Contact-Free Dispensing of Living Cells in Nanoliter Droplets

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Abstract

We present a novel method for automated dispensing of living cells in nanoliter range droplets using a disposable pipette tip combined with an elastic polymer tube. After introduction of an unmetered suspension of cells into a reservoir connected to the pipette tip, a tuneable volume of 10 - 80 nL of cells suspension is issued in a non-contact procedure. Droplet ejection is enabled by a piezostack driven piston squeezing the tube at a defined position. We achieve a reproducibility of the printed cell culture medium volumes better than 5% and survival rate of the cells of 97% directly after dispensing. In addition we demonstrated good culturability and cell differentiation in order to consider potential long term effects of the dispensing process that could harness the cells.

Keywords: nanoliter dispensing, cells, piezo actuation

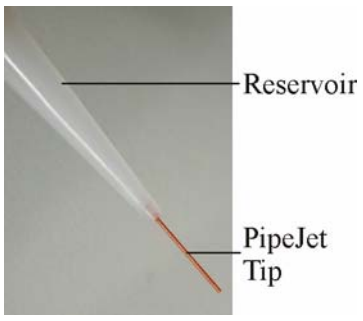
Introduction

Handling liquid volumes from several microliters down to a few nanoliters with high reliability and high accuracy within a single platform is very important in drug development, modern diagnostics, chemistry and biological applications especially for the reduction of assay volumes in high throughput screening applications. For pharmacological tests the influence of pharmacological active substances on cells has to be tested prior to clinical studies. Therefore a systems capable of dispensing cells is needed that reproducibly allows to spot cells without affecting their behaviour or survival rate.
Compared to conventional pipetting systems, where the precision of the dosage volumes below 1 µl is limited by capillary and adhesive forces, dosage systems with free flying liquid jets or drops have the important advantage to avoid these surface interactions. Because of the variety of different fluids used in chemical and biological laboratories with very different properties it is essential that the dispenser provides a dosage volume nearly independent of fluid properties like viscosity, surface tension or density. It is thus not necessary to calibrate the system after every used liquid. For biological or medical purposes it is also recommended that all contaminated parts are disposable and therefore low-cost and easy to handle. Because of the sensitivity of cells to mechanical stress the influence of the piezo-driven droplet ejection on different cell types has to be investigated.

Design

Key element of our experimental setup is the so-called PipeJet™ dispenser [1,2] which clamps an elastomer tube (Fig. 1A) between two jaws along a length of approximately 5 mm in the vicinity of the orifice (Fig. 1B). Upon highly dynamic displacement of a piston connected to a piezostack actuator, liquid is transported towards either end of the tube. Due to the low ratio of the fluidic resistances between actuation zone and the nozzle and the other end of the tube, respectively, most of the displaced volume is issued as a free flying droplet.

A)



B)

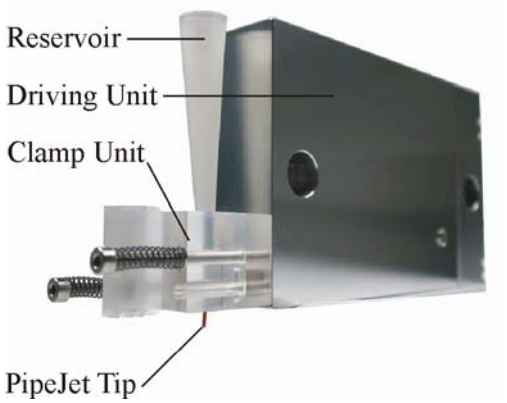


Fig. 1: *(A) The disposable PipeJetTip™ consists of a standard pipette tip that can be used as reservoir and a polymer tube with a diameter of 500 µm. (B) The PipeJet™ module features a clamp unit for fixation of the tip and a driving module with integrated piezo-driven actuator.*

The droplet volume can precisely be defined by the stroke amplitude and velocity of the piston in the driving unit. After shooting, the tube refills by capillary forces as shown in figure 2. The high droplet generation frequencies of up to 150 Hz allow the accumulation of a 100-µL volume in less than 10 seconds (Fig. 3).

