Development of on Chip Devices for Life Science Applications

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Abstract

This work reports on diverse technologies implemented for fabricating microfluidic devices such as biomedical micro sensors, micro pumps, bioreactors and micro separators. UV depth lithography and soft lithography were applied in the fabrication processes using different materials, for example SU-8, polydimethylsiloxane (PDMS), silicon, glass and ceramics. Descriptions of the fabrication process of completed devices and their performance are provided. Experimental tests and results are presented where available.
This work highlights the importance of down scaling in producing efficient devices suitable for life science applications using diverse materials that are compatible with chemical and biomedical applications.

Keywords: Microfluidics, Biosensors, Bioreactors, UV depth lithography, soft lithography.

1. INTRODUCTION

Microfluidics is an exciting field of science and engineering that enables very small-scale fluid control and analysis, and allows instrument manufacturers to develop smaller, cost-effective and powerful systems. It also offers potential benefits in chemistry, biology, and medicine through minimized sample volume, fast detection, usability for non specialized staff, temperature stability, reduced reagents consumption, decreased analysis time, etc [1].

This work presents the optimized process technologies used by the Institute for Microtechnology (IMT) research group for fabricating microfluidic devices (e.g. dispersion microelements, micro pumps, bioreactors, blood separator and Quartz crystal microbalance (QCM)). These devices are suitable for life science applications and can be integrated on chip.

2. SILICON BASED MICROFLUIDIC DEVICES

Nanoparticles gain more and more in importance. A major process during handling of nanoparticles especially for the formulation of pharmaceutical, cosmetic and biotechnological products is the dispersion. While mixing the nanoparticles into a fluid, the nanoparticles agglomerate to each other. Dispersion describes the agglomerate breakup as well as the homogeneous distribution of particles in the surrounding fluid.

The dispersion process within a micro-system offers the following two main advantages: generation of the high energy density required for dispersion of nano-sized particles, and use of extremely low volumes of reactants. Hence, micro-systems afford an excellent approach for pharmaceutical and biotechnological screening applications.

To generate the high stress intensities, which are necessary to disperse agglomerates into primary particles, a new dispersion micro-element has been developed at IMT. The designed micro-elements (Fig.1) consist of a 20 mm long channel with 1 mm diameter inlet and outlet ports at both ends.

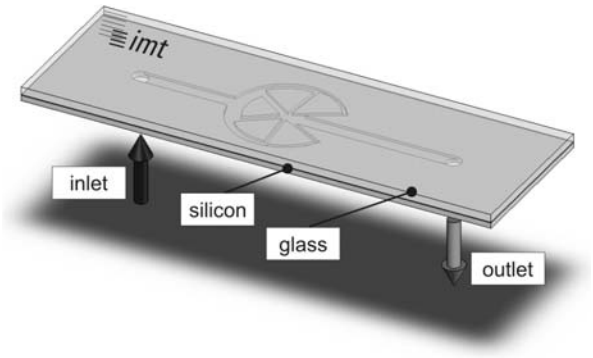

FIGURE 1: Function principle of the dispersion micro-element

The central part of the micro-channels features different geometries varying from elementary angular and circular alternatives to more complex geometries with multiple streams (Fig. 2). Furthermore, diverse barrier structures are also included. In the different designs, the width of the micro channel varies from 76 µm to 1 mm.

In addition to using the dispersion effect of the micro-elements, it is planned to compress the nanoparticle suspension with high pressure, comparable to macroscopic apparatus, through the micro-channels. Therefore a resilient material was needed, that is simultaneously able to be fabricated into a micro-channel with precise and rectangular walls. For these reasons silicon was chosen in combination with dry etching for the fabrication process.

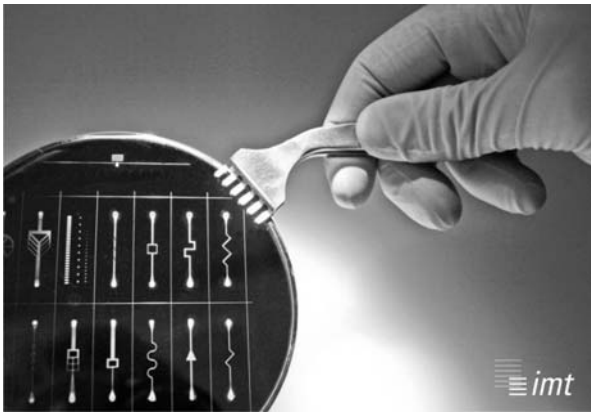

FIGURE 2: Different micro-channel designs

To realize both a leak-proof micro-channel and an unrestricted visibility of the dispersion process in the micro-element, glass was chosen as a coating material. The structuring of glass is a difficult and extensive process, which often entails producing material stresses in the treatment affected zone. In this case, where the inlet and outlet are under high pressure, micro-cracks are especially disadvantageous. To avoid this problem and to achieve a maximum stability in the dispersion micro-element, we used a technique of double-sided etching of silicon.

