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Pneumatically Driven Microfluidic Platform for Micro-Particle Concentration

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TITLE:

Pneumatically Driven Microfluidic Platform for Micro-Particle Concentration

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KEYWORDS:

Microparticle, pneumatic valve, sieve, concentration, polydimethylsiloxane

SUMMARY:

The present protocol describes a pneumatic microfluidic platform that can be used for efficient microparticle concentration.

ABSTRACT:

The present article introduces a method for fabricating and operating a pneumatic valve to control particle concentration using a microfluidic platform. This platform has a three-dimensional (3D) network with curved fluid channels and three pneumatic valves, which create networks, channels, and spaces through duplex replication with polydimethylsiloxane (PDMS). The device operates based on the transient response of a fluid flow rate controlled by a pneumatic valve in the following order: (1) sample loading, (2) sample blocking, (3) sample concentration, and (4) sample release. The particles are blocked by thin diaphragm layer deformation of the sieve valve (Vs) plate and accumulate in the curved microfluidic channel. The working fluid is discharged by the actuation of two on/off valves. As a result of the operation, all particles of various magnifications were successfully intercepted and disengaged. When this technology is applied, the operating pressure, the time required for concentration, and the concentration rate may vary depending on the device dimensions and particle size magnification.

INTRODUCTION:

Due to the importance of biological analysis, microfluidic and biomedical microelectromechanical systems (BioMEMS) technologies[1,2] are used to develop and study devices for the purification and collection of micromaterials[2-4]. Particle capture is categorized as active or passive. Active traps have been used for external dielectric[5], magnetophoretic[6], auditory[7], visual[8], or thermal[9]

forces acting on independent particles, enabling precise control of their movements. However, an interaction between the particle and external force is required; thus, the throughput is low. In microfluidic systems, controlling the flow rate is very important because the external forces are transmitted to the target particles.

In general, passive microfluidic devices have micropillars in microchannels[10,11]. Particles are filtered through interaction with a flowing fluid, and these devices are easy to design and inexpensive to manufacture. However, they cause particle clogging in micro-pillars, so more complex devices have been developed to prevent particle clogging[12]. Microfluidic devices with complex structures are generally suitable for managing a limited number of particles[13-18].

This article describes a method to fabricate and operate a pneumatically driven microfluidic platform for large particle concentrations that overcomes the shortcomings[18] as mentioned above. This platform can block and concentrate particles by deformation and actuation of the thin diaphragm layer of the sieve valve (Vs) plate that accumulates in curved microfluidic channels. Particles accumulate in curved microfluidic channels, and the concentrated particles can separate by discharging the working fluid *via* the actuation of two PDMS seals on/off valves[18]. This method makes it possible to process a limited number of particles or concentrate a large number of small particles. Operating conditions such as the magnitude of flow rate and compressed air pressure can prevent unwanted cell damage and increase cell trapping efficiency.

PROTOCOL:

1. Designing the microfluidic platform for particle concentration

1.1. Design the pneumatic microfluidic platform consisting of one pneumatic valve for fluid flow in the 3D flow network and three pneumatic valves for sieve (Vs), fluid (Vf), and particle (Vp) valve operation (**Figure 1**).

NOTE: Vs blocks concentrate particles from the liquid, and Vf and Vp allow fluid and particle release after concentration. Three pneumatic ports provide compressed air from the fluid/pneumatic supply layer (normally open) and the pneumatic valve light outlet to actuate the valve. The microfluidic channel network is designed with a CAD program[18,19].

1.2. Design the channel to be a pneumatic supply layer and a 3D channel network layer (**Figure 2**).

NOTE: The fluid network is interconnected with the curved channels in the anterior part and the rectangular chamber in the posterior region. Vs block the inlet, and particles accumulate in the collection area of the curved fluid channel. Particle-free fluids (particle-free liquids) are exited through the Qf outlet and the concentrated particles through the Qp outlet (**Figure 3**).

1.3. According to the above conditions, prepare four types of SU-8 molds.

NOTE: The four molds include a mold that allows the valve to be controlled *via* pneumatics, two molds that create fluid channels, and a clean mold without shape (**Figure 4** and **Table 1**). The four types of molds mentioned are fabricated using standard photolithography processes. This mold making consists of a SU-8 mold on a silicon wafer as per previously published reports[18,19]. **Figure 5** depicts the device chip.

2. Fabrication of the microfluidic platform for particle concentration

NOTE: **Figure 6** illustrates the fabrication of a microfluidic platform that concentrates particles.

2.1. Replicate the PDMS layer using a prepared pneumatic valve channel SU-8 mold (step 1.3) for pneumatically controlling the valve.

2.1.1. Pour 10 mL of liquid PDMS and 1 mL of curing agent (see **Table of Materials**) into a prepared pneumatic valve channel mold (step 1.3) and heat-activate at 90 °C for 30 min.

2.1.2. After the PDMS structures are cured, separate the SU-8 mold of step 2.1.1.

2.1.3. Punch three 1.5 mm pneumatic ports (Vs, Vf, and Vp) into the pneumatic valve channel manufactured according to step 2.1.2 using a 1.5 mm puncture (see **Table of Materials**).

2.1.4. Pour 10 mL of liquid PDMS and 1 mL of curing agent into a prepared clean SU-8 mold prepared in step 1.3 and spin-coat at 1,500 rpm for 15 s using a spin coater (see **Table of Materials**). Then heat-activate at 90 °C for 30 min.

2.1.5. After the PDMS structures are cured, separate the SU-8 mold of step 2.1.4.

NOTE: The valve diaphragm layer controls fluid flow according to the pneumatic pressure.

2.1.6. Treat atmospheric plasma (see **Table of Materials**) to the PDMS structures prepared in steps 2.1.3 and 2.1.5 for 20 s.

2.1.7. Align directly plasma-treated PDMS structures from step 2.1.6 according to the channel structure by checking with a microscope.

2.1.8. Bond the aligned PDMS structures prepared in step 2.1.7 by heating at 90 °C for 30 min.

2.1.9. Punch a 1.5 mm diameter hole in the fluid channel inlet (Qfp) and fluid channel outlets (Qf and Qp) within the pneumatic channel part to which the thin diaphragm layer is bonded, using a 1.5 mm puncture.

2.2. Replicate both sides of the PDMS layer using two SU-8 molds to make a microfluidic channel. Use a curved and rectangular microfluidic channel mold on the front and a microfluidic interconnection channel mold on the rear.

2.2.1. Pour 10 mL of liquid PDMS and 1 mL of curing agent into the curved and rectangular microfluidic channel mold and spin-coat at 1,200 rpm for 15 s. Then create molds for the curved fluid chamber and fluid channels by thermal activation at 90 °C for 30 min (**Figure 6A**).

2.2.2. Separate the PDMS layer on which the microfluidic channel is formed, then make a heat-activated mold covering the sealed vent wall by bonding to the glass wafer by treating atmospheric plasma for 20 s (**Figure 6B**).

2.2.3. Pour 3 mL of liquid PDMS into the interconnection channel of the SU-8 mold (**Figure 6C**).

2.2.4. Arrange the structure fabricated in step 2.2.2 with the interconnection channel mold in liquid PDMS on the microfluidic interconnect channel mold, and dry the superimposed structure at 130 °C for 30 min (**Figure 6D**).

NOTE: While curing the rear structure, the PDMS mold fabricated in step 2.2.2 is inflated by the thermal pressure of the air layer, and the deformed PDMS layer is thermally activated (**Figure 6E**)[16].

2.2.5. After curing, remove the front SU-8 mold from the microfluidic channel network layer and carefully peel off the rear PDMS mold (**Figure 6F**).

NOTE: The 3D fluidic network layer allows the creation of an anterior curved fluid chamber and microfluidic channels.

2.2.6. Pour 10 mL of liquid PDMS and 1 mL of curing agent into a clean SU-8 mold. Then heat-activate at 90 °C for 30 min.

2.2.7. After the PDMS structures are cured, separate the SU-8 mold.

NOTE: This step creates the additional sealing layer.

2.2.8. Treat the atmospheric plasma to PDMS structures prepared in steps 2.2.3 and 2.2.7 for 20 s.

2.2.9. Align directly plasma-treated PDMS structures according to the channel structure by checking with a microscope.

2.2.10. Bond the aligned PDMS structures by heating at 90 °C for 30 min.

2.3. Align the PDMS structures prepared in steps 2.1 and 2.2 according to the channel structure and bond them by treating atmospheric plasma for 20 s.

3. Setting up the device

NOTE: **Figure 7** shows fabricating a microfluidic platform that concentrates particles.

3.1. Manually fill the microfluidic channel with bubble-free demineralized water using a 10 mL syringe.

3.2. To control the P_Qfp and the three pneumatic valves (P_Vs, P_Vf, and P_Vp) that control the microbead flow, insert a precision pressure controller with four or more output channels (see **Table of Materials**) for the working fluid (Qfp) into the microfluidic platform.

NOTE: A precision pressure controller with four output channels can be replaced with multiple precision pressure controllers. In this experiment, the operating pressure of P_Qfp was 10 kPa, P_Vs was 15 kPa, and P_Vf and P_Vp were both 18 kPa (**Figure 8** and **Table 2**). **Figure 8** shows the working fluid flow rate over time as particles are concentrated by the microfluidic platform with P_Vs of 15 kPa, and **Table 2** shows the actuation results according to the pneumatic valves.

3.3. Prepare carboxyl polystyrene test particles of various sizes in distilled water (see **Table of Materials**).

NOTE: The particle sizes used in this experiment were 24.9, 8.49, and 4.16 µm; particles of various sizes can be used depending on the pressure of P_Vs.

3.4. To control the flow rate of the working fluid, fill a glass bottle half full with water (working fluid) and connect the glass bottle cap to the controller output channel and microvalve.

NOTE: Connect one tube to the microvalve to receive compressed air from the controller and the other tube to inject water.

3.5. Observe platform operation through an inverted microscope for all platform operations and measure the operating flow rate over time at the outlet by a liquid flow meter (see **Table of Materials**).

4. Operation of the device

4.1. Inject the particle/fluid mixture under pressure at the inlet (Qfp) with Vp (**Figure 9A**).

NOTE: The flow of particles and clean fluid from the outlet through the interconnected channels are controlled *via* Vp and Vf, respectively (**Table 2**).

