Electrohydrodynamic Jet Printing on Hydrogel Substrates for Cell Culturing Applications

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Abstract:

Patterning cell cultures on a biologically compatible surface has the potential to significantly extend cell biology knowledge. Electrohydrodynamic jet (e-jet) printing has the capability to pattern surfaces with various biological compounds. This project focused on the use of an e-jet printer to pattern a protein complex, fibronectin, used for cell attachment, on the surface of polyacrylamide hydrogels. E-jet has the potential to print fibronectin ink droplets as small as 2 μm. Polyacrylamide gel stiffness can be modified to mimic various biological tissues. This research has the potential to make advancements in cellular biology knowledge, three-dimensional cellular patterns or bio-arrays, and tissue engineering.

Introduction:

Cell cultures are affected by many different factors. Being able to control and manipulate the environment (biocompatible substrates) and location of a cell culture can provide important information to cell biology knowledge [1]. The ability to place a single cell on a substrate in a flexible pattern has the potential to affect many areas of research. Electrohydrodynamic jet (e-jet) printing can be used for high-resolution (< 10 μm) free form patterning of biological materials on a wide range of substrates [2]. A schematic of the e-jet printer can be seen in Figure 1. The main components of the system are a charged nozzle and a grounded substrate that create an electric field. As the electric field is increased, the ink in the nozzle tip is drawn into a conical formation and onto the substrate [3]. The system is computer controlled through LabVIEW [2]. The system can be run on a direct current mode for continuous jetting or a pulsed printing mode for drop-on-demand printing [4].

The e-jet printing system was used to pattern fibronectin on the surface of polyacrylamide hydrogels for cell culturing applications.

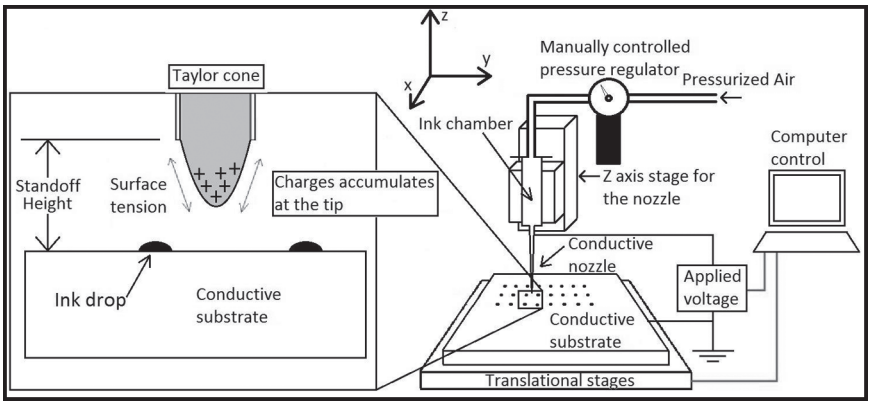

Figure 1: Electrohydrodynamic jet printer schematic [1].

Experimental Procedure:

Fibronectin Production. Fibronectin was made from a 40 μg/mL fibronectin concentrate [1]. The fibronectin was made with filtered 1X phosphate buffered sulfate (PBS) [5].

Polyacrylamide Hydrogel Production and Activation. The hydrogels were made with 8% acrylamide and 0.3% bis-acrylamide solution in double distilled water (ddH_2O) along with 1M HEPES stock solution buffer and 0.2% acrylic acid [1]. To initiate polymerization of the gel, 10% ammonium persulfate and 0.05% tetramethylethylenediamine (TEMED) were added. Then 35 μL of the liquidous (and quickly polymerizing) hydrogel was placed between an indium tin oxide (ITO) microscope slide and a standard glass coverslip. After the gels were fully polymerized, the gels were soaked in an n-hydroxysulfosuccinimide (NHS) and n-(3-dimethylaminopropyl)-n′-ethylcarbodiimide hydrochloride (EDC) bath to activate the surface for printing by reacting with acrylic acid within the gel [1]. The gels were then stored in ddH_2O to keep hydrated before printing.

