A modular diffusion barrier based on phase separation for localized delivery of discrete drug volumes in aqueous environments

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We present a new tool for the precisely controlled transfer of individual picoliter (pL) droplets in the range of 150–950 pL at user defined local positions within aqueous liquid environments while avoiding any leakage by diffusion. This is achieved by a low-cost, disposable and biocompatible cap that can be placed on top of any pL-dispenser and generates a phase-gap between dispensing agent and target liquid when the dispenser is dipped into the latter. We developed two different working modes: (i) the standard mode enables an instant injection ($\ll$ 1 ms) of the droplet into the liquid environment and (ii) the focus mode further increases the spatial resolution from 100 μm to 50 μm at the cost of slowing down the injection time. For the phase-gap we have proven an excellent long-term stability of more than 30 hours against capillary priming.

Introduction

Modern techniques in the field of pharmaceutical or biological applications are based on highly targeted delivery of drugs or biochemical agents.[1] In biological studies, the controlled drug release can be used to locally deliver precise concentrations of biochemicals to a tissue or cells to observe their response.[2,3] A focus of interest is the chemical stimulation of neuronal cells to mimic a synapse since neurons are naturally stimulated by biochemical agents (neurotransmitters).[4,5] This way, a highly specific stimulus could be obtained. The following requirements have to be achieved to realize this[6] (1) delivery of biochemical agents in very precise and defined quantities, (2) at well defined locations, (3) with a high temporal resolution and (4) a temporal control of the release profile. Since diffusion is one of the dominant transport phenomena in microfluidic systems and cells have to be cultured in an aqueous, physiological environment to ensure their survival, uncontrolled diffusion of the drugs in the physiological environment has to be prevented.

Various approaches for the delivery of biochemical agents and for the generation of defined biochemical gradients in microfluidic devices have been presented.[7,8] Most works are mainly based on two basic approaches; multilaminar flow[9,10,11,12] and pulsed release.[13,14,15] Using multilaminar flow large quantities of biochemical agents are disposed and thus increasing the costs extremely. Using a drop on demand approach for the discrete and repeatable release of defined liquid volumes at user defined positions combines less sample consumption with a high temporal and spatial resolution. Concerning microfluidic devices for pulsed drug release *in-vitro* two basic setups are possible. The agents are released through apertures in the bottom of customized cell culture dishes.[13,14,15] Furthermore, a commercially available drop on demand dispenser[16,17,18] could be positioned above a liquid surface to inject individual droplets into the target liquid. Using this configuration, the released droplet could only be injected a few micrometers under the liquid surface. Both methods based on pulsed drug release show a sufficient localized drug release as well as a good temporal resolution. Nevertheless, the drug has to diffuse from the release site to the cell leading to a temporal delay of the drug release, a loss in spatial resolution as well as a distance dependent variation of maximum drug concentration.

In this paper we present a flexible approach for the precisely controlled drop on demand delivery of biochemical solutions in discrete liquid plugs within aqueous liquid environments. This is achieved by dipping a drop on demand dispenser into the liquid environment and avoiding diffusion based leakage by a modular diffusion barrier ("phase-gap"). This phase-gap can be adapted to any drop on demand dispenser. It enables localized drug release at any region of interest within liquid environments (*e.g.* in the neighborhood of an individual cell).

Modular dispensing system

The phase-gap is generated by a disposable cap made of hydrophobic polydimethylsiloxane (PDMS). The cap contains a cavity adapted to the geometry of the dispenser tip and a hydrophobic chamber. Due to the geometrical adaptation the cap could easily be applied to our NanoJet dispenser and is aligned by its geometry (Fig. 1).

