Project Number: 257401

A highly integrated and sensitive POrous SIlicon based lab on a chip for multiple quantitaTIVE monitoring of food allergies at point of care.

Specific Targeted Research Project

Information Society Technologies

Deliverable D5.3R:
Report on the layout and fabrication of fluidic system, and characterisation of fluidic test system.

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Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	X
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

1 About this deliverable

1.1 Introduction

In the POSITIVE project a POC system is developed which can detect specific IgE antibodies in the patient's blood in order to determine the sensitization to a set of food allergies. A microfluidic system incorporated into the POC device receives the blood sample, filters it and brings it to the sensing unit at the heart of the instrument. The sensing unit itself, the porous silicon membrane, is contained in a disposable cartridge which is easily replaced between each allergy test session. This report presents the layout of this fluidic system, the components and test fluidic system that have been fabricated, and characterization of the fluidic test system.

This version of D5.3 is a revision of the previously submitted deliverable as requested by the EC in the review on the 1st reporting period. Specifically:

"D5.3 Report on the layout and fabrication fluidic system, and characterisation of fluidic test system

***Conditionally Approved** Pending the optimized design"*

In relation to optimizing the design and fabrication of the fluidic system we report here on improvements to the system most notably the use of a glass window cell (4.5). We also report further on how the fluidic test system is characterized by detailing the flow setup used for assay testing (6.4).

[All changes from the original version are made in italics.]

1.2 Scope of the deliverable

For the commercialization of the POSITIVE POC system it is of vital importance that the cost per test is kept at a competitive level. Care is taken to minimize the cost of the disposable components in particular. Novel wafer-level microfluidic packaging schemes have therefore been developed in the project and will be used in the fabrication of the prototypes. At the same time test devices such as fluidic test cells have been developed and fabricated in the project using conventional rapid prototyping technology, and the realized fluidic systems have already been put to use as necessary tools to fulfil tasks in other work packages. The integration of the porous silicon (porSi) membrane into the fluidic system has begun, involving the work of other work packages as well (WP3, WP4 and WP6). The porSi membrane is a crucial part of the complete fluidic system and the fluidic properties of the membrane, embedded in a test chip, have been characterized as part of the fluidic test systems.

1.3 Structure of this deliverable

The report is laid out according to the following topics:

2. Layout of the fluidic system
3. Development of wafer-level microfluidic packaging schemes
4. Fabrication of fluidic test cells
    - 4.1 Porous silicon membrane array chip
    - 4.2 Open cell
    - 4.3 Closed cell
    - 4.4 Improved closed cell
    - *4.5 Glass window cell*
5. Design of fluidic cartridge
    - 5.1 System overview

2 Layout of the fluidic system

At the core of the Positive device is the porous silicon membrane where specific binding between allergen-specific anti-bodies in the patient's serum and the functionalized surface takes place, and where the optical transduction mechanism will detect the resulting concentration of bound molecules. The membrane will be a disposable part of the system, to be replaced between each test (patient). It is therefore part of a cartridge which the operator easily can insert into the POC device, and eject and dispose of after the test session is completed.

It is assumed that the binding assay and the associated optical readout require the use of filtered blood plasma in the sensing unit. The necessary filtering of the patient's blood, outlined in Deliverable 5.1, is achieved by a disposable blood filter. This filter is also integrated in the cartridge, both in order to simplify the user operation, and to maintain short distances between the involved fluidic components.

The design of the disposable cartridge is described in detail in section 5.

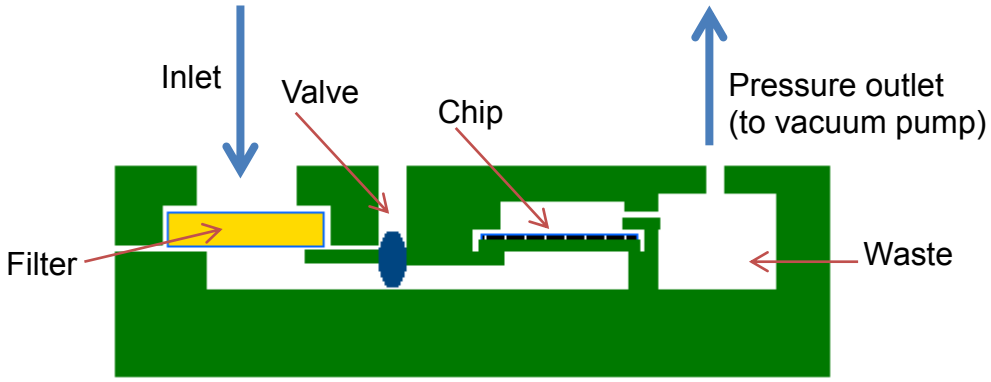

Figure 1: Schematic of the fluidic system (disposable cartridge). The blood sample is filtered before passing through the sensor membrane chip. A hydraulic valve seals of the chip chamber during measurements. Waste fluids are retained in the cartridge.

