UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2021/22

NB: This whole report will be posted on the Society's website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.

Name of student:

Jarren Yi Sheng Lu

Name of supervisor(s):

Dr Stephen Thorpe

Project Title: (no more than 220 characters)

Assessment of Cell-Mediated Collagen Remodelling in Pancreatic Cancer Using Label-Free Imaging

Project aims: (no more than 700 words)

Pancreatic cancer is the 11th most common cancer worldwide. and recorded a staggering incidence rate of 7.6/100,000 people in Western Europe in 2018.[1] Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and is characterised by the appearance of a desmoplastic stroma around pancreatic tumour cells.[2] Collagen is the most abundant protein found in the extracellular matrix (ECM) and therefore contributes significantly to the structure and content of the tumour microenvironment. Desmoplasia, the deposition of fibrous connective tissue by fibroblasts, has been shown to both confer chemoresistance[3] and promote the progression of PDAC.[4] Collagen hydrogels serve as excellent *in vitro* models to study PDAC as pathological processes such as desmoplasia can easily be observed using microscopy. We utilized confocal reflectance microscopy (CRM) to image both collagen fibrils and cancer cell-hydrogel interactions. CRM was chosen not only because it required no staining of collagen hydrogels for imaging, but also because it acquires high-resolution 3D images. To better understand how cancer cells remodel collagen in PDAC pathogenesis, we outline three distinct but complementary objectives.

1. Investigate the effect of collagen gel concentration and polymerization temperature on fibril architecture.

Acellular collagen gels were made at final concentrations of 1, 2, 3, and 5.56 mg/mL. These were incubated at 37 °C for 1 hour. 3 mg/mL gels were additionally incubated at different polymerization temperatures, including 4 °C, and 25 °C. Collagen gels were cast in chamber slides (ibidi), then imaged directly using a Nikon A1 confocal microscope equipped with a 60× oil objective. A 488 nm excitation with a 465-500 nm emission window was used for reflectance imaging. Z-stack images of 10 μm spacing were taken for each gel and analysed using CTFire[5] via MATLAB. Figure 1 shows a representative confocal reflectance image of collagen fibrils taken from a 5.56 mg/mL collagen hydrogel polymerized at 37 °C.

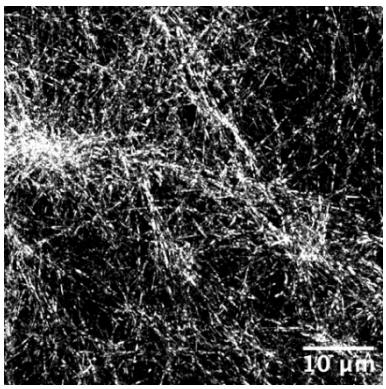

Figure 1. Confocal reflectance image of collagen fibrils taken at 60× magnification.

2. Investigate the effect of collagen gel concentration and polymerization temperature on gel mechanics.

Acellular collagen gels were made as described above. Collagen gels were cast in 1.5 mL Eppendorf tube caps to obtain ∅8 mm × 2 mm thick gels. A rheometer was used to perform strain sweep and frequency sweep tests on each collagen gel to obtain storage and loss moduli.

3. Investigate the effect of collagen gel concentration on cellular morphology and cell-matrix interactions.

Cell-laden collagen gels were made by incorporating SUIT2-007 cells into 1, 2, and 3 mg/mL collagen hydrogels. These were cast in chamber slides (ibidi) and incubated at 37 °C for 1 hour. A live dead assay was

subsequently performed for all cell-gel networks by staining the cells with Calcein-AM and Ethidium Homodimer-1 dyes. The chamber slides were then immediately imaged using a Nikon A1 confocal microscope equipped with a 20× air objective. Figure 2 shows a representative image of a live dead assay taken of cells incorporated into a 3 mg/mL collagen hydrogel. To assess cellular morphology, confocal images were acquired using 60× oil objective, with 488 nm excitation with 495-535 nm emission used for fluorescence imaging of calcein-AM stained cells, and 488 nm excitation with 465-500 nm emission used for reflectance imaging of collagen fibrils. Z-stack images of 10 μm spacing were taken for each cell-gel network. Figure 3 shows a representative image of a cell-gel network within a 3 mg/mL collagen hydrogel, after maximum projection.

