Droplet on demand system utilizing a computer controlled microvalve integrated into a stiff polymeric microfluidic device†

Krzysztof Churski, Jacek Michalski and Piotr Garstecki*

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An integrated microvalve fabricated in a stiff polymeric (polycarbonate) device allows for formation of droplets and bubbles of arbitrary volumes and at arbitrary times of emission. Predesigned protocols of formation of sequences of volumes, intervals between the droplets and their chemical compositions can be practically and reproducibly realised and controlled from a personal computer. The design of the microvalve enables its utilization in chips made in a range of other materials, including plastics and glass and in devices operated both with positive and negative pressure providing a useful module for a range of applications of microfluidic droplet systems.

Introduction

We demonstrate a microfluidic ‘droplet on demand’ system that produces droplets of arbitrary volumes and at arbitrary times of emission. The operation of our device rests on a modular valve of our design that was inspired by the work of Grover et al. Our integrated membrane microvalve has the following features: (i) it is pneumatically controlled with the use of off-the-shelf electro-valves, (ii) it has an interchangeable membrane that can be resistant to the chemistry of use, (iii) it operates successfully in stiff polymeric devices, (iv) it can withstand pressures up to 2 bar, (v) it has a dead volume of less than 10 nL, and (vi) it can switch closed–open–closed in 48 ms yielding frequencies of formation of droplets above 20 Hz. Further, we demonstrate that it is feasible to integrate several such valves onto a single microfluidic chip allowing for formation of predesigned sequences of droplets of different chemical composition.

The advent of droplet microfluidics almost immediately inspired the vision of automated microfluidic chips to perform reactions within (~1 nL) droplets and provide a versatile platform for combinatorial chemical analyses and syntheses to be performed (i) fast, (ii) on small samples of reagents, and (iii) with reliable statistics of the results. This vision is already in part realized in the myriad of demonstrations of chemical and biochemical reactions performed in droplets (droplets).

Performing thousands of different reactions on a single chip will certainly require computer controlled execution of the pre-encoded protocols. This execution necessitates the development of computer controlled modules for sorting (guiding) droplets and—above all—for introducing droplets of predesigned volumes at scheduled times of emission—a technique often called ‘droplet on demand’.

Formation of droplets and bubbles in microfluidic systems at constant forcing (either constant pressure or constant rate of flow) applied to the inlets of the immiscible streams is well understood. The two most commonly used configurations include the T-junction and flow-focusing geometries. The T-junction was first introduced by Thorsen et al., with the mechanism of formation of droplets and bubbles detailed by Garstecki et al., Colin et al., De Menech and Anna et al. The flow focusing geometry was pioneered in an axi-symmetric configuration by Ganan-Calvo and applied to planar microfluidic devices by Anna et al. Droplet systems have found a number of uses, including systems for chemical processing and, for example, centrifugal systems for generation of emulsions. For planar microfluidic chips, the mechanisms of formation of bubbles were characterized by Garstecki et al. and Marmottant and formation of droplets by Kumacheva et al. and Anna et al. In short, in the typical operation of the device at low values of capillary numbers (Ca ≪ 10−2) the process of break-up is dominated by interfacial effects: the growing droplet or bubble obstructs the cross-section of the channel in order to minimize its surface area. This leads to an effective blocking of the flow of the
continuous fluid and to an increase of pressure upstream of the growing bubble/droplet. This in turn leads to ‘squeezing’ of the neck between the inlet stream of the fluid-to-be-dispersed and the growing droplet at a rate that is proportional to the rate of flow of the continuous phase.\textsuperscript{17} This mechanism results in scaling of the volume of the bubbles or droplets V that includes only the ratio of the averaged rates of flow of the two immiscible phases.\textsuperscript{11,17} At higher rates of flow (higher values of Ca) the shear stresses exerted by the continuous fluid on the growing droplet introduce additional effects and a dependence of the volume of the droplets on the value of the capillary number.\textsuperscript{13,14} All the summarized techniques provide for good control of the volume of the droplets and the frequencies of their emission. They are also the basis for construction of droplet on demand systems.