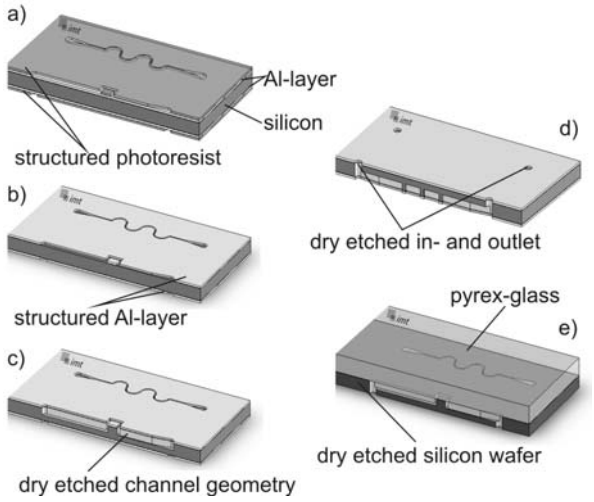

FIGURE 3: Batch fabrication process of the dispersion micro-element

Standard UV photolithography is used to structure the aluminum layers, which are sputtered on both sides of a silicon wafer (Fig. 3a). After wet chemical etching in Al-etching solution, the photoresist layer is removed, whereby the aluminum structures remain (Fig. 3b). The desired micro-structures are now etched into the silicon base material. A deep reactive ion etching (DRIE) process, also known as Bosch process, has been used for this purpose. More details concerning the fabrication process are presented in [2]. The alternating process sequence from etching (with SF_6) and passivation (with C_4F_8) allows extremely high aspect ratios and almost rectangular walls. First the channel geometry is etched from the top side to a depth of 200 µm into the silicon wafer (Fig. 3c). In a following etch step the wafer is flipped and the inlet and outlet are etched from the bottom side until they meet the channel bottom (Fig. 3d). After the aluminum masking layer is removed in a wet chemical etching step, the upper side is coated with a glass wafer in an

anodic bonding process step (Fig. 3e). Finally the dispersion micro-elements are separated with a wafer saw.

Initial experiments with nano-particulate titanium dioxide suspension (AEROXIDE® Evonik Degussa) have shown that the micro-structure could operate smoothly up to pressures of 800 bar. Figure 4 shows a first dispersion experiment with a pre-dispersed 5 %wt TiO_2 suspension in water. The rising agglomerate size at higher inlet pressure depends on re-agglomeration due to the fact that surfactants for stabilization of the suspension had not been added.

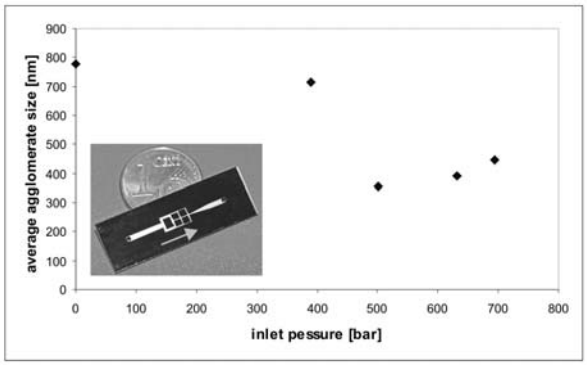

FIGURE 4: Dispersion effect of the dispersion micro-element (the analyzed design is shown in the diagram left below) on a pre-dispersed 5 % by weight TiO_2-suspension in water

Nevertheless the average agglomerate size could be halved, which demonstrates the general aptitude of the micro-elements for the dispersion of nanoparticles. Further measurements of the flow rate and the analysis of the dispersion effect as function of the micro-channel geometry are ongoing.

