A stand-alone and portable microfluidic platform for quantitative multi-step assays

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ABSTRACT

This paper reports an automated platform for quantitative multi-step assays composed of two main components: a microfluidic chip using collapsible chambers pneumatically actuated and a portable instrument to automate the fluidic operations.

To achieve a complete biological assay on a microfluidic chip, major fluidic operations such as volume calibration, fluid transfer, mixing, aliquoting have to be implemented. Moreover, for a quantitative assay, our strategy is to integrate a dilution range to generate an internal calibration. Standards solutions are diluted in the chip to perform a calibration curve and to compare the sample measurement to this curve.

The challenge is to control this multi-step long process as precisely as in laboratory in an autonomous and portable device. To reach this objective, an original technology combining an XY architecture and collapsible chambers has been developed and validated through two protocols.

Keywords: Microfluidics ; Lab on Chip ; Dilutions ; Quantitative assays; Collapsible chambers ; Portable devices.

1. INTRODUCTION

There has been a growing interest in integrated portable and disposable microfluidic systems for point of care applications [1], [2], [3].

These systems are used by minimally trained personnel so a maximum number of steps have to be automatized. To integrate biological assays, most of the microfluidic devices have chosen to simplify the protocol and to limit the test to the sample measurement. Nevertheless, for a quantitative assay, biological protocols recommend to prepare a calibration curve using standard solutions during the test. In fact, depending on the storage conditions, the reagents efficency can vary.

To achieve a calibration curve, range of dilutions have to be performed in the same device. That means to create a range of solutions with different concentrations. Dilutions require however a high precision in volume and a complete mixing. So the challenge is to control precisely volumes and to mix them in a microfluidic device to obtain homogeneous solutions.

Two different approaches have been used for dilutions: continuous flow approach [4] and digital microfluidics [5], [6]. Our objective is not only to prepare standard solutions but also to use it to calibrate the system. So the digital microfluidic approach which control discrete volumes is more adapted.

Another issue is to carry out the operations in parallel to limit the number of actuators and to simplify the automaton. An X-Y architecture is used to actuate each column of valves or chambers simultaneously. The reagents are injected into the Y direction and mixed into the X direction. To reach solutions with different concentrations, the volume of sample chambers increases in the Y direction while that of diluent chambers decreases in a complementary way, keeping the total volume constant in the X direction [8]. However, this architecture involves chamber volumes ranging from microliter to hundreds of microliters in a compact cartridge (a credit card format).

We then propose a new technology based on a hyper elastic membrane to fabricate collapsible chambers with large volumes and particularly with high aspect ratio (such as hemispheric chambers of a few millimeters). This material have been recently characterized and used in our lab as hyper elastic strain sensors [9] and in microfluidic devices for fluid storage [10].

To demonstrate the capabilities of this technology to perform complete quantitative biological assays, a glucose assay and a homogeneous ELISA assay have been developed and validated. These two examples of quantitative and multi-step assays contain all the required functions to perform bioassay protocols, such as aliquoting to manipulate small volumes and mixing with several reagents. Then, an autonomous and portable instrument to control these microfluidic chips has been developped which shows the possibility to perform multi-step and quantitative assays in a stand alone and portable platform.

2. MATERIALS AND METHODS

2.1. Design and architecture

The architecture proposed is an array of collapsible chambers and microvalves. (Figure 1-A). The device is composed by a hyper elastic membrane (Ecoflex, Smooth on) positioned between two Cyclic Olefin Copolymer (COC) micro milled credit card format cards. The membrane is actuated through the pneumatic layer to shift the chambers and valves between two states (Figure 1-B). To close the chambers, compressed air is injected through the pneumatic layer. Then, fluid is transferred where pressure is released.

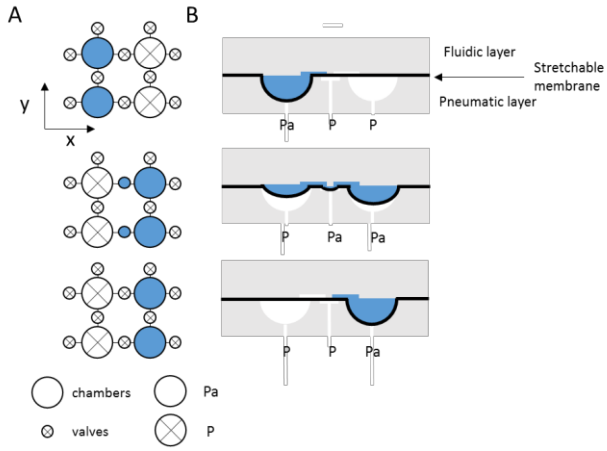

Figure 1: Fluid transfer operation. (A) Design of an array of chambers and microvalves. (B) Cross sectional view of a microvalve between two chambers.

