Sensors and Actuators B 261 (2018) 106–114

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Quantitative biological assays with on-chip calibration using versatile architecture and collapsible chambers

C. Parent [a,*], F. Boizot [a], M. Cubizolles [a], N. Verplanck [a], J.-L. Achard [b], Y. Fouillet [a]

[a] Univ. Grenoble Alpes, CEA, Leti, DTBS, F-38000 Grenoble, France
[b] Univ. Grenoble Alpes, CNRS, LEGI, F-38000 Grenoble, France

ARTICLE INFO

Article history:
Received 13 October 2017
Received in revised form
22 December 2017
Accepted 11 January 2018

Keywords:
Microfluidics
Lab on chip
Dilutions
Quantitative assays
Collapsible chambers

ABSTRACT

A new microfluidic device composed of pneumatically actuated multiple collapsible chambers arranged in an X-Y architecture has been developed. Elementary fluidic functions such as fluid transfer, volumes calibration, mixing, aliquoting and linear dilutions can be parallelized, achieving automatically and rapidly complex operations. The ultimate aim is to perform fully integrated quantitative assays such as complete enzymatic assays and Elisa assays by using existing kits and an on-chip calibration. Both objectives require to manipulate a high range of volumes (from 1 μL to 100 μL) while keeping an excellent accuracy. Furthermore, quantitative assays have to be highly repeatable to be relevant. This challenge has been successfully addressed by combining three original characteristics. First, a versatile architecture has been designed allowing to be adapted to any quantitative protocol. Then, a hyper elastic membrane with a high elongation rate and switching between two polymer solid layers has been used to control precisely the fluid volumes at each inlet and outlet chambers. Finally, repeatability was obtained by including linear dilutions to generate a standard curve and to calibrate the system.

1. Introduction

Because of the increasing number of chronic diseases and number of patients, there has been a growing interest in integrated, portable, disposable microfluidic systems [1,2]. These systems integrate conventional laboratory work, provide fast diagnosis and can be used by minimally trained personnel.

Microfluidics has been widely used to miniaturize immunoassays [3,4,5]. This protein analysis technique can determine the response to infection with viruses or bacteria, biomarkers such as cancer marker or monitor the evolution of a disease, for example.

Microfluidic systems use generally reagents chambers connected by a network of microchannels to perform biological assays. Fluids can be actuated by using gas bubbles produced by electrodes [6], by manual pressure [7,8], by capillary forces [9,10] or by using centrifugal forces [11,12]. These systems are simple and easy to use but they are limited to one measurement point. More recently, a homogeneous immunoassay (Alphalisa) has been performed [13]. In this way, one target analyte can be detected simultaneously for up to eight samples.

For a quantitative assay such as an immunoassay, the results have to be compared to a calibration curve performed in laboratory conditions. As the calibration is external and achieved in different conditions, the test has to be very reproducible and robust. For instance, if the reaction depends on the temperature or on the reagents storage, the calibration has to be done in the same conditions to be relevant. Moreover, in case of a negative result, the test is considered as negative because its success cannot be checked with a single point measurement and no reference. To obtain quantitative and precise results, a calibration curve has to be generated during the test. To achieve that, 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.

Dilutions can be generated and integrated into a microfluidic chip by using a network of microchannels in a branching structure [14,15,16]. These systems are based on a continuous flow approach. The principle is to control the liquid flows to reach the different concentrations. This technique is adapted to handle continuous volumes over a long period but is complicated for a given analyte volume.

Another approach is digital microfluidics, which controls discrete volumes. Fluidic operations such as mixing, splitting and aliquoting are achieved sequentially and combined to perform dilutions. Two digital approaches can be then employed for dilutions. The first technique is iterative. The principle is to dilute several

* Corresponding author at: CEA Leti, 17 rues des Martyrs, 38000 Grenoble, France.
E-mail address: yves.fouillet@cea.fr (C. Parent).

