

Article

Passive Time-Controlled Fluid Delivery in Microfluidic Devices Using One-Shot Dissolvable Poly(vinyl) Alcohol Microvalves

Illya Klyusko ^{1,†} , Mattia Giovanni Scardamaglia ^{1,†}, Stefania Scalise ¹ , Isabella Aquila ² ,
Elvira Immacolata Parrotta ² , Giovanni Cuda ¹ , Carmen Caiazza ³ , Renata Palladino ³, Massimo Mallardo ³ ,
Patrizio Candeloro ¹  and Gerardo Perozziello ^{1,*} 

¹ Department of Experimental and Clinical Medicine, University of Catanzaro, Germaneto, 88100 Catanzaro, Italy; illya.klyusko@unicz.it (I.K.); mattiagiovanni.scardamaglia@studenti.unicz.it (M.G.S.); stefania.scalise@unicz.it (S.S.); cuda@unicz.it (G.C.); patrizio.candeloro@unicz.it (P.C.)

² Department of Medical and Surgical Sciences, University of Catanzaro, Germaneto, 88100 Catanzaro, Italy; isabella.aquila@unicz.it (I.A.); parrotta@unicz.it (E.I.P.)

³ Department of Molecular Medicine and Medical Biotechnology, University Federico II, 80138 Naples, Italy; carmen.caiazza@unina.it (C.C.); renata.palladino@unina.it (R.P.); massimo.mallardo@unina.it (M.M.)

* Correspondence: gerardo.perozziello@unicz.it

† These authors contributed equally to this work.

‡ G. Perozziello is senior author.

Featured Application

Low-cost, passive time-controlled fluid delivery in microfluidic devices without external actuation.

Abstract

Passive microvalves enable autonomous operation of microfluidic devices by allowing time-controlled fluid delivery without external actuation. Here, we report a simple, low-cost, single-use passive microvalve based on poly(vinyl alcohol) (PVA) for time-programmed fluid delivery. The valve operates through dissolution of a solidified PVA plug formed inside poly(methyl methacrylate) (PMMA) microchannels after evaporation of the solvent phase. The valve dissolves when exposed to an aqueous medium, enabling fluid flow. The valve opening time was experimentally characterized as a function of the injected liquid volume using three PVA formulations and three microchannel cross-sections fabricated in PMMA. Experimental results showed a non-linear dependence of the opening time on the injected volume, as well as the influence of PVA formulation, channel geometry, and temperature. In addition, a demonstrative multi-valve device showed sequential valve opening with delays up to 7 h. The proposed approach enables simple and autonomous timed fluid release using inexpensive materials and straightforward fabrication processes, representing a promising strategy for low-cost microfluidic applications.

Keywords: microfluidics; passive microvalves; poly(vinyl alcohol); PVA; timed fluid delivery; PMMA microdevices



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1. Introduction

Microfluidic devices have enhanced research in a plethora of scientific fields, including chemistry [1–3] and biomedicine [4–7]. Miniaturized devices allow economically more affordable, complex procedures, yet performable in an easier manner. Lab-on-Chip devices [8–11] are an excellent example of the alignment with the microfluidics paradigm. In

fact, they are utilized in many applications, such as biomolecular processing [polymerase chain reaction (PCR) [12,13], sequencing [14,15], immunoassays [16,17], biomolecular array for DNA processing [18], cellular culture coupled to advanced analysis equipment [19–21], and organ-on-a-chip (OoC) systems, useful to simulate pathologies such as cancer and study their dynamics [22,23], as well as the functioning of organs' compartments to fine-tune pharmacological procedures [24,25]. It is easy to deduce that over time, devices become more complex and require more automation, since external intervention by operators becomes a less effective option with increasing complexity. It is well established that in microfluidic devices, valves are one of the components that allow flow to be regulated inside the devices. They fall into two active and passive macro categories, depending on whether there are actuators upstream of the flow control. Active valves require external energy to change their open or closed state. Typically, active valves are actuated by pneumatic [26], electrical [27], magnetic and thermal [28] sources. However, they can be overly sophisticated and very challenging in manufacturing [29], while passive valves allow for greater simplification of the devices that house them and are more easily integrated, making them more appealing for various microfluidic applications.

The most popular passive valves in the literature are those whose functioning depends on their geometry, namely, it is shape-dependent, e.g., membrane valves [30–33], ball valves [34,35], and flap valves [36–40]. Additionally, there are valves with actuation of a chemical–physical nature such as the swellable hydrogel valves [41] and burst valves, which simply rely on capillary force by abruptly changing the geometry of the surface type that the liquid encounters [42]. Also, there are siphon valves, which exploit siphon operation through a curved channel connection of two reservoirs and the passage of liquid due to centrifugal force [43,44]. While these valves are effective in specific contexts, each of these options presents some limitations: geometry-dependent valves often require very precise manufacturing; hydrogels can dissolve unexpectedly and have long response times; burst valves depend on surfaces with sophisticated characteristics; and siphon valves require external equipment that can provide rotation, which is the reason why their “passive” classification category may be doubtful.