2.2. Chip fabrication

The microfluidic cartridge is a hybrid card comprised of three polymer cards and a stretchable membrane (Figure 2).

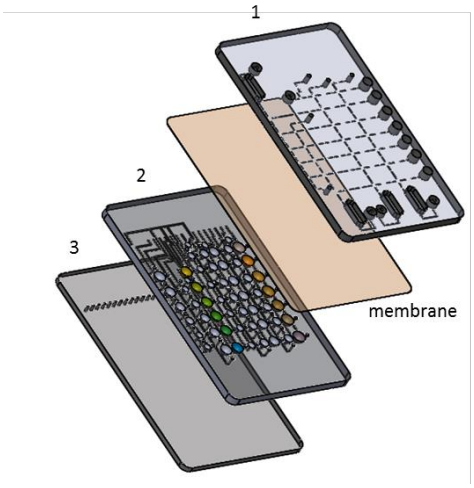

Figure 2: Hybrid card composed of three plastic cards and a stretchable membrane.

The first card contains reagents storage and network channels to connect the fluidic chambers (1). The second card contains pneumatic valves and chambers at the top layer and pneumatic network at the bottom layer (2). The third card allows to close the pneumatic channels (3). The stretch membrane is positioned between the fluidic card (1) and the pneumatic card (2). The microfluidic structures were designed by using Solidworks software. Layers dimensions correspond to a credit card format (85.6 x 54 mm). Microfluidic patterns were directly machined in a 2 mm Cyclic Olefin Copolymer (COC) sheet (TOPAS, US) for the first layer and in a 3 mm COC sheet for the second and the third layer using a DATRON M7HP equipment (DATRON, GE).

Pneumatic channels were machined to a depth of 100 μm and a width of 600 μm. Fluidic channels dimensions are 100 μm in depth and 300 μm in width. Chambers are half-spherical shaped and allows simple sizing. The dimensions depend on the protocol.

The elastic membrane is made by spin coating a 40 μm Ecoflex 00 50 layer (Smooth on, US) on a silanized silicon wafer at 3000 rpm for 6.5 seconds. This bi-component silicone material withstands larger deformations than PDMS. It exhibits a Young's modulus around 200 kPa and a maximal stretching ratio before breaking of 980% (0.8–2 GPa and 7% - 40% for the PDMS). At the end of the filling phase, the elastic membrane must perfectly match the hemispherical surface of the rigid polymer chamber and then adhere to the flat face of this chamber at low pressure (few hundreds of mbar), at the end of the emptying phase. This corresponds to a geometric variation of the membrane surface of 30%. Ecoflex with a stretching rate of 1000% is an ideal candidate to fill these functions, unlike, for example, PDMS, whose stretching rate is too close to the required geometric stretching.

This elastomer is a key material with which the chambers can be filled and emptied totally at low pressures (few hundreds of mbar) which simplifies the pneumatic instrumentation required to actuate the chip. In fact, micropumps can be used to provide the positive pressure.

The second and the third layers are thermally bonded. The membrane is bonded to the second layer and to the first layer by using an adhesive for silicone (Nitto Denko n°5302A). This adhesive was locally opened by a cutting machine (Graphtec, JP) at the chambers and valves places. Valves and the chambers actuation with positive pressures is very efficient, without any pneumatics leaks and repeatable many times.

2.3. Pneumatic actuation

To control the microfluidic cartridge with a portable instrument, a limited number of solenoid valves has to be used for the pneumatic actuation.

This objective is achieved by actuating simultaneously a column of valves or chambers. The chambers and the valves are denoted by the following operators: Ci,j represents the chamber at the row i and the column j and Vi,j, the valve at the row i and the column j. The Figure 3

shows the architecture to perform an enzymatic assay. For a given j and for all i, $C_{i,j}$ or $V_{i,j}$ are actuated at once. V_x refers to the valves between the $C_{i,j}$ and the $C_{i+1,j}$ chambers and are actuated simultaneously during the reagents loading step to isolate each $C_{i,j}$ chambers for a given j.

