Generation of micro-droplet on demand with reduced sizes by a hybrid pneumatic electrohydrodynamic method

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Abstract
Sample deposition based on micro-droplet ejection (MDE) has broad application prospects in the field of biomedicine. As a potential technology option for cell printing, a hybrid pneumatic-electrohydrodynamic (HPEHD) MDE system is built in the laboratory. Strong electric field is established by applying a high voltage between the nozzle and a collector electrode. The pneumatic actuation is realized via a solenoid valve staying outside of the liquid chamber. The solenoid valve is set in conduction for a short period of time Δt; gas of high pressure P_0 enters the liquid chamber, and produces a pressure pulse, which extrudes the liquid slightly out of the nozzle. The liquid is deformed further in the electric field into a cone shape (Taylor cone), and then the end of the Taylor cone breaks to form a micro-droplet. The ejection process is studied using machine-vision and image processing. With sodium alginate (1.0%) as bioink, single droplet per ejection is realized, and the droplet size is reduced by 50% due to the presence of the electric field. It is found that increasing the voltage has little effect on the size of droplets. In contrast, increasing source pressure P_0 or increasing Δt the conduction time of the solenoid valve can change the volume of droplet in a wider range.

Keywords: on-demand, micro-droplet, pneumatic, electrohydrodynamic

1. Introduction

The micro-droplet ejection (MDE) technology has been extended from the popular inkjet printing to many scientific and technical fields such as fabrication of micro/nano structures and microelectronic packaging [1]. Recently in the field of biomedicine, the rapid development of tissue engineering, regenerative medicine, drug testing has opened up new application prospects for precise deposition of biological samples based on the MDE technology [2]. Particularly for cell printing, it is hoped that the size of the droplets can be reduced to achieve higher printing resolution without negative impact on cell viability.

Commercial printers have been modified for cell printing [3, 4]. However, those printers are difficult to clean, and are prone to clogging due to small nozzle diameter. Some single nozzle drop-on-demand (DOD) ejectors were also used for the print of cells. Piezoelectric ejectors can be operated at high frequencies (up to 30 kHz). However, the bioinks can be subjected to high shear stress during the ejection [5]. For another single-nozzle system, known as micro-valve ejector, the liquid chamber is pressured at all time, and the flow is gated by a solenoid actuated plunger at the nozzle [6]. Micro-valve ejectors work well with bioinks of wide range of viscosity. Post-printing cell viability by such bioprinters can reach 90%. Slightly different from the micro-valve ejector, a pneumatic ejector has a high-speed solenoid valve outside of the liquid chamber. Brief conduction of the solenoid valve allows high-pressure gas to rush into the chamber, generating a pressure pulse, pushing the liquid out of the nozzle to form a droplet

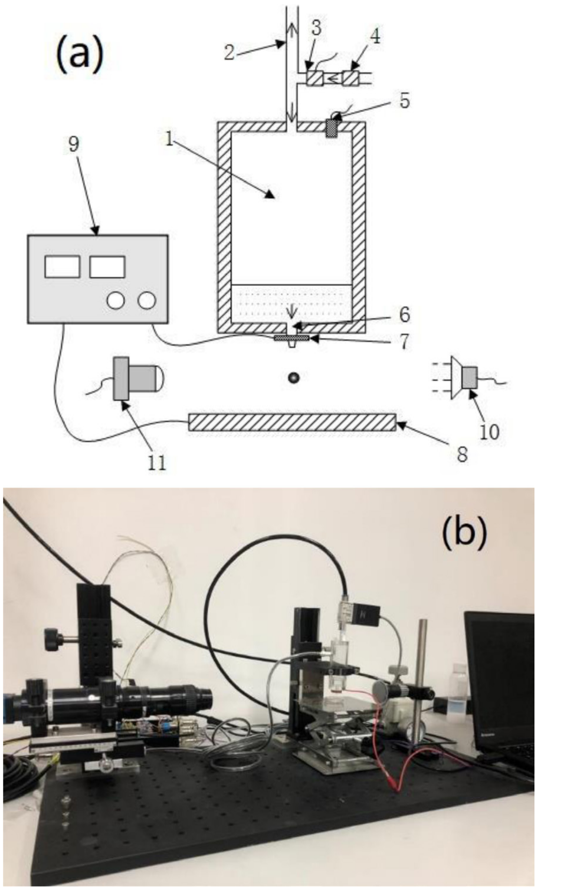

Figure 1. (a) Schematic representation of the HPEHD ejection system, where 1 is liquid chamber, 2 is venting tube, 3 is high speed solenoid valve, 4 is pressure regulator, 5 is pressure sensor, 6 is nozzle, 7 and 8 are respective electrodes attached to the nozzle and collector, 9 is high voltage source, 10 is LED illuminator, and 11 is CMOS camera. (b) Real photo of the ejection system.

