PRE-PROGRAMMED, SELF-POWERED CIRCUITS BUILT FROM MICROFLUIDIC CAPILLARY ELEMENTS

Roozbeh Safavieh*, and David Juncker*
*Biomedical Engineering Department, McGill University, Montréal,
Quebec, CANADA

ABSTRACT
We first present two novel capillary valves: a programmable retention burst valve, in which the local increase in the capillary pressure causes the channels to drain sequentially, and a low aspect ratio trigger valve, in which an abrupt enlargement of a two level valve in combination with using a hydrophobic cover can stop the liquid passively. The liquid would then be triggered with the second stream of the flow when it is desired. Afterwards, we integrate these valves with other previously developed capillary valves and pumps to make a novel capillary circuit, enabling us to preprogram the sequential flow of multiple reagents. Finally, we illustrate how the circuit works in the context of a one-step sandwich immunoassay, which measures the concentration of C-reactive protein in 5 mins.

KEYWORDS
Microfluidic Capillary Circuits, Capillary Flow, Point of Care, Immunoassay

INTRODUCTION
Microfluidic capillary systems are powered by capillary effects and control of fluid flow is structurally and chemically encoded in microscale conduits. Such capillary systems can be entirely self-powered and self-regulated, making them useful for point-of-care applications. The elements used for capillary sytems include microchannels, capillary pumps, capillary retention valves (CRVs) and trigger valves. Using these elements, capillary systems for filling and draining of one sample at a time can be made, but more complex fluidic operations cannot be achieved with these alone[1, 2]. Here, we introduce programmable retention burst valves (pRBVs) and robust, low aspect ratio trigger valves. Using these elements, circuits with autonomous and sequential flow of multiple chemicals at various flow rates along with flow reversal were designed and built. Finally, the circuit was used to perform a sandwich immunoassay for a cardiac marker within 5 minutes on an area of 100 µm ×200 µm.

DEVICE FABRICATION AND CHARACTERIZATION
Circuit masters with two levels of thickness were fabricated in SU-8, replicated into PDMS, and sealed against a flat PDMS cover. Dilute aqueous solutions of food dyes and fluorescein were used to characterize the circuits. For the sandwich immunoassay, a line of anti-C-reactive protein (CRP) capture antibody (250µg/ml) was patterned on the cover and sealed with the PDMS circuit. Various solutions including biotinylated anti-CRP detection antibody (200 µg/ml), 1% phosphate buffered saline (PBS) in DI water, and streptavidin-Alexi Fluor 488(500 µg/ml were preloaded by contacting the tip of a pipette to the side channel vents. Finally, 2.5 µl of various solutions of CRP antigens were used as test samples.

RESULT AND DISCUSSION
Fig. 1A shows the library of elements that can be combined to make various circuits. We selectively combined these elements to make a capillary circuit with flow reversal as shown in Fig. 1B.

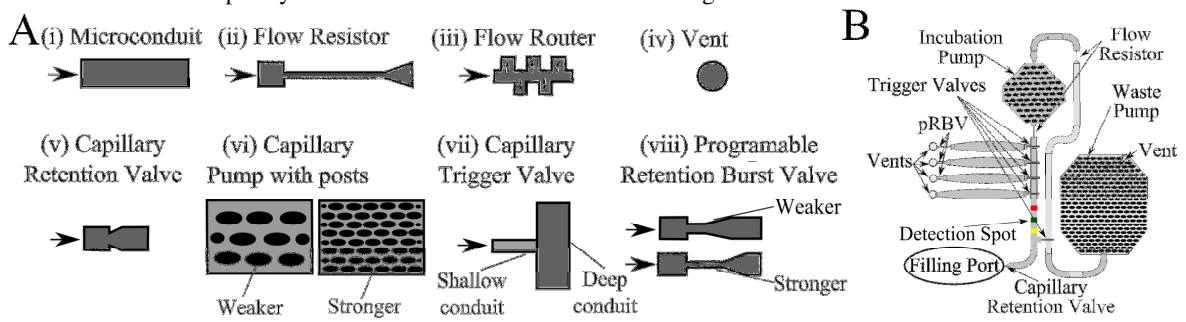

Fig. 1 *Schematic illustrating capillary circuits. (A) The library of capillary elements including the novel two level trigger valve, and a programmable retention burst valve (pRBV). (B) A layout of a microfluidic chip with flow reversal. The chip comprises four capillary reservoirs for the reagents and a filling port to introduce the sample. Various reagents are spontaneously filled by contacting the tip of a pipette through the vent of the reservoirs. The liquids are then held due to the trigger valves at the end of conduits. After introducing the sample the liquid fills the main conduit, activates the trigger valves, and, upon entering the reverse flow pump, the reagents are drained sequentially.*

