NDnano Undergraduate Research Fellowship (NURF)
2015 Project Summary

1. Student name: Jack Casey

2. Faculty mentor name: Dr. Pinar Zorlutuna

3. Project title: Spatially patterned microfabricated co-cultures to study cancer development and progress

4. Briefly describe any new skills you acquired during your summer research:

Over the course of my summer research, I have learned several new things in regards to cell culture and tissue engineering. I learned microwell fabrication using hydrogels, cell encapsulation in hydrogels using GelMA and how to perform new cell viability and metabolic activity assays such as Live/Dead and Alamar Blue. Another useful skill was cell freezing to store cells as a backup in the case of contamination. I also learned advanced microscopy and cell imaging skills including fluorescent imaging and z-stack images.

5. Briefly share a practical application/end use of your research:

A future application of my research is a new and more accurate way to study cancer development. By creating a 3D microenvironment that can better simulate the tumor microenvironment than current research models, cancer and other potential diseases could be studied with more precision.

Begin two-paragraph project summary here (~ one type-written page) to describe problem and project goal and your activities / results:

This research project looks to address the problem of current models used to study cancer development. *In vivo* animal models can simulate physiological conditions, however they fail to model interactions of human cell types. *In vitro* models can use human cells types and have several tunable parameters, however they can fail to model complex interactions and cannot reproduce specific cell crosstalk. Most cancer models are also studied in a 2-D environment. A new 3-D model is necessary for controlling tumor microenvironment and essential for understanding tumor growth and metastasis. This research looks into seeding cells in microwells, a platform to control and study the cell aggregation and proliferation.

The first goal of this project was to determine and optimize parameters for the cancer cell seeding protocol. The cell types studied were HCC-1806, an established cancer cell line which was used as a control for optimization experiments and mouse mammary organoids. The 3D model will use microwells, a patterned array of microscopic wells at equal spacing. Two different spacing lengths were used, one had a distance of 150 microns between each microwell and the other had a distance of 300 microns. The materials used for fabrication of the microwells were polyethylene glycol (PEG) and methacrylated gelatin (GelMA). Three types of PEG, each with different molecular weights, 1kDa, 10kDa, and 20kDa and two types of GelMA, a low methacrylate concentration and a high methacrylate concentration were used in an experiment to test their long-term stability of the microwell arrays. Microwells were fabricated to study each gel material and assess its stability. Brightfield images were taken daily for 14 days and ImageJ was used to analyze roundness of the microwells to determine how the microwells distorted over time. PEG 20k, GelMA low, and GelMA high were used in all further experiments.

To optimize cell seeding density, different densities were used to test their effectiveness of filling the microwells and leaving no cells remaining on the surface of the hydrogel (Figure 1). The optimum density found was 300,000 cells per 30ul. Cell culture media and media temperature were varied as well to further optimize the cancer cell seeding. The optimal seeding parameters were different for PEG microwells and GelMA microwells.