[7]. Similar to the micro-valve ejection systems, the pneumatic ejectors are simple to operate, compatible with liquid of wide range of viscosity. The pneumatic ejection was initially used for cell printing and achieved cell viability closed to 100% [8], making it a potential choice for dispensing cell laden biomedical samples.

All the above-mentioned MDE technologies support the DOD mode. Liquid is extruded from the nozzle to form a liquid ligament, and the liquid ligament breaks to form a droplet. The geometrical size of the droplet is usually larger than the inner diameter of the nozzle [9]. For micro-valves and pneumatic methods, the droplet is larger than that by the piezoelectric method [10]. To obtain smaller droplets for better printing resolution, reducing the nozzle diameter is the most direct approach. However, smaller nozzle requires a much stronger actuation, producing higher shear stress that may hurt biological cells [11]. And the nozzle is more prone to clogging. For piezoelectric MDE, more complex driving functions may produce droplets smaller than the nozzle diameter [12]. For the pneumatic generation, optimizing the nozzle geometry and external atmosphere is helpful to reduce the droplets [13, 14].

For generating a droplet smaller than the nozzle diameter, electrohydrodynamic (EHD) method is sometimes employed [15], where a high voltage is applied between the nozzle and a collector electrode, creating a strong electric field. Under the action of electric stress, the meniscus at the nozzle is deformed into a cone (known as 'Taylor cone') [16]. The end of Taylor cone breaks, and a droplet smaller than the nozzle diameter can be produced, improving the printing resolution, and alleviating the tendency of nozzle blockage. EHD ejection was also used in bio-printing [17, 18]. EHD ejection typically works in a continuous mode, which is affected by many factors, including the electric field distribution and flow rate [19]. Therefore, it is not easy to maintain a stable droplet generation of fixed frequency. EHD ejection in a DOD mode can be achieved to some extent by applying pulsed voltage [20], but high-speed pulsed high-voltage sources are very expensive.

In a hybrid approach, piezoelectric actuation squeeze the liquid out of the nozzle, and then the electric stress continues to produce the droplets [21, 22]. This method has been used for cell printing, but no detailed report on cell viability [23]. It is well known that piezoelectric actuation produces large shear stress, which has adverse effects on cell viability. In contrast, the shear stress amplitude is drastically smaller during the pneumatic actuation. Inspired by those previous works, we propose a hybrid pneumatic EHD (HPEHD) method, achieve ejection of droplets on demand, while the droplet size is largely reduced, due to the presence of the electric stress. Our laboratory recently printed PBMC-laden Sodium alginate bioink with HPEHD method. Short-term cell viability after prining was close to 100%, suggesting HPEHD a potential option for cell printing.

2. Instruments and methods

The homemade HPEHD micro-droplet generator is shown in figure 1. Pressure pulses are generated in a manner similar to that of previous pneumatic ejectors [7]. The key components of this system include a cylindrical liquid storage chamber, with a tiny nozzle (stainless steel nozzle from Musashi-engineering, ID = 0.3 mm, OD = 0.5 mm) at the bottom. The chamber is connected to a high pressure nitrogen gas source via a high speed solenoid valve, and to the ambiance through a venting tube. The source pressure P_0 is tunable with a pressure regulator. To create the strong electric field at the nozzle, a collector electrode (35 mm diameter) is placed 8 mm below the nozzle. And a high electrical voltage is applied between the nozzle and the collector electrode. In this experiment, the function of the collector electrode is realized by a culture dish containing conductive phosphate buffered saline (PBS). The ejected droplets would disperse into the PBS, avoiding the phenomenon of liquid accumulation, which would modify the electric field distribution as the ejection continues. The high-voltage is provided by a DC high voltage supply (Dongwen High-voltage Power Supply Co., Tianjin, model DW-P503-1ACDF), with output voltage adjustable between 0–50 kV,