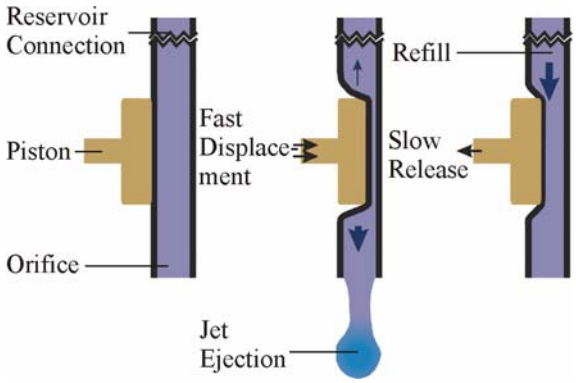


Fig. 2: *Dispensing principle scheme: A piezo actuated piston squeezes the polymer tube and generates a pressure forcing the ejection of a droplet. Slow release of the piston allows capillary filling of the tube.*

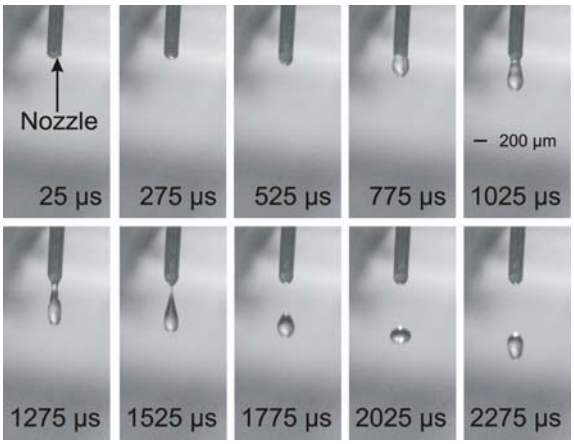


Fig. 3: *(A) Pictures of the droplet ejection from PipeJet™ Tip.*

Results

Gravimetric measurement of the droplet volume with the cell culture medium DMEM (Dulbeccos Modified Eagle Medium) [2] displays a CV of 1.6 % for a volume range of 50-80 nL, only (Fig. 4A). Printed volumes in a range of 10-50 nL show a CV of 6.4 % allowing very precise dispensing in the nL-scale, proving that the PipeJet is able of dispensing cell culture medium with a very high accuracy and reproducibility. In subsequent experiments, two commonly used cell lines are being dispensed with the PipeJet™. We suspended neuronal cells (line PC12) and fibroblast cells (line L929) in DMEM and dispensed a concentration of 50 cells per droplet into a standard Eppendorf tube. The ratio between viable cells before and immediately after the dispensing process is calculated by a living-dead stain method using trypan blue. The appropriate results show a negligible decrease in the amount of living cells of 3% for both cell lines (Fig. 4B), leading to the conclusion that the piezo-driven dispensing processing has no effect on the survival of the cells. In additional experiments PC12 cells in DMEM are being shot into wells of a microtiterplate. The tests reveal that 10 days after printing, the capability of dispensed PC12 cells to proliferate in DMEM is not influenced by dispensing.

