AUTO-ACTUATED SEQUENTIAL RELEASE VALVES FOR LAB-ON-A-DISC SYSTEMS

D. J. Kinahan, S. M. Kearney and J. Ducrée
Biomedical Diagnostics Institute; National Centre for Sensor Research,
School of Physical Sciences, Dublin City University; IRELAND

ABSTRACT

In microfluidic biomedical systems valving is often of critical importance for process control. In centrifugal microfluidics valves are typically actuated through changing the centrifugal force seen by the working liquid. Here we present for the first time a new valving structure (based on dissolvable films) where the entry of liquid into a chamber on the disc can trigger the release of liquid from a chamber located elsewhere on the disc. These valves can be configured such that multiple valves can be released in a sequential manner independent of external inputs.

KEYWORDS

Lab-on-a-disc; centrifugal microfluidics; valving; dissolvable films; auto-actuated; cascading; sequential release

INTRODUCTION

In the past decade the Lab-on-a-Disc concept has been subject to increased interest for biomedical applications including sample preparation, analyte detection, nucleic acid amplification (PCR) [1] and cell analysis [2]. The use of Centrifugal Force (CF) to transport fluid around the disc rather than integrating internal or external pumping strategies makes the Lab-on-a-Disc paradigm particularly applicable to biomedical diagnostics where both low-cost and disposability is often pre-requisites.

However, like all microfluidic systems, an inherent difficulty in these systems is the miniaturization and integration of the valving strategies which permit the execution of complex analytical assays. Valving strategies have been developed which make use of (often complex and expensive) external control mechanisms such as infra-red laser heating [3]. More often valves are used which are actuated through varying the CF experienced by a fluid (through varying the spin rate of disc). Among others these include capillary burst valves, capillary and centrifugo-pneumatically actuated siphoning [4]. Gorkin *et al.* [5] combined a dissolvable film with a pneumatic chamber to create valves where the burst frequency (and hence CF) could be accurately tailored over a wide range.

For those valving strategies actuated through varying the spin rate, the number of valves which can be used is limited by the tunability of a valve to a specific burst frequency, the finite upper limit of the spindle-speeds which can be practically used and the fidelity of manufacture. This is particularly an issue where a biomedical assay requires release of a number of reagents in a defined order.

In this paper we present a new type of DF valve which utilizes the presence of an ancillary liquid at one location to trigger the release of another liquid residing at another location of the disc. For the first time, this event-triggered rather than frequency-actuated mechanism allows to cascade a multi-step liquid handling sequence without the need for any external actuation. Using this technology the number of assay steps that can be sequentially concatenated on a disc-based microfluidic network can be increased independently of the typical limiting factors such as the number of discrete burst frequency bands available.

VALVE DESIGN AND OPERATION

Each Sequential Release Dissolvable Film (SRDF) valve consists of a pneumatic chamber which is sealed by two gas-tight dissolvable film (DF) valves located at discrete locations. The outlet valve through which the retained liquid will be passed is referred to as the Release Valve (RV). The second dissolvable film is referred to as the Control Valve (CV) (Fig 1a).

Centrifugation pressurizes the gas volume in the channel segment enclosed between the liquid in the inlet reservoir and the two dissolvable films RV and CV. The microchannel/pneumatic chamber is shaped such that liquid in the reservoir cannot compress the gas trapped in the pneumatic chamber sufficiently under typical spin-rates to reach the RV (Fig. 1b). In addition the pneumatic chamber is shaped such that the liquid cannot reach the CV even if there is no pneumatic force impeding its flow. This geometry is usually a siphon-like microconduit between the outer CV and the RV.

The valve is actuated through the contact of a liquid with the dissolvable film of the CV. This is typically through the filling a chamber sufficiently so it can come in contact with the liquid. This dissolves the CV and vents the pneumatic chamber to atmosphere. The retained liquid then enters the valve structure and comes in contact with the RV. The siphon-like shape of the valve prevents the liquid from exiting through the CV outlet (Fig 1c). The RV is dissolved and this allows the liquid to exit the valve through the outlet channel (Fig 1d,e).