4.2. Apply pressure to Vs at 15 kPa and Vp at 18 kPa to actuate the valve.

NOTE: At this time, the diaphragm is deformed, the particles of the fluid Qfp are blocked in the contact space between the curved fluid channel and the curved fluid cantilever, and the unwanted Qfp fluid is released through the open Qf (**Figure 9B,C**).

4.3. When the particles are concentrated, apply pressure only to Vf.

NOTE: At this time, when pressure is applied only to Vf, the clogged particles are released through Qp (**Figure 9D**).

REPRESENTATIVE RESULTS:

Figure 8 shows the flow rate of the fluid rates for a four-stage platform operation, as mentioned in **Table 2**. The first stage is the loading state (a state). The platform was supplied with fluid with all valves open, and the working fluid (Qf) and particles (Qp) are almost identical as the microfluidic channel network exhibits structural symmetry. In the second stage (b state), compressed air was transported to Vs to block the particles, and as the Vs diaphragm deformed, the flow path narrowed, and the flow rate measured at the outlet port was reduced by hydraulic resistance. The flow rates of Qf and Qp were almost similar, and the difference was less than 2.67%. In the third stage (c state), compressed air was delivered to Vs and Vp for particle concentration, with Vs and Vp closed and Vf open. The measured Qp was close to zero, and the Qf was about 1.42 times that of the b state. In most cases, the flow rate doubles when both dissipation channels are in operation, but the platform has different types of hydraulic resistance in the main fluid channels and Vs, so the total flow of the working fluid is reduced. Finally (d state), compressed air was delivered only to Vf to collect the concentrated particles, and the flow rates of Qf and Qp were reversed. The flow was zero because Vf blocked Qf, and Qp was about 1.42 times the b state. The concentration ratio of the particles (Qp/(Qf+Qp) × 100) was 3.96-4.53. This shows that the sequential actuation programmed with the pneumatic valve works well due to flow changes.

Figure 9 shows the screen capturing concentrated particles. **Figure 9A** shows the flow state of the fluid with the three pneumatic valves not actuated, **Figure 9B** shows the method used to trap the particles, **Figure 9C** shows the sieve method, and **Figure 9D** shows the ejection of the concentrated beads. Particles were concentrated and accumulated in the collection area when Vs and Vp were closed, and all collected concentrated particles were released within 4 s when only Vf was closed. Therefore, the device successfully collects many particles suitable for particle collection and concentration.

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic diagram of a pneumatic microfluidic platform for microparticle concentration (P, port; Q, flowrate; f, fluid; p, particle; V, valve; s, sieve).

Figure 2: Assembly of the pneumatic microfluidic platform for microparticle concentration.

Figure 3: Schematic of Vs in the pneumatic microfluidic platform for microparticle concentration (P, port; Q, flowrate; f, fluid; p, particle; V, valve; s, sieve).

Figure 4: CAD image of the pneumatic microfluidic platform for microparticle concentration. (**A**)

Pneumatic channel valve. (**B**) Main fluid channel. (**C**) Interconnection fluid channel. (**D**) Cross image of each channel (For the dimensions of 1 to 7, see **Table 1**).

Figure 5: Fabrication image of the pneumatic microfluidic platform for microparticle concentration.

Figure 6: Schematic of the cross-section of the 3D fluidic channel network during fabrication. (**A**) Molds are created for the curved fluid chamber and fluid channel for replica molding. (**B**) Plasma bonding of the PDMS layer after curing to a glass wafer. (**C**) Liquid PDMS is poured into the SU-8 mold to create the interconnection channel. (**D**) The fluid chamber and fluid channel structure are arranged in liquid PDMS on the SU-8 mold. (**E**) The system is inflated by the thermal pressure of the air layer. (**F**) The inflated structure and SU-8 mold are removed.

Figure 7: Schematic of the pneumatic microfluidic platform set up for micro-particle concentration.

Figure 8: The flow rate of the fluid rates for a four-stage platform operation. The Qf and Qp working fluid flow rates following set Vf and Vp operating times (particle concentration times) in a pneumatic microfluidic platform with a Vs of 15 kPa. a–d show the pneumatic microfluidic platform operation state according to **Table 2**. (1) Sample loading, (2) Sample blocking, (3) Sample concentration, (4) Sample release.

Figure 9: Operation of the microparticle concentrator. (**A**) Before the operation. (**B**) Microparticle sieving. (**C**) Microparticle sieve completion. (**D**) Release of concentrated particles.

Table 1: Dimensions of the pneumatic microfluidic platform (1 to 7 in Figure 4).

Table 2: Pneumatic microfluidic platform operation by pneumatic valve operation, shown in Figure 8.

DISCUSSION:

This platform provides a simple way to purify and concentrate particles of various sizes. Particles are accumulated and released through pneumatic valve control, and no clogging is observed because there is no passive structure. Using this device, the concentration of particles of three sizes is presented. However, the operating pressure, the time required for concentration, and the rate may vary depending on the device dimensions, particle size magnification, and the pressure at Vs[18,20,21].

When performing step 3.1, air bubbles may remain on the curved surface of the channel. When the air bubble remains, the environment in the channel changes, so it is necessary to check the channel very carefully through a microscope before operation.

Compared with previous studies, this platform has some advantages and disadvantages. In the dielectrophoretic method, fewer target particles are used[22]. An additional process was required

to prepare particles to enhance the physical interaction between particles and external forces[22,23]. Complex design issues must be considered to increase the separation efficiency in magnetophoretic separation systems[5,22]. This platform showed higher separation efficiency than the ultrasonic method, which can separate samples at high flow rates[24]. However, because this platform does not have a passive structure, no clogging effect[25-27] was observed when beads were trapped and accumulated, unlike the passive method.[7,10] This platform can be used for water pretreatment when concentrating and extracting suspended bio-particles, as the operation is not affected by the properties of the physical particles[18,21].

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DISCLOSURES:

The authors have no conflicts of interest to disclose.

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Figure 1

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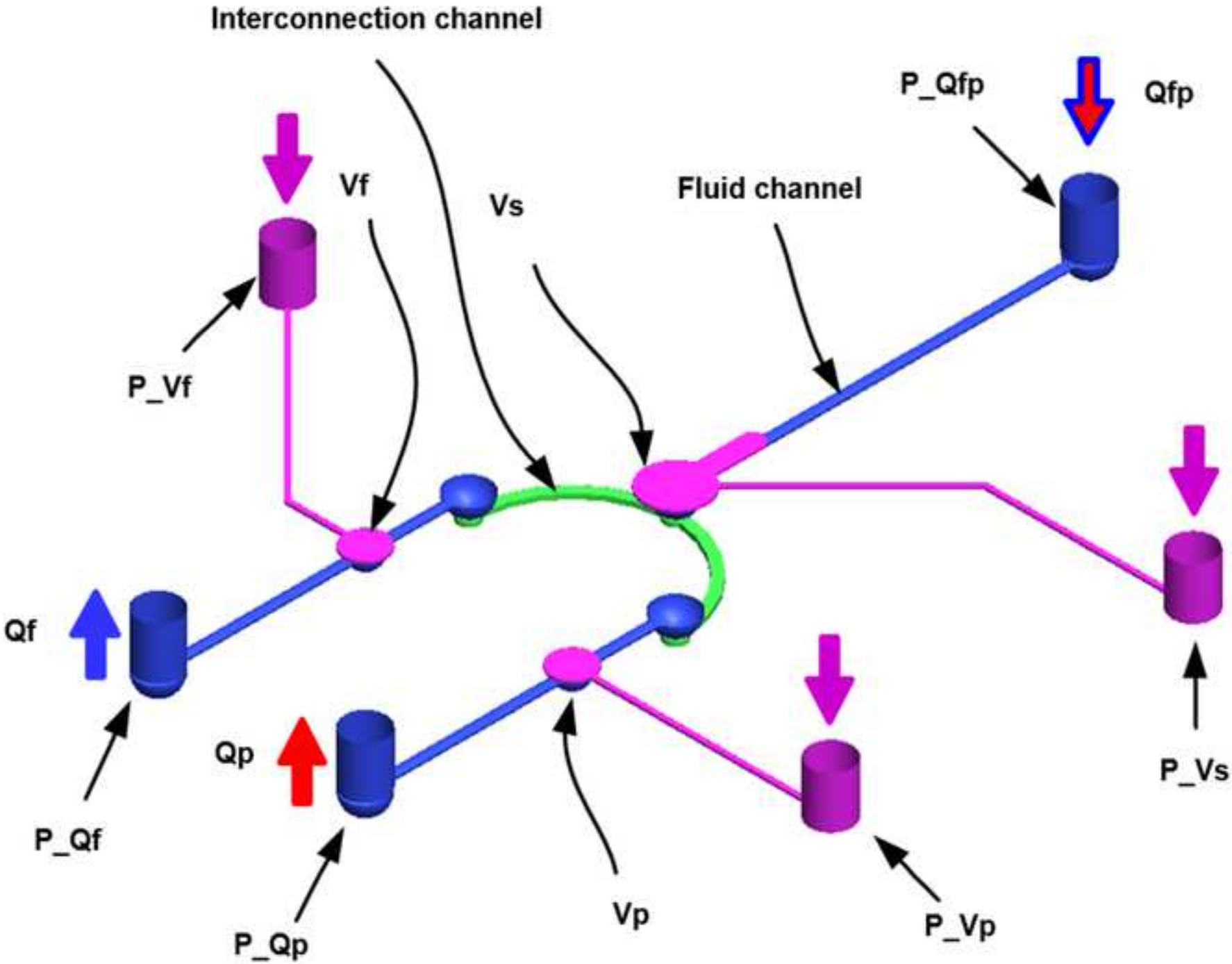

