Manipulating liquid plugs in microchannel with controllable air vents

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An air venting element on microchannel, which can be controlled externally and automatically, was demonstrated for manipulating liquid plugs in microfluidic systems. The element's open and closed statuses correspond to the positioning and movement of a liquid plug in the microchannel. Positioning of multiple liquid plugs at an air venting element enabled the merging and mixing of the plugs. Besides these basic functions, other modes of liquid plug manipulations including plug partitioning, multiple plug mixing, and spacing adjustment between liquid plugs, were realized using combination of multiple elements. The structure, operation, and some functions of the element were demonstrated with a microfluidic chip application. The performances of the element including its failure modes, threshold flow rate, and structural optimization were also discussed. © 2012 American Institute of Physics. [doi:[10.1063/1.3686878](https://doi.org/10.1063/1.3686878)]

I. INTRODUCTION

Microfluidic system is considered to be a promising technology for handling small volumes of liquid in microchannels and reactors for biological, medical, and chemical discovery and applications. It has benefits such as reduced sample consumption, decreased analysis time, increased portability and integration, and high throughput ability.^{1–5} In the past two decades, many active and passive microfluidic components (such as pumps, valves, and mixers), fluidic platforms (such as capillary-driven platform, centrifugal microfluidics, droplet based microfluidics, and pressure-driven systems including microfluidic large scale integration (LSI) etc.) have been developed.^{6–10} The pressure-driven systems are among the most versatile and some have even been commercialized. However, the ability of manipulating small liquid plugs in microchannels is still limited. For example, in a simple task of merging two liquid plugs, e.g., 5 μ l of sample with 5 μ l of reagent in microchannels, liquid plugs are usually combined at a Y junction channel. If one plug from a branch reaches the Y junction before the other one, an air bubble between the two plugs is trapped and they cannot merge together. Thus synchronization of the two plugs for merging is important when using such technique. The synchronization can be accomplished using position or speed sensors, speed control, or by precise balancing of pressures and capillary forces.¹¹ However, these methods are complicated and are not robust. An approach that bypasses the synchronization is the microfluidic LSI which uses pressure to squeeze air bubble into the polydimethylsiloxane (PDMS) chip material which is semi-permeable to gases. However, such an approach has material and volume constraint, and is time consuming. We ascribed the lack of manipulating ability to the lack of powerful control units other than simple valves in the microchannel network.

Air vents for macro pipeline systems such as drain-waste-vent system in plumbing have been used for centuries for releasing air in liquids. However, the application of air vent in microfluidic systems is still rare. One application example reported recently is the use of an air vent as a chip-integrated valve for positioning of a liquid plug.¹² We found that the air vents

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FIG. 1. Positioning and merging of two liquid plugs in a microchannel with an air venting element. (a) and (b) Positioning of a liquid plug at the air venting element. (c)–(e) Merging of two liquid plugs at the position. (f) Merged plug moving forward when the air venting element is closed externally.

can be used in microfluidic systems for accomplishing more versatile functions than the basic positioning function. Instead of acting only as an auxiliary element, combination of multiple air vents in microchannels has the potential to be developed into a microfluidic platform technology.

Fig. 1 illustrates the principle of merging two liquid plugs using an air venting element. The air venting element has two statuses: open and closed which were controlled by an external valve. When the element is open, the liquid plug, driven by air pressure, can pass through the element and then stops with the rear of the plug aligned with the element, since air was bypassed out of the channel through the venting window above the channel, as shown in Fig. 1(b). If a second liquid plug is driven to the air venting element, this plug will merge with the first plug and is then positioned the same as the first. The merging happens because the first plug keeps its position when the second plug is approaching, as shown in Figs. 1(c)–1(e). At any time if the air venting element is closed, the liquid plug moves forward along the channel, as shown in Fig. 1(f). Besides the above functions, combinations of multiple air venting elements and the control of their open and closed statuses enabled us to manipulate liquid plugs in microchannels in various ways such as partitioning, spacing, and distribution, etc. In addition to its versatility of manipulating liquid plug, the air venting element is structurally simple and is easy to implement in microchannel fabrication. Also, the technique facilitates automation, e.g., with external solenoid valves. Combining these abilities, higher level of complexity and integrity of microfluidic platform can be realized.