Similarly to the valves described by Gorkin *et al.* [5], increasing the rotation velocity of the disc will force the liquid to enter the pneumatic chamber. The entry of the liquid into a pneumatic chamber is determined by balancing of the centrifugally induced pressure and the pressure of the gas within the pneumatic chamber. Increasing the spindle speed of the lab-on-a-disc will increase the induced pressure and hence further compress the gas within the pneumatic chamber.

The centrifugally induced pressure, p_ω, is defined by:

$$p_\omega = \rho \Delta r \bar{r} \omega^2 + p_0 \quad (1)$$

where ρ is the density of the fluid, Δr is the radial length of the fluid element, $\bar{r}$ is the central radial location of the fluid element, ω is the rate of rotation and p_0 is ambient

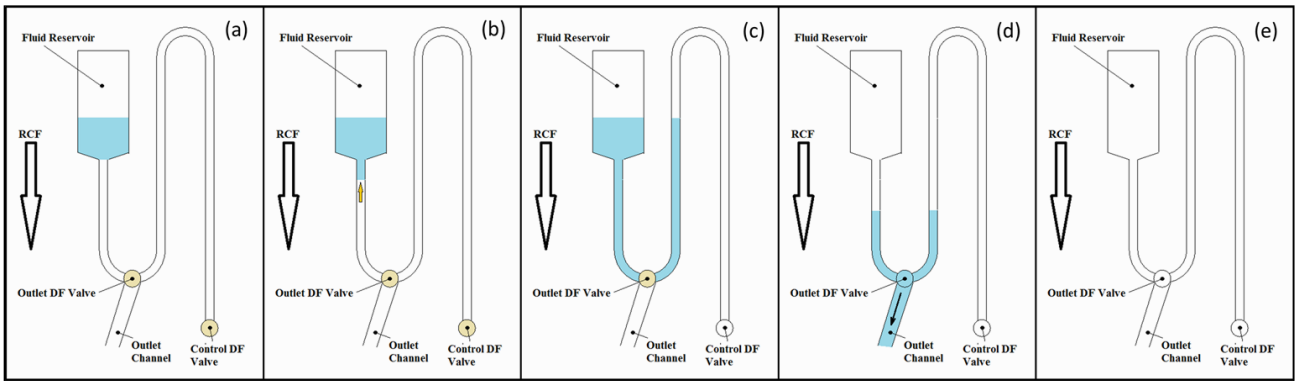

Figure 1: Schematic of Valve Operation. (a) Valve consists of a pneumatic chamber sealed by two dissolvable film valves (b) Under normal centrifugation pneumatic pressure prevents liquid reaching the dissolvable films. (c) Opening the Control DF Valve (CV) permits liquid to reach the DF-sealed outlet Release Valve (RV). (d) RV dissolves and liquid exits through outlet channel. (e) Structure empties completely. Note the shape of valve prevents liquid reaching/exiting through CV.

(atmospheric) pressure [5]. From Boyle's Law the pressure of the gas within the pneumatic chamber, p_c, is

$$p_c = p_0 \frac{1}{1-\Delta V/V} \quad (2)$$

where V is the total volume of the pneumatic chamber and ΔV is the reduction in gas volume due to the ingress of the liquid into the pneumatic chamber [5]. These equations apply assuming temperature remains unchanged, the liquid is incompressible and interfacial tension is sufficiently strong to hold/maintain a stable liquid/gas interface. Applied to centrifugo-pneumatic dissolvable film valving ΔV is the volume the liquid must occupy in the pneumatic chamber in order to come in contact with the dissolvable film and p_c, is the pressure which must be induced on the gas to allow the liquid to occupy this volume.

Similarly to the valves described by Gorkin *et al.* [5], increasing the speed of rotation will force the liquid to enter the pneumatic chamber. In their approach, they used a combination of pneumatic pressure and interfacial tension to keep liquid from entering sufficiently into the pneumatic chambers to come into contact with the dissolvable film. By tailoring the inlet geometry and volume of the pneumatic chamber Gorkin *et al.* could tailor the valves to open at discrete spindle spin-speeds.

