IMBIBITION-MODULATED EVENT-TRIGGERING OF CENTRIFUGO-PNEUMATIC CASCADING FOR MULTI-STAGE DILUTION SERIES

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ABSTRACT

We present a new technique which uses paper imbibition to effectuate time delays between event-triggered valving on a centrifugal microfluidic "Lab-on-a-Disc" (LoaD) platform. This is important to extend the degrees of freedom for the on-disc integration of multi-step, multi-reagent bio-analytical assay protocols in a sample-to-answer fashion. A paper strip is adhered over an array of dissolvable films, each of which seals a pneumatic chamber. Wetting of the paper strip sequentially wets and dissolves the films and thus vents the chambers and triggers the valves. As a pilot study, we demonstrate a 3-stage serial dilution protocol.

KEYWORDS: Lab-on-a-Disc; Serial Dilution; Valves; Paper Microfluidics

INTRODUCTION

The Lab-on-a-Disc concept has been subject to increased interest over the last decade. It has been applied to a number of biomedical applications, particularly in the point-of-care domain, including analyte detection, nucleic acid amplification (PCR) [1] and cell analysis [2] and to other fields such as bioprocess control [3] and environmental monitoring [4]. Since all liquids (e.g. sample and reagents) residing on the rotating substrate experience the centrifugal field, valving schemes directing their release sequence are at the very core of integrated LoaD platforms. This is reflected in the wide variety of technology which has been developed, including strategies based upon external control mechanisms (such as pneumatic inputs or thermal heating) [4-6]. Most commonly used valves on LoaD platforms rely on variations in the spin-rate for actuation; for example capillary burst valves [7], siphon valves [3] and centrifugo-pneumatically actuated siphoning [8]. Recently, Gorkin *et al*. [9] developed a highly flexible and tunable burst-type valve based upon combined a dissolvable film (DF) with a pneumatic chamber.

A drawback of rotationally actuated valves are the finite upper limit of the spindle-speeds paired with smearing of burst frequencies (due to manufacturing tolerances) to limit the number of sequential valving steps which can be practically implemented. While the combination of low-pass valves and high-pass valves can circumvent this limitation to some effect [3] this can be complex, unreliable and can take up significant (valuable) disc real-estate. This limitation on the number of valves has particular effect where a biomedical assay requires the execution of multiple liquid handling steps. To avoid these limitations, efforts have been made to develop valving methods where actuation is independent of both external actors and changes in rotational speed [10].

Recently Kinahan *et al*. [11] introduced a new type of DF valve which is actuated by the presence of fluid at particular points on the LoaD. This mechanism, which is event-triggered rather than frequency-actuated, can allow a multi-step liquid handling sequence to cascade without external actuation. In effect, this means the number of successive assays steps which can be rotationally automated on an LoaD is independent of frequency smearing and is practically only limited by available disc real-estate. However, in a serial triggered configuration, the time between valve actuations is dependent on the time for fluid to move around the disc and the dissolution times of dissolvable films (typically < 60 s). To increase the time between valve actuation to a magnitude more agreeable to typical biomedical applications, a delay element must be interspersed. Here we use well-controllable imbibition of a disc-integrated, commercial grade paper strip to modulate liquid transport to the points on the LoaD where the valves are triggered. The use of paper in microfluidic devices has recently been of major interest, particularly in the applications for point-of-use in resource poor countries [12].