In this paper, we demonstrated the design, fabrication, and use of the air venting elements for manipulation of liquid plugs in microchannels. Upon test and analysis of failure modes of the air venting element, structural optimization and directions for improving the performance were discussed. Then a pressure-driven microfluidic chip utilizing 4 air venting elements for polymerase chain reaction (PCR) sample preparation was presented as an example of application.

II. EXPERIMENTAL

A. Structure of the air venting element

A photo of an air venting element built with a microchannel on a chip is shown in Fig. 2(a). The air venting element together with the chip is made of polymethyl methacrylate

FIG. 2. (a) A photo of an air venting element with fitting and tubing for external control of the opening and closing of the element. (b) A photo of an air venting element without tubing and fitting. (c) Structural diagram of the air venting element.

(PMMA). The structure of the element is illustrated in Fig. 2(c). There is a rectangular shape venting window located above a micro channel. The channel under the window generally has a narrow profile for preventing liquid from flowing up into the venting window. A fitting was placed above the venting window for tubing connection. The Teflon tubing is connected to an air valve for easy control of the opening and closing of the air venting element. A photo of the air venting element without fitting and tubing is shown in Fig. 2(b) for better demonstration of the microchannel and the venting window. In such a case, the closing of the element can be achieved by covering the venting window with a Scotch tape or other sealing methods. The channel within the element demonstrated here has a cross-sectional dimension of $0.5 \text{ mm} \times 1 \text{ mm}$ which is suitable for manipulating liquid sample in microliter range. Smaller channels and air venting elements can be built to handle sample in nano-liter range using the same principle and structure. We have also tested microchannels with smaller sizes such as $200 \mu\text{m} \times 200 \mu\text{m}$.

B. Design and fabrication of a multiple-mixing chip

As a demonstration of multiple functions of the air venting element, a multiple-mixing chip containing four air vents was fabricated and tested. An application of the chip is for sample preparation for multiple PCRs. Inside the chip, DNA sample is divided into plugs and then

FIG. 3. Layout of the multiple-mixing chip for PCR preparation. (Ave1-Ave4): Air venting elements. (Ain1-Ain3): Air pressure inlets. (Pin1-Pin3): Primer solution inlets.

mixed with different primer pairs in sequence, followed by spacing adjustment of the mixed plugs. A structural layout of the chip is shown in Fig. 3. The microchannels in the chip include a sample dispensing channel, primer dispensing channels, and a main channel which contains three sections: a plug partitioning section, a merging section, and a spacing-mixing section. There are four air venting elements Ave1 ~ Ave4 located on the main channel for the functionality of the chip. The position and number of the air venting elements were chosen according to the functionality of the multiple-mixing chip. Among the four elements used, Ave1 and Ave2 are used for partition of a $15\ \mu\text{l}$ liquid plug into 3 plugs of $5\ \mu\text{l}$ each. Ave3 and Ave4 are used for merging and positioning of sample plugs with primer plugs. Since the main channel in the chip is 1 mm deep and 0.5 mm wide, the distance between Ave1 and Ave2 is 10 mm to measure out $5\ \mu\text{l}$, and the distance between Ave2 and the nearest Y junction is also 10 mm. In this example, 2 venting elements are used to divide a plug into 3 plugs. If more partitions were needed, more elements can be used accordingly. On top of the microchannels, there are also access ports for sample inlet, sample outlet, air pressure inlets Ain1–Ain3, and inlets for primer pair solutions Pin1 ~ Pin3.

