METHODS AND TECHNIQUES OF PRODUCING BREAST CANCER TUMOR MODELS

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OVERVIEW

- Background
- MDA-MB-231 cell line
- MCF7 cell line
- Methods and Techniques:
  - cell culture & collagen matrix
  - Diskoids
  - Spheroids
  - Bioprinting
- Results
- Discussion

BACKGROUND

- Breast cancer is the leading cause of cancer death among females and the most frequently diagnosed cancer
- MDA-MB-231 cell line: invasive
- MCF7 cell line: noninvasive
- Collagen
  - Structural protein of the extracellular matrix
- Making tumor models: cells + collagen
  - Experiments study micromechanics

MDA-MB-231 CELL LINE

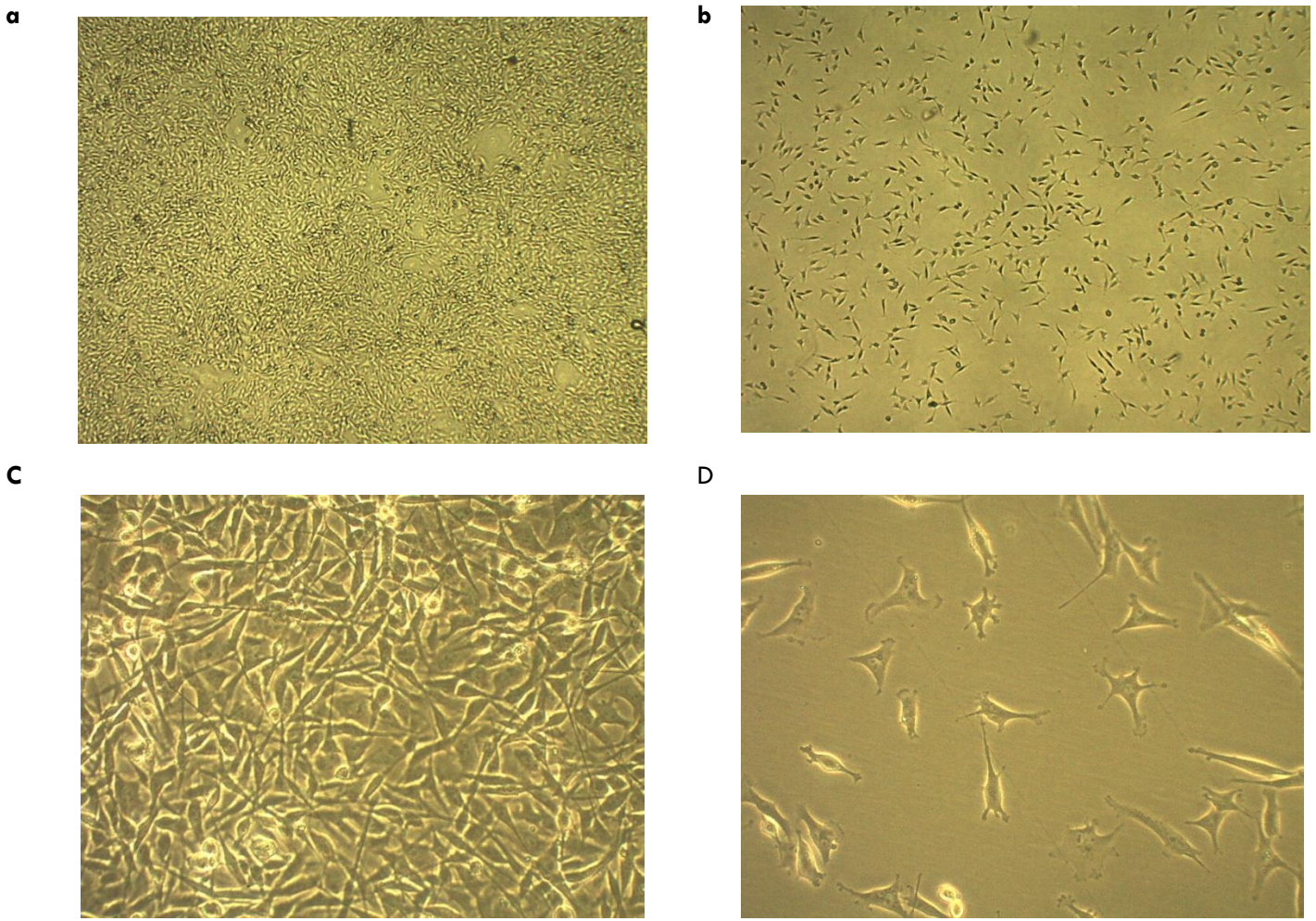
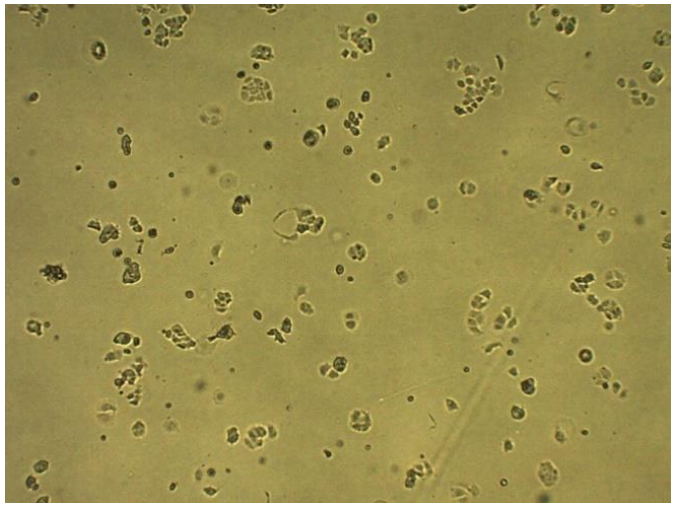


