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PAPER
Roozbeh Safavieh and David Juncker
Capillarics: pre-programmed, self-powered microfluidic circuits built from capillary elements

Featuring work from the Single Molecule Biophysics Laboratory of Hermann E. Gaub, Center for Nano-Science, Ludwig-Maximilians-Universität München, Germany.

Title: Protein–DNA force assay in a microfluidic format

Protein–DNA interaction forces are studied using a miniaturized and multiplexed molecular force assay on a microfluidic MITOMI chip and with a new confocal analysis method.

As featured in:

See Marcus Otten et al., Lab Chip, 2013, 13, 4198.
Capillarics: pre-programmed, self-powered microfluidic circuits built from capillary elements†

Roozbeh Safaviehab and David Junckerabc

Microfluidic capillary systems employ surface tension effects to manipulate liquids, and are thus self-powered and self-regulated as liquid handling is structurally and chemically encoded in microscale conduits. However, capillary systems have been limited to perform simple fluidic operations. Here, we introduce complex capillary flow circuits that encode sequential flow of multiple liquids with distinct flow rates and flow reversal. We first introduce two novel microfluidic capillary elements including (i) retention burst valves and (ii) robust low aspect ratio trigger valves. These elements are combined with flow resistors, capillary retention valves, capillary pumps, and open and closed reservoirs to build a capillary circuit that, following sample addition, autonomously delivers a defined sequence of multiple chemicals according to a preprogrammed and predetermined flow rate and time. Such a circuit was used to measure the concentration of C-reactive protein. This work illustrates that as in electronics, complex capillary circuits may be built by combining simple capillary elements. We define such circuits as “capillarics”, and introduce symbolic representations. We believe that more complex circuits will become possible by expanding the library of building elements and formulating abstract design rules.

Introduction

Lab on a Chip (LOC) devices have emerged as a powerful tool for a variety of applications including bio-analysis1,2 and point of care diagnosis.3 Central to microfluidic applications is the control of liquid flow within microconduits. Most microfluidic systems depend on peripheral equipment to effect and control the flow of liquid. As the complexity of these circuits increased, the similarities to electronic circuits were exploited, most notably in electrokinetic and electrophoretic microfluidics. In addition, centrifugal and hydrophobic microfluidic systems were also built where the advancement of the liquid was controlled by a combination of external control, and pre-embbeded restrictions and hydrophobic patches.4 The most complex microfluidic circuits developed to date are the ones made by multilayer soft lithography.5 More recently, digital microfluidics that use electrical fields and capacitances to move droplets on arrays of electrodes, effectively create a connection between electronics and microfluidics, which may facilitate subsequent scaling up.6 Owing to the great complexity of active microfluidic systems, abstract level of representation were developed.7,8 Passive microfluidics, which have not been developed to the same level of complexity, have not been represented at a symbolic level. Here, we first briefly review passive microfluidics in this introduction, and then present novel capillary fluidic elements and their use for making an advanced capillary microfluidic circuit. Moreover, we propose a symbolic representation of capillary elements (see Table 1) to facilitate the representation and design of capillary circuits.

Passive and capillary microfluidics

So-called passive microfluidic systems circumvent the need for peripheral equipment and extract the energy to move the liquids from the system, thus only requiring minimal user interventions. The flow in passive microfluidics is typically driven by capillary effects, and they have in fact long been used in diagnostics in lateral flow tests.9 Recently, paper–based microfluidics have attracted renewed interest from academia and microfluidic circuits with channels and reservoirs patterned in a paper or nitrocellulose substrate using photolithography, printing or laser cutting are being developed.10 To advance the functionality of these circuits, various valves including (i) flow rate regulators by patterning dilute solutions of a water soluble wax,11 or sugar barriers12 in paper, and (ii) metering valves using sugar bridges12 or expandable polymer actuators13 combined in paper have been developed to control the timing of the fluid delivery. In addition, we and others showed that thread and yarn can be used to build fluidic circuits from the bottom up14 and can be used for immunoassays.15

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3lc50691f
The heterogeneity of fibrous microfluidics, however, makes miniaturization difficult, implying that larger samples are required, and multiplexing will be limited. Furthermore, the limit of detection of paper and thread based microfluidics don’t match the performance of classical ELISA and micro-fabricated microfluidic systems until now.