In the approach presented here it was critical to design the valves so that they would not burst at typical operating spin rates. Therefore the ratio of ΔV to V is critical. In the approach of Gorkin *et al.* this achieved through reducing the over-all volume of the gas chamber V relative to the inlet microchannel into the pneumatic chamber (ΔV). However, in the approach described here the increased volume associated with using two dissolvable films in the structure and the need to have a linking microchannel means that this approach will not be sufficient.

Therefore the approach used here is to increase the volume the fluid must occupy (ΔV) to reach the first dissolvable films (RV) relative to the total volume. This can be implemented by increasing the length of the inlet channel (which can take up increased space on the disc and can be unstable) or by increasing the cross-sectional area of the inlet channels. Yet, increasing the cross-sectional area will reduce the stabilizing effect of interfacial tension on the liquid/gas interface and allow fluid to 'drip' onto the dissolvable film.

The need for this stabilizing effect can be removed by turning the inlet microchannel radially inward before increasing its cross-sectional area. This inverts the liquid/gas interface so the greater density of the liquid will act in concert with interfacial tension to maintain a stable liquid/gas interface (Fig. 2a). A drawback to this approach is that it can result in a dead-volume where a small proportion of the liquid can be trapped within the valve.

MATERIAL AND METHOD

In the structure presented here (Figs. 2 and 3) we actuate a sequence of three valves using a single, conventional centrifugo-pneumatic valve [4]. This valve releases dyed water (red food dye) into a chamber where the first CV is dissolved. The actuation of the first valve automatically releases a second reservoir of dyed water (blue) which in turn sequentially triggers the remaining reservoirs (green and red respectively).

The disc is manufactured from aligned laminae of transparent PMMA plastic (1.5 mm) (4 layers) and double-sided PSA (pressure sensitive adhesive, 0.086 mm) (4 layers). Chambers, microchannels and vertical vias (links between layers) are created by removing material from the layers of PSA and PMMA. The top layer (Layer 1) is composed of PMMA and contains loading and venting holes. Layer 2 is PSA and the removed material allows optical access to the reservoirs and defines the microchannels which link reservoirs. Layer 3 is composed of PMMA and removed material defines the reservoirs and also the vertical vias used to link microchannels in different layers. Layers 4 and 5 are cut from PSA. These layers contain holes which link the vertical vias. In addition Layer 5 contained spaces into which the DF tabs could be fitted while Layer 4 was placed over these tabs to provide additional support and sealing.