3. SU- 8 BASED MICROFLUIDIC DEVICES

SU-8 is a negative photoresist that has several interesting and useful material properties (e.g. transparency to visible light, thermal stability, biocompatibility and the possibility of fabricating high aspect ratio structures) [3]. Recently, it has been used as an alternative material for fabricating new microsystem designs and was integrated in various applications, such as micro grippers [4] and microfluidic chips [5, 6]. It can also be used in casting master features for soft lithography processes. Its average value of Young's modulus was found to be about 5.6 GPa [7].

3.1. Fabrication Process

The fabrication process is illustrated with the steps for fabricating spiral micro disks. The final features aimed at spiral channels that are carried on a base as depicted in Fig. 5.

The fabrication process begins with a sacrificial layer of 3 nm Cr and 10 µm Cu deposited onto the substrate (Fig. 6, I). According to the required structure height, multilayer of SU-8 can then be deposited. The base layer of SU-8 is spun on the ceramic substrate, dried, exposed totally, and then post exposure baked (PEB) for 45 minutes using ramped temperature 60-95 ^{o}C (Fig. 6, II). The spiral protrusion layer is fabricated by spinning a new layer of SU-8 above the first exposed one, and then dried, exposed to UV light under the spiral mask, and PEB (Fig. 6, III).

After that the SU-8 layers were developed in 4-hydroxybutyric acid lactone used as pre-developer and 1-methoxy-2-propylacetat to remove the unexposed material, and the sacrificial layer is etched and the SU-8 patterns are removed (Fig. 6, IV). The fabricated SU-8 patterns are of 380 µm channel height, and 100-1200 µm widths.

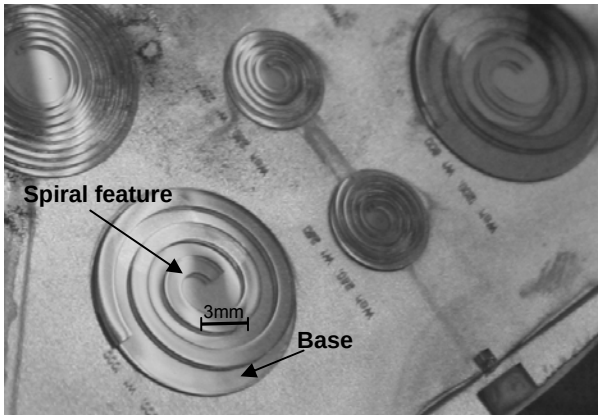

FIGURE 5: Micro fabricated spiral disks.

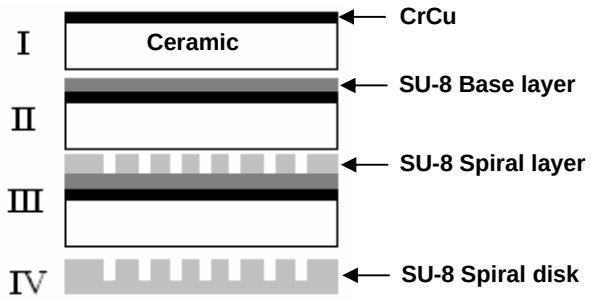

FIGURE 6: Fabrication procedure of SU-8 using UV depth lithography.

3.2. SU-8 Microfluidic Devices

The standard fabrication process was used for fabricating spiral channel micropump and several micromixers. Spiral disks of SU8-50 photoresist (Fig. 5) with 3 mm outer radius, 385 μm height, and 150, 250, and 500 μm widths have been successfully tested for pumping glycerin. The spiral disks are glued to Aluminum shafts, and connected to an external driving motor. The micropump comprises a flat plate cover, a spiral disk, pump housing, and inlet and outlet ports. Example of results of flow rate measurements at different rotational speeds is shown in Fig. 7.

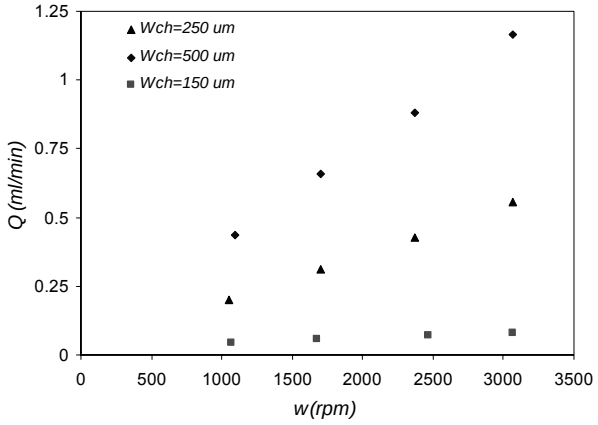

FIGURE 7: Flow rate against rotational speed for spiral micropump.