Another subgroup of passive valves is what might be called “one-shot”, since the flow is regulated by valves made of dissolving materials which are biodegradable and biocompatible, such as polyethylene glycol (PEG) and polyvinyl alcohol (PVA). Such materials dissolve or break upon contact with the triggering liquid and are therefore not reusable. This approach is more convenient in cases where it is necessary to have disposable microfluidic devices in the biomedical field or analysis procedures where the integration of passive valves, which was discussed above, leads to less than justifiable waste and higher costs. Single-use valve materials include paraffin valves that remain closed until they are subjected to a temperature of about 70 °C to dissolve. These valves are well suited for PCR analysis, but their implementation in the biological field is particularly challenging, considering that they require an external PCB board to operate and a high activation temperature [28]; in addition, there are thin films in PVA that dissolve upon contact with the liquid. Although they do not require external sources for implementation and are, in theory, compatible with numerous types of experiments, these valves do not allow high channel-closing times (maximum 11 min, if the films are placed in a series of 19 units), making them ineffective for longer experiments [45]. Similarly, PEG has also been used in the implementation of microchemomechanical microfluidic systems (μ CHEMS) [46], used to embody data in the form of chemical concentrations. The liquid flow was regulated by valves made of PEG to allow flow after its dissolution. However, opening times are observed in a time range between 20 s and 600 s (10 min), narrowing the application range of such valves.

Although several passive and dissolvable microvalves have been reported, many existing solutions rely on thermally triggered phase-change materials, prefabricated dissolvable films, serial valve configurations, or platform-specific actuation mechanisms. These approaches can increase fabrication complexity or limit the accessible delay range. Therefore, a need for simple, low-cost, and externally actuator-free strategies enabling programmable delayed fluid delivery in microfluidic devices remains. In this work, this gap is addressed by forming a dissolvable PVA plug directly inside PMMA microchannels via injection and drying of an aqueous polymer solution. This approach avoids thin-film transfer and external thermal actuation, while allowing the valve opening time to be tuned through polymer formulation, injected volume, channel geometry, and temperature.

Here, we present a multipurpose PVA valve. We fill polymethylmethacrylate (PMMA) microchannels with a PVA solution (alcohol–water mix) and allow the solvent to evaporate so that only a solid PVA plug remains, blocking the channel. When the aqueous sample is later added, the plug dissolves after a delay, opening the channel to flow. As with other dissolvable-film valves, this device requires no external actuation beyond the fluid itself. It is inherently one-shot: once the PVA dissolves, the valve cannot re-close. The opening time can be tuned by the volume of the PVA solution inserted inside the microchannel, PVA formulation, namely by tuning the geometry of the microchannels in terms of cross-section, concentration, molecular weight, hydrolysis degree, and temperature, as the higher it is, the faster it accelerates dissolution; in addition, temperature can act as trigger by using a high hydrolysis degree of the polymer [47]. The valve is inexpensive and easy to realize. These features make it suitable for automated assays (e.g., sequential reagent delivery in biological tests) where timed flow control is needed without manual intervention and reduced contamination risk.

2. Materials and Methods

2.1. Materials

2.1.1. PVA Valves

All the PVA variants were purchased from Sigma-Aldrich and are listed in Table 1. The selected variants differ in molecular weight and hydrolysis degree.

Table 1. PVA variants used in the present study.

PVA Variant	Average Molecular Weight (kDa)	Hydrolysis Degree (%)
PVA 1	9–10	80
PVA 2	13–15	87–89
PVA 3	13–23	87–89
PVA 4	31–50	87–89
PVA 5	85–124	87–89
PVA 6	13–23	98
PVA 7	146–186	99+

2.1.2. Microfluidic Devices

PMMA was purchased from Röhm Italia SRL, Settimo Milanese, Italy. DESKAM 2000 version 5.1.5.11 was provided by the Minitex Machinery Corp, Norcross, CA, USA. Kyocera end-mills were purchased from NTW Sales SRL, Casalnuovo di Napoli, Italy. Absolute anhydrous ethanol and isopropanol were purchased from Sigma-Aldrich, St. Louis, MI, USA.

2.2. Fabrication of PVA Valves

PVA valves were prepared with MilliQ water and different PVA variants, which span from a low molecular weight and hydrolysis degree, respectively, of 9–10 kDa and 80%, to high molecular weight and hydrolysis degree, respectively, of 146–186 kDa and 99+%. PVA was mixed inside a beaker with water at a temperature of 70 °C with a magnetic stirrer on a heated plate. To maintain a precise concentration of PVA in water and prevent its evaporation, the beaker is sealed. After the complete dissolution of PVA in water, the solution was allowed to cool down at room temperature and then transferred into a Falcon.

Three types of valves were prepared for characterization inside the microfluidic devices, two of which were colored with food colorants. Of these three valves, one was realized with fully hydrolyzed (99%+) PVA colored with red colorant, which will be referred to as rPVA; the other two valves were realized with 87–89% hydrolyzed PVA, one of which was colored with a green colorant and will be referred to as gPVA, and the other was kept transparent, which will be referred to as tPVA. After the insertion of the valve into a microchannel, the liquid phase evaporates, and a PVA plug remains inside the channel, thereby occluding it. Due to the hygroscopic nature of PVA, the drying step was preferably carried out under low-humidity laboratory conditions to allow more uniform solvent evaporation and reproducible plug solidification.

