INVESTIGATIONS OF BIO-INK RHEOLOGICAL PROPERTIES FOR DROP ON DEMAND PIEZOELECTRIC CELL PRINTING

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ABSTRACT

In this paper, the effects of increasing the viscosity of density matching bio-inks on the reliability of printing MCF-7 breast cancer cells using piezoelectric inkjet nozzles are investigated. The cell printing reliability was characterized by the frequency of occurrence of cell reflection in the nozzle: in cell reflection cells moved further away from the orifice after an ejection cycle. To decouple the effects of fluid density and viscosity on cell trajectory, three solutions were used: 1) phosphate-buffered saline (PBS) which has low viscosity and has lower density than cells; 2) low viscosity, density-matching sodium polytungstate (SPT) solution; and 3) high viscosity, density-matching Ficoll sucrose polymer solution. While cell reflection was observed for PBS and SPT solutions, it was not observed for high viscosity Ficoll solution. Propagation of pressure waves induces the transient motion of cells, and higher viscosity of solution causes increased viscous forces on the cells and prevents cell reflection.

INTRODUCTION

Inkjet cell printing has received considerable interest due to its capability to engineer tissues and organs in intricate three dimensional orientations [1, 2]. This technology has potential for applications in tissue engineering [3], cell based assays [4], stem cell research [5] or therapeutics [2]. In particular, the drop on demand (DOD) printing technology enables to precisely and accurately place biological materials (i.e., cells, extracellular matrix) onto substrates. Piezoelectric inkjet systems generate mechanical pulses to squeeze liquids out of the nozzle orifice with high speed, resulting in the formation of well controlled droplets [6]. However, the commercialization of such systems for cell dispensing remains challenging due to less controlled cell behavior.

A particle (e.g., a cell) suspended in a fluid is subjected to hydrodynamic forces. For low Reynolds number (Re), viscous force dominates over inertial force such that the Stokes drag force is the dominant force in dynamics of particle motion. However, during droplet ejection the Reynolds number in an inkjet system is high ($Re > 10$ [7]) and during this transient event the inertial force could be no longer neglected [8]. Both inertia and viscosity act on a cell and makes the cell dispensing more complex, resulting in low reliability [9]. For example, cells sometimes move far away from the orifice (termed as cell reflection) after a droplet ejection [8].

Our previous work indicates that the use of high viscosity, density-matching Ficoll sucrose polymer solution improves cell printing reliability [7][8] by reducing cell sedimentation within the suspension, giving a good match to the expected Poisson distribution of cells within printed droplets [7]. However, this previous work did not separate the effects of fluid viscosity and fluid density. In addition, high viscosity solutions may fall outside the window of operability of some inkjet nozzles. In this paper, we decoupled the effects of fluid density and fluid viscosity on cell printing by using low viscosity, density-matching sodium polytungstate (SPT) solution and respectively investigated their effects on cell motion in a piezoelectric inkjet nozzle.

MATERIAL AND METHODS

Piezoelectric Inkjet Nozzle

A piezoelectrically actuated inkjet (Microfab Technologies Inc., Suite, MJ-ABP-0) was used to dispense cells within droplets. The inkjet consists of a 21.7 mm long glass cylindrical tube with an inner diameter of 0.475 mm which tapered to the orifice with a diameter of 80 μm . The middle of the glass tube was surrounded by a piezoelectric element, which consists of a sandwich structure of inner electrode/piezoelectric material/outer electrode. When applying a voltage difference, the electrical field is generated between the inner and outer electrodes, resulting in the deformation of piezoelectric (PZT) actuator. In this way the piezoelectric element enables to produce pressure wave and squeezing fluid through the orifice to generate droplets. The piezoelectric element was actuated by a bipolar waveform (Fig. 1). An arbitrary waveform generator (Agilent 33220A) was used to provide the command signal input to a high-voltage amplifier (Trek Model 603) with a DC voltage gain of 50 V/V. The amplitude and frequency of the electrical waveform depend on the rheological properties. The SPT printing used a bipolar waveform with a peak-to-peak voltage of ± 30 V and a frequency of 60 Hz.