3 Wafer-level microfluidic packaging schemes

For the microfluidic integration of the porous silicon, we have adopted a dual approach: in one we have focused on creating packages for initial porous structures so that they can be handled and tested in terms of their fluidic and optical properties; in the other we have focused on a generic wafer-level microfluidic packaging approach for the porSi layers. The activities related hereto are all described in this section.

3.1 Wafer-level biocompatible microfluidic packaging

For the wafer-level microfluidic packaging, we have developed two methods, both are based on the OSTE material system that the KTH partner group recently developed [Carlborg2011]. A first method focuses on the microfluidic bonding of a polymer layer to a silicon/silicon-oxide surface, in which the surface is functionalised using isocyanate or vinyl prior to bonding.

The second method utilises the bonding of the OSTE microfluidic layer directly and covalently to the copolymer surface that is used as the surface chemistry for bonding of the probes in POSITIVE. Both methods provide biocompatible bonding techniques.

3.1.1 →Wafer-scale bonding on an isocyanate or vinyl treated surface:

Here we introduced and developed a novel wafer-level bonding concept designed for permanent attachment of micromolded polymer structures to functionalized silicon substrates, using isocyanate and vinyl chemistries in an initial demonstrator to explore the wafer-level manufacturing capability of the method. The method, designed for simultaneous fabrication of many identical lab-on-chip devices, utilizes a chemically reactive polymer microfluidic structure, which rapidly bonds to a functionalized substrate wafer via “click” chemistry reactions. The microfluidic structure consists of an off-stoichiometry thiol-ene (OSTE) polymer with a very high density of surface bound thiol groups and the substrate is a silicon wafer that has been functionalized with common biolinker molecules. The main features of the “click” bonding technique here presented are: 1) low temperature biocompatible process conditions: 2) no need for solvents or plasma; 3) covalent bond formation; and 4) no formation of volatile compounds. The method is biocompatible and is well suited for wafer-level microfluidic packaging of pre-functionalized surfaces. We demonstrate void free, fast curing (~45 s UV-exposure) and low temperature (<37ºC) bonding in the fabrication of a complete batch of microfluidic devices consisting of a microfluidic OSTE polymer layers bonded to a silane functionalized silicon wafer. The diced devices showed a burst pressure exceeding 4 bars, are compatible with most organic solvents, are easily surface modified and have excellent solvent barrier properties.

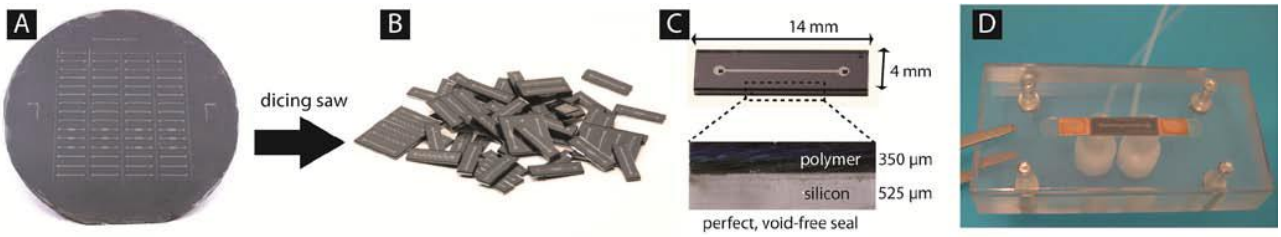

Figure 2: Pictures from OSTE processing and pressure test set-up: (A) bonded layer of OSTE on a silicon wafer; (B) diced chips; (C) 1st demonstrator chips, demonstrating void-free seals; and (D) the “1st demonstrator” chip in the holder used for pressure tests.

This work has been published at the MicroTAS 2011 conference [CarlborgSaharil2011] and has been submitted to Lab-on-a-Chip [Saharil201X]. These two manuscripts are attached in Appendix A1 and A2.

3.1.2 Bonding on a copoly surface:

Thereafter we continued our developments using copoly-functionalised surfaces. We presented a one-step, reversible, and biocompatible bonding method of a stiff patterned microfluidic "Biosticker", based on off-stoichiometry thiol-ene (OSTE) polymers, to state-of-the-art spotted microarray surfaces. The method aims at improving and simplifying the batch back-end processing of microarrays. We illustrate its ease of use in two applications: a high sensitivity flow-through

protein assay; and a DNA-hybridization test. Read-out was performed in a standard high-volume array scanner, and showed excellent spot homogeneity and intensity. The Biosticker is aimed to be a plug-in for existing microarray platforms to enable faster protein assays and DNA hybridizations through mass transport optimization.