Layer 6 is composed of PMMA and contains vertical

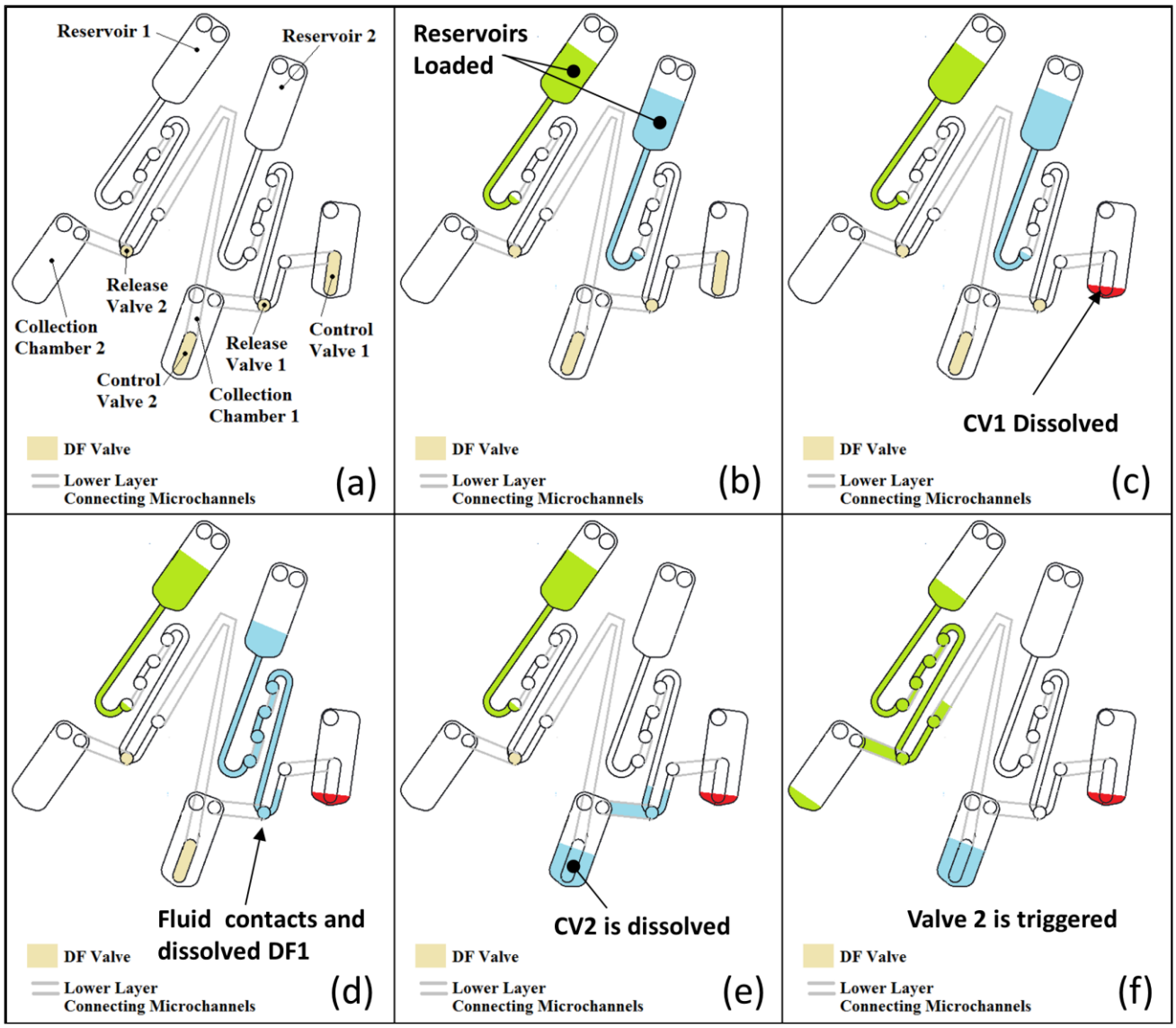

Figure 2: Auto-Cascading of Valve Sequence (a) Valves configured for sequential release. (b) Under typical spinning frequencies, liquid in reservoirs cannot reach Release Valves (RVs) (c) Fluid comes into contact with and dissolves CV1 (d) Fluid enters the pneumatic chamber/microchannel and comes in contact with RV1 (e) Fluid drains from Reservoir 1 into Collection Chamber 1 and dissolves CV2. (f) Reservoir 1 fully empties and Valve 2 is actuated.

vias. It also provides structural support to the DF valves.

Layer 7 is cut from PSA and contains a lower layer of linking microchannels. Layer 8 is made of PMMA and is used as a backing layer. The dissolvable film tabs are manufactured from low-cost dissolvable films commonly used for embroidery and sewing. They are manufactured using the technique described by Gorkin *et al.* [5].

Figure 2 shows a schematic of the structure used and the alignment of multiple layers. Of note, the inward turn of the microchannel results in the inversion of the gas/liquid interface during centrifugation. The volume of the chamber the fluid must occupy to come into contact with the RV due to centrifugation alone (ΔV) is greatly increased by directing the chamber back and forward through vertical vias (which at 1 mm diameter and ~3 mm depth occupy significant volume relative to microchannels cut from PSA). These valves in this configuration will not burst at spindle-rates greater than 100 Hz. Note also the elongated DF tabs used for the CV valves means that gas will not be trapped within the valve (which can occur if the fluid fills the collection chambers before the valves are fully dissolved).

DISCUSSION AND OUTLOOK

The valving strategy presented in this proof-of-concept is uniquely compatible with many of the biomedical assays which are executed using defined, sequential steps. These applications could potentially include serial dilution studies [6], cell enumeration and on-disc ELISA [7]. The technology presented circumvents many of the limitations of other valving strategies on centrifugal platforms, particularly as these valving sequences (or cascades), once initiated, can continue independently of external inputs.