The piezoelectric actuator generates pressure waves using the deformation of PZT under the change of electrical voltage. Notethat the deformation only occurs during the transition period (rise and fall) and ceases during the constant voltage (dwell) period. On the rising (or falling) edge of the waveform, the annular PZT expands (or contracts) its circumference, resulting in an outwards (or inwards) motion of the inner glass material and consequently a negative (or positive) pressure (with respect to the equilibrium). The pressures travels in fluid as an acoustic wave at a certain speed. Bogy and Talke examined the one-dimensional wave propagation along the glass tube (vertical to the motion of the glass) [11]. For the longitudinal acoustic wave, the displacement of the liquid is in the same direction as (or the opposite direction to) the direction of travel of the wave. The wave travels to the orifice and the supply end, where the wave was reflected and moves back towards the orifice. The backwards wave reinforces the positive pressure wave [12]. In this case the pressure wave makes fluid overcome the liquid-air interface, resulting in the ejection of droplets [13]. The motion of the cells and the surrounding fluid as well as the droplets dispensing were recorded by using a high speed camera (Phantom® Miro 4 or Phantom v611).

The back end of the nozzle was connected to a syringe reservoir through tubing (Tygon® AAC00001; OD: 2.38 mm, ID: 0.79 mm; Saint-Gobain). The pressure at the back end was continuously measured by a differential pressure sensor (PX139, Omega) and read by a microcontroller (Uno R3, Arduino). The back pressure was maintained at -500 Pa by adjusting the relative height of the external reservoir to the inkjet nozzle during the printing.

oscillate about the equilibrium position at the orifice. This oscillation process took about 1617 μs and then the liquid-air interface returned back to the equilibrium (see Fig. 2d). After 3080 μs , the oscillation of the liquid-air interface occurred again.

On the falling edge (from 30 V to -30 V), the piezoelectric material contracted and resulted in a positive pressure ($t = 0.01$ s). The positive pressure squeezed liquid out of the orifice and make the formation of droplets. The formation of SPT droplets could be categorized as four phases, i.e., (e) liquid extrusion; (f) thread thinning, and consequently (g) break-off; and (h) primary and satellite droplets formation and meniscus damping. Afterwards, the meniscus damping takes around 160 μs and subsequently the liquid-air oscillation would occur twice during the dwell period as same as at the constant negative voltage. The volume of the primary droplet was measured to be 82 pL and the satellite droplet was measured to be 15 pL. The volume of the primary (or satellite) droplet was determined by detecting the droplet edges and fitting to a sphere (or cylinder). Note that the satellite droplets did not merge with the primary droplets and that is undesirable. An actuation profile with lower peak to peak voltage would probably enable optimization to avoid forming satellites.

Figure 2: High speed images of a meniscus oscillation (a-d) and jet formation including (e) liquid extrusion, (f) thread thinning and (g) break off, and (h) breakup of liquid thread into primary drop and satellites and meniscus damping. The images were captured with a lateral magnification of $5\times$ objective and a numerical aperture of 0.15 at 130,232 frames per second with a pixel resolution of 256×128 pixels. This imaging system has an interframe resolution of $7.7 \mu\text{m}$ and a spatial resolution of $0.65 \mu\text{m}/\text{pixel}$.

Cell behavior

The positive pressure waves squeezed fluid out of the orifice, resulting in the formation of droplets (see Fig. 2e-h). During the droplet formation, liquid moved within the nozzle such that cells moved. Fig. 3 shows the high speed images and the corresponding cell trajectory for various cell behaviors. In Fig. 3a, a cell (indicated by the black arrow) with a diameter around $12 \mu\text{m}$ approached the nozzle orifice with a distance around $40 \mu\text{m}$. During liquid extrusion, the cell moved towards the orifice. After the arrival to the orifice, the cell merged with the meniscus (Fig. 3b) and subsequently the droplet containing the cell was dispensed out of the orifice (termed as cell ejection, see Fig. 3c). Fig. 3d recorded the cell's vertical position (along Y-axis) away from the orifice ($y=0$) as a function of time. Fig. 3e-g visualized the travel process of a cell. In this case, the cell (with a diameter around $12 \mu\text{m}$) moved closer to the orifice ($\Delta y = -18 \mu\text{m}$, Fig. 3h)

during a droplet ejection, but the cell was not dispensed. This cell behavior is termed as cell travel. In Fig. 3i-k, a cell with a diameter around $20 \mu\text{m}$ moved far away from the orifice ($\Delta y = 34 \mu\text{m}$, Fig. 3l) and this behavior is termed as cell reflection. During the droplet ejection, liquid moved towards orifice, however the cell was reflected towards the opposite direction. In this case, cell reflection indicated that the fluid is unable to control the motion of cells well, making the cell printing less reliable.