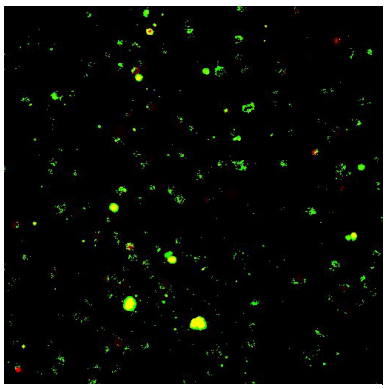

Figure 2. Live/dead assay of SUIT2-007 cells in a 3 mg/mL collagen hydrogel. Live cells are stained green with Calcein AM; dead cell nuclei are stained red with Ethidium Homodimer-1.

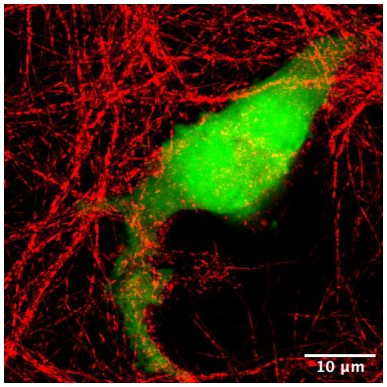

Figure 3. A SUIT2 cell within collagen hydrogel. A superimposed, maximum projection of both confocal fluorescence and confocal reflectance images.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Project Outcomes

1. Collagen concentration significantly alters hydrogel structure and mechanics

Collagen fibrils decrease in length as collagen concentration increases; collagen fibrils increase in width and straightness with collagen concentration (Figure 4).

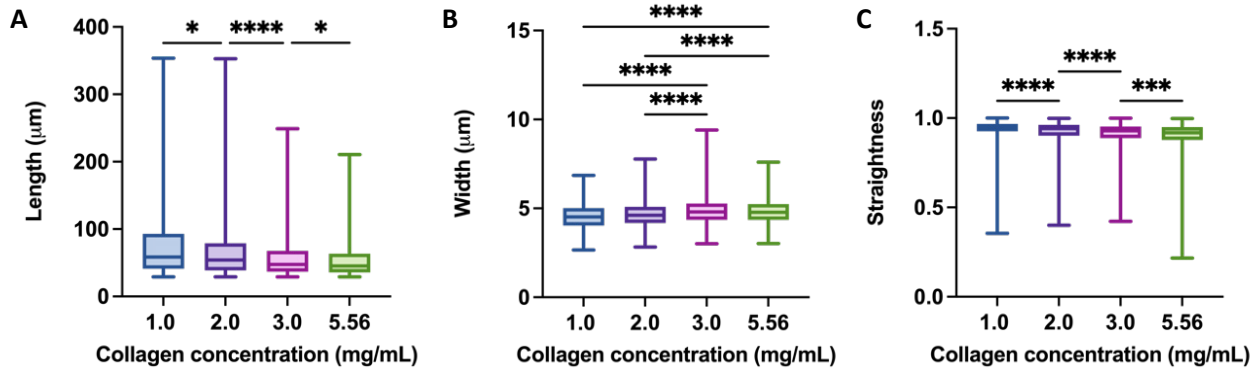

Figure 4. Collagen fibril length (A), width (B), and straightness (C) against collagen concentration. n = 488-1,960 collagen fibrils, Kruskal Wallis test with Dunn's multiple comparisons, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

The storage modulus (G', elastic stiffness) of collagen gels at 0.1 Hz increases with collagen concentration (Figure 5).

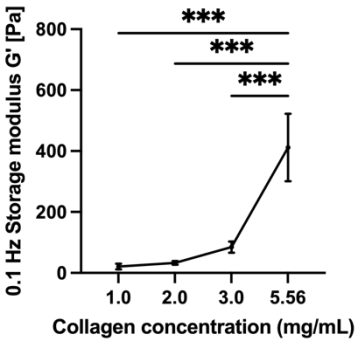

Figure 5. Storage modulus (G') at 0.1 Hz against collagen concentration. n = 2-4 gels, One-way ANOVA with Tukey's multiple comparisons *** $p < 0.001$.

