

FINAL LAB REPORT

Name: Shaik Mohammad Aslam

ID-11656882

Background

Micropattern cell culturing is a specialized laboratory technique for cell culturing on surfaces using precise geometrical patterns. These generated patterns can affect cell morphology, behavior, and interactions, providing the researchers with a controlled environment to further study and gain insights into various cell physiology and processes. The micropattern cell biology technique is where cells are cultured on a substrate patterned with adherent micrometer-sized domains with geometric characteristics. The importance of cell culturing on micropatterns aligns with its significance in resolving complex feature details of cell function and behavior. This method of controlling the environment of cells enables researchers to understand and gain insights into cell differentiation, cell migrations, and cell-to-cell communication. This technique is essential in understanding how cells react to unique geometric signals and is critical in cancer treatment, regenerative medicine, and tissue engineering. Furthermore, cell culture models contribute to the innovation of more advanced tools for disease diagnosis and other treatment strategies. In this

project on cell culture on micropatterns, we developed the hypothesis that micropatterning cell culture with specific geometric patterns affected cell morphology and intracellular signaling, resulting in evident changes in the behavior of cells. We predicted that the geometric models would modify cell behavior, cell migration, cell adhesion, and the ability of the cell to stimulate various signaling pathways related to the cell response. This research aimed to gain insights into how spatial organization affects cell behavior concerning its implications for regenerative medicine, tissue engineering, and disease diagnosis.

Methods

Cell Culture

The cells from the thawing stage were then humidified in an incubator at 37°C and 5% Carbon (iv) oxide; these conditions created a conducive environment for the optimal growth of the cell. The cell growth was regularly checked and assessed with microscopic techniques to monitor the cell confluence. Immediately when the cell growth reached between 70-80%, Trypsin-EDTA was used to detach the cells after the cell incubation time was over.

Matrigel Coating

To Prepare Matrigel-diluted PBS solution for coating stencil. In a 1.5 ml Eppendorf tube, 298.5 microliters of PBS and 1.5 microlitres of Matrigel were combined to create a solution, which was then chilled. Then, 40 µl of the solution was piped into the holes to ensure no leakage outside the stencil. The plate was put back into the incubator once all six stencils were covered with the solution. The Matrigel-coated stencils were prepared for cell seeding after 30 to 45 minutes.

Cell Thawing:

The biohood is cleaned before the cells are thawed. Following that, prepare a 15 ml centrifuge tube and fill it with 9 ml of full media that you will label "cells." Put the necessary information on the flask's label, such as the date, passage number, lab section, and cell line. Take out the cells, set them on ice, and record the passage number. For about a minute, thaw the frozen vial in a water bath at 37°C. To collect all the cells, pipette 1 ml of culture medium into the vial three times and add the cells from the vial to the 9 ml of media. For 4 minutes, centrifuge the tube containing the cells at 280 g. Remove the supernatant with caution so as not to disturb the pellets. Gently pipette 10 ml of fresh complete culture media to resuspend the cells.

Cell Counting:

In a fresh tube, combine 10 µl of cells and 10 µl of Trypan Blue for cell counting. Transfer 10 µl of this mixture to the inlet of the cell counting slide. Count the number of live (transparent) and dead (Trypan Blue-stained) cells in each of the four corner squares while using a bright field view microscope. To determine the final live and dead cell density, calculate the average cell count, multiply it by 10,000, and then adjust for the Trypan Blue stain's 1:2 dilution.

Cell Seeding:

Finished marking the flask with the passage number so that cells can be seeded inside the culture dish. After the flask reaches 85–90% confluency, place it in the incubator and replace the medium every three days.

Using a tweezer, open the autoclave bag inside the biohood. Using the same tweezer, open the jar containing the PDMS stencils, and place each stencil in one well of a 6-well plate with a different hole size. Store the plate in the biohood until the following week, loosely wrapping it in aluminum foil.

Cell Passaging:

Remove all culture media from the flask and rinse it twice with 5 ml PBS. In the flask, add 2 ml of trypsin enzyme/PBS solution and incubate for 7 minutes. Trypsin should be neutralized with 8 ml of complete culture media, swirled to release cells, and transferred to a labeled 15 ml tube. Centrifuge the tube for 4 minutes at 1200 rpm with a balance tube, then remove the

supernatant without touching the pellet and resuspend the cells in 1 ml of culture media. Counting cell density with Trypan Blue, then dilute the cells with complete culture media to achieve the desired density. Rinse the culture flask with 10 mL PBS, then remove the PBS and reseed with 1 mL of diluted cells plus 9 mL of complete media and keep in the incubator.

Cell Fixing

First remove the stencil and then the medium in order to fix the cells. After adding 1 mL of PBS, take it out. Next, add 1 milliliter (mL) of 4% PFA, and let it sit at room temperature (RT) for 15 minutes before taking it out. Then add 1 mL of PBS, let it settle for 2–3 minutes, and take it out. Go through this process twice more. After that, add 1 mL of 0.5% Triton X-100, let it sit at room temperature for 10 minutes, then remove it and rinse with 1 mL of PBS. Next, cover the cells with aluminum foil, add 1 mL of 4% BSA in PBS, and incubate at room temperature for 20 minutes. During the incubation phase, work on ice, turn out the light, and get ready for F-actin working solution was created by pipetting 7 μ L of F-actin stock into 1 mL of PBS. After the BSA incubation is finished, take out the BSA, give the cells a washing with 1 mL PBS, and then add 50–100 μ L of the F-actin working solution. After 30 minutes of RT incubation, remove the dye and wash three times with PBS, letting the between-washes interval of two minutes. Place a coverslip on each cell, add three drops of DAPI, and then wrap the cells in foil. Place the cells in an environment that is 4°C at the end. Examine the cells under a microscope before wrapping them in foil.

