ROTATIONAL-PULSE ACTUATED DISSOLVABLE-FILM VALVES FOR AUTOMATED PURIFICATION OF TOTAL RNA FROM E. COLI

D. J. Kinahan[1], E. Clancy[2,3], N. A. Kilcawley[1], N. Dimov[1,4], T.J. Smith[2,3] and J. Ducrée*[1]

[1]*Biomedical Diagnostics Institute, School of Physical Sciences,*
Dublin City University, IRELAND
[2]*Molecular Diagnostics Research Group, National University of Ireland, Galway, IRELAND*
[3]*Biomedical Diagnostics Institute Programme,*
National University of Ireland, Galway, IRELAND
[4]*Department of Biochemical Engineering, University College, London, UNITED KINGDOM*

ABSTRACT

In this work we report for the first time on a repertoire of valving technologies which are combined to enable automated purification of total RNA from cell homogenate. Process control is implemented using rotational-pulse actuated dissolvable-film (DF) valves; where the order of valve actuation is determined by the disc architecture while the timing of valve actuation is governed by pulses in the spin rate. Selective liquid routing is enabled by combining a heavy, inert and immiscible liquid plug with a DF. The combination of these technologies enables bead-based extraction of amplifiable RNA, with a yield comparable to gold-standard bench-top protocols.

KEYWORDS: Lab-on-a-Disc; Valving; Dissolvable Films; Nucleic Acid; Sample Preparation

INTRODUCTION

Over the recent decade so-called "Lab-on-a-Disc" (LoaD) platforms have been increasingly leveraging biomedical point-of-care diagnostics [1-2]. On these centrifugal microfluidic systems a disc-shaped cartridge is rotated at defined frequency protocol by a robust and low-cost spindle motor. This simple actuation renders the LoaD platform independent of external pressure sources and their pneumatic interfaces, thus ensuring ease-of-use and minimum maintenance. However, to orchestrate multi-liquid, multi-step flow control as well as preconditioning of sample and reagents within the ubiquitous centrifugal field, high-performance valving schemes are pivotal.

Flow control technologies may be broadly divided into two categories. Instrument actuated schemes typically use complex support instrumentation to open or close valves on the disc. While highly reliable and flexible, these valves tend to compromise the conceptual simplicity of the LoaD platform and increase the complexity of both the instrument and the disc cartridges. The second category are rotationally actuated valves, such as centrifugo-pneumatic dissolvable films [3-4], which yield by increasing

