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A Microfluidic Approach to Pulsatile Delivery of Drugs for Neurobiological Studies

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Abstract

We present an innovative microfluidic approach to transcranial delivery of small quantities of drugs in brief time pulses for neurobiological studies. The approach is based on a two-stage process of consecutive drug dispensing and delivery, demonstrated by a device featuring a fully planar design in which the microfluidic components are integrated in a single layer. This 2-D configuration offers ease in device fabrication and is compatible to diverse actuation schemes. A compliance-based and normally closed check valve is used to couple the microchannels that are responsible for drug dispensing and delivery. Brief pneumatic pressure pulses are used to mobilize buffer and drug solutions, which are injected via a cannula into brain tissue. Thus, the device can potentially allow transcranial drug delivery and can also be potentially extended to enable transdermal drug delivery. We have characterized the device by measuring the dispensed and delivered volumes under varying pneumatic driving pressures and pulse durations, the standby diffusive leakage, and the repeatability in the delivery of multiple pulses of drug solutions. Results demonstrate that the device is capable of accurately dispensing and delivering drug solutions 5 to 70 nL in volume within time pulses as brief as 50 ms, with negligible diffusive drug leakage over a practically relevant time scale. Furthermore, testing of pulsatile drug delivery into intact mouse brain tissue has been performed to demonstrate the potential application of the device to neurobiology.

Index Terms

Drug delivery; microfluidic; neurobiology; pulsatile; transcranial; transdermal

I. Introduction

Neurobiological research focuses on central and peripheral nervous systems of biological organisms [1] to understand specific physiological functions associated with different neural substrates, and investigates how these functions are influenced at levels ranging from molecules to entire systems [2]. An effective approach to neurobiological studies is to apply stimuli directly to specific functional brain zones of mammalian animals, using methods

involving electrical probes [3], magnetic fields [4], and chemical molecules [5]. In particular, chemical stimulation, via transcranial delivery of drug molecules to specific sites in the nervous system, is highly attractive due to its broad applicability and high selectivity.

Drug delivery for neurobiological studies is challenging because of its stringent spatial and temporal requirements. The spatial dimensions of effective signaling zones among neurons, on the order of hundreds of micrometers, are small and hence require chemical drug compounds to be delivered in minute amounts with microscale spatial precision. In addition, the temporal domain covered by signaling among neurons is highly acute, typically on the order of milliseconds, requiring delivery of chemicals to be completed within comparably brief time periods. Conventionally, neurobiological drug delivery is performed using small injectors coupled with external actuation modules. Stereotaxic stages are usually required to position injectors for accurately localized injections. For the small-dimensional scale of neurobiological samples (e.g., mouse brains), such drug delivery tools are cumbersome and inadequate. Specifically, drug injections have long startup times, require prolonged injection time intervals and are limited by large injected liquid volumes. These limitations have significantly hindered the use of chemical stimulation in neurobiological studies.