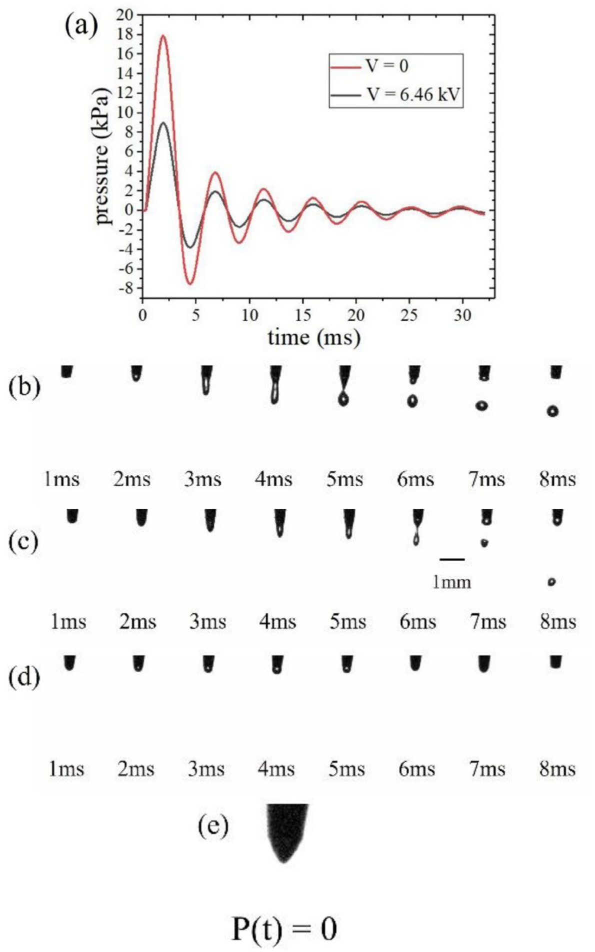

Figure 2. (a) Pressure waveforms for producing single droplet per ejection at $V = 0$ and $V = 6.46\,\text{kV}$ (b) ejection process for $V = 0$, (c) ejection process for $V = 6.46\,\text{kV}$. (d) Deformation of the meniscus for the $P(t)$ shown as black curve in panel (a), and at $V = 0$. (e) Meniscus at $V = 6.46\,\text{kV}$, without pneumatic actuation.

maximum output current 1 mA. In the experiment, the positive electrode is connected to the stainless steel nozzle, and the grounding electrode is connected to the collector electrode. As can be seen in figure 2, the nozzle is slightly tilted, but it does not affect the main results of our studies.

During the operation of the HPEHD printer, the high voltage is applied at all time, and the ejection is executed with the high-speed solenoid valve. By setting the solenoid valve in conduction for Δt, high-pressure gas (pressure P_0) enters the chamber, a pressure pulse $P(t)$ is generated in the chamber. With a high-speed pressure sensor (acquisition frequency 10 kHz, measuring range -20 kPa to $+20$ kPa), typical pressure waveforms $P(t)$ in the liquid chamber are shown in figures 4(a) and 5(a). The amplitude of $P(t)$ increases with the increase of P_0. The width of the first positive lobe increases with the increase of Δt [24]. The oscillating behavior originates from the Helmholtz resonator structure composed of the liquid chamber and the venting tube [25]. The positive pressure (especially the first positive lobe) forces the liquid out through the nozzle. For the HPEHD method, unlike ejection relying solely on pneumatic actuation, the positive pressure only needs to squeeze the liquid slightly out of the nozzle, and the electric stress will continue and make the ejection happen, similar to that occurs in an ordinary EHD ejection. The high pressure gas in the chamber is then rapidly released from the venting tube, and creating un-abiding negative pressure in the chamber. This negative pressure may help to withdraw the ligament back into the chamber, suppressing further ejection. Data presented in this paper are collected using a 40 mm long venting tube of 2.8 mm inner diameter. We have tried a longer (80 mm) venting tube, and its influences on ejection behaviors can be ignored.

A photographic system was used to evaluate the uniformity and consistency of the droplet generation process by the HPEHD method. In order to ensure high signal-to-noise ratio imaging of the moving droplets, the system adopts the mounting structure, with the nozzle staying between the camera and the light source, to image the light and shadow of the droplets. In order to clearly capture the image of the micro-droplet (with a velocity of 1 m s^{-1}, diameter of 0.1 mm–0.6 mm), a monochromatic CCD camera (Daheng Optoelectronics, Model MER-125-30UM) is chosen with a resolution of 656×492 and a shutter time as short as 10 μs. Camera is triggered by a pulse delayed by a certain amount relative to the rising edge of the driving current for the solenoid valve, and the images are uploaded to a PC through a USB interface. By setting a set of delays, a complete ejection process can be studied, as shown in figures 2(b)–(d). For the imaging optics, a Navitar 6.5 $\times$ zoom lenses (magnification of 0.7–4.5$\times$) is used. As the information obtained from the machine vision method is sensitive to the magnification, the imaging system is always calibrated based on the fact that the outer diameter of the nozzle is 500 μm.

