Towards Robotic Bioprinting Directly onto Moving, Stretching Anatomy

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INTRODUCTION

Biomedical engineering has combined tissue engineering with additive manufacturing, yielding the ability to 3D print a tissue scaffold for human anatomical organs available for transplant [1]. Traditionally, bioprinting techniques are open-loop processes which are limited to printing on a stationary rigid surface. Recently, O'Neill et al. demonstrated the ability to 3D print hydrogel onto a moving human hand [2][3]. While this work exhibited adaptation to unpredictable motion of the print surface, it was unable to account for any print surface deformation. However, by accounting for the motion, stretch, or shear of a print surface, bioprinting could be used to apply therapy directly onto a biological surface, such as human skin or tissue. Additionally, the work presented in [2] stayed within the bounds of printing non-cellular materials, which is an unrealistic expectation for clinical bioprinting applications.

With these bioprinting challenges in mind, the objective of this paper is twofold. There are two experiments presented in this paper, each of which use the same piezojet robotic system. Experiment 1 demonstrates the level of cell viability attained upon 3D printing cellular fibroblasts suspended in a gelatin methacrylate (gelMa) solution, and additionally demonstrates the level of cell survival upon curing the gelMa solution via ultraviolet (UV) exposure. Experiment 2 introduces a closed-loop bioprinting procedure which accounts for the motion, shear, and stretch of the print surface.

MATERIALS AND METHODS

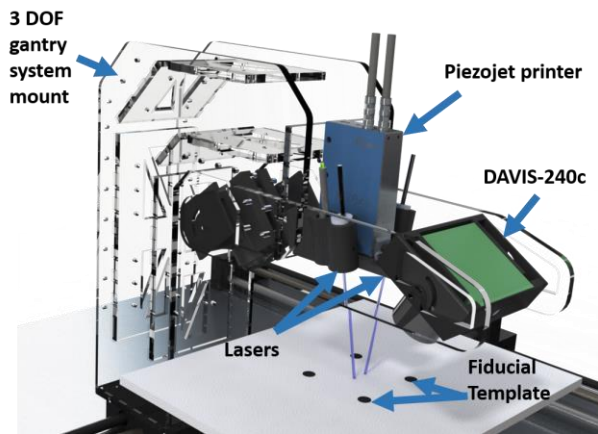*Experiment 1: Printing fibroblasts suspended in gelMa*
In the first experiment, a gelMa solution was prepared by mixing 0.05g of LAP photoinitiator and 1g of gelatin into 10mL of phosphate buffered solution (PBS) at 37°C. Fibroblast cells were seeded on collagen and cultured in Dulbeccos modified Eagles medium (DMEM) with 5% fetal bovine serum (FBS) at 37°C until 80% confluent in 50 mL flasks. Fibroblast cells were printed on passage 3. On the day of the print, cells were suspended in 5mL of trypsin and ethylenediaminetetraacetic acid (EDTA) for 5 minutes at 37°C, after which 5mL of DMEM was added to the flask to neutralize trypsin. The cell suspension was then transferred to a 15mL tube and centrifuged at 1200 RPM for 5 minutes. GelMa was prepared as described previously and filtered under a biohood for sterilization. For control samples (-UV/-inkjet), LAP was not added to the gelMa. After removing the supernatant from the centrifuged cells, the gelMa solution was added to the left-over cell mass. The cells were then resuspended in gelMa using a low level vortexer and allowed to sit in the water bath to remove the bubbles. The gelMa/cell solution was then loaded into the inkjet printer and ejected onto 10 mL petri dishes. UV curing was induced after the print for 10 seconds. 1mL of DMEM was then added to the resulting gelMa/cell construct, and cells were cultured for 2 hours, 24 hours. After the period of cell culture, 2 drops of NucBlue(R) Live and NucGreen(R) Dead (ThermoFisher Scientific, Carsbad, CA) dyes were added to each petri dish sample to quantify cell viability. Fluorescence imaging was performed using a Leica fluorescence microscope.