2. Polymerization temperature has minimal effect on hydrogel structure or mechanics

Results for hydrogel structure show a decrease in fibril width at 25 °C compared to 4 °C or 37 °C (Figure 6B). However, polymerization temperature did not significantly affect fibril length and straightness (Figure 6A,C).

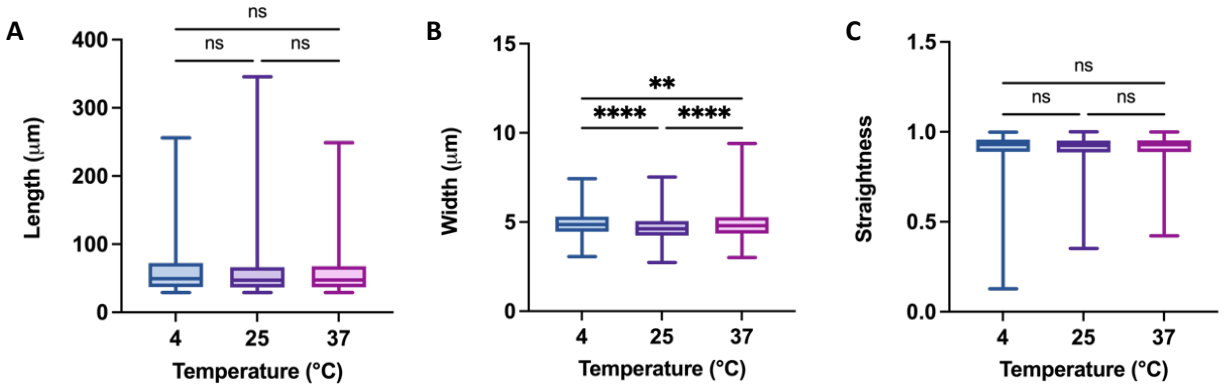

Figure 6. Collagen fibril length (A), width (B) and straightness (C) against collagen concentration. *n* = 488-1,960 collagen fibrils, Kruskal Wallis test with Dunn's multiple comparisons, ** $p < 0.01$, **** $p < 0.0001$, ns = not significant.

Storage modulus (G') at 0.1 Hz of collagen gels was not significantly affected by polymerization temperature.

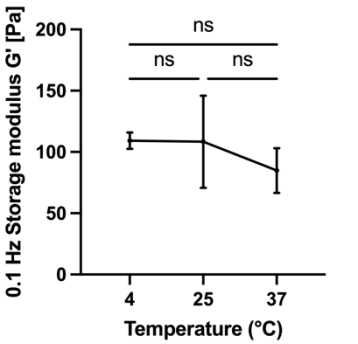

Figure 7. Storage modulus (G') at 0.1 Hz against collagen concentration. *n* = 2-4 gels, One-way ANOVA with Tukey's multiple comparisons, ns = not significant.

3. Collagen hydrogel concentration modulates cell morphology

Cell-associated collagen pixel intensity and collagen gel concentration exhibit a biphasic relationship with collagen pixel intensity greatest at 2 mg/mL, as shown in Figure 8.

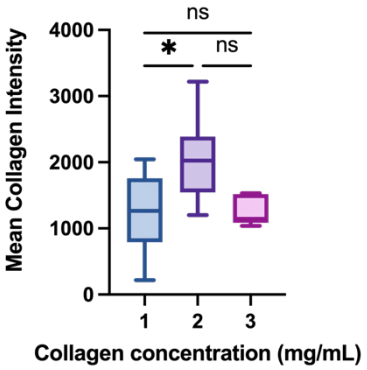

Figure 8. Box and whisker plot of cell-associated collagen (mean intensity) against collagen gel concentration. *n* = 5-11 cells, Kruskal Wallis test with Dunn's multiple comparisons, * $p < 0.05$, ns = not significant.