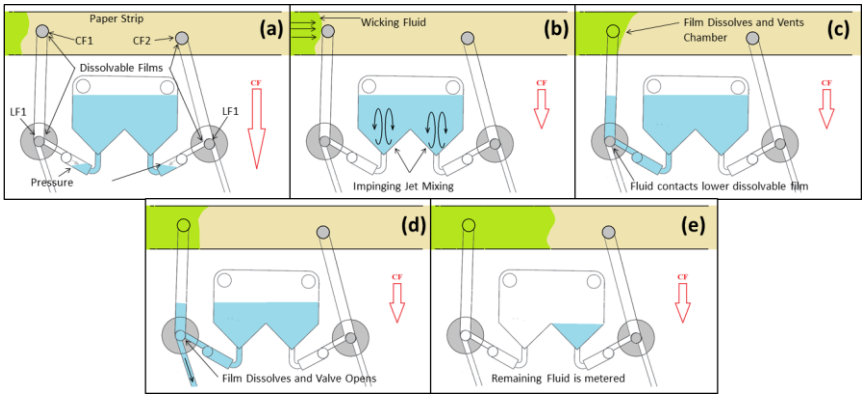

Figure 1: A schematic of a metering and mixing module sealed by two outlet valves. (a) Each valve consists of a pneumatic chamber valved by two dissolvable-films (DFs). (b) Varying the rate of disc rotation induces jet-based stirring (c) When the wicking fluid wets the first control film (CF) the valve pneumatic chamber is vented. (d) This allows the liquid to contact the lower load film (LF) and thus open the valve. (e) Fluid exits and the remaining fluid is accurately metered.

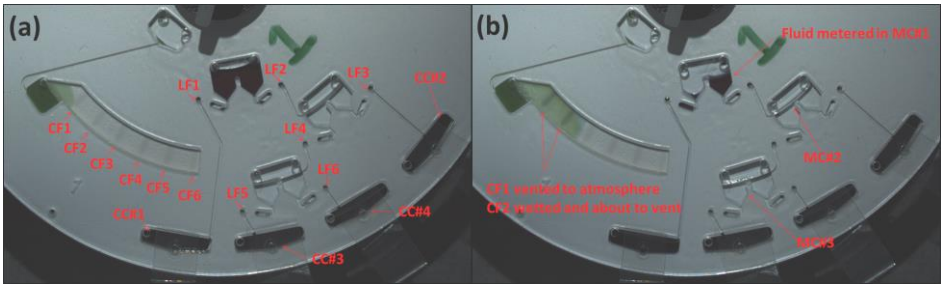

Figure 2: (a) Disc in its initial configuration just after reagents have been loaded and the paper-strip has been wetted by dyed water (green). (b) CF1 has been wetted and thus opened. This has removed food dye to Collection Chamber (CC) #1 and a metered volume remains in the Metering / Mixing Chamber (MC) #1. CF2 has wetted and will vent as soon as the dissolvable film loses its integrity.

VALVE DESIGN AND OPERATION

As described previously [11], each Event-triggered Dissolvable Film Valve (EDFV) consists of a pneumatic chamber which is sealed by two gas-tight dissolvable film (DF) valves located at discrete locations. As these valves can be considered akin to an electrical relay, these films are referred to as the control film (CF) and load films (LF). The pneumatic chamber is shaped such that, under typical centrifugation rates, the restrained liquid cannot compress the gas trapped in the pneumatic chamber sufficiently for the fluid to contact (and dissolve) the LF. Thus the valve actuation is independent of disc speed of rotation. In addition the pneumatic chamber is shaped such that the liquid cannot reach the CF even if there is no pneumatic force impeding its flow. This is implemented by ensuring the microchannel linking the LF to the CF extends along a path radially inwards of the restrained fluid.

The valve is actuated through a liquid contacting the dissolvable CF and thus venting the pneumatic chamber. Open to atmosphere, the restrained fluid can now enter the chamber and wet the LF film, thus opening the valve, but cannot escape through the CF orifice. In this work we use wicking of fluid through a paper strip to transport liquid from a reservoir to the CF films and so actuate the valves. The speed of fluid wicking, which is typically slower than microchannel flow, controls the timing of valve actuation. Locating the CF films for multiple valves along a paper strip results in these valves being opened in a sequential manner as the fluid wets the paper strip (Figure 1).

Additionally, correct design of these valves [11] ensures that, at normal spin rates, these valves are opened independent of disc rotational rate. However, increasing the speed of rotation will force the liquid to enter the pneumatic chamber of the valve and subsequent decreasing the speed of rotation will expel the fluid. This feature can be used to good effect for mixing as the valves can have an additional role to inducing impinging jet/pneumatic stirring.

MATERIALS AND METHOD

The discs are manufactured using the method previously described [11]. Briefly, it is manufactured from aligned laminae of transparent 4 layers of PMMA (1.5 mm) and 4 layers of double-sided PSA (pressure sensitive adhesive, 0.086 mm). The multi-layer architecture allows channels to cross each other and creates greater flexibility in design.

