Design of a Three-Dimensional (3D) Adipose-laden Hydrogel Model to Study Breast Cancer Metastasis

Rachel Yu, Cheryl T. Gomillion, Ph.D
School of Chemical, Materials & Biomedical Engineering, University of Georgia College of Engineering, Athens, GA

Statement of Purpose: Triple negative breast cancer is a highly aggressive form of cancer with great metastatic potential. It has been reported that up to 90% of cancer-related deaths result from metastasis, yet contributors to the metastatic cascade are still poorly understood [1]. *In vitro* three-dimensional (3D) models have vast potential for evaluating cancer metastasis because their 3D nature exhibits greater physiological similarity to the human body. A primary goal of our work is to fabricate a 3D model system suitable for observing the effects of adipocytes and adipocyte-secreted factors on breast cancer cell metastasis. In particular, breast tissue is composed of a high number of adipocytes that release cell-signaling molecules. Additionally, increased adipose tissue surrounding tumors has been shown to increase an inflammatory response by the body, which induces pro-metastatic behavior [2]. In this work, 3D-printed constructs were used to cast composite cell-laden hydrogels used for studying breast cancer metastasis. Adipose-laden hydrogels were prepared to mimic physical and biochemical properties of the breast microenvironment. Hydrogel-based tumor microspheres were formed and centrally-placed in the microenvironment hydrogels to monitor cancer cell migration, towards demonstrating efficacy of our model approach.

Methods: Murine bone marrow stromal cells were grown to confluence and differentiated for 7 days in adipogenic differentiation media. Conditioned media from the resulting adipose cells was collected at Day 7 for later experimental use. SolidWorks® was used to design casting molds that were 3D printed and used to form the microenvironment hydrogels. All hydrogels consisted of a blend of alginate and gelatin (porcine) in a 3:2 ratio (5% w/v). Adipose-laden microenvironment hydrogels were prepared by combining a suspension of differentiated adipocyte cells (1 million cells/mL) in low serum media (2% FBS, 1% P/S) to make up the liquid portion of the hydrogel. Microenvironment hydrogels were also prepared using either low serum media (LSM) or adipose-conditioned media (CM) only for experimental comparison. For all samples, the hydrogel solutions were cast into the 3D printed molds and cooled for 10 minutes at 4°C, followed by crosslinking in calcium chloride. Rheological characterization of the adipocyte-laden hydrogels was performed using an Anton-Paar rheometer. Two cancer cells lines, MCF-7 and MDA-MB-231 cells, were grown to confluence and labeled with CellTracker Green CMFDA. Tumor microspheres were created for both cancer cell types by adding the hydrogel suspension containing cells dropwise to calcium chloride. The microenvironment hydrogels and tumor microspheres were cultured (n=3) for 7 days in 12-well plates with low serum media. The tumor microspheres were then removed, and the migrated cancer cells were imaged at three random fields of view (FOV) and counted using ImageJ (NIH).

Results: Rheological analysis showed ideal storage and loss modulus for a hydrogel. The calculated Young's modulus for adipocyte-laden hydrogels was 4.7 kPa, which is slightly higher than reported literature values for *in vivo* adipose tissue (2.9±0.5 kPa) [3]; however, this may be more representative of a pro-metastatic microenvironment according to a study on breast density and breast cancer risk [4]. Based on the average cell counts from image analysis, there was a statistically significant difference in the amount of MCF-7 and MDA-MB-231 cell migration in the co-culture when compared to the LSM control and CM treatment groups. Due to the 3D nature of this model, imaging at three random FOV were representative of migratory trends for each treatment group, but not a complete count for all cells that had migrated.

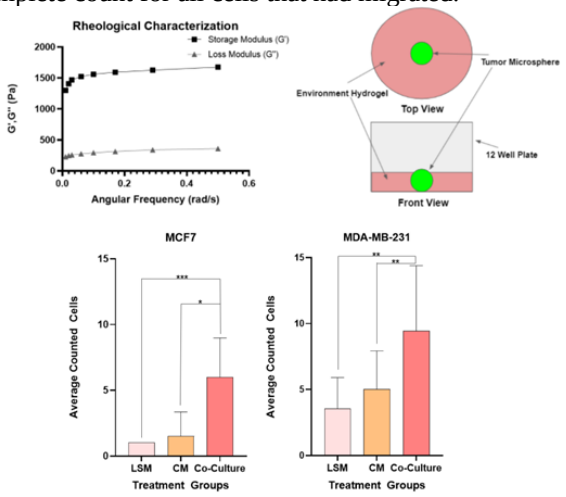

Figure 1. (A) Rheological characterization of adipose-laden hydrogels. (B) Schematic of experimental set up in each well of 12 well plate. (C) Average number of migrated MCF-7 (left) and MDA-MB-231 (right) cells in 3D hydrogel metastasis models (n=5, * indicates p<.05, ** indicates p<.006, *** indicates p<.0025).

Conclusions: Both cancer cell lines in this proof-of-concept study were able to migrate from the tumor microspheres into the environment hydrogels. Based on the total migration results, it can be inferred that the presence of adipocytes, and not just their secreted factors, tends to promote cancer cell migration for the MCF-7 cells and MDA-MB-231 cells. Future work will further optimize this model to increase cell permeability, while maintaining a physiologically-relevant tumor microenvironment. An effective model has significant translational impact for the development of personalized cancer treatments and therapeutics, among other applications.

References: [1] Jin, X., et al. Breast Cancer: Basic and Clinical Research 1 (2015). [2] Nyga, A., et al. J of Cell Comm and Signal 5(3) (2011). [3] Louis, F., et al. Cyborg and Bionic Systems (2021). [4] McCormack, V., et al. *Cancer Epidemiology Biomarkers & Prevention* 15(6) (2006) 1159-1169.