The valves vary in composition in terms of concentration, degree of hydrolysis, and molecular weight. Table 2 shows the main characteristics of the three varieties of valves used for characterization.

Table 2. Three types of solutions and PVA concentration utilized for valve implementation in microfluidic devices.

	Concentration [PVA (g)/MilliQ (mL)]	Avg. Molecular Weight (kDa)	Hydrolysis Degree (%)
rPVA	1/10	146–186	99+
gPVA	1/10	13–23	87–89
tPVA	1/5	13–23	87–89

2.3. Fabrication of Microfluidic Devices

Micro-milling was chosen for the fabrication of the devices, although several fabrication techniques are available [48,49], as it satisfies most of the requirements of this study. It is an inexpensive technique allowing rapid prototyping—as long as the devices are not very complex—reproducibility, high precision, and considerable design flexibility. For instance, complex channel geometries can be achieved with commercially available end-mills, offering a high span of shapes, such as semi-circular, square, rectangular, etc.

Compared to other fabrication techniques, including laser engraving, photolithography and replica molding, micro-milling offers several benefits. Laser engraving is much faster, but limited to very simple 2D structures and has poor reproducibility and a high risk of feature deformation in thermoplastic materials such as PMMA. Photolithography offers excellent feature resolution and surface quality, although it requires expensive photomasks and cleanroom facilities. Replica molding with PDMS allows good structural fidelity, but it relies on a master mold and may introduce limitations related to hydrophobicity, gas permeability, and adsorption of small molecules, resulting in reduced material compatibility. Micro-milling allows direct machining of thermoplastics (PMMA, cyclic olefin copolymer, etc.), offering acceptable surface roughness in the 0.1–2 µm range [50–52] and good chemical stability. These characteristics make micro-milling suitable for rapid prototyping and small-scale production.

The Mini-Mill/GX, Minitech Machinery Corp, micro-milling machine was used for manufacturing devices in the present study, and 3 mm thick PMMA layers were machined by securing them onto the working plane. The spindle was controlled by a Nakanishi E300C controller. FreeCAD version 0.21 was used to create 2D and 3D models (Figure 1A,E). DESKAM 2000 software was used for G-code generation for the milling process. End-mills 0.25 mm, 0.50 mm, 1 mm, and 3 mm in diameter were used. All channels had a square-shaped cross-section. Post-fabrication cleaning was performed with the first nitrogen blowing of PMMA chips, MilliQ water and soap washing, 2' immersion in isopropanol for any residual soils, and one last nitrogen blow for their elimination. PMMA layers were subsequently assembled via solvent-assisted bonding by using absolute anhydrous ethanol and a P/O/Weber Laborpresstechnik column lab manual press.