Each module of the 3-stage cascade microfluidic cascade consists of a metering / mixing chamber (MC), each sealed by two event-triggered, centrifugo-pneumatic valves (Figure 1). The first chamber is loaded a dyed water (blue) while the subsequent chambers are loaded with DI water. To initiate the experiment dyed water (green) is introduced as an ancillary fluid. As the test cycle begins this fluid wets one end of the paper strip and wicking begins. Both outlet valves on each chamber are initially closed and defined variations in the spin rate (alternating between 10 Hz to 25 Hz in 8-s cycles) induces jet-induced stirring of the liquids in the chambers. Figure 2 and 3 show, respectively, the valve actuations in stages and visualization of centrifugo-pneumatically induced mixing / metering.

RESULTS, DISCUSSION AND OUTLOOK

Figure 4 demonstrates a successful, 3-stage serial dilution by absorption measurements on samples removed from the LoaD. Samples were pipetted from the disc into a standard well plate and read using a commercial plate-reader (Tecan). As illustrated in Fig. 4c, the samples recovered from the collection chamber are successively more dilute. However, it is clear further optimization is required to ensure dilution occurs in a log-linear fashion; in particular the mixing times and volumes of successive mixing chambers will require attention.

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. The use of paper, a well-characterized medium, to mediate the actuation of the valves allows the timing of fluid release to be well controlled and compatible with biomedical assays. Additionally, the capability to use a single event – the wetting of the paper – to sequentially actuate multiple valves can greatly simplify the layout of disc architecture.

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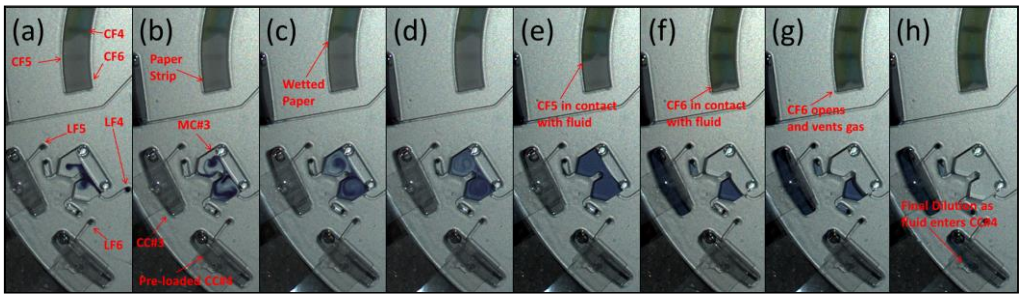

Figure 3 – (a-e) on-disc mixing by rotational acceleration (Euler Force) and centrifugo-pneumatically propelled impinging jets. Note also the wicking of fluid along the paper strip. (f-h) Final metering steps which result in the 3rd and 4th stage serial dilutions.

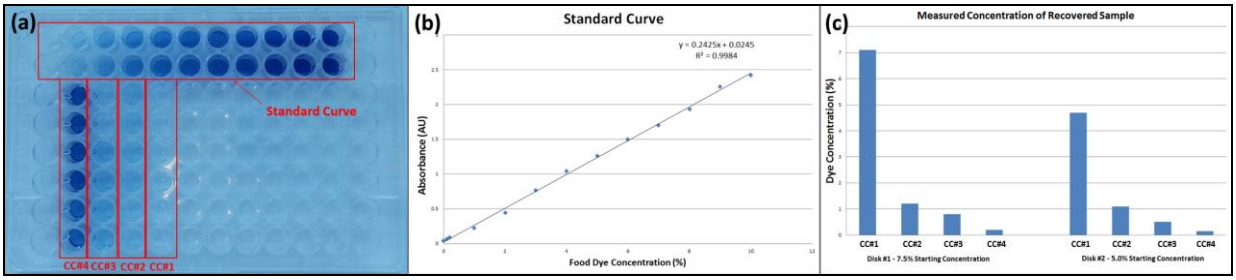

Figure 4: (a) Microtitre plate showing standard curve and fluid collected from sample discs. (b) Standard curve obtained using a Tecan plate-reader. (c) Dye concentrations from recovered samples.