Microfluidics technology can potentially address the challenges in neurobiological drug delivery [6]–[8]. In recent years, microfluidics has been actively applied to various drug delivery applications. Drugs have been loaded in responsive polymers [9] and micro- or nanoscale particles [10] within microfluidic devices, so that they can be released *in vivo* through intravenous or oral routes for chemotherapeutic applications. Microfabricated needles [11], [12] have also been used to realize minimally invasive transdermal delivery of drugs in small volumes [13]. With these devices, the delivery rate, i.e., the time rate of flux at which drugs are delivered, is small and cannot be controlled. There is also a lack of control in the timing of drug release (for example, the time at which the release of drugs begins or ends). In addition, diffusive drug leakage, or unintended release of drugs by diffusion from the micro- or nanoparticles, may occur. To offer improved control, some microfluidic devices have allowed prespecified drug volumes and delivery times, by using tubing cartridges filled with plugs of reagents separated by air spacers [14], or hydrophobic surfaces that digitize a continuous liquid stream into discrete segments [15]. These approaches are still limited by small delivery rates, as well as by relatively large volumes of delivered drugs. Air bubbles are also injected into tissue, which can potentially lead to tissue damage. Other microfluidic devices have been developed to allow for controlled delivery of drugs in small volumes. Osmosis [16] and localized electrokinetic effects [17] are used in microfluidic devices to enable the control of drug delivery rates and release timing. Implantable probes with integrated microfluidic ports [18] and convection-enhanced infusion [19] can deliver chemical compounds into neurobiological samples *in vivo* at specified times and locations. These devices, however, usually have complicated structure and fabrication, only allow relatively small delivery rates, require long response times to control signals, and also have problems in unintended drug release due to diffusion. The use of discrete droplets would minimize diffusive drug leakage while allowing delivery of minute drug volumes [20], but their inability to deliver drugs at specified locations in tissue is undesirable for neurobiological studies. Moreover, microfluidic devices integrating microflow regulation components [21] and drug delivery routes [22] are used for local delivery of drugs with the dosage varied *in situ*. However, most existing microsystems of this type are designed for chronic drug delivery. Thus, they deliver relatively large volumes of drugs over extended time intervals and are not well suited to pulsatile delivery of neurobiological drugs.

Aiming to meet the strong need in neurobiological studies for drug delivery in small volumes over brief time periods, this paper presents a microfluidic approach for pulsatile

delivery of chemical compounds into mouse brain tissue, a functionality that to the best of our knowledge has not been previously available. To address the limitations of existing drug delivery devices that involve extended-time or uncontrolled drug release, relatively large drug volumes, unintended diffusive drug leakage, and a lack of precisely determined delivery route, our approach uses a two-stage strategy in a planar design so that the drug delivery process is partitioned into a dispensing stage and a delivery stage. The fine control afforded by this approach can potentially lead to effective tools for transcranial pulsatile delivery of drugs in neurobiological studies and neurological therapeutics, as well as for transdermal drug delivery in other basic science and therapeutic applications.

II. Microfluidic Design

This microfluidic drug delivery device mainly consists of two channels for drug dispensing and delivery, respectively (Fig. 1). The two channels are coupled by a planar check valve (top view shown in the inset of Fig. 1) to regulate the direction and volume of the drug flow during the dispensing and delivery stages. This device is entirely planar; that is, all microfluidic components, including the dispensing and delivery microchannels and the interconnecting check valve, are integrated in a single layer (Fig. 1). The use of such a single fluidic layer offers ease of fabrication and integration with any additional functional elements. The fluidic layer is fabricated from poly(dimethylsiloxane) (PDMS) to exploit the large compliance of the polymeric material and sandwiched between two glass plates. The drug and buffer inlets are defined through one of the glass plates to connect the dispensing channel to an external line filled with drug solution and the delivery channel to another external line filled with buffer solution. Coupled into the end of the delivery channel is a cannula that can be inserted into the mouse brain for transcranial drug delivery. While this design allows for the use of various actuation methods, pneumatic actuation is used in this work for demonstration of principle. Pneumatic pressure pulses are generated externally and applied directly to each of the drug and buffer lines.

The key dimensions of the device are as follows (Fig. 1). The fluidic layer in the device ($1 \times 1 \text{ cm}^2$) has a uniform thickness of $250 \text{ }\mu\text{m}$. The delivery channel is $200 \text{ }\mu\text{m}$ wide and 3 mm long, corresponding to a drug storage volume of $0.15 \text{ }\mu\text{L}$ that is considered adequate for practical neurobiological studies. The dispensing channel is $500 \text{ }\mu\text{m}$ wide and 3 mm long. The check valve, located within the dispensing channel, has a $420 \times 60 \text{ }\mu\text{m}$ flap separated from a $420 \times 500 \text{ }\mu\text{m}$ stopper by a gap $30 \text{ }\mu\text{m}$ wide. The cannula, with an inside diameter (I.D.) of $99 \text{ }\mu\text{m}$ and outside diameter (O.D.) of $191 \text{ }\mu\text{m}$, is 10 mm in length (total internal volume: approximately 75 nL), 2 mm of which is inside the PDMS fluidic layer.