The novel trigger valves consist of a shallow conduit intersecting a deep one (Fig. 2). pRBVs were made by adjusting the size of the cross-section and the length of the narrow section. The negative pressure required to burst a valve increases as the channel cross-section narrows. The minimal cross-section is constrained by the pressure of the capillary pump, which needs to be sufficient to drain the liquid. Fig. 3 shows time-lapse images of the sequential drainage of six side channels filled with fluorescein.

978-0-9798064-5-2/µTAS 2012/$20©12CBMS-0001

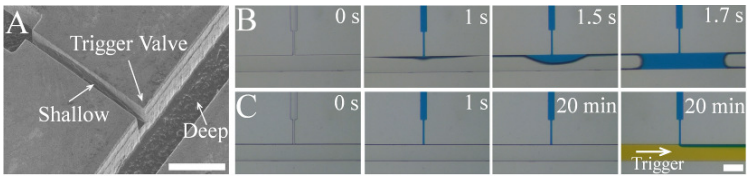

Fig 2 *A capillary trigger valve used to passively stop the liquid. The microstructures are hydrophilic (plasma activated PDMS). The sealing layer is hydrophobic PDMS. (A) SEM micrograph of the two-level valve. (B) Timelapse images showing that abrupt enlargement of the one level trigger valve fails after only 1.5 seconds;(C) images showing that the abrupt enlargement of the two level trigger valve together with the use of a hydrophobic cover were effective to stop liquids for periods of 20 min, and could be triggered at any time by flowing a sample in the deep conduit. The scale bars are 300 µm.*

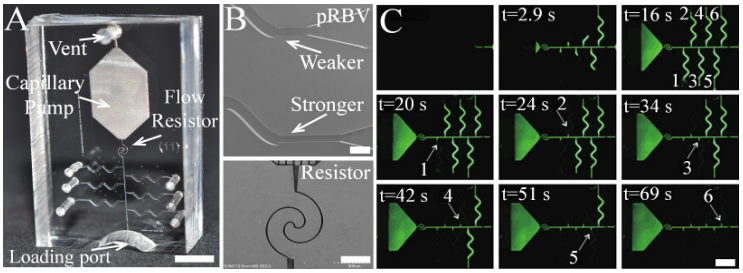

Fig. 3 *Operation of the programmable retention burst valves (pRBVs). (A) Micrograph of a circuit to test the pRBVs. Enlarged SEM micrographs of the pRBVs to drain two conduits sequentially, and a flow resistor. (C) Time lapse images showing sequential fill and drain of six lateral channel reservoirs, each with a pRBV at the distal end and a CRV at the intersection with the main channel. The retention pressure of the CRV exceeds the pressure of the capillary pump. There are six pRBVs programmed with increasing capillary pressure that indicate the sequence of liquid draining from the side channels. The height of the microconduits is 100 µm. Scale bars in A & C are 3 mm. The scale bars in B are 300µm.*

Various elements were used to design a capillary circuit with pre-programmed sequential flow of reagents, controlled flow rates, and flow reversal. Fig. 4 illustrates the fabricated circuit.