By using an automatic threshold segmentation method (Otsu's method) to binarize the image, and a mathematical morphology closed operation to fill the hole in droplet caused by light refraction, a 2D image of the droplet is obtained [24]. The width of the image w is measured versus z (vertical distance from the nozzle). Both w and z are measured in numbers of pixels. The volume of the droplet is calculated via $V = \sum_z \pi[w(z)/2]^2$. Then, it is converted to volume in nanoliter.

3. Experiments and discussions

Here, the liquid is Sodium alginate (SA) solution (1%), a popular material for bio-printing. The viscosity of the SA solution is 0.1 Pa $\cdot$ s. At zero voltage, the system is just a pneumatic ejector. For reliable ejection at $V = 0$, a typical pressure pulse is shown as red curve in figure 2(a). The ejection process is shown in figure 2(b). It is seen clearly that the liquid is gradually extruded from the nozzle as a liquid ligament which is broken into two parts, producing a single droplet. The droplet diameter is confirmed by the photographic system to be around two times of nozzle diameter, consistent with the research results and theoretical analysis of Cheng *et al* [7].

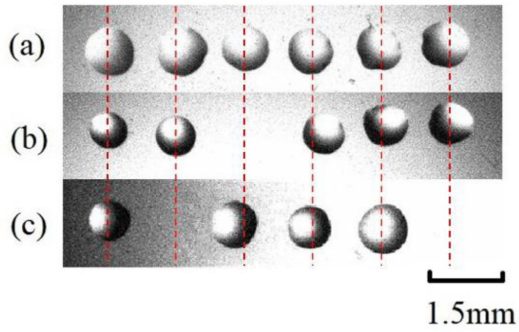

Figure 3. Demonstration of the operation mode of DOD ejection. Photograph of micro-droplets sprayed on a glass slide. (a) All ejections are enabled, (b) the third ejection is disabled, (c) the second and sixth ejection are disabled.

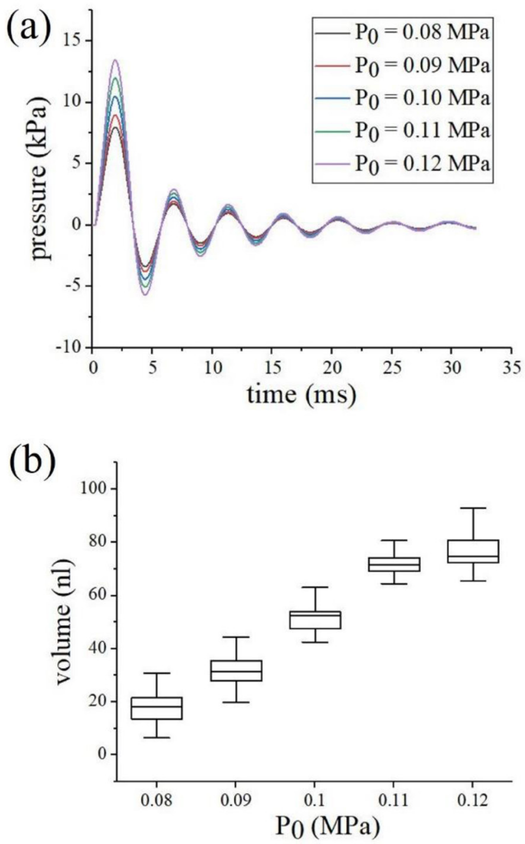

Figure 4. (a) Pressure pulse $P(t)$ in the liquid storage chamber, and (b) droplet volume, for a set of source pressure P_0 at the front-end of the solenoid valve, where $\Delta t = 1.5$ ms, $V = 6.46$ kV.

Then, applying 6.46 kV voltage, with a much weaker pressure pulse (shown as black curve in figure 2(a)), the ejection process is shown in figure 2(c). One droplet per ejection is realized. Compared with the ejection relying solely on the pneumatic actuation, the required amplitude of the pressure pulse can be reduced by 50%, and the diameter of the droplet decreases by nearly 50%, due to the effect of the electric field.