Figure 1: a) MDA-MB-231 GFP at 4x magnification at high confluency. B) MDA-MB-231 GFP at 4x magnification at low confluency. C) MDA-MB-231 GFP at 20x magnification high confluency. D) MDA-MB-231 GP at 20x low confluency.

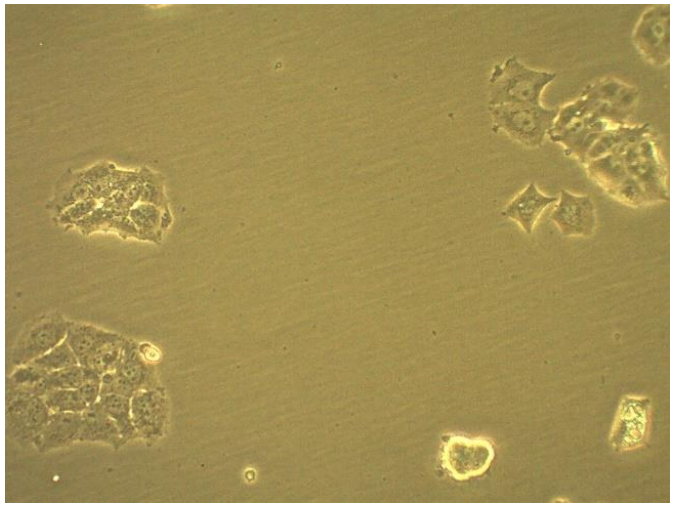
- Spindle shaped
- Spread out at high confluency (many cells)
- Invasive cell line
- High confluency: high percentage of cells
- Low confluency: low percentage of cells
- Can be used for experiments to study invasion of cells into collagen

MCF7 CELL LINE

a



b



- Round shaped
- Form clusters at high confluency
- Noninvasive
- Can be used for experiments to study how the cells pull collagen toward it

Figure 2: a) MCF7 cells at 4x magnification and low confluency. b) MCF7 cells at 20x and low confluency.