The NanoJet[19] is a single channel dispenser based on a piezo-stack actuated direct volume displacement. The dispenser features a large working volume range from V = 150–950 pL suitable for cell applications with a very high linearity between the deflection of the piezo-stack and the dispensed volume (R^2 = 0.995) as well as an excellent reproducibility of the dispensed droplet volumes ($CV \leq 3.5\%$) (Fig. 2). In Fig. 2 each

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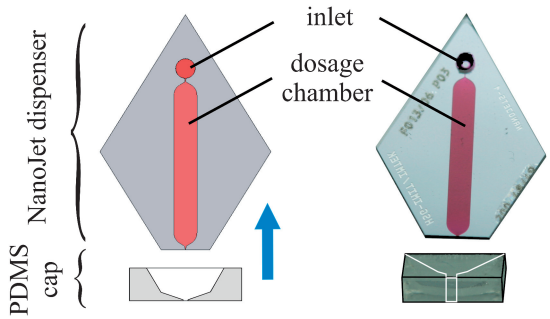

Fig. 1 Concept of the modular dispensing system consisting of a disposable PDMS cap which is easily applied and self-aligned to the tip of the NanoJet dispenser.

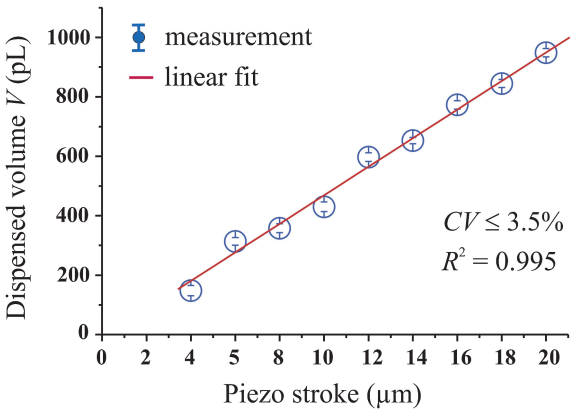

Fig. 2 The piezo-stack actuated NanoJet dispenser spans a large working range (V = 150–950 pL) with an excellent reproducibility of $CV \leq 3.5\%$ and shows a good linear increase ($R^2 = 0.995$) of the dispensed droplet volume in respect to the piezo-stroke.

measurement point presents the mean value and standard deviation of 50 measurement cycles derived from gravimetrical measurements (*microbalance SC2, Sartorius AG*). Because of the low mass of a single droplet, 50 droplets were averaged per measurement cycle.

When the modular dispensing system is dipped into the liquid environment, the air in the hydrophobic chamber is trapped and two stable menisci at the nozzle and the phase-gap outlet are formed. This way, the phase-gap is created separating the dispensing agent and the target liquid (Fig. 3). It works as a reversible burst valve whereas only ejected droplets with sufficient kinetic energy to overcome the surface tension can cross the phase-gap and reach the target liquid. Any leakage by diffusion is efficiently prevented.

Two working modes of the phase-gap

We developed two different setups primarily optimized for increased temporal resolution (standard mode) or spatial resolution (focus mode) (Fig. 4). The standard mode is characterized by an outlet width w larger than the droplet diameter d ($d < w$). Due to its high kinetic energy, the ejected droplet passes the phase-gap, impinges on the liquid surface and is directly injected into the aqueous solution. In this respect, the focus mode is

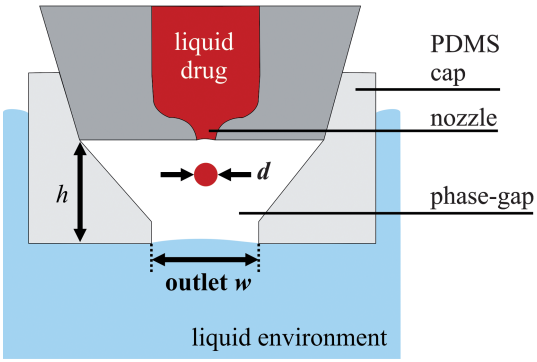

Fig. 3 Working principle of the phase-gap. By inserting the modular dispensing system into a liquid environment, a phase-gap, *i.e.* an air-trapping hydrophobic chamber (chamber height h) with two stable menisci is formed preventing diffusion based leakage. Only ejected droplets with sufficient kinetic energy can overcome the phase-gap.

characterized by an outlet width smaller than the droplet diameter ($d > w$). The ejected droplet impinges on the PDMS cap. Since PDMS is highly hydrophobic the ejected droplet will not stick on the PDMS. Due to the conical shape of the phase-gap in combination with the kinetic energy of the droplet, the droplet will slide down along the wall of the phase-gap towards the outlet. As soon as the droplet gets in contact with the liquid environment it is released *via* capillary forces and diffusion. This approach results in a spatially focused drug release according to the smaller outlet diameter.

