Directed Assembly of Human Bone Marrow Stromal Cell-Laden Gels for Studying Paracrine Signaling

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Statement of Purpose: The development of simple, functional, and patternable technologies for the study of cell signaling in three dimensions (3D) is of great importance in engineering complex tissues. Significant research has already been performed on cellular signaling within patterned two dimensional systems, yet the transfer of this technology into 3D has proven difficult. Towards this end, the Khademhosseini lab [1] has utilized hydrophobic forces to direct the assembly of poly(ethylene glycol) (PEG) hydrogels. Using this technique, we have optimized fabrication parameters to yield high fidelity directed gel assemblies that retain maximal cell viability. In the future, we aim to use these directed assembly approaches to study the effects of scaffold material properties on paracrine signaling of hBMSCs during bone tissue regeneration.

Methods: Photopolymerization of PEG-dimethacrylate (PEGDM) was used to fabricate hydrogel building blocks for directed assembly. Many parameters were varied to optimize gel assembly versus cell viability and the optimized protocol follows. PEGDM prepolymer solutions in phosphate buffered saline (10 % to 20 % mass/vol with 1 % mass/vol Irgacure 2959 photoinitiator) were photopolymerized (365 nm, 2 min to 4 min, 2 mW/cm^2) in molds made from coverslides. RGD peptides (1 mM, GCGGGRGDS, synthesized by Fmoc solid-phase chemistry) were simultaneously photocross-linked into gels via the cysteine thiols to provide sites for cell adhesion. A photomask was used to obtain desired cross and rod shaped microgels. Directed assembly of gel building blocks was performed by transferring gel blocks to mineral oil and agitating with a pipette tip. Additional UV exposure (1 min) following assembly resulted in secondary crosslinking between gels to stabilize the constructs. MC3T3-E1 osteoblasts (Riken Cell Bank) were used to test viability during optimization of the fabrication/assembly protocols and then hBMSCs (Tulane Center for Gene Therapy, cultured according to supplier protocols) were used for final testing of optimized methods. For encapsulation, MC3T3-E1's or hBMSCs were suspended in sterile-filtered PEGDM monomer solutions (10^6/mL, containing Irgacure and RGD) prior to photopolymerization. Cell viability was measured using the calcein AM and ethidium homodimer-1 Live/Dead kit (Molecular Probes). In some cases, cells in gels were fixed and stained with Sytox green.

Results: The following fabrication parameters were optimized to yield the protocol described in the Methods: UV dose (distance, time), photoinitiator concentration (Irgacure), monomer concentration (PEGDM), RGD concentration, shapes on the photomask (geometry, size) and gel thickness. The methods yielded cross and rod shaped-gels whose assembly could be directed with hydrophobic driving forces. Typical assemblies had 50 % to 100 % cross openings filled by rods, but 100 % assembly was obtained by agitation with a pipette tip (Fig 1). Directed assembly was tested using a 10-fold excess of rods to crosses. MC3T3-E1's were 65 % viable after 1 d culture in 10 % PEGDM crosses or rods; this dropped to 55 % through 28 d. hBMSCs were 60 % viable after 1 d culture in 10 % PEGDM crosses or rods and viability dropped to approximately 50 % at 28 d. 10 % PEG gels generally showed 2 % to 5 % higher viability for hBMSCs (at all time points) than 20 % gels, though the gel shapes were the same. hBMSCs adopted a spherical morphology in the 3D gel cultures. Characterizing the effect of mineral oil, secondary crosslinking, and RGD on hBMSC viability is ongoing.

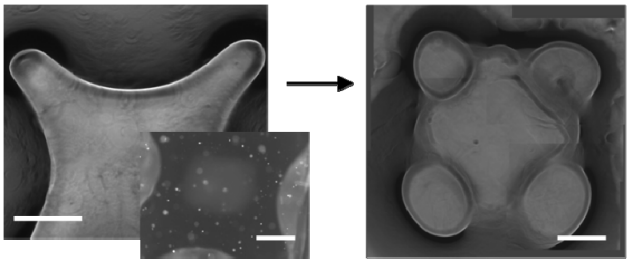

Figure 1: Cross-shaped gel building block before and after assembly with rod-shaped gels. Gels are 1 mm thick 10 % PEGDM. Left inset shows encapsulated hBMSCs at 21 d (Sytox green stained nuclei). Scale bars are 250 μm.

Conclusions: By carefully tuning the concentration of PEG, photoinitiator and RGD, and the size and shape of gel building blocks, we identified fabrication parameters that optimize the balance between hBMSC viability and integrity of the directed gel assemblies. Directed assembly proceeds with high efficiency where all crosses bind 4 rods in the presence of a 10-fold rod excess. Cell viability ranges between 50 % and 65 % depending on culture time, cell type and gel composition. Further development of this system will allow generation of more complex constructs with organized mechanical and chemical properties. We aim to use this technology to study the effect of material properties on paracrine signaling of hBMSCs during bone tissue regeneration.

Acknowledgements: C.T. was supported by a National Academies of Science/National Research Council Research Associateship. This work was supported by NIH/NIBIB R21 EB006497-01. Thanks to K. Chatterjee, G. Kumar, and N. Moore for critical input.

References:

1. Du, Y. PNAS. 2008; 105: 9522-9527.