METHODS

- Culture cells to grow cells to use in cancer model
  - Let cells grow in a flask
  - Remove a % of cells and put in new flask to grow
  - Use cells from a flask to create cancer model
- Create collagen gel
  - Create a collagen solution with NaOH, 10x PBS, and growth medium
  - Collagen added last because it gels quickly at room temperature
  - Pipette solution onto dish

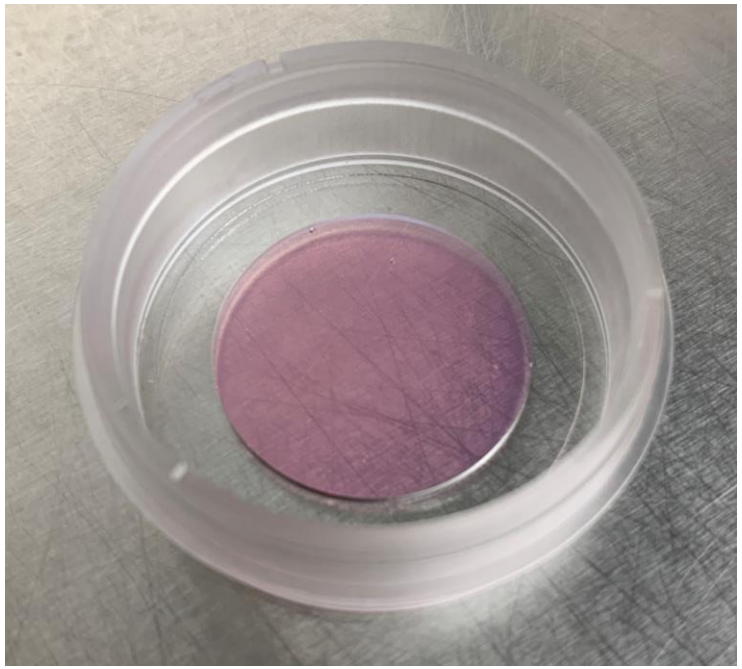


Figure 3: Sample of a collagen gelled in a dish.

METHODS

- Diskoids
  - One method to use
  - Create collagen gel like normal
  - Pipet around small rod
  - Remove rod carefully
  - After collagen gels, pipet cells into hole where rod once was
  - A cylindrical tumor is created