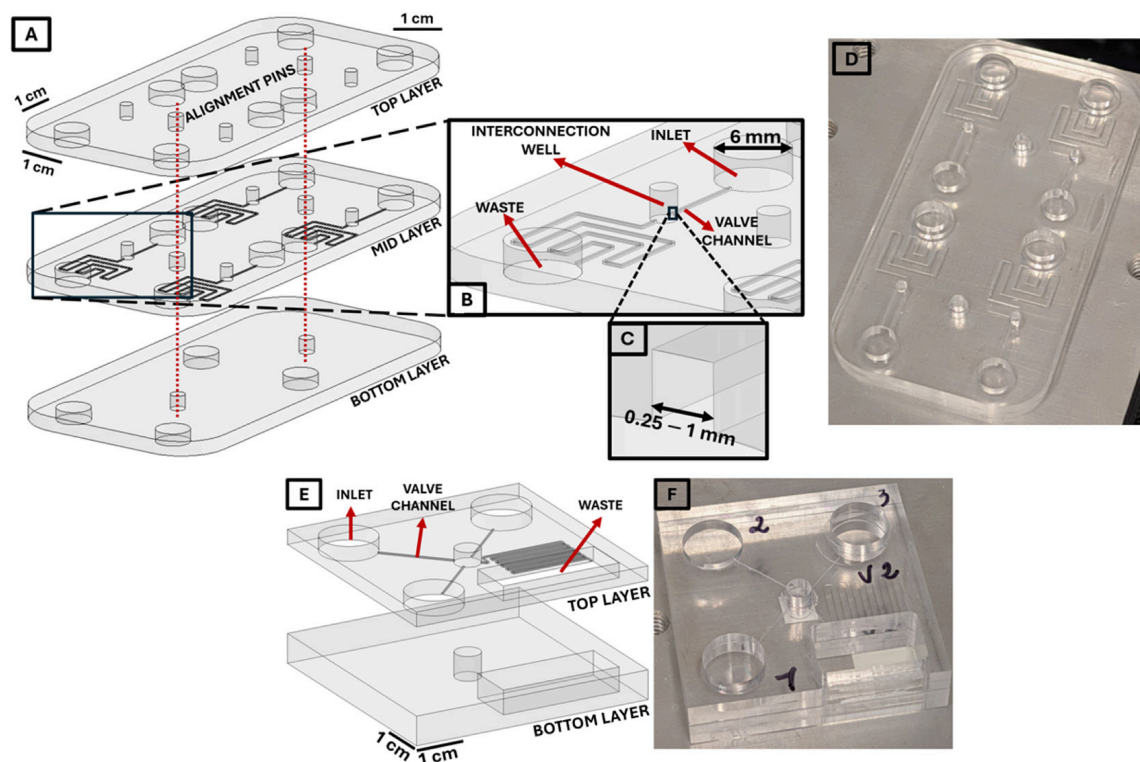


Figure 1. (A–D): Detailed view of the device type employed for PVA valve characterization. (A) Exploded view of the three 3 mm thick layers; (B) detailed view of the inlet–channel–waste hydrodynamic circuit. The interconnection well between the valve channel and the serpentine was realized to prevent the dissolved PVA plug from flowing into the serpentine microchannel as the dissolved PVA flowed to the top, thanks to buoyancy; (C) detailed view of the squared cross-section of the valve channel; (D) photograph of the final PVA valve characterization device. The only parameter varied across the different devices was the cross-section of the valve channel. (E,F): detailed view of the device employed in the time-lapse imaging to demonstrate the functioning of the PVA valve. (E) Exploded 3D CAD of the layers composing the device: all the microchannels have a squared cross-section of side equal to 0.25 mm and are 15 mm long; (F) photograph of the finished device.

2.4. Preliminary Evaluation of PVA Valves Dissolution: Experimental Setup

The use of PVA as a passive microvalve was first investigated by testing different combinations of formulations of PVA with deionized water; namely, 13 specimens of PVA valves were prepared by varying concentration, molecular weight and hydrolysis degree. The specifications of the 13 specimens are described in Table S1. Also, the aim of the preliminary evaluation was to investigate the effective impact of the temperature on dissolution in terms of the opening time of the different PVA formulations. Therefore, the dissolution

of each specimen was tested at room and 37 °C temperatures. For each PVA formulation and temperature condition, dissolution time measurements were independently repeated five times using 100 µL of PVA solution. Each experiment was conducted by placing 100 µL of the liquid PVA solution inside a small silicon tube and the liquid phase was allowed to evaporate so that only a PVA plug remained. The silicon tube was then connected on both sides to two Tygon tubes. One of the Tygon tubes was connected to an uplifter syringe containing water in order to generate flow through it. Water was stopped from flowing by the PVA valve. To bring the PVA valve to 37 °C, the tube was in turn immersed in water inside a Pyrex plate placed on a heated plate.

2.5. PVA-Valve Testing Devices: Rationale

Four PMMA microfluidic devices were fabricated in this study to characterize PVA-based passive valves with different channel dimensions. Three of the four devices were used for quantitative valve characterization (Figure 1D), each featuring a distinct microchannel cross-section in the valve region (Figure 1C), while the fourth device was employed exclusively for time-lapse visualization of valve opening to visually confirm the transition from the closed to the open state upon liquid introduction (Figure 1F). The latter is composed of three bigger circular reservoirs for colored water injections, one smaller circular reservoir for hosting water flowing from opened microchannels when microvalves dissolve, and a rectangular reservoir for waste collection. The microchannels of the latter have a $0.25 \times 0.25 \text{ mm}^2$ cross-section, and their wells were numbered to more easily identify the corresponding channels. The volumes of the PVA solution (identical in composition to the tPVA valve) inserted inside the microchannels of the device for time-lapse visualization device were as follows: microchannel 1, PVA volume was 0.25 µL; microchannel 2, PVA volume was 0.18 µL; and microchannel 3, PVA volume was 0.14 µL. The device was placed on a temperature-controlled heated plate to bring the water to 37 °C.

The characterization devices were designed to host the three different valve configurations and for each valve configuration, microchannel geometry, and injected PVA volume, opening time measurements were independently repeated three times: rPVA valve was tested inside a microchannel with a square cross-section of $1 \times 1 \text{ mm}^2$ and 10 mm length; gPVA valve was tested in a channel with a cross-section of $0.5 \times 0.5 \text{ mm}^2$ and 10 mm length; and tPVA valve was tested in a channel with a cross-section of $0.25 \times 0.25 \text{ mm}^2$ and 10 mm length. The microchannel cross-section in the valve region was the only geometrical parameter that varied across the characterization devices and the temperature at which valves were characterized was equal to 37 °C by means of a heated plate.

Different PVA formulations were selected for each channel's dimension to ensure opening times were compatible with the temporal resolution of the experimental setup. For the largest channel cross-section, a fully hydrolyzed, high molecular weight PVA formulation was employed to avoid excessively rapid valve opening.

2.6. Working Principle of the PVA Microvalve

A PVA solution is first deposited into the microchannel using a micropipette (Figure 2A). Upon evaporation of the liquid phase in low-humidity environmental conditions, a solid PVA plug forms and occludes the channel (Figure 2B). When the device is subsequently brought into contact with an aqueous medium (Figure 2C), the polymer dissolves, enabling fluid flow through the microchannel and resulting in valve opening (Figure 2D).