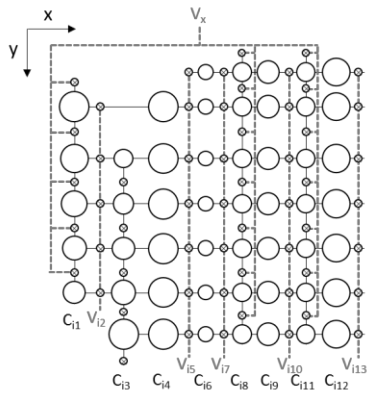

Figure 3: Schematic top view of valves and chambers array for the enzymatic assay architecture.

3. RESULTS AND DISCUSSIONS

3.1. Glucose assay results

Figure 4 shows the fluidic operations required for a glucose assay (GAGO-20, Sigma Aldrich). The first step is to dilute the standard solution for the calibration. A solution of glucose of 0.1 mg/mL is diluted at different concentrations from 1 to 1/5 by using this architecture.

Then, 5 μL of each solution is aliquoted to continue the protocol on a small volume. After that, the solutions are mixed with 10 μL of enzymes. The glucose solutions change to orange depending on the glucose concentrations. After 5 min of incubation time, 10 μL of stop reagent (sulfuric acid) are mixed with the solutions in a 25 μL chamber. The color changes from orange to pink. Qualitatively, the intensity depends on the expected glucose concentration.

Finally, to validate and to precisely quantify the results, the solutions are transferred to the exit wells.

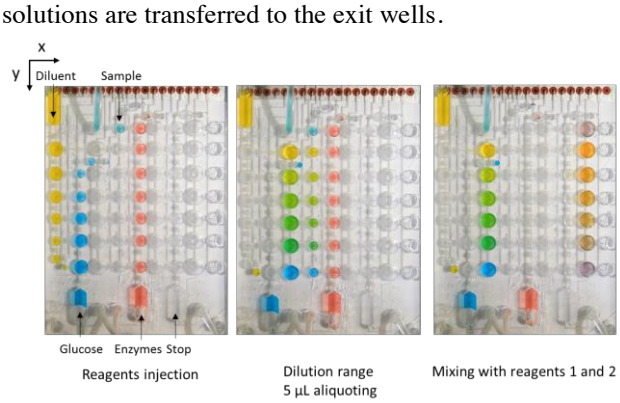

Figure 4: Protocol steps of a glucose assay validated with dyed water.

To quantify the results, the absorbance of the different solutions has been measured at 540 nm (Figure 5). The calibration curve shows a high linearity (R^2 =0.985). The absorbance of the sample which is represented by a solution diluted per 2 corresponds to the expected value. The concentration expected is 0.05 mg/ml and the result given by the calibration curve is 0.047 ± 0.02 mg/ml. So, this architecture is efficient to compare a sample measurement with an internal calibration.

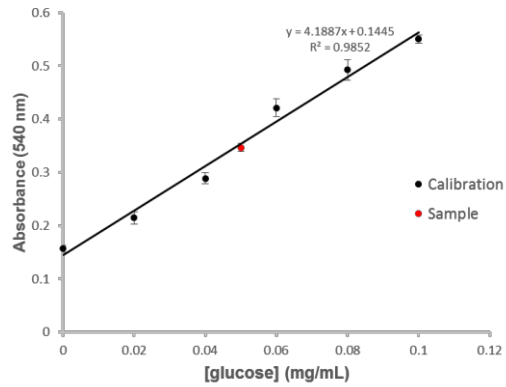

Figure 5: Validation of on chip enzymatic assay. Plot of absorbance intensities depending on the glucose concentration. Mean values with standard deviation bars of 5 repeats have been plotted.

3.2. Homogeneous ELISA test results

Another example of quantitative and multi-step assay is a homogeneous ELISA assay. Dilutions ranging from 1 to 1/64 have been prepared in a chip to perform a calibration curve for a glucagon assay (Cisbio assay, n°62SGLPEF). After the generation of the dilution range, the aliquoting step allows to proceed the protocol with 10 μL. Then, the solutions are mixed with 5 μL of each antibody. As for the enzymatic assay, protocol steps has been first validated with dyed water (Figure 6).