**Autonomous capillary microfluidic systems**

A landmark paper by Delamarche and colleagues highlighted the potential of self-filling microfluidics based on capillary effects.\(^{16}\) Arrays of hydrophilic 2 μm-wide microfluidic channels, formed by sealing structured poly(dimethylsiloxane) (PDMS) onto silicon wafers, were used to spontaneously draw in liquids into the conduits and pattern proteins on surfaces; however, solutions could not be rinsed or exchanged. Common chemical and biochemical reactions do require addition and exchange of multiple solutions in sequence. An autonomous microfluidic capillary system (AMCS) that was self-powered and self-regulated – hence autonomous – was proposed and designed by combining a capillary pump (CP) and a capillary retention valve (CRV).\(^{17}\) In AMCSs the capillary pressure is encoded in the geometry and surface properties (free surface energy of both solid surface and the liquid), which drives and controls the liquid flow on the chip. AMCS allows filling and flushing multiple solutions by simply delivering them sequentially to an inlet, without any need for removing solutions,\(^{17,18}\) making them well adapted for conducting immunoassays that require the delivery of multiple solutions.\(^{19}\)

**One-step immunoassays**

The ideal devices to conduct immunoassays and point-of-care diagnostics would only require a single manipulation, namely the addition of sample, to complete the assay, so-called one-step immunoassays.\(^{20}\) Efforts to realize such systems have progressed along two directions. One is to keep fluidic functionality simple and implement the sequential delivery of chemicals by controlled dissolution or the geometry of the system. For example, Gervais et al.\(^{21}\) pre-dried reagents in a dead-end conduit thus controlling the release, although this system was unable to sequentially deliver multiple reagents required to amplify the signals and enhance the detection limit of the assay.\(^{21,22}\) Another simple approach featured three open and linked reservoirs that drain sequentially, although limited cross-talks between reagents occurred, and flow rates were not individually controlled, because of the flow resistance, the reservoirs are drained in sequence.\(^{23}\) The second strategy was to expand the functionality of AMCSs to further enhance assay performance and versatility and emulate the functionality normally obtained using active systems.

**Capillary valves**

An important group of elements required for making advanced circuits are valves, but developing them is one of the challenges for building capillary systems, as on one hand they need to be self-filling and moving parts are not usable, and yet the liquid should stop. However, to stop the liquid in capillary systems, one approach is to design an abrupt increase in microchannel cross-section, to make it energetically unfavor-
able for the liquid to flow from the narrow to the wide channel due to the large increase in liquid-air interface at the filling front.24 Such geometric valves can then be triggered by flowing a sample through the wide channel. Although the concept was proposed long ago,25 and even multi-liquid valving was shown,26 an implementation that is robust and reliable for everyday use has been difficult to achieve. The channel enlargement is often only produced laterally within plane, and thus the liquid tends to creep along bottom and cover of the channel. High aspect ratio valves mitigate this issue, but they are difficult to microfabricate, even in Si, and cannot be transferred to other materials, while still occasionally failing.24 In a follow up study, Zimmermann et al.27 added new functionalities to their CPs and improved their performance. More recently, valves have been made in low aspect ratio structures using less hydrophilic surfaces, for example using a PDMS cover that can be depressed manually for liquid activation.28

Hybrid capillary systems

Autonomous sequential delivery of samples has been demonstrated by trapping air bubbles between liquid plugs, mirroring an approach initially implemented with tubes and a syringe pump,29 but using capillary effects to drive the flow.30 However, bubbles create high flow resistance, and varying bubble sizes will affect the flow resistance and may get stuck, which will thus affect the reproducibility and reliability of such circuits.

Capillary microfluidics can also operate with positive pressures formed by hemispheric droplets dispensed atop of the inlet.31 The simplicity of this approach makes them appealing for generating concentration gradients for example; however, the flow rate is low, and changes as the droplets are shrinking, thus only affording limited control over the flow rate. Recently, positive pressure fluidics have also been combined with capillary stop valves for more advanced fluidic operations,32 however, the timing and triggering of liquid flow was not fully pre-programmed, and in fact required multiple timed user interventions, reminiscent of the constraints of the original AMCS.