The chip is made by bonding of three layers of PMMA sheets: 2-mm thick top layer, 1-mm thick middle layer, and 1-mm thick bottom layer. The bottom layer forms the floor of the microchannels. The middle layer contains the microchannels. The top layer has windows for the air venting elements and openings for attachment of fittings for air pressure and liquid connections. Each layer was designed using CORELDRAW. The drawings were input into a laser-cutting machine (VersaLaser, USA) which cut out the patterns on PMMA sheets. The three layers were then aligned and thermally bonded at 125°C for 30 min.

C. Pressure driving and test setting

Air pressure was used to drive liquid plugs inside the microchannels. Air pressure can be generated by syringe pump, peristaltic pump, or air compressor. If using air compressor, generally the generated air pressure is too powerful for moving of the liquid in the microchannel. This was solved by regulating the pressure to a low value, and using a pulsed operation with a high speed pneumatic valve (Lee Valve LHD series) of response time 1 ms. Speed control of the liquid plug in microchannel was accomplished by adjusting duty ratio of the valve. This pressure driving method accompanied with externally controlled solenoid valves for the opening and closing of the venting element can form an automatic microfluidic platform.

Air pressure out of a pump was adjusted to 0.01 bar by a precise regulator (SMC ITV001-3BL) and then delivered to an array of 3 high-speed solenoid pneumatic valves. Air inlets on

the chip were connected to the valves through soft tubing. The valves were operated at a duty time of 5 ms with an interval of 1 s. The flow rate of liquid in the microchannels under such operation is about $2 \mu\text{l/s}$. For easier observation of the flow and mixing of liquid columns, the liquid samples were colored magenta and blue.

III. RESULTS AND DISCUSSION

A. Basic manipulations: Moving, positioning and merging of liquid plugs

The basic function of the air venting element is the positioning of liquid plug in microchannel. When multiple liquid plugs were positioned at the same element, they will merge together. Fig. 4 shows a sequence of photos taken on top of a microchannel with an air venting element. Four liquid plugs that were dispensed into the channel were moved by air pressure from the left of the channel and positioned at the air venting element. In the process, the air venting element was kept open. The four small liquid plugs eventually passed through the air vent and merged into a big liquid plug. The microchannel in this test has a $200 \mu\text{m} \times 200 \mu\text{m}$ square cross-section.

B. Partitioning, multiple mixing and spacing adjustment of plugs using air vents in a chip application

Combining multiple air venting elements, functional microfluidic chips can be made. Fig. 5 illustrates working flow of the multiple-mixing chip containing four air venting elements (see Fig. 3). For better demonstration, fitting and tubing are not attached on top of the venting windows, and we used Scotch type to close the venting windows when required. Initially, liquid sample represented by blue color was loaded into the sample dispensing channel and three different reagents (primer solutions) represented by magenta color were loaded into the primer

FIG. 4. Sequential merging of four liquid plugs at an air venting element.

FIG. 5. Partition of a sample into three plugs and mixing with three reagents in sequence followed by spacing adjustment of the mixed plugs on a chip, using four air venting elements.

dispensing channels, as shown in Fig. 5(a). A sample volume of 15 μ l was dispensed into the main channel and then pushed forward by air pressure from Ain1. The sample plug passed through Ave1 and stopped after the venting window since it was open. Meanwhile, 5 μ l of primer solution 1 was dispensed to the branch channel before Ave3, as shown in Fig. 5(b). The plug of primer solution 1 was pushed by air from Ain3 and stopped after passing Ave3. Then air from Ain2 divided the 15 μ l sample plug into two parts (5 μ l part and 10 μ l part) with a 5 μ l of plug being pushed forward in the main channel, as shown in Fig. 5(c). This first sample plug kept moving forward and mixed with the plug of primer solution 1 and positioned after the Ave3 since it was open, as shown in Fig. 5(d). After this, the Ave3 was closed and the merged plug was pushed forward through a winding microchannel for a thorough mixing and stopped after the last air venting element Ave4 on the chip. Then the Ave1 was closed and the remaining plug of 10 μ l of sample was pushed forward by air until it stopped after Ave2. Meanwhile, 5 μ l of primer solution 2 was dispensed to the branch channel before Ave3, as shown in Fig. 5(e). The Ave3 was then opened and again the plug of primer solution 2 was pushed by air from Ain3 and stopped after passing the Ave3. Air from Ain2 divided the 10 μ l of sample plug into two parts (5 μ l each) with a 5 μ l of plug being pushed forward in the main channel, as shown in Fig. 5(f). The second sample and primer plugs were then mixed after the Ave3 and moved forward with operations of close/open of the Ave3 and Ave4, as shown in Fig. 5(g). The distance between the first and second mixed plugs was determined by the distance between the Ave3 and Ave4. Finally, the remaining plug of 5 μ l of sample was mixed with primer solution 3 and the multiple mixed samples are ready for next stage of analysis.