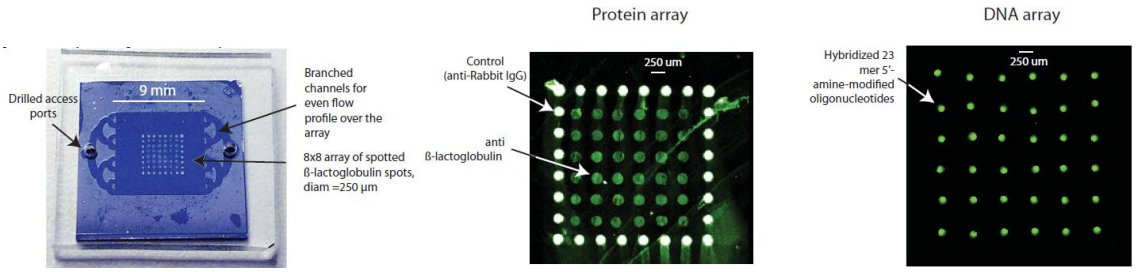

Figure 3: Left: A Biosticker flow cell attached to the protein microarray. The spots are visible though the polymer. To guarantee an even flow profile over the spots, branched inlet and outlet channels are used. Middle and right: The results of the scanned microarrays. The results from the ß-lactoglobulin protein assay and the DNA hybridization are very promising and show homogenous spots and excellent intensity. With an optimized fluidic protocol the Biostickers will not only increase the performance of microarrays but also greatly improve and simplify the processing compared to previously demonstrated microfluidic integrations.

This work has been published at the MicroTAS 2011 conference [CarlborgCretich2011], which manuscript is attached in Appendix A3.

3.2 Fabrication of chip-level microfluidic packages of the porSi membranes

In a next development, we focused on bonding to porous silicon specifically. By utilizing OSTE(+), we demonstrate dry transfer bonding of porSi membranes from their source wafer directly to a polymeric chip (Fig. 1). The novel and unique features of the method include: i) not requiring adhesives; ii) not requiring additional surface treatment; hence being iii) fast; and iv) simple. Our novel method enables the integration of nano-engineered porSi materials in cost-sensitive applications, such as next-generation single-use in-vitro diagnostic tests. The method is enabled by the unique dual cure process of OSTE(+): after the first stage UV cure of the prepolymer, and its subsequent demolding, the OSTE(+) features a compliant surface containing epoxy groups for bonding; after the second stage thermal cure of the bonded structure, the OSTE(+) hardens to provide the mechanical stiffness, stability, and chemical inertness, needed for microfluidic applications.

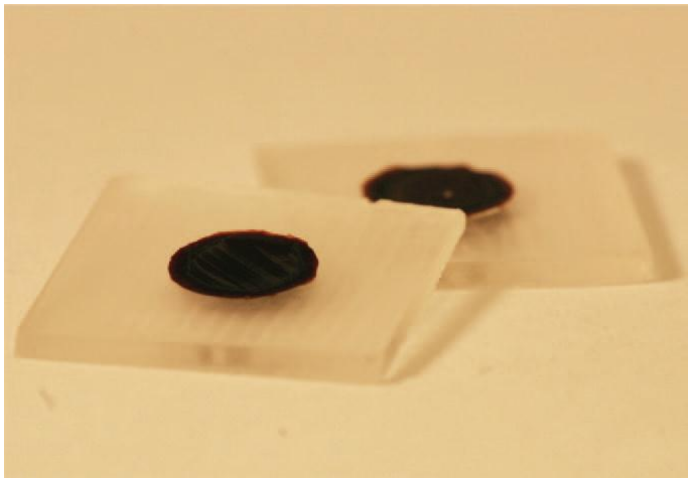

Figure 4: Photo of the transferred porous silicon membrane onto OSTE(+) chips.

This work has been accepted to the IEEE MEMS 2012 conference [Saharil2012] against a 35% acceptance ratio, and the conference abstract is attached in Appendix A4.

Early results on the application of OSTE(+) to porSi membranes were also reported in D4.2 (section 2.3).

4 Fabrication of fluidic test cells

Both for characterization of the flow in the porSi membrane chips, and for performing functional assays on functionalized porSi chips, fluidic test cells have been designed and fabricated. For functionalization experiments where individual porSi membranes on the silicon chip must be accessed, for instance by a micro spotter, an open cell model was developed. A closed model was developed for flow-through experiments with parallel flow through the membrane array on the silicon chip.

4.1 Porous silicon membrane array chip

The fabrication of a silicon chip with an array of porous silicon membranes was done partly done in the scope of WP 3 and partly within the present work package. Full integration of a sensor array is scheduled for WP 7 (from M13), however several designs have already been fabricated to allow important experiments to be performed and valuable experience to be gained as early as possible in the project.

The early porous membranes fabricated in WP 3 were attached by glue along their rim to a membrane holder directly after the pore etching process and subsequently released from the bulk silicon substrate by pulling them out. In a second iteration the membranes were released from the substrate silicon by laser cutting in order to obtain a cleaner cut edge, for then to be transferred onto the membrane holder. In both cases relatively large (5 mm diameter) freestanding single membranes were obtained. These membranes did not have the mechanical stability to permit any extensive fluidic characterization. The development therefore moved on to an integrated membrane-support concept, where only a small aperture of the membrane is made freestanding. The membrane aperture is defined by etching the silicon substrate wafer from the backside using reactive ion dry etching (RIE). The RIE etch is patterned by photolithography and arrays of membranes are defined on one silicon chip.