Capillary elements and capillarics

Over the last few years, the functionality of capillary microfluidic systems has been expanded significantly. Here, we introduce additional capillary components, namely a robust trigger valve (TV) and a retention burst valve (RBV). Furthermore, we introduce capillarics, in analogy to electronics, denoting both the complex capillary microfluidics as such, and the modularity of their architecture allowing them to be designed and assembled hierarchically by combining basic building elements selected from a library. The elemental building blocks are called capillary elements or capillaric elements depending on the context and their complexity. To underline this idea, we introduce a symbolic representation akin to the standardized symbolic representation widely used in electronics. To illustrate potential of this concept, a capillaric circuit was designed that upon flowing a sample, reverses the flow and flushes four different chemicals in a predetermined sequence with a different flow rate. This circuit is then applied to measure the concentration of C-reactive protein.

Materials and methods

Chemicals and materials

Sylgard® 184 silicon elastomer kit (PDMS) was purchased from Dow Corning (Midland, MI, USA). Prepolymers, i.e. curing agent and polymer base, were manually mixed at a ratio of 1 : 10, and cured for 8 h in an oven (Lindberg Blue M, Fisher Scientific) at 60 °C. A 1 mg mL⁻¹ solution of Fluorescein sodium salt (C₂₂H₁₄Na₂O₅) (fluorescein dye, Sigma-Aldrich, USA) in water (Milli-Q purified water, Millipore, USA) was used for characterizing the retention burst valves. Food dyes (McCormick & Co., MD, USA) were purchased from local stores and 50% diluted solutions were used to test the capillaric circuit. Experiments were performed at the room temperature of 23 ± 2 °C. Phosphate buffered saline (PBS) tablets (Sigma-Aldrich, USA) were reconstituted in deionized (DI) water to form 1% PBS in water. Bovine serum albumin (BSA) (Jackson ImmunoResearch, PA, USA) was dissolved at a concentration of 3% in DI water. Human C-reactive protein (CRP) antigen, capture anti-CRP, and biotinylated detection anti-CRP were purchased from R&D Systems (Minneapolis, MN, USA). Streptavidin-Alexa Fluor 488 (Invitrogen, Burlington, ON, Canada) was used as a label in the immunoassay.

Chip design and fabrication

Circuits were designed in a layout editor software, CleWin (CleWin 5, WieWeb software, Netherlands), and the designs were printed in a 7” × 7” chrome mask with 65 000 dots per inch resolution (Thin metal parts, Colorado Springs, USA). A soft lithography technique was followed as described previously elsewhere.33 Briefly, 2 level moulds were fabricated in SU-8 30 (Microchem, Massachusetts, USA), and replicated into PDMS. The elements and circuits are 100 μm deep, except the two level trigger valves, which are only 50 μm deep. The reaction chamber was designed in an oval shape with 200 μm width and 2000 μm length. A razor blade (single edged razor blade, Fisher Scientific, Canada) was used to cut the chips from the replica. The vents of the chip were fabricated using a biopsy punch (1.5 mm puncher, Ted Pella Inc., USA). The chip was rendered hydrophilic with an air plasma (Plasmaline 415, Tegal Inc., US) for 45 s with 250-mTorr pressure and 150-mW power. A flat 2 mm thick PDMS cover was used to seal the chip.

Capture antibody patterning on PDMS

We used a microfluidic capillary system (CS)34 with 16 parallel microchannels to pattern capture anti-CRP on the PDMS cover. Each microchannel was 2000 × 100 × 100 μm³ in size, and was separated from the neighboring microchannel by a 200 μm gap. The CS was activated with air plasma to render it hydrophilic, and sealed reversibly to the PDMS cover, orthogonal to the future reaction chamber that was outlined with a pen. 2 μL of anti-CRP capture antibody (250 μg mL⁻¹) were pipetted into the loading port and spontaneously filled
the microchannels. The PDMS was incubated for 45 min at room temperature in a humidified closed Petri dish containing a wet tissue to prevent evaporation. The liquid was then drawn out using a clean room paper, the PDMS removed, rinsed with PBS for 15 s and blocked with a 3% BSA solution for 30 min to avoid nonspecific binding, rinsed with DI water, dried, and stored for later use.