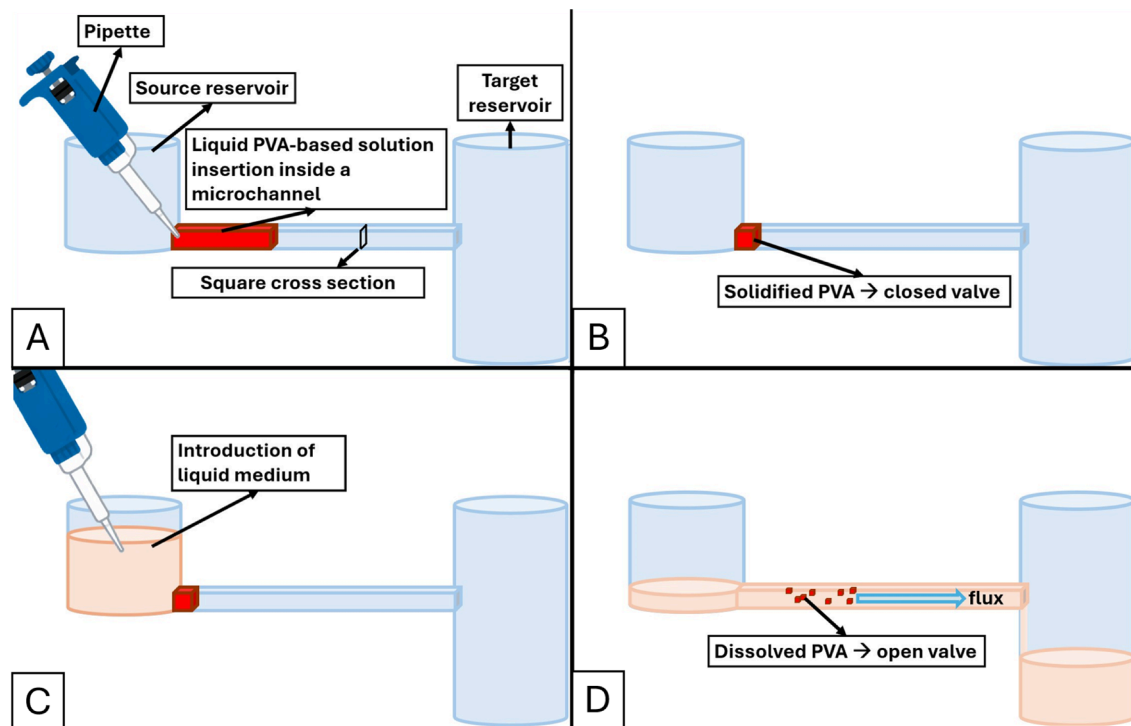


Figure 2. Conceptual schematic of the use of the PVA-based valve. Source reservoir is the reservoir from which the liquid must flow to the target reservoir through the microchannel. (A) A microchannel is partially filled with the desired volume of the PVA solution via a pipette. (B) The liquid phase of the PVA solution evaporates, while the solid phase remains, forming the PVA plug. (C) When the source reservoir is filled with liquid, it comes into contact with the solid PVA, and (D) gradually dissolves it in time, allowing the liquid to flow through the channel.

3. Results

3.1. Preliminary Evaluation of PVA Valves Dissolution: Results

The dissolution of the solidified PVA plugs was evaluated preliminarily before their implementation inside the microchannels. The trend in the opening time of a fixed volume as a function of a different PVA composition and temperatures showed evident dependence on the temperature of the medium (Figure 3). Highly hydrolyzed PVA valves are not reported in this figure.

3.2. Valve Opening Time as a Function of Injected Volume

The opening time of the passive microvalves was quantitatively measured as a function of the injected liquid volume for the three valve configurations. Opening time was characterized as a function of volume for rPVA, gPVA, and tPVA valves (Figure 4), each integrated into microchannels with different cross-sectional dimensions.

Despite the common non-linear dependence on volume, distinct opening behaviors were observed for the different valve configurations. For the rPVA and tPVA valves, the second-order polynomial trends revealed an increasing opening time with increasing injected volume, with a progressively steeper increase at higher volumes. For all valve configurations, the opening time exhibited a clear dependence on the injected volume. However, the relationship between opening time and volume was non-linear over the range investigated. In all cases, the experimental data were best described by second-order polynomial trend lines.