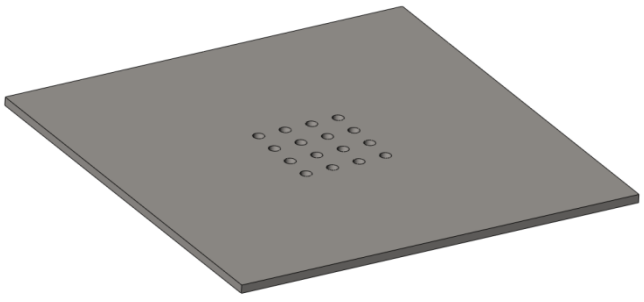

Figure 5: Porous silicon membrane array chip (CAD model).

4.2 Open test cell

The open cell design has an open window on the upper side of the silicon chip, through which liquids can be pipetted or spotted onto individual membranes. The lower side is sealed off with a transparent (non-machined) polymer window, with an output channel which can be connected to a vacuum pump in order to suck the dispensed liquids through the membrane.

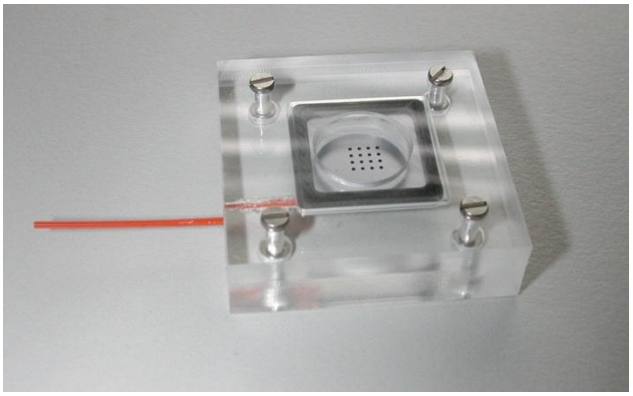

Figure 6: Fluidic test cell (open type) for accessing the membranes from one side.

4.3 Closed test cell

The closed test cell is sealed off on both sides of the silicon chip with channels leading to an input and output tubing. The cover plates are non-machined polymer surfaces to provide transparent windows to the chip surface. Special care was taken to reduce the volumes of the compartments between chip and windows, so that less sample is needed to fill the cell before it enters into the membrane. This was achieved by placing the sealing gasket in a recess on the cover plates, so that the compressed gasket leaves the chip hanging just above the cover plate window.

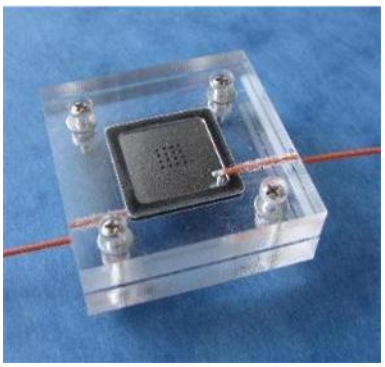

Figure 7: Fluidic test cell (closed type) for liquid flow through.

4.4 Improved closed cell

The first test cells were designed to accommodate large arrays of membranes on the chip surface. Since the current membrane chips have focused on a smaller array of 16 membranes extending over a surface area of only 4 mm x 4 mm, the area inside the sealing gasket has been reduced

accordingly to further minimize the compartment volumes and been shaped to avoid dead volumes during sample flow. Both sides of the cell have two diametrically opposed inlet/outlet channels to allow filling or flushing of each chip surface without flow through the membrane.

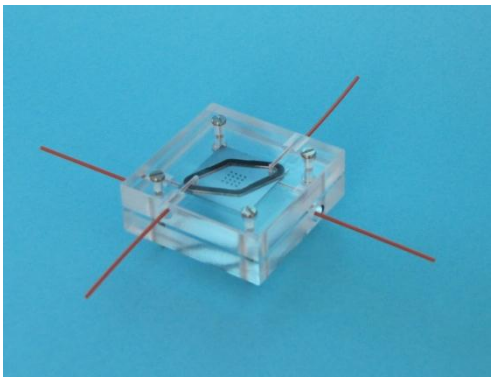

Figure 8: Fluidic test cell (closed type) for liquid flow bypass and/or through, with minimized cell volume.

4.5 Glass window cell

The glass window cell was conceived to provide optimized conditions for the first optical readout experiments. The use of glass windows improves on transparency and mechanical stability and thus increases the signal to noise ratio of the optical signal. Also the thermal conduction and stabilization was enhanced by the use of brass plates in the outer packaging. This cell was mounted inside the temperature chamber described in D8.1.