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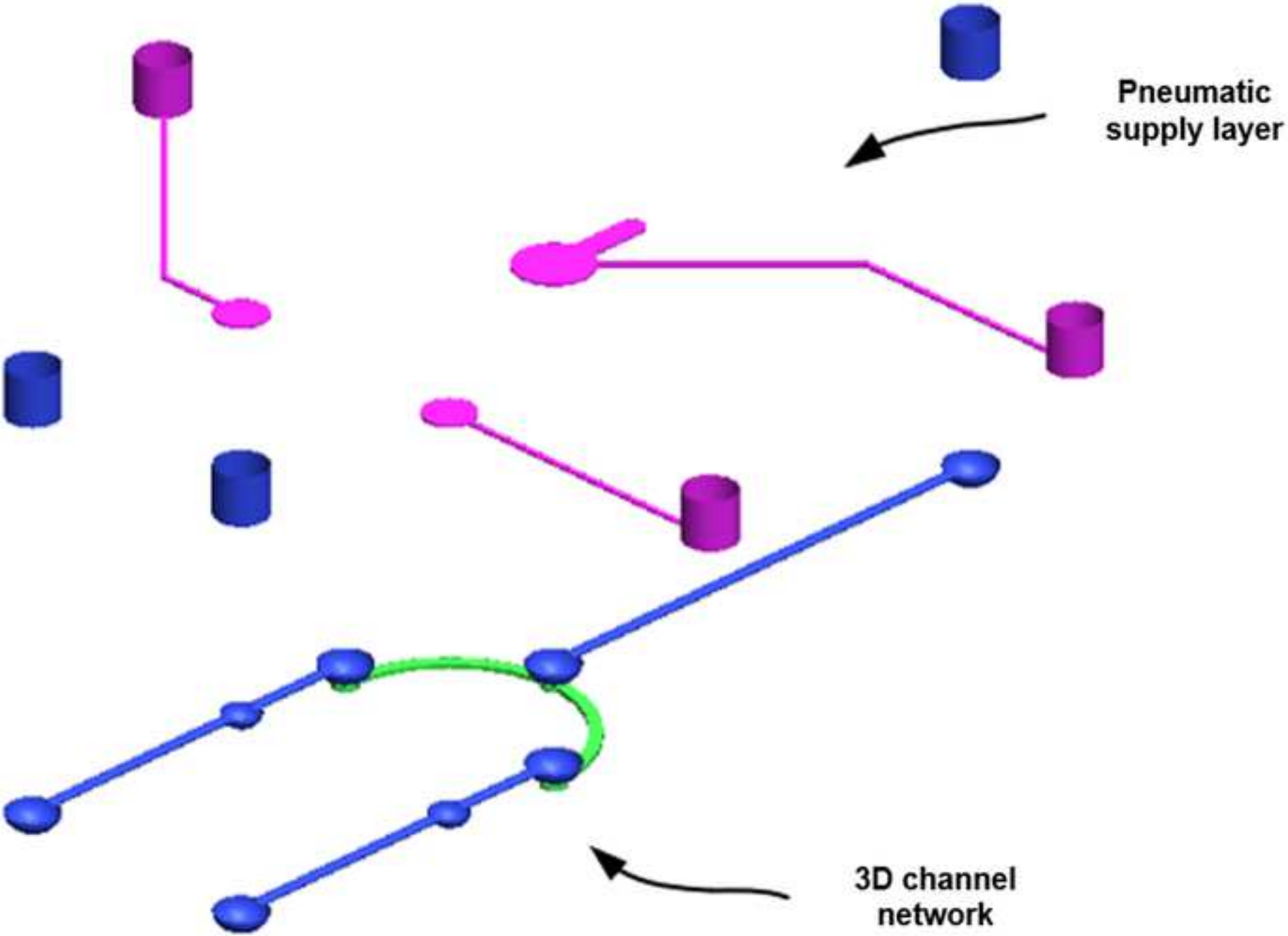

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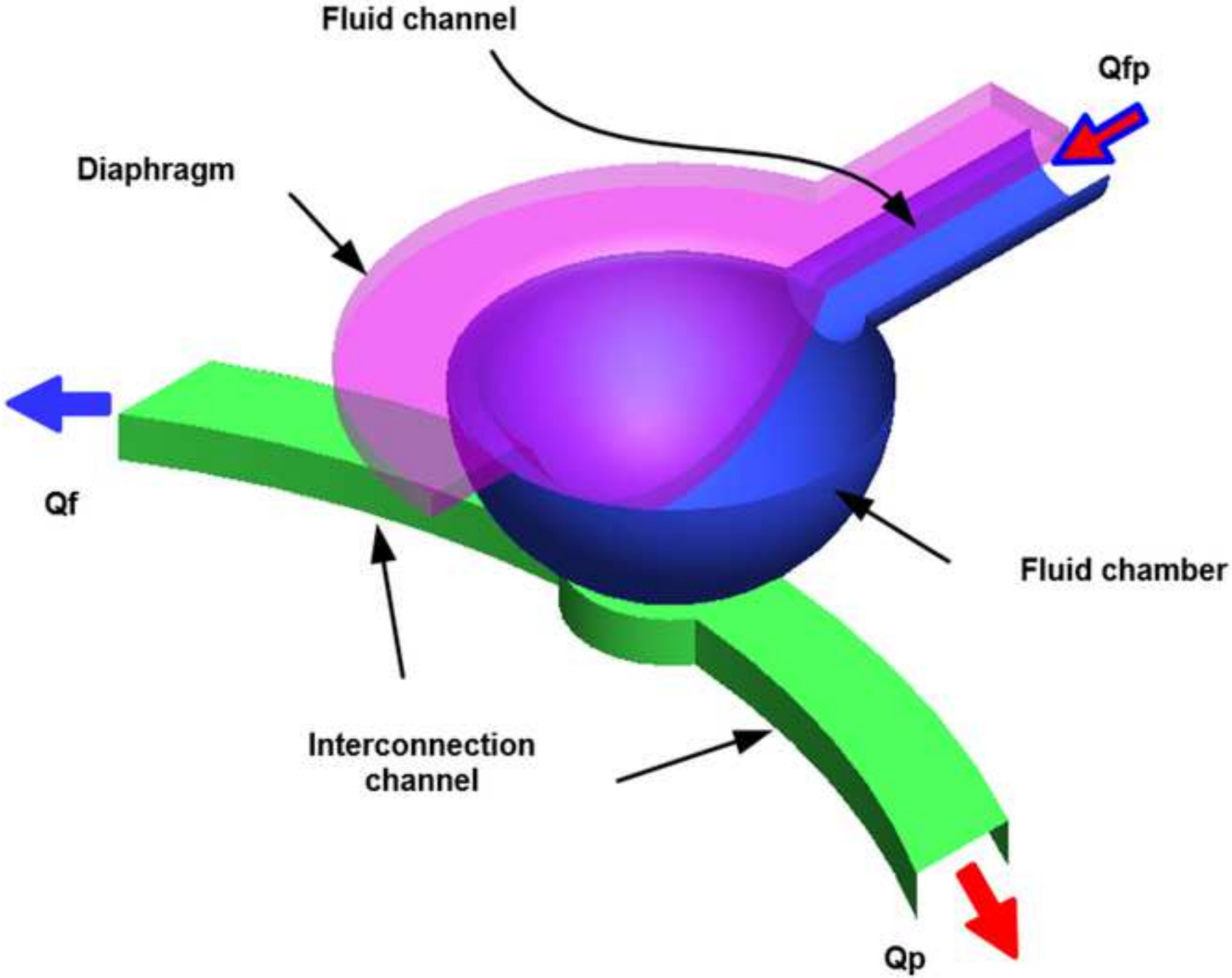

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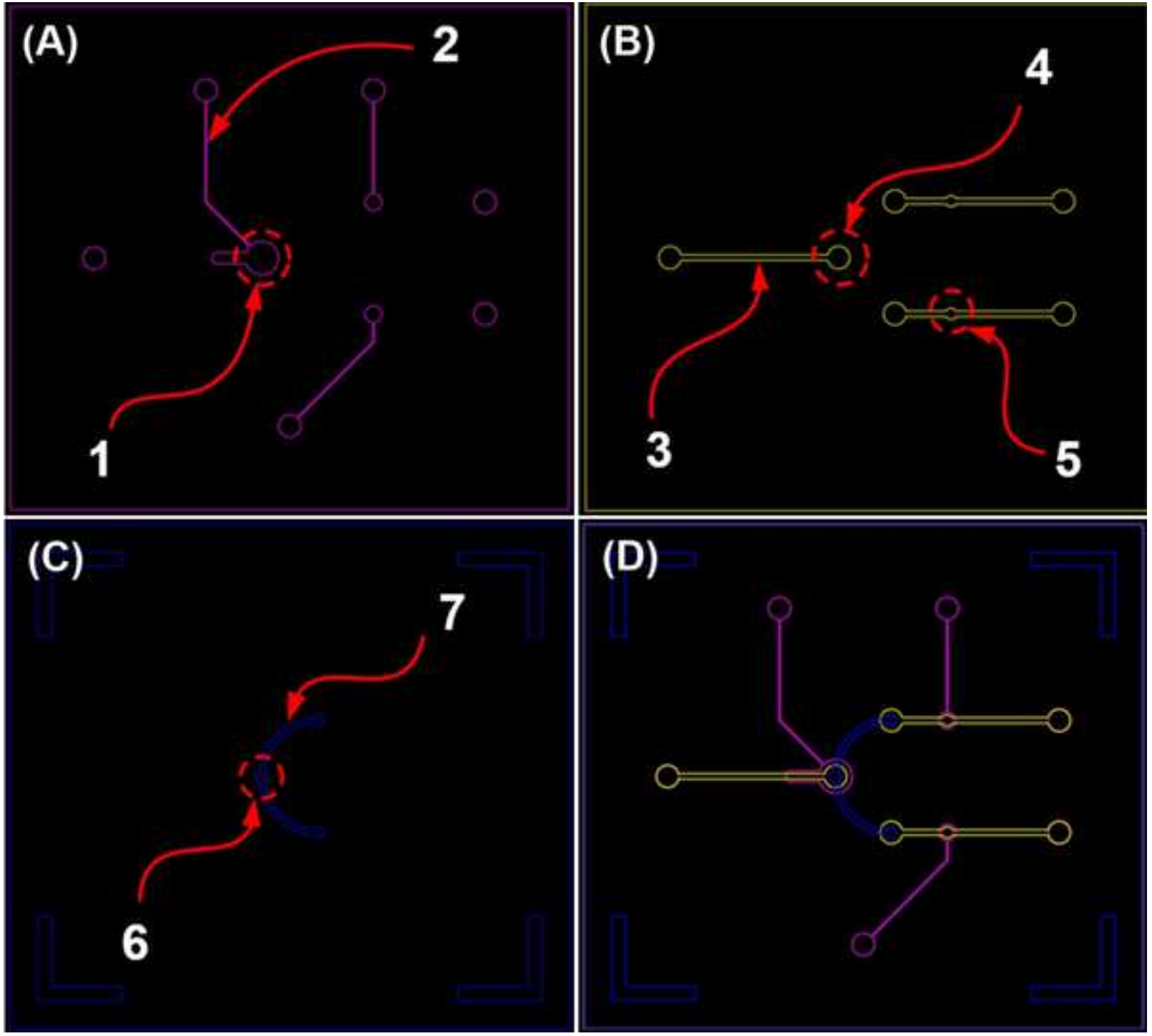