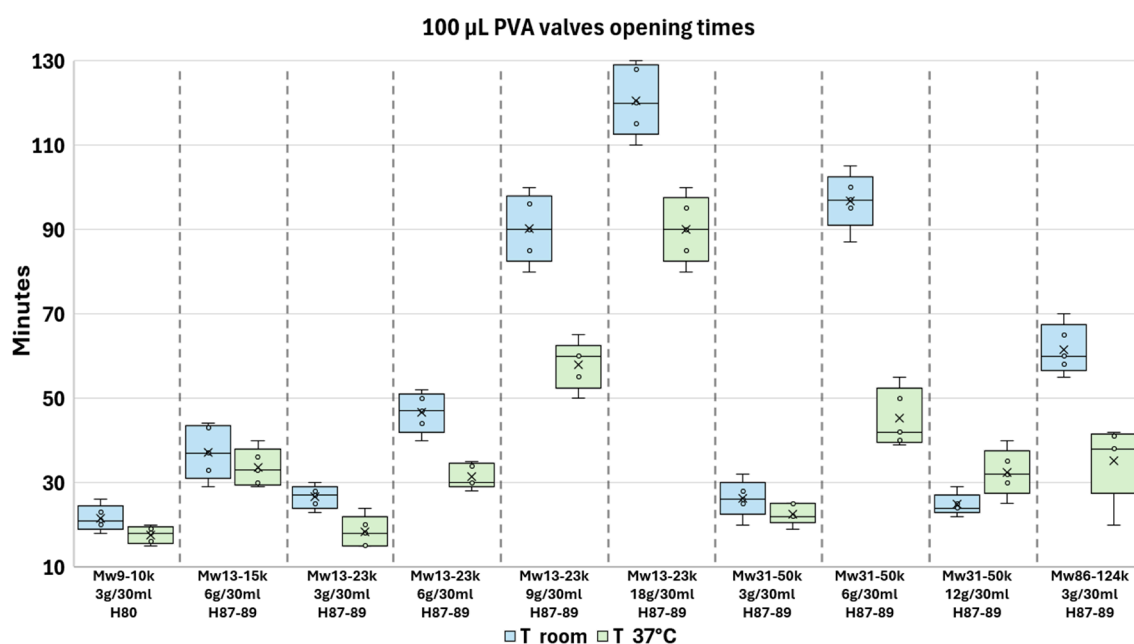


Figure 3. Box plots of dissolution times of different combinations of PVA plugs. Each formulation was independently tested five times using 100 μL of PVA solution. Dissolution was evaluated at room temperature and 37 $^{\circ}\text{C}$ in order to investigate the dissolution behavior of the plugs. Different compositions were utilized to investigate the trend in dissolution depending on the PVA type. On the x-axis, compositions of the specimens are reported and are to be interpreted in the following manner: first row—molecular weight in kDa; second row—PVA concentration in grams in deionized water in mL; third row—hydrolysis degree in percentage.

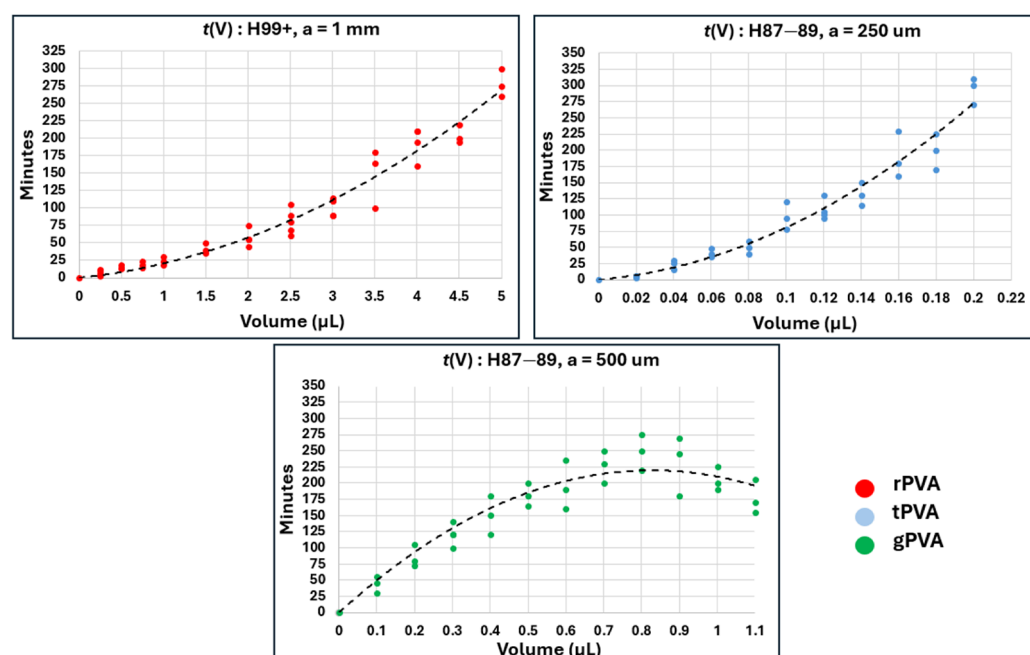


Figure 4. Opening time as a function of injected PVA volume for the three different valve configurations and corresponding microchannel cross-sections. Each experimental condition (valve type, channel geometry, and injected volume) was independently tested three times at 37 $^{\circ}\text{C}$. Red dots refer to the rPVA valve; light blue dots refer to the tPVA valve; and green dots refer to the gPVA valve. Dashed lines represent second-order polynomial fits used as guides to the eye to describe the experimental trends.

In contrast, the gPVA valve exhibited a different behavior. While the opening time initially increased with increasing volume, the second-order polynomial trend suggested a tendency toward reduced opening times at higher injected volumes. This behavior was observed within the explored volume range.