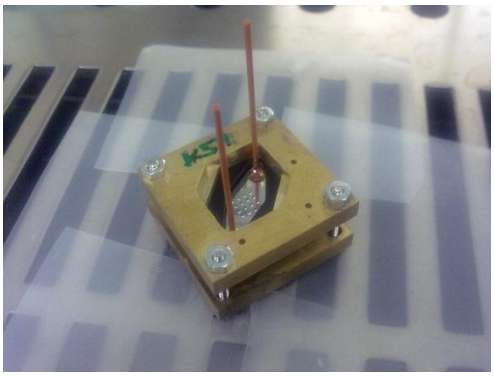

Figure 9: Fluidic test cell (glass window type) for liquid flow through/bypass, with brass package.

5 Design of fluidic cartridge

5.1 System overview

The fluidic cartridge design aims to combine all disposable components into one easily handled unit, and to profit from tight integration to reduce the needed fluid volumes. The main components are the inlet reservoir, the blood filter unit, the porSi membrane chip compartment, and the waste reservoir. A dry, filtered outlet from the waste chamber will be connected to the vacuum pump inside the POC main unit upon cartridge insertion.

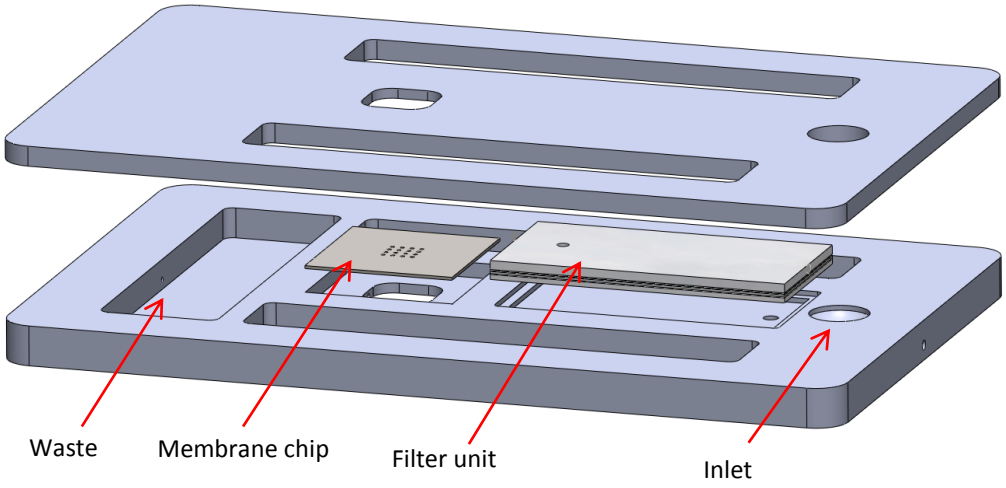

Figure 10: Overview of fluidic cartridge, showing the four main modules: Inlet reservoir, blood filter, chip compartment for the pSi chip, waste reservoir.

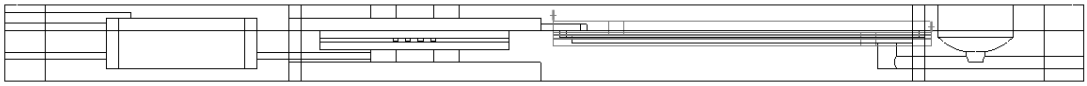

Figure 11: Cartridge design, transparent side view

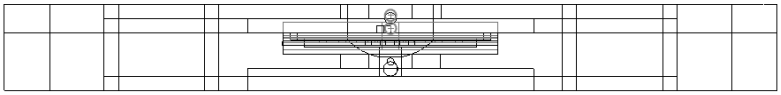

Figure 12: Cartridge design, transparent front view

5.2 Inlet reservoir

The inlet reservoir receives the blood sample from the patient as described in D5.1 on sample injection. It is shaped as a rounded sink with a channel at its bottom leading to the blood filter unit. The reservoir has a capacity of 90 µL.

5.3 Blood filter unit

The blood filter is integrated in the fluidic cartridge. At the core, is uses a commercially available blood separation membrane from Pall, Inc. The membrane pore size is progressively smaller from the inlet side to the outlet side, which helps to hold back particles along the whole cross-section and minimize blocked flow. The blood entering the filter module is spread over the filter surface by a network of channels. The channel walls maintain the membrane at a distance from the lid so that the membrane does not collapse against the lid which would block the flow in the lateral direction.

For testing purposes, a free-standing blood-filter module was designed and manufactured. The lateral dimensions of the filter are 24 mm x 9 mm.

