Heparin Hydrogels Promote Mineralization of Encapsulated Mesenchymal Stem Cells in Coculture with Osteoblasts

Song P. Seto, Ryan C. Adams, Johnna S. Temenoff.

Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA.

Introduction: Mesenchymal stem cells (MSCs) are often found in the proximity of mature tissues, such as bone, and may undergo dynamic signaling events that alter their differentiation (Zhou H. J Biol Chem. 2008; 283:1936-1945). One mode of communication between cell types is secretion of soluble factors. Heparin, a glycosaminoglycan found in the extracellular matrix of many tissues, has been implicated in sequestering growth factors with heparin-binding domains, thereby affecting their bioavailability (Capila I. Angew Chem Int Ed Engl. 2002; 41:391-412). Our laboratory is investigating the role that heparin and coculture can have in MSC differentiation in a 3D environment for the purpose of priming cells for delivery to defects in orthopaedic tissues. Utilizing a novel hydrogel system of oligo(poly(ethylene glycol) fumarate) (OPF) and heparin methacrylamide (heparin MAm) as an MSC carrier, markers of osteoblastic differentiation were measured as a function of heparin content when the constructs were exposed to coculture with osteoblasts for up to 21 days.

Methods: N-(3-aminopropyl) methacrylamide was conjugated to heparin to form heparin MAm by EDC/NHS chemistry. To fabricate hydrogels, heparin MAm was incorporated into PEG-diacrylate/OPF hydrogels at 0%, 1%, and 10% dry wt./total dry wt. An acrylated adhesive peptide, RGD, was incorporated at a concentration of 1µmole/g of hydrogel. Human MSCs were encapsulsted in macromer solutions at a final concentration of 10×10^6 cells/mL and photocrosslinked into 6mm disks with Irgacure D2959 at ~10mW/cm^2 UV light for 15 min. Human osteoblasts were seeded into 12-well plates at a density of 10,000 cells/cm^2 and allowed to proliferate for 7 days prior to the start of the study. Co-culture medium consisted of 10% serum, 10mM β-glycerolphosphate, 50µg/mL ascorbate-2-phosphate, 1% amphotericin B and 0.1% gentamicin. Hydrogels with MSCs were placed into transwells with 0.4µm pores and placed over osteoblasts for up to 21 days. On days 1, 7, 14, and 21 hydrogels were retrieved and homogenized for DNA content by the PicoGreen assay, alkaline phosphatase (ALP) activity by the p-nitrophenol assay, and calcium content by the Arsenazo III assay. Control samples, consisting of MSCs in coculture medium with no osteoblasts, were analyzed on day 21. Day 21 samples were also cryosectioned and stained with von Kossa.

Results: ALP activity was significantly higher in the 10% heparin samples by day 21 compared to all other formulations (Fig. 1). ALP activity did not significantly change over time in the 0% heparin hydrogels, while activity in the 10% heparin hydrogels increased from day 14 (2.4±0.5 nmol/min/µg DNA) to significantly higher on day 21 (5.0±0.5 nmol/min/µg DNA). By day 21, ALP activity in the 10% heparin group was significantly greater than all other conditions, including the controls (3.3±0.3, 2.3±0.3, and 0.7±0.3 nmol/min/µg DNA for 0%, 1%, and 10% heparin controls respectively).

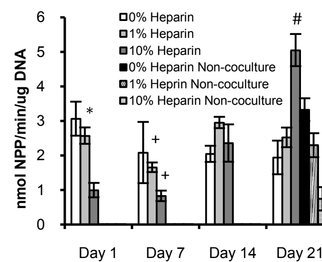

Figure 1. ALP activity in hydrogels up to 21 days. # indicates that ALP activity is significantly higher in the 10% samples vs. other formulations on day 21. * d1 is higher than d7 for the same formulation. + d7 is lower than d14 and d21 of the same formulation. n=3±S.D., $p<0.05$.

Calcium concentrations in the gels were not significantly different on day 1 across all formulations, but increased over time. By day 21, all coculture formulations reached a significantly higher calcium concentration than all previous days (Fig. 2). At day 21 there was at least a 15-fold increase in calcium concentration over day 14, with average concentrations of 7.0±3.1, 21.4±13.5, 65.6±26.1 µg calcium/ng DNA for 0%, 1% and 10% heparin gels respectively. Non-cocultured controls exhibited low levels of calcium deposition at 0.2±0.3, 0.4±0.5, and 0±0 µg calcium/ng DNA for 0%, 1%, and 10% heparin gels.

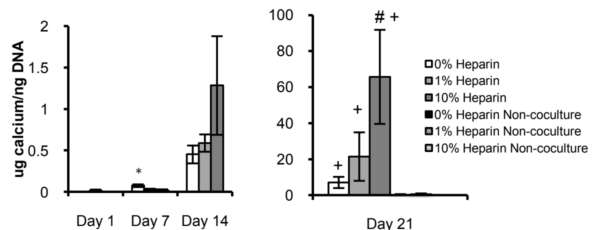

Figure 2. Calcium concentration levels in hydrogels up to 21 days. There was significant accumulation of calcium in the 10% heparin hydrogel samples by d21 vs. all other formulations. # indicates significantly higher calcium in 10% heparin gels vs. all other samples on d21. * calcium in 0% samples significantly higher than levels in 1% and 10% samples on d7. + d21 calcium levels are significantly higher than all other time points for the same gel type. n=3±S.D., $p<0.05$. Note different Y-axis scale for d21.

Mineralization of hydrogels with 10% heparin was macroscopically apparent after 14 days of coculture. However, non-cocultured controls did not exhibit significant mineralization. Von Kossa staining (not shown) revealed mineralization around cells across all cocultured hydrogels, but with more intense mineral deposits on the edges of the 10% heparin hydrogels.

Conclusions: By day 21, hMSCs cocultured in hydrogels with the greatest amount of heparin exhibited the highest calcium concentration and ALP activity. Non-cocultured controls showed negligible calcium deposition and low ALP activity. These results support a potential method of using biomaterials to enhance the effects of coculture. Although this proof-of-concept study explores only the effects of osteoblasts on MSCs, this concept can be extended to coculture with other types of cells in which dynamic signaling is important to develop specific phenotypes from stem cells.

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