DATA ANALYSIS

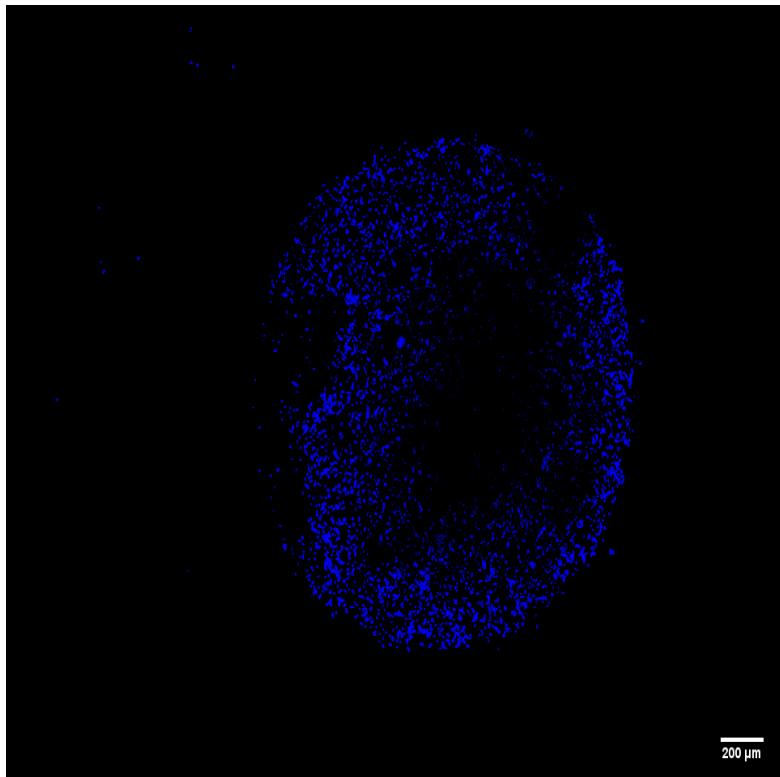
For this research, data analysis was done with types of software programs. The principal techniques comprised:

Cell images were analyzed using programs such as ImageJ Data was organized and interpreted using spreadsheet programs such as Excel, guaranteeing a clear comprehension of the trends and patterns noticed.

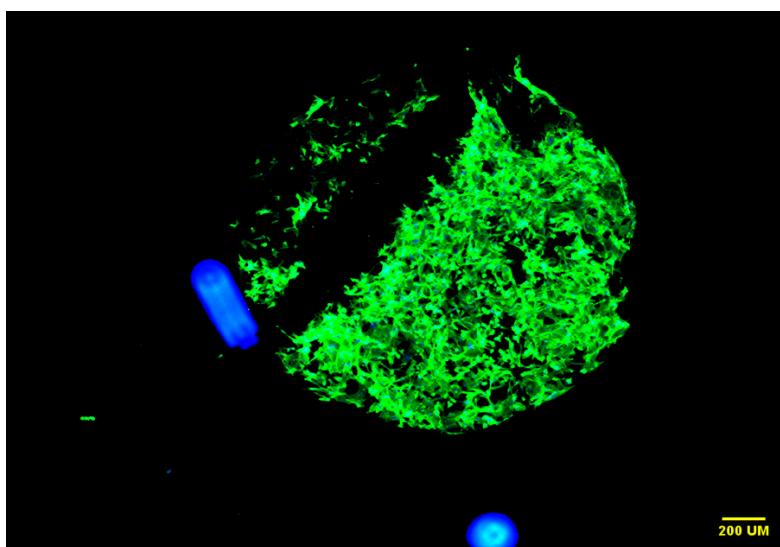
The procedure is to add the Images in the Image J tool and should follow the methods from the guide of image j tool and this tool collect the image and we can do any type of editing's in that tool from measuring the length width depth and brightness, contrast and much more this is also able the merge images into one single image and shows the calculations of it. With these calculations we can create bar graphs and errors graphs and much more