https://doi.org/10.1016/j.snb.2018.01.111
0925-4005/

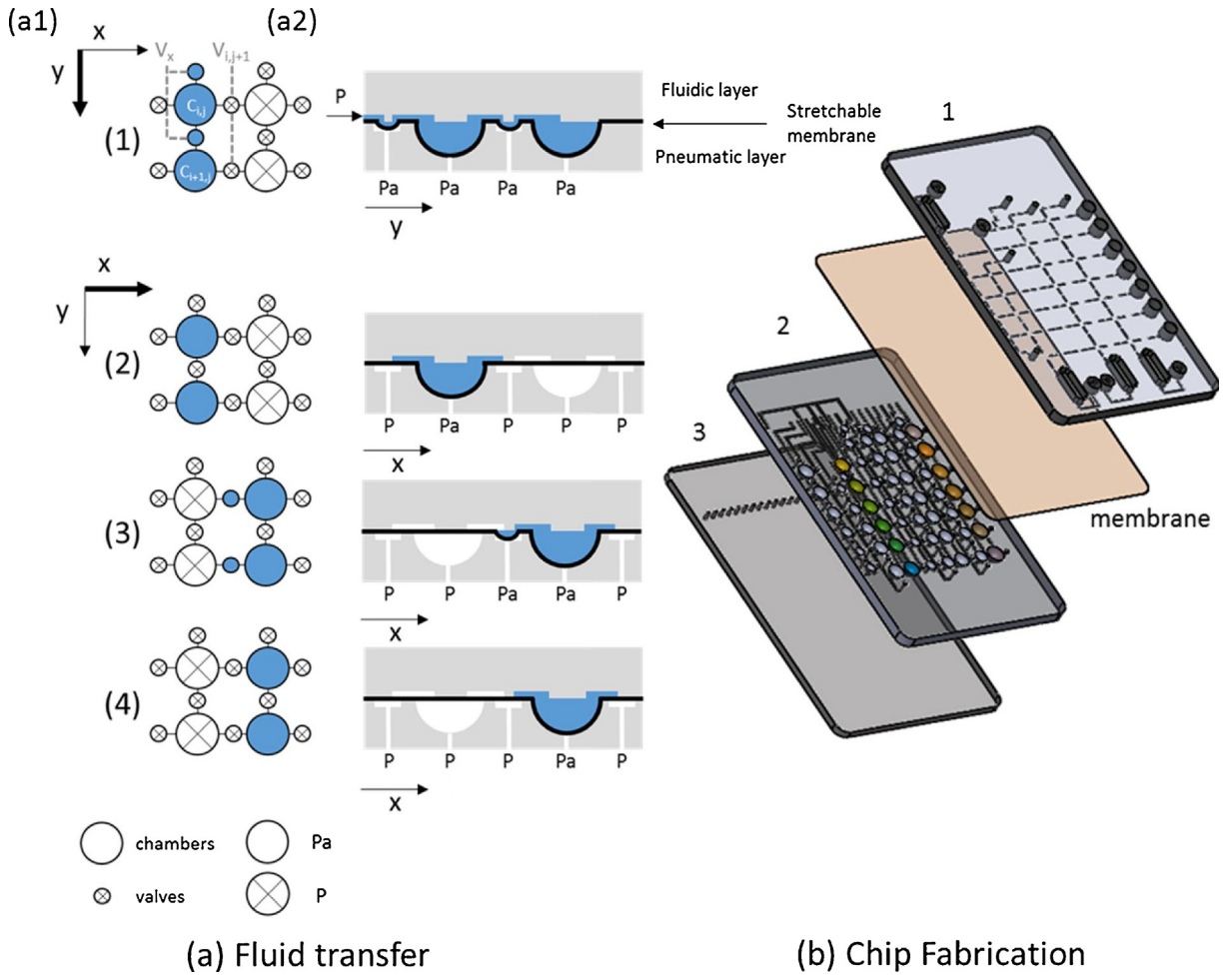

Fig. 1. (a1) Schematic top view of the architecture, design of an array of $C_{i,j}$ chambers and $V_{i,j}$ microvalves. (a2) Cross sectional view of a microvalve between two chambers. (b) Hybrid card composed of 3 plastic cards and a stretchable membrane.

times the same chamber to obtain the concentration required. A volume is first diluted and separated in two volumes. The first volume is diluted again to reach the desired dilution ratio, and the second volume needs to be stored in a specific chamber. This method is based on the control of a unit volume and can be performed by electrowetting [17,18]. This operation can also be achieved by pneumatic actuation on a PDMS chip. This technique uses the deflection of a PDMS membrane to transfer fluid volume from one chamber to another [19,20]. This technology is well adapted because reagents can be efficiently mixed by combining valve operations. Several fluidic architectures use this principle including loop structures [21], ladder structures [22] and a rectilinear array of chambers [23,24]. An iteration process requires many successive steps to perform serial dilutions. Consequently, the final concentration, in particular for high iteration numbers, is strongly affected by cumulated deviations at each single dilution step. The second approach using digital microfluidics is linear dilutions. For each ratio, the dilution results in only one mixing step. This is realized by mixing two discrete volumes (e.g. reagent and diluent) with a ratio corresponding to the expected concentration [25]. As the mixing steps are independent, these operations can also be parallelized. An X-Y architecture can be used to achieve a full dilution range in parallel. 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 [26]. This protocol is simple and requires only a few steps. An additional advantage is that the chip is totally passive, avoiding the use of external pumps. Thus, the fluid control is managed by a reconfigurable layer in one way and passive Laplace valves in another way. The fluid actuation is performed by manual pipetting.

Our objective is not only to achieve a range of dilutions but to use it to provide a standard calibration curve in quantitative biological assays. So the dilutions need to be integrated in a more complex microfluidic chip. Our intention is to use an X-Y architecture while integrating a fully automatized pneumatic actuation. One specificity, which is also a challenge, is that linear dilutions involve chamber volumes ranging from microliter to hundreds of microliters in a compact cartridge (a credit card format). Using classical chambers with such volumes leads to uncomplete filling and emptying that will affect the dilution precision. To avoid this, it may be possible to use elastomeric films such as PDMS to fabricate collapsible chambers which are easy to fill and to empty [24]. However, the elongation of this material is not able to produce the large volume chambers we need to actuate. 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 [27] and in microfluidic devices for fluid storage [28].

To demonstrate the capabilities of this technology to perform complete quantitative biological assays, we have developed and