Companion diagnostic test using digital microfluidics

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ABSTRACT

This paper reports an automated platform using a new digital microfluidic approach based on pneumatic actuation. The originality of this approach is in the use of a hyper-elastic material that allows the manipulation of a wide range of volumes from μl to several hundreds of μl in the same disposable device. Basic operations such as dispensing, mixing and dilution are successfully validated. These programmable operations are integrated in a fluidic cartridge and operated with simple pneumatic instrumentation working on low pressure (typically a few hundreds of mbar). Our technology is therefore suitable for the development of portable diagnostic assays that require relatively complex protocols that include multiple mixing steps. As an example of a suitable application, this paper describes the integration of a commercial diagnostic assay dedicated to monitoring the medication of patients suffering from a cardio-vascular condition.

Keywords: Digital microfluidic, lab on a chip, point of care application, stretchable microfluidic.

1 INTRODUCTION

Companion diagnostics are an indispensable part of personalized medicine, providing information that is essential for the safe and effective use of a corresponding therapeutic drug. However, the development of devices in companion diagnostics faces various technical challenges. Such devices need to be robust, transportable and easy to use. Moreover, the bio-fluidic protocol can be complex with multiple operations in series and in parallel that make integration in a portable format challenging. Our objective is to develop an automated platform using an original digital microfluidic approach in order to ease the integration of such complex protocols.

Since several years microfluidics has emerged as enabler for medical point-of-care diagnostics. Many different fluidic technology were developed in order to integrate microfluidic protocols in disposable devices. Because it is often advantageous to physically separate reactions from one another, digital microfluidic is now widely used in lab-on-chip application. In digital microfluidics, discrete volumes of fluid can be independently dispensed, mixed, stored, measured, manipulated in a programmable fluidic device. Various protocols with high complexity have been successfully validated by using EWOD actuation [1][2][3]. Other protocols with large scale integration capability [4] are also performed by soft lithography technology in PDMS material [5]. However, these mature technologies are designed for the manipulation of reaction volumes ranging from nl to μl, but are limited when aiming higher fluid volumes. Our technology provides a solution for those applications that have a clear need of precisely handling discrete volumes ranging from μl to ml in a single consumable device. The originality of this approach is in the use of a hyper-elastic material that allows the integration of collapsible chambers having wide range of sub-microliter volumes in a same disposable device. Furthermore, due to the softness of the membrane the pressure required for fluidic actuate are low and the platform will be easily miniaturized.

This paper describes the integration of a commercial diagnostic kit (PLT VASP/P2Y12®, Stago, France) which is used for monitoring patients under the administration of anti-coagulation drugs by measuring the platelet responsiveness to ADP via the P2Y12 platelet receptor. Based on this application, our novel digital microfluidics approach was developed and successfully validated with whole blood samples and reagents from the kit.

2 MATERIALS AND METHODES

2.1 VASP protocol

The PLT VASP/P2Y12 Test kit is qualified to monitor specific platelet ADP receptor (P2Y12) antagonists. The blood sample is first incubated with PGE1 alone or with PGE1 + ADP for 10 minutes. Afterwards, the cells are permeabilized, and the phosphorylated VASP is labelled by indirect immunostaining with a specific monoclonal antibody (clone 16C2). The platelets are counterstained with an anti-CD61 specific antibody. Dual color flow cytometry analysis allows to compare the two tested conditions and to evaluate for each sample the capacity of ADP to inhibit VASP phosphorylation.

Starting from a 15 μL sample of whole blood, the kit runs 3 tests in parallel, each consisting of four mixing and incubation steps and one dilution step. Finally, the result is quantified by flow cytometry. At the current stage of our study, for raisons of simplification, only one of the three parallel tests will be considered. Hence, the fluidic protocol consist of 4 successive mixing steps with 4 reagents (respectively R2, R3, R4, R5) and a final dilution step with diluent R6.

2.2 Chip fabrication

The integration of the fluidic protocol requires a combination of elementary function such as, volume dispensing, splitting, and 5 successive mixing steps with a volume starting from 5μl going up to to 200μl for the final dilution. This has been achieved by defining collapsible chambers using a hyper-elastic silicone membrane (Ecoflex 00 50Smooth on, Macungie, PA, USA). This material was recently used as well for other novel applications like soft robots [6] or hyper elastic strain sensors [7]. This bi-component silicone material withstands deformations much larger than PDMS. It exhibits a Young's modulus around 200 kPa and a maximal stretching ratio before breaking of 980%. The membrane is fabricated by spin coating of a 100-200 μm layer.

