ON-CHIP AEROSOL GENERATION FOR ORGANS-ON-CHIPS

Karel Domansky[1], Michael Karpelson[1], Robert J. Wood[1,2], and Donald E. Ingber[1,3]*

[1] *Wyss Institute for Biologically Inspired Engineering at Harvard University, USA*

[2] *Harvard School of Engineering and Applied Sciences, USA and*

[3] *Children's Hospital, Harvard Medical School and Harvard School of Engineering and Applied Sciences, USA*

ABSTRACT

A vibrating mesh nebulizer for delivering aerosol to cells at the air-liquid interface in organs-on-chips is demonstrated. The piezoelectrically actuated nebulizer forms the top surface of a microchannel and is used to deposit liquid droplets, magnetic beads, and solid particles on the bottom of the channel. This arrangement produces a short, unobstructed flow path for aerosol, enabling high deposition efficiency, accurate dose control, and significant waste reduction. Droplet exit velocity is controlled by driving the piezoelectric element intermittently, making this method suitable for depositing aerosol from a very short distance without damaging the cells at the air-liquid interface.

KEYWORDS: Aerosol, Drug Delivery, Air-Liquid Interface, *In vitro*, Cell Culture, Organs-on-Chips

INTRODUCTION

Many drugs appear promising in animal studies but fail in human clinical trials. As a result, there is a pressing need to develop *in vitro* models capable of reconstituting the key structural and mechanical features of whole organs as an alternative to animal studies. Recently, we demonstrated this capability with two organs-on-chips: the human peristalsing gut-on-a-chip and the human breathing lung-on-a-chip [1,2]. In order to study the response of lung cells to drugs in the latter device, these drugs need to be delivered without perturbing the air-liquid interface. Previously, we presented a method for generating aerosol off-chip, delivering it to the chip through a capillary, and depositing it in the microchannel along its long axis [3]. In the current work, liquid microdroplets or solid particles are generated on-chip and delivered from the top surface of the microchannel. Generating aerosol on-chip in close proximity to the cells enables accurate dosing and localized aerosol deposition. Furthermore, because this methods uses very small quantities of solutions or powders, and leaves virtually no remaining waste, toxic compounds can be tested in various types of organs-on-chip with significantly reduced risk.

Piezoelectric droplet ejectors [4] have been studied for a number of years and are used in commercial nebulizers for pulmonary drug delivery. The novelty of our approach lies in integrating the vibrating nozzle plates into microfluidic devices. Positioning the nozzle plate several hundred microns above the cells allows for localized deposition of various entities of controlled size and distribution; the same nozzle plate also can potentially be used for cell seeding, extracellular matrix coating and drug delivery.

EXPERIMENTAL

The vibrating mesh nebulizer comprises a nozzle plate, a piezoelectric ring, and a dual medication chamber (Fig. 1a). The diameter of the nozzles in the plate approximately determines the size of the generated microdroplets. The nebulizer is fabricated by attaching a laser-machined PZT ring to a polyimide film with photolithographically patterned copper electrodes (Fig. 1b). The nozzle plate is bonded to the bottom of PZT/electrode assembly, and a medication chamber is cast on top using a mold (Fig. 2). Because the medication chamber is mechanically coupled to the nozzle plate and the piezoelectric ring, it has to be fabricated from an elastomeric material that does not impede vibrations in the plate while reliably encapsulating the ring and electrodes.

To demonstrate localized deposition, the medication chamber has dual compartments separated by an elastomeric wall 400 µm in thickness. Each medication chamber has a volume of 9 µL and a 400 x 400 µm bottom footprint. The nebulizer is attached to a PDMS block, where it serves as the top surface of a 400 µm wide and 120 µm deep microchannel. The distance from the bottom of the nozzle plate to the bottom of the channel is ~320 µm. The PDMS block is supported from the bottom by a cover slip. The deposition is imaged through the bottom of the microchannel using an inverted Zeiss microscope in transillumination mode. High-speed imaging of the droplets is performed using the same microscope rotated by 90º and equipped with a Photron FASTCAM SA4 500K-M2 high-speed camera operating at 3600 frames per second.

RESULTS AND DISCUSSION

The medication chambers are filled by pipetting liquids or dispensing solid powders. The amount loaded can be used to control the dose. To deposit aerosol on the cells from a short distance above them without harmful effects, it is critical to control the exit velocity of the microdroplets. Our approach relies on precisely timed intermittent driving of the piezoelectric material. Figure 3a depicts streams of microdroplets ejected from a nebulizer actuated continuously at the resonant frequency. Figure 3b shows a high-speed camera image of a stream of droplets generated using intermittent actuation. The droplets are ~10 µm in diameter and have an average spatial separation of approximately 50 µm. An analysis of multiple high-speed video trials shows that the droplet velocity is ~25 mm/s.