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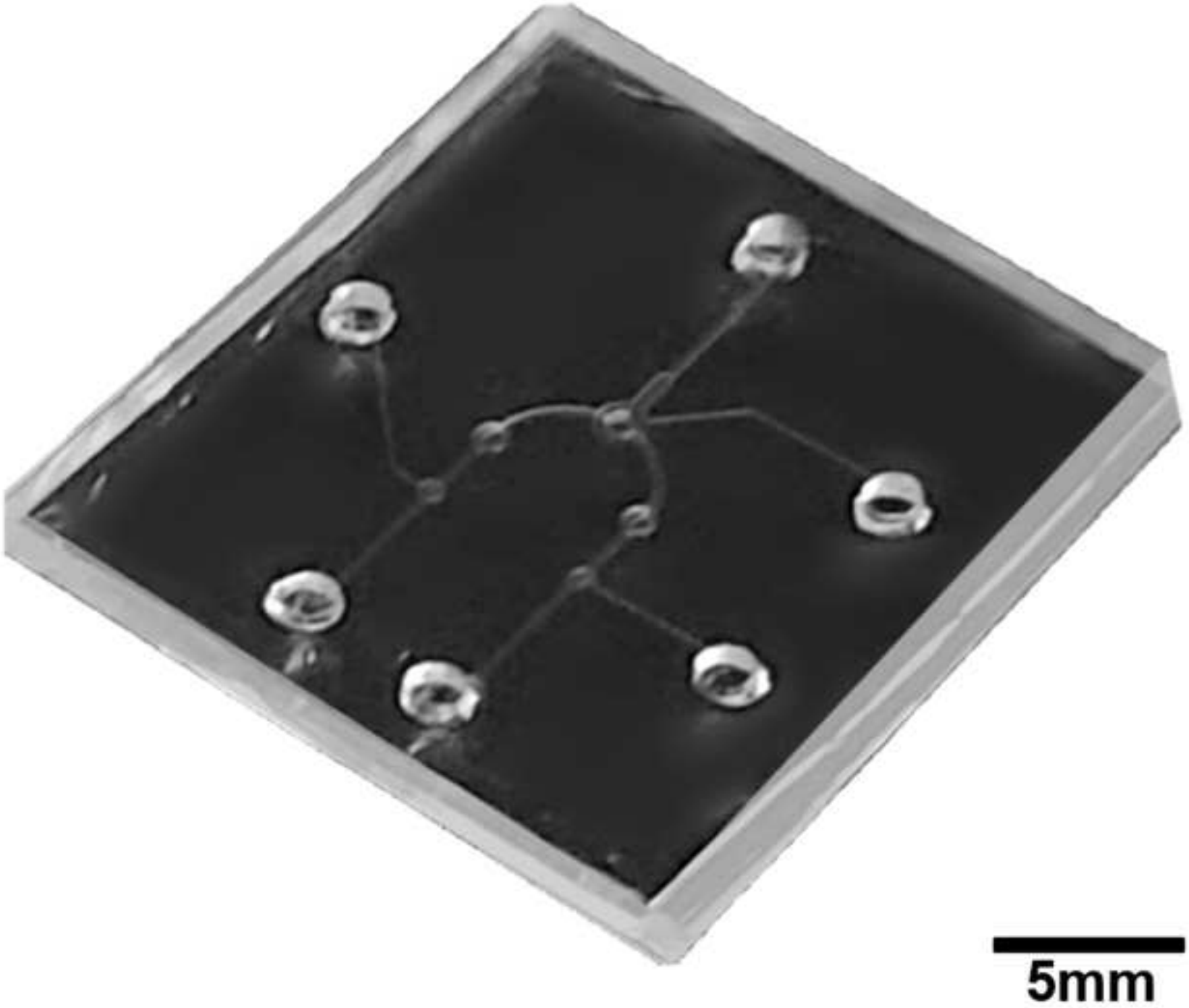

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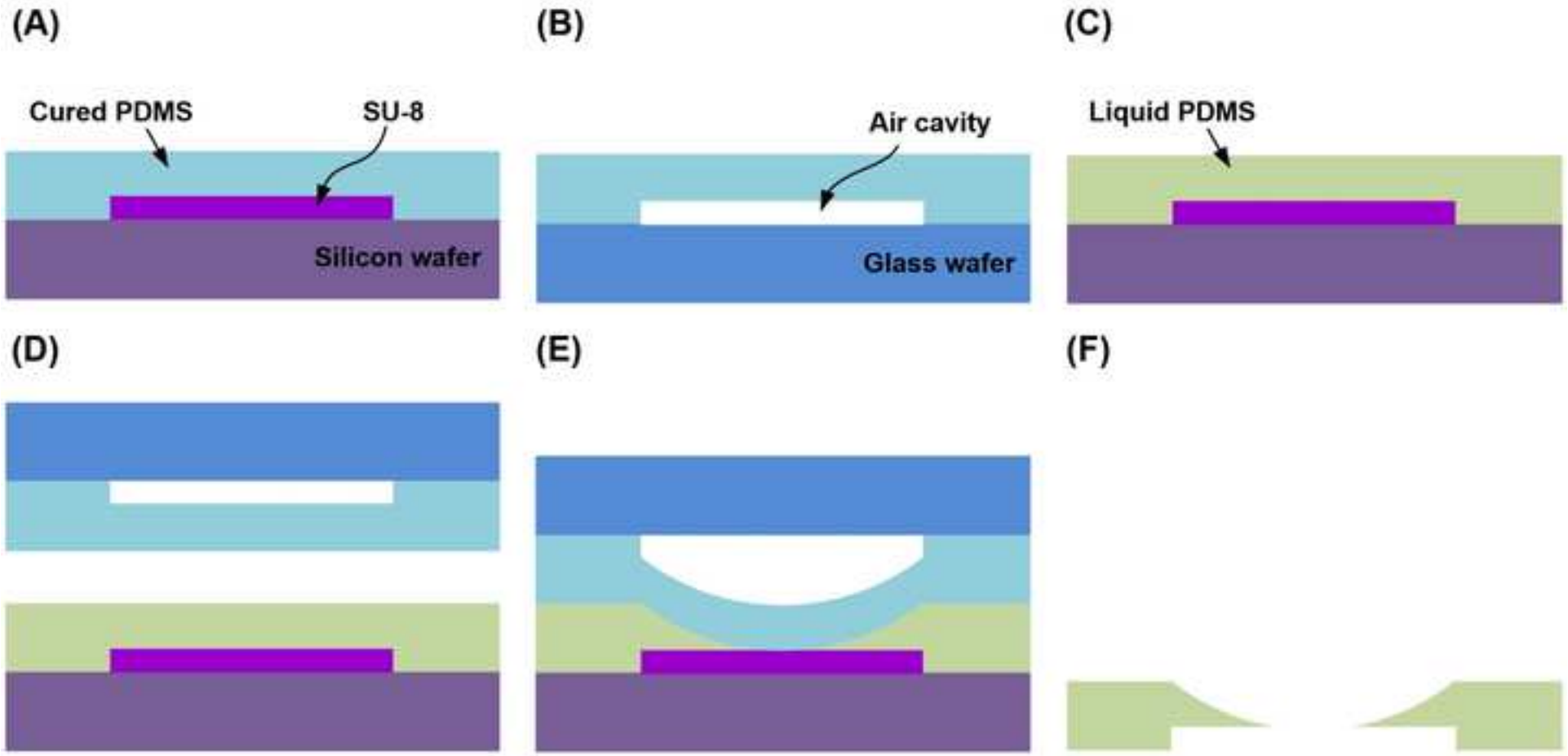