The microfluidic device operates as follows for pulsatile drug delivery. In standby [Fig. 2(a)], the dispensing and delivery channels are filled with drug and buffer solutions, respectively, and separated by the check valve. During dispensing [Fig. 2(b)], a measured volume of drug solution is metered through the check valve and introduced into the delivery channel to form a plug. The check valve closes upon completion of dispensing. In the following delivery stage [Fig. 2(c)], a pressure pulse is applied to the buffer reservoir, allowing the drug solution plug to be ejected through the cannula into the mouse brain.

The check valve, which plays a key role in coupling the drug dispensing and delivery stages, is based on a compliant thin flap that is perpendicular to the flow (Figs. 1 and 2) [23]. In the valve, the as-fabricated flap is in contact with a stiff block (called a stopper) via spontaneous adhesion. Under reverse pressure, the flap is maximally sealed against the stopper, allowing for almost zero leakage under reverse pressure [23]. On the other hand, forward flow readily passes through the valve as the hydrodynamic force from the flow deflects the compliant

flap away from the stopper with minimal resistance. The check valve is located close to the cannula/channel interface to reduce the buffer preceding the drug plug.

III. Experimental

A. Materials

For device characterization, red ink (Higgins) was used as a model drug solution and deionized (DI) water as a model buffer solution, while for drug delivery performed with mouse brain tissue, a green dye (Kation Scientific) was used as a model drug for visualization. The ink or dye solution, as well as the DI water, was filtered by a 0.2- μm syringe filter (Nalgene) and degassed using a vacuum pump (Gast Manufacturing) before it was introduced into the device. Mouse brains were extracted when mice were first anesthetized with barbiturate (Nembutal) and then their heads placed in a stereotaxic device (Kopf 900). The extracted mouse brains were quickly frozen at $-20\text{ }^{\circ}\text{C}$ for preservation. Before drug delivery experiments, the mouse brains were allowed to thaw for approximately 5–10 min.

B. Device Fabrication

We fabricated this device using standard PDMS-based replica molding techniques [23]. Briefly, to obtain the fluidic layer defining the microchannels and check valve, a PDMS prepolymer (Sylgard 184, Dow Corning) mixed with a curing agent (weight ratio: 10: 1) was cast on a SU-8 (MicroChem) mold [Fig. 3(a)], which was then sandwiched between a transparency film and a thick, cured PDMS sheet. This stack was clamped tightly between two aluminum plates, and placed on a hotplate at $80\text{ }^{\circ}\text{C}$ for 2 hours, so that the PDMS prepolymer on the SU-8 mold was cured [23]. The resulting PDMS fluidic layer [Fig. 3(b)] was next peeled off the SU-8 mold and transparency film, with the flap establishing and maintaining contact with the stopper by spontaneous interfacial interaction. Following an oxygen plasma treatment of the interfaces, this PDMS layer was bonded to two glass plates [Fig. 3(c)], including the upper and lower edges of the compliant flap. Finally, a glass capillary tubing (Polymicro Technologies) used as a cannula was inserted into the drug delivery channel, and the plastic tubings (Tygon PVC, used as buffer and drug lines) were coupled to the drug and buffer inlet holes that were pre-drilled through one of the glass plates. Fig. 4 shows a fabricated PDMS fluidic layer before bonding to glass, as well as a fully packaged device.

C. Characterization Setup and Method

The experimental setup [Fig. 5(a)] included pneumatic actuation and microscopic monitoring. Pneumatic actuation was based on a compressed N_2 tank: pulsed pressures were generated through a pressure regulator (ITV-2030, SMC) controlled by a waveform generator (33220A, Agilent) for drug dispensing, and through a high-speed electromagnetic valve (EV-3, Clippard) driven by a computer-controlled power supply (E3631A, Agilent) for drug delivery. During experiments, the microfluidic device was mounted on the stage of an inverted microscope (Diaphot 300, Nikon) equipped with a high-speed CCD camera (PL-B742U, PixeLINK).