1. Partition of a liquid plug

In the above application, we demonstrated that a liquid plug can be equally divided into three smaller plugs in a microchannel by combination of two air venting elements and an air inlet channel, as shown in Figs. 5(b), 5(c) and 5(f). Position arrangement of the air venting elements determines the volumes of the partition. The partitioning of a plug into more parts can be accomplished by simply adding more air venting elements.

2. Multiple mixing of liquid plugs

Multiple mixing of sample with different ingredients is accomplished at the merging section (see Fig. 3) of the chip. The merging section contains a Y junction and an air venting element (Ave3) placed after the junction. As shown in Figs. 5(b), 5(c) and 5(f), sample plugs from one branch of the Y junction and reagent plugs from the other branch were mixed at the Ave3 in sequence, without requiring synchronization. The multiple-mixing is realized by combining functions of two venting elements, Ave3 and Ave4 on the card. In the beginning, both Ave3 and Ave4 were open. After partition of the sample, the sample plug 1 was driven forward by air pressure coming from Ain2, to merge with primer plug 1 positioned at Ave3, as shown in Fig. 5(d). Ave3 was then closed and the merged plug 1 was pushed forward, passing the curved channel for mixing, and then stopped behind Ave4, as shown in Fig. 5(e). In the next step, Ave3 was opened for merging of sample plug 2 and primer plug 2, as shown in Fig. 5(f). After this second merging, both Ave3 and Ave4 were closed thus both merged plug 1 and merged plug 2 were driven forward. After these two plugs passed Ave4, air pressure was stopped and then Ave3 and Ave4 were opened, and ready for the next mixing of sample plug 3 and primer 3, as shown in Fig. 5(g). Finally, after merging of sample plug 3 and primer plug 3 at Ave3, both Ave3 and Ave4 were closed and all the three merged plugs were pushed forward together to the outlet.

3. Spacing adjustment of liquid plugs

The application example also demonstrated how to adjust spacing between liquid plugs by air venting element. As shown in Figs. 5(e)–5(g), since the first mixed plug was positioned at the Ave4 and the second mixed plug was positioned at the Ave3, the distance between these two liquid plugs was determined by the positions of the Ave3 and Ave4. The space between the second and the third mixed plugs was determined by the same two air venting elements.

C. Performance of the air venting element

The basic function of the air venting element is as follows: when it is open, liquid plug can pass through it and stop with its rear aligned with the venting window, and when it is closed, liquid plug will move on. The function when the window is closed is simple and can be easily accomplished. Thus, the performance of the air venting element is mainly about the functionality of positioning the liquid plug when the venting window is open. The performance can be influenced by channel shape and dimension, shape and size of the venting window, hydrophilicity of the material, and some structural variations. We will discuss some major factors here from the failure modes of the air venting element.

1. Failure modes

Failure of the air venting element means failure in positioning of liquid plug with the element. There are two main failure modes. The first one is overflow, which means liquid goes up into the chamber of the venting window. Such a failure can happen when the flow rate (speed) of the liquid plug is high and above the capacity of the element. A higher aspect microchannel, a hydrophobic venting window surface, and a bigger venting window can all help to increase the flow rate capacity of the element. A threshold flow rate for different sizes of venting window will be discussed in Sec. III C 2.