Because of its importance for micro total analysis systems (μTAS) applications, micromixers are other vital components. Several passive micromixers were fabricated as shown in Fig. 8.

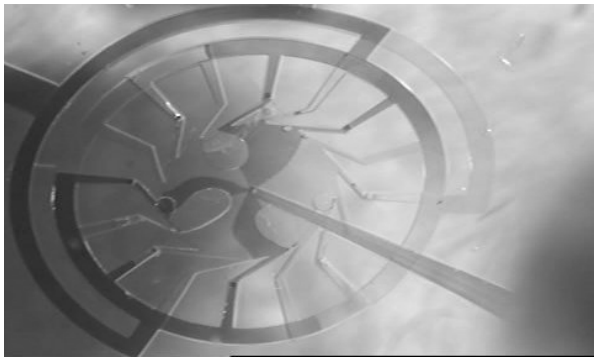

FIGURE 8: Micromixer [8].

4. PDMS BASED MICROFLUIDIC DEVICES

Due to it is diverse advantages (e.g. transparency between 240 and 1100 nm [9], biocompatibility [10], low cost and the possibility to pattern a relief structure from a mold master [11]) PDMS is an essential design material for fabricating microsystems. Moreover, it can be covalently bonded to itself and to several other materials such as glass or silicon and to thin film materials such as silicon nitride [12].

4.1. Fabrication Process

At first, a ceramic wafer is spin-coated with the photoresist SU-8 (Micro-Chem), for planarization which is then exposed to UV light. The actual casting SU-8 master features can be realized in heights ranging from a few µm to 720 µm. This SU-8 photoactive layer is first exposed photolithographically to UV light and then developed as described above (Fig. 9, A I-A II). To fabricate the microstructured PDMS layer the pre-polymer (Sylgard 184, Dow Corning) composed of silicone based elastomeric pre-polymer and curing agent in a ratio 10:1, respectively, is poured on the SU-8 master (Fig. 9, A III). After cross-linking the polymerized PDMS is peeled off the master (Fig. 9, A IV). Time for polymerization depends on the applied heating conditions: the cross linking is finished at room temperature after 24 h and at 80 °C after 10 min resulting in different stiffness. Rigidities of PDMS can also be modified by applying different ratios of the oligomer and curing agent. As depicted in Fig. 9 the micro structured PDMS are bonded irreversibly with another PDMS-part (C1) or with a glass bottom (C2) after oxygen plasma activation (85 W, 30 s).

4.2. PDMS Microfluidic Devices

A wide range of microfluidic components for different applications can be fabricated using the above process. Diverse PDMS based systems that have already been developed and fabricated are the following.

4.2.1. Bioreactor

Micro bioreactors are increasingly applied in environmental and pharmaceutical biotechnology as screening tools for interesting microorganisms, as innovative cultivation techniques for bioprocess development or as chip based biosensors.

The advantage of using a hybrid micro fluidic chip composed of a glass bottom and PDMS top layer featuring the reactor geometries is that the integration of different online analysis is possible when structuring glass with electrodes e.g. made of titanium/gold or chromium/gold where the first metal serves as an adhesive layer (Fig. 9, B I-III). In doing so, tools for measuring the velocity, temperature or retention time can easily be implemented into the system. Electrodes can also be used for cell separation based on dielectrophoresis, in form of heating coils as integrated heaters or for amperometric detection of metabolites via capillary electrophoresis [13].

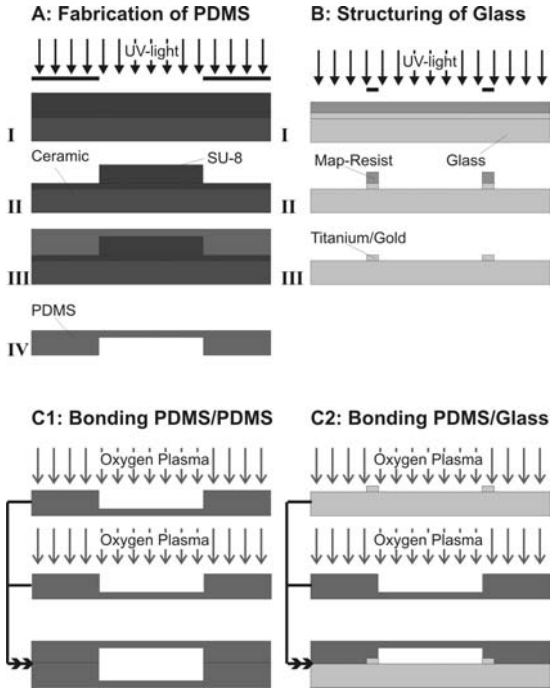

FIGURE 9: Fabrication procedure using UV-depth lithography and soft lithography.

