Biofabrication and Cell by Cell Deposition

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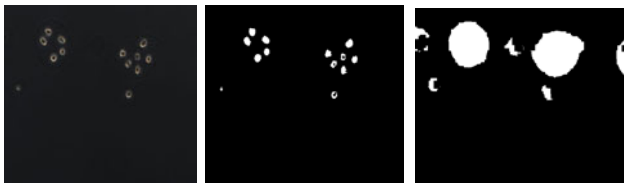
Statement of Purpose: Tissue engineering may be used for the design of *in vitro* physiological models to aid in the study of disease pathogenesis and development of suitable drug therapies. Every organ in the body is composed of a number of cell types and the phenotypic expression of each cell is governed by the cell-extracellular matrix interactions, cell-cell interactions, the growth factors, physical forces and other external stimuli.[1] The inkjet printer is an inexpensive microfabrication tool which can be used to construct 3D tissues; however, it clearly was not designed with the goal of dispensing biological elements and requires considerable refinement before the development of 3D tissues will be realized.[2] The objective of this study was to assess the potential of an inkjet printer in realizing cell by cell deposition of a cellular solution.

Methods: Mouse mesenchymal stem cells were cultured in tissue culture flasks at 5% CO_2 and 37°C. Cellular concentrations of 4, 6, 8, and 10 million cells/mL, suspended in serum free media and Hank's buffered salt solution, served as the bio-ink.[3] An HP 540 inkjet printer, modified for bioprinting, was used for the study with a cartridge that was cleaned and sterilized for bioprinting. A pattern of 6 rows of 10 evenly spaced dots was created using Adobe Photoshop 7.0 where each drop was one pixel. The pattern was printed on microscope glass slides. Each row was one print pass and the printer was paused for 3 minutes between rows. A total of 15 minutes elapsed between the first drop and the last drop. Images were taken at 100X magnification using a Zeiss inverted microscope. Image processing was conducted using MATLAB. The images were read through the MATLAB program where image processing tools were used to count cell numbers and drop size. A typical microscope image with two drops is shown in Figure 1. In the middle image of Figure 1 the image was contrasted and dilated to identify the individual cells. In the right-hand image of Figure 1 the image has been contrasted and dilated in order to estimate drop area. The drop areas and cell locations were then correlated to identify the number of cells in a specific drop and to reduce noise.

Figure 1: Original Image (left), Image Processed for Cell Counting (middle), and Image Processed for Drop Area Estimation (right).



Results: The cell count data (Figure 2) suggest that the cell number per drop approached a constant value in each time period for each cellular concentration and the number of cells per drop increased with increase in time. Also, higher cell concentrations led to a higher number of cells per drop (Figure 3). Cell aggregation was also observed, particularly in the first drop of each pass.

Figure 2: Number of Cells Ejected Per Drop, Starting Solution 6M Cells/mL.

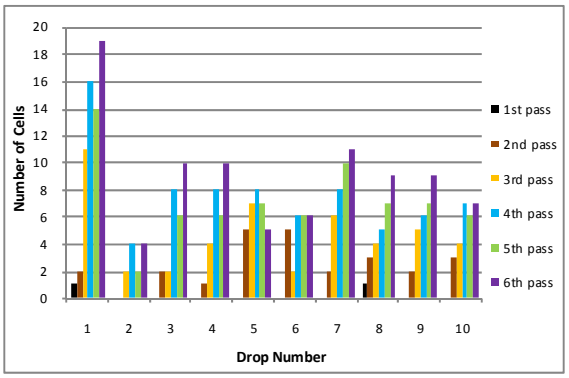
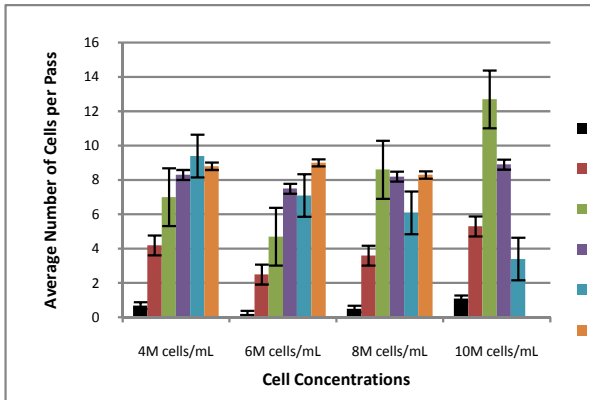


Figure 3: Number of Cells Ejected Per Pass at 4, 6, 8, and 10M Cells/mL.



Conclusions: The increase in the number of cells per drop with increase in the time period is attributed to cell settling and a gradual change in the solution concentration. This indicates that the cells need to be maintained in suspension for the consistent ejection of cells per drop. Ongoing work is addressing the effect of the solution on cell aggregation and drop by drop dispensing potential; for example, the precision deposition capacity of cells suspended in a biomaterial.

References: [1] C.T. Gomillion et al., *Biomater. 27:* 6052-6063, 2006. [2] B. Derby, *J. Mater. Chem.,* 18, 5717-5721, 2008. [3] C.A. Parzel et al., *JTERM* 3(4):260-268, 2009.

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