FIG. 6. Threshold flow rate for air venting elements with different widths and lengths of venting window.

The second failure mode is film formation. Thin liquid film may form above the microchannel and below the venting window when liquid passes through the venting window. The film will block the air vent, preventing air to flow out. Because of this blockage, the droplet will keep moving forward. This phenomenon causes the droplet to stop not at the desired position but further than the desired position. The film formation problem can be mitigated with a wider microchannel, a lower flow rate, and can be prevented by some structural film breaking mechanism. We will describe some of these mechanisms later.

2. Threshold flow rate with different sizes of venting window

Flow rate of the liquid plug is a crucial factor for proper functioning of the air venting element. To find out the threshold flow rate and suitable venting window size for the element, various sizes of venting windows were made for a microchannel of size of $200\ \mu\text{m} \times 200\ \mu\text{m}$. There are 3 different lengths and 4 different widths used to construct the venting window. The lengths used are 1.5 mm, 3 mm, and 4.5 mm, while the widths are 1 to 4 mm. Combining all possible widths and lengths, there are twelve different air venting elements that were created.

FIG. 7. (a) A structure of air venting element with improved channel profile to prevent overflow and a micro needle to prevent film formation. (b) A photo of the elements with the improved structure.

TABLE I. Performance comparison of the original and improved structure of the air venting element.

	Threshold flow rate before overflow ($\mu\text{l/s}$)	Failure rate due to film formation when test with pure water	Failure rate due to film formation when test using water with surfactant (0.1% Triton X-100)
Original structure (Fig. 2)	40	5%	20%
Improved structure (Fig. 7)	70	0%	0%

The experiment was conducted with different flow rates of the liquid plug until the threshold flow rate is found. Threshold flow rate is defined as the maximum flow rate that will be able to transfer liquid plug without overflowing into the air vent. The flow rate higher than this threshold flow rate will cause the droplet to overflow. The maximum flow rate was recorded as threshold flow rate of the element versus the size of venting window, as shown in Fig. .

It can be seen that the bigger the venting window, the faster the threshold flow rate. This characteristic is predictable since as the well becomes bigger, more space is provided for delaying the liquid to over flow into the air vent. However, it is not that, the bigger the venting window the better the air venting elements. The length of the liquid plug should also be longer than the length of the venting window for the air venting element to function properly. A bigger venting window limits the ability to handle smaller liquid plug. Thus there is a compromise of the size of the venting window.

3. Structural improvement of the air venting element

Besides the dimensional optimization, another way to increase the threshold flow rate and prevent failure is the fine-tuning of the structure of the air venting element. As we have mentioned, hydrophobic coating of the venting window can increase its resistance to overflow. Also the profile of the microchannel in the air venting element plays an important role in the performance of the element from our observation. Fig. 7 shows an improved structure of the air venting element, which we preferred after many rounds of tests. The micro channel is narrower at the liquid entrance side of the element and is wider at the exit side. A narrow entrance increases resistance of overflow at this point. A wide exit reduces resistance when liquid flows into the outlet channel, which again prevents overflow. In addition, a micro needle placed at the exit of the element is found to be the most effective way to break the air bubble and prevents failure due to film formation. In a test using water added with surfactant (which increases its tendency to form thin film), reliable performance of the air venting element was observed. The performance improvements of this structure when compared with the original in Fig. 2 are listed in Table I.

IV. CONCLUSIONS

The air venting element on microchannel is structurally simple, yet has many capabilities in a microfluidic system. Although it is not suitable for handling very small droplets, it performed well in manipulating liquid plug of micro-liter or nano-liter range in microchannels. It is suitable for automation of microfluidic workflow since it can be actively controlled outside. This paper demonstrated structure, basic function, and applications of the element when combining multiple elements. The performance of the element has been discussed and some improvements have been applied upon tests. We are exploring more functions and applications of the element, and are trying to propose a microfluidic platform using the versatility of the element.

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