

## Development of a Heterogeneous *In Vitro* Three-Dimensional Breast Tissue Model

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**Statement of Purpose:** Past cellular response research has been largely conducted using two-dimensional tissue cultures. Emerging research has shown that three-dimensional (3D) systems allow a better understanding of cellular behavior in the native environment and a better indicator of cellular response in *in vivo* processes. The objective of this work was to develop a heterogeneous *in vitro* 3D breast tissue model. This model will be used as a proof of concept as a modular research model system. Gels of different compositions and concentrations, seeded with mammary epithelial cells and incorporated with polylactide microspheres, were used to develop the heterogeneous 3D breast tissue model. Cell viability and proliferation was evaluated as a result of changes in the surrounding matrix, specifically in response to heterogeneity induced by polylactide microspheres.

**Methods:** The gel component of the 3D breast model was produced, following the work conducted by Swamydas and colleagues [1], using agarose, collagen and Matrigel™ either alone or in combination. To determine the optimal stromal components, a base layer of gel was deposited in an 8-well chamber slide and allowed to solidify for 30 minutes. Normal mammary epithelial cells (MCF10A), at a concentration of 100,000 cells/well, were seeded in a second gel layer. Gel constructs were allowed to solidify overnight and DMEM supplemented with 10% FBS, 1% fungizone, 5% antibiotic/antimycotic, and MEGM® Singlequot® supplements including: 0.5 mL hEGF, 0.5mL hydrocortisone, and 0.5 mL insulin, was added at 24 hours post seeding. Media was changed every 3 days. Gels were harvested at Day 10. Live/dead assay and cell cluster size were used to determine the optimal gel for the 3D breast model. Collagen/Matrigel™ gels of 1.6, 2.4, 3.2, and 4.0 mg/mL concentrations were seeded with MCF10A cells and evaluated at Day 10 for cell viability to determine the optimal gel concentration. To develop a heterogeneous 3D breast model, collagen and Matrigel™ gels were seeded with either MCF10A cells or human cancerous mammary epithelial cells (MCF7), incorporating polylactide microspheres in the second gel layer. Gel constructs were maintained in culture; following culture, a Live/Dead assay was used to ascertain cell viability of the heterogeneous model.

**Results:** Live/dead analysis revealed that stromal conditions yielded differences in cell viability and morphology. Conditions with cell viability under 75% included agarose (Figure 1, left image), agarose/collagen, and collagen. Conditions with cell viability above 75% were further analyzed for cell cluster number and size (Figure 2). The greatest number of cell clusters were found in 3D gels composed of collagen/Matrigel™ and agarose/Matrigel™. Cell clusters in these systems also had the highest average size compared to cluster sizes in other conditions. Collagen/Matrigel™ was the only

environment where cell clusters were ductal-like in structure (Figure 1, center image). Preliminary results indicate that cell viability and cell cluster distribution throughout 2.4 mg/mL gels is the most consistent as compared to gels of 1.6, 3.2, and 4.0 mg/mL.

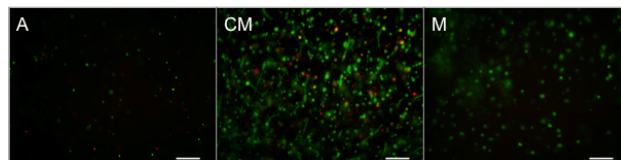


Figure 1. Live/Dead images demonstrate cell viability in each condition (scale bar = 200µm); (A) Agarose condition – lowest cell viability, (CM) Collagen/Matrigel™ condition – ductal-like structures, (M) Matrigel™ condition – highest cell viability.

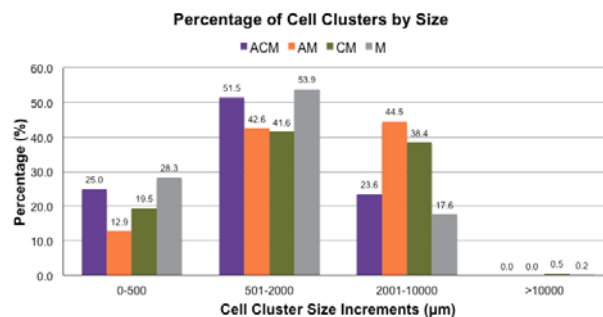


Figure 2. Percentage of cell clusters by size in conditions with cell viability above 75%. ACM = Agarose/Collagen/Matrigel™, AM = Agarose/Collagen, CM = Collagen/Matrigel™, and M = Matrigel™.

Preliminary live/dead assay results of the heterogeneous 3D breast models indicate no adverse effects on cell viability when polylactide microspheres are incorporated into the collagen/Matrigel™ stromal component.

**Conclusions:** Optimal cell growth occurs in 3D construct combinations of collagen and Matrigel™, thus these components will be used as the stromal component of the modular, heterogeneous 3D breast tissue models. Further studies will need to be conducted to better understand how the combination of gel components and polylactide microspheres affect cell viability. Future work should be conducted to evaluate how incorporation of polylactide spheres in the heterogeneous breast tissue model influences normal and cancerous cell behavior.

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**References:** [1] Swamydas M, Eddy JM, Burg KJ, Dreau D. Matrix compositions and the development of breast acini and ducts in 3D cultures. *In Vitro Cell Dev Biol Anim* 2010;46(8):673-84.