Ex Vivo Tissue Test Systems: Novel Layered Scaffold Design Offers Unique Analysis

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Introduction: Tissue engineering addresses reconstructive/regenerative purposes, i.e. replacement or enhancement of a damaged tissue or organ, as well as bench-top modeling purposes, i.e. therapeutic development, developmental cell biology and disease prevention. For the past two decades, cells have been cultured in 3D extracellular matrix hydrogels to create *in vitro* models to more accurately understand the behavior of cells *in vivo* [1]. Current iterations of these 3D scaffolds are limited because they lack complexity and tissue-like heterogeneity and rely mostly on delicate and low-throughput analysis methods, such as microtomy and microdissection. A recent method of biofabrication has been introduced, "cells-in-gels-in-paper" or CiGiP, in which 3D cell cultures can be rapidly assembled and disassembled [2]. Biochemical processing of these constructs to date are limited, and do not provide insight into cellular organization, morphology or phenotype. A study was conducted to investigate the feasibility of other biochemical processing techniques, as well as cell extraction efficiency, for lysate and ribonucleic acid (RNA)-based assays used to analyze cells in these CiGiP systems.

Materials and Methods: Heterogeneous scaffolds were fabricated, consisting of grade 2 chromatography paper and type I bovine collagen. These papers were precisely cut into circles of 18-mm diameter using a laser printer, allowing samples to fit in a 12-well plate. A volume of 100-150µL of collagen/cell suspension was deposited on top of each paper circle, creating one composite layer (CL). In Study 1, D1 multipotent bone marrow stromal precursor cells were tagged with the fluorescent probe Cell Tracker Green, and then suspended in collagen. After 1 day of incubation, scaffolds consisting of 3-CL's were de-stacked and collagenase was introduced to each layer. Cells were retrieved during initial collection as well as during a phosphate buffered saline wash. Fluorescent imaging was performed during each step of the de-stacking and cell extraction process. Flow cytometry was performed to evaluate number of cells retrieved. In Study 2, 1-CL scaffolds were fabricated and incubated for 1 day. A modified live/dead assay was performed. In Study 3, cells were retrieved from 2-CL scaffolds, as described above. RNA was extracted using TRIzol® and the nucleic acid concentration was obtained using the nanodrop 2000.

Results and Discussion: 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. Figure 1 reveals that cells can be extracted from the layered construct for use in more informative assays such as real time polymerase chain reaction. The nanodrop results in Study 3 showed that an adequate amount of RNA, 213.9 ± 77.7 ng/µl, was extracted from the CiGiP scaffold. 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.

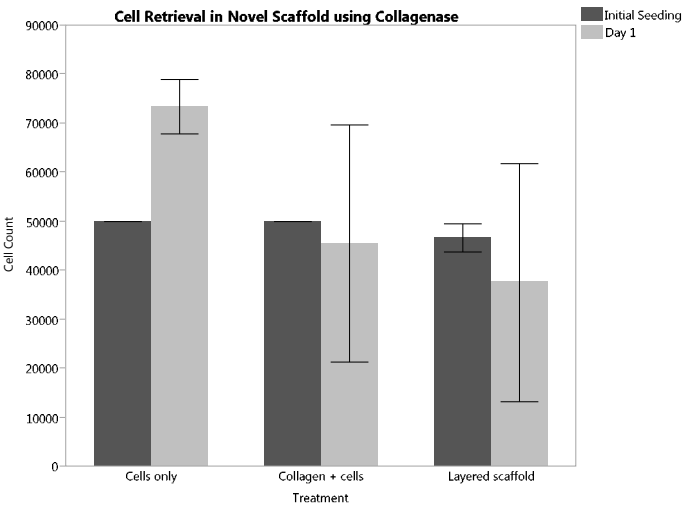

Figure 1. Flow cytometry used to assess cell retrieval technique. Controls used to account for cell proliferation.

Conclusions: This preliminary data provides the foundation for analysis of a novel *ex vivo* tissue test system. Our results thus far show that de-stacking the layers is a viable, expeditious method of analysis for this layered construct. Future studies include incorporating other fluorescent markers pre-seeding, e.g. employing RNA transfection for key protein expression. These analysis methods will create a more powerful, reliable "big picture" of this tissue engineered construct, including better insight into the cellular organization taking place.

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References: [1] Pampaloni F. Nat. Rev. Mol. Cell Biol. 2007, 8, 839-345 [2] Deiss F. Anal. Chem. 2013, 85, 8085-8094