Figure 9 demonstrates a negative correlation between cell circularity and cell-associated collagen at 1 and 2 mg/mL, but not at 3 mg/mL. Although results suggest a significant difference only for 1 mg/mL, and not 2 and 3 mg/mL, there were insufficient data points collected and used, hence further work is required to avoid bias.

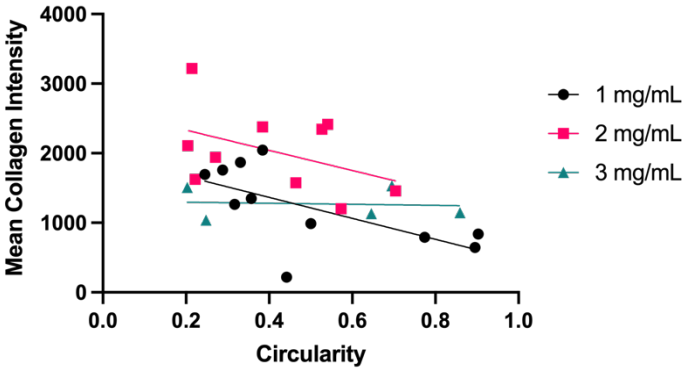

Figure 9. Cell-associated collagen plotted against cell circularity for collagen hydrogel concentration. *n* = 5-11 cells, linear regression.

In summary, collagen concentration and polymerization temperature alter fibril architecture and hydrogel mechanics to varying extents. Confocal reflectance imaging serves as a useful technique in imaging collagen fibrils and cellular interactions with these fibrils. Metastatic PDAC SUIT2-007 cells resembling a mesenchymal morphology (low circularity) are associated with a higher degree of collagen indicative of matrix remodelling than those of epithelial morphology (high circularity).

Experience

This summer project gave me the opportunity to acquire various laboratory skills including:

1. Producing acellular collagen gels
2. Setting up, configuring, and operating the A1 Nikon confocal microscope to:
   - Perform confocal fluorescence imaging
   - Perform confocal reflectance imaging
3. Setting up, configuring, and operating a rheometer to:
   - Perform frequency sweep tests
   - Perform strain sweep tests
4. Performing a live dead assay
   - Staining of cells with special dyes, including Calcein AM, and Ethidium Homodimer-1

However, I believe it is of greater importance that I've also learnt many transferable skills such as:

1. Properly planning my experiments by preparing relevant protocols (including useful stoichiometry calculations) and listing down discussion points prior to starting any experiments.
2. Optimizing protocols through trial and error.
3. Conducting literature reviews to either optimize current protocols or adopting a different approach/experiment to achieve the same primary objective.
4. Learning how to use open-source software for useful measurements/interpretation of data collected. For example, Fiji, and CT Fire via MATLAB.
5. Plotting graphs and performing statistical analyses using GraphPad Prism 9.
6. Most importantly, learning how to work closely with other lab members to continually improve on current laboratory skills and having meaningful discussions.

Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

Winter Meeting 2023

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

Aim

- To assess the role of collagen concentration and gelation conditions on fibril architecture and hydrogel mechanical properties
- To assess the effect of collagen concentration on pancreatic cancer cell morphology and cell-matrix interactions

Background
- Collagen type I is the most abundant protein found in the extracellular matrix of pancreatic ductal adenocarcinoma (PDAC)
- Desmoplasia confers chemoresistance and promotes the progression of PDAC
- Collagen hydrogels serve as good *in vitro* models to study diseases like PDAC
- Confocal reflectance microscopy (CRM) has increasingly been used to image collagen fibrils
- The main objective of this study is to assess collagen remodelling in the vicinity of SUIT2-007 cells using CRM

