Epithelial cyst phenotype is modulated by synthetic hydrogel elastic properties and adhesive ligand density

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Statement of Purpose: To enable more detailed interrogation of extracellular matrix (ECM) instructive cues in epithelial morphogenesis, a synthetic hydrogel platform has been developed that precisely controls adhesive ligand density, tunes elastic properties, and supports cyst morphogenesis. Organization of epithelial cells into polarized, three-dimensional (3D) tissue structures is critical to the barrier, secretion, and exchange functions of organs including lung, kidney, intestine and salivary gland [1, 2]. Disruption of the 3D structure or polarity in epithelial tissues occurs with significant morbidity and mortality [3]. 3D cell cultures in natural ECM extracts, including type I collagen and Matrigel™, enable observation of epithelial morphogenesis, a hard-wired, multicellular differentiation program that integrates cell-ECM adhesion, cell proliferation, and ECM remodeling to achieve functionally differentiated structures like hollow cysts and tubules [4]. Nevertheless, critical limitations of natural ECM extracts, including clinical incompatibility, lot-to-lot variability and undefined presentation of cell adhesion motifs, motivate development of a synthetic ECM analogue. Here, a multi-arm poly (ethylene glycol) (PEG)-maleimide macromer (PEG-4MAL) is covalently functionalized with adhesive peptides and crosslinked with proteolytically-cleavable structural peptides in the presence of epithelial cells [5].
Methods: Madin-Darby canine kidney, type II (MDCK II) epithelial cells were encapsulated and cultured for 10 days in defined matrices to investigate the effects of PEG-4MAL density (3.5% - 5.0%, w/v) and RGD adhesive ligand density (0 – 2 mM) on polarized cyst morphogenesis. After 1-2 days, proliferation was assessed by Click-iT® EdU uptake assay. Apoptotic cells were labeled with an antibody against cleaved caspase-3. At day 10, cultures were fixed and labeled with antibodies against gp135 (apical polarity marker) and laminin (LM) (basement membrane component). Actin filaments were stained with rhodamine phalloidin and nuclei were stained by Hoechst 33342. After staining, cysts were embedded in agarose and imaged on a Nikon C1 laser scanning confocal microscope. Images were analyzed using ImageJ. Categorical data was analyzed by chi-squared test with Tukey-like test for multiple comparisons.
Results: Whereas matrices across the range of PEG density supported high viability, significant proliferation was observed only at low PEG density (≤ 4.0%) and high RGD density (≥ 1 mM) (data not shown). After four days, apoptotic cells were detected in the hollowing cores of cell clusters and indicated a mode of lumen formation observed in classical collagen gel cultures (data not shown). At day 10, cysts grown in synthetic matrix were evaluated on the bases of single-lumen formation, apical-basal polarity, shape, and peripheral laminin (LM) deposition and compared to cysts grown in collagen gels (Fig 1). Cysts grown in 4.0% PEG had normal apical-basal polarity, circular cross-sections, and complete LM deposition, thus matching collagen gels, but were significantly more likely to have multiple lumens. Interestingly, cysts in 3.5% PEG departed significantly from collagen gels and 4.0% gels in that they had inverted apical-polarity, oblong cross-sections, incomplete LM deposits, and multiple lumens (Fig 2).

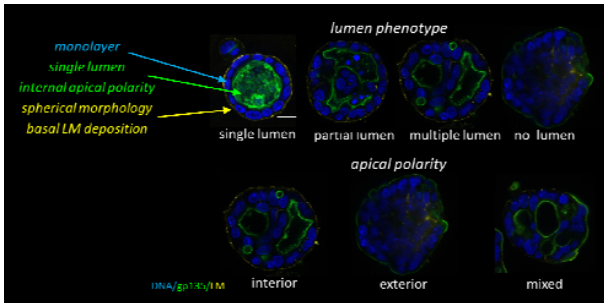
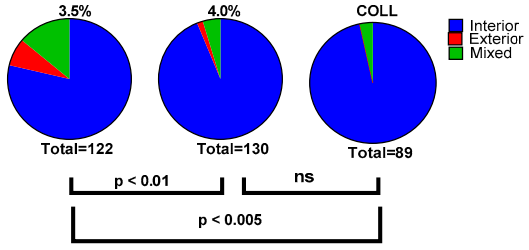


Figure 1. Cyst lumen phenotypes and apical polarity phenotypes. Normal cyst phenotype features single lumen, interior apical polarity, circular cross-section, and complete basal LM deposition. Scale bar 20 μm.

A



B

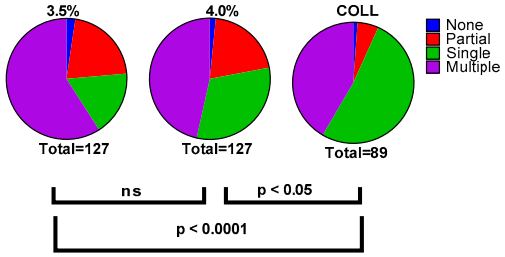


Figure 2. Distribution of apical polarity phenotypes (A) and lumen phenotypes (B).

Conclusions: These results demonstrate that our synthetic hydrogel platform assembles appropriate instructive cues to modulate epithelial cyst morphogenesis.

References: Bryant, D.M., et al. Nat Rev Mol Cell Biol. 2008. 9: 887-901; O'Brien, L.E., et al. Nat Rev Mol Cell Biol. 2002. 3: 531-537; Wang, C.-C., et al. WIREs Syst Biol Med. 2012. 4: 51-78; Rodríguez-Fraticelli, A.E., et al. Curr Opin Cell Biol. 2011. 23: 638-646; Phelps, E.A., et al. Adv Mats. 2012. 24: 64-70.