The essential component of a droplet-on-demand system is a valve: formation of droplets on command requires that the flow of the fluid-to-be-dispersed is stopped over arbitrarily long spans of time and can be switched on with an external signal. Analogously to the classification of conventional hydraulic valves that can be either ‘normally open’ or ‘normally closed’, there are two ways to construct a DOD system: one is to actively force the flow of fluid with external stimuli at predefined instants, while normally the fluid rests. Second is to use systems that normally (without the temporally modulated stimuli) allow for flow, and have to be actively stopped. There are several types of forces that can be used to force or stop the flow: electric (dielectrophoresis or a direct electrostatic force on a charged interface), magnetic, capillary, electrolytotic, Marangoni (e.g. thermocapillary flow) or mechanical (i.e. with the use of valves).

For example, Jones et al.\textsuperscript{21} demonstrated a system with two coplanar and parallel electrodes that allowed for dragging of liquid with the use of dielectrophoresis, and demonstrated control of generation of droplets with pulses of an alternating electric field.\textsuperscript{22} Malloggi et al.\textsuperscript{23} utilized electrolytotic to form single droplets. The major drawback of this technique lies in typically extended times of generation of droplets (e.g. 500 ms).

He et al.\textsuperscript{24} applied an electric field along the axis of formation of water droplets in oil (between the continuous stream of water upstream of the droplet generator and the outlet stream downstream of the junction). They demonstrated that short (e.g. 10 ms) pulses of applied potential (e.g. 800 V) can generate a Taylor cone and formation of droplets via an electro-hydrodynamic mechanism.\textsuperscript{25} The droplets were typically polydisperse (3–25 μm) and it was difficult to produce single droplets. Also Weitz et al.\textsuperscript{26} demonstrated control over volumes and frequencies of formation of droplets with the use of an electric field.

Thermocapillarity can also be used to control the flow of immiscible phases: Baroud et al.\textsuperscript{27} demonstrated control over formation of droplets and guiding them with a laser beam.\textsuperscript{27} Conceptually—although not necessarily fabrication-wise—the simplest approach to microvalving is to construct micro-mechanical valves. The two most popular types of microvalves, both utilizing the deformation of elastic membranes in soft (PDMS) systems,\textsuperscript{28} were proposed by Quake et al. and, in rigid (glass) chips,\textsuperscript{3} by Grover et al.

Although it might be surprising when one considers the common availability of droplet on demand technology in ink jet printers, there is only a handful of reports on microfluidic DOD systems. Prakash and Gershenfeld\textsuperscript{8} utilized a DOD system based on a thermocapillary effect in their droplet logic systems. In their design the inflow of the fluid-to-be-dispersed into the flow focusing junction was blocked by Laplace pressure. A Pt thin film heater (200 nm) placed under the junction lowered the interfacial tension and allowed the stream of gas to enter the junction to create a single bubble for each 100 ms pulse of current. Attinger et al.\textsuperscript{29} also used the Laplace blocking and a piezoelectric actuator glued to a PDMS reservoir of the fluid-to-be-dispersed to force its flow through the flow focusing junction. This system generated uniform droplets of average volume of ~1 nL at 6.2 Hz; at larger frequencies, however, the droplets were polydisperse. Bransky et al.\textsuperscript{30} reported the same concept but with the piezoelectric actuator integrated in the PDMS device, with slightly better results. The main disadvantage of these reports is the strongly limited range of pressure that can be applied to the discontinuous phase, as it cannot be larger than the Laplace pressure (of the order of ~10 mbar).