978-0-9798064-5-2/µTAS 2012/$20©12CBMS-0001

Localized deposition of liquid droplets, magnetic beads, and solid particles is shown in Figures 4a, b, and c. The droplets evaporate and coalesce as they are deposited. Figure 4a shows large coalesced droplets at the end of a deposition. Figure 4b depicts magnetic beads deposited from suspension before complete evaporation of the liquid. Figure 4c shows iron particles deposited from dry iron powder.

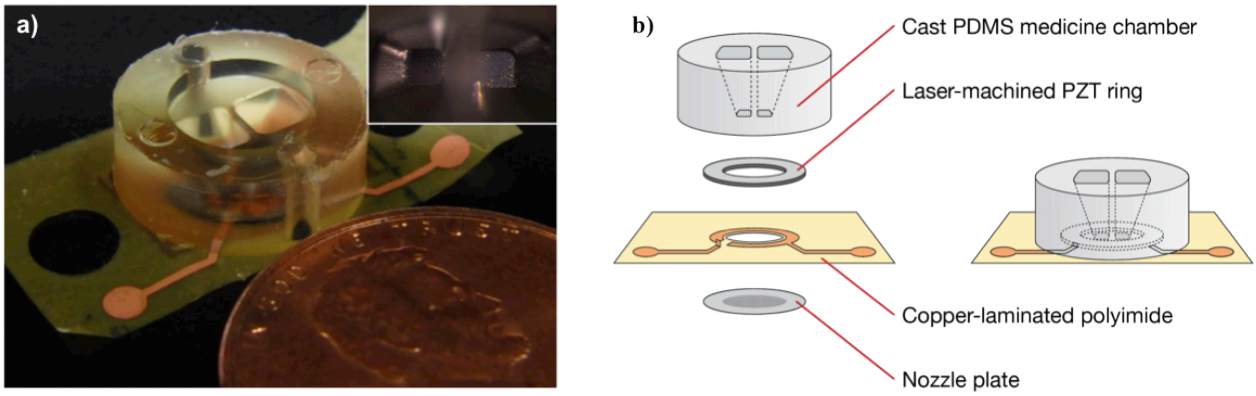

Figure 1: a) *Vibrating mesh nebulizer with dual medication chamber. The volume of each medication chamber is 9 µL. The insert shows the nozzle plate at the bottom of the chambers, which are separated by 400 µm. b) Exploded view and schematic diagram of the nebulizer.*

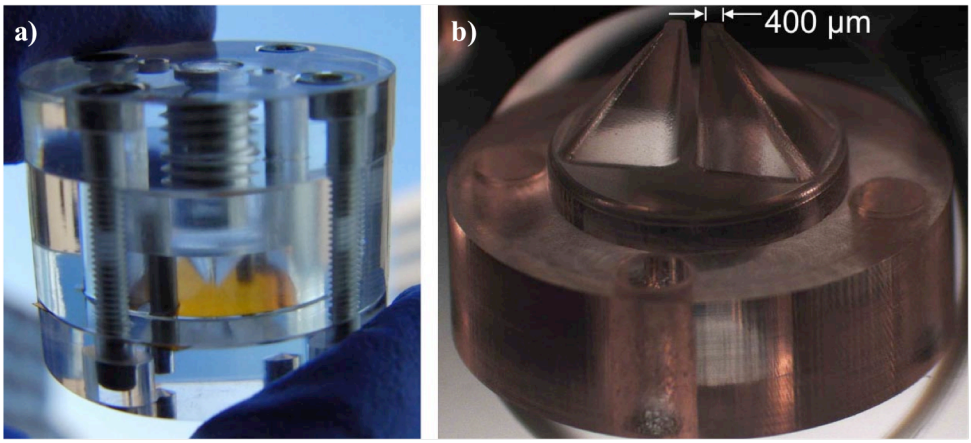

Figure 2: a) Mold for casting the dual elastomeric medication chamber on top of the nozzle plate. b) The mold insert with two half-pyramid shapes used to form the medication chambers.