Figure 1 (a) Rotational-pulse actuated valves. Initially, at low and high spin rates, the valve remains closed. Dissolving a control film (CF, yellow tab) by an ancillary liquid vents part of the pneumatic chamber. This effectively lowers the burst frequency of the valve to within the ranges typical for LoaD systems. Concatenated, each pulse in the spin profile will, based on the disc architecture, open only a predetermined valve. (b) Liquid-selective routing. Initially a heavy, inert liquid (FC-40, pink) is loaded and plugs the microchannel before the DF. Cell homogenate and washes emanating from the upstream bead chamber cannot contact the DF and are thus routed into a waste chamber. A small volume of aqueous 'ancillary' liquid can wet the DF the downstream side and thus opens a route from the bead chamber to the sample collection chamber. The FC-40 and ancillary liquid can also be sequestered in a dead-end chamber. The elution buffer is now preferentially diverted from the bead chamber to the sample collection chamber.
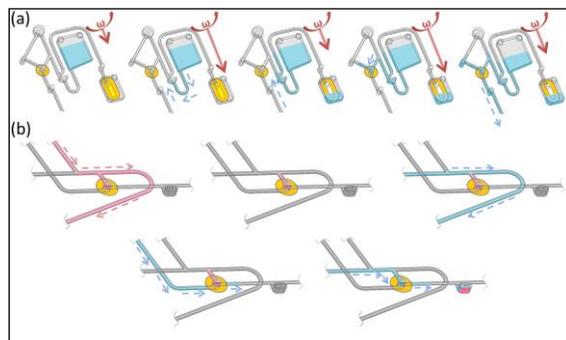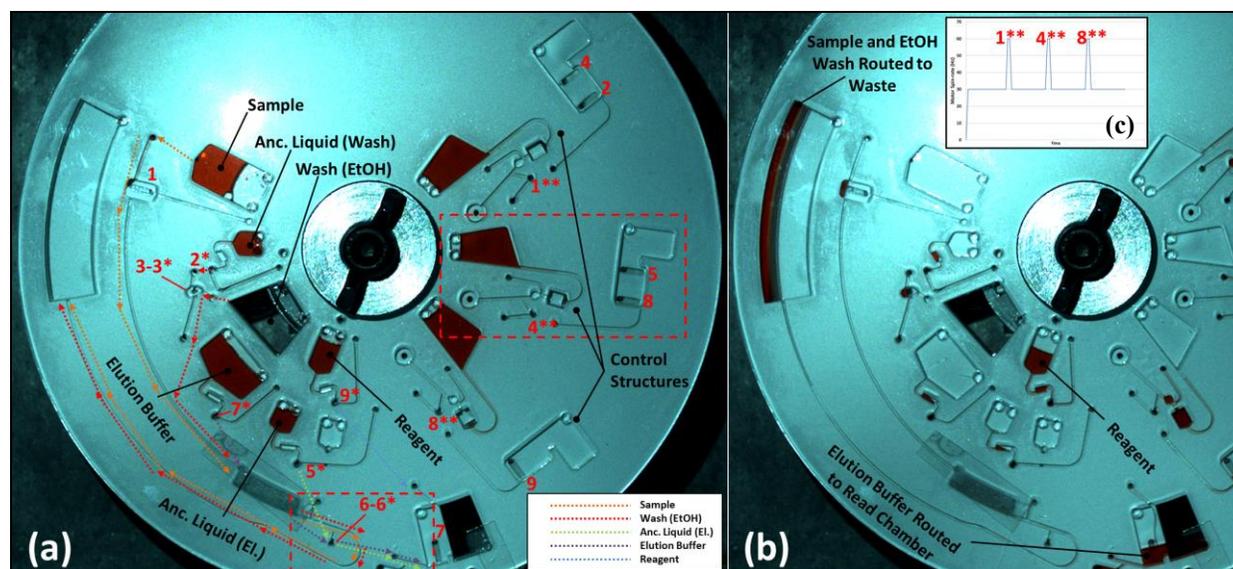
Figure 2 - (a) Video still showing integrated disc loaded with dyed water (for visualisation) and EtOH. The movement of the liquids are shown by coloured arrows. The DF valves are numbered 1 to 9 where an un-starred number indicates a CF and a starred number indicates the corresponding LF. The numbers with two stars indicate a pulse-actuated valve where two conditions must be met; the CF must be dissolved and the motor frequency increased (pulsed). (b)Video still showing the final step in the liquid handling sequence: the addition of the reagent to the elution buffer. Note that sample (cell homogenate) and EtOH wash have been routed to a waste chamber while the elution buffer has been directed to a read chamber. (c) Representation of the spin envelope used in this experiment. The first pulse opens Valve 1** and releases the EtOH wash, the second pulse opens Valve 2** and triggers routing to the read chamber and the release of the elution buffer; the third pulse opens Valve 3** and adds the temperature sensitive reagent to the read chamber. Note that dashed red boxes highlight technology shown in schematics in Figure 1.

or decreasing the spin rate. A drawback of these valves is that manufacturing tolerances burst frequencies limits consequently also the number of discrete valve steps which can be implemented on disc. Recently, a new paradigm, called event-triggered valving [6], combines DFs with centrifugo-pneumatic control. Akin to an electrical relay, opening the so-called Control Film (CF) triggers the release of liquid through the other DF termed the Load Film (LF) at a distal location on the disc. These valves are reliable and function independently of spin rate; however, a limitation is their burst times are dependent on dissolution times of DFs.

Here we synergetically pair two technologies built upon DF valving to enable extraction of total RNA from *E. Coli*. Firstly, pulse-actuated valves are introduced which are a hybrid of DF burst valves [3-4] and event-triggered valves [5]. The valve is configured such that, with the CF intact, the valve will not open at any practical spin rate. However, with the CF dissolved, the valve can be actuated using pulses in the rotational frequency (Fig. 1a). Configured in an interconnected network, the sequence of valve actuation is now determined by the disc architecture while valve opening is governed by the spindle motor.