The membrane is inserted between a fluidic card on top and a pneumatic card on bottom. Inlet/outlet wells and channels are patterned on the fluidic card and spherical caps are defined on the pneumatic card. Both rigid polymer card are made of 3 mm thick Cyclic Olefin Copolymer (COC) sheet (TOPAS, US) and have the size of a credit-card (85.6 × 54 mm). All patterns were directly machined using a DATRON M7HP (DATRON, GE), which allows very fast prototyping.

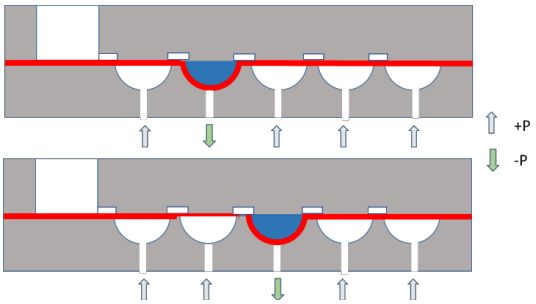

Figure 1: Working principle of our digital microfluidics approach.

2.3 Working principle

Depending on the pneumatic pressure applied to the hyper-elastic membrane the chamber can have two states. At state 0 a positive pressure pushes the membrane onto the fluidic card: the chamber is collapsed and the chamber is at state 0. When a negative pressure is applied, the chamber is completely opened and therefore completely filled, corresponding to state 1. The elementary volume of fluid is calibrated by the chamber geometry and can flow to an adjacent chamber through a small channel which has a volume that is very small compared to the volume of the chamber. This displacement is done by switching the state of the two adjacent chambers (Figure1). Due to the softness of the membrane the pressure of actuation is very low. Negative and positive pressure are -250 mbar and +150 mbar respectively.

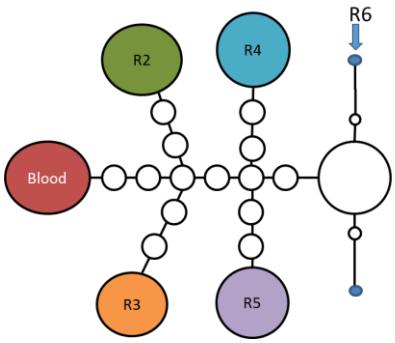

Figure 2: Microfluidic architecture used for the VASP protocol.

2.4 Chip design

The spherical cap cavities (4 mm in radius and 0.8 mm in height) defined in the pneumatic card calibrate an elementary volume of 5μl. Each chamber presents the same geometry. In order to minimize dead volume, the fluidic channel between two adjacent chambers is designed to be 0.1 μl in volume.

To integrate the protocol with one sample and 4 reagents the network presents five branches and five inlet wells. A larger chamber of 200 μl is also added for the final dilution (Fig2) with fluidic input for the injection of diluent (R6) and output for collecting the final solution.

3 RESULTS AND DISCUSSIONS

Initially, all chambers are placed at state 0. Several tens of microliters are manually pipetted in each well, after which all the following step are performed automatically by pneumatic actuation only.

3.1 Dispensing and displacement

The creation of a single elementary volume is depicted in figure 3. Two adjacent chambers near the input wells are activated so that the displacement of the membrane pumps the liquid from the well (Fig 3-b and c).

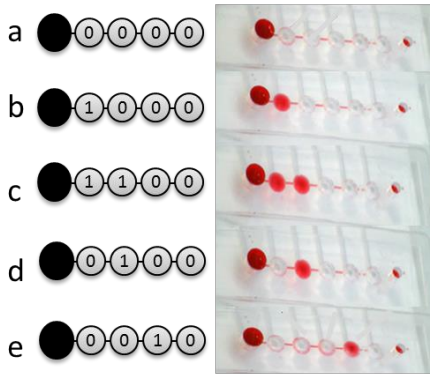

Fig 3 Dispensing and transporting an elementary volume.

When the first chamber is closed an elementary volume of 5 μl is created (Fig 3-d). The dispensing operation can be repeated for defining successive elementary volumes until the inlet well is empty. Excellent reproducibility (CV < 3% for 10 consecutive volumes) of the dispensing operation from the wells was evaluated by fluorescence imaging.

Volume displacement from chamber to chamber (Fig 3.d,e), is very fast because of the hemispherical shape of the chamber and the short pneumatic response (few 100 ms) of the hyper-elastic membrane.

