Developmentally Inspired Zone-Specific Chondrogenic Differentiation of Human Mesenchymal Stem Cells

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Statement of Purpose: Articular cartilage is organized into multiple zones including the superficial, pre-hypertrophic middle/deep, and hypertrophic calcified zones with distinct cellular and extracellular components to impart the desired functions of lubrication, compressive strength, and load transmission to the underlying bone tissue. During embryonic chondrogenesis, differentiating mesenchymal condensations arrange into multiple morphologically distinct zones as a result of spatiotemporal gradients of matrix stiffness and chondrogenic signaling factors. We hypothesized that the zonal organization of articular cartilage is formed and maintained by the combination of gradients in matrix stiffness and zone-specific growth factors. The hypothesis was tested by measuring the response of human mesenchymal stem cells (hMSCs) encapsulated in resorbable PEG-based gels simulating the stiffness of the superficial, middle and calcified zones of articular cartilage supplemented with zone specific growth factors.

Methods: The acrylated lactide-chain-extended polyethylene glycol (SPELA) macromer was used for simulating the zonal modulus of the articular cartilage. The hydrogel with 15% SPELA in aqueous solution (10% lactide) was used to simulate the 80 kPa modulus of the superficial zone; the gel with 50% SPELA (7.5% lactide) was used to simulate the 2.1 MPa modulus of the middle zone; and the gel with 35% SPELA (5% lactide) laminated with aligned poly(L-lactide) nanofiber microsheets oriented perpendicular to the surface of the articular surface was used to simulate the 230 MPa modulus of the calcified zone. Human MSCs at a density of $60x10^6$, $20x10^6$, and $15x10^6$ cells/mL were encapsulated in the gel simulating superficial, middle, and calcified zones, respectively. The gels simulating the superficial, middle, and calcified zones were loaded with BMP-7 (100 ng/mL), IGF-1 (100 ng/mL), and nanoapatite crystals (3%), respectively. All gels were loaded with TGF-β1 (100 ng/mL). The cells-encapsulated and growth factor-loaded gels were incubated in basal medium for 21 days. At each time point, the constructs were evaluated with respect to cellularity, GAG content, total collagen, alkaline phosphatase (ALP) activity, and the expression of aggrecan (AGC), collagen type II (COL II), Col X, ALP, superficial zone protein (SZP), and Sox-9. The morphology of the encapsulated cells were assessed histologically by staining with Alcian blue (GAG) and H&E.

Results: Images in Figure 1B-D compare GAG stained histological sections (Alcian blue) of hMSCs encapsulated in Superficial (B), Middle (C) and Calcified (D) gels after 21 days incubation in chondrogenic medium. The rounded cells increased in size from Superficial to Middle and Calcified gels compared to that cultivated in basal medium (not shown). The cells in Calcified gel were in isolated Lacunae consistent with their morphology in the native calcified layer of articular cartilage tissue. GAG content of the samples progressively increased from Superficial to Middle and Calcified gels compared to those gels cultured in basal medium.

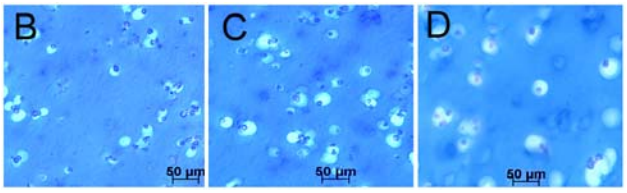

Figure 1. GAG) stained sections of the hMSCs encapsulated in Superficial (B), Middle (C), and Calcified (D) gels.

The hMSCs in the simulated superficial zone gel showed up-regulation of Sox-9, the early-stage marker of chondrogenesis, and SZP (Figure 2A) whereas hMSCs in the simulated calcified zone gel showed up-regulation of Col X (Figure 2C), the late-stage marker of hypertrophic chondrocytes, and ALP. Further, hMSCs in the simulated middle zone gel had highest total collagen content (Figure 2B) and highest expression of Col II, AGC, and GAG markers among all three gels.

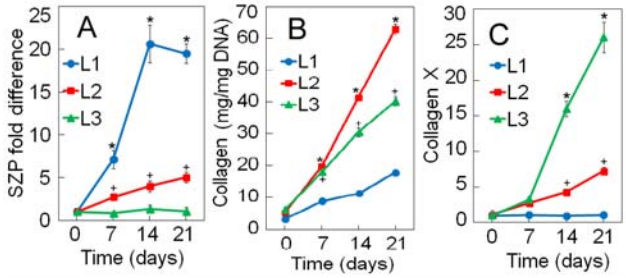

Figure 2. SZP (A), total collagen (B), and COL X expression of the hMSCs encapsulated in Superficial (L1, blue), Middle (L2, red), and Calcified (L3, green) gels with incubation time in medium supplemented with zone-specific growth factors.

Conclusions: Results demonstrate that a developmental approach with gradients in cell density, matrix stiffness, and zone-specific growth factors can potentially regenerate zonal structure of the articular cartilage.

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