The two working modes of the phase-gap concept were investigated by observing the release of ink dye by a high speed camera (*Fastcam PCI-R2, Photron Inc.*). In both cases droplets of $V = 500$ pL volume with a corresponding diameter of $d = 100$ µm were ejected at $t = t_0$. The phase-gap widths were either larger ($w = 500$ µm) or smaller ($w = 50$ µm) than the droplet diameter. For outlet widths larger than the droplet diameter ($d < w$), the whole drug volume is immediately injected into the liquid environment, making the initial drug concentration c_0 available in a time frame $\Delta t \ll 1$ ms (limited frame rate of high speed camera) (Fig. 4A). The penetration depth of the droplet was observed to be 100 µm while the spherical shape is maintained. After injection the dye is distributed by 3-dimensional diffusion. Thus, the concentration profile could precisely be controlled in space and time according to the diffusion constant of the released agent. This setup could be useful for neural stimulation where highly dynamic neural activity is initiated. If the application demands higher spatial resolution, the focus mode characterized by ($d > w$) can be used (Fig. 4B). In our example, the droplet is spatially focused by a factor of two compared to the standard mode, while a temporal delay of the drug release is observed. Due to the diffusion of the dispensed dye out of the outlet the concentration continuously increases and reaches a maximum concentration after 200 ms. The dye diffuses while the concentration remains constant until the whole droplet volume is released into the liquid environment. Like in the presented methods so far,[13,14,15] the delayed release profile makes the temporal and spatial prediction of the concentration profile more complicated compared to the standard mode. The spatial- and time-resolved concentration profiles in Fig. 4 results