Figure 3: High speed images and cell tracking of cell ejection (a-d), cell travel (e-h) and cell reflection (i-l) in SPT cell suspension.

Furthermore, the coordinates of cells immediately before the liquid extrusion were recorded for more than 300 printed droplets (Fig. 4). For low viscosity, non-density-matched saline (PBS) solution (Fig. 4a), we observed three cell behaviors: cell travel, cell ejection and cell reflection. For low viscosity, high density SPT solution the cell reflection was also observed (Fig. 4b) which was completely avoided for high viscosity, high density Ficoll solution (Fig. 4c). Note that Fig. 4a (for PBS) and Fig. 4c (for Ficoll) were captured using Phantom Miro 4 high speed camera with an objective lens (Nikon CFI LU Plan Fluor $5\times$, 0.3 NA). The Phantom v611 camera allows us to record around 100 droplet ejection events in one single video whereas Miro 4 is normally 4 ejection events. In this case, we recorded the continuous motion of cells towards the orifice step by step (see Fig. 4b). At the region where the nozzle has a diameter of $180 \mu\text{m}$ (with a distance of $150 \mu\text{m}$ to the orifice), the step is $4 \mu\text{m}$ on average. In this case, the volume of the liquid is around 100 pL, which roughly equals that of a droplet (see Fig. 2).

Figure 4: Cell mapping for (a) PBS, (b) SPT and (c) Ficoll within the inkjet nozzle indicating cell travel, ejection and reflection.

Cheng *et al.* experimentally determined the fluid velocity within the same Microfab nozzle (MJ-ABP-0) using the micro particle image velocimetry [8]. The velocity of liquid could be up to $1 \text{ m}\cdot\text{s}^{-1}$ during droplet ejection and induce a large Reynolds number (>10). For this high Re regime, both inertia and viscosity may act on cells. The Stokes number (St) correlates a particle response time with the fluid flow characteristic time, and it is given by

$$St = \frac{\tau}{t_f} = \frac{\rho_p \cdot d^2}{18 \cdot \mu} \quad (1)$$

with τ the time constant of a cell (s), t_f the fluid flow timescale (s), ρ_p the cell density (kg/m^3), d the cell diameter (m). The time scale (t_f) of SPT solution was determined as the half of the meniscus oscillation period (T , see Fig. 5). The t_f of PBS and Ficoll were obtained from our previous work [8]. The corresponding Stokes numbers (St) were calculated according to eq. (1) (see Table. 1). The relatively large St values were found for PBS (0.67) and SPT (0.38). For $St > 0.1$, particles slip with respect to the fluid streamline is expected [15]. In this case some cells probably moved from a high velocity region (close to the nozzle) to a lower velocity region, that is, these cells slip with respect to the surrounding fluids. The slip behavior results in the motion of the cells to a position further away from the nozzle (compared to its position at the onset of droplet ejection), which is the so-called cell reflection behavior. On the other hand, Ficoll solution has a smaller St value (i.e., 0.09), indicating a shorter response time that a cell moves with fluid at an equivalent velocity, contributing to the elimination of cell reflection. Ongoing work will evaluate the statistical distribution of cells in SPT within printed grids.

Table 1: Rheological properties of the inks, i.e., PBS, 10% w/v Ficoll PM400, 0.02 M Sodium polytungstate (SPT) and the corresponding response time of cells.

Ink	ρ (g/mL)	μ (cP)	τ (μs)	t_f (μs)	St
PBS	1.000	1.00	13.4	20	0.67
SPT	1.068	1.00	13.4	35	0.38
Ficoll	1.050	4.86	2.8	30	0.09

Figure 5: Meniscus oscillation: (a) Meniscus position away from the orifice as a function of time after droplet break-off ($t = 0$) as determined in (b). Fluid flow timescale (t_f) equals the half of meniscus oscillation period.

CONCLUSION

In this work, we compared the effects of solution density and solution viscosity on the trajectory of cells during inkjet printing.

The use of a neutral buoyancy salt solution did not prevent the occurrence of cell reflection, while the use of a neutrally buoyant, high viscosity solution did suppress incidents of cell reflection. Future work will examine the effect of cell diameter on cell reflection in the PBS suspensions. Future work will also examine the effect of nozzle geometry on cell reflection.

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