Recently and independently Lin and Su\textsuperscript{31} and Wang et al.\textsuperscript{32} demonstrated fully operational DOD systems based on pneumatically actuated valves\textsuperscript{28} made in multilayer PDMS devices. Both demonstrations included the control of the volumes of the droplets (~1 nL and above) and of the times of their emission with frequencies approaching 15 Hz. The only drawback of these demonstrations lies in that they rely on the elastic properties of the material (PDMS) from which the device is made, and PDMS is known\textsuperscript{33} not to be compatible with a wide range of solvents.

Here we report the first demonstration of a DOD system in a stiff polymeric device based on an integrated microvalve.\textsuperscript{1} In the following sections we present the details of fabrication, characterization of the flow focusing and T-junction DOD and BOD (bubble on demand) systems and demonstrate formation of (i) heterogeneous sequences of drops of same and (ii) different chemical content, (iii) synchronization of four DOD lines, and (iv) operation of the system in the suction mode allowing for DOD formation from small samples of fluids.

**Materials and methods**

We fabricated the chips via direct milling in polycarbonate sheets (Macrolane, Bayer, Germany) of various thicknesses using a CNC milling machine (MSG4025, Ergwind, Poland). The CNC machine has a reproducibility of positioning of 5 μm, and allows for using milling bits as small as 50 μm in diameter.

Fig. 1a shows a reproduction of the experimental setup—the microchip with integrated microvalve connected to two electromagnetic valves, EM$_{\text{HIGH}}$ and EM$_{\text{LOW}}$. EM$_{\text{HIGH}}$ controls high pressure (usually 1 bar) which we use to close the microvalve. EM$_{\text{LOW}}$ controls the low pressure (usually vacuum or atmospheric pressure) that opens the microvalve.

We constructed the microvalve from three polycarbonate slabs: bottom of thickness 1.5 mm with microchannels of cross-section of 200 × 200 μm, middle of thickness 0.25 mm with two circular through-holes of diameter 200 μm and an upper plate of thickness 5 mm with a large hole for a polycarbonate cork. The cork has a circular hole for tubing of diameter 1.21 mm on one side while on the other side it has an elliptic hole (200 μm by 600 μm). After registering the three polycarbonate plates we gently stretch a nitrile membrane over the hole for the cork and push the cork down. In this simple manner we obtain a system with an
First the press was set to a temperature \( p = \frac{T}{C^1_{14}} \) and allowed the plates to bond via diffusion of the polymer chains for 15 min. This procedure typically results in good bonding of polycarbonate to polycarbonate. We found that the valving chuck, in spite of the fact that it was separated from the rest of the device with a nitrile membrane, was also firmly positioned in its place. After gluing in the tubing (PE60, Becton and Dickinson, USA) the device was ready to use.

We used three variants of the DOD system (Fig. 2): (i) a T-junction system, (ii) a flow-focusing system, and (iii) a double T-junction system that forms droplets of two different chemical compositions into one channel. All designs were prepared in MasterCam, translated into CNC code and used to program the milling machine.

We performed two sets of experiments: (i) formation of aqueous droplets in 2% (w/w) solution of Span 80 (Sigma Aldrich) in hexadecane (Alfa Aesar) in a T-junction, and (ii) formation of bubbles of nitrogen (technical grade, Linde Gas) in a 2% (w/w) solution of Tween 20 (Sigma) in water, in a flow-focusing geometry.

The continuous phases (either hexadecane or water) were supplied to the chip at a constant rate of flow from a syringe pump (Harvard Apparatus PHD2000). In the first experiment, water was supplied from a pressurized tank (custom made). In the second experiment, nitrogen was supplied directly from a pressurized tank through a pressure reduction valve.

The integrated valves were controlled with compressed air via a pneumatic installation involving two standard electromagnetic valves (V165, Sirai, Italy) controlling the connection of the integrated valve to either the container with pressurized air (valve closed) or atmosphere (valve open); see Fig. 1. The operation of the electromagnetic valves was controlled with a computer programmable voltage sequencer (LabSmith, model HVS448 3000D) interfaced with a custom made circuit that transformed logical—low current—signal from the sequencer into the high current (150 mA) signal controlling the electromagnetic valves. We measured that the electromagnetic valves had different lags between the application of the current and the actual events of opening (4 ms) and closing (24 ms). These lags were the main factor limiting the maximum frequency of our devices.