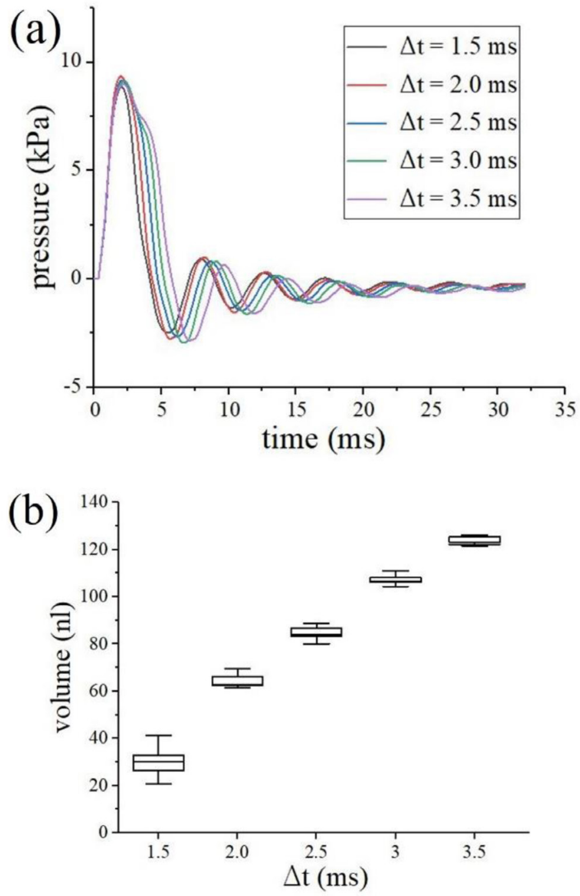

Figure 5. (a) Pressure pulse $P(t)$ in the liquid chamber, and (b) droplet volume, for a set of Δt, where the source pressure is fixed at $P_0 = 0.09$ MPa, $V = 6.46$ kV.

Further optimization of ejection parameters may improve the performance of the system.

If no voltage is applied, the droplets cannot be generated with the pressure pulse shown in the black curve of figure 2(a). For such a weak actuation, as shown in figure 2(d), the liquid ligament elongate at the nozzle, reaches its maximum at time 4–5 ms. Subsequently, the ligament returns to the liquid chamber and the whole system restores its equilibrium. On the other hand, if the pressure pulse is not applied and only a high voltage, for example $V = 6.46$ kV in figure 2(e), is maintained, there will be no droplet ejection. At high voltage, the electric stress may pull the liquid out of the nozzle to form a cone (Taylor cone), as shown in figure 2(e). High frequency oscillation of the meniscus occurs, but without droplet ejection or jetting. Those behavior has been observed in the previous experiments [26]. Hydrophobic treatment of the nozzle is common in the MDE. For the pneumatic method, the actuation required for an ejection is stronger (see figure 2(a)), and after the generation of a droplet, the liquid ligament recoils and tends to accumulate at the outer rim of the nozzle. Therefore, a hydrophobic coating (Fluororesin-based, YCP0012 from Sysmyk, Guangzhou China) self-cured at room temperature is applied for steady ejection. For the HPEHD method, both the

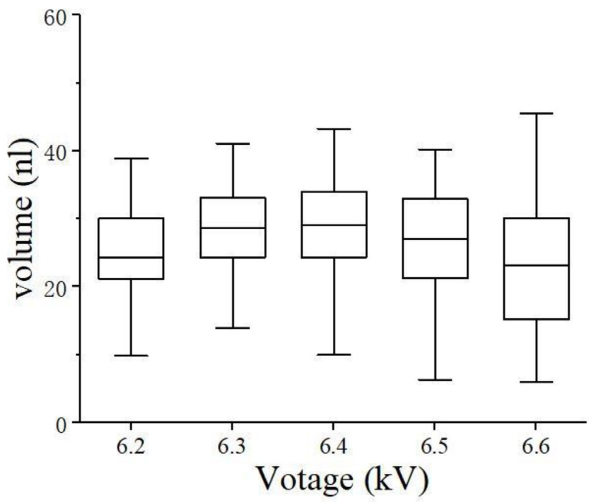

Figure 6. Droplet volume ejected under a set of voltage between the nozzle and the collector electrode. The pneumatic actuation is kept unchanged by setting $P_0 = 0.09$ MPa and $\Delta t = 1.5$ms.

untreated nozzles and the nozzles with hydrophobic coating have been tested. Consistent ejection behaviors are achieved. For the HPEHD method, the actuation can be much weaker, smaller amount of liquid is extruded out of the nozzle. The chance that the recoiled liquid accumulates at the nozzle rim is rather small. This might be the reason that hydrophobic coating is not really necessary. The data presented in the rest of this paper are collected with untreated nozzles.