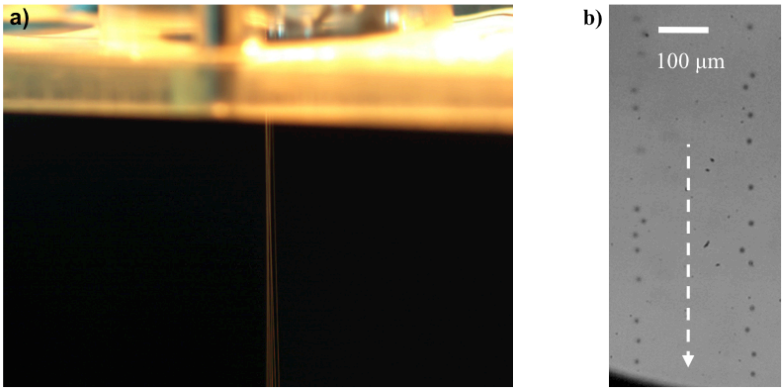

Figure 3: a) Streams of aerosol ejected from a nebulizer with a medication chamber. b) High-speed camera imaging of aerosol exiting the nozzles. Two streams of droplets are visible on both sides of the arrow. The droplets are ~10 µm in diameter. Average spatial separation between the droplets is ~50 µm.

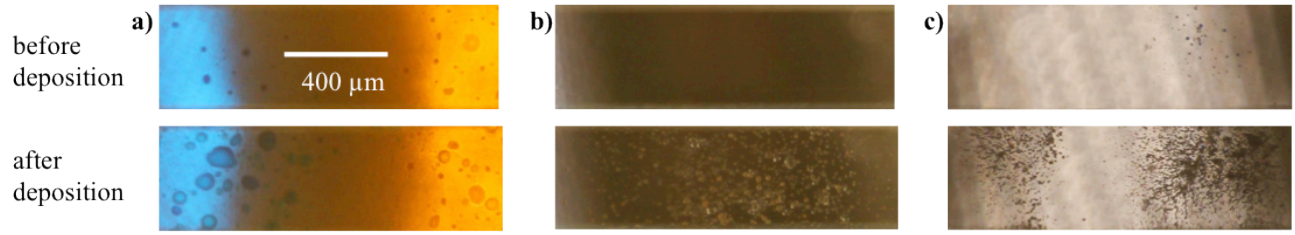

Figure 4: Localized deposition of liquid droplets, magnetic beads, and solid particles into a 400 µm wide and 120 µm deep PDMS microchannel. Deposition is performed from 10-µm nozzles positioned ~320 µm above the bottom of the channel. a) Dyed liquid droplets ~10 µm in size deposited from differently colored solutions. The droplets evaporate and coalesce as they are deposited. b) Liquid droplets containing 1-µm magnetic beads deposited from a bead suspension. Image taken after evaporation of the liquid. c) Solid iron particles (<9 microns) deposited from dry powder.

CONCLUSION

We have fabricated a vibrating mesh nebulizer with a dual medication chamber and demonstrated localized deposition of liquid droplets, magnetic beads, and solid particles into a microchannel. We have achieved low droplet exit velocity by intermittent driving of the piezoelectric element, enabling the deposition of droplets with low impact forces from very short distances. The device makes it feasible to perform localized delivery of aerosolized drugs to cells at the air-liquid interface in lung-on-a-chip. Due to short, unobstructed flow path of the droplets, virtually all of the solution loaded into the device is nebulized and deposited on the target region, allowing for accurate dose control and waste reduction.

ACKNOWLEDGEMENTS

This work was supported by a grant (U01 NS073474) from the NIH Common Fund, through the Division of Program Coordination, Planning, and Strategic Initiatives (DPCPSI), Office of the Director, NIH and by the Food and Drug Administration (FDA), and by DARPA cooperative agreement W911NF-12-2-0036.

REFERENCES

[1] H. Kim et al., *Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow*, Lab Chip, 12, pp. 2165-2174 (2012).
[2] D. Huh et al., *Reconstituting organ-level lung functions on a chip*, Science, 328, pp. 1662-1668 (2010).
[3] D. Leslie et al., *Aerosol delivery for lung-on-a-chip*, Micro Total Analysis Systems 2011, Kluwer Academic Publisher, Dordrecht, The Netherlands, p. 97-99 (2011).
[4] G. Percin and B. T. Khuri-Yakub, *Piezoelectric droplet ejector for ink-jet printing of fluids and solid particles*, Review of Scientific Instruments, 74, pp. 1120 - 1127 (2003).

CONTACT

*don.ingber@wyss.harvard.edu