We recorded still micrographs and videos using interchangeably two high speed cameras (monochromatic Photron 1024 and color Photron 1024C). The cameras were mounted either on
a direct microscope (Nikon E200) or on a stereoscope (Nikon SMZ1000). In order to retrieve the lengths of the droplets formed in our systems we used an image analysis script written in MatLab.

Results

The key element of our droplet-on-demand system is the integrated pneumatic microvalve. The valve is schematically depicted in Fig. 1b. A nitrile membrane separates the flat middle plate (with two through-holes that connect to the microfluidic channel controlled by the microvalve) and the chamber milled in the valving chuck. The volume of the chamber is approximately 10 nL, while the actual dead volume of the microvalve depends on the deflection of the membrane. From the observation of the operation of the device we guess that the dead volume of the valve is on the order of single nanolitres. The microvalve interconnects two microfluidic channels—one that supplies the fluid from a pressurized container (kept at pressure \( p_{\text{dispersed}} \) with the dispersed phase being either water or nitrogen depending on the type of the experiment), and a second that guides the fluid to the droplet (or bubble) generator (either a T-junction or a flow-focusing module). The valving chamber is connected via electromagnetic valves (EMV\(_{\text{HIGH}}\) and EMV\(_{\text{LOW}}\)) to two reservoirs of high (maintained at a pressure of \( p_{\text{HIGH}} \)) and low (\( p_{\text{LOW}} = \) atmospheric or vacuum) pressure. All pressures are given with respect to the ambient (atmospheric) pressure \( p_0 \). Applying \( p_{\text{HIGH}} \)—by opening EMV\(_{\text{HIGH}}\) and closing EMV\(_{\text{LOW}}\)—pushes the membrane down against the flat intermediate polycarbonate plate and closes the pneumatic valve. Closing EMV\(_{\text{HIGH}}\) and opening EMV\(_{\text{LOW}}\) results in venting of the upper chamber to the atmosphere or vacuum and in opening of the integrated microvalve.

The first characteristic of a valve is its ability to stop the flow. Fig. 3 illustrates minimal overpressure applied to the valve that is sufficient to stop the flow of water or flow of gas. We observed that in both cases the value of \( p_{\text{HIGH}} \) needed to close the valve increased linearly in \( p_{\text{dispersed}} \). In the case of water \( p_{\text{HIGH}} \) had to be significantly larger than \( p_{\text{dispersed}} \). We associate this observation with the asymptotically slow closure of the valve at the final stages when the liquid has to be drained from the thin gap between the membrane and the flat intermediate plate.

**Formation of droplets in the flow-focusing chip**

We first report a T-junction system that generates droplets of water on demand. A flow-focusing (FF) system (Fig. 2a) comprises the main channel that delivers the aqueous phase, and two perpendicular channels that supply the oil at a constant rate of flow \( Q \). We positioned the valve on the channel that supplies the aqueous phase, 4 millimeters upstream of the junction. We set the protocols for opening the EMVs in such a way (for details of the protocol please consult the supplementary information†) as to obtain (i) simultaneous closure of EMV\(_{\text{HIGH}}\) and opening of EMV\(_{\text{LOW}}\), and (ii) the opposite action of opening EMV\(_{\text{HIGH}}\) and closure of EMV\(_{\text{LOW}}\) after a delay that we call \( t_{\text{open}} \) as it corresponds to the time that the pneumatic microvalve stays open.

