In Vitro Model Alveoli From Photodegradable Templates And Primary Lung Epithelial Cells

Katherine J. R. Lewis,[1] & Kristi S. Anseth.[1,2,3]
[1]Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO USA
[2]Howard Hughes Medical Institute, University of Colorado, Boulder, CO USA
[3]BioFrontiers Institute, University of Colorado, Boulder, CO USA

Statement of Purpose: Given the 3D nature of native tissue architecture, recapitulating those cellular structures *in vitro* is often necessary to demonstrate how cells respond to stimuli. Much of the work done with alveolar epithelial cells, for example, has been done with monolayers grown either on TCPS or on protein-coated transwell membranes, and therefore a spherical model that captures the essential features of a single layer of cells surrounded by extracellular matrix (ECM) and enclosing a hollow lumen would greatly benefit the lung community. Our previous work with primary mouse type II pneumocytes (ATII cells) revealed that these cells form aggregates when cultured within wells patterned into hydrogels[1]; thus we developed a templating procedure to create hollow spheres of cells within a matrix using photodegradable microspheres as sacrificial scaffolds.

Here, freshly isolated mouse ATII cells were coated onto poly(ethylene glycol) (PEG) microspheres containing ECM proteins and encapsulated within collagen gels, followed by degradation of the microsphere template with light.

Methods: Microsphere templates with uniform diameter (200 microns) were synthesized within microfluidic devices with mineral oil as the carrier phase and the aqueous polymer solution as the disperse phase. The polymer droplets consisted of PEG-di(photodegradable acrylate)[2], PEG-monoacrylate, photoinitiator, and laminin, and they were crosslinked within the microfluidic device using 405 nm light. The laminin became entrapped within the microsphere network, enabling cell adhesion to the particles.

To test the method, alveolar epithelial cancer cells (A549s) were incubated with microspheres suspended in DMEM media containing 10% FBS on an orbital shaker at 37°C and 5% CO_2. After 24 hours of attachment and proliferation, cell-coated microspheres were encapsulated within 2.7 mg/mL bovine collagen gels. These pre-cysts were cultured for several more days to form attachments to the surrounding collagen and then exposed to 365 nm light at 13 mW/cm^2 for 15 minutes to erode the microsphere templates. Cysts were cultured further before being fixed for analysis.

Subsequently, ATII cells were isolated from mice and incubated with microspheres suspended in DMEM/F12 media supplemented with 10% FBS and 10 ng/mL FGF-7 to promote proliferation. After several days of attachment and growth, these pre-cysts were encapsulated in collagen gels and cultured in the same manner as the A549 cysts

Results: The photodegradable microspheres are effective templates for creating a hollow shell of epithelial cells, which maintain their central lumen over time when encapsulated within a surrounding matrix. First, robust cysts were successfully formed with A549s encapsulated in collagen gels (Figure 1), demonstrated by bright staining of the nucleus and the actin fibers in the cytoskeleton. These cancer cells proliferated out into the surrounding collagen matrix, forming multilayered cysts, but did not fill in the hollow central lumen, even after 5 days in culture following the dissolution of the microsphere template.

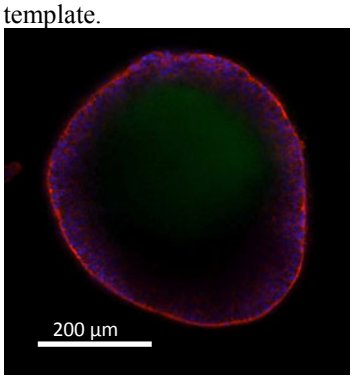

Figure 1. Model alveolus composed of A549 cells encapsulated in collagen gel. Confocal microscope image of a cyst cross-section showing nuclei (blue), actin (red), and residual microsphere template (green).

Moving on to primary cells, freshly isolated mouse ATII cells were used in the templating procedure. These slower-growing cells were allowed 5 days to attach and fully coat the microsphere templates, with FGF-7 in the media to induce proliferation. Cell-microsphere constructs lived through the encapsulation process, continued to thrive in the collagen matrix, and survived template degradation with the central lumen intact.

Conclusions: This model has the potential to transform how alveolar development and disease are studied by replacing a flat monolayer with a physiologically relevant 3D structure. Alveolar epithelial cells take on the spherical shape of the microsphere template and maintain a hollow lumen after the template is removed with light. Future work will focus on the ATII cells and the assessment of epithelial polarity, differentiation to type I pneumocytes (ATI cells), and presence of tight junctions. Immunostaining for basolateral and apical proteins as well as tight junction proteins will confirm polarity of the epithelial layer. Cell phenotype will be assessed by immunostaining for ATII and ATI specific markers and RT-PCR for phenotype-specific genes,. Further utility of the model will be demonstrated with endothelial cells co-encapsulated within the collagen gels next to the epithelial pre-cysts. In addition, FGF-10 will be added to the media after cyst formation to induce budding of the epithelial layer. Further studies will determine the effect of growth factor location on the directionality of budding.

Acknowledgements: HHMI and NSF (DMR 1006711).

References: (1) Kloxin A.M., *et al.* Integr. Biol., 4, 1540, 2012. (2) Kloxin A.M., *et al.* Science, 324, 59, 2009.