Tolerances in centrifugally actuated valves, combined with the limited rotational speed envelope which can be practically used, restricts the number of valves which can be discretely activated through step-wise increments of rotational speed. While it is possible to extend the number

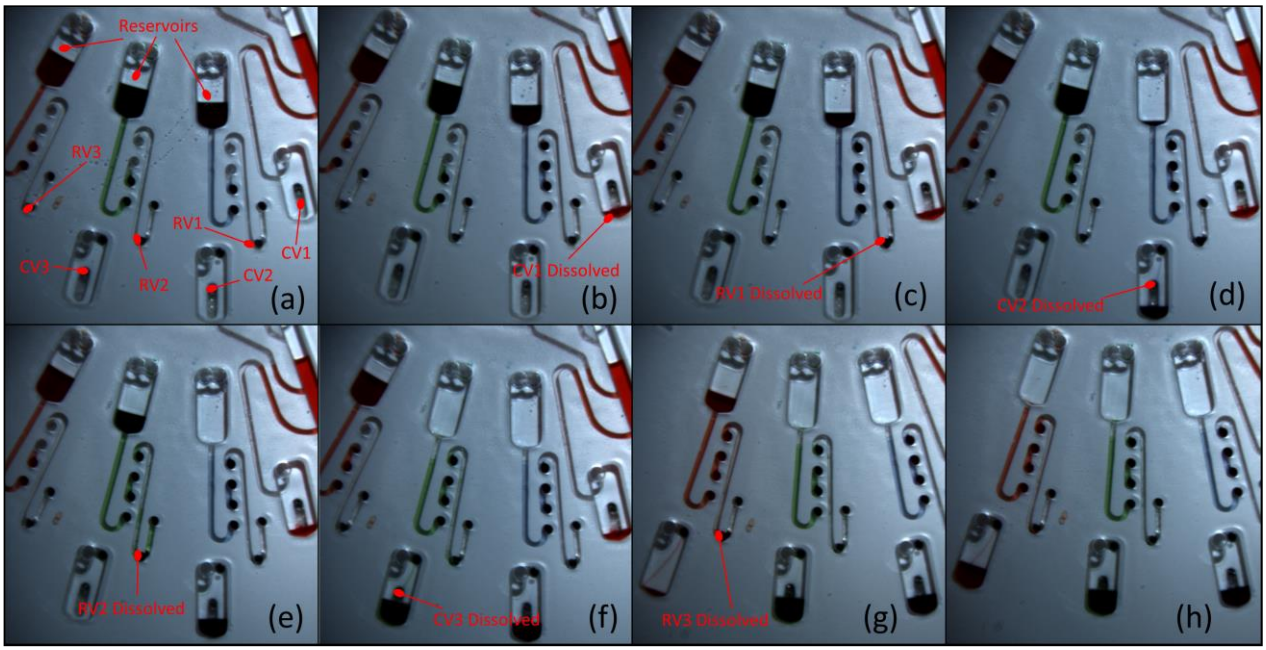

Figure 3: Sequential release of three valves auto-triggered by centrifugo-pneumatic siphon valve (a) Under centrifugation liquid is contained in reservoirs. (b) Valve is triggered and CV1 is dissolved. (c) Liquid in Reservoir 1 reaches and dissolves RV1. (d) Collection chamber 1 is filled and CV2 is dissolved. (e) Fluid in Reservoir 2 reaches and dissolves CV3. (f) Collection Chamber 2 is filled and CV3 is dissolved. (g) Liquid in Reservoir 3 reaches and dissolved DV3. (h) All Reservoirs are emptied.

of discrete actuations by combining low-pass valves (such as siphon valves) with high-pass valves (like centrifugo-pneumatically actuated DF valves) in practice this can be complex and make excessive use of limited real estate on the disc. The valves presented here have no such limitation and have proven to be highly reliable; the number of independent release steps which can be implemented by this technology is effectively limited only by disc real-estate.

It is also envisaged that interactions of greater complexity beyond serial actuation are possible. These could include implementation of AND relationships (where two or more upstream conditions (fluid release events) must have occurred for a valve to open) or OR relationships (where a valve can be triggered by one of two or more fluid release events).

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CONTACT

J. Ducrée T: +353 1 7005377 E: jens.ducree@dcu.ie
D. Kinahan T: +353 1 7005889 E: david.kinahan@dcu.ie