Using Photo-polymerizable Collagen to Characterize Panc-1 Behavior Under Simulated Fibrotic Conditions *In vitro*

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Statement of Purpose: Pancreatic ductal adenocarcinoma (PDAC) will emerge as the second deadliest cancer in the U.S. by the next decade. Over time, interest has shifted from sole focus on the cellular constituents of the tumor microenvironment (TME), to the influence of the acellular components on cell behavior. PDAC is characterized by a fibrotic extracellular matrix (ECM) that contributes to malignant cell transformation, tumor progression, and altered cell-cell communication. Attempts have been made to recreate a fibrotic TME *in vitro*, but they either: 1) fail to recapitulate in situ stiffening on a relevant timescale or 2) accomplish progressive stiffening with non-fibrillar ECM materials. Also, pancreatic tumor cell behavior when cultured within a progressively stiffened ECM vs. culture at a static stiffness (single stiffness value) is understudied. Here, we used photo-polymerizable type I collagen that can undergo progressive stiffening to represent the fibrotic ECM surrounding PDAC tumors and compare static and progressively stiffened samples. Cells were evaluated on changes to proliferation, viability, and morphology under static and progressively stiffened conditions.

Methods: Methacrylated type I collagen was combined with a lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photoinitiator to enable crosslinking with 405 nm light. A PDAC tumor cell line (Panc-1) was seeded within hydrogels and cultured for 5 days. Static stiffness samples were exposed to light (90 sec. on, 60 sec. off, 4 cycles) immediately following initial polymerization and remained at one stiffness level. Progressively stiffened samples were polymerized and cultured for 3 days before exposure to the same light conditions. Cell metabolic activity was measured with alamarBlue™ to track proliferation daily (endpoints: Days 1, 3, 5). Separate experiments were performed to determine the most appropriate time to stiffen static samples after initial polymerization to avoid compromising viability from the start. Static samples were stiffened 1-, 2-, 4-, or 24 hours after initial polymerization before tracking proliferation over 5 days. To assess changes in morphology, samples were fixed on Day 5 for nuclear and F-actin staining and imaged in fluorescence using a Keyence BZ 810 microscope. Cell morphology (eccentricity) was measured in (CellProfiler) as a marker of invasive potential. Significance was analyzed using a one-way ANOVA with Tukey's post hoc analysis or Kruskal-Wallis analysis of variance for non-parametric data ($p < 0.05$).

Results: Progressive samples and no light control samples expressed the highest cell viability, which were significantly greater than static stiffness samples (not shown). Delaying the onset of stiffening by a few hours increased cell survival in static stiffness cultures when compared to samples stiffened samples immediately after collagen polymerization (fig A). No light control samples and progressive samples showed increased cell clustering

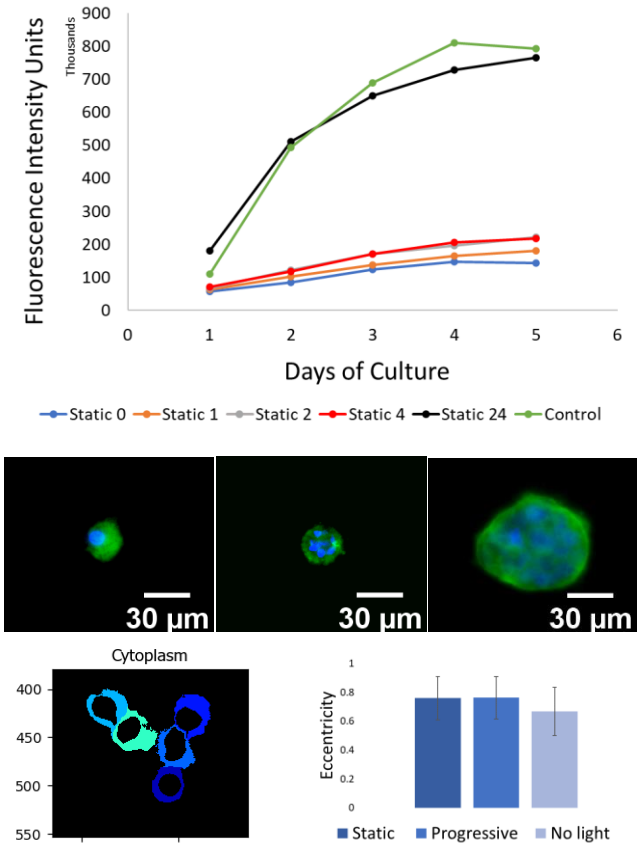

***Figure 1**. **(A)** Cell proliferation with delayed stiffening by 0,1,2,4 and 24 hours. **(B)** Immunofluorescence images of static (left), progressive (middle), and no light control samples (right) (blue=nucleus, green=F-actin; 20x magnification, 2x digital zoom, Z-stack projection, 10 µm section, 0.5 µm pitch). **(C)** Representative images of thresholding and mask generation during Eccentricity (0- irregular, 1 – circular) analysis using CellProfiler.*

(fig B), while static samples primarily grew as single cells. Cell eccentricity (fig C) was measure for static (0.759±0.15), progressive (0.763±0.14), and control (0.668±0.17) samples with no significant difference.

Conclusions. Delayed onset of stiffening is beneficial to cell viability by allowing cells to adjust to their culture environment prior to photopolymerization. Proliferation results also indicate that the timing of light exposure can be further tuned to mimic a more gradual onset of fibrotic conditions, which supports the suitability of this system for a fibrotic tumor model. Morphological changes in Panc-1 cells were seen between the experimental groups, but eccentricity alone did not detect differences. More comprehensive analysis is needed to capture and quantify changes. Future work will also expand to include more replicates, additional PDAC cell lines, and evaluate changes in PDAC specific markers. **References:** 1. Baskaran J. Apl Bioengineering, 2020;4;026105