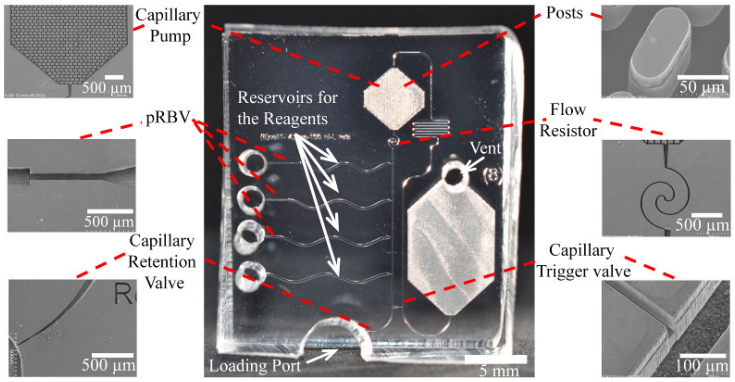

Fig. 4 *Optical micrograph of a microfluidic capillary circuit for flow reversal; the chip is made out of PDMS, and has a size of 19 mm×21 mm. The chip is hydrophilized using plasma activation and covered with a flat peace of PDMS, which is hydrophobic by nature. The chip consists of four reservoirs for the reagents and various programmable retention burst valves (pRBVs), capillary trigger valves, flow resistors, and pumps, all of which are shown as SEM micrographs.*

The flow sequence is self-powered and triggered by adding the sample to the inlet (Fig. 5). A preloaded chip can thus be triggered by a single operation.

To illustrate potential applications of capillary circuits, a system was designed for conducting a sandwich immunoassay to measure the concentration of CRP-antigen solutions. The assay steps are shown in Fig. 6A. We first patterned capture anti-CRP antibody on the cover surface of the chip using a capillary microfluidic network. The patterning lines were aligned perpendicularly to the reaction chamber. We then filled biotinylated anti-CRP antibody in conduit 1, the washing buffer in conduits 2 & 4, and streptavidin-Alexa Fluor 488 in conduit 3. Afterwards, a sample containing CRP antigen was applied to the main inlet, flowed through the reaction chamber into a metering capillary pump and, upon filling the pump, triggered the main burst valve, inducing flow reversal and sequential drainage of the assay reagents stored in the side channels into the waste pump. The flow rates of the pumps and the reservoirs for the buffers and streptavidin are controlled by to the resistance placed in front of the pumps. The designed sequence for the flow rates maximizes assay sensitivity while minimizing assay time. Fig. 6B shows the binding of the CRP antigen over the patterned captured antibody. Finally, Fig. 6C illustrates a standard curve fitted in a set of 3 independent experiments.

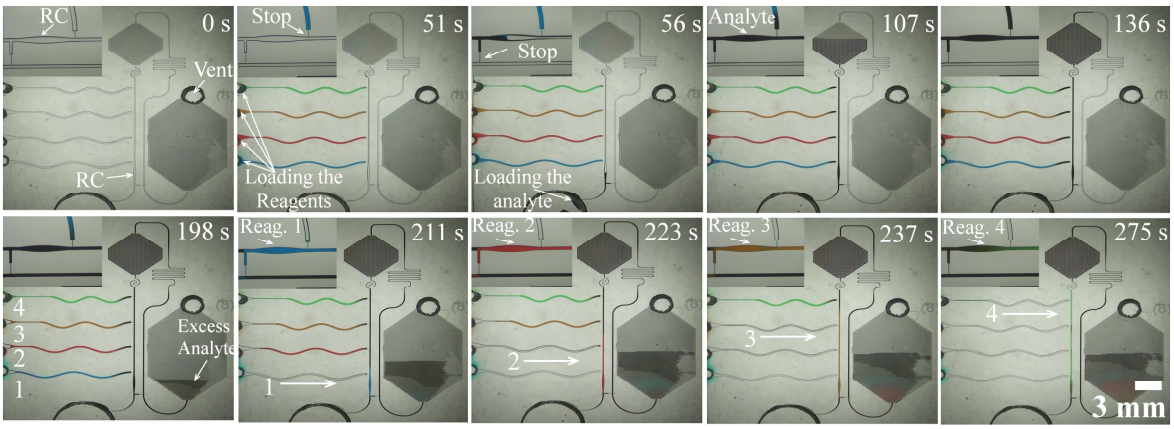

Fig. 5 *Capillary circuit with flow reversal and differential flow rates. Four food dyes, blue, red, orange, and green were dispensed to the channel reservoirs. 1µl of a black food dye representing a sample was introduced into the loading port. The black food dye flowed for ~ 150s through the metering pump (800nl), and after flowing back and activating the main trigger valve, went into the waste pump. Subsequently, the excess amount of sample was drained and the reagents in the side reservoirs were drained sequentially and flowed in the opposite direction through the reaction chamber.*