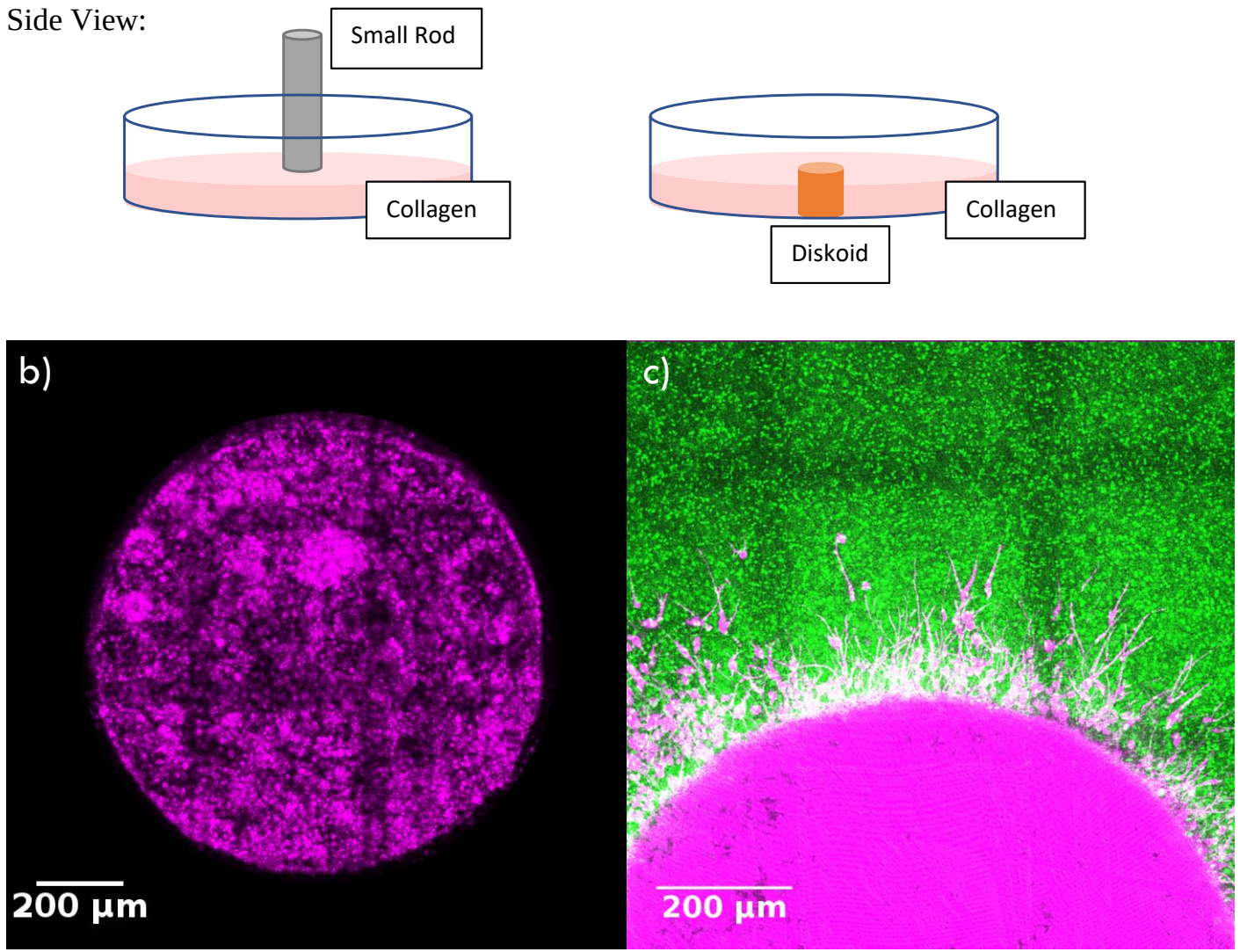


Figure 4: a) Diagram of the diskoid model b) MDA-MB-231 GFP cell tumor diskoid at 4x magnification on Day 0 c) Same discoid at 10x on Day 1. Images b) and c) taken by Austin Naylor

METHODS

- Spheroids produced in well plates
  - Place cells in V-shaped wells
  - Cells grow and form spheroids
- Use bioprinter to precisely place spheroids into collagen gel
- Can also pipet spheroids into collagen gel