Results

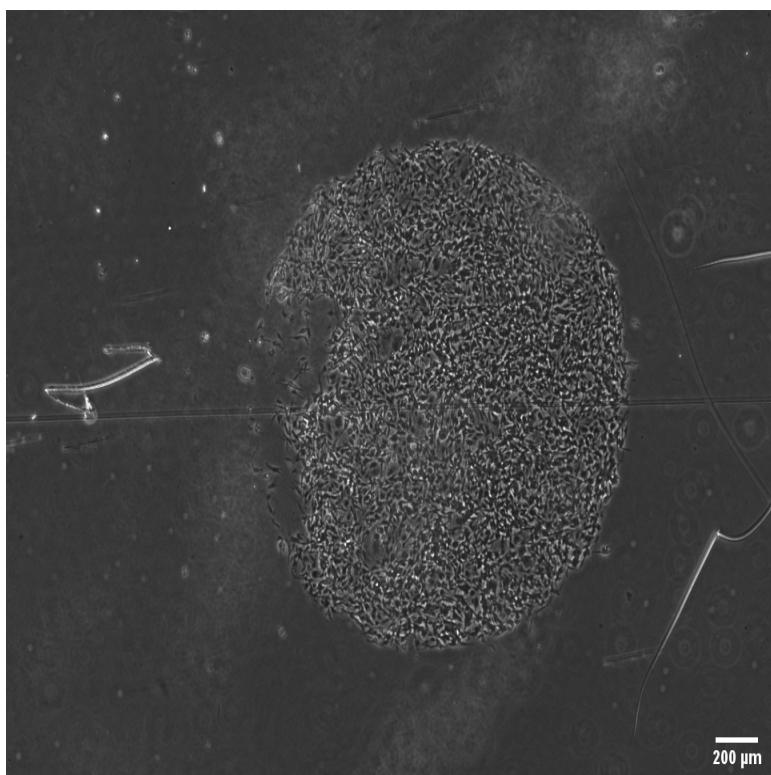
Figure 1



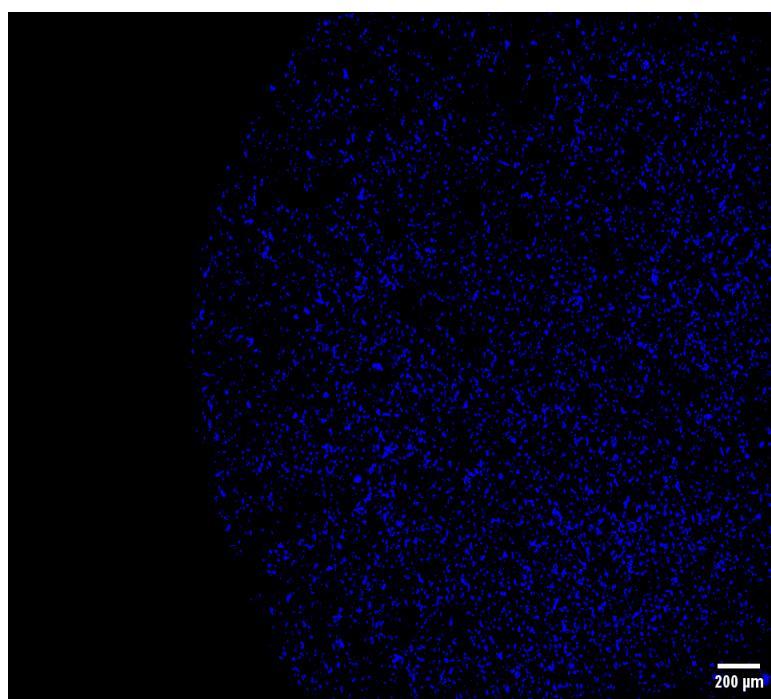
4X 2mm DAPI



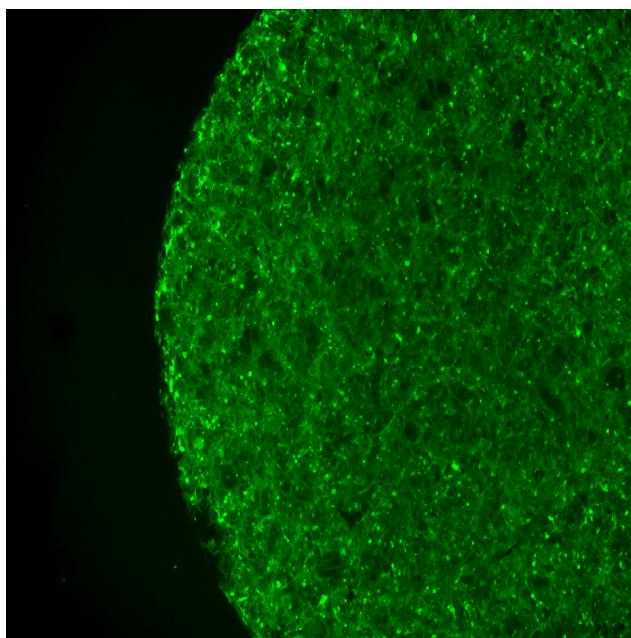
4X 2mm F-ACTIN



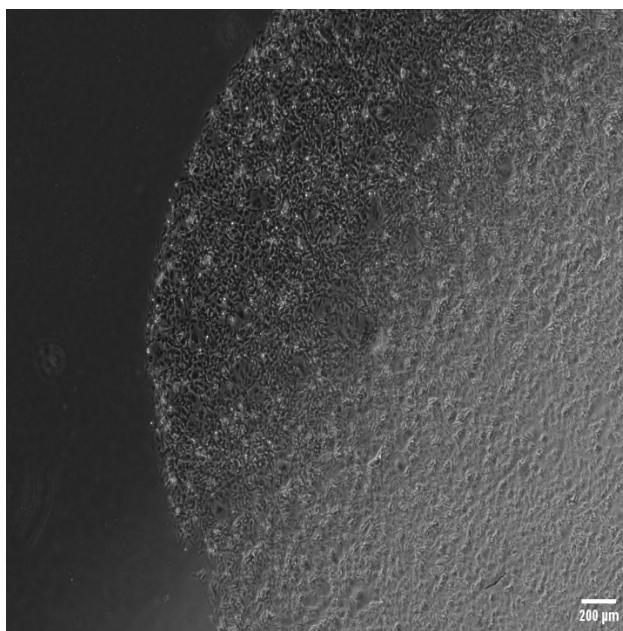
4X 2mm Phase



4X 3mm DAPI

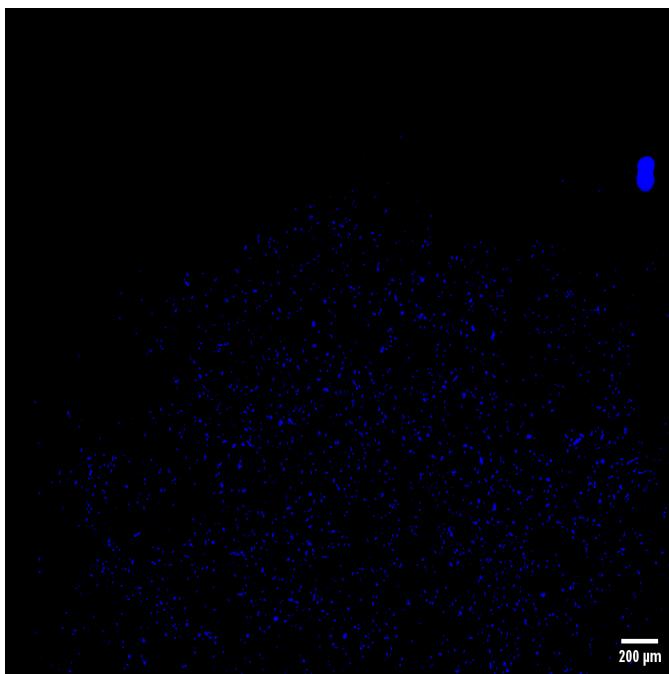


4X 3mm F-ACTIN

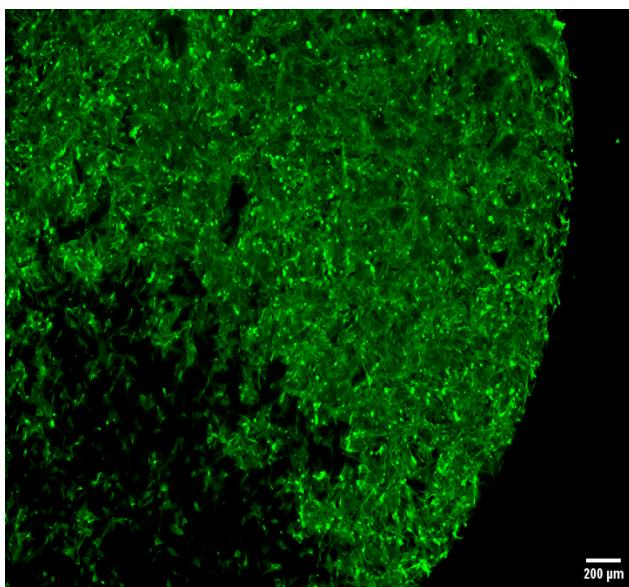


4X 3mm PHASE

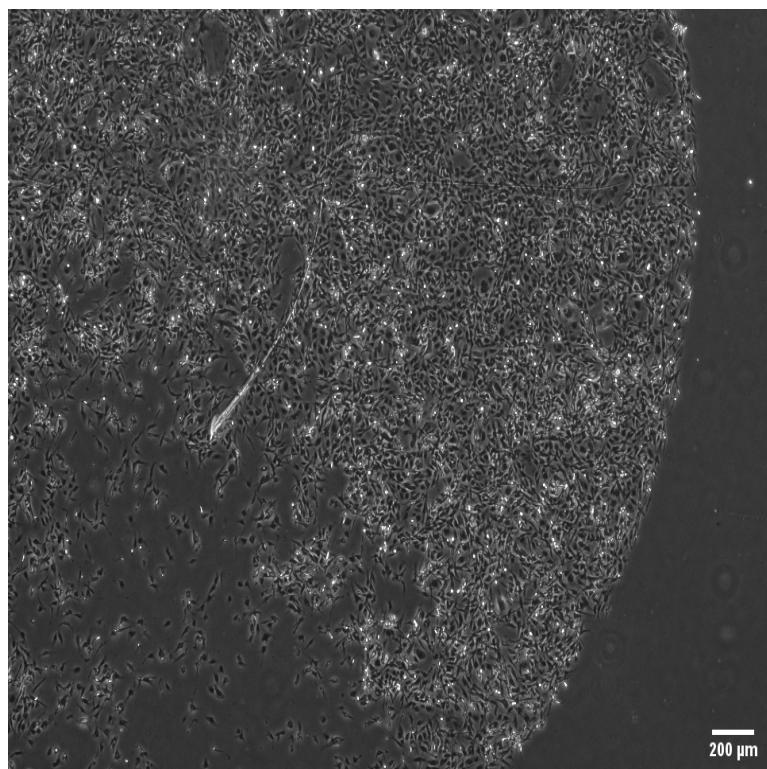
4x4 mm



4X 4mm DAPI

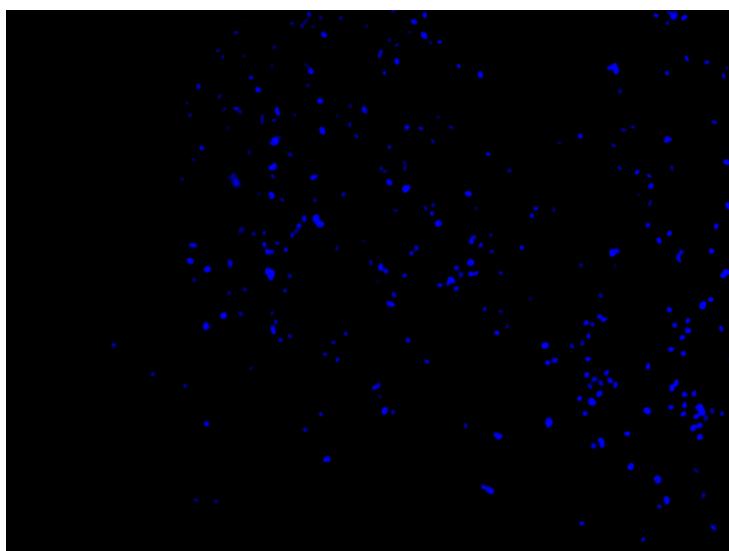


4X 4mm F-ACTIN

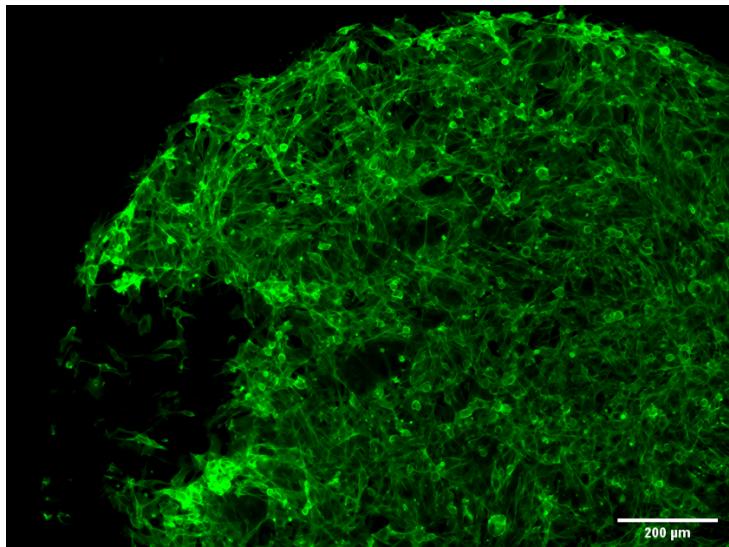


4X 4mm PHASE

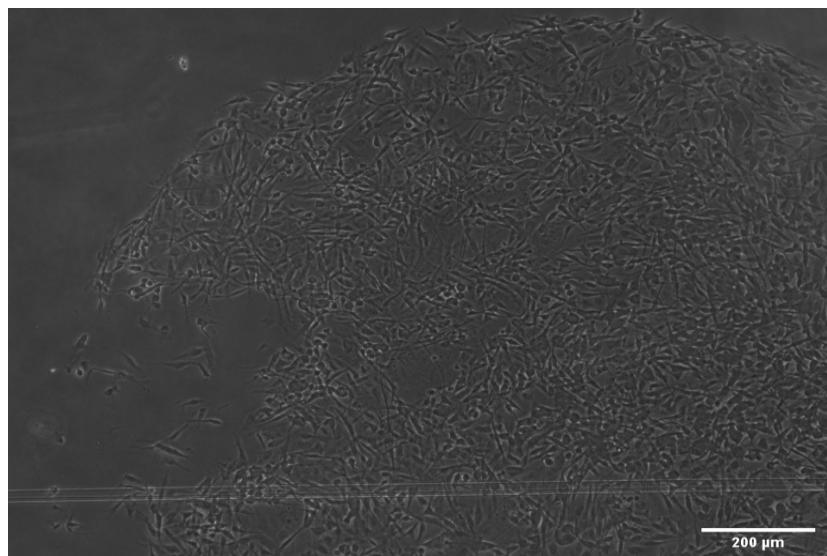
10X 2MM



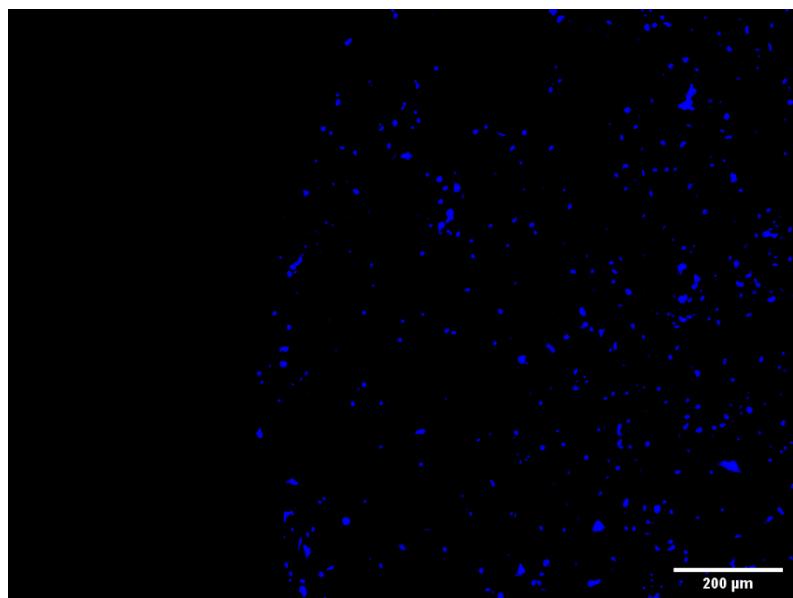
10X 2mm Dapi



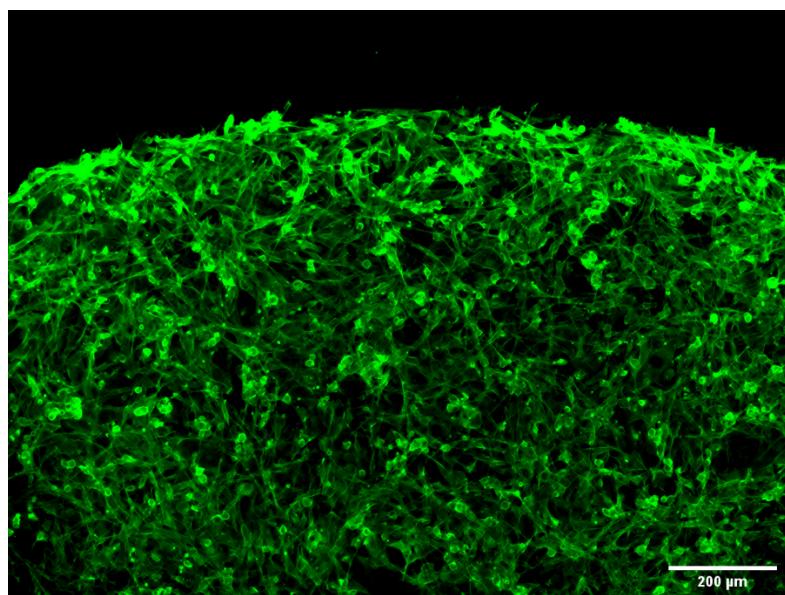
10x2mm F-ACTIN



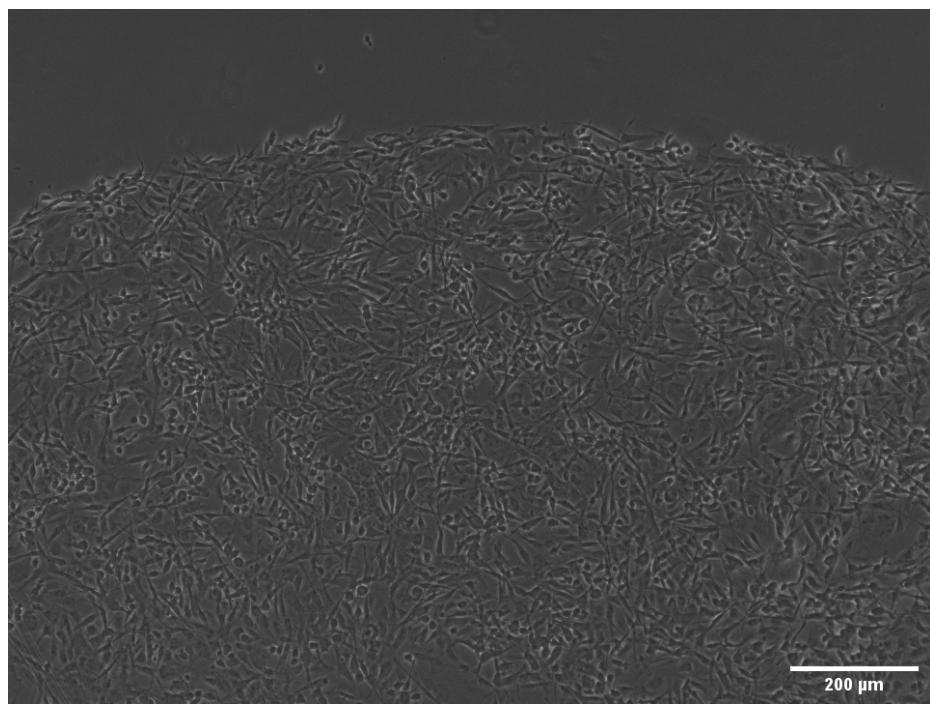
