes neocartilage matrix deposition that mimics native cartilage zonal organization; type II collagen (top row) and aggrecan (bottom row). (Scale bar = 50 um).