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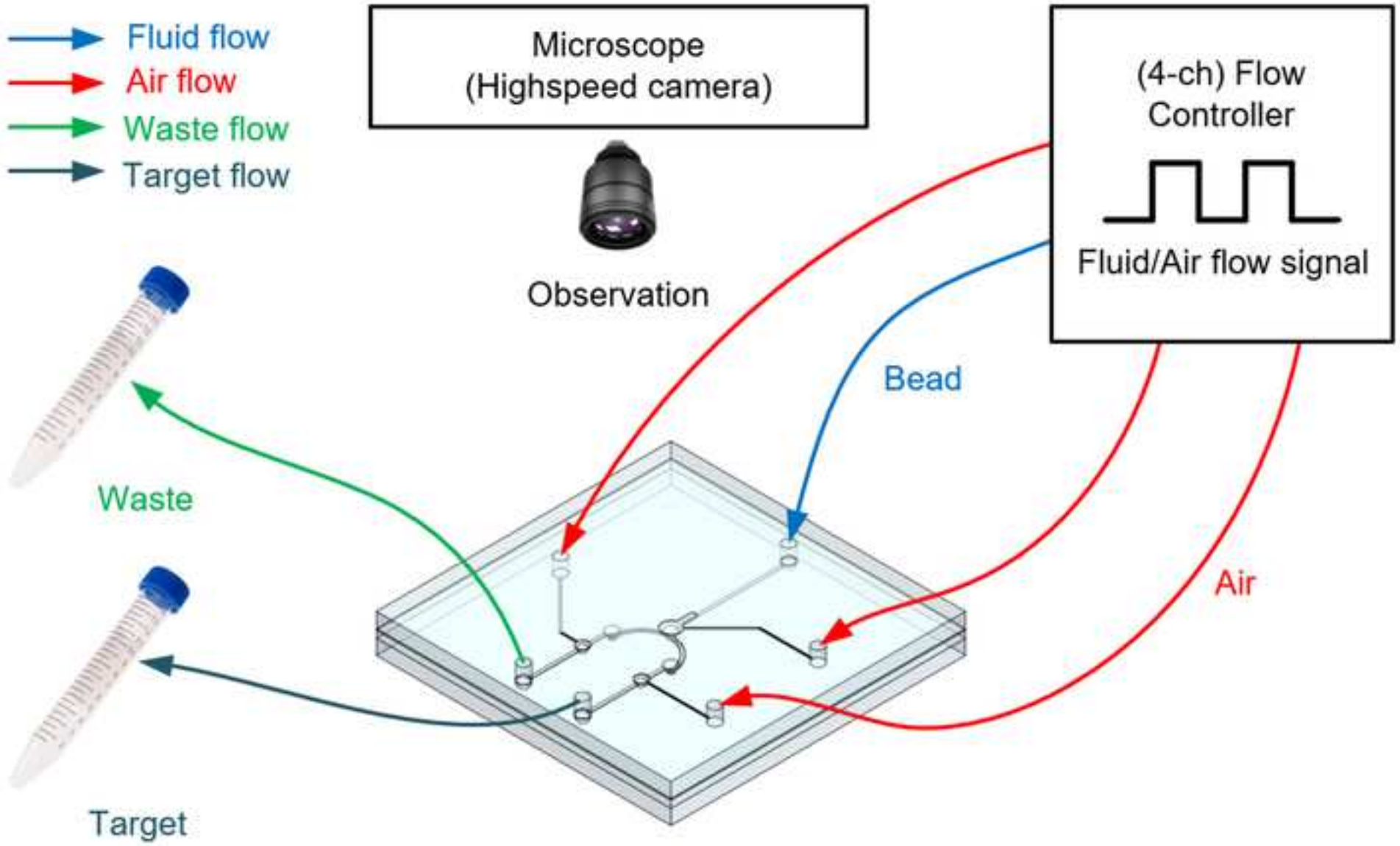

Figure 8

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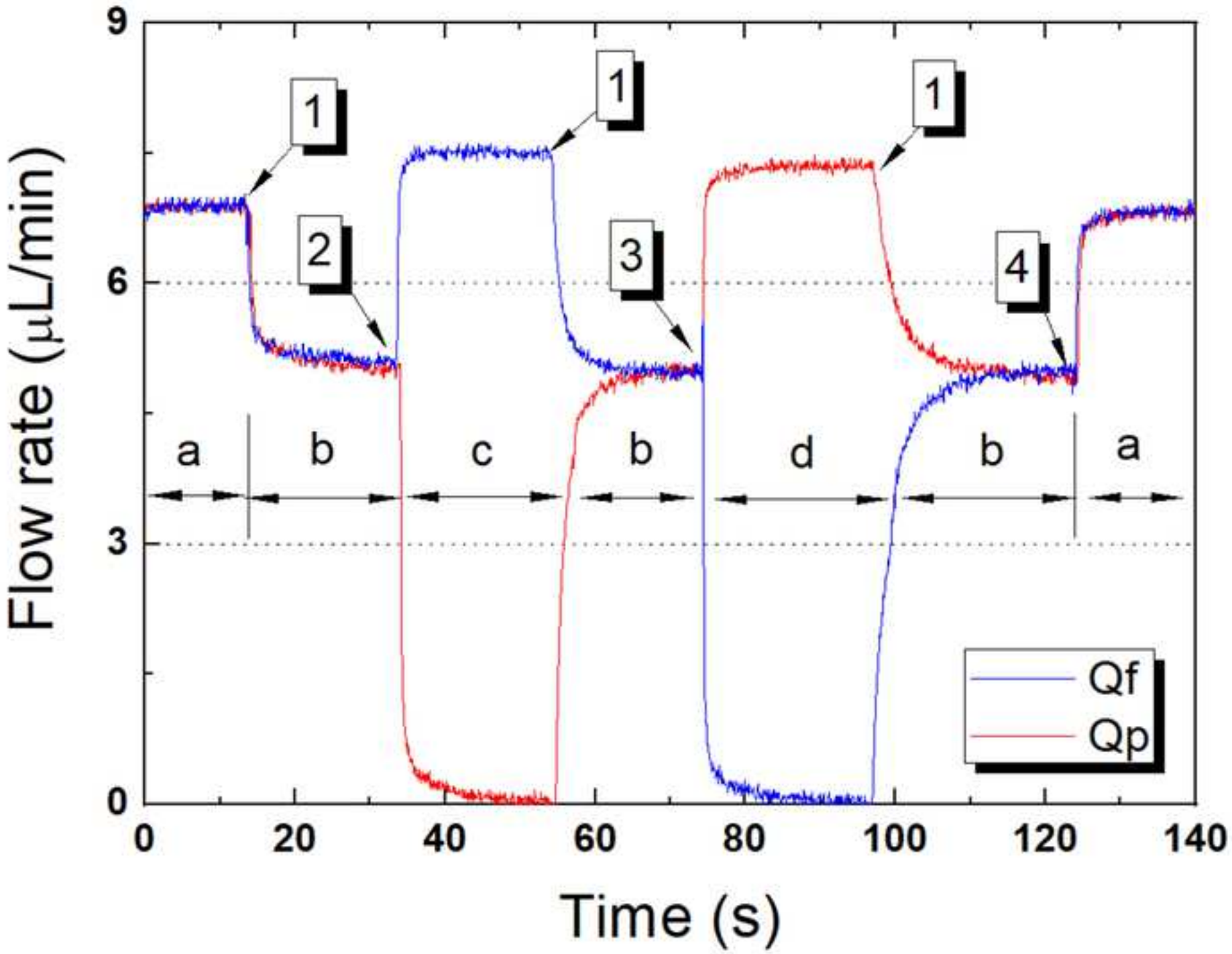

Figure 9

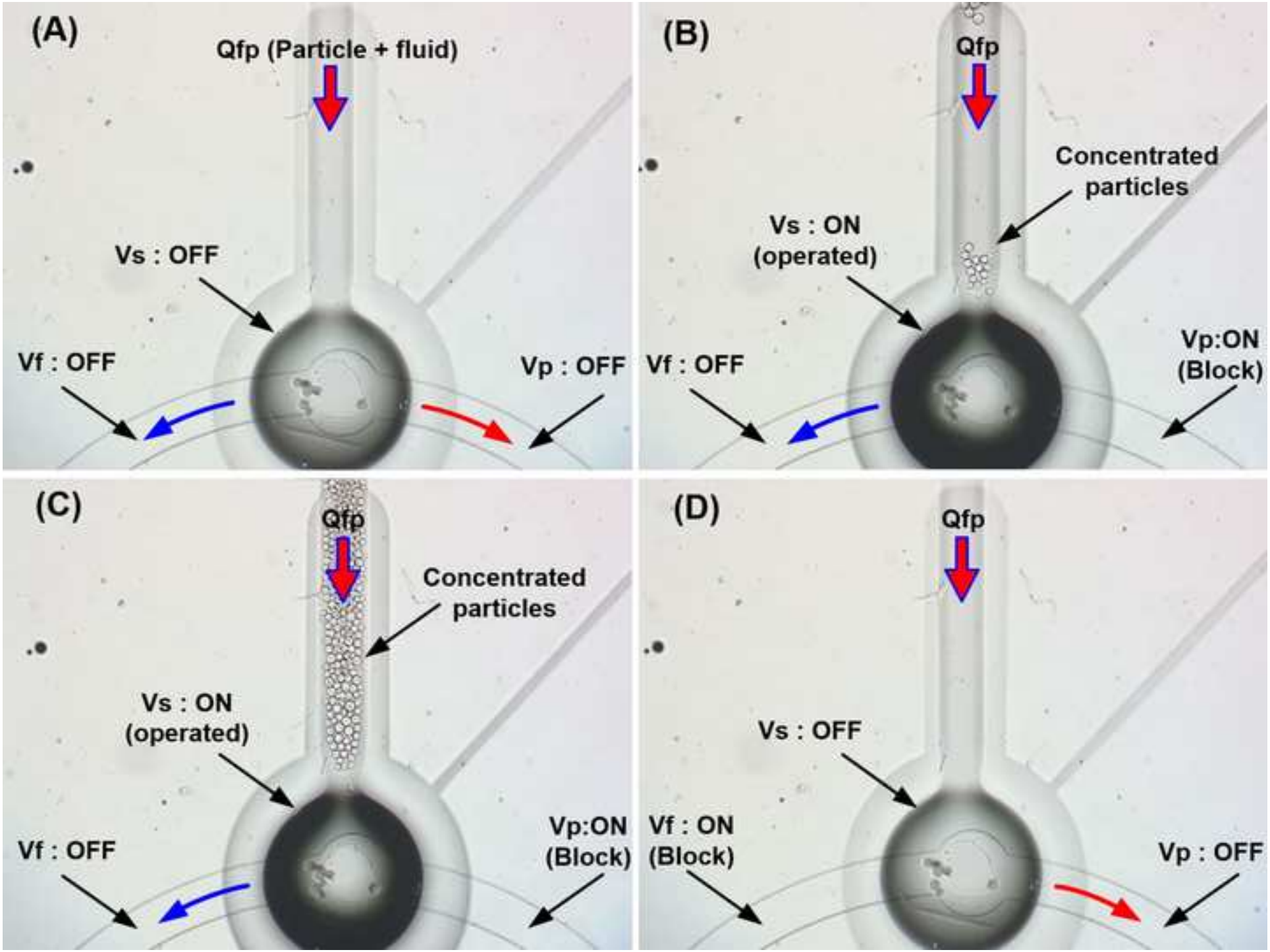

Table 1

Number	Structure	Width (W) or diameter
1	Pneumatic chamber	1200 (D)
2	Pneumatic channel	50 (W)
3	Fluid channel	200 (W)
4	Fluid chamber for Vs	800 (D)
5	Fluid chamber for Vp (Vf)	400 (D)
6	Interconnection chamber	400 (D)
7	Interconnection channel	200 (W)

r (D), (µm)

Table 2

State	Pneumatic Microfluidic Platform	Pneumatic Valve Operation			
		Signal	Vs	Vf	Vp
a	Loading	4	OFF	OFF	OFF
b	Blocking	1	ON	OFF	OFF
c	Concentration	2	ON	OFF	ON
d	Release	3	OFF	ON	OFF

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We thank you for referees' helpful comments. We carefully revised the manuscript on the basis of the reviewers' comments. We have improved our manuscript and believe that we have fully addressed the referees' recommendations.

