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ARTICLE

Programmable V-type Valve for Cell and Particle Manipulation in Microfluidic Devices

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A new microfluidic valve or "v-type valve" which can be flexibly actuated to focus a fluid flow and block a specific area of a microchannel is demonstrated. Valves with different design parameters were fabricated by multilayer soft lithography and characterized at various operating pressures. To evaluate the functionality of the valve, single microparticles (Ø 7 µm and Ø 15 µm) and single cells were trapped from flowing suspensions. Continuous processes of particle capture and release were obtained by controlling the actuation and deactuation of the valve. Integration of the v-type valve with poly(dimethyl siloxane) (PDMS) monolithic valves in microfluidic devices was demonstrated to illustrate the potential of the system in various applications such as the creation of a solid phase column, the isolation of a specific number of particles in reactors, and the capture and release of particles or cells in the flow of two immiscible liquids. We believe that this new valve system will be suitable for manipulating particles and cells in a broad range of applications.

Introduction

Handling micro-sized particles or cells in fluid flow plays important roles in various chemical and biological applications such as particle synthesis,[1-3] separation/extraction columns,[4-7] biosynthesis,[8] cell biophysics,[9,10] cell-drug response,[11,12] cell-cell interaction,[13,14] single cell analysis,[15] and circulating tumor cell isolation and analysis in blood.[16,17] Previous particle separation systems have been developed by adapting bio/chemical adhesives,[9,18] hydrodynamics,[19-21] dielectrophoresis,[22,23] magnetophoresis,[24,25] acoustophoresis,[26] and optical trapping.[27,28] Although the reported systems show good performance for trapping particles, they are either costly in terms of materials or equipment, or quite complex, whereas implementation of additional sample preparation steps with these systems remains challenging. Integration of the techniques that these systems employ with an additional fluid handling system is necessary for further analysis of the captured particles or cells, for example, for the purpose of PCR [29,30] or cell drug dose response.[11,12]

For the full implementation of particle sorting systems for screening and diagnostic applications, researchers introduced microfluidic devices combined with microstructures such as micro-cups,[13,17,31] micro-pillars,[32,33] micro-filters,[34] and micro-valves.[7] The microfluidic platforms showed not only a high capturing efficiency but also the capability of further analysis of the particles by generating fluid flows of various reagents over the capture structures. These separation systems using microstructures face the lack of flexibility to capture particles heterogeneous in size and require large numbers of capturing structures to meet the capacity of the sample, without the possibilities of selection and eventual release of the (individual) particles. Metering and sequential addition of reagents is often necessary for the study of complicated biological systems on individual particles or cells. Microfluidic systems based on multilayer soft lithography have shown the capability of performing multiple parallel reactions with particles and cells by controlling the fluid flows of various reagents with the integration of PDMS monolithic valves in a single device.[4,5,7,8,11,12,29,30] Sieve valves that are produced by the combination of two kinds of fluidic channel profiles, rounded and unrounded channels, showed the possibility of impeding the flow of particles, such as chromatography beads or cells, in a microfluidic channel while allowing liquid solution to pass through the valve.[7] Most reported sieve valves, however, are not capable of capturing particles of a desired size or size range, or providing a specific number of particles into a separated reactor to isolate trapped particles from the flow of particle suspension.

Here, we developed a flexible microfluidic v-type valve system, which can trap particles or cells in a fluid in a microchannel. The v-type valve was designed to generate a focused flow in the center of a channel by the flexible actuation of a PDMS membrane. The position of the valve and the degree of its blockage are adjustable. The system therefore allows to control over the size of the particles that are blocked by the

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valve. Furthermore the v-type valve not only allows to catch particles from a liquid stream in a microchannel and trap particles in the adjustable valve, but also enables the release of the particles into a second microchannel. This second channel could then be connected to a reactor in which a process involving the captured particles can be performed, e.g. a catalytic process, an adsorption process, or (if the particle is a cell) a biological experiment. We developed and tested various designs of the v-type valve to optimize the actuation of the valves and evaluate the functionality of the valve to capture and release particles and cells in a fluid flow. We also demonstrated the smart uses of a v-type valve in combination with general monolithic PDMS valves for 1) the generation of a solid phase column, 2) the isolation of the desirable number of particles in microreactors, and 3) the separation of particles from a liquid droplet in two immiscible fluid flows.