To investigate the suitability of the check valve design for use in the microfluidic drug delivery device, we tested a check valve (with the same dimensions given above) fabricated in a single microchannel. Pressurized nitrogen was used to drive DI water through the check valve in either forward or reverse direction, while the flow rate through the valve was obtained from the timed movement of the water meniscus in a plastic tubing connected to the exit end of the microchannel [24].

Upon check valve testing, the entire microfluidic drug delivery device was characterized as follows. We investigated the diffusive leakage from the dispensing channel to the delivery channel in the device's idle state, as well as the reverse flow to the dispensing channel through the check valve in delivery stage, via time-resolved microscope images of red ink solution in the small regions located at the exit in the delivery channel and at the entrance in the dispensing channel, respectively. The mean color intensity in the observation regions was calculated and compared with the color intensity of prefilled red ink solution in the dispensing channel.

To determine the dispensed drug volume, ink solution was dispensed into the delivery channel, forming a cuboid plug. The plug length was immediately measured and used to calculate the dispensed drug volume. To test the repeatability of this dispensed volume, multiple cycles of dispensing and delivery of red ink were performed, and the dispensed dye volumes in the individual cycles were compared.

The time duration and volume of delivered drug pulses were also characterized. To determine the delivery pulse duration, the drug delivery process was imaged in real time by the CCD camera at 200 frames per second (fps), and the acquired frames were analyzed. To determine the volume of delivered drug pulses, a thin polyethylene sheet was used to receive the droplet of red ink ejected from the device. On the hydrophobic polyethylene surface, the droplet formed a spherical cap, whose image was taken by a digital camera (Cyber-shot 6.0, Sony) through a 10 $\times$ amplification lens (Thorlabs) [Fig. 5(b)]. Analysis of the image allowed calculation of the volume of the droplet, and hence the total volume of delivered red ink and accompanying DI water.

D. Testing in Intact Mouse Brain

Neurobiological drug delivery using this device was tested on intact mouse brain tissue as follows. The mouse brain was located on the microscope stage using a custom-built holder. The protruding cannula penetrated into the tissue at the target spot for a depth of approximately 2–3 mm, followed immediately by a brief pulse to deliver the green dye solution into the tissue. Multiple injections were performed symmetrically on the two hemispheres of the prefrontal cortex of the mouse brain. After all injections were accomplished, the mouse brain was removed from the device and preserved in formalin solution. For observation, the mouse brain was carefully sliced on a microtome, throughout the anterior-posterior range used for the cannula placements, and imaged using the microscope.

IV. Results and Discussion

A. Characterization of Microflow Control

We first characterized the efficacy of the check valve for coupling the drug dispensing and delivery channels. Measurement of the forward and reverse flow rates through the check valve as a function of applied pressure showed that the reverse flow rate was smaller than 0.2 $\mu\text{L}/\text{min}$ over a wide range of reverse pressures and became smaller than 0.05 $\mu\text{L}/\text{min}$ at pressures higher than 30 kPa. This demonstrates minimal leakage through the check valve, which is attributed to the as-fabricated in-contact configuration of the compliant flap and stiff stopper [23]. In contrast, the forward flow rate increased from 2 to 16 $\mu\text{L}/\text{min}$ as the pressure increased from 5 to 30 kPa. The forward flow resistance of the check valve is mainly determined by the widening of the gap between the flap and the stopper. While the forward flow resistance generally decreased with pressure in the full range, we noticed that under driving pressures ranging 0–40 kPa, the forward flow rate increased almost linearly with pressure, showing a relatively stable flow resistance of 1.9 kPa/($\mu\text{L}/\text{min}$). This property enables the accurate control of the drug dispensing stage.

