Photodegradable Microspheres as Templates for Model Alveoli Formation

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Statement of Purpose: A major challenge in tissue engineering is recreating native 3D tissue archetecture, such as hollow tubules and cysts, within synthetic scaffolds. Typically, researchers have relied on spontaneous formation of such structures, either within naturally-derived Matrigel materials or through the use of physical patterns. However, user control of biochemical and mechanical signals can facilitate discovery of important cues for tissue morphogenesis and pathology, making bioinert and tailorable synthetic hydrogels desirable for *in vitro* models. Since lung epithelial cells do not follow the same lumen-formation process that was key for physical patterning success, we have developed a procedure for creating model alveoli within a poly(ethylene glycol) (PEG) hydrogel using photo-degradable microspheres as templates for the hollow cystic structure.

Here, microspheres were coated with alveolar epithelial cells, encased in a second hydrogel, and photodegraded to create cysts. Microsphere degradation and diffusion profiles within these gels were characterized, as well as the cell coating timeline and final cyst size distribution. Finally, basic biologic function was assessed for tight junction formation and mucin production.

Methods: Extracellular matrix (ECM) proteins (laminin, fibronectin) were entrapped within photodegradable PEG-based microspheres, incubated with A549 cells to coat the surface, encapsulated in a peptide-functionalized PEG hydrogel, and degraded with cytocompatible UV light, leaving a hollow shell of cells within the surrounding hydrogel (Figure 1). Microspheres were synthesized by the Michael-type addition of PEG-tetrathiol and PEG-di(photodegradable acrylate)[1] in an inverse phase microsuspension, as previously described.[2] The ECM protein and an AF488 C5 maleimide dye (Life Technologies) were added to the aqueous phase, enabling entrapment of the protein and reaction of the dye with PEG-tetrathiol end groups.

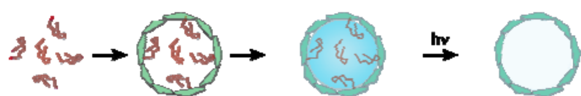

Figure 1. Schematic of cyst-making procedure. ECM proteins (red) were entrapped within microspheres (blue sphere), which were seeded with cells (green), entrapped within a second hydrogel (blue rectangle), and degraded with light.

A549 cells were incubated with microspheres suspended in DMEM media with 10% FBS at 37°C with 5% CO_2 in an ultra-low adhesion well plate on an orbital shaker. Brightfield microscopy was used to document cell coating over time. Cell-sphere constructs were added to a macromer solution containing 8-arm, 40 kDa PEG-norbornene, a di-cysteine MMP-cleavable peptide (for enzymatic network degradation), CRGDS (for adhesion), and a photoinitiator (LAP[3]). This solution was placed between Sigmacote-treated glass slides and polymerized with 365 nm light at 10 mW/cm^2. After incubation for at least 24 hours, degradation of spheres was induced with flood irradiation (365nm at 10 mW/cm^2).

Time-lapse confocal microscopy was used to image the diffusion of the degradation products through the surrounding gel, with and without the presence of cells. Cells were stained for cell nuclei (DAPI) and actin (phalloidin-TRITC), and image analysis (ImageJ) was used to characterize the size of the final cysts. Biologic function was assessed by immunostaining for cell-cell junction markers (β-catenin, E-cadherin, ZO-1) and mucin proteins secreted by these cells (MUC5AC).

Results: Using time-lapse confocal microscopy, the diffusion profiles of fluorescent degradation products were quantified from individual particles, which were described with a statistical-kinetic model of particle degradation and macromolecule diffusion.

Microspheres containing laminin or fibronectin were successfully coated with A549 cells and used to create alveolar epithelial cysts within biofunctionalized PEG hydrogels (Figure 2). These cysts persisted for several days with positive staining for DAPI and actin. Typical cyst diameters were ~200 μm. Cells form tight and adherens junctions, as evidenced by immunostaining and fluorescent imaging, hallmarks of a healthy epithelium. These cells also secrete mucin proteins, evidenced by immunostaining, which is characteristic A549 behavior.

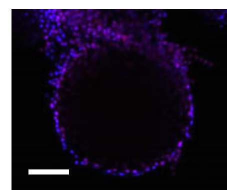

Figure 2. Model alveolus encapsulated in functionalized PEG gel. Single slice taken on confocal microscope showing nuclei (blue) and actin (red). Scale bar = 100 μm.

Conclusions: Photodegradable microspheres are effective templates for directing lung epithelial cell cyst formation within synthetic hydrogels. This platform offers new opportunities to study lung development and disease, and could be expanded to allow co-culture with other cell types in the surrounding gel.

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References:

1. Kloxin A.M., *et al.* Science, 324, 59, 2009.
2. Tibbitt M.W., *et al.* JBMR A, 100A, 1647, 2012.
3. Fairbanks, B., *et al.* Biomaterials, 30, 6702, 2009.

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