Engineering bioartificial matrix characteristics to modulate epithelial cyst phenotypes

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Statement of Purpose: A bioartificial matrix is employed to identify extracellular matrix (ECM) characteristics that influence Madin-Darby canine kidney (MDCK) epithelial cyst morphogenesis. MDCK cells grow as contiguous, polarized cell sheets (epithelia) and, when dispersed in natural ECM extracts, form hollow, spherical monolayers of polarized cells that surround a fluid-filled lumen (cysts). Thus, MDCK and other epithelial cell lines have been used extensively to study epithelial morphogenesis, a hard-wired differentiation program that involves ECM-to-cell signaling and cell-driven ECM remodeling to produce functional tissue structures including sheets, tubes, and cysts. Although most insights into MDCK cyst development have come from three-dimensional (3D) cultures in type I collagen gels and laminin (LM)-rich ECM extracts like Matrigel, the utility of these materials is limited by lot-to-lot quality variations, uncontrolled cell adhesive interactions, and growth factor contamination. Consequently, we have adapted poly (ethylene glycol) (PEG)-based hydrogels that present cell adhesive molecules and enzymatic degradation substrates [1] to serve as well-defined bioartificial matrices that promote MDCK epithelial cyst differentiation *in vitro* [2].

Methods: To rapidly assess MDCK cell behaviors elicited by bioartificial matrix, cells were cultured on the surfaces of thin hydrogels prepared with a 4-arm PEG-vinyl sulfone (VS) macromer at 7.5 or 15% (w/v) (a range of stiffness values). The hydrogels incorporated the Arg-Gly-Asp (RGD) cell adhesion motif, a non-adhesive scrambled peptide (RDG), full-length LM protein, LM-derived peptide EIKLLIS, or a combination. The gels were crosslinked with either the fast-degrading GCRDGPQG ↓ IWGQDRCG ("GPQ") peptide (↓ indicates enzymatic cleavage site) or a scrambled, non-degradable peptide. After 24 hours, the surfaces were washed, the attached cells labeled with calcein-AM, and the cell morphology assessed by fluorescence imaging.

To assess the influence of PEG-VS weight percentage (gel stiffness) and matrix adhesivity on cyst development, single MDCK cells were embedded in 50 μL hydrogels formulated with 5, 10, 15 or 20% PEG-VS, 2.0 mM RGD or 2.0 mM RDG, and crosslinked with the fast-degrading GPQ peptide. After 15 days, the cultures were fixed and imaged using DIC optics. The size of MDCK cysts and presence of lumens were evaluated manually using NIS elements software (Nikon; Melville, NY). Epithelial polarity markers were visualized by confocal immunofluorescence imaging.

Results: Our results indicate that 5% gels with RGD elicit cyst morphogenesis behavior that differs from stiffer gels. Whereas the frequency of cyst formation and the size of cysts were highest in 5% gels, the frequency of lumen formation was lowest in 5% gels. Whereas the frequency of cyst formation trended lower with increasing stiffness, cyst size and frequency of lumen formation reached a plateau above 5%. RGD appears to enhance the number of cysts and frequency of lumen formation versus RDG. In the two-dimensional (2D) assay, RGD mediated significantly higher cell attachment than RDG after 24 hours.

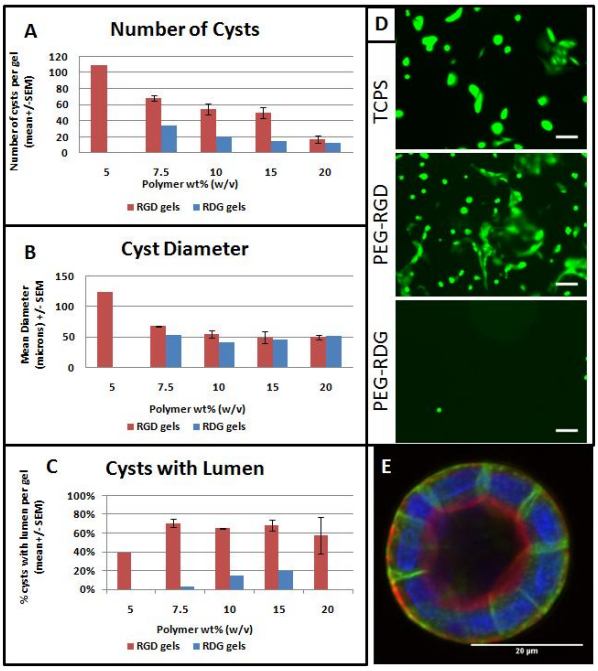

Figure 1. A: Number of cysts, B: cyst diameter, and C: frequency of cysts with lumens among 3D cultures of MDCK cells in PEG hydrogels. D: Representative images of calcein-labeled MDCK cells cultured on surface of tissue culture plastic (TCPS) or degradable 7.5% (w/v) PEG hydrogels. Scale bar 100 μm. E: Representative confocal fluorescence image of polarized MDCK cyst with lumen grown in 7.5% gel with RGD: f-actin (red), β-catenin (green), nuclei (blue). Scale bar 20 μm.

Conclusions: The increased frequency of cyst formation in 5% gels suggests that MDCK cells can more easily remodel a low-density network and make room for proliferation. This would also explain the increased size of cysts. The decreased frequency of lumen formation in 5% gels may indicate that a lesser amount of tethered ligand is available to cells at low polymer concentration. As low attachment on the surface of gels incorporating RDG agrees with low frequency of cyst formation and lumen formation in 3D, two-dimensional cell attachment and proliferation assays may be good screening tools for identifying cell adhesion ligands that modulate MDCK cyst morphogenesis in bioartificial matrix.

References: [1] Lutolf MP. Adv Mater 2003; 15:888-92. [2] Chung, IM. Biomaterials 2008; 29:2637-45.