We then characterized the drug dispensing and delivery stages by varying the amplitude and duration of the driving pressure pulse. The dispensed volume during the drug dispensing stage is shown in Fig. 6 as a function of dispensing pulse duration at varying pneumatic pressure amplitudes of 7, 12, 17, 22, and 27 kPa, in which error bars represent the standard deviation computed from five measurements. As we can see from these results, at a fixed pressure amplitude and as the pressure pulse duration varied from 100 to 250 ms, the dispensed volume increased linearly, reflecting that the time response of the check valve was sufficiently fast for controlling the drug flow from the dispensing channel to the delivery channel. Additionally, there is a highly linear dependence of the dispensed volume on pneumatic pressure amplitude in the range of 5–30 kPa, which can be explained by the relatively stable flow resistance within this pressure range as mentioned above. Throughout the characterization of the dispensing stage, we observed no appreciable flow through the outlet of the cannula, indicating that the pressure in the delivery channel induced by the dispensing pressure pulses was insignificant. These results were used as a basis to choose the amplitude and duration of pressure pulses (Fig. 6) to produce dispensed volumes in the 5–70 nL range.

We also characterized the drug delivery stage with varying pneumatic pressure pulses. As the dispensing channel is shut off by the coupling check valve in this stage, the total delivered volume of drug and buffer solution is solely controlled by the pneumatic pressure pulse applied to the delivery channel. Measurement of the total delivered volume as a function of pressure amplitude at pulse durations of 50 and 65 ms is shown in Fig. 7, where error bars again represent standard deviations from five measurements. Note that this volume is larger than the dispensed drug volume because the buffer before and immediately after the drug plug was also ejected from the cannula. A linear dependence of the total delivered volume on pressure amplitude can be seen, demonstrating a constant flow resistance of approximately 0.14 kPa/(μ L/min) in the delivery channel. These results demonstrate that drug delivery in this device can be readily accomplished within pressure pulses as short as 50 ms in duration. The duration of delivery pulses is mainly limited by the response time of the electromagnetic valve (nominal on/off response time: 8 ms), and the communication time between the computer and the power supply.

B. Characterization of Drug Delivery

The characterization of drug delivery in this device began with an investigation of the unintended drug release by diffusion from the dispensing channel to the delivery channel. With red ink filling the dispensing channel for a given time duration ranging from 15 s to 3 h, the mean color intensity in the delivery channel over an observation region near the exit of the coupling check valve was obtained, from which the background, i.e., the mean color intensity when DI water filled in the dispensing and delivery channels, was subtracted. This diffusive leakage color intensity was then normalized with respect to the mean color intensity, also corrected with background subtraction, in the ink-filled dispensing channel (Fig. 8). It can be seen that diffusive leakage in the device was insignificant over experimentally relevant time durations. For example, over a time period of 5 min, or 60 times the 5-s duration of each dispensing and delivery cycle, the normalized diffusive leakage color intensity was less than 4%, indicating an estimated dye concentration decrease of also less than 4%. An analysis of the diffusion process involved indicated that the leakage path could be represented equivalently by a microchannel with a uniform cross-sectional area of $0.6 \times 250 \mu\text{m}^2$ and a length of $50 \mu\text{m}$, assuming the dye diffusivity to be $1 \times 10^{-9} \text{ m}^2/\text{s}$.

We then investigated the reverse flow through the check valve during the delivery stage, which would dilute the subsequently dispensed solution. The region in the dispensing channel near the coupling check valve was observed, with red ink and DI water again filling

the dispensing channel and delivery channel, respectively. We measured the mean color intensity over this region as a function of delivery pressure amplitude (pulse duration fixed at 50 ms) at time durations of 0 s and 1 min, and again normalized the intensity with respect to the ink-filled dispensing channel after background subtraction (Fig. 9). As shown in Fig. 9, the normalized mean color intensity had a change of less than 2% upon delivery pulse and recovered within 1 min, indicating that the dye concentration in the dispensing channel was slightly diluted (also by less than 2%) by the reverse DI water flow. This dilution due to reverse flow decreased as delivery pressure increased, due to the check valve's larger diodicity at higher reverse pressures [23]. Thus, it was concluded that the reverse flow through the check valve during the delivery stage was negligible for practical purposes.