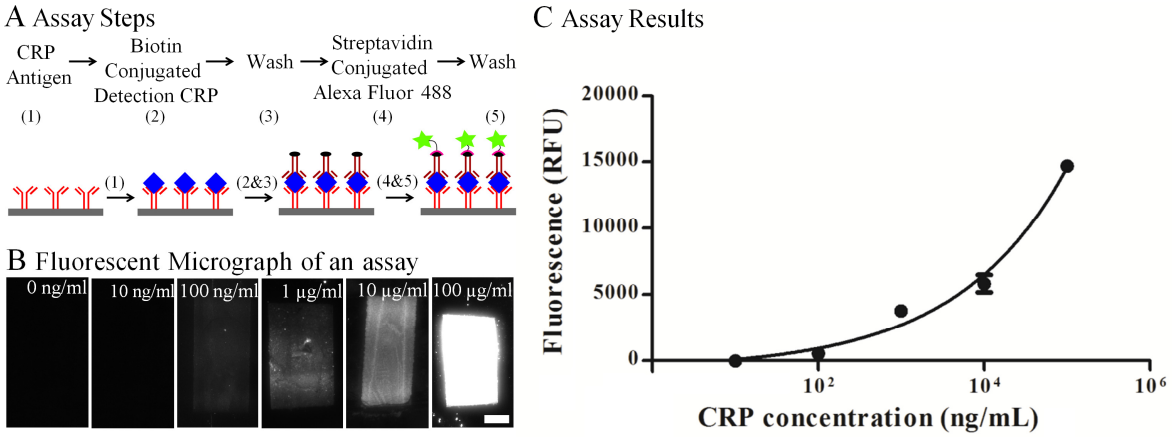

Fig. 6 *Sandwich immunoassay to detect CRP antigen, using the capillary flow reversal platform. (A)The sandwich assay consists of five steps. First, CRP antigen is captured on the reaction chamber where the capture antibody is immobilized. Afterwards, the biotinylated detection CRP, washing buffer, fluorescent streptavidin, and second washing buffer were flushed from the side channels sequentially. Finally, the stamp was imaged with a fluorescence microscope. (B) The fluorescence micrographs (vertical stripes) correspond to the signals of various concentrations of CRP antigen. The scale bar is 50µm. (C) Standard curves of the fluorescent signals obtained from three independent sets of experiments. In each experiment, we tested six chips and measured the average intensities of the fluorescent signals in two patterned reaction zones in each chip. We then fitted a curve the set of data.*

CONCLUSION

We have introduced a library of microfluidic capillary elements including a novel two level trigger valve and a programmable retention burst valve. We then selectively assembled these elements to make a capillary circuit in which various reagents are filled and then drained sequentially, controlled only by the capillarity. Finally, we used a capillary flow reversal circuit to perform a sandwich immunoassay for measuring the concentration of C-reactive protein. We believe this class of fully preprogrammed capillary circuits can be used in a variety of applications, either as research tools for chemists or biologists, or as biosensors for point of care diagnostics.

ACKNOWLEDGEMENT

We would like to acknowledge funding from NSERC, CIHR and CFI, and the assistance of the McGill Nanotools Microfab Laboratory (funded by CFI, NSERC and VRQ).R.S Wishes to thank V. Laforte, H. Li, S. Bergeron, and M. Akbari for their help and discussion. DJ acknowledges support from a Canada Research Chair.

REFERENCES

1 Juncker, D. et al. Autonomous microfluidic capillary system. Analytical Chemistry 74, 6139-6144, doi:10.1021/ac0261449 (2002).

2 Gervais, L., de Rooij, N. & Delamarche, E. Microfluidic Chips for Point-of-Care Immunodiagnostics. Advanced Materials 23, H151-H176, doi:10.1002/adma.201100464 (2011).

CONTACT

hosseinali.safavieh@mail.mcgill.ca, and David.Juncker@mcgill.ca