Engineered Cellular Hydrogels for Zonal Regeneration of Articular Cartilage

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Statement of Purpose: Osteoarthritis (OA) affects nearly 27M Americans with joint pain, loss of function and disability. Current treatment methods for cartilage injury rarely restore full function to the articular cartilage. Conventional constructs for cartilage regeneration do not take into account the zonal complexity of articular cartilage, leading to long-term degeneration and fibrocartilage formation. The complexity of cartilage structure is rooted in embryonic development of the tissue in which mesenchymal stem cells (MSCs) begin to arrange in morphologically distinct zones that reflect the spatiotemporal gradient of chondrogenic signaling factors [1]. The differentiated cells form three distinct zones, namely, the superficial zone for lubrication, middle zone for dimensional stability, and calcified zone for load transmission to the underlying bone matrix [2]. Each zone is maintained by a distinct combination of cells, matrix, and growth factors. The objective of this work was to develop composite hydrogels for encapsulation of MSCs and their lineage-specific differentiation to the superficial, middle, and calcified zones.

Methods: For the superficial zone with 80 kPa compressive modulus, human MSCs were encapsulated in star lactide-chain-extended polyethylene glycol (SPELA) macromer and loaded with a combination of TGF-β (3 ng/mL) and BMP-7 (300 ng/mL); for the middle zone with 2.1 MPa modulus, MSCs were encapsulated in SPEAL gel and loaded with a combination of TGF-β (30 ng/mL) and IGF-1 (100 ng/mL); and for the calcified zone with 320 MPa modulus, MSCs were encapsulated in SPELA gel laminated with poly(lactide) (PLA) nanofibers and loaded with TGF-β (30 ng/mL). Nanofibers were aligned in the thickness direction to mimic the morphology of the fibers in calcified zone. Cell densities of 60M, 20M, and 10M cell/mL were used for the superficial, middle, and calcified zones, respectively. The three hydrogels were incubated in chondrogenic medium. After incubation of the cell-encapsulated hydrogels in chondrogenic medium, the expression of markers specific to each zone (SOX9 and ZSP for superficial [3], aggrecan for middle [4], and ALP for calcified zone [5]) was measured with incubation time.

Results: Figure 1a, b, and c show schematic diagram of the cell-encapsulated gels in the superficial (blue), middle (pink), and calcified (green) zone, respectively. For the calcified zone, the gel was laminated with PLA fibers parallel to the direction of thickness, similar to that of native calcified cartilage. The total collagen content of the middle load-bearing zone (orange) was higher than the other two layers (blue and purple), as shown in Figure 1d and the GAG content of the middle and calcified zones (orange and purple) was higher than the superficial zone (blue) (Figure 1e). The expression of SOX9 and SZP genes in hydrogels simulating the superficial zone increased with time (Figure 1f); aggrecan showed significant increase in the hydrogels simulating the middle zone (Figure 1g); ALP activity increased significantly in hydrogels simulating the calcified zone (Figure 1h). These results demonstrate that the unique combination of growth factors and hydrogels leads to expression of markers specific to each cartilage zone by the encapsulated MSCs.

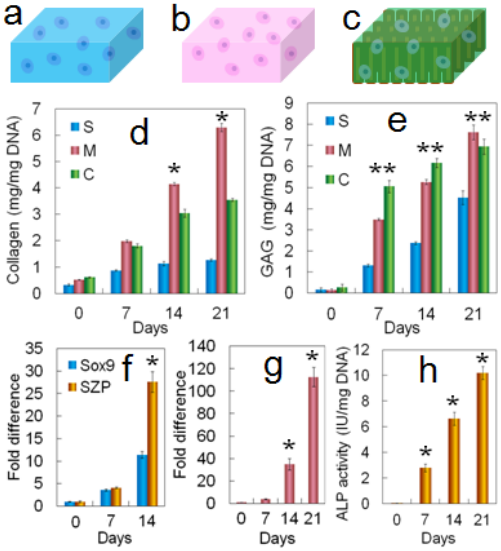

Figure 1. Schematics of the gels in the superficial (a), middle (b), and calcified (c) zones; total collagen (d) and GAG (e) content of the superficial (S, blue), middle (N, orange), and calcified (C, purple) gels; expression of SOX-9 and SZP mRNA (f), aggrecan mRNA (g), and ALP activity in gels corresponding to superficial, middle, and calcified zones, respectively.

Conclusions: Novel gels are developed with tunable stiffness and degradation for encapsulation of MSCs and their differentiation to individual zones of articular cartilage. The results show that combination of zone-specific growth factors and zone-specific matrices can direct differentiation of MSCs to superficial, middle, and calcified phenotypes.

References: [1] Akiyama H. J Bone Miner Metab. 2011;29:290-395. [2] Silverberg JL. J Orthop Res. 2013;31:686-691. [3] Andrades JA. Arthritis Res Ther. 2012;14:R72. [4] Las Heras F. Orthop Clin North Am. 2012;43:155-171. [5] Herlofsen SR. Tissue Eng A. 2011;17:1003-1113.

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