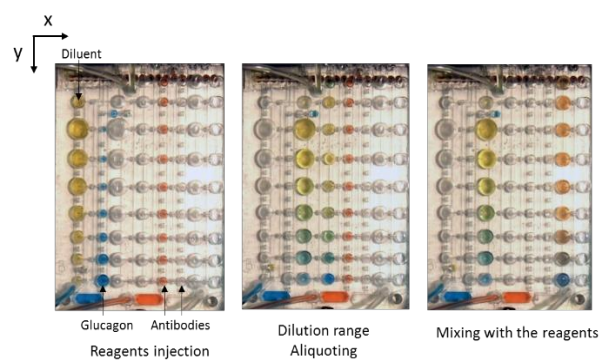

Figure 6: Protocol steps of a homogeneous ELISA test validated with dyed water.

Compared to the enzymatic assay, this architecture includes different volumes chambers for different dilution factors and also more chambers for a wider range. On this chip, nine chambers are analysed in parallel. Eight chambers are the solutions prepared on chip for the

calibration curve and one chamber is for the sample to be analysed. To quantify the results, the HTRF® signal has been measured (Figure 7).

The experiment has been repeated three times with the same chip and the results have been normalized to be compared. The calibration curve was generated by measuring the HTRF® signal for the eight solutions prepared on chip. The signal for the sample (a solution diluted per 2 off chip) was also measured and compared to this calibration curve. The result for the sample was 866 pg/ml ± 150 pg/ml, instead of 1000 pg/mL. The errors can be due to the sensitivity of the detection and also to the pipetting operations required to transfer the solutions from the chip to an adapted microwell plate.

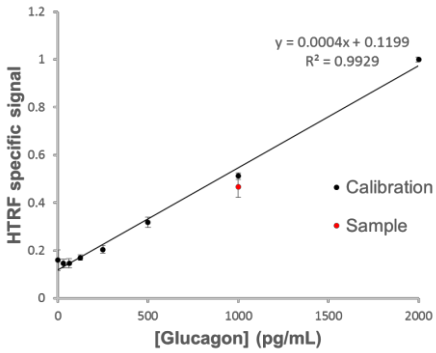

Figure 7: Validation of a homogeneous ELISA assay. Plot of HRTF signal depending on the glucagon concentration.

3.3. Stand alone platform

The technological choices have simplified the development of a generic and portable platform dedicated to the previous microfluidic chips (Figure 8). The portable instrument is only 10 x 14 x 24 cm (height x width x depth) and work with a 24 volt power supply. The main components operated by an Arduino mega microcontroller include micropumps, pressure sensors and solenoids valves. Micropumps can delivered a pressure up to 800 mBar which is comptatible with the collapsible chambers actuation.

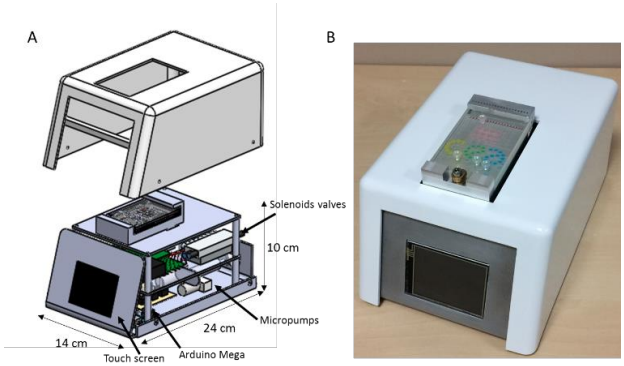

Figure 8: Portable instrument for fluid actuation for complex and quantitative assays.

The inputs, the outputs and the chips holder are standardized to use the same instrument for different microfluidic chips. The user can select and start a protocol through the touch screen. The next step will be to integrate an optical detection module.

CONCLUSION

In conclusion, this microfluidic platform composed of multiple collapsible chambers arranged in an X-Y architecture enables the integration of complex multi-step assays with a calibration. First, the fluidic actuation by a stretchable membrane offers the possibility to handle volumes from microliter to hundreds of microliter and allowing precise fluid filling and draining at low pressure. Second, the X-Y architecture simplifies the integration of dilutions in the chip and allows to synchronize the valves and chambers actuation.

Finally, the technology combining this architecture and collapsible chambers offers the possibility to perform multi-step and quantitative assays in an autonomous and portable platform.

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