In order to show that the system can realize the ejection of droplet on demand, we set the state of the solenoid valve once per second. If a droplet is expected, Δt is set to be 1.5 ms; if no droplet is expected, Δt is set to be very short (e.g. less than 0.3 ms). For the second setting, the solenoid valve can hardly reach the conduction state, so there is no enough liquid squeezed out of the nozzle, therefore no droplet is ejected. In this experiment, the collector electrode is served by an electrically grounded glass slide, moving at a speed of 1.5mm s^{-1}. As shown in figure 3(a), one droplet is ejected per second. In figure 3(b), the third ejection is artificially disabled. Similarly, shown in figure 3(c), the second and sixth ejections are disabled. Although not shown here, DOD ejection can be realized at higher rates.

In the next experiment, the effects of main ejection parameters on the size of droplet are studied. Firstly, as shown in figure 4(a), by changing the source pressure P_0 at the front end of the solenoid valve, the amplitude of the pressure pulse can be adjusted, where the conduction time of solenoid valve is set to be $\Delta t = 1.5$ ms, and $V = 6.46$ kV. As shown in figure 4(b), by enhancing P_0, the volume of droplet can increase nearly five times. This result indicates that, for HPEHD method, adjusting P_0 is an effective way to adjust the volume of droplet.

Secondly, by increasing Δt, the conduction time of solenoid valve, as shown in figure 5(a), the first positive pressure lobe widens, where the source pressure is fixed as $P_0 = 0.09$ MPa, $V = 6.46$ kV. As shown in figure 5(b), the volume of the droplets increases with the conduction time. For controlling the volume of the droplet, comparable tuning capabilities can be achieved via adjusting P_0 or Δt.

Finally, the voltage V between the nozzle and the collector electrode is changed, while the pneumatic actuation is fixed by setting $P_0 = 0.09$ MPa and $\Delta t = 1.5$ms. As can be seen from figure 6, the volume of the droplets remains stable over a wide range of voltage. This behavior is well consistent with the experimental results of the hybrid piezoelectric-EHD droplet ejection [22]. It is worth noting that for the experiment presented in figure 6, there are always some ejections, which generate droplets significantly smaller than the average (in volume). Those ejections account for only 2%–5% of the total ejections, making the lower bounds of data points different from those in the figures 4 and 5. But their influence on the average volume can be ignored.

Similar experiments are performed with two nozzles of smaller inner diameters, 100 μm and 200 μm respectively. The smallest droplets ejected are, correspondingly, 101 μm and 210 μm in diameter. As is obtained from figure 4(b), the smallest droplet with the 300 μm nozzle is 18 nl in volume, or 324 μm in diameter. Therefore, the smallest droplet realized by this method is roughly 1.0–1.1 times the inner diameter of the nozzle. Limited by experimental conditions, we did not try even smaller nozzles.

All the data presented in this paper are collected at an ejection frequency of 10 Hz. As can be seen from figure 2, it takes about 20 ms for the pressure in the liquid chamber, and consequently the meniscus at the nozzle, to reach equilibrium. It is also worth noting that the time required for the formation of a droplet is in the order of several milliseconds, rather long comparing with, for example, the piezo-electric method. Therefore, ejection at a rate significantly higher than 50 Hz is not reasonable. The highest ejection frequency we have tried in the experiment is 60 Hz, and stable ejection is confirmed using a high speed camera (Fastcam mini AX from Photron).

4. Conclusions

A HPEHD MDE system is introduced and established. With 1% Sodium alginate as bioink, single droplet per ejection is realized. By applying a high voltage between the nozzle and the collector electrode, both the required pneumatic actuation and the droplet size are significantly reduced, due to the effect of the electric field. This not only reduces the shear stress during the ejection, but also improves the printing resolution. In a recent experiment not shown in this paper, as evaluated by flow cytometry and microscopy, the adverse effect of ejection on the short-term viability of human cells is shown to be negligible, suggesting that HPEHD ejection is a promising technical option for the cell printing. DOD ejection mode can be reliably realized. Droplet volume can be increased, by increasing either P_0 or Δt. However, enhancing the voltage between the nozzle and the collector electrode has much less effects on the droplet volume.

Acknowledgments

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