Production of Highly Porous Bioactive Hydrogels by Self-Assembly of Phase Separated Poly(ethylene glycol) Microspheres in the Presence of Cells

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Statement of Purpose: We have recently developed methods to form poly(ethylene glycol) (PEG) microspheres by a coacervation process using kosmotropic salt solutions.[1] The formed microspheres can be separated from the salt solution and centrifuged with cells to form modular, porous scaffolds.[2] However, the mechanical weakness of scaffolds made by this method has impeded further research. We have found that the elastic properties of the formed scaffolds can be vastly improved by increasing the concentration of the kosmotropic salt solution. Microspheres form, phase separate from the salt solution, and then crosslink together to form the scaffold. However, this method cannot be used in the presence of living cells due to the high salt concentration. Alternatively, aqueous two phase systems (ATPS) of PEG and dextran are commonly used to separate living cells.[3] Cell-binding ligands may be used to help drive cells into the PEG phase.[4] We now demonstrate the encapsulation of cells in PEG microsphere scaffolds of improved strength through the use of a PEG/dextran ATPS. High cell viability is maintained during and after scaffold formation.

Methods: ATPS scaffolds were made by two methods: 1) *microgel scaffolds*: PEG-octa-vinylsulfone (PEG-OVS, MW 10K) and PEG-octa-amine (PEG-OAm, MW 10K) were synthesized as previously described.[1] The PEG derivatives were dissolved in PBS at 20% (w/v) at a 2:1 VS:Am ratio and crosslinked at 37ºC until d_{PCS} = 50 nm particles were observed by light scattering ("microgels"). The PEG-OAm was fluorescently labeled with Dylight-633 (Pierce) as previously described.[1] Dextran was added to give final polymer concentrations of 25% PEG and 15% dextran. The mixture was incubated at 37ºC for 2 h to allow phase separation and crosslinking. 2) *RGD-microsphere scaffolds:* To promote cell attachment, microgel solutions were reacted for 1 h with 5mM RGD peptide (GCGYGRGDSPG) (Genscript). Afterwards, microspheres were formed by a 45 minute incubation in 0.6 M sodium sulfate, followed by buffer exchange into PBS as previously described.[2] The microspheres were then suspended in medium with human foreskin fibroblasts and incubated for 2 h at 37ºC to allow the cells to bind the RGD on the microsphere surfaces. The cells and microspheres were subsequently centrifuged then resuspended in dextran to give final polymer concentrations of 25% PEG and 15% dextran. Two hours were allowed for PEG/dextran phase separation and additional microsphere crosslinking. Cell viability assays (Invitrogen) were performed.

Results: Preliminary studies without cells revealed that PEG crosslinking could be achieved at 37ºC, pH 7.4, and at osmotic pressures favorable to cell survival (e.g., in PBS or DMEM). Polymer concentrations of >10% (w/v) PEG and >10% dextran were required to attain thorough crosslinking. ATPS scaffolds formed by the microgel method were tough enough to be handled with forceps for at least 32 days (Figure 1a). In comparison, the scaffolds formed by the previously used centrifugation method could not be handled with forceps.[2] We were able to visualize the highly porous nature of the ATPS scaffolds through confocal microscopy of fluorescently labeled PEG (Figure 1b). The preference of cells for the PEG-OVs-RGD phase was analyzed in systems that did not crosslink (i.e. PEG-OAm was eliminated). Fibroblasts preferred the PEG phase when both dextran and PEG concentrations were >10%. Cell encapsulation and maintenance of high viability was possible using both of the described scaffold fabrication methods. However, a more homogenous distribution of cells within the scaffold was obtained by starting with microspheres rather than microgels. When ATPS scaffolds were fabricated from RGD-derivatized PEG microspheres in the presence of fibroblasts, viability was >99% at 24 and 48 hours after scaffold formation (n = 4). Figure 1c shows a live/dead assay of fibroblasts in an RGD-microsphere scaffold at 24 hours after scaffold formation. No dead cells were present in this sample. Furthermore, it is possible to maintain high viability for at least 5 days in culture.

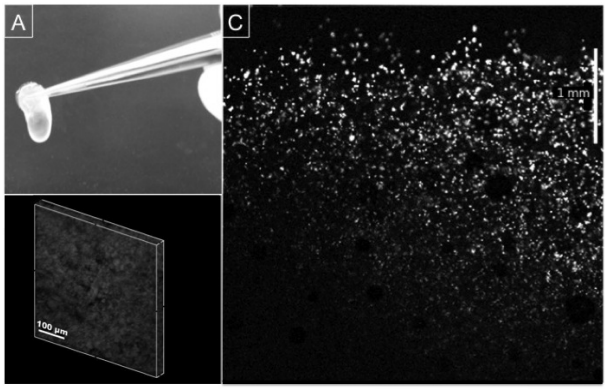

Figure 1: a) microgel scaffold, b) microgel scaffold, 20x, labeled with Dylight-633 c) calcien labeled cells in a microsphere-RGD scaffold

Conclusions: Scaffold formation via a PEG/dextran ATPS allows for the assembly of 100% PEG scaffolds that are modular and highly porous, yet also tough. This method permits cell encapsulation at the time of crosslinking and retention of high cell viability in culture. Future ATPS scaffolds will likely include our previously described degradable microspheres.[2] These will increase porosity, providing additional routes for vascularization.

References:

1. Nichols MD. Biomaterials. 2009;30:5283-91.
2. Scott EA. Acta Biomater. 2009;doi: 10.1016/j.actbio.2009.07.009
3. Nam KH. Biomed Microdevices. 2005;7:189-95.
4. Hamamoto R. J of Ferm and Bioeng. 1996;82:73-76.