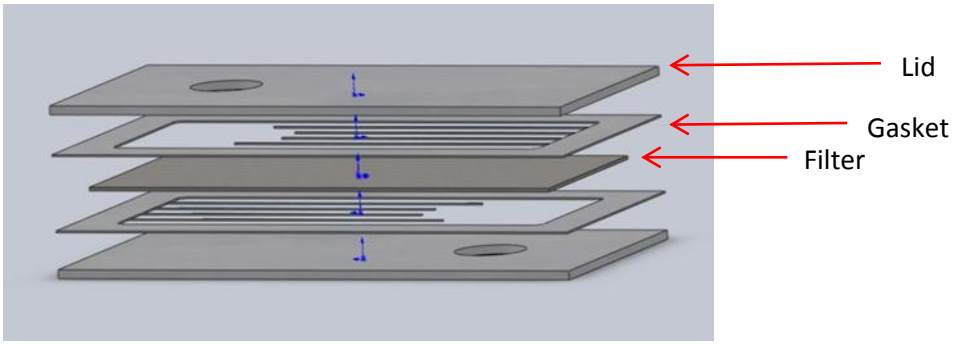

Figure 13: Blood filter unit composed of five layers. The filter membrane in the middle is embedded by channel gaskets and top/bottom lids. The channel gaskets distributes the flow over the filter membrane are while maintaining a minimal volume between membrane and the inlet/outlet.

5.4 Membrane array chip compartment

The chip with the array of porSi membranes is seated inside the cartridge in a similar way as in the flow test cells in Section 4. The cell is closed off on both sides of the chip with small glass windows to provide maximum optical transparency for the readout. A thin gasket seals the glass window to the chip while at the same providing an inlet channel.

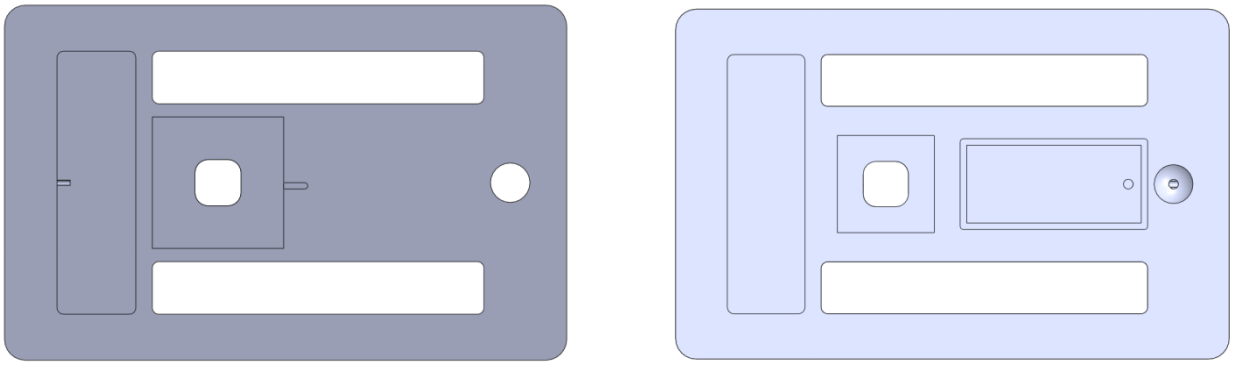

Figure 14: Cartridge design details. Left: Upper part (lid) seen from below. Right: Bottom part (base) seen from above.

5.5 Waste reservoir

The waste reservoir collects the sample and buffer solution that flow through the membrane array. The total volume of the chamber is 2 mL. An absorbing layer, as well as a filter on the outlet, prevents any fluid from escaping the cartridge and entering the POC base device.

6 Characterization of the fluidic test system

For measuring the flow-through characteristics of the test devices and structures a setup was used which allowed application of a controlled overpressure on the inlet of the device. A differential pressure controller from Alicat Scientific, Inc. (Tucson, Arizona, USA) of model “PCD 0-15 PSIG” was used to maintain pressures up to 15 psi (1 bar) at high precision. In experiments where higher pressures were needed, pressurized air (from CSEM laboratory infrastructure) at 10 bar was used

in combination with a manually operated pressure-reducing valve, which allows variable pressures up to 8 bar, although at lower precision.

The flow driven by this pressure was measured by both visual means (displacement of liquid front over time) and a flow sensor. The visual readout permits a precise average flow over several minutes, and can be performed simultaneously before and after the flow cell in the flow path. Any leakage in the flow cell or fluidic connectors can therefore be seen as a discrepancy between these two measurements. The flow sensor on the other hand, is fast and sensitive and is able to capture the dynamics of the flow in real-time. However, the sensor is easily disturbed by microbubbles in the flow passing through the sensor. So both methods are used in parallel.

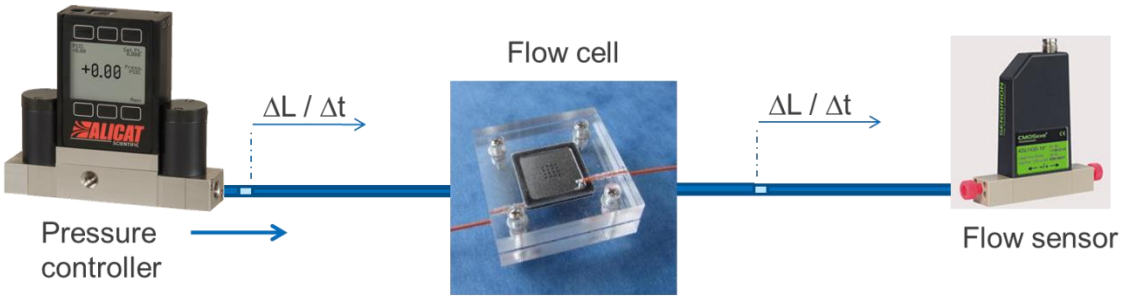

Figure 15: Test setup for flow-through characterization of fluidic components.