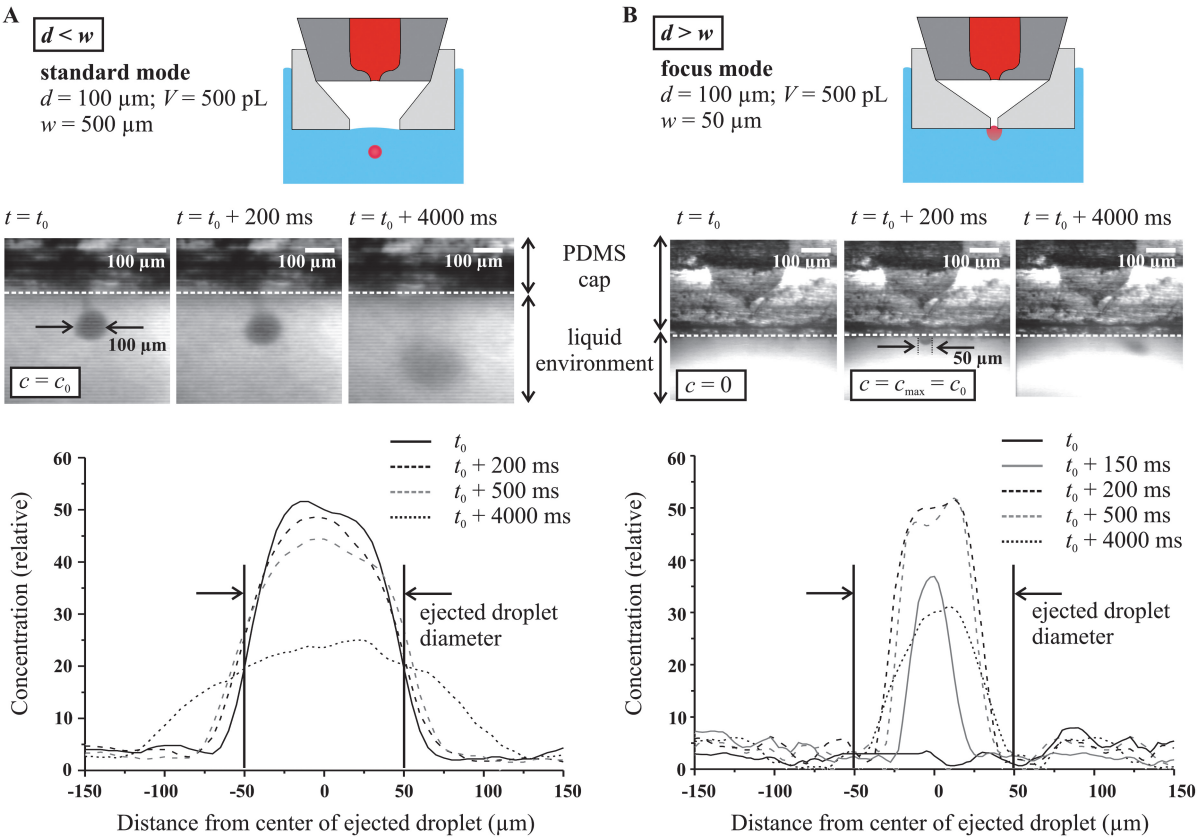

Fig. 4 The two working modes of the phase-gap concept presented with two characteristic examples. The graphs show the time-resolved concentration profile (inverted grey level) of the released dye according to the presented images (scale bar 100 µm). (A) Standard mode enables an instant injection of the droplet into the liquid environment. (B) Focus mode is further increasing the spatial resolution to the outlet width of 50 mm.

from inverted grey level values of the released dye based on the recorded images using the open source software *ImageJ*.

To demonstrate the functionality the dispensing system was positioned within the cell media slightly above a dense L929 mouse-fibroblast cell culture. A single droplet (V = 600 pL, d = 105 µm) of saturated Pam3-TAMRA-Lipopeptid (*EMC microcollections GmbH*) solution in DI-water was ejected through the phase-gap onto the cell culture and starts diffusing. The fluorescence signal of the labeled cells was investigated using an inverse fluorescence microscope (*Olympus CKX41*) and a camera setup (*Zeiss AxioCam MRc*). Fig. 5 presents the fluorescence signal of the labeled cells 5 s after the droplet release where no further distribution of the fluorescence signal was observed.

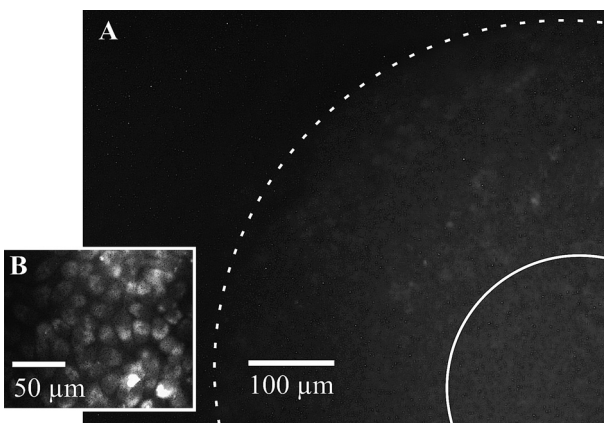

Fig. 5 Fluorescence image of L929 cells labeled with Pam3-TAMRA-Lipopeptide 5 s after release of a 600 pL droplet. (A) Image of the concentration gradient (— droplet release area, --- effective drug area). (B) Enlargement of the diffusion gradient across individual cells.