Printing Protocol. Hydrogels on ITO slides were placed on the vacuum chuck of the e-jet printer. The syringe was filled with 1-3 drops of fibronectin and initialized by pressurized air pushing the fibronectin to the end of the nozzle. The nozzle was positively charged until jetting occurred and parameters were set

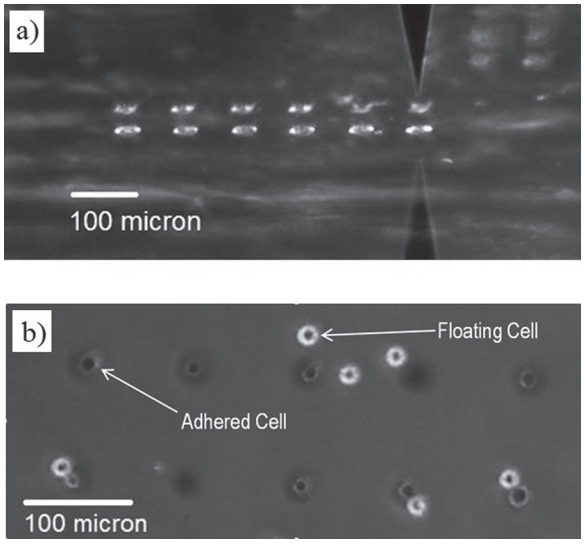

Figure 2: Fibronectin patterning during; a) printing process, and b) cellular seeding.

Figure 3: 'NNIN' pattern.

for printing [2]. Figure 2a shows the printing process underway, while a dot matrix on fibronectin is patterned. All gels were stored in ddH_2O until time of imaging or cellular seeding.

Cellular Seeding Protocol. Gels were cultured with retina pigment epithelial (RPE) cells with 10% fetal bovine serum, 50x 1M HEPES and 200x penicillin-streptomycin. All samples seeded with cells were stored in a 37ºC incubator until imaging was needed [6]. Images of cells seeded on hydrogels patterned with fibronectin can be seen in Figure 2b.

Results and Conclusions:

The fibronectin was successfully patterned on the hydrogel surface and cells were subsequently seeded on those patterns, Figure 3. Various patterns were developed using both electronic and manual techniques. Ink droplet size was characterized using an Olympus BX51 microscope. The droplet size was varied by changing either the stand-off height (the distance between the charged nozzle and the substrate) or the pulse width (the duration of time that the nozzle is charged in one location). The pulse width provided more consistent size variations. Figure 4 shows the relationship between pulse width and droplet size. As the pulse width increased the droplet size increased exponentially. The vertical bars on this graph show the standard deviation from the average droplet size. As can be seen, the standard deviation increased as pulse width increased; mostly likely, a function of increased variation due to the larger number of droplets released in a given period.

Future Work:

The next phase of this project will focus on cell culturing. During this research phase, the cell cultures did not uniformly adhere to the patterned fibronectin. This requires further investigation, including looking into different ink materials like collagen. Printing quality and properties using variations in hydrogel stiffness and water content is another potential research avenue.

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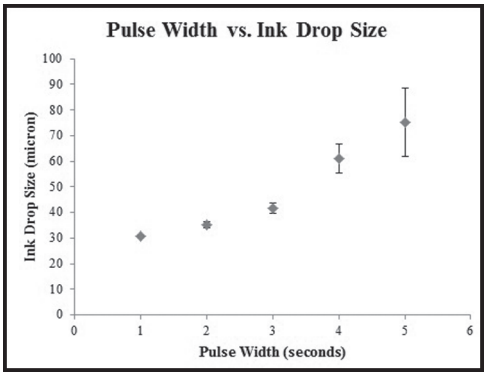

Figure 4: The relationship between pulse width and ink drop size.