6.1 Flow measurement on silicon-packaged test chips

The first series of porSi membrane array chips are packaged as described in Section 4.1 and contains an array of 16 membranes. The membrane thickness is 35 µm, and the nominal pore diameter is 160 nm.

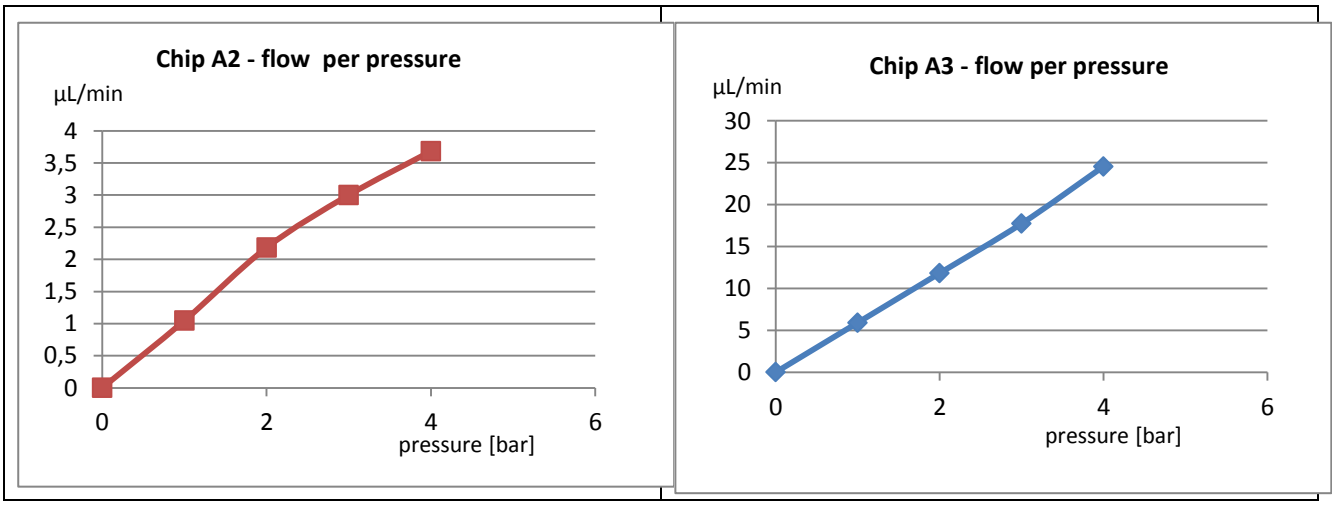

Figure 16: Flow as function of pressure through two "A" series membranes.

The flow increases linearly with the driving pressure, as is expected. However, the flow decreased over time. In the case of the "A3" chip, a reduction of 30% was observed after 30 minutes. This explains the relatively large discrepancy in flow rates between the "A2" and "A3" chip shown in Figure 13 where the pressure test was performed after some hours of testing in the case of "A2". It is believed that the flow reduction was caused by particles in the fluid clogging the pores. Initial flow rates reach 16 µL/min/bar, which is not far off the theoretical value of 26 µL/min/bar.

6.2 Flow measurement on OSTE-packaged test chips

The newer generation chips make use of the chip-level OSTE(+) packaging as described in Section 3.2. The membranes are 35 µm thick and the nominal pore diameter is 75 nm, with a porosity of 40-50%. The four membrane apertures on each chip are defined by holes in the OSTE(+) support layer. The theoretical flow (Bernoulli/Darcy) would be 9 µL/min/bar. However, only lower flows, on the order of 0.5 µL/min/bar, were measured. This deviance is still under investigation.

6.3 Tests on blood filter module

The blood filter unit described in section 5.2 was tested in a standalone package.

A sample of 100 µL whole blood was driven through the filter module with a pressure of 3 psi (200 mbar). The resulting volume of plasma was 20 µL.

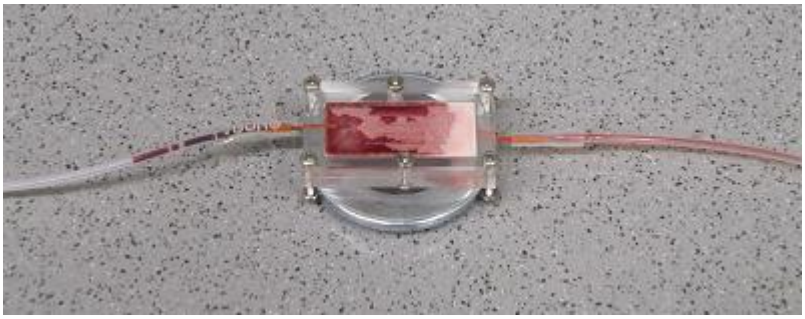

Figure 17: Blood filter module under test. Whole blood is injected through the left hand side tubing, and the filtered plasma exits on the right hand side.

