Cyclodextrin-based Tuning of PEG Hydrogels for Improved Chondrogensis of Mesenchymal Stem Cells

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Statement of Purpose: Mesenchymal stem cells (MSCs) hold great promise in cartilage tissue engineering. However, to enable translation of stem cell therapies, there is a significant need to generate materials that can modulate and enhance differentiation. Here, we have developed a simple strategy to modify poly(ethylene glycol) (PEG)-based hydrogels, which resulted in significantly improved chondrogenesis of encapsulated MSCs. α-Cyclodextrin (α-CD) was introduced to form inclusion complexes with PEG-diacrylate (PEGDA) in pre-gel solution, followed by photopolymerization to prepare PEGDA-α-CD composite hydrogels.

Methods: PEGDA (Mw 3400 Da, Pdi 1.1) from SunBio (Orinda, CA); Bone-marrow derived gMSCs, Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Gaithersburg, MD), 365-nm ultraviolet (UV) light at 4 mW/cm^2 for photopolymerization of PEGDA were used. Live dead assay was performed by using Live/Dead Viability Kit (Molecular Probes, Eugene, OR); biochemical assay was performed for collagen content, DNA and GAG; cell morphology was studied by hematoxylin and Eosin staining; proteoglycan content was determined by Safranin-O/fast green staining and statistical analysis was performed with the SPSS (version 10.0; SPSS, Chicago, IL) software package.

Results: Based on histological and biochemical analyses, synthesis of cartilaginous extracellular matrix (ECM) including glycosaminoglycan (GAG) and type II collagen by encapsulated goat bone-marrow derived MSCs (gMSCs) in the composite hydrogels with less than 5% α-CD was significantly increased after a 3-week induction in chondrogenic medium and addition of 1% α-CD produced maximal chondrogenic induction. In a further 42-day *in vitro* study by comparing constructs with 0% and 1% α-CD, constructs with 1% α-CD supported greater survival and proliferation of gMSCs. Chondrogenic differentiation of gMSCs in 1% constructs became significant at as early as day 7 based on histostaining for GAG and type II collagen and resulted in more than two-fold increase afterwards in both GAG and collagen production reaching 62 and 41 μg/μg of DNA at day 42, respectively. An earlier upregulation in gene expression of Sox-9 was observed. Moreover, no such effects were observed when primary bovine chondrocytes were encapsulated in these composite hydrogels, which suggested that the specific interactions of the microenvironments created with the addition of α-CD with MSCs.

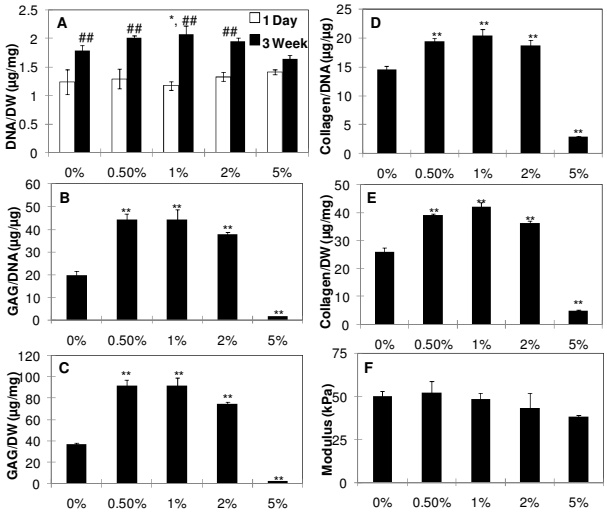

FIGURE 1. Comparison of DNA content, cartilaginous ECM components and gene expression of chondrogenic markers in constructs with gMSCs encapsulated containing different amounts of α-CD as indicated (0%, 0.5%, 1%, 2% and 5%, respectively) cultured in chondrogenic medium with TGF-β1 for 3 weeks. DNA content of constructs on day 1 and after 3 weeks were measured and normalized by the dry weight of the respective constructs (μg/mg) **(A)**. GAG amount was quantified by DMMB assay and normalized to the DNA content (μg/μg) **(B)** for comparison as well as by the dry weight as well (μg/mg) **(C)**. Total collagen content was also determined by hydroxyproline assay and normalized to the DNA content (μg/μg) **(D)** and by the dry weight of the respective construct (μg/mg) **(E)**. The equivalent moduli of the constructs were also compared after 3 weeks **(F)**. All data were presented as mean ± standard deviation (n = 3). **P*<0.05 and ***P*<0.001, compared to 0% constructs; $^{\#\#}P$<0.001, compared to day 1 of the respective group.

Conclusion: In conclusion, by the addition of α-CD, the chondrogenesis of MSCs could be dramatically improved in PEG-based hydrogels. This simple strategy for modifying PEG had a significant on cell behavior and also opens a new strategy. Further chemically-modified CDs and their effect on stem cell differentiation are currently under investigations with PEG and other biopolymer-based hydrogels.