3.2 Mixing

Mixing occurs when two elementary volume are brought together in adjacent chambers. A back-and-forth actuation movement results in homogenous mixing after only several movements. The reason for this effective mixing is that the fast displacement generates recirculation in the chambers that are well observed with fluorescent dyes (see Fig 4).

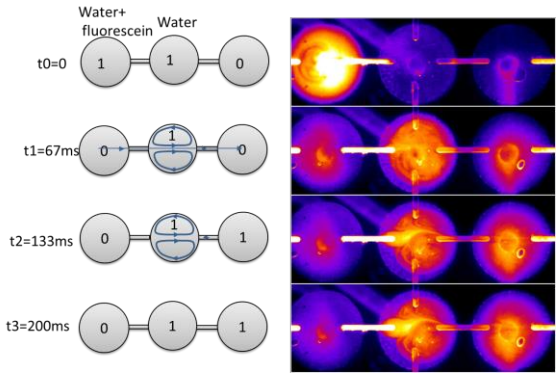

Fig 4 Visualization in false color of mixing during fluid displacement using a solution of fluorescein

3.3 Dilution

Many protocols require a dilution step. Because it is generally difficult to handle both large and small volumes on a same chip, dilution is generally achieved by iteration [8] of multiple smaller steps with the risk of accumulating error in the precision. A major advantage of our technology is the possibility to handle a wide range of volumes and a variety of chamber geometries. The membrane with a very high stretching ratio before breaking allows deflection up to several millimeters into cylindrical chambers. The advantage is twofold: first, the footprint of high volume chambers can be reduced, and second, it is easy to induce strong internal flow patterns because viscous damping is drastically reduced compared to chambers with a very large aspect ratio.

The VASP protocol requires a dilution ratio of 40X, which is achieved by combining a 5 μl and a 200μl cylindrical chamber (Fig5). The dilution is thus performed in only one step. For mixing enhancement, internal flow is generated in the large chamber by activation and deactivation of the adjacent 5μl. About ten pneumatic switches are required to achieve homogeneous mixing in less than 30 seconds.

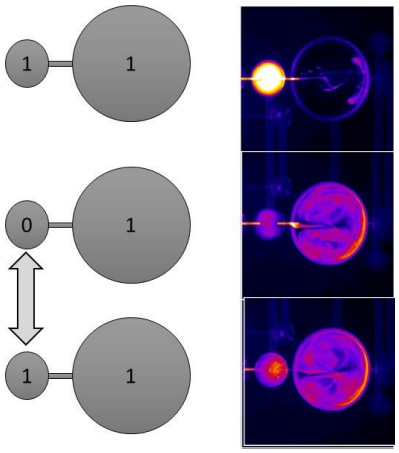

Fig 5 Visualization of dilution using a solution of fluorescein.

3.4 Fluidic validation with blood and reagents

Fluids of interest such as whole blood have complex properties and protocols can require reagents with high concentration of surfactant. Because this can have a large influence on the fluidic behavior, all fluidic operation described above were also validated with blood and the various reagents of the VASP kit. Since pneumatic actuation can generate sufficient force to overcome viscous and capillary forces, the fluidic behavior of the cartridge is not affected by the presence of bubbles. Also, transport and mixing kinetic are similar between water and blood.

3.5 Fluidic validation of the VASP protocol and perspectives

The complete protocol, including 4 successive mixing steps and one dilution, were optimized with dyed solutions and subsequently validated with blood and reagents. Blood and reagent are pipetted in the dedicated wells as depicted in Fig 2. Then elementary volumes of blood and reagent R1 are created and mixed. After 5 min of incubation, a 5 μl volume of reagent R2 is created and then mixed with the incubated 10 μl volume (blood+R1). Similar operations of mixing and incubation are performed subsequently with R3 and with R4 so that the final reaction volume is 20 μl. Mixing is performed each time by a back-and-forth switching operation of the chamber state as described before. Finally, a 5 μl volume is aliquoted and dispensed in the 200μl chamber for a 40X dilution.

The overall protocol is achieved in a fully automated way with 15 collapsible chambers of 5 μl and one of 200 μl on the same chip (Fig 6). With 130 instructions for the pneumatic actuation, the protocol takes 30 min including all incubation times.

After dilution, the reaction volume is collected in a tube and characterized by flow cytometry and compared to a reference of the same protocol executed completely in–tube using the conventional manual method. We observe similar levels of florescence and comparable dot plots between the microfluidic and the conventional method. These preliminary results demonstrate that the double labeling of platelets was successfully performed in our digital microfluidic device.