We first tested the ability of the system to control the volume of the droplets formed at a constant frequency of \( f = 1 \text{ Hz} \) by varying the value of \( t_{\text{open}} \) for five different values of the pressure \( p_{\text{water}} \) applied to the reservoir of water and for a constant rate of flow of oil \( (Q = 1 \text{ ml h}^{-1}) \). We observed that (i) the volume of the droplets increases linearly with increasing \( t_{\text{open}} \) (Fig. 4), (ii) the rate of this increase was proportional to \( p_{\text{water}} \) (inset in Fig. 4). The droplets were almost perfectly monodisperse with standard deviation of their length well below 5%. The smallest droplets had volume of \(-100 \text{ nL} \). The volume of the droplets depended also on the rate of flow \( Q \) of the oil phase: the larger the value of \( Q \) the smaller the droplets. We associate this effect with a higher value of pressure at the junction at higher values of \( Q \), due to the finite resistance to flow in the outlet channel, and hence a smaller pressure drop between the reservoir of water (maintained at \( p_{\text{water}} \)) and the junction. Interestingly, for a given \( p_{\text{water}} \) and \( t_{\text{open}} \) there is a limiting value of \( Q \) above which the droplets are broken off before the whole portion of the dispersed phase—as administered by the microvalve—is pushed through the junction. This is exemplified in Fig. 5 for \( Q = 6 \text{ ml h}^{-1} \), where two droplets are formed: the volume of the first one is

![Fig. 3](image-url)

**Fig. 3** The graphs show the minimum pressure that needs to be applied to the microvalve in order to close the flow of either water or nitrogen as a function of the pressure applied to the containers with either of the two fluids. One can note that water requires a significant overpressure to be applied to the valve in order to effectively close it.

![Fig. 4](image-url)

**Fig. 4** Quantitative characterization of the process of formation of droplets in a flow-focusing DOD system. The main graph shows the length of the droplets \( L \) (normalized by the width of the channel \( W \)) as a function of the time that the microvalve stayed open for five different values of the pressure \( p_{\text{water}} \) applied to the container with water. The width of the channel was 400 μm. The inset shows the slope of the relation of \( L(t_{\text{open}}) \) as a function of \( p_{\text{water}} \). The micrographs illustrate the droplets formed with the parameters \( t_{\text{open}}, p_{\text{water}} \) marked with the symbols D1 through D3 on the main graph. The droplets were formed at a frequency of 1 Hz and a flow rate of continuous phase of 1 ml h\(^{-1} \), \( p_{\text{HIGH}} = 1 \text{ bar} \), \( p_{\text{LOW}} = -1 \text{ bar} \).
Fig. 5 Dependence of the length ($L$) of the droplets normalized by the width of the channel ($W = 400 \mu m$) on $t_{\text{open}}$ for different values of the rate of flow of the continuous phase ($Q$) in a FF system. We observe linear increase of $L$ with increasing $t_{\text{open}}$. The inset shows that the slope of this dependence decreases with increasing $Q$. At high enough values of $Q$ the droplets are broken before they are completely formed and as a result one droplet of volume (or length) depending only on the value of $Q$ is formed and a second one with a volume increasing linearly with $t_{\text{open}}$. The symbols D1 through D5 mark the parameters corresponding to the droplets shown in the micrographs. The pressure applied to the reservoir with water was set to $p_{\text{water}} = 0.041$ bar, $p_{\text{HIGH}} = 1$ bar, $p_{\text{LOW}} = -1$ bar.

\[ L = \frac{Q}{W} \]  

independent of $t_{\text{open}}$, as its volume is controlled by the ‘natural’ time of breaking off the droplets\(^4\) ($t_{\text{break}} \sim Q/W$), while the volume of the second droplet remains controlled by the microvalve (and is proportional to $t_{\text{open}}$ - $t_{\text{break}}$)