One step immunoassay
For the sandwich immunoassay, we positioned a plasma activated chip on a patterned cover. The orientation of the reaction chamber was orthogonal to the capturing stripes patterned on the PDMS cover. We then preloaded 4 reagents including: (i) 1% PBS in DI water, (ii) biotinylated anti-CRP detection antibody (200 µg mL\(^{-1}\)), (iii) 1% PBS in DI water, and (iv) streptavidin-Alexa Fluor 488 (500 µg mL\(^{-1}\)) in four reservoirs in the chip by contacting the tip of a pipette to the side channel vents. Subsequently, we loaded 2.5 µL of a buffer with CRP protein, and repeated the entire experiment with a series of different concentrations of CRP. Upon the completion of the assay, we separated the cover from the chip, rinsed it with DI water for 15 s, and dried it using a nitrogen gun, followed by fluorescence imaging of the PDMS cover using a microscope.

Imaging analysis and signal quantification
To characterize the fabricated capillaric elements and the circuits, we used both optical (LV150 industrial microscope, Nikon, Japan) and scanning electron microscopy (S-3000N variable pressure SEM, Hitachi, Japan). Images of the liquid flow in the chip were captured using a stereomicroscope (Leica MZ8, Leica Microsystems, Switzerland) outfitted with a CCD camera (DS-Fi1, Nikon, Japan). As for the immunoassay, we used a customized fluorescence confocal microscope (C1si Nikon Inverted Confocal microscope, Nikon, Japan) connected to a CCD camera (CoolSNAP HQ\(^2\), Photometrics, USA) to quantify the binding of the assay. The CRP binding curve was calculated from three independent experiments. Average colour intensities of the fluorescence images were extracted using Image J (NIH, Bethesda, MD), and binding curves fitted with a four-parameter logistic curve (GraphPad Prism 5, GraphPad Software Inc., USA), Fig. 6. In each experiment, we tested six chips with six different concentrations of CRP antigen and measured the average fluorescent intensities of two stripes.

Results and discussion
Library of capillaric elements
Although active fluidic and microfluidic systems benefited from conceptualization and symbolic representation of the various elements,\(^7,8\) no symbolic representation has been developed for capillary and passive microfluidics to date. Together with technological advances proposed here, we introduce a symbolic representation for capillary elements including (i) microchannels, (ii) fluidic resistors, (iii) vents, (iv) capillary pumps (CPs), (v) trigger valves (TVs), (vi) capillary retention valves (CRVs), (vii) novel retention burst valves (RBVs), (viii) closed reservoirs as well as (ix) open reservoirs. The elements are shown in both schematic and symbolic forms in Table 1.

Microchannels are transporting the liquids and while they also generate a flow resistance, it can often be neglected, and they thus represent the equivalent of electrical lines in electrical circuits. Fluidic resistors are typically microchannels with reduced cross-sections. Because the flow resistance scales as \(R^{-2}\) (radius) in channels with circular cross-section, small changes in size can have significant effects, and a few small sections along the flow path can dominate the overall flow resistance of the circuit, as in electrical circuits. Vents are conduits that are connected to the atmosphere. It is noteworthy that the capillary pressure is proportional to \(R^{-1}\) and in practice becomes negligible when the smallest dimension in a circular or rectangular conduit is \(> 1\) mm; thus vents should be big enough and hydrophobic to minimize surface tension.

CPs generate a constant capillary pressure similar to a voltage source in an electronic circuit. Microstructured reservoirs with posts serve as CPs and the gaps between the posts define the capillary pressure; the smaller the gap, the larger the pressure drop and the stronger the pump.\(^27,35\) TVs stop the flow of a first liquid at a point until it is triggered by the flow of a second liquid entering through a second conduit, as discussed previously.\(^24\) RBVs are a modification of the CRVs, and both are formed by a localized reduction of the channel cross-section, which prevents the liquid from draining due to a large capillary pressure. CRVs are meant to retain the liquid permanently, while the RBVs act as release valves, once the capillary pressure exceeds a threshold value. A series of RBVs with increasing threshold can thus be used to control the sequence of liquid being delivered.