The next step of our study is to perform the three tests of the VASP kit in parallel on the same cartridge (including positive and negative control) in order to obtain test results that can be compared to the conventional manual in-tube method.

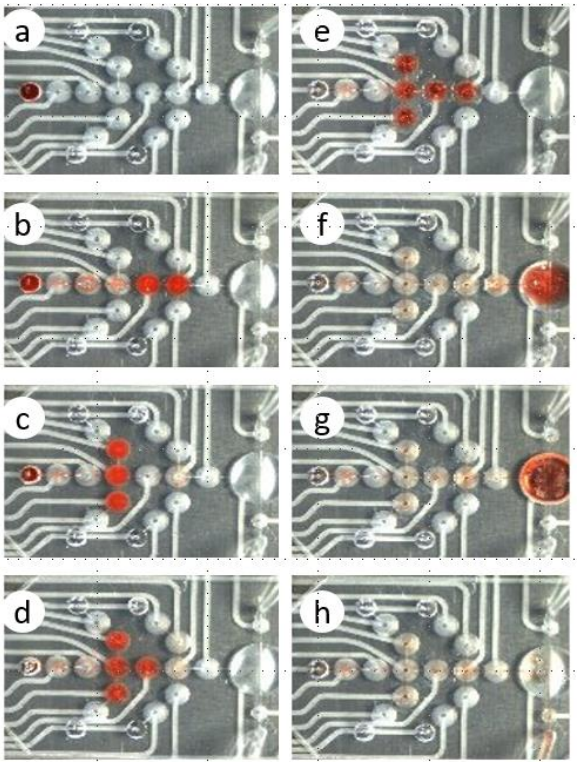

Figure 6: Snapshots of the VASP protocol carried out in the microfluidic cartridge. The starting volume of 5 μl blood is increased with an additional 5 μl or reagent at each of the four mixing steps (a-e), and diluted in volume of 200 μl (f).

4 CONCLUSION

A novel digital microfluidic approach based on pneumatic actuation of a stretchable membrane was developed. Basic fluidic operations of dispensing, transport, mixing, and dilution were demonstrated with dyed solutions, blood, reagents. The working principle is simple and robust. Compared to other digital microfluidic technologies, the cartridge does not require a complex diphasique fluid system (water-oil) and no sophisticated layer stack (conductor-dielectric-hydrophobic layers). Furthermore a wide range of volumes can be operated on a same chip using pneumatic actuation only. In particular, a challenge for the integration of the VASP assay is that the fluidic system needs to manage different reaction volumes (5, 10, 15, 20 and 200 μL) in the same disposable fluidic device. This is possible because of the high elasticity of the membrane which presents a maximal stretching ratio before break of more than 100 times higher than PDMS. Due to the softness of the membrane, the required pressure for actuation is less than 500mbar, which allows the pneumatic system to be miniaturized easily. The instrument contains no wetted parts because all fluidic operations are performed by pneumatic actuation only. Current research and development efforts are focused on the integration of a complete VASP protocol (including positive and negative control) and the integration of an optical detection system for the flow cytometry analysis.

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REFERENCES

[1] S.J Cho , S.K Fan , H. Moon , and C.J Kim, Journal of Microelectromechanical Systems, 70-80, 2002.

[2] R. Sista, Z. Hua, P. Thwar, A. Sudarsan, V. Srinivasan, A. Eckhardt, M. Pollack, V. Pamula, Lab Chip 8 (12), 2091-2104, 2008.

[3] Y. Fouillet, D. Jary, C. Chabrol, P. Claustre, C. Peponnet, Microfluid. Nanofluidics, 4, 159–165 (2008).

[4] T. Thorsen, S.J. Maerkl, S.R. Quake, 298. Science, Vol. 298, Issue 5593, 580-584, 2002.

[5] M.A. Unger, M.A. Unger, H. Chou, T. Thorsen, A. Scherer, S.R. Quake, Science, Vol. 288, Issue 5463, 113-116, 2009.

[6] M. Wehner, R.L. Truby, D.J. Fitzgerald, B. Mosadegh, G.M. Whitesides, J.A. Lewis, R.J. Wood, Nat. Publ. Gr. 536 451–455, 2016.

[7] F. Pineda, F. Bottausci, B. Icard, L. Malaquin, Y. Fouillet, Microelectron. Eng. 144 27–31, 2015.

[8] E.C. Jensen, P. Bhat, R.A. Mathies, Lab Chip 6. 685–691, 2010.