Finally, we tested the ability of the microvalve to control the time of emission of the droplets and the intervals between them. We performed a scan of the frequencies of formation of the droplets with all other parameters constant. When we used vacuum as the low pressure, we observed that as we increased the frequency, the actual value of $p_{\text{LOW}}$ also increased (due to the limited efficiency of our vacuum pump). As the pressure drop across the membrane in the microvalve decreased at higher frequencies, the droplets had smaller volumes: see SI\(^\dagger\) for these results. On the other hand, when we used atmosphere as a reservoir of $p_{\text{LOW}} = 0$ bar, the volume of the droplets increased with increasing frequency. We associate this effect with the dynamics of drainage of the liquid from the thin space between the membrane and the PC plate upon closing of the valve. As the thin film of liquid drains (and fills) asymptotically slowly, the spacing between the membrane and the plate can depend on (even large) values of $t_{\text{close}}$. Conversely, the cross-section available to flow within the time $t_{\text{open}}$ depends on the starting separation between membrane and plate. As a result, for small values of $t_{\text{close}}$, the film is not completely drained from the microvalve, and subsequent opening of the valve for $t_{\text{open}}$ generates higher flow than at larger values of $t_{\text{close}}$. We found that for $p_{\text{HIGH}} = 0.3$ bar $p_{\text{LOW}}$ can be tuned to a value of approximately $0.15$ bar to compensate the two opposing effects and to yield monodisperse droplets regardless of the interval between their emission (see SI\(^\dagger\) for these results). Alternatively, having characterized the operation of the valve, one can compensate for dependence of the volume of the droplet on $t_{\text{close}}$ by a correct choice of $t_{\text{open}}$. This is exemplified in Fig. 6 for $p_{\text{LOW}} = 0$ bar.

Fig. 6 Dependence of the lengths of the droplets ($L$) normalized by the width of the channel ($W = 400 \mu m$) on $t_{\text{open}}$ for different frequencies of formation $f = 1/(t_{\text{open}} + t_{\text{break}})$. One can observe that the longer is the delay between formation of consecutive droplets the smaller they are. The symbols D1 through D3 mark the parameters corresponding to the micrographs shown as an inset to the figure. $Q = 3$ ml h\(^{-1}\), $p_{\text{water}} = 0.270$ bar, $p_{\text{HIGH}} = 1$ bar, $p_{\text{LOW}} = 0$ bar.

**Formation of bubbles in a T-junction chip**

We also tested formation of bubbles on demand (BOD) in a microfluidic T-junction (T) system (Fig. 2b). Similarly to the liquid–liquid FF system, the gas–liquid BOD T system produces monodisperse bubbles in a finite region of the $(p_{\text{HIGH}}, p_{\text{gas}})$ space (Fig. 3), and at frequencies up to 20 Hz.

Without the pneumatic valve, a T geometry forms bubbles of volume $V$ that is proportional to the pressure ($p_{\text{gas}}$) applied to the stream of gas and inversely proportional to the rate of flow of the gas $Q$.

\[ V = \frac{Q}{p_{\text{gas}}} \]

Fig. 7 Formation of bubbles in a T-junction system controlled with a microvalve. Dependence of the lengths of the bubbles ($L$) normalized by the width of the channel ($W = 400 \mu m$) on $t_{\text{open}}$ for different pressures applied to the stream of gas. The inset shows the dependence of the slope of the linear increase of $L$ with $t_{\text{open}}$ as a function of $p_{\text{gas}}$. The symbols B1 through B3 mark the parameters corresponding to the micrographs shown as an inset to the figure. $Q = 1$ ml h\(^{-1}\), $p_{\text{HIGH}} = 1$ bar, $p_{\text{LOW}} = 0$ bar, frequency of formation = 1 Hz.
continuous liquid (here water; $Q$). Fig. 7 shows the dependence of the lengths of bubbles ($L$) as a function of $t_{\text{open}}$ for constant $Q = 1$ ml h$^{-1}$ and for a set of values of $p_{\text{nitrogen}}$. Similarly to the case of droplets in the FF system, we observe that the volume (here parameterized by length) of the bubbles increases linearly with $t_{\text{open}}$. The rate of this increase is proportional to $p_{\text{nitrogen}}$ (inset to Fig. 7). The smallest generated bubbles had a volume of $\sim 350$ nL.