Previously, we and other researchers have introduced some of these capillary elements, shown in Table 1, including CPs, flow resistors, vents, closed and open reservoirs.\(^25,36\) We introduce a symbolic representation that follows the representation from electronics for some elements. Also we present novel symbols for some elements such as capillary pumps along with numerical indication of the strength and both open and closed reservoirs. The symbolism of the valves was developed on the ones commonly used for macroscopic valves,\(^7\) while introducing an arc representing the curved capillary TVs and the RBVs. The elements are shown as a rectangle with a symbol mimicking a voltage source along with a scale bar indicating their strength. In the following sections we explain the functionality of the two novel capillaric elements introduced here, namely the two-level capillary TVs and the RBVs.
Two-level capillary trigger valve (TV)

Conventional capillary TVs that expand only laterally lack robustness, and are prone to spontaneous triggering. A single layer TV with an aspect ratio of depth/width = 3 was made of plasma-activated, hydrophilic, PDMS and closed with native, hydrophobic, PDMS cover. This leaked in less than two seconds, Fig. 1A and Movie S1 in electronic supplementary information (ESI). To overcome the lack of reliability, we propose a two-level capillary TV, which consists of a shallow conduit intersecting a deep one and a hydrophobic cover, Fig. 1B. The abrupt enlargement in the cross-section of the microchannel occurs laterally and vertically at the bottom, while the hydrophobic cover prevents the liquid from creeping along the top. A two level TV with the aspect ratio, depth/width = 1.5, stopped the liquid for more than 20 min, Fig. 1C and Movie S2 in ESI. These valves robustly stopped liquids for over 20 min, and we did not observe a single failure in 50 experiments.

Retention burst valves (RBVs)

CRVs and RBVs are formed by constrictions in the microchannel that produce high capillary pressure. The CRVs and RBVs shown here were all engineered in deep channels, while varying the width to gradually increase the capillary pressure. A schematic illustrating a step by step operation of an RBV is presented in Fig. 2A. If the capillary pressure of a CP is weaker than the one of the constriction, a CRV is formed. If the capillary pressure of the pump is stronger than the constriction, then an RBV is formed that will retain the liquid until the pressure at a point within the circuit drops below the capacity of the valve. Thus, as long as there is flow in the circuit, the pressure drop across the microchannel makes the hydrodynamic pressure of any point in the vicinity of the RBV weaker than the receding capillary pressure of the RBV itself, $|\Delta P|_x < |\Delta P|_{RBV}$. As a result the liquid stops at the valve, but once the flow stops, the circuit acts as a hydraulic system, and the capillary pressure of the pump is transmitted throughout, causing $|\Delta P|_x > |\Delta P|_{RBV}$, and eventually the RBV bursts. Thus, if multiple retention burst valves with different thresholds are included, the weakest one will burst first and the liquid stored downstream will be drained, and then the second weakest one will burst and the liquid will be drained, and so on.

The burst pressure of the valve is related to its dimensions according to the rules of capillary pressure. However, while the advancing contact angle is present during filling, it is the receding contact angle that arises during draining. Hysteresis between advancing and receding contact angles thus requires that the dimensions of the CP be significantly smaller than the one of the RBVs to burst it. Moreover, although not reflected in the equations, we found that the length of the RBV also contributed to the strength of the RBV. We then designed a series of RBVs with varying width and length, and integrated them in a simple circuit to illustrate sequential drainage of 6 valves, Fig. 2B&C. The RBVs are located at the extremity of each side arm. CRVs with a capillary pressure exceeding the one of the CP were included at the junction of each side arm and the main conduit, as well as on the inlet side to prevent drainage of the main conduit. Fig. 2D and Movie S3 in ESI show time-lapse images of the filling and sequential drainage of the six side arms with a solution containing fluorescein. The liquid was added to the loading port (not visible) and then started filling the microchannels and the CP. A flow resistor in front of the CP ensured that all side-arms were filled despite the large capillary pressure of the CP. Next, as the liquid in the loading port is drained, it is pinned and stopped at the CRV0. The CRVs are numbered in the schematic to facilitate the discussion, but they have no functional difference except the fact that they are activated at different time points. As all flow stops at this moment, the pressure of the CP acts throughout the entire circuit, and bursts the RBV1, followed by drainage of the first conduit until the liquid is pinned at the CRV1 next to the main channel. Then, the RBV2, is burst, and so on, until all 6 side arms are drained.

