Maximizing Cellular Pattern Repeatability in Biofabrication

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Statement of Purpose: This project focuses on enhancing the functionality of a bioprinting system to build tissues and tissue test systems. Drop-on-demand microfabrication, often referred to as bioprinting, depends on a high level of precision placement of both cellular and non-cellular materials. When printing cellular material, it is important to control the number of cells that are printed per drop.[1] Previous studies show that as time evolves during a printing cycle, the number of cells per drop increases, likely due to gravitational cell settling of cells in the printhead reservoir. Accordingly, we proposed the design of a device that could be readily integrated with a bioprinting unit and which would apply gas flow and prevent cell settling during the printing process. We postulated that the ideal device must function without physically vibrating a bioprinter cartridge, must be easily moved between cartridges, and must be sterilizable.

Methods: A device was designed based on adapting the parameters of a device proposed for agitating cells in a nuclear magnetic resonance tube.[2] Figure 1 shows the device design which is comprised of two concentric tubes. The top of the inlet tube is connected to a gas source and the side openings of the aperture tube are placed at the liquid line of the cell suspension. Gas is applied, forming a bubble that escapes upward through the aperture. The movement of the gas upward creates a flow in the liquid that moves the cell suspension from the bottom into the tube.

A prototype was built using a pipette tip as the aperture tube; the aperture was created using a grinding tool. An 18-gauge syringe needle was pushed through the modified pipette tip to serve as the inlet tube. In order to test this design, experiments were performed using collagen coated Solohill microcarriers, sizes 125-212 µm (Sigma Aldrich) suspended in a cuvette with Rhodamine B fluorescent die. The device was connected to a pulsatile flow bioreactor pump and tested at different flow rates. Images were taken on a light microscope using ImagePro Plus software. Matlab (The Mathworks, Inc.) was used for analysis by sectioning each image and averaging the intensity of each section.

Results and Discussion:

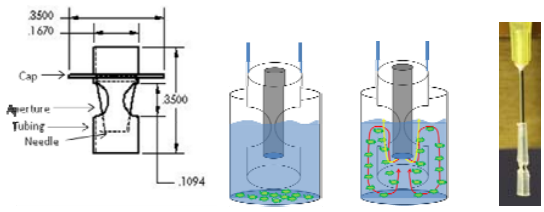

Figure 1. Solidworks drawing of the device (left); schematic depicting cells (green) settled at bottom of container (middle); movement of cells (red arrows) once air (yellow arrows) is ejected from center tube; photograph of prototype (right).

The average intensities were plotted for each image, which is depicted in Figure 2, as a progression over time. Figure 2 shows that over time during the study, the density of the microcarriers begins to level throughout the image sections. As seen in Figure 2, while the density is not completely level through the images, there is an improvement in the distribution over time.

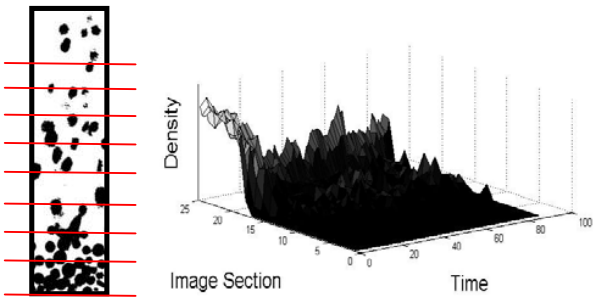

Figure 2. Sample image converted to binary from Matlab depicting microcarriers in cuvette being mixed by device (red lines represent image binning); graph represents density of microcarriers throughout set of images taken over 90 seconds. Each image is binned into sections and average microcarrier density of each section is plotted over time. The bottom of the cuvette is bin 0.

Conclusions: Initial studies indicate that cell settling is a likely cause for the increase in number of cells printed per drop. The studies performed using the device prototype designed to overcome this biofabrication challenge show that the device allows a more uniform density of microcarriers throughout a sample. Studies conducted for longer periods of time using a refined prototype and at higher flow rates will be performed to optimize the amount of mixing. Further studies will also be performed using cells to replace the microcarriers. Also, a viability study will be performed with the cells to ensure that the device does not cause cell lysis.

References: 1. Burg, T. et al., *Phil Trans R Soc A*. 2010; 368.1917: 1839-1862.
2. Zakhartsev, B., et al., *Anal Biochem*. 2010; 397: 244-246.

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