High Resolution Cell Patterning and Co-Culture Using a Custom BioPrinter

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Statement of Purpose: We have proposed the use of precision biofabrication for the development of *in vitro* tissue test systems [1]. For example, the current "gold standard" for tumor modeling involves the suspension of cells within gel-like matrices, mainly matrigel [2]; though these systems appear to be superior to traditional 2-D models, they lack the rigidity necessary to allow normal functioning of anchorage dependent breast cells, specifically adipocytes [3]. Biofabrication strategies may be employed in the development of *ex vivo* breast tissue models that are more structurally similar and therefore have enhanced potential for use in testing regimens of drug therapies and vaccines. Researchers can also study the physical and chemical interactions that occur among cells and extracellular matrix components, e.g. to better understand the progression of transformed epithelial cells into tumors. Conventional cell seeding methods are inadequate in the development of *in vitro* tissue test systems because they involve the random placement of cells, and therefore lack the accuracy and precision necessary for spatial control. Inkjet-based microfabrication can provide a preliminary foundation for developing such *in vitro* models. In this study, we present a custom bioprinting system that can be utilized to print micron-scale patterns with multiple cell types.

Methods: To prepare cell-based bio-inks for printing, D1 murine mesenchymal stem cells and 4T07 murine mammary tumor cells were suspended in serum-free DMEM (SF-DMEM) at a density two times the desired final concentration. All cell suspensions were filtered using a 40µm sterile cell strainer. Just prior to printing, 75µL of the cell suspension was combined with 75µL of HBSS containing 1.06mM EDTA, and was subsequently deposited into the HP26 cartridge well [4]. Collagen coatings (2.0mg/mL) were prepared on tissue culture polystyrene microscope slides using aseptic techniques. The patterns shown in Figures 1-3 were created using MATLAB software and then each pattern was printed onto a separate collagen coating. Following monoculture printing, D1 cells were allowed to attach in an incubator for 15 minutes, after which they were covered in 10% serum-inclusive DMEM. An image of each cell pattern was captured at timepoints 0, 4, 20, 24, 96, and 120 hours to show stages of cell attachment and spreading on the collagen substrates (all timepoints not shown). To print cells in co-culture, D1 cells were first pipetted into an HP26 cartridge well, after which a designated portion of the pattern was printed onto a collagen coating. Next, a different HP26 cartridge was used to print the remainder of the pattern with 4T07 cells.

Results:

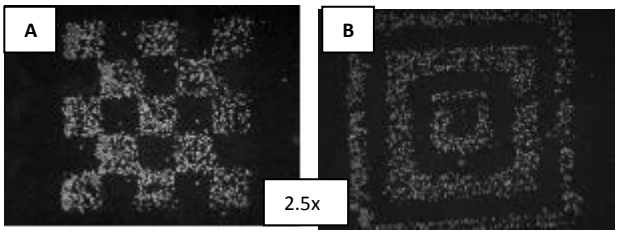

Figure 1. Images of D1s in monoculture, captured immediately after printing (2.5x objective). A) Checkers, B) Concentric Squares.

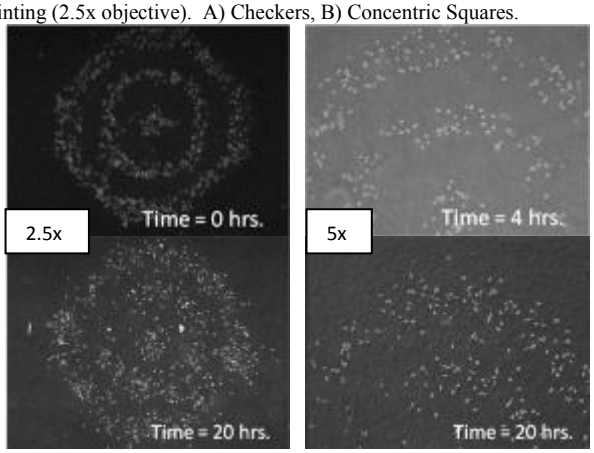

Figure 2. Images of D1s, originally printed in a bullseye, were captured from 0-120 hours to show stages of cell attachment, spreading, and proliferation on a collagen substrate.

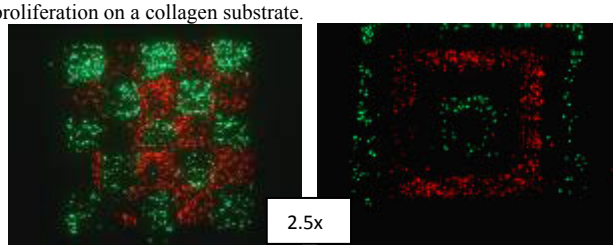

Figure 3. Images, captured immediately after printing (2.5x objective), of D1 (green) and 4T07 (red) cells printed onto collagen substrates.

Conclusions: Collagen coatings improved the viability of D1 cells following printing (compared with polystyrene surfaces pre-coated in serum). Collagen prevented the disruption of co-culture patterns by allowing more rapid attachment at each point of cell ejection. Co-culture patterns were achieved at a resolution of ~85µm using the custom bioprinting system.

References:

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