Very truly yours,

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- **Ln 79-83:**

  Note: The fluid network is interconnected with the space of the curved oil channels in the anterior part and the rectangular chamber in the posterior part. The inlet is blocked by Vs, and particles accumulate in the collection area of the curved fluid channel. Particle-free fluids (particle-free liquids) are exited through the Qf outlet and the concentrated particles through the Qp outlet (see Figure 3).

- **Ln 202-203:**

  3.5. Observe platform operation through an inverted system microscope for all platform operation and measure the operating flow rate over time at the outlet by a liquid flow meter.

3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

[Response] The personal pronouns have been revised.

- **Ln 27, 54:** we introduce -> this article introduces
- **Ln 30:** Our devices operate -> This device operates
- **Ln 305:** Our platform -> This platform

4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your

manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.
For example: µFlucon, Spherotech, SLI 1000, Olympus

[Response] The revised manuscript is thoroughly proofread and all commercial languages are removed from our revised manuscript.

5. Please revise the Introduction to include all of the following:

a) A clear statement of the overall goal of this method

b) The rationale behind the development and/or use of this technique

c) The advantages over alternative techniques with applicable references to previous studies

d) A description of the context of the technique in the wider body of literature

e) Information to help readers to determine whether the method is appropriate for their application

[Response] Introduction steps have been modified carefully as advised.

6. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

[Response] Protocol steps have been modified carefully as advised.

7. Please add more details to your protocol steps:
Step 1: Please provide an image of the CAD file of the microfluidic device used along with the dimensions as a Supplementary Figure to help the readers understand the exact architecture of the device. Please a reference the figure in the manuscript and provide a legend having Title and description in the Figure/Table label section.

[Response] It has been modified. And, Figure 4 and Table 1 have been added in the revised manuscript.

- **Ln 74**: The microfluidic channel network is designed with a CAD program.[18,19]
- **Ln 88-90:** (see Figure 4 and Table 1). The four types of molds mentioned are fabricated using common photolithography processes. This mold making consists of a SU-8 mold on a silicon wafer. [18,19]

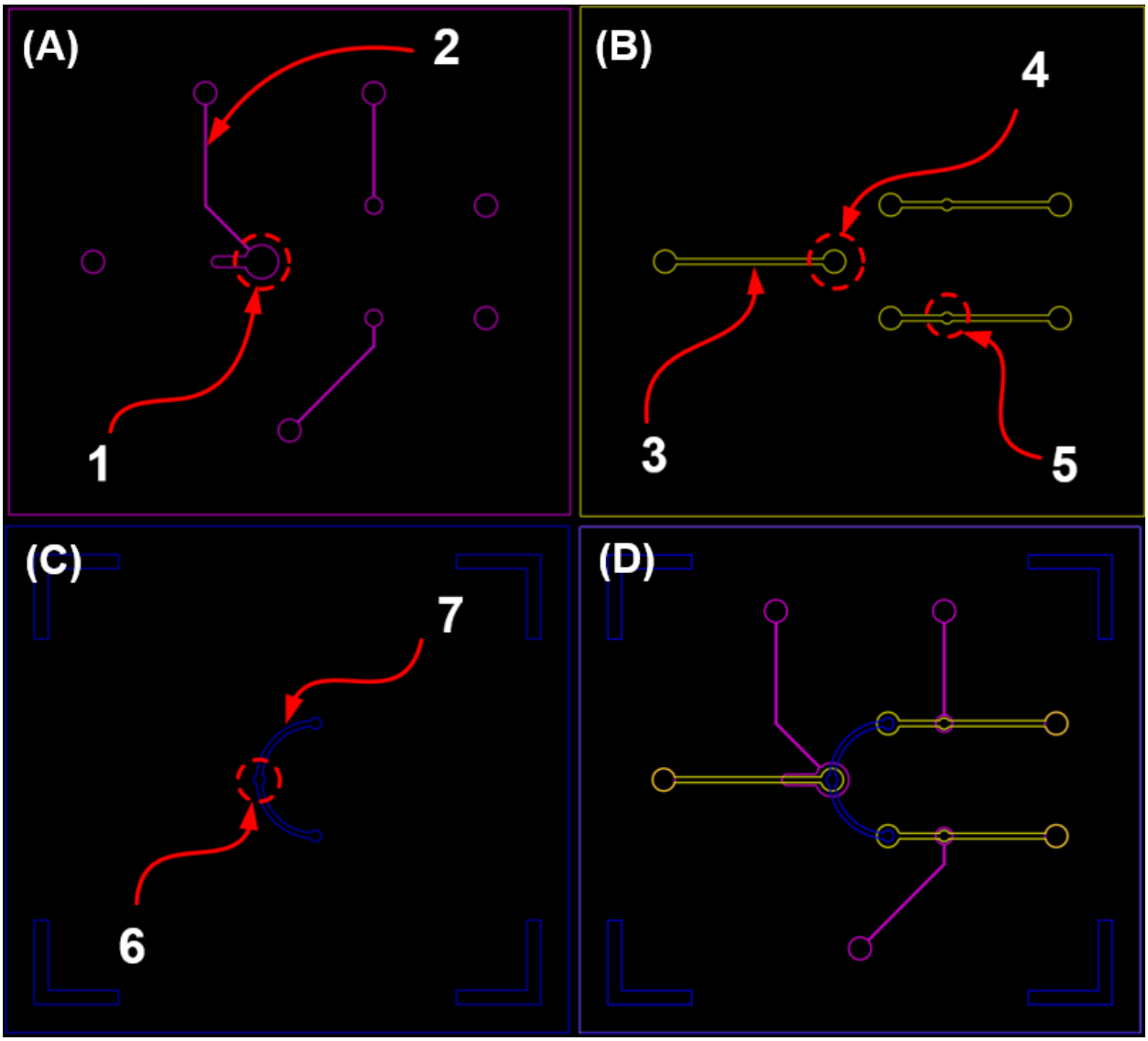

Figure 4. CAD image of the pneumatic microfluidic platform for microparticle concentration. (a) Pneumatic channel valve, (b) Main fluid channel, (c) Interconnection fluid channel, and (d) Cross image of each channel. (Dimensions of 1 to 7 in Figure 4 see Table 1).

Table 1. Pneumatic microfluidic platform detail dimensions of 1 to 7 in Figure 4.

Number	Structure	Width (W) or diameter (D), (μm)
1	Pneumatic chamber	1200 (D)
2	Pneumatic channel	50 (W)
3	Fluid channel	200 (W)
4	Fluid chamber for Vs	800 (D)

5	Fluid chamber for Vp (Vf)	400 (D)
6	Interconnection chamber	400 (D)
7	Interconnection channel	200 (W)

Step 1.3: Please mention how the SU-8 molds are created. Please include citations from published references. Also please include the details of SU-8 in the Table of Materials.

[Response] It has been modified. And, one reference has been added in the revised manuscript.

- **Ln 74**: The microfluidic channel network is designed with a CAD program.[18,19]
- **Ln 88-90:** (see Figure 4 and Table 1). The four types of molds mentioned are fabricated using common photolithography processes. This mold making consists of a SU-8 mold on a silicon wafer. [18,19]

18. Jang, J.H., Jeong, O.C. Fabrication of a pneumatic microparticle concentrator. Micromachines. 11, (1), 40 (2020).

19. McDonald, J. C., et al. Poly(dimethylsiloxane) as a material for fabricating microfluidic device. Accounts of chemical research. 35, (7), 491-499 (2002).

Step 2.1.3/2.1.7: Please mention how the punching was done. If commercial punctures are used, please include the details in the Table of Materials.

[Response] It has been inserted. See the Step 2.1.3 and 2.1,9. However, puncture is not commercial, it is not included details in the Table of Materials.

- **Ln 107-108**: 2.1.3. Punch three 1.5 mm pneumatic ports (Vs, Vf and Vp) into the pneumatic valve channel manufactured according to step 2.1.2, using a 1.5 mm puncher.
- **Ln 124-126:** 2.1.9. Punch a 1.5 mm diameter hole in the fluid channel inlet (Qfp) and fluid channel outlets (Qf and Qp) within the pneumatic channel part to which the thin diaphragm layer is bonded, using a 1.5mm puncher.

Step 2.1.6/2.2.8: How was the alighment done? Was any mask aligner used? If yes, then please include that in the Table of Materials.

[Response] It has been inserted. See the Step 2.1.6 ~ 2.1.8/2.2.8 ~ 2.2.10.

- **Ln 117-122**:

  2.1.6. Treat the atmospheric plasma to PDMS structures made in step 2.1.3 and 2.1.5.

  2.1.7. Align directly plasma-treated PDMS structures according to the channel structure with checking by microscope.

  2.1.8. Bond the aligned PDMS structures by heat-activate at 90°C for 30 min.

- **Ln 164-169:**

  2.2.8. Treat the atmospheric plasma to PDMS structures made in step 2.2.3 and 2.2.7.

  2.2.9. Align directly plasma-treated PDMS structures according to the channel structure with checking by microscope.

2.2.10. Bond the aligned PDMS structures by heat-activate at 90°C for 30 min.

Step 4.2: Please mention how much and how the pressure was applied.

[Response] It has been modified. See Step 4.2.

- **Ln 213:** 4.2. Apply pressure to Vs at 15 kPa and Vp at 18 kPa to actuate the valve.

8. Please include one line space between the protocol steps and highlight in yellow up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

[Response] It has been modified and inserted highlight in yellow about the essential steps of the protocol for the video.

9. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next and also is in-line with the Title of the manuscript. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in the imperative tense. However, the NOTEs cannot be filmed, so please do not highlight them.

[Response] Ensured.