The bioreactor (reactor volume of 50 µL) is optimized and developed to achieve both a specific flow characteristic as well as a selective wash out of biomass during its continuous process mode. The oxygen supply of the culture medium is ensured by diffusion through the gas permeable PDMS which is enhanced by decreasing the membrane thickness. Experimental investigations like measuring average retention time via electrodes and dissolved oxygen concentration is carried out online, whereas cell density and metabolite concentrations in growth medium are characterized offline with conventional analysis instruments. All experimental data are used to verify the simulation results done with CFD RC-ACE + (Ansys) for different inlet configurations and geometries of passive barrier elements. In optimized micro reactors no concentration gradients occur along the entire reactor width. However, there are significant concentration differences along the reactor length because of the design configuration similar to plug flow.

The average retention time in different reactor geometries can be determined with micro structured titan/gold electrodes (50 µm wide) in the in- and outlet of the micro device shown in Fig 10. The measurement procedure is based on the variation in resistance between two electrodes (disruption/ response principle) by flushing the water filled system with a 1 % KBr solution at operation conditions of 1 mL/h [14].

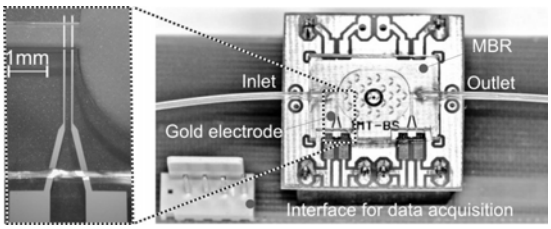

FIGURE 10: Setup for conductivity measurements in the in- and outlet of the micro bioreactor (MBR).

Due to strongly hydrophobic interactions between reactor materials and cell surfaces, the used model organism Saccharomyces cerevisiae DSM 2155 tends to reactor wall growth resembling a biofilm. Due to this reason a high dilution rate up to 20 h-1 is possible. The maximum specific growth rate of adsorbed cells could be estimated with 0.1 h-1 in comparison to 0.32 h-1 for the submers cultivation in a 1 L-chemostat reactor [15].

A wide range of custom designed surface treatments exist for the modification / functionalization of materials such as glass and PDMS. Depending on the applied treatment cell adhesion can either be enhanced or avoided. To achieve cell growth in submers culture without unspecific cell and protein adhesion on the reactor wall materials, a surface hydrophilization would be advantageous [16].

4.2.2. Quartz crystal microbalance (QCM)

In medicine and biotechnology there is an increased requirement in quantification methods for analytes in liquid medium. Common detection methods, which mostly depend on special labels for an indirect detection of an immune reaction need specialized staff, are time consuming and expensive [17]. A further advantage of micro systems is their small size and the resulting small sample volume needed. The detection with QCM occurs directly with a frequency shift Δf due to the mass deposition Δm of an analyte on the surface [18, 19] and does not need special markers. With this mass sensitive device it is possible to detect products for example of a bioreactor or a certain substance in a blood serum sample. [20, 21]

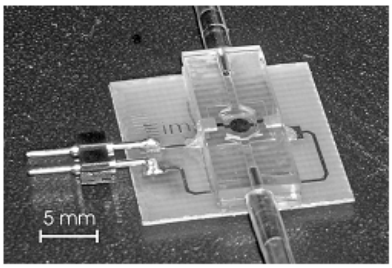

FIGURE 11: Quartz crystal micro balance (QCM)-Sensor [22]

In Fig. 11 the micro QCM sensor is illustrated. The resonator consists of a thin AT-cut quartz wafer with gold electrodes patterned on opposite sides. The electrode, which is in contact with the liquid, has to be coated with a detection layer especially designed to bind the analyte to be measured [20, 21].
We obtain AT-cut quartz blanks with the dimension of 38.1x38.1 mm^2 and a thickness of 128 µm. For the purpose of a sufficient mechanical stability for handling, only a part of the quartz is thinned down to the desired thickness, forming a thin membrane with a thick, mechanically stable outer ring. This is done by photolithography, etching and deposition steps [23].