Capillary circuit with flow reversal

To illustrate the possibility of making complex systems using a variety of capillaric elements, we designed a capillaric circuit for performing one-step immunoassays, Fig. 3A&B. The chip is sealed with a hydrophobic PDMS cover with vents and loading ports to add the reagents and a sample. The circuit comprises 4 side-arms, each comprising an RBV at the extremity close to
the loading ports, and a TV that connects each arm to the main channel, and that simultaneously acts as a CRV. The side arms are preloaded with 4 different reagents that are applied to the respective loading ports, Fig. 3C, (t1–t2). A filled pipette tip is brought into contact with the chip, and the reagents are spontaneously drawn by capillary pressure into the side-arms up to the TVs1–4 (t3). A sample applied to the main loading port flows via a channel, a CRV, fills a short side-conduit stopping

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Fig. 2 Operation of the RBVs. (A) schematic illustrating a step by step operation of an RBV as used in this demonstration. (t1) First, the RBV is filled by capillary flow. (t2) While the pressure in the capillary circuit in the vicinity of the RBV (indicated by X) is weaker than the capillary burst pressure of the RBV (|ΔP|X < |ΔP|RBV), the liquid remains pinned by the RBV. (t3) If and when the pressure in the circuit rises beyond the withholding burst pressure, |ΔP|X > |ΔP|RBV, the liquid starts draining the narrow channel of the RBV until it eventually bursts (t4), resulting in rapid drainage of the (closed) reservoir downstream of the RBV. (B) symbolic representation of a circuit comprising 6 RBVs with incremental burst thresholds, 7 CRVs, a flow resistance, a CP and an inlet (not shown). SEM micrographs of RBV3 (weaker) and RBV5 (stronger) are shown on the left. (C) Micrograph of the circuit to test the RBVs. (D) Time lapse images showing the sequential filling and draining of reservoirs according to the pre-defined sequence dictated by the incremental burst threshold of the RBVs. Each of the serpentine side-reservoirs comprises an RBV and a CRV. The CRVs were placed at the junctions of the side-arm and the main conduit, and have a stronger capillary retention pressure than that of the CP. The RBVs were at the end of each side arm, and have a weaker capillary pressure than that of the CP. By adjusting the length and the width of each RBV, the sequence of draining of 6 side-reservoirs follows the pre-defined sequence. The depth of the microchannels is 100 μm. The scale bar in (B) is 400 μm and the scale bars in (C) and (D) are 3 mm.
at a TV$_5$ ($t_3$), and progresses to the reaction chamber. The liquid then flows past the 4 side-arms, through a resistor and into the incubation CP that draws a precise volume, flushing it through the reaction chamber. Upon filling the incubation CP ($t_5$), the sample moves continuously, and activates the TV$_5$, which is located upstream of the reaction chamber. Following activation, excess sample flows through the TV into the waste CP until the entire excess sample is depleted and the flow stops at CRV$_5$ ($t_6$). The capillary pressure in the circuit then rises to the level of the waste CP, and triggers the first RBV ($t_7$) and drains the reagent from the first side-arm, which flows back through the main channel and the reaction chamber to the CP until the CRV stops the drainage. Each arm is drained in sequence according to the threshold of each RBV ($t_8$–$t_{10}$). The flow path through the incubation CP remains open but, because the flow resistance is $\sim 55$ times higher than that through the reaction chamber, virtually all reagents flow back through the reaction chamber at a preprogrammed flow rate. This circuit was designed for performing immunoassays.

The capillaric circuits were made out of PDMS using a two level SU8 mould as a $19 \times 21$ mm$^2$ chip. The chips were plasma activated to render them hydrophilic, and a hydrophobic PDMS with vents and loading ports was used as the cover layer, Fig. 4.