Fabrication process

To ensure a flexible fabrication process, a silicon master featuring the geometry of the dispenser tip and the phase-gap was fabricated *via* dry etching and fixed without any additional surface treatment in a drilled aluminium mold. The mold was filled with degassed PDMS (*Sylgard 184*, mixing ratio 1 : 10). A 1 mm thick polymethylmethacrylate (PMMA) plate, which can easily be separated from cured PDMS was put carefully onto the Sylgard 184 compound. During curing of the PDMS by T = 70 °C for t = 90 min a force of F = 95 N was applied to generate defined outlets of the phase-gap (Fig. 6).

Afterwards, the PDMS cap was peeled off the mold and the silicon master is simply unplugged. This way, modular PDMS caps are fabricated in a cost-efficient manner. Outlet widths down to w = 50 µm were realized very precisely (Δw < 2 µm), where the length of the outlet is defined by the thickness of the silicon master (Fig. 7A). Since PDMS is highly hydrophobic (measured contact angle θ = 109°), no further surface treatment

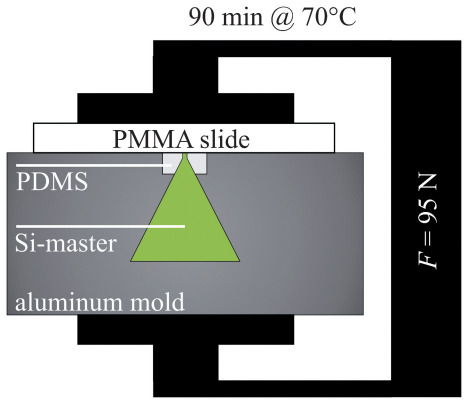

Fig. 6 The flexible fabrication process based on silicon master featuring the geometry of the dispenser and the phase-gap. The master is inserted into a mold form, cast into PDMS (Sylgard 184) and cured by applying a pressure of $F = 95$ N.

is required to ensure the stability of the phase-gap. Furthermore, PDMS shows a sufficient biocompatibility for microfluidic applications in the field of life science.[20,21] According to the characteristics of the NanoJet dispenser, a minimum height of the hydrophobic chamber of $h = 300$ µm is needed to ensure a droplet formation. An impact for larger heights (investigated up to 1 mm) concerning the functionality of the phase-gap principle or the NanoJet dispenser was not observed. Due to the modular nature of the PDMS cap and the flexible fabrication process, this phase-gap technology can easily be applied to other dispensers, just by adapting the geometry of the silicon mold master. According to the dry etching of the silicon master various outlet geometries *e.g.* rectangles or circles could be realized whereas no effect of the working principle of the phase-gap was observed. We already fabricated phase-gap systems for our Pico-Injector[22] dispenser as well as for commercial dispensers, *e.g.* from a microdrop.[16] Furthermore, this fabrication process also offers the possibility to fabricate phase-gaps in an array format adapting to array dispensers like TopSpot®,[23,24] or ink-jet printheads (Fig. 7B).

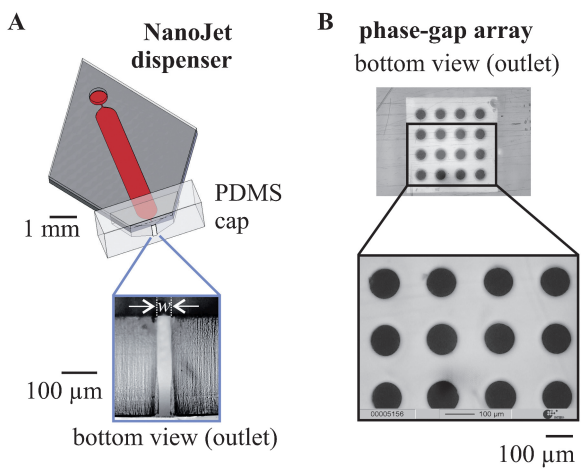

Fig. 7 Fabricated PDMS caps with integrated phase-gaps. (A) Outlet width down to $w = 50$ µm are realized with very good quality ($\triangle w < 2$ µm). (B) PDMS caps with multiple phase-gaps and outlets in an array format.