Drug delivery in the device was performed with multiple cycles of dispensing and delivery using pressure pulses 24 kPa in amplitude. Each cycle was 5 s long with a 1-s separation between the dispensing and delivery pressure pulses (Fig. 10). The pressure pulse duration was 150 ms for dispensing and 50 ms for delivery, with the resulting dispensed red ink solution having a volume of 30 nL. Corresponding to this pressure waveform, each cycle started with the delivery channel filled with DI water and the dispensing channel with red ink and ended with the completion of drug ejection from the device. From images of a typical drug dispensing and delivery cycle (Fig. 11), no red ink in the delivery channel was observed until the start of the dispensing stage ($t_1 = 3.80$ s). During this stage, 30 nL of red ink was dispensed into the delivery channel within 150 ms ($t_2 = 3.95$ s), forming a measurable plug approximately 800 μm long ($t_3 = 4.00$ s). Next, the delivery stage started ($t_4 = 4.95$ s) with a pneumatic pressure pulse applied to the delivery channel, and ended ($t_5 = 5.00$ s) with the ejection of the drug plug. The small amount of dye visible at ($t_5 = 5.00$ s) would be swept away and ejected by the residual flow that existed briefly after the pressure pulse vanished. Thus, the delivery channel was entirely clear for the next drug dispensing and delivery cycle ($t_6 = 5.01$ s). Again, throughout the drug dispensing and delivery process, no significant diffusive leakage was observed.

We further characterized the repeatability of the dispensed volume in multiple cycles of drug dispensing and delivery. The dispensed volumes of 50 consecutive drug dispensing and delivery cycles are shown in Fig. 12. The standard deviation of dispensed volume with a target volume of 30 nL was smaller than ± 1.8 nL, or repeatable within $\pm 6\%$. This repeatability is considered adequate for practical applications [7], and can be attributed to the capability of the coupling check valve to function reliably with a consistent flow resistance and time response, which is critical to ensuring a nearly constant dispensed volume and minimizing the interference between consecutive dispensing and delivery cycles. Although the total volume of dye solution and accompanying DI water delivered (and thus the concentration of dye solution) may vary slightly, the total amount of dye to be delivered, given by the dispensed volume, still remains consistent (Fig. 12) during the multiple-cycle drug delivery process.

C. Neurobiological Drug Delivery in Intact Mouse Brain Tissue

Drug delivery into intact mouse brain tissue using this microfluidic device was further performed to demonstrate the potential for neurobiological studies. By adjusting the pneumatic actuation parameters based on the characterization results above, drug volumes in the 5–70 nL range were delivered into mouse brain tissue samples with pressure pulses with a duration down to 50 ms. Fig. 13 shows a representative image of a slice (thickness: 40 μm) of prefrontal cortex in a mouse brain with two spots that had green dye injected symmetrically. The dispensed volume of green dye solution was 30 nL, and the delivery was accomplished in a single time pulse of 50 ms using a pneumatic pressure of 24 kPa. This resulted in a total delivered volume of approximately 125 nL, which is considered acceptable to neurobiological experiments. It can be seen that the multi-cycle drug delivery

in the mouse brain tissue was repeatable in the sense that the two spots were almost identical in size and dye intensity. Each spot had a typical area of less than 1 mm^2 , as appropriate for neurobiological studies on the prefrontal cortex. Also, the cannula insertion created a noticeable injection path in the tissue (not visible in Fig. 13) that indicated the accuracy of the drug delivery target zone. Notably, we observed virtually no green dye along this injection path, suggesting that the injected green dye solution was delivered to the target zone with negligible leakage. It is also interesting to note that while this test has aimed at transcranial delivery of neurobiological drugs, the device can be potentially extended to transdermal drug delivery applications.