The quartz resonator is totally embedded in PDMS. For a permanent bonding between the quartz crystal microbalance, the PDMS flow cell and the PDMS bottom layer, a bonding procedure has been used based on combination of method C1 and C2 depicted in Fig. 9. Upon completion the sensor flow cell has a volume of 14 µL.

4.2.3. Affinity cell

For some applications it is advisable for an effective detection to add a purification unit as well. For this purpose we designed an affinity cell which consists of a PDMS reaction chamber filled with agarose beads (Fig. 12). The beads can be coated with a sensitive layer in the same way as the sensor. The beads are retained in the purification unit with a PDMS fence structure. The purification step works like an affinity chromatography as substances of interest are consequently bound to the beads while unbound substances are washed away. The purified analyte can be

subsequently detected with the quartz resonator with less interfering interactions from impurities [17].

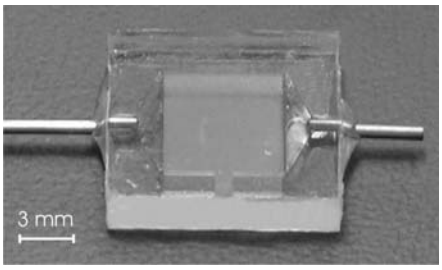

FIGURE 12: Affinity cell [17]

4.2.4. Blood separation system

It is important for the detection of serum proteins and to prevent the microstructure from blocking, that the blood serum is free of blood cells. To guarantee this, special structures have been developed and realized in PDMS as described in [24].

4.2.5. Hydro-Gel Actuated Microvalve

To handle different fluids needed for pre-purification and detection, special valves have to be used. In general blood proteins and biomolecules are very temperature sensitive so that the used valves may not warm during operation process. To guarantee this, valves with a pH sensitive hydrogel actuator were fabricated. The valve is composed of 5 PDMS layers as can be seen in Fig. 13. Layer 1 and 3 feature fluidic channels with height and width of 200 µm which are connected by a 200 µm hole in layer 2. This hole can be blocked by the expanding hydrogel pressing the 40 µm thick membrane (layer 4) down. The circular chamber for the hydrogel actuator has a diameter of 1500 µm.

The hydrogel consists of the monomer 2-hydroxyethyl methacrylate (Sigma-Aldrich) and the copolymer acrylic acid (Sigma-Aldrich) in a 4:1 molar ratio. Ethylene glycol dimethacrylate (1 wt%, Sigma-Aldrich) was added as a crosslinker and Irgacure 651 (3 wt%, Sigma-Aldrich) as photoinitiator. Irgacure 651 is the registered name of 2,2-dimethoxy-2-phenyl acetophenone (Ciba Speciality Chemicals). A 5 mW UV-source with a wavelength of 366 nm was used for the exposure of the hydrogel through a mask in the microfluidic system.

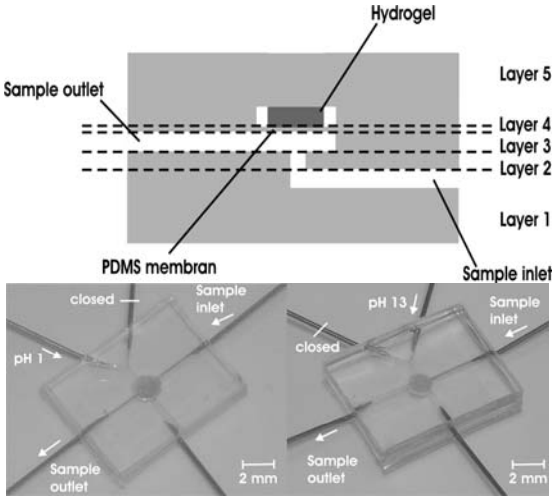

FIGURE 13: Schematic and Picture of PDMS valve [24, 25]

To open and close the micro valve, pH 1 and pH 13 standard solutions have to be pumped through the hydrogel chamber, respectively. In Figure 13 a dye was injected into the fluid network to demonstrate function of the valve. [25, 26]

5. CONSLUSION

The possibility of implementing available fabrication technologies at IMT for fabricating microfluidic devices using different materials has been described. Optimized processes showed high capability in handling several microfluidic applications under diverse conditions. This work highlights the advantages of micro technologies in biomedical applications.

ACKNOWLEDGEMENT

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