10X 2mm PHASE



10X 3MM DAPI

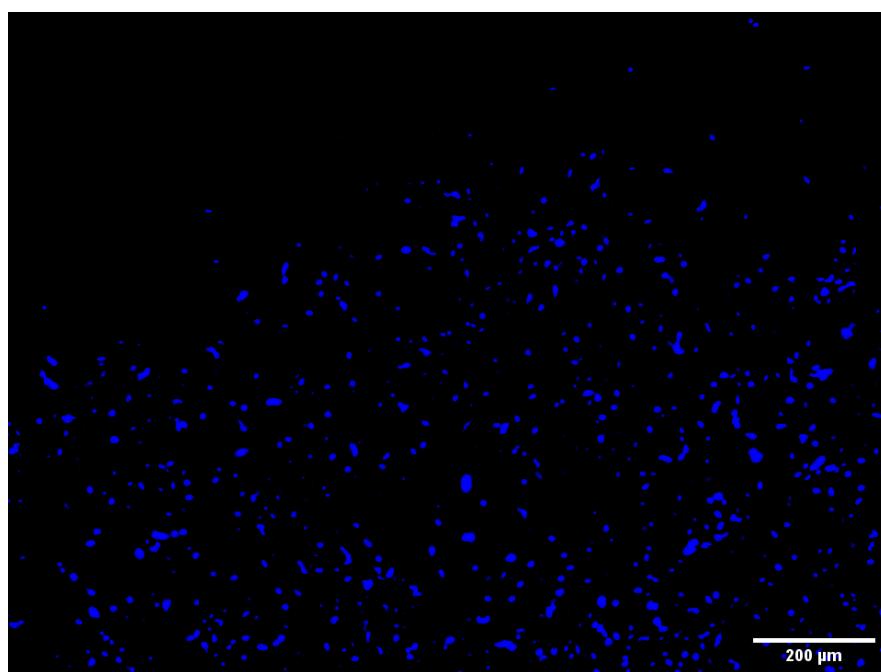


10X 3MM FACTIN

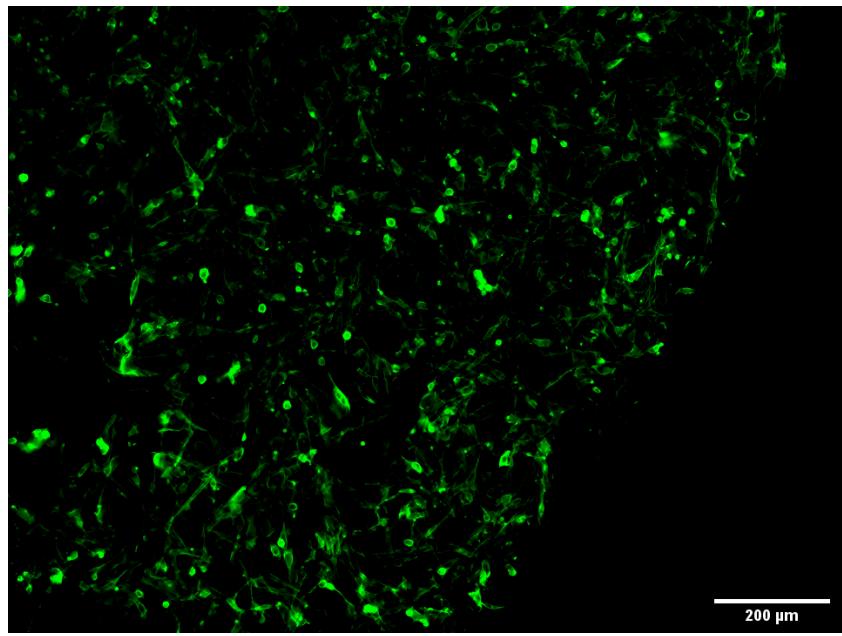


10X 3mm PHASE

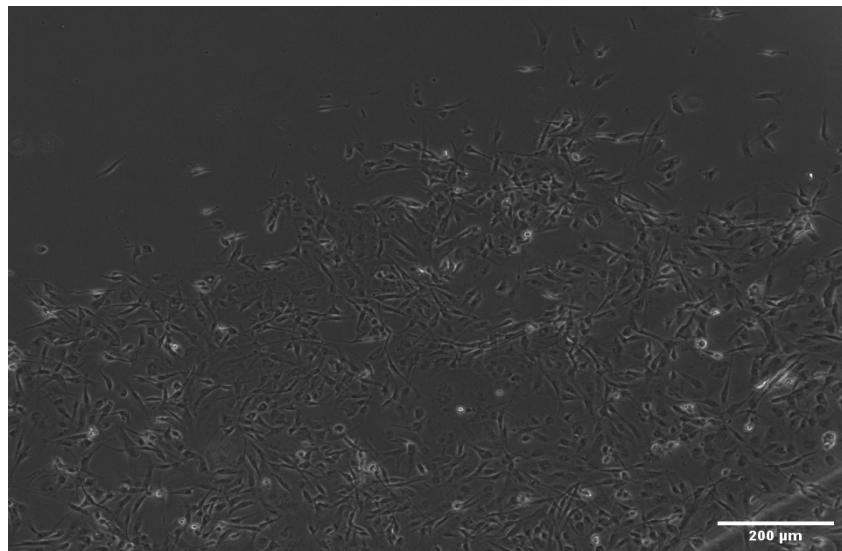
10X4mm



10X 4mm DAPI



10X 4MM F-ACTIN



10X 4mm PHASE

Figure -2

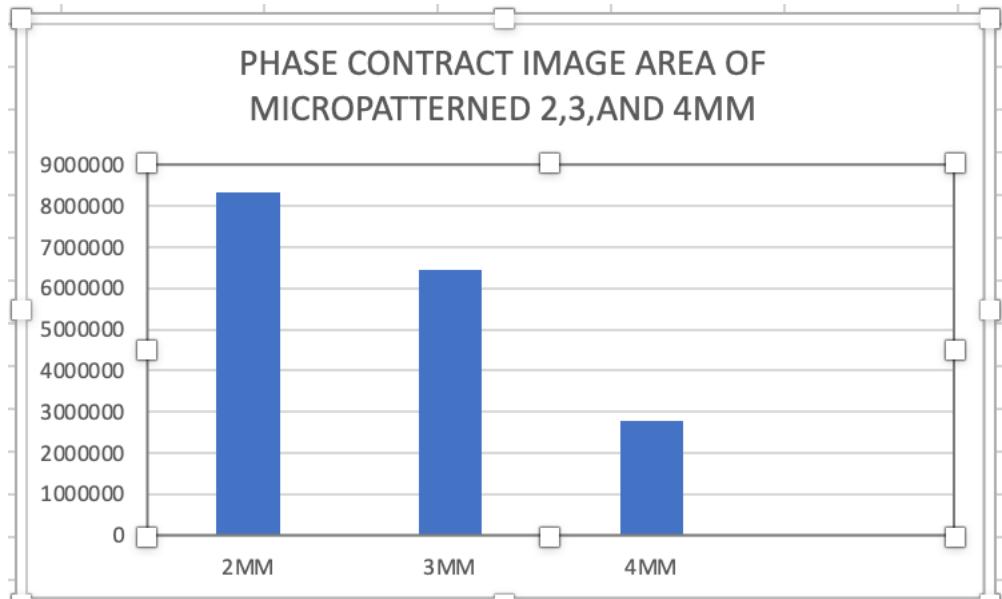


FIGURE 3

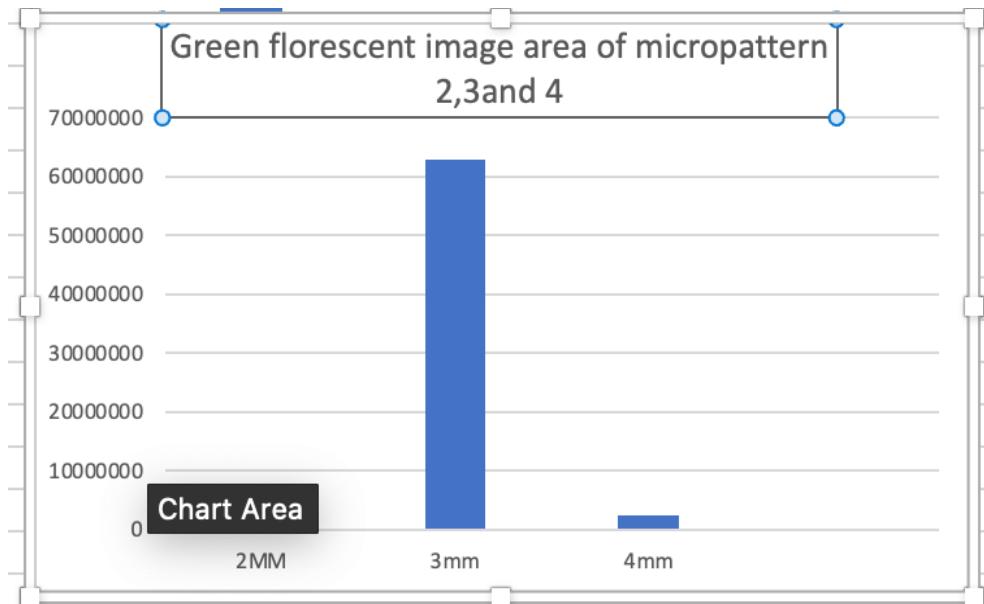


Figure 4

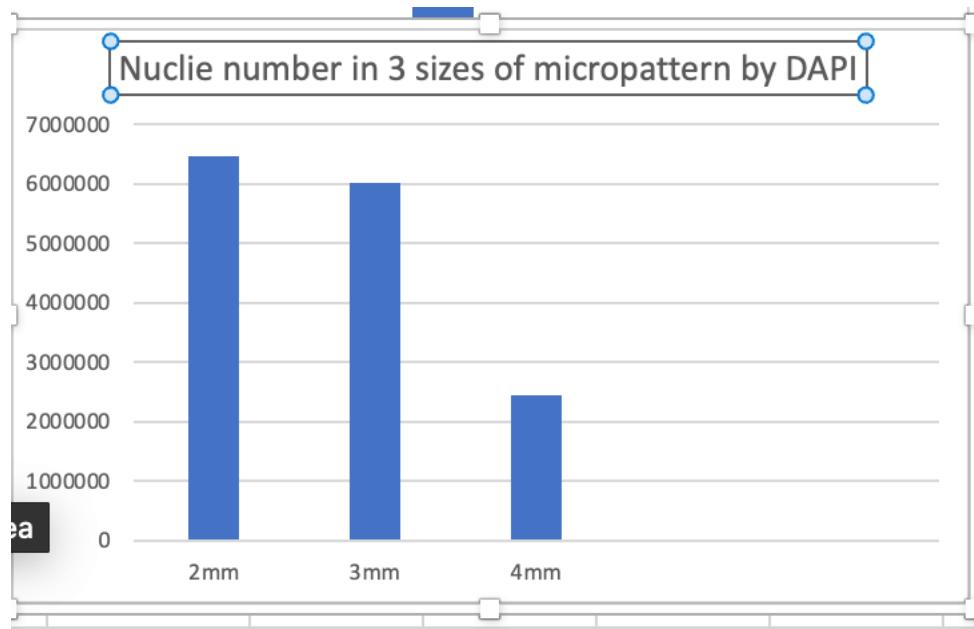


Figure 5

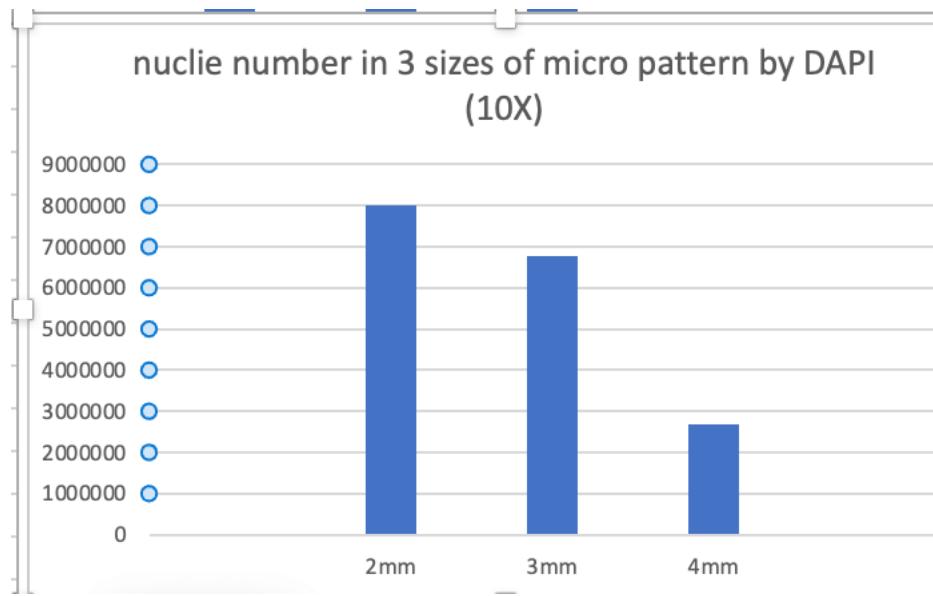
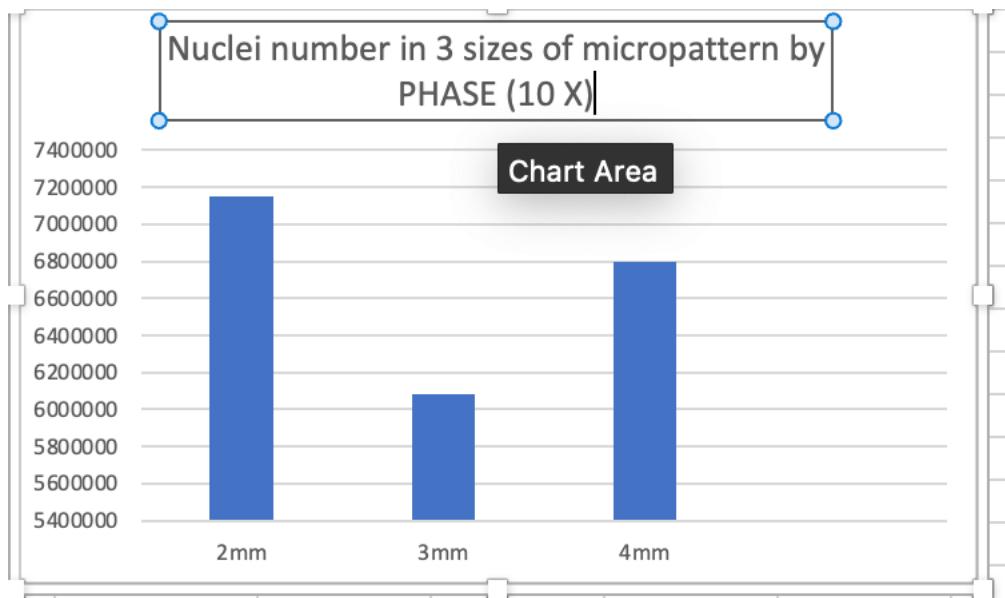


Figure 6



Discussion

Talking of this whole procedure and methods has done in the image J and using this tool helps to maintain the all the measurements and editing things for maintaining graphs and the exact portion