V. Conclusion

This paper has reported a microfluidic approach to pulsatile transcranial drug delivery for neurobiological studies. Aimed at addressing the spatial and temporal requirements for neurobiological drug delivery, our approach is based on a two-stage principle consisting of drug dispensing, in which a small amount of drug is metered, and drug delivery, in which the measured drug is delivered within a brief time frame. This approach was demonstrated by a proof-of-concept device consisting of a dispensing channel and a delivery channel that are coupled by a compliance-based check valve. The check valve features an in-channel flap perpendicular to a stiff stopper, which are in contact as-fabricated, to regulate a unidirectional flow with minimized leakage. This device is fully planar in that all microfluidic components are integrated in a single layer for simple fabrication and integration with other functional elements. In a demonstrative driving scheme, brief pressure pulses are generated by pneumatic lines as dictated by a waveform-controlled pressure regulator and a high-speed electromagnetic valve.

We have systematically characterized the device in terms of the dispensed and delivered volumes of drug solution under varying pneumatic driving pressures and pulse durations. Drug volumes from 5 to 70 nL were metered and delivered within time pulses as brief as 50 ms, with negligible diffusive leakage and reverse flow through the check valve over an experimentally relevant time scale. Further characterization of this device showed that the dispensed drug volume was repeatable within $\pm 6\%$ for 50 consecutive drug dispensing and delivery cycles. Delivery of a dye as a model drug into mouse brain tissue has demonstrated that this approach can potentially lead to a tool that offers functionalities important to transcranial and transdermal neurobiological drug delivery and thus is of general utility to neurobiological studies.

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Biographies

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Fig. 1. Schematic of the microfluidic drug delivery device consisting of two microchannels coupled by a check valve.

Fig. 2. Device operation: the device is in (a) standby, (b) drug dispensing stage, and (c) drug delivery stage.

Fig. 3. Device fabrication process. A PDMS sheet defining the microfluidic geometry is bonded between two glass plates.

Fig. 4. Images of (a) a fabricated PDMS fluidic layer, and (b) a packaged microfluidic drug delivery device.

Fig. 5. Experimental setup. (a) Testing of microfluidic drug delivery by pneumatic pressure pulses. (b) Measurement of the delivered volume of drug solution by ejection of the solution from the microfluidic device onto a polyethylene sheet.

Fig. 6. Dispensed volume as a function of the dispensing pulse duration (pneumatic pressure pulse amplitude: 7, 12, 17, 22, and 27 kPa).

Fig. 7. Delivered solution volume as a function of the pressure pulse amplitude (pulse duration: 50 and 65 ms).

Fig. 8.
Normalized mean color intensity of red ink that diffused through the closed check valve into the delivery channel from the dispensing channel.

Fig. 9. Normalized mean color intensity of red ink in the dispensing channel when a 50-ms delivery pressure pulse with varying amplitude was applied. Data were taken at time durations of 0 s (initially) and 1 min.

Fig. 10.

Pressure pulse waveform for a drug dispensing and delivery cycle of duration T , starting at time t_0 and ending at time t_6 . The dispensing and delivery pulses corresponded to the time intervals (t_1, t_2) and (t_4, t_5) , respectively. (For clarity, the time axis is not drawn to scale).

Fig. 11. Images of the delivery channel and check valve (the flap and a small portion of the stopper) at different times during a typical drug dispensing and delivery cycle (Fig. 10). The dispensed volume was 30 nL.

Fig. 12.
Dispensed volumes from 50 consecutive drug dispensing and delivery cycles.

Fig. 13.

Micrograph of a slice of mouse brain tissue (thickness: $40\ \mu\text{m}$) into which a green dye was delivered using the microfluidic device (green dye solution volume: 30 nL, and delivery pulse duration: 50 ms).