Experiment 2: Bioprinting onto a non-rigid surface
In the second experiment, an IniLabs DAVIS-240C sensor was used to record the motion of four circular fiducials marking the boundary of the print surface. A Nordson EFD PICO Pμlse piezo jetting system was used to deposit a 2% Sodium Algenate solution onto the print surface. The DAVIS-240C sensor and the Pico Pμlse printer were both mounted onto a robotic gantry system. Two lasers were also fixtured onto the gantry system, and were oriented such that they projected onto the print surface (see Fig. 1).
The DAVIS-240C captured the motion of the projected lasers relative to the fiducials on the print surface, and the relative displacement between the projected lasers and fiducials was computationally determined. Using this relative displacement, the robotic gantry system was used to compensate for any motion of the print surface. The Pico Pμlse was used to deposit the print material onto the print surface whenever it was in the right position relative to the printer. Three different fiducial templates were used: a square template, a rectangular template, and a parallelogram template (see Fig. 3.e-f). The desired print geometry was a solid square lying within the boundary of the print surface (see Fig. 3.a). The same print geometry template was used to print on each of the three fiducial templates.

**Fig. 1** Experimental setup of experiment 2

RESULTS

Experiment 1:

The bar plots given in Figure 2 show the cell viability results of fibroblasts suspended in gelMa for 3 different scenarios: the control sample which was neither printed with the inkjet nor cured with UV (blue), a sample which was cured using UV but not printed with the inkjet (orange), and a sample which was both UV cured and printed via the inkjet (grey).
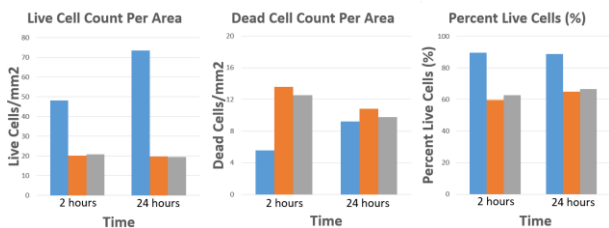
Fig. 2 Live/dead cell count measured after 2 hrs and 24 hours of printing. Blue indicates - UV/- Inkjet (control), orange indicates +UV/- Inkjet, and grey indicates + UV/+ Inkjet

Experiment 2:

The results of printing onto different fiducial templates are shown in Fig. 3. Fig. 3.a represents the original print template provided for each trial, Fig. 3.b-c depicts the anticipated print deformation given the change in fiducial geometry, Fig. 3.d depicts printing on an undeformed surface (square fiducial template), Fig. 3.e depicts printing on a template mimicking a stretched surface (rectangular template), and Fig. 3.f depicts printing on a template mimicking a sheared surface (parallelogram template).
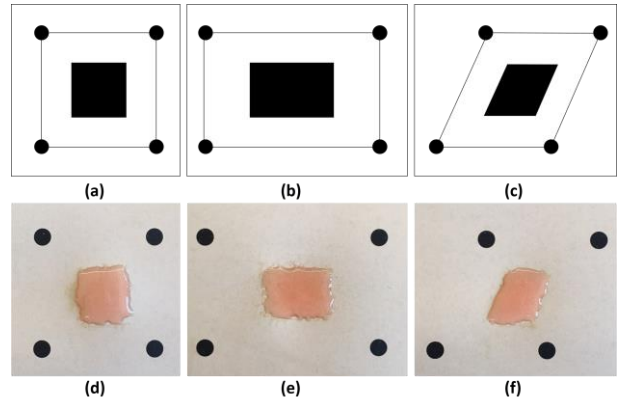
Fig. 3 Results of printing on each fiducial template. (a) original print template, (b)-(c) expected print deformation corresponding to (e)-(f), (d) non-deformed control case, (e) template mimicking surface stretch, and (f) template mimicking surface shear

DISCUSSION

As summarized in Fig. 2, there was no significant decrease in cell viability due to ink jetting versus that caused by UV curing, which is a process already ubiquitously used in bioprinting.

Additionally, from the images shown in Fig. 3, it can be seen qualitatively that the printing algorithm used is able to adapt to different fiducial geometries. While the same square print template was provided for each of the fiducial templates, the print algorithm was able to adapt the print such that it mimicked the deformation of the fiducial template. The adaptation of the print algorithm to fiducial template deformation indicates that it may be feasible to print on a surface which undergoes some type of deformation mid print. This could prove extremely valuable in clinical applications where it is necessary to print on non-rigid biological tissue.

These results suggest that, in conjunction with previous work, bioprinting viable cells onto moving, stretching, and shearing anatomy is feasible, and merits further investigation.

REFERENCES

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