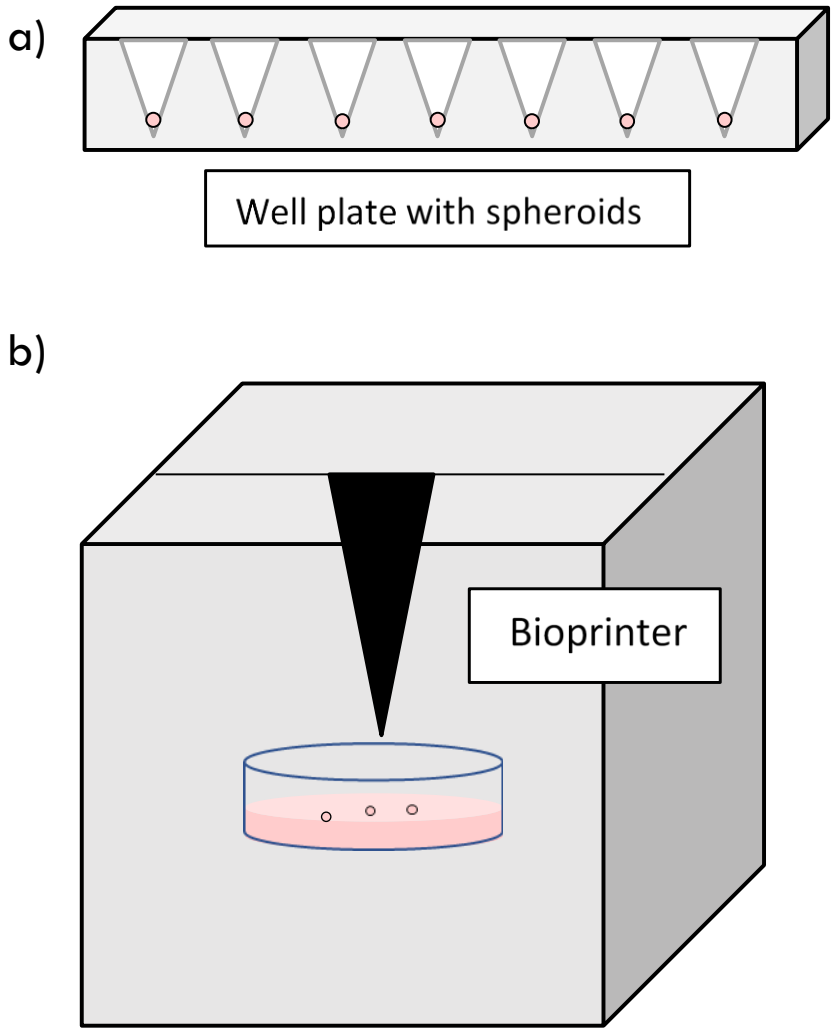


Figure 5: a) diagram of well plate growing spheroids in V-shaped wells b) diagram of a bioprinter printing spheroids into collagen