Materials and Method

Materials

Source 15Q (Ø 15 µm particles based on rigid polystyrene /divinyl benzene polymer matrix) were purchased from GE Healthcare Life Sciences (GE Healthcare Europe GmbH, Eindhoven, The Netherlands), and diluted 1:1000 in Milli-Q water for testing particle capture and 1:100 for testing particle packing. CountBright Absolute Counting Beads (Ø 7 µm particles for flow cytometry) were obtained from Molecular Probes (Life Technologies Europe BV, Bleiswijk, The Netherlands). 100 µM of resorufin (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) solution was prepared in Milli-Q water to visualize the actuation of valves in microchannels. We used mineral oil (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands) and Source 15Q suspension in Milli-Q water to generate droplets in oil flow.

Cell culture and preparation

The prostate cancer cell line, PC3 (PC-3 (ATCC CRL-1435), was cultured in RPMI-1640 media (Sigma Aldrich, Zwijndrecht, The Netherlands), supplemented with 10 % Fetal Bovine Serum (Sigma Aldrich) and 1% Penicillin-Streptomycin (Sigma Aldrich) at 37 °C in 5 % CO_2 atmosphere. Before experimentation, cells were stained with CellTracker Orange CMTMR (Molecular Probes, Breda, The Netherlands) at 37 °C for 30 min and detached them from the culture flask by 0.05 % of Trypsin/EDTA (Gibco, Paisley, UK). Thereafter, cells were washed once with the culture medium and re-suspended in PBS solution at a concentration of 500,000 cells/ml.

Chip fabrication

The microfluidic chips consist of a PDMS fluidic layer and a PDMS control layer; and were fabricated by multilayer soft lithography technique.[35,36] To prevent non-specific binding of fluorescent molecules and particles on PDMS surfaces, fluidic channels were treated with copolymer pluronic 10 g/L (Millipore, Zug, Switzerland) for 5 min and washed with Milli-Q water for 30 min.

Characterization of the device profile

Fabricated devices were sliced by a surgical blade to measure average values of the channel height and membrane thickness. The microscope images of the cross-sectional view of the sliced device were acquired by an inverted microscope (Leica DMI 5000M) and analyzed by Leica imaging software (Leica Application Suite, Leica Microsystems BV, The Netherlands). We obtained an average channel height of 37.6 ± 0.4 µm and an average membrane thickness of 14.7 ± 0.5 µm from 15 microscope images of three different devices.

Chip operation

The microfluidic devices were controlled by a pneumatic control system. Micro-valves were operated by applying compressed nitrogen gas into control channels. The pneumatic control system was automated by combining precision pressure regulators, 3/2-way solenoid valves, and EasyPort USB digital I/O controller (all from Festo, Festo BV, The Netherlands). We controlled the pneumatic system by a custom-built LabVIEW (National Instruments Co.) program. For loading reagents into a microchannel we applied a constant pressure from the backside of the solution.[37] In our previous study we calibrated the coefficient for the relationship between applied pressure and flow rate as 0.033 µl/min·mbar.

Data processing

We used an inverted fluorescent microscope (Leica DMI 5000M, 10X, 20X, and 40X Objectives, Leica Microsystems BV, The Netherlands) equipped with an automatic XY-stage (Oasis PCI XY control unit), and a digital camera (Leica DFC300 FX, Leica Microsystems BV, The Netherlands) for acquisition of images to monitor the actuation of valves. The fluorescent signal from resorufin was observed by a Leica N 2.1 filter cube (excitation: BP 515 - 560 nm; emission: LP 590 nm) and the fluorescent images of Source 15Q and CountBright Absolute Counting Beads were acquired by a Leica I3 filter cube (excitation: BP 450 - 490 nm; emission: LP 515 nm). All the acquired images were processed and analyzed by the image calculator and interactive 3D surface plot of Image J software (http://rsb.info.nih.gov/ij/).

Result and Discussion

Principle of a v-type valve

A comparison of a general push-up monolithic PDMS valve and a v-type valve is shown in Fig. 1. Fig. 1A shows a scheme of the general push-up shut-off valve in a top view (left) and a cross-sectional view (right). The valve system consists of two PDMS layers, a top fluidic layer and a bottom control layer. Applying pressure to the control channel actuates the PDMS membrane between the two layers and the membrane blocks the cross sectional area of the fluidic channel wherein the valve is located. Because the displacement of the membrane increases with the increase of the applied pressure, the fluidic channel is completely blocked when the applied pressure is higher than a certain threshold pressure.[37,38]

