Hydrogel Effects on Long-Term Maturation of Chondrocyte and MSC-Laden Hydrogels

Isaac E. Erickson, Cindy Chung, Alice H. Huang, Ryan T. Li, Jason A. Burdick, Robert L. Mauck
Departments of Orthopaedic Surgery and Bioengineering, University of Pennsylvania, Philadelphia, PA
lemauck@mail.med.upenn.edu

Introduction: Mesenchymal stem cells (MSCs) hold great promise for cartilage tissue engineering (TE) given their ease of isolation, rapid in vitro expansion, and ability to undergo robust chondrogenic differentiation in 3D culture when exposed to defined media conditions [1-2]. We have previously reported, however, that MSC-seeded agarose hydrogels form constructs with lower mechanical properties than those from donor matched chondrocytes (CH) [2]. Similar results were found in alginate [3] and in a self-assembling peptide hydrogel [4] (Puramatrix) (which is permissive to chondrocyte-mediated mechanical maturation [5-6]). Taken together, these findings suggest an inherent limitation in matrix forming capacity by differentiated MSCs. To further this inquiry into hydrogel effects on chondrogenesis and functional maturation, this study examined donor-matched MSC chondrogenesis in 3 different hydrogels; agarose (Ag), Puramatrix (Pu), and in a novel photo-crosslinkable hyaluronic acid (HA) gel. This HA gel has previously been shown to support CH-mediated maturation in subcutaneous culture [7-9], though MSC chondrogenesis in this gel has not previously been reported. We hypothesized that MSCs would differentiate in each hydrogel, and that hydrogel type and properties would influence tissue maturation, with MSCs producing lower properties in all cases compared to their chondrocyte-laden counterparts.

Materials and Methods: Bovine CH and donor-matched MSCs were isolated and seeded (20 million cells/ml) in type VII agarose (20%w/v; Sigma) [2], methacrylated HA (290w/v) with 0.05%w/v I2959 photoinitiator [7], or peptide hydrogel (0.56%w/v, BD Bioscience). Cylindrical constructs (2.25mm thickness; 5mm diameter) were cultured in chondrogenic medium (1ml/construct) with TGF-β3 [2] for a total of 8 weeks. Cell viability was assessed throughout this time course using the Live/Dead and MTT assays (Invitrogen). Mechanical testing in unconfined compression was carried out bi-weekly to determine equilibrium and dynamic properties [2]. Total DNA, sulfated glycosaminoglycan (GAG), and collagen contents were determined at these time points as in [2]. Sections were stained with Alcian Blue for proteoglycan and Picrosirius Red for collagen. Significance was determined by ANOVA with Tukey’s post hoc tests.

Results: Overall, significant gains in modulus, GAG, and collagen were achieved for each hydrogel and cell combination by day 56 (p<0.001 vs. day 0). Ag-CH hydrogels attained modulus values several fold higher than HA-CH and Pu-CH (Fig. 1A). When seeded with MSCs, no differences were observed at 8 weeks between gel types (Fig. 1B). Interestingly, biomechanical properties and biochemical content were improved in both HA-MSC and Pu-MSC compared to their CH-laden counterparts (p<0.05), while Ag-MSC values were lower than Ag-CH (p<0.01; Fig. 1). Ag-CH hydrogels accumulated >3% GAG with lesser amounts in Pu-CH and HA-CH (p<0.001; Fig. 1C). The GAG content of Pu-MSC was higher than other MSC groups, reaching ~4% (p<0.001; Fig. 1C). Collagen content showed similar trends for all CH hydrogels (p<0.001; Fig. 1D). Pu-MSC accumulated more collagen than both HA-MSC (p<0.001) and Ag-MSC groups (p<0.01; Fig. 1D). However, this is likely due to the significant contraction observed in the Pu-MSC hydrogels. While Pu-MSC constructs decreased in diameter (by ~30%) and wet weight (~60% less than Ag-MSC) over the time course, these parameters remained constant or increased in all other groups. DNA content (Fig. 1E) and viability (not shown) of MSC groups was stable, while DNA in CH groups increased by 2-4 fold. Histological analysis confirmed GAG and collagen and DNA contents were improved in both HA-MSC and Pu-MSC compared to their CH counterparts (p<0.05). Pu-MSC showed similar trends for all CH hydrogels (p<0.001; Fig. 1D). Pu-MSC showed similar trends for all CH hydrogels (p<0.001; Fig. 1D). Pu-MSC showed similar trends for all CH hydrogels (p<0.001; Fig. 1D). Pu-MSC showed similar trends for all CH hydrogels (p<0.001; Fig. 1D).

Discussion: Consistent with previous findings [2] this study showed that Ag-CH constructs improved in mechanical and biochemical properties, and did so to a greater extent than Ag-MSC gels. Contrary to previous findings [4], Pu-CH gels showed poor maturation with properties emerging only at later time points. Consistent with in vivo findings, articular CH fared poorly in HA gels [10]. While perhaps not ideal for CH, seeding both HA and Pu with MSCs resulted in robust growth, equivalent to that observed for Ag-MSCs. This suggests that the biomaterial properties of each hydrogel system regulate and enable growth, depending on the cell type employed. However, regardless of hydrogel type, no MSC groups

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Fig. 1 Modulus (mean ± SD) of CH (A) and MSC-seeded (B) Ag, HA, and Pu with culture (n=3-5). Hydrogel comparisons significant (p<0.05) except noted (ns) (*ns vs. HA and Pu). Percent GAG (C) and collagen (D) on day 56 (mean ± SD; n=3-5). Inter-hydrogel comparisons were significant for GAG (p<0.001) and collagen (p<0.01) except noted (ns). All CH-MSC comparisons significant (p<0.05). Day 0 to 56 fold change in DNA (E).

Fig. 2 Day 56 Alcian Blue of CH (A-C) and MSCs (D-F) in Ag (top), HA (middle), and Pu (bottom). Picrosirius Red of CH (G-I) and MSCs (J-L) in Ag (top), HA (middle), and Pu (bottom).