10. Please modify the Result section to include all the observations and conclusions you can derive from the Figures. The Results section should focus on the effectiveness of your technique backed up with data.

[Response] It has been modified at representative result section

- **Ln 226-249:** Figure 8 shows the flow rate of the fluid rates for a four-stage platform operation as shown in Table 2. The first stage is the loading state, where the platform is supplied with fluid with all valves open, and the working fluid (Qf) and particles (Qp) are almost identical as the microfluidic channel network exhibits structural symmetry. In the second stage (b state), compressed air was delivered to Vs to block the particles, and as the Vs diaphragm deformed, the flow path narrowed and the flow rate measured at the outlet port was reduced by hydraulic resistance. The flow rates of Qf and Qp were very similar, and the difference was less than 2.67 %. In the third stage (c state), compressed air was delivered to Vs and Vp for particle concentration, with Vs and Vp closed and Vf open. The measured Qp was close to zero and the Qf was about 1.42 times that of the b state. In most cases, the flow rate doubles when both dissipation channels are in operation, but the platform has different types of hydraulic resistance in the main fluid channels and Vs, so the total flow of the working fluid is reduced. Finally (d state), compressed air was delivered only to Vf to collect the concentrated particles, and the flow rates of Qf and Qp were reversed. Because Qf was blocked by Vf, the flow was zero, and Qp was about 1.42 times the b state. The concentration ratio of the particles at this time ($Qp/(Qf+Qp) \times 100$) was 3.96 - 4.53. This shows that the sequential actuation programmed with the pneumatic valve works well with the result of the change in flow. Figure 9 shows the screen capturing concentrated particles. Figure 9a shows the flow state of the fluid with the three pneumatic valves not actuated, Figure 9b shows the method used to trap the particles, Figure 9c shows the sieve method, and Figure 9d shows the ejection of the concentrated beads. Particles were concentrated and accumulated in the collection area when Vs and Vp were closed, and all collected concentrated particles were released within 4 seconds when only Vf was closed. Therefore, the device

successfully collects a significant number of particles, making it a suitable platform for particle collection and concentration.

11. As we are a methods journal, please ensure that the Discussion covers the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) Significance with respect to existing methods
e) Any future applications of the technique

[Response] we revised the discussion part, and Reference.

- **Ln 307-320:** This device, only the results for the concentration of particles of three sizes are presented. However, the operating pressure, the time required for concentration, and the concentration rate may vary depending on the device dimensions, particle size magnification, and the pressure at Vs.[18,20,21] Compared with previous studies, this platform has some advantages and disadvantages. In dielectrophoretic method, fewer target particles are used.[22] An additional process was required to prepare particles to enhance the physical interaction between particles and external forces.[22,23] In order to increase the separation efficiency in magnetophoretic separation systems, complex design issues must be considered.[5,22] This platform showed higher separation efficiency than the ultrasonic method, which can separate samples at high flow rates.24 In addition, because this platform does not have a passive structure, no clogging effect[25,26,27] was observed when beads were trapped and accumulated, unlike the passive method.[7,10] This platform can be used for water pretreatment when concentrating and extracting suspended bio-particles, as the operation is not affected by the properties of the physical particles.[18,21]

5. Jung, Y., et al. Six-stage cascade paramagnetic mode magnetophoretic separation system for human blood samples. Biomedical microdevices. 12, (4), 637–645 (2010).

7. Lin, Y.H., Lee, G.B. Optically induced flow cytometry for continuous microparticle counting and sorting. Biosensors and Bioelectronics. 24, (4), 572–578 (2008).

10. Yin, D., et al. Multi-stage particle separation based on microstructure filtration and dielectrophoresis. Micromachines. 10, (2), 103 (2019).

18. Jang, J.H., Jeong, O.C. Fabrication of a pneumatic microparticle concentrator. Micromachines. 11, (1), 40 (2020).

20. Brivio, M., et al. A MALDI-chip integrated system with a monitoring window. Lab on a Chip. 5, (4), 378-381 (2005).

21. Jeong, O. C., Konishi, S. The self-generated peristaltic motion of cascaded pneumatic actuators for micro pumps. Journal of Micromechanics and Microengineering. 18, (8), 085017 (2008).

22. Taff, B.M., Voldman, J. A scalable addressable positive dielectrophoretic cell-sorting array. Analytical chemistry. 77, (24), 7976–7983 (2005).

23. Pamme, N., et al. On-chip free-flow magnetophoresis: Separation and detection of mixtures of magnetic particles in continuous flow. Journal of magnetism and magnetic materials. 307, (2), 237-244 (2006).

24. Harris, N.R., et al. Performance of a micro-engineered ultrasonic particle manipulator. Sensors and Actuators B: Chemical. 111, 481-486 (2005).

25. Yoon, Y., et al. Clogging-free microfluidics for continuous size-based separation of microparticles. Scientific reports. 6, (1), 1-18 (2016).

26. Beattie, W., et al. Clog-free cell filtration using resettable cell traps. Lab on a Chip. 14, (15), 2657–2665 (2014).

27. Cheng, Y., et al. A bubble- and clogging-free microfluidic particle separation platform with multi-filtration. Lab on a Chip. 16, (23), 4517–4526 (2016).

12. Figure 4: Please include a scale bar.

[Response] The scale bar for images has been inserted.

13. Figure 5/7: Please label the figure panels (A), (B), (C), etc., in capital letters. Accordingly, please upload revised Figures in your editorial manager account.

[Response] we revised the Figure 5/7 panels in capital letters.

14. Please upload each figure individually in the Editorial Manager account. Please ensure a high resolution for each figure.
[Response] It has been modified as advised.

15. Please upload Table 1 as xls/xlsx file instead of embedding it in the manuscript text.

[Response] We provide the additional Tables which are formed with .xlsx.

16. Please ensure that the Table of Materials includes all the supplies (reagents, chemicals, instruments, equipment, software, etc.) used in the study.
[Response] Ensured.

Reviewers' comments

Reviewer #1

In this article, authors have reported a Pneumatically driven microfluidic platform for micro-particle concentration. My review decision is to accept with revisions as per the comments.

1. I would suggest authors to do more in-depth analysis along with different operation configurations in detail.
[Response] It has been modified at representative results

- **Ln 226-249:** Figure 8 shows the flow rate of the fluid rates for a four-stage platform operation as shown in Table 2. The first stage is the loading state, where the platform is supplied with fluid with all valves open, and the working fluid (Qf) and particles (Qp) are almost identical as the microfluidic channel network exhibits structural symmetry. In the second stage (b state), compressed air was delivered to Vs to block the particles, and as the Vs diaphragm deformed, the flow path narrowed and the flow rate measured at the outlet port was reduced by hydraulic resistance. The flow rates of Qf and Qp were very similar, and the difference was less than 2.67 %. In the third stage (c state), compressed air was delivered to Vs and Vp for particle concentration, with Vs and Vp closed and Vf open. The measured Qp was close to zero and the Qf was about 1.42 times that of the b state. In most cases, the flow rate doubles when both dissipation channels are in operation, but the platform has different types of hydraulic resistance in the main fluid channels and Vs, so the total flow of the working fluid is reduced. Finally (d state), compressed air was delivered only to Vf to collect the concentrated particles, and the flow rates of Qf and Qp were reversed. Because Qf was blocked by Vf, the flow was zero, and Qp was about 1.42 times the b state. The concentration ratio of the particles at this time (Qp/(Qf+Qp) × 100) was 3.96 - 4.53. This shows that the sequential actuation programmed with the pneumatic valve works well with the result of the change in flow. Figure 9 shows the screen capturing concentrated particles. Figure 9a shows the flow state of the fluid with the three pneumatic valves not actuated, Figure 9b shows the method used to trap the particles, Figure 9c shows the sieve method, and Figure 9d shows the ejection of the concentrated beads. Particles were concentrated and accumulated in the collection area when Vs and Vp were closed, and all collected concentrated particles were released within 4 seconds when only Vf was closed. Therefore, the device successfully collects a significant number of particles, making it a suitable platform for particle collection and concentration.

2. More data from experimental analysis should be included.
[Response] It has been modified at representative results and discussion. More data from experimental results has inserted in the additional result part, and comparative analysis in the discussion part.

3. A comparative graph of the authors system to that of already existing system should be mentioned. Also, should explain how this system performance is better compared to the already existing system.

[Response] It has been modified at discussion for compare of this system to that of already existing system.

- **Ln 308-318:** Compared with previous studies, this platform has some advantages and disadvantages. In dielectrophoretic method, fewer target particles are used.[22] An additional process was required to prepare particles to enhance the physical interaction between particles and external forces.[22,23] In order to increase the separation efficiency in magnetophoretic separation systems, complex design issues must be

considered.[5,22] This platform showed higher separation efficiency than the ultrasonic method, which can separate samples at high flow rates.24 In addition, because this platform does not have a passive structure, no clogging effect[25,26,27] was observed when beads were trapped and accumulated, unlike the passive method.[7,10]

Reviewer #2

Manuscript Summary:

The authors describe the fabrication of a microfluidic device on a PDMS-based platform that makes use of a deformable membrane to block the flow of particles carried by a flow stream. This effectively results in a sieving mechanism that concentrates the particles in the main channel. The particles can be released into a channel different from the discharge channel by differential valve actuation. This is likely a useful device for particle and cell separation applications.

Major Concerns:

1. It is necessary to provide black and white, 2-dimensional drawings with specifications for each of the mold layers. The objective of the article is to facilitate construction; provide the masks' drawings to prepare the SU-8 molds. This is the main obstacle for someone to replicate a functional device when following the instructions.

[Response] It has been inserted. See the Figure 4 and Table 1.