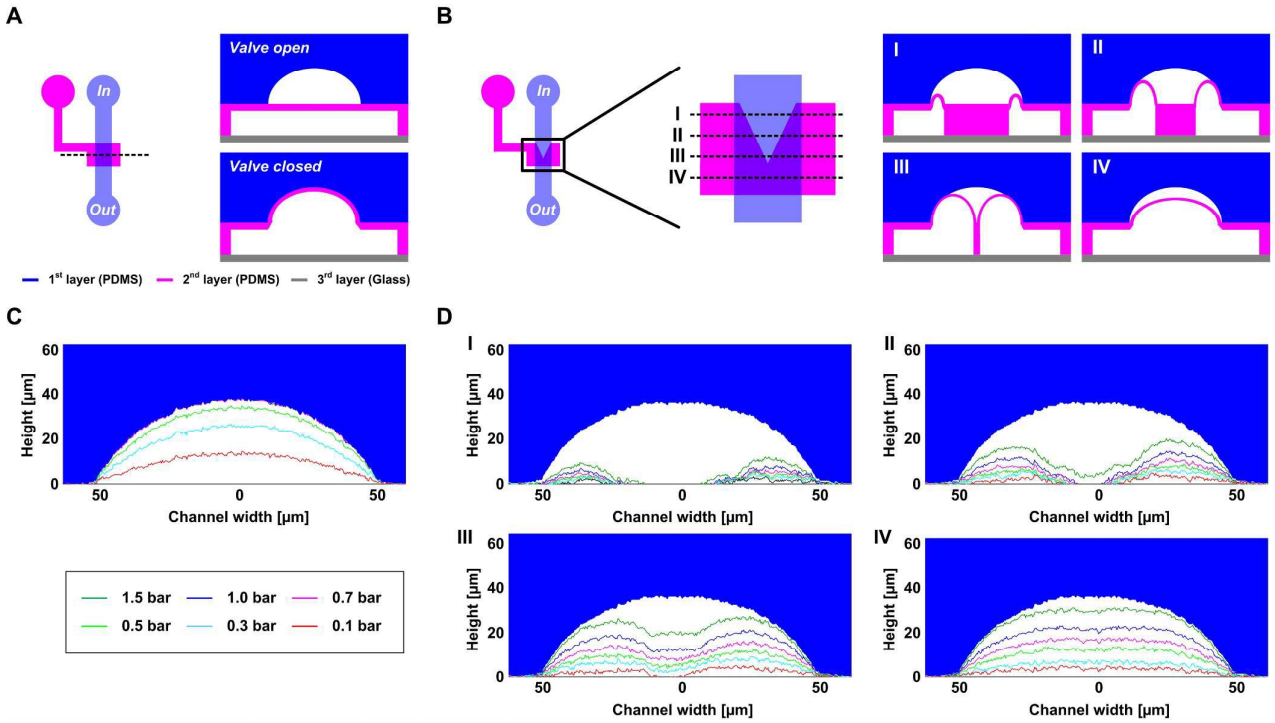


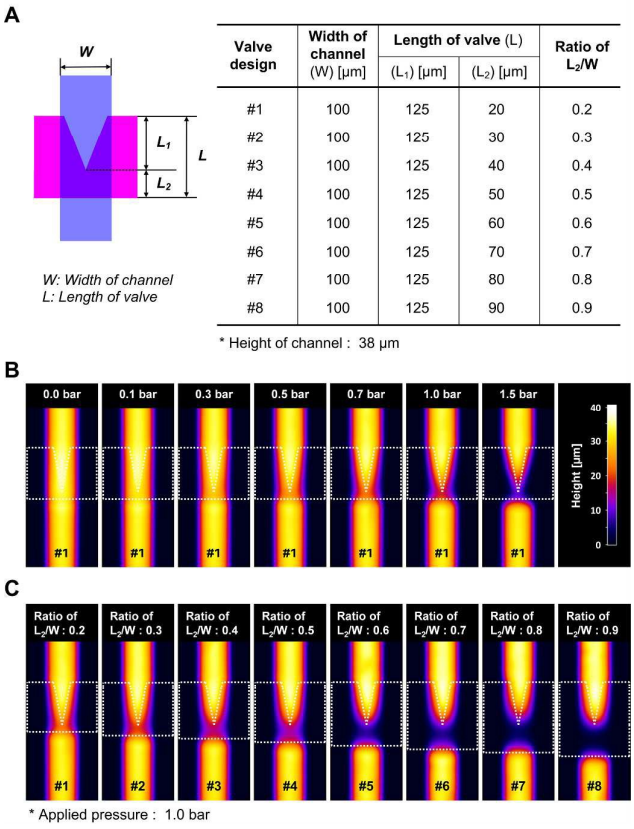
Fig. 1 Comparison of a shut-off valve and a v-type valve. A. Design (top view) and operation (cross-sectional view) of the shut-off valve, B. Design (top view) and actuation (cross-sectional views at the location of I, II, III, and IV) of the v-type valve, C. Measured height of the shut-off valve at various applied pressure, and D. Measured height of the v-type valve at various applied pressure.

