Chondroitin sulfate based hydrogel niche for chondrogenic differentiation of Mesenchymal stem cells

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Statement of Purpose: Tissue engineering approaches that utilize mesenchymal stem cells (MSCs) in conjunction with three-dimensional scaffolds have great potential for repairing critical cartilage defects. Synthetic hydrogels such as poly(ethylene glycol) diacrylate (PEGDA) permit chondrogenic differentiation of MSCs by providing a three-dimensional (3D) support (1) despite being completely devoid of crucial cell-matrix interactions. However, biologically active scaffolds are required to yield efficient lineage-specific differentiation of MSCs owing to their multilineage differentiation ability. To test the above, we have created a bio-instructive scaffold by introducing chondroitin sulfate (CS) moieties into the PEGDA network via copolymerization and investigated its effect on chondrogenic differentiation of MSCs. Chondroitin sulfate was chosen because it promotes both chondrogenic activity and integration of engineered tissue with native tissue.

Methods: Mesenchymal stem cells were isolated from femurs of 3 to 3.5 years old goats as described elsewhere (1). We used trypsinized passage five cells for this study. Cells were encapsulated in two types of hydrogels: PEGDA and PEGDA/CSMA. PEGDA hydrogels were synthesized as described in Ref. (1). PEGDA/CSMA hydrogels were synthesized according to the procedure described in Li et al. (2). Twenty million cells were dispersed per ml of polymer solution and then photo-polymerized for 5 minutes. The cell-laden hydrogels were cultured using defined chondrogenic conditions in the presence of 10ng/ml TGF-β1 (1). Chondrogenic differentiation was evaluated by histological, biochemical and RNA analysis for cartilage specific genes.

Results/Discussion: We have synthesized hydrogels from two different precursors: PEGDA homopolymer and a mixture of PEGDA/CSMA (50/50) polymers. Photo-polymerization of PEGDA/CSMA mixture created an interpenetrating network (IPN) with structural properties comparable to PEGDA network. The hydrogels were photo-polymerized without cells for studying their gelation ability and swelling behavior. Both PEGDA and PEGDA/ CSMA solutions resulted in 'solid' gels within five minutes of UV light exposure indicating that they have similar polymerizing abilities.

The CSMA/PEGDA hydrogels had a higher swelling ratio (~18) compared to their PEGDA counterparts (~12). This might be either attributed to pendant sulfate groups of disaccharide repeat units in chondroitin sulfate, or to the low percentage of methacrylate groups. The swelling behavior of hydrogels provides useful information on their crosslink density, mechanical properties, and degradation profile. Cylindrical hydrogels with 6mm × 4mm reached their equilibrium swelling within 36 hours. The hydrogels retained the absorbed water content for the next 6 weeks without any significant change, indicating that they did not undergo any degradation during the experimental time.

Live/dead staining indicates that most of the cells were viable after photo-polymerization, and the presence of CS moieties in the network do not have any adverse effect on cell viability. MSCs encapsulated within PEGDA/CSMA network aggregated to form cell clusters, which grew with time and produced cartilaginous tissues (Fig1) as the network degraded. Cells displayed limited mitosis in PEGDA/CSMA hydrogel compared to its PEGDA hydrogels (Fig. 1). Quantification of matrix components showed higher GAG and collagen production, which was further supported by their respective gene expression. Moreover, a significant downregulation in typeX collagen was observed in CS/PEGDA scaffold indicating that CS may play a role in inhibiting or delaying hypertrophic differentiation of chondrocytes.

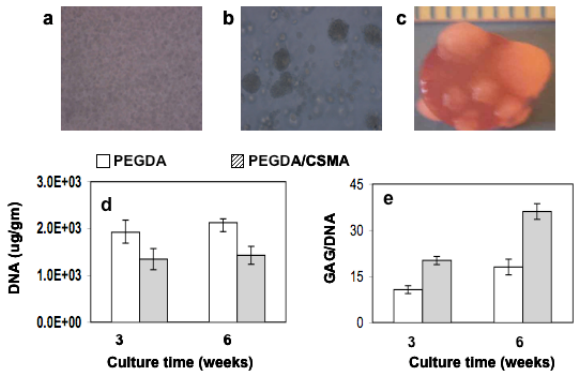

Fig.1. (a) Cells are distributed uniformly within the PEGDA/CSMA hydrogels, (b) aggregation of encapsulated cells after 3 weeks of culture (c) hydrogel containing grown cell aggregates next to a millimeter ruler, (d) & (e) quantification of DNA and GAG content

Conclusion: The results presented here indicate that CSMA/PEGDA scaffold promotes aggregation of encapsulated MSCs. This leads to enhanced chondrogenic differentiation and subsequent matrix production of MSCs. In sum, CS-based scaffolds provide a chondroinducive environment to MSCs that stimulates natural extracellular matrix.

Reference:
1. Williams C G et al. Tissue Eng. 2003, 9, 679
2. Li Q et al. J. Biomed. Mater. Res. A. 68, 28 (2004)