Novel Analysis of in Vitro Breast Tissue Test System

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Statement of Purpose: Current 3D systems are limited because they lack complexity and tissue-like heterogeneity and, on the whole, are significantly limited to bulk analyses. A recent type of tissue model has been introduced, "cells-in-gels-in-paper" or CiGiP, in which 3D cell cultures can be rapidly assembled and disassembled [1]. Analysis of these constructs to date are limited, and do not provide insight into cellular organization, morphology or phenotype. We have conducted a number of feasibility studies to investigate the ability of this layer-by-layer assembly approach for use in the breast cancer research setting. These studies include the evaluation of mechanical properties, cell extraction efficiency, and cell imaging techniques, in order to better understand the capabilities and bounds of this new tissue model.

Methods: A total of 228 single-layer CiGiP systems consisting of a paper support material and type I bovine collagen were fabricated. Paper support materials under consideration included lens paper, and various grades of chromatography paper (2, 4, 20, 2668) with thicknesses of 35, 180, 210, 170 and 900 µm, respectively. The paper support materials were precisely cut into circles of 18-mm diameter using a laser cutter, allowing samples to fit in a 12-well plate. Instron mechanical compressive testing was completed with a 50N load cell in order to determine if parameters of media submersion time (0, 1, 7, and 14 days) and support paper type had a significant effect on elastic modulus. Next, CiGiP systems were seeded with MCF10A cells, a breast tissue epithelial cell line. Cell retrieval was performed at Days 1,7, and 14 and flow cytometry completed to determine cell count. CiGiP systems were seeded with MCF10A cells, and a live/dead assay was performed at Days 1, 7, and 14 followed by imaging using Zeiss Axiovert 135 widefield fluorescence and Olympus confocal microscopy. CiGiP systems were also seeded with Cell Tracker Green labeled-MCF10A cells, and the systems were imaged at Days 1, 7, and 14 using a Zeiss Axiovert 135 widefield fluorescence microscope. For experiments containing cells, controls of cells only and cells in collagen were included.

Results: Mean values in Figure 1 reveal that cells can be extracted from the single-layer CiGiP systems for use in more informative assays such as real time polymerase chain reaction. In general, both timepoint and paper support material had significant effects on cell extraction. Significant differences in cell extraction can be seen within the later timepoints (7 days and 14 days). Chromatography papers performed similarly, and resulted in less cells retrieved than the other treatment groups. Mean values in Figure 2 show that amount of time cultured in media and paper support material selected do have significant effects on mechanical properties of the CiGiP system. Cell Tracker Green loses its fluorescent capabilities after a few days, rendering it not useful for long-term studies. However, this analysis is still valuable in the early stages of fabrication, in which the assessment of initial cell deposition and distribution is useful. Following modification of the incubation time, the live/dead assay proved to be a valuable tool to better understand cell location and viability in the scaffold. Using confocal microscopy, it was possible to incrementally step through the CiGiP single-layer sample and obtain an increased resolution of 3D cell location.

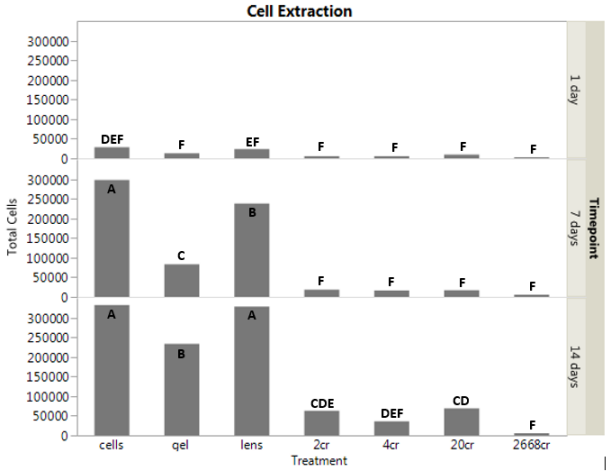

Figure 1. Cell Extraction for single-layer CiGiP (n=5)

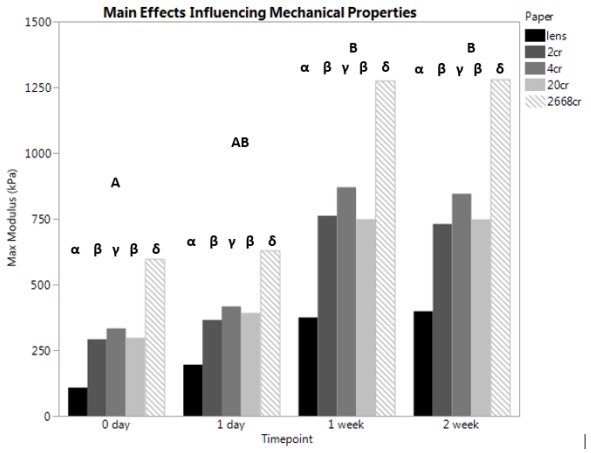

Figure 2. Mechanical testing for single-layer CiGiP (n=3)

Conclusions: This work is essential to developing a 3D stacked system that can be readily de-stacked to investigate cellular behaviors within specific layers. Knowledge gained from this project provides critical information regarding the feasibility and limitations of studying heterogeneous structures and cellular behaviors in the interior of millimeter or more thickness tissue systems. Significant potential exists for tissue engineered 3D *in vitro* models to bridge the gap between 2D cell culture models and pre-clinical and clinical reality.

References: [1] Derda R. PNAS. 2009;106:57-62

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