In addition, we establish the use of an ancillary liquid to open a DF via the downstream end which is restraining a 'non-aqueous' liquid. This technology is first used to release a non-aqueous 75% ethanol (EtOH) as part of the experimental protocol. This 'reversed' actuation method is also at the core of selective liquid routing [5] of liquids where a plug of an inert, heavy (specific weight: 1.85) fluorocarbon liquid (FC-40) is placed between any incoming liquid and a DF, thus initially diverting any incoming liquid (aqueous or non-aqueous) past the liquid plug to a specific reservoir. However, after removing the DF from the backside, a subsequently incoming liquid is routed to through this new opening (Fig. 1b).

We combine these novel, DF-based valving technologies with our previously established DF burst valves [3-4] and event-triggered flow control [5] to demonstrate a fully automated, solid-phase purification of RNA from *E. Coli* homogenate on an integrated microfluidic disc (Fig. 2).

MATERIALS AND METHODS

The disc is manufactured using a previously established, multi-lamination method [5]. To stage an integrated multi-step, bead-based extraction [4-6], the cartridge in Fig. 2 exhibits three pulse-actuated valves in series. After the cell homogenate washes through the beads, these valves initiate specific steps of the extraction process (Figs. 2, 3); the release of an EtOH wash and its direction to waste, the release of an elution buffer and its routing to a read chamber and, in a forward looking step, the potential addition of a reagent to the eluate in the read chamber (thus enabling use of this cartridge for NASBA amplification) . To exemplify the applicability of this LoaD to downstream nucleic acid analysis, we designed an RT-qPCR assay to amplify the *E. coli* tmRNA transcript.
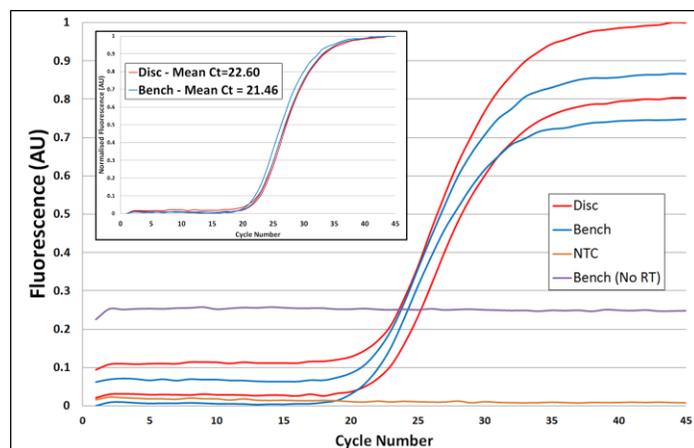

Figure 3 - RT-qPCR results acquired using the disc shown in Figure 1. Cell homogenate (from 2 x10^6 E. Coli cells) is prepared using the Trizol extraction method [7] and processed on-disc using nuclease-free water as the elution buffer. Bench samples are prepared by standard techniques. The eluate is then DNAse-treated followed by a reverse transcription (RT) step. The resulting cDNA is then amplified by real-time PCR. Results show amplification of tmRNA in samples prepared on-bench and on-disc. No-Template-Controls (NTCs) and a bench sample which was not subject to the RT step did not amplify. The inset shows internally normalised s-curves for amplified samples and the mean Ct values calculated using commercial software.

RESULTS AND CONCLUSION

This work represents a significant advancement on existing, rotationally-actuated flow-control techniques for LoaD platforms. Pulse-actuated valves feature the strengths of event-triggered valving (reliability and independence from the spin rate) with the temporal control of conventional, rotationally actuated valving. Similarly, the presence of the non-aqueous liquid plug increases the reliability of previously established, selective liquid routing while the capability of storing and releasing non-aqueous reagent using water-dissolvable films has also been established. These technologies have jointly enabled the extraction of nucleic acid from cell homogenate with comparable yield to the bench-top method (Fig. 3)

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CONTACT * J. Ducrée: +353-1-700-5377; jens.ducree@dcu.ie