Again, similarly to the case of formation of droplets, the volume of the bubbles could also be controlled with the rate of flow $Q$ of the continuous phase: increase of $Q$ causes decrease of the size of the bubbles. In contrast to the case of formation of droplets, we found that it was not practically possible to control the size of the bubbles with a microvalve controlled by negative $p_{\text{nitrogen}}$: the flow of gas through the open microvalve was too rapid to generate reasonably small bubbles within the available range of values of $t_{\text{open}}$.

**Formation of sequences of droplets**

An operational and well characterized droplet on demand system is a crucial building block for automated miniaturized laboratories to perform reactions within droplets. Here we demonstrate the ability of our system to generate sequences of droplets of the same chemical composition but of varied volume and intervals between their emission (Fig. 8). Such sequences can be used in larger, integrated systems, to merge droplets of different chemical composition (drawn from different reservoirs) to form sequences of mixtures of reagents in a predefined controlled manner, e.g. for fast screening of reaction conditions.

In another example, we demonstrate (Fig. 9) sequences of droplets of two different compositions (here either pure water or water stained with black ink) formed in a range of sequences of their volumes and times of emission.

**On demand drawing of droplets from wells by suction**

Importantly, the microvalve and DOD systems that we describe here are also compatible with forcing the flow by a negative pressure. Such systems are important in that they provide for a facile way of formation of droplets from small samples of solutions—it is possible to form a small well of volume of several hundreds of microlitres at the inlet to the channel that guides the fluid-to-be-dispersed, and draw fluid from this well by an application of a negative pressure in the channel. In Fig. 10 we show four micrographs of different sequences of droplets generated with negative pressure.

**Fig. 8** Micrographs of sequences of droplets produced in a T-junction DOD system. The graphs show: (a) view of monodisperse droplets ($t_{\text{open}} = 28$ ms) produced at a constant interval ($t_{\text{close}} = 404$ ms), (b) monodisperse droplets ($t_{\text{open}} = 26$ ms, $t_{\text{open}} = 24$ ms) formed at two different intervals ($t_{\text{close}} = 604$ ms, $t_{\text{close}} = 204$ ms), (c) packs of three monodisperse droplets ($t_{\text{open}} = 26$ ms, $t_{\text{open}} = 24$ ms, $t_{\text{open}} = 24$ ms) separated by a longer interval ($t_{\text{close}} = 604$ ms, $t_{\text{close}} = 204$ ms, $t_{\text{close}} = 204$ ms), (d) droplets of two different volumes ($t_{\text{open}} = 31$ ms, $t_{\text{open}} = 26$ ms) all produced at the same intervals ($t_{\text{close}} = 804$ ms) and (e) packs of three droplets ($t_{\text{open}} = 24$ ms, $t_{\text{open}} = 22$ ms, $t_{\text{open}} = 31$ ms), with the first two droplets having one volume, and the third droplet having a different volume ($t_{\text{close}} = 404$ ms, $t_{\text{close}} = 404$ ms, $t_{\text{close}} = 804$ ms). $Q = 0.5$ ml h$^{-1}$, $p_{\text{water}} = 0.2$ bar, $p_{\text{HIGH}} = 1$ bar, $p_{\text{LOW}} = 0$ bar.