RESULTS: COLLAGEN AT 25° C VS 37° C

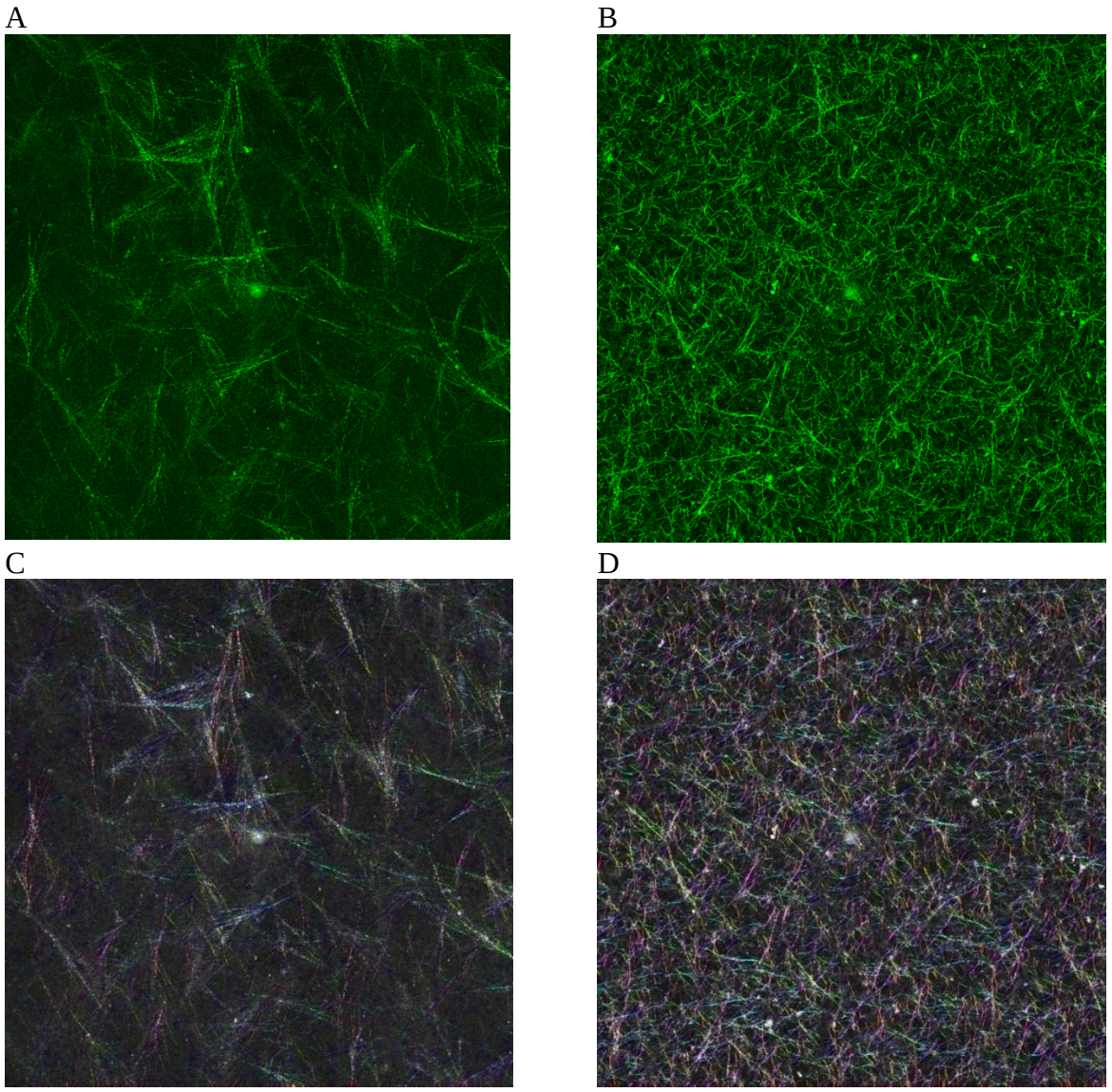
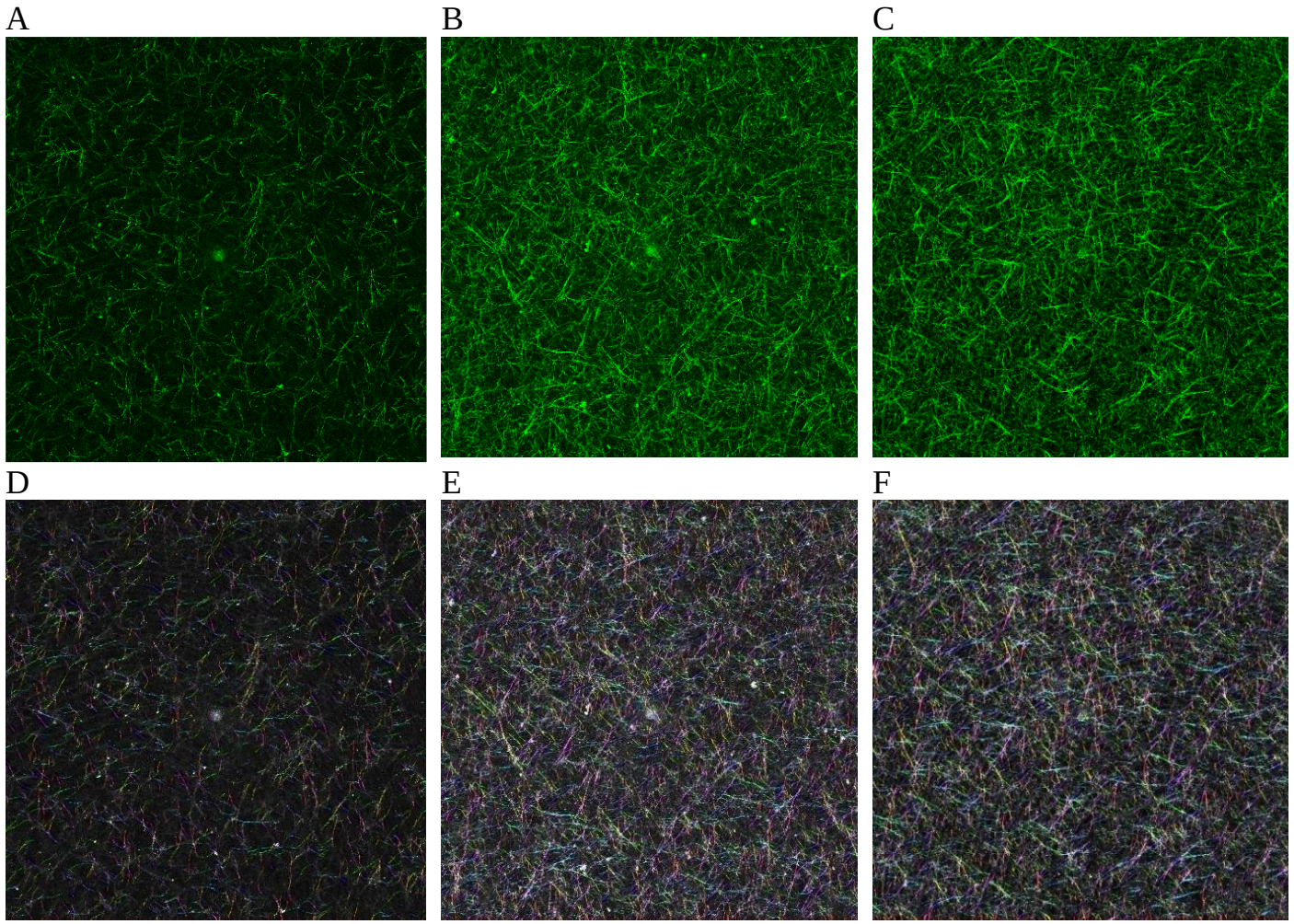