of the cells . Cellular responses are influenced by micropatterning, which regulates cell arrangement at the micrometer scale. Size-regulated fibronectin patterns affect cell shape and behavior. All things considered, size-controlled micropatterning provides information on intercellular communication, differentiation, adhesion, and proliferation that is useful for tissue engineering and regenerative medicine.

Limitations and Future Directions

While the experimental procedure for this project remains flowing, particular focus is critical during cell seeding and staining because the reagents and chemicals used during the process could sometimes be very toxic to the cells, which can easily affect the cells, thereby interfering with the process. DAPI has been related to health problems mostly during the membrane cell

attachment; therefore, proper washing is always required during this process to allow all removals of DAPI, Moreover, the use of various chemicals during cell fixation, such as Organic solvents requires much focus and careful attention as poor preparation of such chemicals could lead to cell apoptosis. In conclusion, the future of this area of study can mainly focus on understanding innovative ways that involve the use of fewer chemicals to prevent the risks of cell death or interference during the process.

Conclusions

In conclusion, this project focused deeply on understanding the cell biology technique of micropattern cell culturing. This technique allows researchers to understand various cell components, such as cell interactions, morphology, and behavior. With the data obtained significant result, the outcome of this study could contribute to the field of cell biology and other related fields by offering deep information from this experiment to address challenges facing cell research and other challenges related to a few reagents found to be of toxicity during this experiment.

References

Shaik Mohammad Aslam BMEN 5210 lab report 1,FALL 2023

Shaik Mohammad Aslam BMEN 5210 lab report 2,FALL 2023

Shaik Mohammad Aslam BMEN 5210 lab report 3,FALL 2023

Park, D., Lee, J., Lee, Y., Son, K., Choi, J. W., Jeang, W. J., ... & Jeon, N. L. (2021). Aspiration-mediated hydrogel micropatterning using rail-based open microfluidic devices for high-throughput 3D cell culture. *Scientific Reports*, 11(1), 19986.

Singh, A. V., Chandrasekar, V., Laux, P., Luch, A., Dakua, S. P., Zamboni, P., ... & Gemmati, D. (2022). Micropatterned neurovascular interface to mimic the blood–brain barrier’s neurophysiology and micromechanical function: a BBB-on-CHIP model. *Cells*, 11(18), 2801.

Roukos, Vassilis, et al. “Cell Cycle Staging of Individual Cells by Fluorescence Microscopy.”

Nature News, Nature Publishing Group, 29 Jan. 2015,
www.nature.com/articles/nprot.2015.016

Yang, H. (2023). BMEN 5210 Cell Culture instructions [Word documents on canvas page of BMEN 5210 biomedical laboratory fall 2023 course]