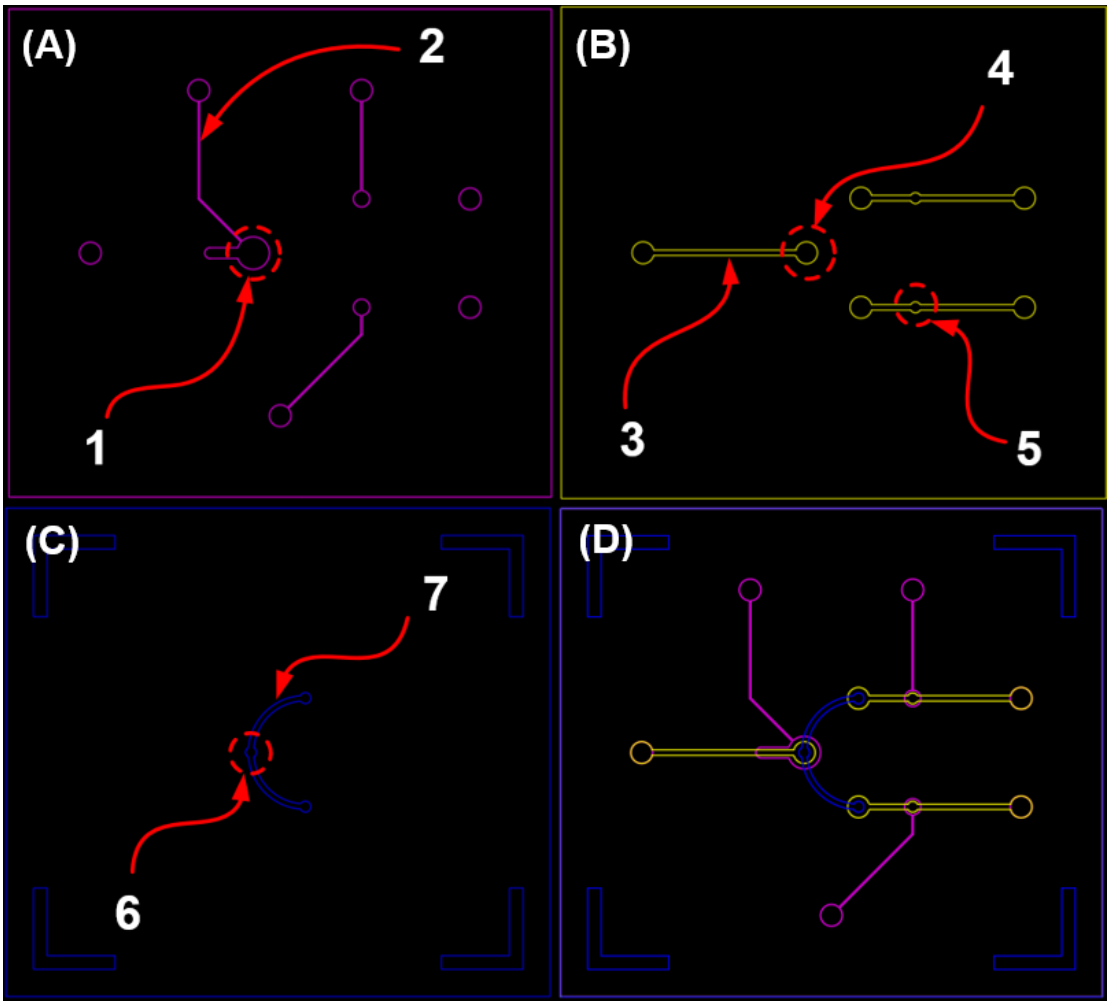

Figure 4. CAD image of the pneumatic microfluidic platform for microparticle concentration. (a) Pneumatic channel valve, (b) Main fluid channel, (c) Interconnection fluid channel, and (d) Cross image of each channel. (Dimensions of 1 to 7 in Figure 4 see Table 1).

Table 1. Pneumatic microfluidic platform detail dimensions of 1 to 7 in Figure 4.

Number	Structure	Width (W) or diameter (D), (μm)
1	Pneumatic chamber	1200 (D)
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3	Fluid channel	200 (W)
4	Fluid chamber for Vs	800 (D)
5	Fluid chamber for Vp (Vf)	400 (D)
6	Interconnection chamber	400 (D)
7	Interconnection channel	200 (W)

2. The number 2 section of the instructions is naturally complicated. It would probably be useful to assign numbers to the layers to make it clearer when referring to each one of them.

[Response] The number 2 section has been modified to assign numbers to the step layers to make it clearer when referring to each one of them.

3. Use of the precision pressure controller is only lightly explained. Perhaps an image of the set up is necessary.

[Response] It has been inserted at Figure 7.

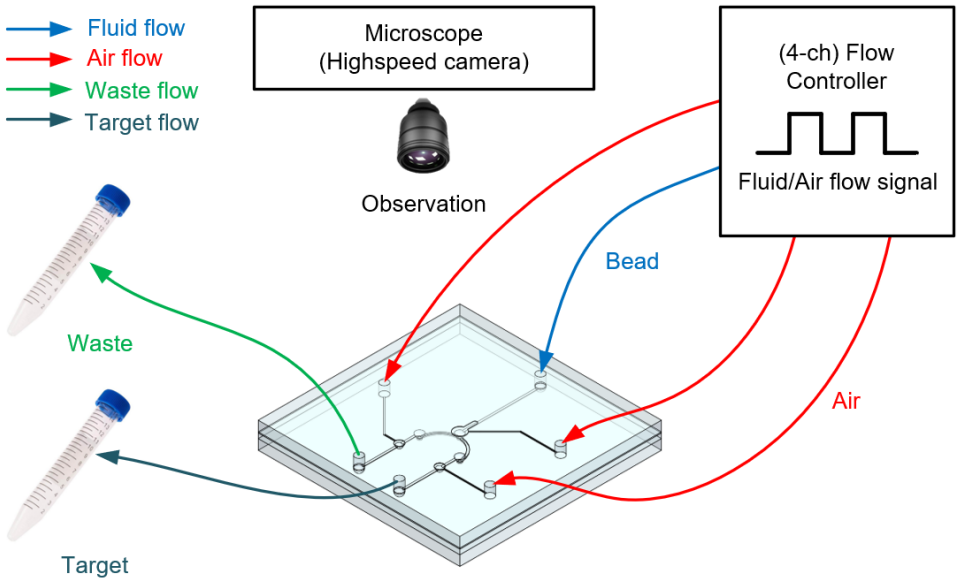

Figure 7. Schematic of pneumatic microfluidic platform set up for micro-particle concentration.

Minor Concerns:

1. Phrasing in some instances can be improved to ensure proper communication and understanding by the reader (e.g. line 227-228) Overall, is understandable.

[Response] It has been revised the discussion part, and reference.

- **Ln 307-320:** This device, only the results for the concentration of particles of three sizes are presented. However, the operating pressure, the time required for concentration, and the concentration rate may vary depending on the device dimensions, particle size magnification, and the pressure at Vs.[18,20,21] Compared with previous studies, this platform has some advantages and disadvantages. In dielectrophoretic method, fewer target particles are used.[22] An additional process was required to prepare particles to enhance the physical interaction between particles and external forces.[22,23] In order to increase the separation efficiency in magnetophoretic separation systems, complex design issues must be considered.[5,22] This platform showed higher separation efficiency than the ultrasonic method, which can separate samples at high flow rates.24 In addition, because this platform does not have a passive structure, no clogging effect[25,26,27] was observed when beads were trapped and accumulated, unlike the passive method.[7,10] This platform can be used for water pretreatment when concentrating and extracting suspended bio-particles, as the operation is not affected by the properties of the physical particles.[18,21]

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18. Jang, J.H., Jeong, O.C. Fabrication of a pneumatic microparticle concentrator. Micromachines. 11, (1), 40 (2020).

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25. Yoon, Y., et al. Clogging-free microfluidics for continuous size-based separation of microparticles. Scientific reports. 6, (1), 1-18 (2016).

26. Beattie, W., et al. Clog-free cell filtration using resettable cell traps. Lab on a Chip. 14, (15), 2657–2665 (2014).

27. Cheng, Y., et al. A bubble- and clogging-free microfluidic particle separation platform with multi-filtration. Lab on a Chip. 16, (23), 4517–4526 (2016).

2. From Abstract, this sentence is unclear, does it refer to particle SIZE? "The working pressure, time required for concentration, and rate of concentration depend on the particle magnification"

[Response] The above sentence mentions that when the technology we introduce is applied, the operating pressure, the time required for concentration and the concentration rate may vary depending on the device dimensions and particle size magnification. To avoid confusion, the sentence has been modified, and additional sentences and references related to this content have been inserted in the discussion part.

- **Ln 35-37:** When this technology is applied, the operating pressure, the time required for concentration, and the concentration rate may vary depending on the device dimensions and particle size magnification.
- **Ln 307-310:** This device, only the results for the concentration of particles of three sizes are presented. However, the operating pressure, the time required for concentration, and the concentration rate may vary depending on the device dimensions, particle size magnification, and the pressure at Vs.[18,20,21]

3. The use of the word rubber in line 58 is a bit confusing. Rubber is not mentioned again the document.

[Response] Rubber of line 59 has been modified to PDMS.

4. Step 2.2.4 is somewhat unusual so it needs a good explanation. The choice of colors in figure 5 seems wrong, the green color changing to blue from panel f to the next?

[Response] Previously, in Figure 5, Cured PDMS was shown in blue and Liquid PDMS in green, but the color of F was changed to green to avoid confusion (see Figure 6).

5. The legend in figure 6 has a last sentence in parenthesis referring to figure 7, but without using the number 7, as if figure 7 was part of figure 6.

[Response] The sentence has been modified.

- **Ln 287-288:** (Figures 8 a–d show the state of the pneumatic microfluidic platform operation according to Table 2).

6. Line 224. There can be confusion with this sentence, since the device seems to concentrate all particles at the same time, with no size discrimination. If this can be changed by changing the pressure at Vs, it should be noted clearly.

[Response] It has been modified the discussion part, and Reference.

- **Ln 307-310:** This device, only the results for the concentration of particles of three sizes are presented. However, the operating pressure, the time required for concentration, and the concentration rate may vary depending on the device dimensions, particle size magnification, and the pressure at Vs.[18,20,21]

20. Brivio, M., et al. A MALDI-chip integrated system with a monitoring window. Lab on a Chip. 5, (4), 378-381 (2005).

21. Jeong, O. C., Konishi, S. The self-generated peristaltic motion of cascaded pneumatic actuators for micro pumps. Journal of Micromechanics and Microengineering. 18, (8), 085017 (2008).

7. Table 1 -state d- is wrong. It says that Vs is ON, but it should say OFF.

[Response] Table 2 - State d- Vs pressure has been modified to OFF.