3.3. Time-Lapse Demonstration of Valve Opening

The opening behavior of the PVA-based passive microvalves was qualitatively assessed by time-lapse imaging. The complete time-lapse video is available in the Supplementary Material. A representative sequence illustrates the transition from the closed state, characterized by a solid PVA plug occluding the microchannel, to the open state following the liquid introduction (Figure 5). Upon dissolution of the PVA plug, liquid flowed through the microchannel into the central reservoir, confirming the irreversible, one-shot opening mechanism of the valve. PVA valves' opening occurred sequentially as follows: microchannel 3 (0.14 μL valve) after 112 min, microchannel 2 (0.18 μL) after 180 min, followed by microchannel 1 (0.25 μL) after 420 min.

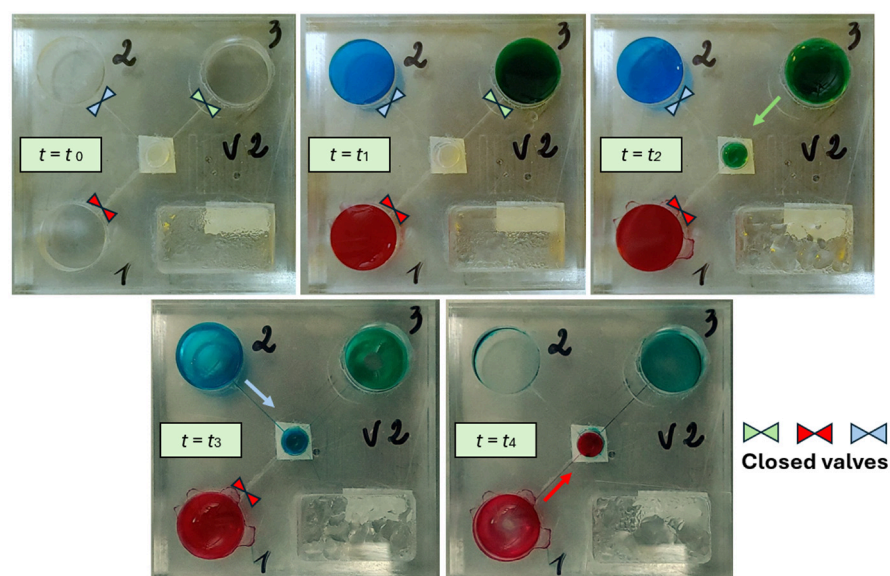


Figure 5. Illustration of the relevant time instants of the process in the time-lapse imaging. The experiment was performed at 37 °C. Instant $t = t_0$ refers to the system at rest, with no liquids and only with solidified PVA plugs within microchannels; instant $t = t_1$ refers to the moment right after the introduction of the colored water inside the circular reservoirs numbered as 1, 2, and 3; instant $t = t_2$ refers to the instant of the opening of the PVA valve corresponding to the reservoir 3; instant $t = t_3$ refers to the instant of the opening of the PVA valve corresponding to the reservoir 2; and instant $t = t_4$ refers to the instant of the opening of the PVA valve corresponding to the reservoir 1.

4. Discussion

In the present study, a passive PVA-based microvalve was presented. Results show that the dissolution time of the microvalve inside the microfluidic channels can be tuned by varying the volume of the liquid PVA solution injected, the temperature of the medium in contact with the solidified PVA plug, and the type of PVA utilized, namely its molecular weight, hydrolysis degree and composition (PVA/water ratio). The configurations preliminarily tested (Figure 3) exhibited dissolution times tendentially depending on the chemo-physical properties of the PVA, where smaller molecular weights and concentrations of PVA exhibited faster dissolution both at room and 37 °C temperatures. For the configurations tested inside the microfluidic channels, the relation between the opening time

and injected volume showed a non-linear trend, satisfactorily described by a second-order polynomial trend in the range of the investigated volume.

In the preliminary tests, it emerged that highly hydrolyzed PVA ($M_w = 13\text{--}23$ kDa and %H = 98 and $M_w = 89\text{--}98$ kDa and %H = 99+) did not dissolve at room temperature, while at 37°C , at certain point, it detached from the walls of the silicon tube and clogged the flow in the adjacent tube, leading to the impossibility of correctly continuing the experiment. This is the reason why opening times of highly hydrolyzed PVA valves are not reported in the preliminary evaluation. The same PVA was then implemented inside the microfluidic devices, and it was able to properly dissolve without detaching from the walls, thanks to the roughness of the micro-milled PMMA microchannels.

For the rPVA and tPVA valves, the opening time increased with respect to the injected volume, particularly with higher volumes. This might be due to a higher concentration of the tPVA valve and smaller cross-section of the microchannel; meanwhile, in the case of the rPVA, the prolonged dissolution time might be attributed to the higher molecular weight of the PVA and hydrolysis degree. Although no quantitative dissolution model was developed in this study, the non-linear trend observed is coherent with the complex interplay between polymer hydration and concentration, molecular weight, mass transport and channel geometry inside the confined microfluidic environments. The gPVA valve exhibited a qualitatively different behavior compared to the other configurations. While an initial increase in opening time with increasing injected volume was observed, a deviation from monotonicity occurred at higher volumes. A possible explanation may involve reduced solidification homogeneity of longer PVA plugs formed in small cross-section microchannels. In such conditions, solvent evaporation during the drying of the liquid phase may become less efficient, leading to localized regions with higher residual water content and lower compactness. Upon contact with the aqueous medium, these regions may facilitate faster liquid penetration and localized dissolution, resulting in shorter opening times than expected.

