Biomimetic microenvironments for controlling morphogenesis of human pancreatic ductal epithelial cells

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Statement of Purpose: Biomimetic poly(ethylene glycol) or PEG-based hydrogels incorporated with defined instructive cues are excellent matrices for three-dimensional (3D) cell culture [1-3]. We are interested in using PEG hydrogels as a platform to study pancreatic ductal epithelial cell (e.g., PANC-1) fate in 3D due to the multiple roles of this particular cell type. PANC-1 cells are immortalized pancreatic epithelial cells that have been widely used to study pancreatic cancer cell metastasis. In addition, studies have shown PANC-1 cells, when given appropriate soluble cues, can be differentiated into insulin secreting cell clusters that serve as an alternative cell source to treat type I diabetes [4]. No prior attempt has been made to establish a well-defined and highly tunable matrix for controlling morphogenesis and differentiation of PANC-1 cells in 3D. The objectives of this study are to evaluate the effects of hydrogel matrix compositions on PANC-1 cell proliferation and morphogenesis, from which to establish conditions for 3D endocrine differentiation.

Methods: PANC-1 cells were encapsulated in hydrogels formed by step-growth thiol-ene photopolymerization using 4-arm PEG-norbornene (PEG4NB) and *bis*-cysteine crosslinker such as dithiothreitol (DTT), CGGY↓C (chymotrypsin-sensitive peptide linker) and KCGPQG↓IWGQCK (MMP-sensitive peptide linker). Gels were functionalized with ECM-mimetic peptides (e.g., fibronectin-derived CRGDS and laminin-derived KCYIGSR) to evaluate the effect of bioactive motifs on PANC-1 cell morphogenesis. Encapsulated cells were cultured in high glucose DMEM with 10% FBS. Cell survival and proliferation were quantified by AlamarBlue reagent. Cell morphology was observed using Live/Dead staining and immunostaining. Protein expressions were detected using western blotting and RT-PCR.

Results: Thiol-ene photo-click hydrogels provide a cytocompatible environment for 3D culture of PANC-1 cells. PANC-1 cell viability immediately following photo-encapsulation was ~92% (live/dead cell counts). In contrast to an epithelium like monolayer when cultured on a 2D surface, PANC-1 cells formed clusters in 3D thiol-ene hydrogels (Figure 1B). Although PANC-1 cells proliferated in all hydrogel formulations studied, the degree of proliferation, size, and morphology of cell clusters were significantly influenced by hydrogel compositions. When encapsulated in MMP-sensitive gels, cells expressed high level of F-actin and β-catenin in the core of the clusters (Figure 2A, 2B). PANC-1 cell clusters formed in DTT and CGGYC crosslinked gels appeared smaller and spherical, while cells encapsulated in MMP-sensitive peptide crosslinked gels formed polydispersed cell clusters, some with large cysts or irregular processes (Figure 2C). The differences in cell cluster morphology can be explained by the fact that gels crosslinked by DTT and CGGYC are only hydrolytically degradable, which restricted cell-material interactions. On the other hand, gels crosslinked by MMP-sensitive peptides permitted cell-mediated matrix remodeling. PANC-1 cell morphogenesis was also affected by immobilized ECM-derived bioactive motifs in thiol-ene hydrogels. In the presence of fibronectin-derived RGD peptide, cells formed large cyst-like clusters compared to small spherical aggregates in YIGSR containing gels. These results are crucial in identifying culture conditions for future endocrine differentiation.

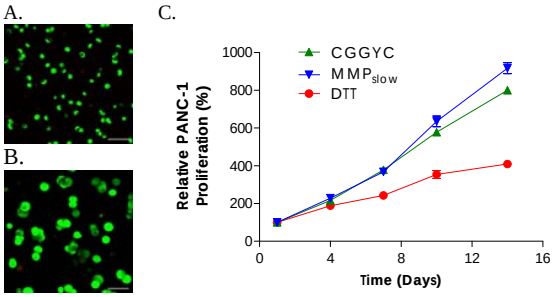

Figure 1. (A-B) Live/Dead images of PANC-1 cells immediately following photoencapsualtion (A) and after 10 days of culture in thiol-ene gels (B). (C) Relative proliferation profiles of PANC-1 cells in thiol-ene gels with different crosslinkers.

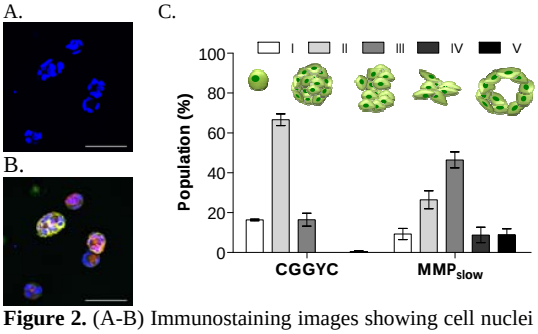

Figure 2. (A-B) Immunostaining images showing cell nuclei (blue), F-actin (red), and β-catenin (green) expression patterns in thiol-ene hydrogel crosslinked by MMP sensitive linker. (C) Cell morphology analysis based on live/dead staining results.

Conclusion: In conclusion, thiol-ene hydrogels provide a cytocompatible environment for 3D culture of pancreatic ductal epithelial cells. The formation of cell clusters was affected largely by hydrogel formulations, including types of peptide crosslinkers and ECM-mimetic bioactive cues. These fundamental studies have established PEG-peptide hydrogels formed by thiol-ene photo-click reaction as a suitable platform for studying pancreatic epithelial cell morphogenesis in 3D. Ongoing work is focused on generating insulin-secreting artificial islets for the treatment of type I diabetes.

References: [1] Fairbanks BD, et al. *Adv Mater.* 2009;21:5005-5010.[2] Schwartz MP, et al. *Integr Biol.* 2010;2:32-40.[3] Weiss MS, et al. *Biomaterials.* 2012;33:3548-59.[4] Hardikar AA, et al. *Proc Natl Acad Sci USA.* 2003;100:7117-22.