Fig. 1B schematically depicts a top view (left) of a v-type valve and cross-sectional views (right) of the adjustable valve respectively represented at the locations of I, II, III, and IV. The v-type valve is designed to trap a particle of a predetermined minimum size while it never completely blocks the fluid flow channel. The adjustable valve has a funnel shape when it is actuated by applying pressure in a control channel. Because of this shape, the adjustable valve does not block the cross sectional area of the fluid flow channel at the upstream side of the valve, whereas the blockade of cross sectional area of the fluid flow channel increases, and thus the effective passage way area gradually decreases, in the direction towards the downstream side of the valve as can been seen in the images I to IV of Fig. 1B. The three-dimensional schematic illustration of the v-type valve operation is shown in supplemental Fig. S1. Fig. 1C and Fig. 1D show the membrane actuations of the shut-off valve and the v-type valve according to various applied pressures ranging from 0.1 bar to 1.5 bar. The shut-off valve was completely closed by applying a pressure higher than 0.7 bar, but the v-type valve was still partially opened at 1.5 bar. The thickness of the membrane was 15 μm and the width and height of the fluidic channel were 100 μm and 38 μm, respectively.

Optimization of the design of a v-type valve

For the realization of the functionality of a v-shaped valve, v-type valves with various dimensions were been designed and tested. The mask design of a microfluidic device is shown in supplemental Fig. S2. Fig. 2A shows the dimensions of 8 different v-valve designs. The valve width (W) equals the width of the fluidic channel at the intersection. The length of the valve (L) comprises the first valve section having a length (L_1) and the second valve section having a length (L_2). The first valve section comprises the upstream side of the valve, whereas the second valve section includes the downstream side of the valve. The actuation of the membrane at a constant applied pressure is dependent on the valve area where a membrane forms between fluidic and control layers. Hence, if the second valve part length (L_2) is very long, the valve may completely block the fluid flow in the fluidic channel at the second valve section, whereas it may also be possible that the second section length (L_2) is too short to provide the blockage of the fluidic channel required to block a particle. To evaluate the dimensions of the v-type valve, 8 different designs of v-valves with various ratios of the length of the second valve part (L_2) and the valve width (W) were been designed and tested. The ratio of L_2 and W ranged from 0.2 to 0.9.

The actuations of the 8 different v-type valves at various applied pressures are shown in supplemental Fig. S3. For the visualization of the displacement of the valves, 100 μM of resorufin was introduced into the fluidic channel by applying 0.1 bar to the backside of the solution. Next we applied compressed nitrogen gas into the control channel and monitored the fluorescent signal of resorufin in the fluidic channel to achieve the fluidic channel profile according to the membrane actuation. The range of applied pressure for the membrane actuation was from 0 bar to 2.0 bar with an increment of 0.1 bar. Fig. 2B shows the fluidic channel profiles of the valve design #1 at various applied pressures from 0 bar to 1.5 bar.



Valve design	Width of channel (W) [μm]	Length of valve (L)		Ratio of L_2/W
		(L_1) [μm]	(L_2) [μm]	
#1	100	125	20	0.2
#2	100	125	30	0.3
#3	100	125	40	0.4
#4	100	125	50	0.5
#5	100	125	60	0.6
#6	100	125	70	0.7
#7	100	125	80	0.8
#8	100	125	90	0.9

* Height of channel : 38 μm

Fig. 2 Optimization of the dimensions of a v-type valve. A. Dimensions of 8 different v-type valve designs. Valves with 8 different ratios of L_2/W were designed and tested at constant membrane thickness, channel height, and channel width. B. Fluidic channel profile with the actuation of a v-type valve (valve design #1) at various applied pressure from 0 bar to 1.5 bar. C. Fluidic channel profile with the actuations of v-type valves with 8 different designs at a constant pressure of 1.0 bar.

Fig. 2C shows the comparison of the valve actuation of 8 different valve designs at a constant applied pressure, 1.0 bar. At a given pressure and depending on the ratio L_2/W the actuation of the valve starts from the two sides of the fluidic channel, therefore a focused flow was generated at the center of the channel. It was observed that with increasing L_2/W focused flow manifested at decreasing pressure, whereas at a constant pressure the intensity of the actuation at the center of the fluidic channel showed an increase with increasing L_2/W, eventually leading to a substantial complete blockage of the fluid flow channel. In Fig. 2C, fluidic channel profiles of design #5, #6, #7, and #8 shows the valves closed the fluidic channels, while fluid still flowed in the channels with valve design #1, #2, #3, and #4 at 1.0 bar.