The demonstrative device was utilized to qualitatively exhibit the functioning of the PVA valve. Three microchannels with equal cross-section were utilized to host three valves identical to the tPVA valve in terms of composition. It is possible to note that dissolution times of PVA for the three channels match the tendency demonstrated for the tPVA valve. The three valves opened sequentially after approximately 112, 180, and 420 min, respectively, showing the possibility of achieving temporally separated opening events over several hours. These dissolution times are consistent with the trend observed for the tPVA valve during quantitative characterization.

Although no persistent channel obstruction or downstream clogging due to residual PVA was observed during the experiments, the concentration of dissolved PVA released after valve opening was not quantified in this study. Therefore, the potential influence of residual PVA on specific downstream biological or biochemical assays should be evaluated according to the intended application.

It is worth noting that, regarding the valve characterization process, the three valve configurations were intentionally tested in microchannels with different cross-sectional dimensions. This choice was driven by practical considerations related to the polymer properties: the rPVA formulation, characterized by higher molecular weight and degree of hydrolysis, was required to achieve sufficiently long opening times in larger channels, whereas less hydrolyzed and lower molecular weight PVA formulations resulted in excessively fast dissolution in the same geometry. Consequently, a direct comparison of absolute opening times across different varieties of valves is not the objective of this study. Instead, the results highlight the tunability of the valve opening time through a combined selection

of polymer formulation, concentration, channel geometry, temperature, and injected PVA solution volume.

To better contextualize the proposed approach, Table 3 compares the present PVA-based microvalve with significant passive and dissolvable microvalves reported in the literature in terms of actuation mechanism, fabrication complexity, achievable delay time, and target application.

Table 3. Comparison of passive time-delay microvalves reported in the literature and the present work.

Valve Type	Actuation	Max Delay Reported	Fabrication Simplicity	Target Application	Limitation
Paraffin valve [28]	Thermal	Thermally triggered (~80 °C)	Moderate	PCR microdevices	Requires heating/PCB
PVA thin-film [45]	Dissolution	~11 min	Simple	ELISA/lateral-flow assays	Short opening window
PEG valve [46]	Dissolution	~10 min	Simple/moderate	μCHEMS/bioanalytical systems	Short opening window
Present work	PVA plug dissolution	Up to 420 min/volume-dependent	Simple, low cost	Biomedical microfluidics	Assay-specific validation needed

Compared with PEG- and thin-film PVA-based valves, the proposed approach allows valve fabrication through water and PVA mixing, and direct injection and drying of the polymer solution inside the microchannel, avoiding multilayer assembly, thin-film deposition, or external actuation. Furthermore, valve opening can be tuned through the adjustment of polymer formulation, injected volume, channel geometry, and temperature.

Beyond the comparison reported in Table 3, the proposed valve concept may be useful for microfluidic systems requiring delayed or sequential fluid delivery without external actuation, such as autonomous reagent addition, incubation steps, washing sequences, or long-term assays. The demonstrative device suggests that multiple PVA valves can be integrated within the same platform to obtain temporally separated openings. However, more complex multi-valve networks would require careful design, since the effect of the cumulative amount of dissolved PVA, local flow redistribution, and interactions between sequentially opened channels may affect device operation. Moreover, since valve opening is governed by hydration of the dried PVA plug and removal of dissolved polymer from the valve region, the opening time may depend on the composition of the liquid phase. Therefore, buffers, culture media, salts, proteins, pH, viscosity, and temperature should be evaluated according to the specific application.

In addition, the long-term stability of the dried PVA valves under different storage humidity and temperature conditions was not specifically investigated in the present study. Considering the hygroscopic nature of PVA, environmental conditions during storage may influence the properties of the dried plugs and consequently their dissolution behavior.

5. Conclusions

In this work, a single-use passive microvalve based on dissolvable PVA plugs was developed for time-controlled fluid delivery in microfluidic devices. The valve is fabricated by direct injection and drying of an aqueous PVA solution inside PMMA microchannels, without external actuators, moving parts, or complex fabrication steps. The results demonstrated that valve opening time can be tuned by adjusting the injected polymer

solution volume, polymer formulation, channel geometry, and temperature. In a demonstrative multi-valve device, sequential valve opening was achieved with delays up to 420 min (7 h), showing the potential of the approach for long-term and time-programmed microfluidic operations.

The proposed strategy provides a simple and low-cost solution for passive temporal flow control in microfluidic systems. Future work will focus on improving the quantitative understanding of the dissolution process, evaluating long-term storage stability under different humidity and temperature conditions, testing the valve response with different buffers and biological media, and assessing compatibility with specific downstream biochemical or biological assays.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16146878/s1>, Table S1: Specimen characteristics used for preliminary tests of dissolution and behavior at different temperatures for evaluation of the PVA plug inside the 100 uL silicon tube discussed in Section 3.1; Video S1: time-lapse video of the full experiment related to the Section 3.3 Time-lapse demonstration of valve opening.

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