Methodology
Fabrication of collagen gels
- Acellular collagen gels were made at final concentrations of 1, 2, 3, and 5.56 mg/mL
- Acellular collagen gels were polymerized at 4 °C, 25 °C, and 37 °C
Confocal Reflectance Microscopy
- Collagen gels were cast in a chamber slide (ibidi)
- A Nikon A1 confocal microscope was used to acquire z-stack images at 10 µm spacing of both acellular and cell-laden collagen gels
- Collagen fibrils were analysed using CTFire via MATLAB
Rheology
- Collagen gels were cast in 1.5 mL Eppendorf tube caps to obtain Ø8 mm × 2 mm thick gels
- A rheometer was used to perform strain sweep and frequency sweep tests on each collagen gel

Results
- Collagen concentration changes hydrogel structure and mechanics
- Polymerization temperature has minimal effect on hydrogel structure or mechanics
- Collagen hydrogel concentration modulates cell morphology

Discussion
- Collagen concentration and polymerisation temperature alter fibril architecture
- CRM serves as a useful technique in imaging collagen fibrils
- PDAC SUIT2-007 cells resembling a mesenchymal morphology are associated with a higher degree of matrix remodelling than those of epithelial morphology

Brief Resume of your Project's outcomes: **(no more than 200-250 words)**.
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

Assessment of cell-mediated collagen remodelling in pancreatic cancer using label-free imaging

Collagen plays a significant role in the development of pancreatic cancer. More specifically, desmoplasia has been shown to both confer chemoresistance and promote the progression of pancreatic ductal adenocarcinoma (PDAC). *In vitro* models like collagen hydrogels serve as a good platform in studying PDAC because processes such as desmoplasia can easily be observed. The objective of this study was to assess collagen remodelling in the vicinity of SUIT2-007 (pancreatic cancer) cells via label-free imaging.

Acellular collagen gels were made from type I bovine telocollagen at final concentrations of 1, 2, 3, and 5.56 mg/mL. Collagen gels were imaged with confocal reflectance microscopy and images analysed with CTFire using MATLAB to quantify fibril architecture (Figure 1). To characterise collagen gel mechanical properties, rheology was conducted with both strain and frequency sweep tests performed. Later, SUIT2-007 cells were incorporated into hydrogels of different collagen concentrations and imaged with confocal reflectance microscopy. The pixel intensity of collagen fibrils was analysed to assess collagen contraction around cells.

Collagen fibril length decreased with increasing collagen concentration, whilst no significant differences were found in fibril width. The storage modulus at 0.1 Hz was found to increase with collagen concentration ranging from 21.04±9.09 to 411.39±110.66 Pa for 1 and 5.56 mg/mL respectively. Elongated cancer cells were associated with condensation of collagen fibrils indicative of matrix remodelling.

This study suggests elongated cells resembling a mesenchymal morphology have a larger impact on their surroundings compared to those of epithelial morphology.

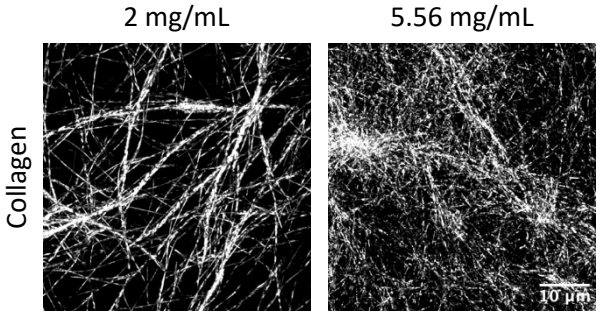

Figure 10. Collagen fibril architecture imaged using confocal reflectance imaging at 2 and 5.56 mg/mL collagen gel concentration.

Other comments: (no more than 300 words)

Acknowledgements

I would like to thank my supervisor, Dr Stephen Thorpe, for the immense support he has shown throughout my summer research project. His guidance and advice have been taken to heart. I would also like to thank all Thorpe Lab members, including Michelle Fox, Leah Fallon, Maëla Mars, Shanu Xavier, Ciara Doyle, and Anwesha Sarkar for help and useful discussions. Also, Rijian Song, for teaching me how to use the rheometer.

References

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Signature of student.....Jarren Yi Sheng Lu ..... Date 9/9/2022

Signature of supervisor…Dr Stephen Thorpe ..... Date 15/9/2022

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