In Vitro 3D Colorectal Cancer Model Using PEG-Fibrinogen Hydrogels

Andrew Moore, Iman Hassani, and Elizabeth Lipke

In areas such as drug study, results show that a 2D environment for cancer cells does not supply an accurate representation of a cancer tumor. In order to provide an appropriate system for drug studies, a 3D model must be fabricated to mimic a true cancer tumor. In this project, 3D colorectal cancer tissues using three different cancer cell lines (HCT 116, HT 29, and SW480) were created and tested over the course of 29 days to determine if this fabrication method was a successful model.

Cancer tissues, or hydrogels, were created using an encapsulation process, where cancer cells were mixed with a photo crosslinking mixture composed of poly(ethylene glycol)fibrinogen (PEG-fibrinogen), n-vinylpyrrolidone (NVP), triethanolamine (TEOA), and EoSinY. Ten microliters of this mixture were pipetted into a polymer mold. The mixture was then photo crosslinked for two minutes using a fluorescent light source. The light emitted interacts with the photo initiator, in this case EoSinY, to produce free radicals that initiate the polymerization of the hydrogels. The hydrogels were kept in media that was changed every other day to let the tissues grow and proliferate. Various batches of hydrogels for each of the three cell lines were encapsulated in order to perform separate experiments throughout the project.

Multiple tests and experiments on the hydrogels were performed on days 1, 8, 15, 22, and 29, with day 0 being the day of encapsulation, to determine the viability and success of the tissues in mimicking known characteristics of native cancer tumors. Phase contrast images were taken using 2X, 4X, 10X, and 20X magnification to monitor growth and morphological changes. To see the number of live cells, dead cells, and nuclei, a series of images were taken from top to bottom of the hydrogel after a live/dead stain was performed. Mechanical stiffness testing was done using a microsquisher device to track the differences in stiffness over 29 days. Immunostaining using several primary antibodies (Ki67, CD44, N-cad, E-cad, alpha SMA, Ck20, SNA1, Vimentin, twist) was used to visualize certain aspects such as proliferation, migration, and cell-cell adhesion. Flow cytometry was carried out to see the percentages of various cell populations within the hydrogels. Tissues were fixed for later testing with scanning electron microscopy and histology to gather morphological, compositional, and anatomical information; tissues were also flash frozen using optimal cutting temperature molds while others were frozen for gene expression testing.

So far, results for colony growth within the hydrogels show an increase in area over time, indicating the viability of the model. Phase contrast images also show a dense outer rim forming as time progresses, attesting to the proliferation and migration of the cells from the hydrogels. Data analysis for colony area, live/dead, mechanical stiffness, and flow cytometry is still ongoing. Results from these experiments will be compared with cancer tumors from literature to fully determine the success of this 3D model for the three cell lines.

The importance of this research project is its potential impact on cancer research in areas such as drug studies. An *in vitro* 3D model can provide more accurate results for these studies if it can successfully mimic native tumors. This project has helped create a base model for HCT 116, HT 29, and SW480 cells that hopefully closely imitate cancer tumors.