**Fig. 9** The micrographs show droplets produced in a double T-junction system: (a) view of monodisperse droplets produced alternately from clear and "black" (stained) water ($t_{\text{open}} = 30$ ms, $t_{\text{close}} = 470$ ms, $Q = 0.5$ ml h$^{-1}$), (b) view of packs of white droplets and two larger monodisperse black droplets ($t_{\text{open}} = 30$ ms, $t_{\text{close}} = 30$ ms, $t_{\text{close}} = 1470$ ms, $t_{\text{close}} = 470$ ms, $t_{\text{close}} = 970$ ms, $Q = 0.3$ ml h$^{-1}$), and (c) view of packs of five droplets—two black and three white—in different intervals ($t_{\text{open}} = 30$ ms, $t_{\text{close}} = 32$ ms, $t_{\text{close}} = 30$ ms, $t_{\text{close}} = 31$ ms, $t_{\text{close}} = 31$ ms, $t_{\text{close}} = 970$ ms, $t_{\text{close}} = 468$ ms, $t_{\text{close}} = 970$ ms, $t_{\text{close}} = 968$ ms, $t_{\text{close}} = 1409$ ms, $Q = 0.2$ ml h$^{-1}$). Dispersed phases are: (i) clear (pure) water and (ii) black (water with black ink). $p_{\text{clear}} = 0.2$ bar, $p_{\text{black}} = 0.181$ bar, $p_{\text{HIGH}} = 1$ bar, $p_{\text{LOW}} = 0$ bar.

**Fig. 10** The photographs show droplets formed in a flow-focusing system powered by a negative pressure at the outlet. Both continuous phase—hexadecane—and discontinuous—water with blue ink—were pulled using a syringe pump. The micrographs show (a) a view of monodisperse droplets ($t_{\text{open}} = 70$ ms) produced at a constant interval ($t_{\text{close}} = 120$ ms), (b) monodisperse droplets ($t_{\text{open}} = 70$ ms) formed at two different intervals ($t_{\text{close}} = 60$ ms, $t_{\text{close}} = 120$ ms), (c) droplets of two different volumes ($t_{\text{open}} = 40$ ms, $t_{\text{open}} = 70$ ms) all produced at the same intervals ($t_{\text{close}} = 90$ ms), (d) view of a pack of five droplets of increasing volume ($t_{\text{open}} = 40$ ms, $t_{\text{open}} = 60$ ms, $t_{\text{open}} = 80$ ms, $t_{\text{open}} = 100$ ms, $t_{\text{close}} = 120$ ms) all produced at the same intervals ($t_{\text{close}} = 70$ ms). $Q_{\text{pub}} = -0.6$ ml h$^{-1}$, $p_{\text{water}} = 0$ bar, $p_{\text{HIGH}} = 0.2$ bar, $p_{\text{LOW}} = -0.2$ bar.

**Conclusions**

Herein, we have reported a DOD/BOD generator with pneumatically operated microvalve fabricated in a stiff polymeric (polycarbonate) material. This system produces either droplets or bubbles in both the flow-focusing and the T-junction...
geometries. The system (i) forms droplets of well controlled volume and (ii) at well controlled time of emission. The droplets can be either formed at constant volume (with a standard deviation below 5% of their volume) or in arbitrary sequences of different volumes. The volume of the droplets depends both on the parameters of the operation of the microvalve (length of the intervals during which it stays open/closed and the control pressures applied to the membrane) and on the parameters of flow (pressure applied to the stream of the fluid-to-be-dispersed and the rate of flow imposed on the continuous fluid).

With the electromagnetic valves that we used, the frequency of formation of droplets (bubbles) could be tuned to a value slightly higher than 20 Hz. Faster electromagnetic valves and an optimized pneumatic system could readily allow for larger frequencies. We estimate that the microvalve itself can operate at frequencies above 100 Hz.

Importantly, the microvalves are integrated into the microfluidic chips, which allows for formation of droplets from small wells with the whole device powered by vacuum, and allowing for high-throughput, or combinatorial tests to be performed on small samples of fluid. The demonstration of the droplet on demand system in a stiff polymeric (polycarbonate) device opens this technology to use in microfluidic systems fabricated in a range of plastics and in glass. Stiff plastics offer a convenient material for fabrication of multilayer microfluidic systems, and a choice of chemical compatibility of the material. The microvalve itself can be tuned to be compatible with various chemistries by an appropriate choice of the membrane. This in turn opens the potential of combinatorial droplet systems for uses in organic and bio-chemistry, important areas of applications of microfluidic systems.

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