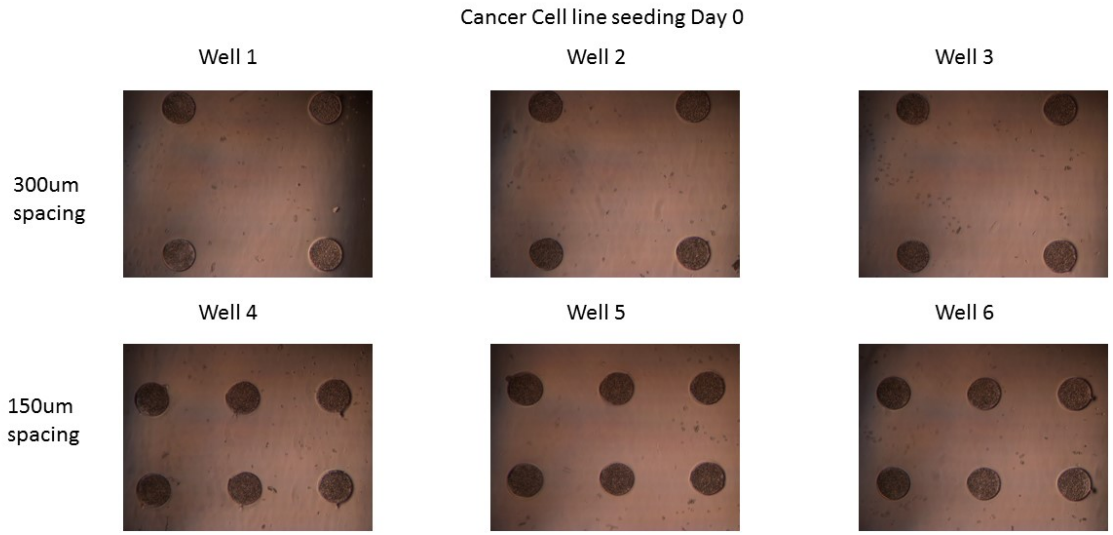

Figure 1: Brightfield images of PEG microwells on Day 0, after seeding with HCC1806 cells.

Live/Dead assays were performed to assess cell viability after the cells had formed aggregates and proliferated within the microwells. The live/dead assay was done on Day 3 after cell seeding as this was when most of the cell aggregates had filled the entirety of the microwells. Fluorescent images were taken of the assay samples and the quantity of green or red cells was assessed to find the proportion of living cells (Figure 2).

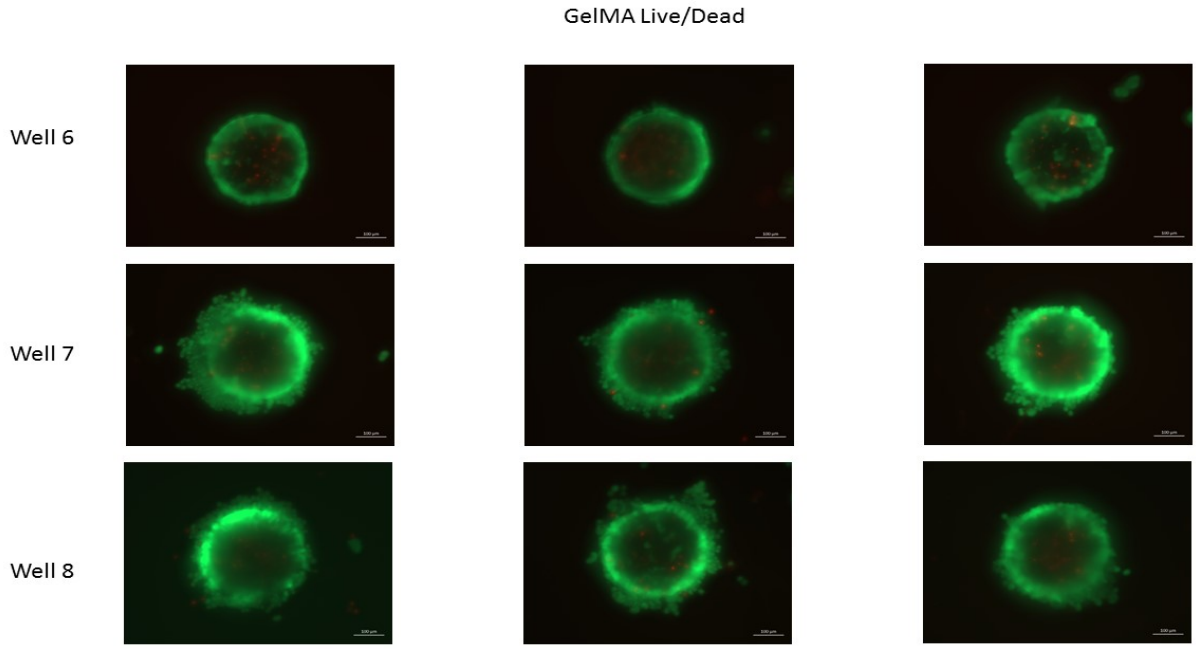

Figure 2: Live/Dead Assay of HCC1806 cells in GelMA microwells. Migration into the gel is visible from the microwells in several samples.

Mouse mammary organoid seeding in microwells and their encapsulation in hydrogels were also studied. This was another experiment to optimize and develop the protocol for studying mammary organoids. Organoids in microwells and gel encapsulations were imaged for seven days with a media change on Day 4. A live/dead assay was performed on Day 7 using confocal imaging to assess cell viability. Majority of the cells were found to be viable. This is an ongoing experiment.