Figure 6: Collagen gelled at 25 °C versus 37 °C: a) Collagen at 2mg/ml density at 25 °C. B) Collagen at 2 mg/ml density at 37 °C. C) Orientation at 25 °C. D) Orientation at 37 °C.

Temperature at Gelation (°C)	Average Pore Size (μm^2)	Average Fiber Length (μm)
25	458.6	50.29
37	25.77	8.831

- 25°: mean room temperature
- 37°: mean body temperature
- Average pore size and average fiber length was greater at 25 °C.
- Orientation appears random for at both temperatures.

COLLAGEN AT 1, 2, AND 3 MG/ML AT 37° C



Density (mg/ml)	Average Pore Size (μm^2)	Average Fiber Length (µm)
1	48.76	12.41
2	25.77	8.831
3	14.31	5.801

- As the density increases, average pore size and average fiber length decreases.
- Orientation appears random at all three densities.

Figure 7: a) Collagen at 1, 2, and 3 mg/ml at 37 °C: a) 1 mg/ml of collagen b) 2 mg/ml of collagen c) 3 mg/ml of collagen d) 1 mg/ml of collagen alignment e) 2 mg/ml of collagen alignment f) 3 mg/ml of collagen alignment

IMAGING & ANALYSIS

- Images were taken using
  - Leica TCS SPE confocal microscope
  - Hood scope: Motic AE2000 and camera: Moticam 5.0MP
- ImageJ (Fiji) was used for analysis
  - Average fiber length was determined by finding an average fiber and measuring the length
  - Average pore size was determined by finding an average pore and measuring the area of the pore
- Cellink BIOX bioprinter used for bioprinting

RESULTS FROM COLLAGEN, DISKOIDS, SPHEROIDS, AND BIOPRINTING

- Collagen
  - As the density increases, average pore size and average fiber length decreases.
  - It appeared that all samples had fibers with random orientation.
- Diskoids: Collagen gelled before inserting cells.
- Spheroids:
  - Dense and round
  - 5/16 viable.
  - Sometimes difficult to remove from the well
- Bioprinting: Did not print one drop but entire ink in the syringe.

DISCUSSION

Collagen:

- 25°C vs 37°C : Smaller pore size with 37°C needed for certain experiments
- 1, 2, and 3 mg/ml: Control density to also control pore size and fiber length
- Orientation should remain the same

Diskoids:

- Not as physiologically relevant compared to the spheroids.
- Difficult to produce.

Spheroids:

- More physiologically relevant than diskoids.
- Studies should be done to perfect this method to produce more viable spheroids in a batch and easier ways to remove spheroids from well plate.

Bioprinting:

- The cost of a bioprinter can be high.
- The speed and pressure of printing must be finetuned.
- Bioprinting provides accuracy for more research.

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