High Resolution Inkjet Printing as a Tool for Creating Tissue Test Systems

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Statement of Purpose: Previous *in vitro* studies have demonstrated the importance of spatial alignment of cells in culture when attempting to accurately mimic the *in vivo* microenvironment, where the latter includes cell-cell and cell-ECM contacts in addition to physical forces and soluble factors [1,2]. The ability to constrain the cell-surface contact areas can lead to better predictions of cell function and an overall increase in the stability of cell phenotype. Conventional cell seeding methods are inadequate in the development of an *in vitro* tissue test system because they involve the random placement of cells, and therefore lack the precision necessary for spatial control. Conversely, microfabrication tools allow the creation of select biomaterial surface variations as well as the precise placement of cellular components. We have selected the modified inkjet printer as a tool for creating 3-dimensional *in vitro* models because it offers an inexpensive and high-throughput solution to microfabrication, and because the printer can be easily manipulated to produce varying tissue attributes. The objective of this research is to manipulate our current methodologies for creating 3-D breast tissue engineering devices to design a 3-D *in vitro* breast tissue testing system [3]. The hypothesis is that the precise placement of cell and biomaterial components within a 3-D culture system using a modified inkjet printer will result in a more physiologically relevant microenvironment that can sustain normal cell morphology and function.

Methods: An HP-540 series inkjet printer was reverse engineered, and a novel cell printer was manufactured to allow control over single nozzles of an HP-26 ink cartridge. To demonstrate the resolution capabilities of this inkjet cell printing technology, D1 (murine mesenchymal stem) cells were suspended in serum-free culture medium at a concentration of $5x10^6$ cells/mL and printed in a straight line pattern onto glass slides using a line spacing of 200µm. Additionally, a 5x250 matrix of single pixel-sized dots was printed in triplicate using cell suspension concentrations including 3, 6, and 12 million cells per mL. Data was collected by counting exactly how many cells appeared in each printed dot.

Results: The line pattern (Figure 1) was retained on slides when lines were separated by a distance of 200µm. The number of dots containing at least one cell continued to increase with increasing cell suspension concentrations. Less than 10% of dots printed using a 12 million cell/mL suspension concentration contained zero cells, while approximately 66% of dots printed using a 3 million cell/mL suspension concentration contained zero cells. Furthermore, the average number of cells ejected in each dot increased as a result of increasing cell suspension concentrations. Average cell counts per dot (excluding dots with zero cells) for 3, 6, and 12 million cells per mL were 1, 2, and 3 cells respectively.

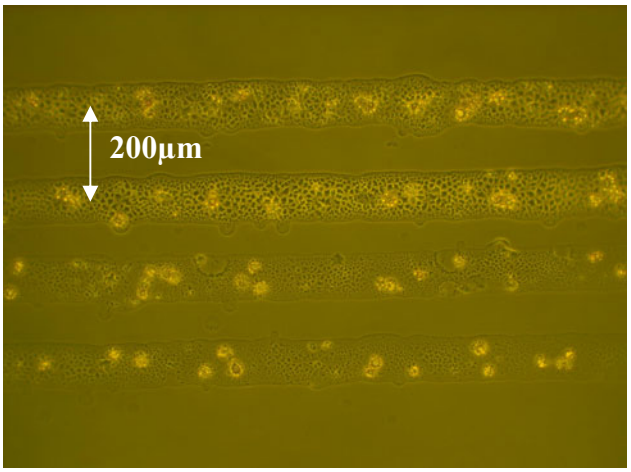

Figure 1. D1 cells printed in a straight line pattern using 50µm pixel spacing and 200µm line spacing. (100x)

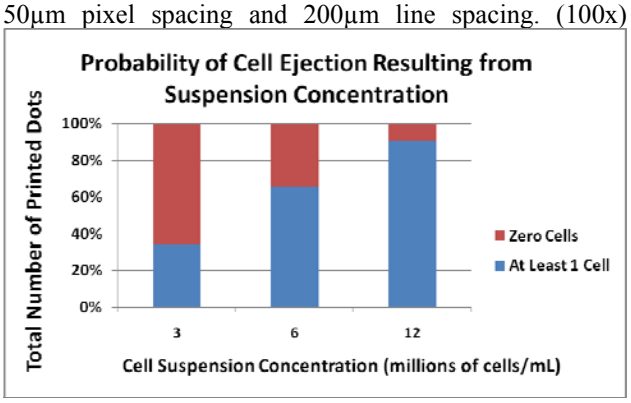

Figure 2. D1 cells were printed from a single nozzle in a dot matrix pattern using various cell suspension concentrations, and probability of cell ejection was evaluated.

Conclusions: High resolution spatial control can be achieved using inkjet printing technology. Figure 1 shows the potential for accurate and precise spatial control of cells, ECM components, soluble factors, and drugs, among other 3-dimensional culture additives. According to the data describing the probability of cell ejection, it will be difficult to predict exactly how many cells will be printed. Particular attention should be paid to remove serum from the cell pellet to avoid aggregation of cells in suspension. A mechanism to apply continuous agitation to the cell suspension might be useful to retain a homogeneous solution.

References: [1.] Bissell, MJ, MA Labarge. Cancer Cell, 2005. 7(1):17-23.
[2.] Falconnet D, *et al.* Biomaterials. 2006. 27(16):3044-3063.
[3.] Burg, KJL, CT Gomillion, CA Parzel**.** Encyclopedia of Biomaterials and Biomedical Engineering. *Tissue Engineering, Breast*. 2007.

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