Particle capture by a v-type valve

To optimize the operating pressures of v-type valves to capture particles of a desired size, the actuation of valves at various applied pressures was studied with v-type valve designs #1, #2, #3, and #4, see supplemental Fig. S4. At a constant operating pressure, the focused flow is created at the center of the channel where the actuation of a v-valve is the lowest. Hence the size of particles that can be captured by v-type valves depends on the height of the membrane at the center of the channel. Fig. 3A (a) shows the height of the actuation of v-type valve design #1 in the center of the channel (L'L'') at various applied pressures. We measured the height of the channel and the actuated valve heights of v-type valve designs #1, #2, #3, and #4 according to operating pressures ranging from 0.1 bar to 2.0 bar. Fig. 3A (b) shows the valve openings calculated from the measurements.

Fig. 3B shows examples of particle capture with a v-type valve (Movie S1 in the Supporting Materials). We used the v-type valve with valve design #4 to capture a Ø 15 μm particle, a Ø 7 μm particle, and a single PC3. We pushed particle suspensions with 0.1 bar into a fluidic channel while the valve was actuated by applying 0.7 bar and 1.0 bar to capture Ø 15 μm particles and Ø 7 μm

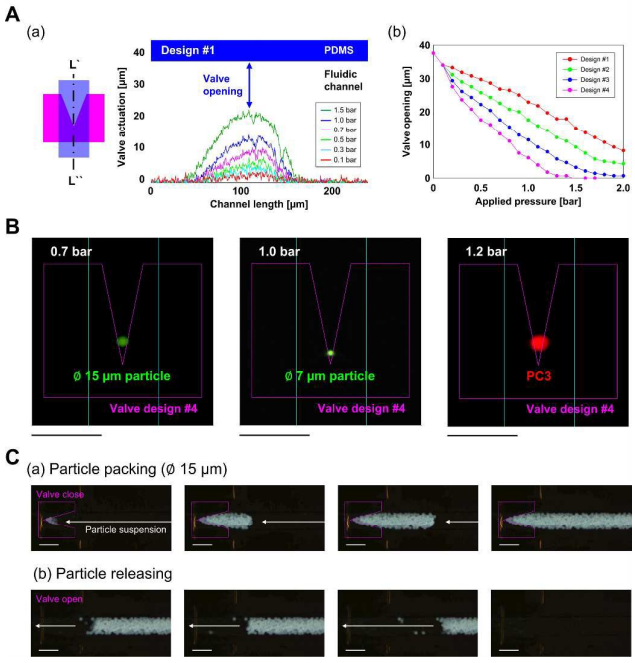


Fig. 3 Particle capture and sieving by a v-type valve. A. Valve actuation and opening of v-type valves. (a) The blockage of a fluidic channel by actuation of the v-type valves was measured at various applied pressures in the center of a fluidic channel (L'L'') and (b) the openings of the channel were calculated and plotted. The height of fluidic channel was 38 μm. B. Single particle captured by a v-type valve (design #4). Particle suspensions were loaded in the fluidic channel while the valve was actuated by 0.7 bar, 1.0 bar, and 1.2 bar for Ø 15 μm particles, Ø 7 μm particles, and PC3 cells, respectively. C. Flexible particle packing and releasing in a microchannel. (a) Ø 15 μm particles were trapped and packed in the fluidic channel when the v-type valve (valve design #1) was actuated by 1.6 bar and (b) released by deactuating the valve. 100 μm scale bars are shown.

particles. In the case of PC3 capture, we applied 0.02 bar and 1.2 bar for loading a cell suspension and actuating the valve. The process of particle capture and release was repeated more than 100 times to test the robustness of the system. During the processes, more than one particle or cell was captured from suspensions with high concentration. Still not a single particle or cell was not captured by the v-type valve. The applied pressures to capture Ø 15 µm and Ø 7 µm particles matched with the pressures to create valve openings smaller than 15 µm and 7 µm as is shown in Fig. 3A (applied pressures to capture Ø 15 µm and Ø 7 µm particles with valve design #1, #2, #3, and #4 are summarized in supplemental Fig. S3 (b)). However, a higher pressure was required to generate a smaller valve opening to capture PC3 cells as compared to the openings for particle capture. This can likely be attributed to the greater flexibility of cell membranes.[39] Debris and damaged cells passed through the valve as their sizes were smaller than the opening area of the channel adjusted by the actuation of the valve.