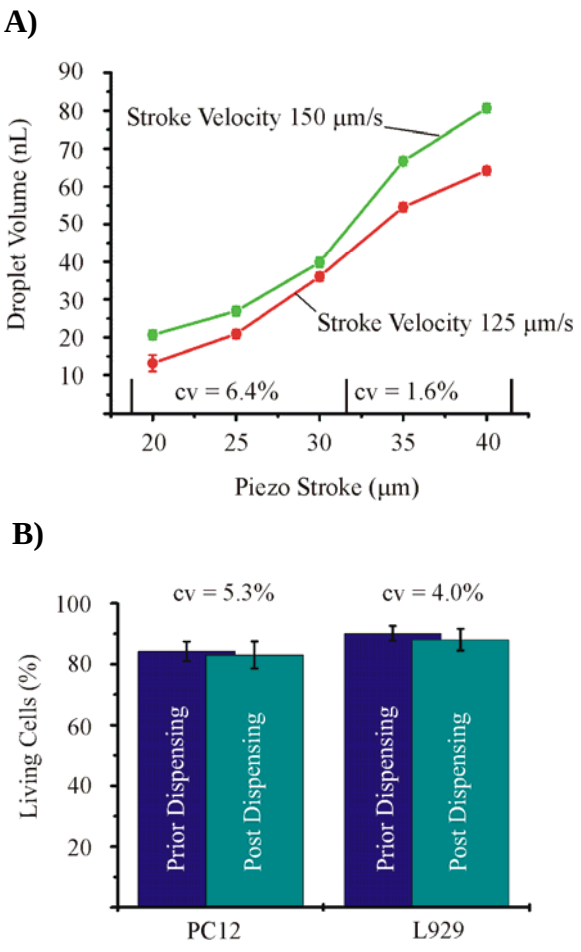


Fig. 4: *(A) Correlation between the DMEMS-droplet volume and the piezo stroke for two different stroke velocities. The droplets exhibit high reproducible volumina from 10 nL to 80 nL. (B) Comparison of the percentages of living cells prior to and after dispensing. Only a small fraction of both tested cell lines ares affected by the dispensing process.*

The number and shape of the printed and control group cells are highly compliant. The fourth day after cell dispensing the cell culture medium was changed to DMEM containing 5 % NGF [3] causing to cells to differentiate into neurons. As can be seen on figure 5 on day 10 the cells show typical structures for neuronal cells like dendrites and axons indicating no influence of PipeJet™ dispensing on cell differentiation.

References

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[2] Biofluidix GmbH, http://www.biofluidix.com
[3] DMEM (+4500mg/L Glucose, +GlutaMAX, +Pyruvate), GIBCO, No. 31966-021
[4] NGF 7S, Invitrogen, No. 13290-0101

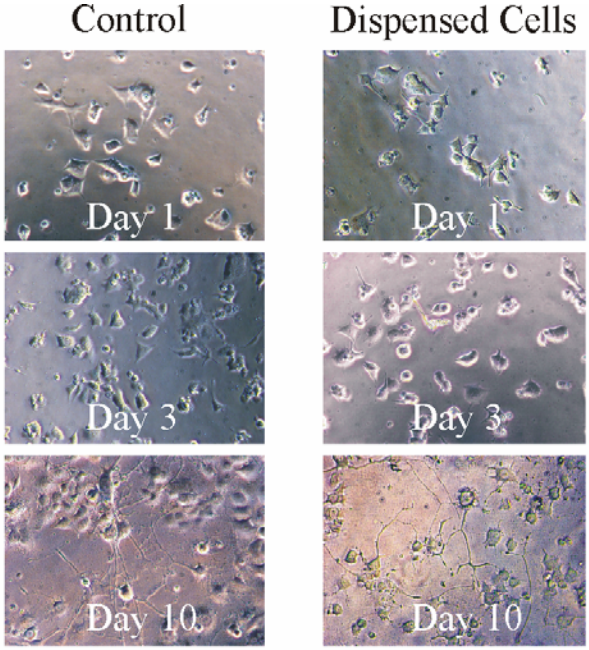

Fig. 5 *PC12 cells are printed into a 96-well microtiterplate using the PipeJet™. The control group is added into the wells by a standard pipette. The cells are then monitored over a period of 10 days to analyze their culturability and differentiability. Cells are treated for 3 days with DMEMS cell culture medium for cultivation and subsequently from day 4 to 10 with DMEMS containing 5% NGF for differentiation. Pictures captured on day 1 and 3 exhibit good culturability of the cells. NGF-treatment leads to the differentiation of PC12 cells which can be recognized by the formation of axons and dendrites.*

Conclusion

We introduced a novel dispensing technology for the printing of living cells in a volume range of 10 nL to 100 µL. The dispensed volume features a high accuracy and reproducibility. Furtheron the influence of the piezo-driven printing process on the two prominent cell lines L929 and PC12 is investigated regarding viability, culturability and differentiability of the cells. The dispensing process has no negative effect on the viability of both cell lines. It is also proved that PC12 cells can be cultivated after dispensing and show a normal proliferation behavior. Further experiments showed the ability of PC12 cells to differentiate into neuronal cells leading us to the conclusion that no negative effect of the printing process on the cells differentiability can be seen. Our method is hence suitable for dispensing of cells in microtiterplates for highly parallelized cell screening experiments.