Gradient Formation Mediated By Density Differences Of Polyethylene Glycol Microspheres

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Statement of Purpose: The formation of complex gradients in growth factors, drugs, cell adhesion peptides, degradability, mechanical properties and even cells may be useful for a variety of tissue engineering applications. We have previously developed novel methods to form micron-scale poly(ethylene glycol) (PEG) microspheres with different functionalities (cell adhesion, degradability, drug delivery).[1] The microspheres are produced by reacting multiarm PEG derivatives following a thermally induced phase separation. The microspheres retain some functional groups and crosslink to form scaffolds following centrifugation. The length of incubation following gelation determines the density (buoyancy) of the microspheres.[2] We now demonstrate that, using microspheres of different densities, centrifugation produces multi-layered scaffolds. Scaffolds with up to five distinct layers of PEG microspheres have been formed. By incorporating cell adhesion peptides and allowing cells to adhere to the microspheres before centrifugation, cells may also be incorporated into these scaffolds.

Methods: PEG-octaamine (PEG-OAm, MW 10K) was reacted with 10 mg/mL solutions of Dylight-488 NHS-ester, Dylight-633 NHS-Ester, or Dylight-549 maleimide at 1600:1, 20:1, and 200:1 mol ratio, respectively, and incubated at 25˚C overnight in the dark. Labeled PEG_8-amine was combined with PEG_8-octavinylsulfone (PEG-VS, MW 10K) at a 1:2 mol ratio and diluted to 20 mg/mL PEG with PBS and PBS+ 1.5 M sodium sulfate to a final sodium sulfate concentration of 0.6 M. The PEG_8-VS/PEG_8-amine solutions were then incubated above the cloud point at either 70˚C or 95˚C for various times. Suspensions of microspheres were subsequently buffer exchanged into PBS 2x to remove the sodium sulfate by: (1) diluting the microsphere solution 3:1 with PBS and titurating, (2) centrifuging at 14,100g for 2 min, (3) removing the supernatant. PEG-VS/PEG-OAm microspheres were also formed in the presence of 0.2 mM RGD peptide (GCGYGRGDSPG) to attach the peptide to PEG-VS by a Michael-type reaction. To aid in fluorescence microscopy, scaffolds were formed in glass Pasteur pipettes. To prevent adhesion of the microspheres to the glass, Pasteur pipettes were first filled with 2% PLL-g-PEG solution, incubated for 30 seconds, and washed wish DI water. The tips of the pipettes were then sealed with silicone aquarium sealant. To form scaffolds, microspheres were resuspended in cell medium with 10% FBS and combined with other labeled microsphere solutions within the Pasteur pipette. The pipette was placed in a 15 mL conical vial and spun at 1000g for 10 min and then incubated overnight at 37˚C before imaging by confocal microscopy.

Results: Serum proteins present in the medium promote the crosslinking of PEG-OVS/OAm microspheres into structurally stable scaffolds following compaction by centrifugation. To facilitate visualization by confocal microscopy, the scaffolds were formed in glass Pasteur pipettes. Coating the pipettes with PLL-g-PEG was found to be necessary to prevent adhesion of microspheres to the pipette walls, likely due to the cationic amine groups in the microspheres interacting with anionic silanol groups on the glass. After PLL-g-PEG passivation, microspheres separated based on densities during centrifugation. Microspheres that were incubated for longer times during formation settled towards the bottom of the scaffold. The magnitude of the difference in incubation times determined the sharpness of separation between microspheres layers. A three-tiered gradient with sharp separation between layers is shown in Figure 1. Cells well mixed with microspheres in a scaffold are shown in Figure 2.

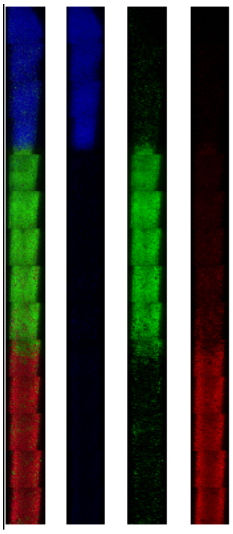

Figure 1: **Three-tier Gradient.** Scaffold fabricated with Dylight-549 (red) labeled microspheres incubated for 45 minutes during microsphere formation, Dylight-488 (green) labeled microspheres incubated for 17 minutes during microsphere formation, and Dylight-633 (blue) labeled microspheres incubated for 11 minutes during microsphere formation. From left to right: (1) combined, (2) blue only, (3) green only, (4) red only. Up to five tiers could also be generated using incubation times of 11, 12, 16, 26, and 45 minutes (not shown).

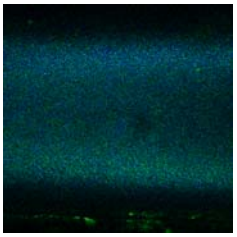

Figure 2: By attaching RGD peptide to the microspheres, the ≈3 micron diameter microspheres (blue) adhered to the human fibroblasts (green). Following centrifugation, the cells segregated based on the density of microspheres to which the cells were attached.

Conclusions: **S**caffolds with multiple layers were formed based on density differences in PEG microspheres. The ability to form layers by a self-assembly process may allow for the formation of complex gradients in growth factors, drugs, cell adhesion peptides, degradability, mechanical properties and cells.

References:

1. Scott, E.A. et al., Acta Biomaterialia, doi: 10.1016/j.actbio.2009.07.009
2. Nichols, M.D. et al., Biomaterials, 30:5283, 2009