Fig. 3C shows an example of the generation of a solid phase column in a fluidic channel with a v-type valve. We actuated the v-type valve with valve design #1 by applying 1.6 bar and loaded Source 15Q (Ø 15 µm particles for anion exchange) suspension. When the v-type valve was actuated the particles in the fluid flow of the suspension were sieved and packed (Fig. 3C (a)) and the packed particles were released by opening the valve (Fig. 3C (b)). The process of packing and releasing particles could be repeated by controlling the v-type valve (Movie S2 in the Supporting Materials). Such a column could be used for a variety of applications by integrating with PDMS monolithic valves.[7,8]

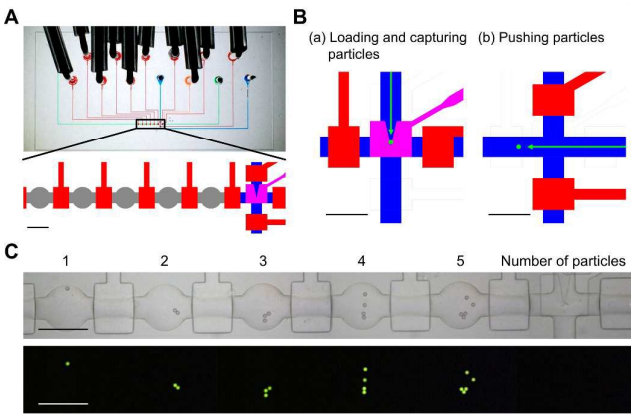


Fig. 4 Isolation of particles in chambers by a v-type valve. A. Photo of the device in connections with control ports (top) and design of capture unit and isolation chambers. B. Operation of the particle capture unit. (a) A particle was captured from the fluid of a particle suspension by a v-type valve, and (b) the captured particle was pushed in a chamber by changing the direction of fluid flows. C. Microscope images of isolated particles in chambers. Scale bars represent 200 µm.

Isolation of a desirable number of particles in a reactor

To demonstrate the isolation of particles captured by a v-type valve in separate reactors for further analysis on or with the particles we designed a microfluidic device with isolation chambers in connection with a v-type valve. The photolithographic mask design is shown in supplemental Fig. S5 (a). Fig. 4A shows a photo and design of the device that consists of two main units, a capture unit and a chamber unit. In the capture unit a v-type valve is arranged at the intersection of two fluidic channels and two shut-off valves are located at opposite sides of the intersection in each fluidic channel. A fluid flow in the fluidic channel could be completely blocked by closing the two shut-off valves in the channel and could be controlled in the direction of a fluid flow in the intersection by controlling the four shut-off valves. The process of capture and pushing particles in a reactor is shown in Fig. 4B. A particle suspension was loaded in the first fluidic channel by opening shut-off valves in the first channel and closing the two shut-off valves in the second channel. A particle having a larger diameter than the minimum opening passageway could be captured from the flow in the first flow channel by actuating the v-type valve. Then the fluid flow in the first fluidic channel was blocked by closing the two first channel valves; and a fluid flow was started in the second fluidic channel by opening the second channel valves, the particle could be introduced in the fluid flowing in the second channel once the particle was released from the adjustable valve. The number of captured particles could be determined by controlling the closing time of the v-type valve. To evaluate the ability of the system to isolate a desired number of particles to be transferred into reactors we captured defined numbers of Ø 15 µm particles and pushed them into chambers in defined positions. The detailed operation is shown in supplemental Fig. S5 (b). Fig. 4C shows microscope images of isolated particles in chambers by bright field and fluorescent illuminations. We captured and isolated one, two, three, four, and five particles in the first, second, third, fourth, and fifth chambers, respectively.

Sieving particles out of a droplet in the flow of two immiscible fluids

To demonstrate the feasibility of a v-type valve for sieving particles in two-phase droplet flows, we designed a droplet-based microfluidic device with a v-type valve. Fig. 5A shows the photo of the device in connection with control ports. The photolithographic mask design is shown in supplemental Fig. S6. The fluidic and control channels were filled with food dyes to visualize the layout of the device. The device contains three control ports for shut-off valves, one control port for a v-type valve, two inlets, and two outlets.

The device comprises a droplet generator, a sieving unit, and a separating unit. The droplet generator was designed to control the size of droplets by mechanical cutting of a flow of a dispensed phase by a shut-off valve.[40,41] We used mineral oil as the carrier fluid and a Source 15Q suspension as the dispersed phase. We introduced the particle suspension into a dispersed phase channel and mineral oil in a main fluidic channel by applying pressures to

