3D Vascularized Tissue Printing by Piezoelectric Droplet Generation

Jeremiah Cohen[1] and Grace Lee[1], Gokhan Percin[2], Ali Khademhosseini[1], Seung-Schick Yoo[3], Utkan Demirci[1,2]

[1]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA
[2]GP Consulting, Los Gatos, CA
[3]Center for Biomedical Engineering, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA
[4]Harvard Medical School, Cambridge, MA

Statement of Purpose:
The objective of our study is to use novel, fluid-sensitive, directional, piezoelectric droplet generation technology to print 3D vascularized tissues. Printing was done one cell at a time in an accurate, rapid, and reliable manner in a high throughput manner that maintained cell viability and with a submicron printing resolution.

Methods:
In piezoelectric droplet generation, a sinusoidal voltage signal is applied through a piezoelectric material that is attached to a fluid reservoir. Our droplet ejector comprises of an aluminum reservoir hollowed to a diameter of 9mm with a 25µm thick brass disk as the base (Figure 1). The brass disk has a small orifice ranging from 50µm to 300µm in diameter for varying droplet sizes. Attached to this disk is a 25µm thick piezoelectric disk. By performing harmonic analysis under certain fluid loading, we can isolate the resonance frequency inherent to the ejector geometry. Once the resonance frequency is known, we sinusoidal voltage signal is applied to the piezoelectric disk. The disk vibrates just enough to break the surface tension of the fluid, generating a precisely determined droplet size. Thus piezoelectric droplet generation is easily and repeatably fabricated, and adds to the reliability of drop-on-demand actuation. In our previous work, we have successfully demonstrated our ability to generate droplets from a piezoelectric ejector as described[1]. The use of sensitive piezoelectric droplet generation will enable us to achieve accurate printing of single cells onto surfaces by single ejectors. This method of printing realizes high cellular viability in 2D printed tissues. The next step is to print vascularized 3D cardiac tissue with the help of an automated XYZ stage. To do this we will incorporate microfluidic channels among layers of tissue made from biodegradable materials. Cells can be printed to resemble blood vessel structures in order to sustain 3D tissues over long periods of time. Afterwards, the channels can be replaced with actual vasculature created by endothelial cells initially surrounding the channel. This contributes to the printed tissue's viability, paving the way for transplantable organs.

Results/Discussion:
Our experiments demonstrate the device's ability to consistently generate identical droplets in drop-on-demand and continuous modes of operation. The ejections are very controllable, generating droplets of equal diameter that leave the orifice with equal velocity. Shipley 3612 sensitive polymers such as photoresist and hydrogels were utilized. The ejected polymer drop size was 85% of the orifice size, where the orifice size was 110 µm. The polymer was successfully printed in patterns of individual droplets and lines of the polymer, created by overlapping droplets. By similarly overlapping the droplets, we demonstrated that a 3 inch diameter silicon wafer could be coated with the polymer with zero waste. We also succeeded in printing live cardiac cells in a random pattern (see Figure 2). Temperature and pressure did not increase enough to harm the cells or polymer during the experiments.

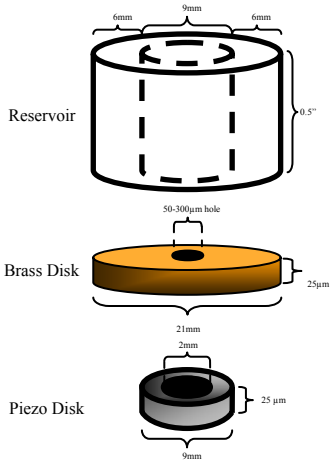

Figure 1.

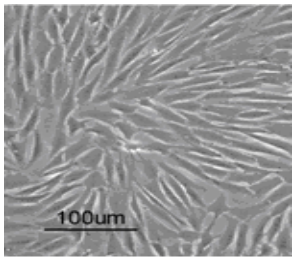

Figure 2.

Figure 1: Piezoelectric droplet generator schematic. **Figure 2:** Cardiomyocytes randomly printed

Conclusion:
This piezoelectric ejector demonstrates several improvements from conventional cell printing devices. It enables control over droplet size and directionality, independent of a nozzle. The device is simple, reliable, and easily repeatable. Also, it does not cause significant increases in temperature and pressure that could harm sensitive liquids and cells. This acoustic droplet ejector could be used for many applications, such as three-dimensional live cell printing for tissue engineering.

References
[1]Percin G. Rev Sci Instrum. 2003;74:1120-1127.