To validate the design, solutions of food dyes and DI water were filled as outlined in Fig. 3, and recorded, Fig. 5 and Movie S4 in the ESI.1 The side-arms were filled by simply contacting the extremity of the channel with a pipette tip. 1 µL of a black food dye, serving as a sample, was introduced into the main loading port. The flow pattern replicated the steps outlined above accurately.
**Sandwich immunoassay in a capillaric circuit**

To show the potential applications of capillaric circuits, a sandwich immunoassay for CRP was performed as outlined in Fig. 6A. We first patterned anti-CRP capture antibody on the cover of the chip using a CS perpendicular to the reaction chamber. Later on, biotinylated anti-CRP antibody was filled into side-arm 1, washing buffer in side-arms 2 and 4, and streptavidin conjugated to Alexa Fluor 488 in side-arm 3. Next, 1 µL of phosphate buffer saline spiked with CRP was applied to the main loading port, flowing and triggering the flow of all the other reagents. Fig. 6B shows the assay results for triplicate measurements of the CRP concentrations between 0.01 and 10 µg mL⁻¹ as well as a negative control. Two fluorescence micrographs (vertical stripes) in a reaction chamber, corresponding to the signals of 10 µg mL⁻¹ concentration of CRP antigen were also illustrated as an inset of the graph. The scale bar of the micrograph is 100 µm. To achieve higher sensitivity, it will be necessary to increase the incubation time of the sample, and optimize the flushing time for the reagents, while also improving the stability of the surface, because the PDMS used slowly reverted to a hydrophobic state.

**Conclusion**

We introduced the concept of capillarics, in analogy to electronics, as a more complex form of capillary systems introduced a decade ago. Whereas one can draw a general conceptual analogy to electronics, there are also deep differences, because with capillaric circuits the fluid logic is realized with the advancement and retreat of the wetting front of the liquid in different parts of the circuit, in a time and space dependent manner. We demonstrated that capillaric circuits can be made from a number of capillary and capillaric elements including CRVs, RBVs, resistors, vents, TVs, CPs, closed and open reservoirs. We notably designed closed reservoirs with RBVs at the extremity and a combined TV/CRV at the intersection with the main channel, so that reagents filled in each reservoir would be drained according to a pre-programmed sequence, regardless of the order with which they were filled. These elements were integrated into a capillaric circuit for a one-step immunoassay with flow reversal implementing distinct flow rates for the sample and subsequent reagents, and comprising two CPs for sample metering, and one acting as a final waste collector. Considering that the maximum aspect ratio in the capillary elements and circuit presented in this paper is 3, these capillary components can be replicated in polymers using injection molding for making low cost microfluidic liquid handling platforms. The concept of flow reversal, and the capillary valves including retention burst valves (RBVs) and the capillary trigger valves (TVs) presented in the paper can be integrated with other microfluidic platforms including centrifugal microfluidics and vacuum assisted filling to enhance their functionalities. The combination of various elements allows creating new fluidic logic operations and circuits, which may be designed at an abstract level, using symbols such as the ones introduced in this manuscript, and then realized physically according to design rules yet to be established. We believe that many more fluidic circuits may be built both as microfabricated structures that afford great control, but also using porous supports such as paper that are inexpensive, or possibly by combining the best of both worlds so as to obtain a better control at a reduced cost. However, challenges remain. For example the number of RBVs that can be reliably operated in parallel are limited by imperfections and hysteresis in wetting, which commands the use of significant differences in retention pressure, while requiring CPs with significantly higher capillary pressure than the highest RBV to ensure its
drainage. Scaling up of capillaric circuits will depend on overcoming such limitations. However, as the examples shown here demonstrate, circuits with moderate complexity can already be designed and built. Whereas we demonstrated a one-step immunoassay, improvement such as long term reagent storage are still needed to permit pre-filling of chips independently of usage. Furthermore, a more convenient assay readout is also needed, and may be implemented using for example silver amplification, permitting assay readout using a cell phone, or an electrochemical detection using simple electronics only. We thus believe that capillarics will be useful for point-of-care diagnostic applications, as well as for many chemical and biochemical processes that require the sequential addition and removal of multiple reagents.

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