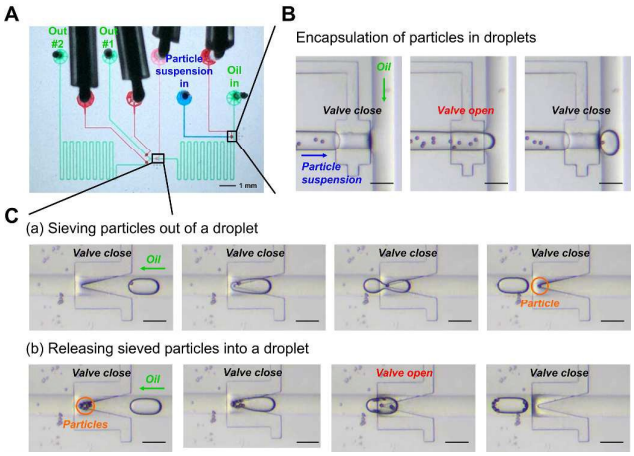


Fig. 5 Design and operation of complete microfluidic device for sieving particles out of droplets in the flow of two immiscible liquids. A. Photo of the device in connections with control ports, B. Operation process of encapsulation of particles in droplets, and C. Particle sieving and releasing.

the backsides of the liquids, 0.1 bar for the suspension and 0.2 bar for mineral oil. A flow of the particle suspension was blocked by closing the shut-off valve arranged in the dispersed phase channel in the T-junction of two channels. When the valve was opened the suspension started to flow into the main channel; and once the valve was closed a droplet was created. We generated droplets contained particles by operating the valve with the dispensing time of 1 sec. Fig. 5B shows time series microscope images of the encapsulation of particles in droplets. The formed droplets flowed into the sieving unit wherein the v-type valve is located. When the v-type valve was actuated particles in droplets could be sieved in the valve area and the trapped particles could be released in droplets by opening the valve (Fig. 5C). The droplets with or without particles could be collected separately in two different outlets by controlling two shut-off valves at the Y-junction of two channels to outlets (Movie S3 in the Supporting Materials).

Conclusions

In conclusion, we established a new programmable v-type valve to capture particles or cells in a fluid flow by focusing the flow in the center of a microchannel. The working principle and proper design of the v-type valve were investigated to provide the design parameters of the valve for the realization of flexible particle trapping function into a microfluidic device. The minimum size of particles trapped by the v-type valve could be determined by the controllable actuation of the valve and release of particles from the valve could be obtained by deactuating the valve. The designs of the v-type valve in combination with monolithic valves can offer the multilayer microfluidic devices suitable for chemical and biological processes wherein particles or cells are involved. Examples for such processes are adsorption/desorption experiments, chromatography, a solid phase extraction, the evaluation of drug response to cells, and single cell analysis. Further integration of the valve platform with detection techniques to fully automate all the operating processes for cell or particle capture, selection, isolation, and performing reagent interactions can now be performed to fully exploit the potential of v-type valves in microfluidic devices.

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References

1 K. W. Bong, D. C. Pregibon and P. S. Doyle, *Lab Chip*, 2009, **9**, 863-866.
2 S. A. Khan, A. Günther, M. A. Schmidt and K. F. Jensen, *Langmuir*, 2004, **20**, 8604-8611.
3 S. A. Khan and K. F. Jensen, *Adv. Mater.*, 2007, **19**, 2556-2560.
4 J. Huft, C. A. Haynes and C. L. Hansen, *Anal. Chem.*, 2013, **85**, 1797-1802.
5 J. Huft, C. A. Haynes and C. L. Hansen, *Anal. Chem.*, 2013, **85**, 2999-3005.
6 K.-Y. Hwang, S. H. Kwon, S.-O. Jung, K. Namkoong, W.-J. Jung, J.-H. Kim, K.-Y. Suh and N. Huh, *Anal. Chem.*, 2012, **84**, 7912-7918.
7 C.-C. Lee, G. Sui, A. Elizarov, C. J. Shu, Y.-S. Shin, A. N. Dooley, J. Huang, A. Daridon, P. Wyatt and D. Stout, *Science*, 2005, **310**, 1793-1796.
8 C.-C. Lee, T. M. Snyder and S. R. Quake, *Nucleic Acids Res.*, 2010, gkq092.
9 Y. Cui, F. M. Hameed, B. Yang, K. Lee, C. Q. Pan, S. Park and M. Sheetz, *Nature communications*, 2015, **6**.
10 D. R. Gossett, T. Henry, S. A. Lee, Y. Ying, A. G. Lindgren, O. O. Yang, J. Rao, A. T. Clark and D. Di Carlo, *Proceedings of the National Academy of Sciences*, 2012, **109**, 7630-7635.
11 J. Dai, S. H. Yoon, H. Y. Sim, Y. S. Yang, T. K. Oh, J. F. Kim and J. W. Hong, *Anal. Chem.*, 2013, **85**, 5892-5899.
12 M. Mehling, T. Frank, C. Albayrak and S. Tay, *Lab Chip*, 2015, **15**, 1276-1283.
13 A. M. Skelley, O. Kirak, H. Suh, R. Jaenisch and J. Voldman, *Nat. Methods*, 2009, **6**, 147-152.
14 C. Zheng, L. Zhao, G. e. Chen, Y. Zhou, Y. Pang and Y. Huang, *Anal. Chem.*, 2012, **84**, 2088-2093.
15 J. F. Swennenhuis, A. G. Tibbe, M. Stevens, M. R. Katika, J. van Dalum, H. D. Tong, C. J. van Rijn and L. W. Terstappen, *Lab Chip*, 2015, **15**, 3039-3046.
16 H. W. Hou, M. E. Warkiani, B. L. Khoo, Z. R. Li, R. A. Soo, D. S.-W. Tan, W.-T. Lim, J. Han, A. A. S. Bhagat and C. T. Lim, *Scientific reports*, 2013, **3**.
17 Y. Yang, H. S. Rho, M. Stevens, A. G. Tibbe and L. Terstappen, *Lab Chip*, 2015.
18 K. R. King, S. Wang, D. Irimia, A. Jayaraman, M. Toner and M. L. Yarmush, *Lab Chip*, 2007, **7**, 77-85.
19 M. Yamada, M. Nakashima and M. Seki, *Anal. Chem.*, 2004, **76**, 5465-5471.