6.4 Flow setup for assay testing

To perform optical readout experiments in combination with a full biological assay, a semi-automated fluidic setup was developed. The glass window cell described in Section 4.5 is inserted in a temperature controlled chamber. Functionalized biochips (refer to WP6) are mounted inside the cell. The different assay liquids, such as washing buffer and sample solution, are selected from their inlet reservoirs by a Scivex gear-actuated selection valve (Idex, USA) (see flow diagram in Figure 18).

The outlet of the flow cell is connected to a vacuum pump (Nitto Kohki, Japan) through a separation bottle which holds back the liquid.

In this laboratory setup with tubing interconnecting the components, the internal volume of the tubing is not negligible. For instance, after switching input liquid, a relatively large volume needs to be pumped before the new liquid has passed through the membrane. For membrane chips with low flow-through rates, this adds considerably to the total assay time. Therefore a by-pass valve is inserted between the auxiliary inputs on each side of the flow cell. When this valve is opened, liquid can flow through the tubing and the cell chambers on each side of the membrane, without going through the membrane, and thus quickly replace the liquid outside the membrane. It remains only to flush the liquid through the membrane, which due to the small porous volume of the membrane takes little time. Our typical membranes have a porous volume of a few nanolitres, which is replaced in seconds with flow rates in the microliter per minute range. On the other hand some tens of centimetres of tubing would take several minutes to flush. A full assay time could therefore be reduced from 40-60 minutes to 5-10 minutes.

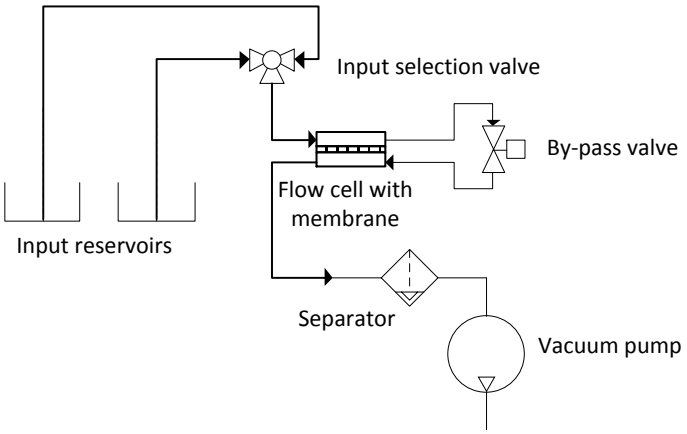

Figure 18: Flow diagram of semi-automated microfluidic setup for biological assay

7 Conclusion

The overall layout of the fluidic system has been presented. A disposable, fluidic cartridge containing blood filter unit, sensor chip and a waste reservoir was designed.

Fluidic tests were performed on test structures and devices. Flow-through characteristics of the porous silicon membrane were found to agree with the values predicted from the known geometries. Only the membranes with the smallest pore size (75 nm) exhibited unexpectedly lower flow. Particles clogging the pores reduce the flow over time; however, the observed flow degradation is not critical over the time span considered for the final application.

The flow setup for sequential feeding of different fluids in a biological assay was described. The integration of an auxiliary by-pass channel on each side of the flow cell connected to a valve improved the liquid exchange rate and thus reduced the total assay time by a factor 10 in this setup.

The blood filter unit was proven to filter out blood plasma even at low driving pressure.

Chip packaging concepts using the new OSTE(+) polymers were developed and applied to the packaging of porous silicon membrane array chips.

8 Glossary

POC – Point of care, medical testing at or near the site of patient care

porSi – Porous silicon

OSTE – Off-stoichiometry thiol-ene

PSI – Pound per square inch, equals 6,894 Pa. Example: 1 bar = 14.5 psi

9 Appendix List

A1 – "Low temperature "click" wafer bonding of off-stoichiometry thiol-ene (oste) polymers to silicon"

A2 – "Dry transfer bonding of porous silicon membranes to oste(+) polymer microfluidic devices"

A3 – "Biostickers: patterned microfluidic stickers for rapid integration with microarrays"

A4 – "Click" Wafer Bonding for Microfluidic Devices"

10 References

[Carlborg2011] C. F. Carlborg, T. Haraldsson, K. Öberg, M. Malkoch and W, van der Wijngaart, "Beyond PDMS: off-stoichiometry thiol-ene (OSTE) based soft lithography for rapid prototyping of microfluidic devices", Lab on a Chip, 11 (2011) pp. 3136–3147.

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