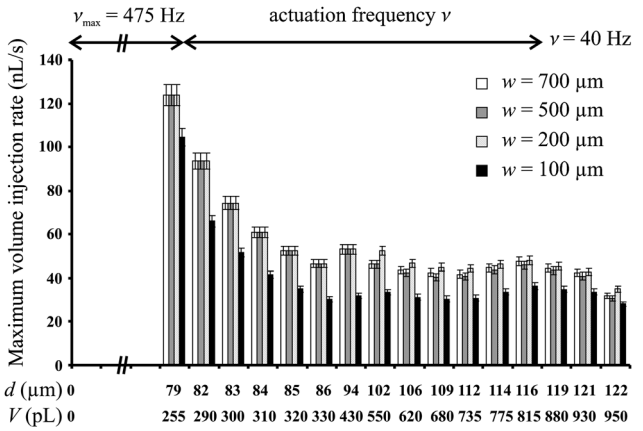

Fig. 8 The system exhibits extremely high maximum volume injection rates where no flooding of the phase-gap is observed. We achieved up to 137 nl/s with a resolution of 250 pL at the maximum actuation frequency of $\nu = 475$ Hz (limited by driving electronics).

Phase-gap investigations

We have proven long-term stability of the phase-gap in liquid environments for more than 30 hours for inactive dispensers. Theoretical investigation[25] of the phase-gap stability derived from the Young–Laplace equation predict a maximum pressure that the hydrophobic chamber can resist which is equivalent to a water column of 24 mm in height (240 Pa) for a outlet width of $w = 700$ µm. These values were also successfully proved by experiments. Additionally different media like cell culture media as well as standard buffers like carbonate buffer or phosphate buffered saline (PBS) were investigated and did not influence the phase-gap stability. Thus, the stability against capillary priming is sufficient for various applications. We also characterized the maximum ejection frequency which is possible without influencing the phase-gap stability for different droplet diameters d and phase-gap outlets w. The maximum dispensing frequency ν before flooding of the phase-gap occurs was documented and the maximum injection rate is calculated ($\nu \times V$) (Fig. 8). For droplet volumes V up to 255 pL no flooding occurs even by actuation with the maximum dispensing frequency of $\nu = 475$ Hz (limit of driving electronics). We achieved maximum ejection rates up to 137 nL/s. If the droplet volume increases the actuation frequency has to be reduced to avoid flooding of the phase-gap resulting in a decreasing volume injection rate. Since the diameter of droplets in the picoliter range are much smaller than 200 µm the droplets are directly injected into the liquid environment for outlets ≥ 200 µm (standard mode). Therefore, no influence for larger outlet widths (200 µm–700 µm) concerning the performance of the phase-gap was observed. The temporal delay of the drug release using the focus mode leads to reduced flow rates compared to the standard mode. Nevertheless, the phase-gap exhibits an extremely high stability concerning possible ejection rates while the achieved rates extend typical values used for chemical stimulation of cells (< 0.1 nL/s)[13,15] by far.

Conclusions

We successfully presented a new method for temporal and localized discrete chemical release in liquid environments. Using

a hydrophobic air chamber as a reversible burst valve and combining it with a drop-on-demand dispenser, this method can discretely release picoliter volumes of chemicals within aqueous liquid environments. Significances of this technology include versatile controllability, leakage free operation, high resolution, and fast response. The functionality of this method was presented using a modular system which is simple to handle. The flexible fabrication process applies this technology to a wide range of dispensers and applications. Thus, the presented approach is promising to be a key technology for the controlled delivery of drugs and neurotransmitters to muscle cells or neurons in pharmacological research, or for generation of chemical microgradients in cell cultures and tissue regrowth. By assembling array based drug release systems stimulation of complex cell cultures like retina tissues could also be possible. In addition to *in vitro* applications, *in vivo* approaches for acute drug delivery could also be possible.

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17 GeSiM Gesellschaft fuer Silizium-Mikrosysteme mbH, www.gesim.com.
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