20 M. Yamada and M. Seki, *Anal. Chem.*, 2006, **78**, 1357-1362.
21 S. Choi, S. Song, C. Choi and J.-K. Park, *Lab Chip*, 2007, **7**, 1532-1538.
22 I. Doh and Y.-H. Cho, *Sensors and Actuators A: Physical*, 2005, **121**, 59-65.
23 M. Javanmard, S. Emaminejad, R. W. Dutton and R. W. Davis, *Anal. Chem.*, 2012, **84**, 1432-1438.
24 S. M. Berry, E. T. Alarid and D. J. Beebe, *Lab Chip*, 2011, **11**, 1747-1753.
25 N. Pamme and C. Wilhelm, *Lab Chip*, 2006, **6**, 974-980.
26 F. Petersson, A. Nilsson, C. Holm, H. Jönsson and T. Laurell, *Lab Chip*, 2005, **5**, 20-22.
27 M. MacDonald, G. Spalding and K. Dholakia, *Nature*, 2003, **426**, 421-424.
28 J. Prikulis, F. Svedberg, M. Käll, J. Enger, K. Ramser, M. Goksör and D. Hanstorp, *Nano Lett.*, 2004, **4**, 115-118.
29 A. K. White, M. VanInsberghe, I. Petriv, M. Hamidi, D. Sikorski, M. A. Marra, J. Piret, S. Aparicio and C. L. Hansen, *Proceedings of the National Academy of Sciences*, 2011, **108**, 13999-14004.
30 Y. Yang, J. F. Swennenhuis, H. S. Rho, S. Le Gac and L. W. Terstappen, 2014.
31 D. Wlodkowic, S. Faley, M. Zagnoni, J. P. Wikswo and J. M. Cooper, *Anal. Chem.*, 2009, **81**, 5517-5523.
32 H. Mohamed, J. N. Turner and M. Caggana, *J. hromatogr.*, 2007, **1162**, 187-192.
33 S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, M. R. Smith, E. L. Kwak, S. Digumarthy and A. Muzikansky, *Nature*, 2007, **450**, 1235-1239.
34 H. M. Ji, V. Samper, Y. Chen, C. K. Heng, T. M. Lim and L. Yobas, *Biomed. Microdevices*, 2008, **10**, 251-257.
35 Y. Xia and G. M. Whitesides, *Annu. Rev. Mater. Sci.*, 1998, **28**, 153-184.
36 M. A. Unger, H.-P. Chou, T. Thorsen, A. Scherer and S. R. Quake, *Science*, 2000, **288**, 113-116.
37 H.S. Rho, Y. Yang, H. Veltkamp, and H. Gardeniers, *LOC Chips and Tips*, 2015, http://blogs.rsc.org/chipsandtips/2015/10/09/.
38 P. Fordyce, C. Diaz-Botia, J. DeRisi and R. Gomez-Sjoberg, *Lab Chip*, 2012, **12**, 4287-4295.
39 F. Coumans, G. van Dalum, M. Beck and L. Terstappen, *PLoS ONE*, 2013, **8**, e61770.
40 W. S. Lee, S. Jambovane, D. Kim and J. W. Hong, *Microfluid. Nanofluid.*, 2009, **7**, 431-438.
41 S. Zeng, B. Li, X. o. Su, J. Qin and B. Lin, *Lab Chip*, 2009, **9**, 1340-1343.