Cytotoxicity of Lithium phenyl-2,4,6-trimethylbenzoylphosphinate on Panc-1 cells in Methacrylated Type I Collagen

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Statement of Purpose: Pancreatic ductal adenocarcinoma (PDAC) has a 5 year survival rate of less than 10% [1]. Desmoplasia (fibrosis) is thought to promote cancer progression in PDAC and lead to progressive tissue stiffening [1], [2]. Yet current *in vitro* PDAC models lack temporal changes in stiffness to adequately mimic progression [3]. Photo-crosslinkable hydrogels, composed of reactive methacrylated materials, are tunable and allow for temporal stiffening when combined with photoinitator and exposed to light [4], [5]. However, Type I photoinitators undergo a photopolymerization reaction that can lead to cytotoxicity concerns [4]. Studies have evaluated cytotoxicity of traditional photoinitiators, such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), on a variety of cell types. However, results are mixed, appear to be cell dependent, and effects on pancreatic cancer cells are underexplored [5]–[7]. Our objective was to evaluate the cytotoxicity of LAP on Panc-1 cells, a PDAC cell line commonly used in tumor studies, in methacrylated collagen hydrogels. We studied the effects of LAP concentration, culture format and time, and light exposure on cell proliferation and also looked at the whether alamarBlue™ is appropriate to assess LAP cytotoxicity.

Methods: Cytotoxicity of LAP on Panc-1 cells (ATCC) was examined in 2D on tissue culture plastic for 2 days (short) or 5 days (long) and 3D culture within PhotoCol® (Methacrylated type I collagen, 8 mg/mL; Advanced BioMatrix) for 2 days. In 2D, adherent Panc-1 cells ($3x10^4$ cells/well; 96-well plate) were exposed to LAP at 0%, 0.1%, 0.25%, 0.5%, 1% and 2% in cell culture medium (2% is the recommendation for PhotoCol®). Silver nitrate (30 µM, Alfa Aesar) was the positive control. Cells in 3D were suspended in neutralized PhotoCol® with LAP at $3x10^5$ cells/mL and polymerized at 37°C (30 min.). Samples were exposed to no light or 405 nm light under sustained (10 min.) or pulsed (1 min. on, 1 min. off, 5 cycles) conditions. AlamarBlue™ (ThermoFisher) was added and fluorescence intensity was measured at 4-, 24-, and 48 hours for 2 days and 4-, 24-, 48-, 72-, 96-, and 120 hrs for 5 days. Experiments were also performed without Panc-1 cells to determine if LAP interferes with alamarBlue™ assay. These samples were exposed to light treatments and read at 2 day time points.

Results: Short- and long-term 2D cultures showed increased proliferation over silver nitrate control at all LAP concentrations (Fig. 1A, B). At 2 days (Fig. 1A), pulsed light appeared to have a negative impact on proliferation up to 0.5% LAP compared to no light and sustained light. However, there appeared to be no difference in proliferation between the type of light exposure after 5 days, and 0-0.5% LAP plus light appeared to negatively impact proliferation (Fig. 1B). For cells in 3D PhotoCol® (Fig. 1C), the type of light exposure did not appear make a difference at any LAP concentration. Proliferation appeared to be higher for exposed samples compared to no light at all LAP concentrations except 0.25%, but standard deviation was high for that group and others. Fluorescence intensity of culture medium with 2% LAP, no cells, and light exposure was also significantly higher ($p<0.0001$) than medium without LAP and no light (Fig. 1D).

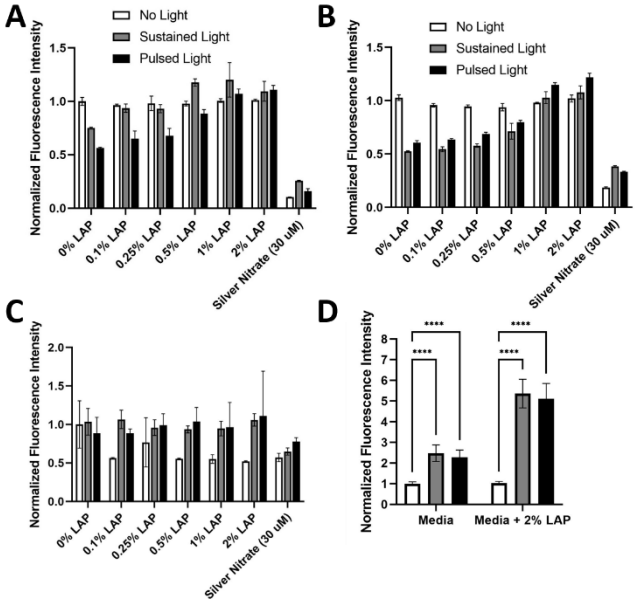

*Fig 1: Endpoint (normalized to no light) for 2 day **(A),** 5 day **(B)** Panc-1 cells on 2D. **(C)** 2 day for cells within 3D PhotoCol®. All were exposed to 0-2% LAP (A-C). **(D)** 2 day without cells at 2% LAP. No light (white), Sustained (gray), Pulsed (black). Bars= mean±SD. (A-C: n=3; N=1; D: n=5, N=3, ****p<0.0001, two-way ANOVA).*

Conclusion: In general, LAP toxicity does not appear to be a large concern for Panc-1 cells in initial studies, since proliferation with 2% LAP (working concentration for PhotoCol®) was comparable to the no light control and greater than the silver nitrate control. 3D culture format also appeared to support Panc-1 cells when exposed to light. However, there are concerns about whether LAP interference with alamarBlue™ may be a factor. Overall, these preliminary results demonstrate potential for using LAP as the photoinitiator for 3D *in vitro* models of pancreatic cancer with progressive stiffening. Future studies are needed to increase replicates, assess long-term 3D culture, and consider an alternate or companion viability assay for a more comprehensive assessment.

References: [1] Huang C. Int. J. Mol. Sci. 2021;22:4970.; [2] Thomas D. Mol. Cancer. 2019;18;14.; [3] Chhetri A. Front. Mol. Biosci. 2021;8:22.; [4] Choi. J. R. BioTechniques. 2019;66:40–53.; [5] Nguyen A. K. Polymers. 2020;12:1489; [6] Williams C. G. Biomaterials. 2005;26:1211–1218.; [7] Lim K. S. Macromol. Biosci. 2019;19:1900098.