SPIE Conference on Microfluidic Devices and Systems II, Vol. 3877, Santa Clara, California, pp. 101 –109 (1999).

Resolving chemical/bio-compatibility issues in microfluidic MEMS systems

Shekhar Bhansali*, Arum Han, Manoj Patel, Kwang W. Oh, Chong H. Ahn and H. Thurman Henderson

Center for Microelectronic Sensors & MEMS
Department of Electrical and Computer Engineering and Computer Science
University of Cincinnati
PO Box 210030 Cincinnati OH 45221-0030

Keywords: : MEMS, microfluidic system, bio-compatible coating, microreservoir, low temperature bonding, spin-on Teflon, CYTOP, non- specific adsorption.

ABSTRACT

We are currently developing a generic microfluidic system (on chip) for the detection of bio-organisms. Numerous bio/chemical compatibility issues arise in development of these chip based microfluidic systems. The resolution of bio/compatibility issues often necessitates a change in materials and, on occasions, leads to redesigning of the system itself. We have successfully decoupled the fabrication and compatibility issues that arose in the fabrication of a generic microfluidic system for chemical detection. We have successfully developed techniques for coating the offending surfaces with a Teflon®-like amorphous fluorocarbon polymer CYTOP™ and assembling the coated components. In this paper we briefly discuss the microfluidic system being developed by us and the bio/chemical compatibility issues that need to be addressed in this system. Next we discuss the material CYTOP and its application to surfaces and devices. The bonding technique developed to bond the polymer coated structures and some of the components fabricated using this material are also discussed.

INTRODUCTION

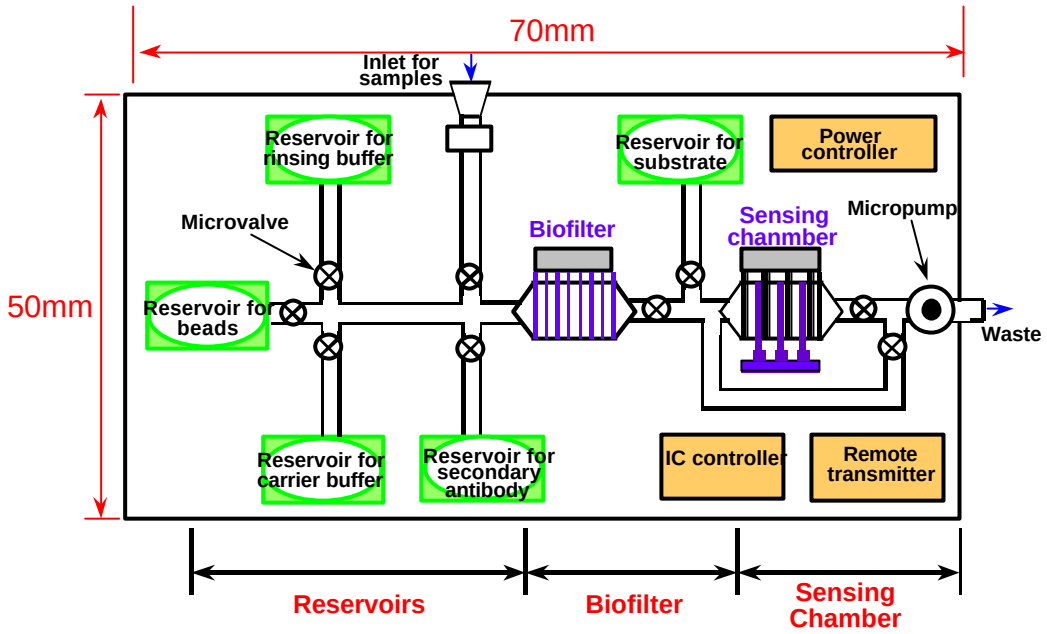
Microfluidic systems for complete analysis are now increasingly under development and have whole conferences dedicated to their development [1]. Most of the microfluidic components and systems under development can broadly be classified into two groups based predominantly on the material of fabrication. They are (a) Si/glass based and (b) polymer based. Both of these material groups allow us to fabricate microfluidic structures and allow integration of diverse components such as reaction chambers, optics, cell electrodes etc.. The resulting systems successfully handle the desired fluids and gases for which they have been designed. However both the Si/glass systems and the polymer systems have specific advantages and disadvantages over the other, as enumerated below.

The components and systems fabricated using Si and glass (a) use established classical MEMS fabrication techniques that allow us to use the existing infrastructure and knowledge base. (b) They can be subjected to high processing temperatures and thus are CMOS compatible. This allows us to fabricate components, devices and systems with integrated electronics. (c) The mechanical strength of these materials is significantly superior to the polymeric components, resulting in very long mean lifetimes to failure (MTF) for active devices such as valves and pumps; and (d) they have excellent bonding strength and benefit from well established bonding techniques for various interfaces (glass-glass, silicon-silicon and silicon-glass). However (a) the Si/glass surface may not be compatible with many of the liquids and bio-molecules in complex systems. The bio-molecules tend to adsorb on the surfaces of the devices. This results in reduced efficiency in the function of the devices and even clogging of the channels. (b) Also some rather complex shapes are hard to fabricate using conventional MEMS technologies.

The polymers, especially fluoropolymers (like Teflon®) overcome the limitations of the Si/glass systems. These fluoropolymers (a) are inert to most solvents and chemicals and are bio/chemically compatible; and (b) complex structures have been fabricated with ease in fluoropolymers [2]. However, (a) the polymers are very difficult to bond and hence device/system fabrication becomes difficult; and (b) they cannot withstand elevated temperatures. Moreover, (c) they are not CMOS compatible, thus making electronic integration impossible.

*Correspondence: e-mail: shekhar.bhansali@uc.edu, Phone: (513) 556-0903, Fax: (513) 556-7326

We are currently developing a rather complex MEMS based micro-fluidic system for detection of bio/chemical organisms in liquids, using silicon, glass and polymers. This self-contained portable microfluidic device consists of reservoirs for onboard reagents [3], valves [4], flow sensor [5] filterless particle separators and immunosensors [6], electronics and controls. As designed, this system will be able to detect very low concentrations of target microorganisms. The analytical chemistry and the concept of this system has been discussed earlier [7;8]. This paper briefly describes the microfluidic system and the basic chemistry of the process to introduce the specific bio/chemical compatibility problems which require attention and are being addressed. We then discuss the material and its application. The bonding techniques and fabrication of reservoirs by integrating MEMS and polymer processing are also discussed.



THE MICROFLUIDIC SYSTEM

Figure 1 schematically illustrates the configuration of the micro-fluidic system being developed at the University of Cincinnati. The system consists of the following discrete components: reservoirs, valves, sampling/reaction chamber, detection chamber, flow sensors and a micro pump. These micro fluidic components are "mounted" on a micro fluidic "motherboard" containing fluidic and electrical connections. Although this board is designed for the bio/chemical detection system, the modular scheme would allow us to "re-configure" the system toward many other applications.

Figure 2 schematically illustrates the steps involved in completing of the assay. Antibody coated super paramagnetic beads, suspended in a buffer solution, are introduced on the filterless particle separator in the sampling/reaction chamber and separated by applying magnetic fields. While holding the antibody coated beads, the sample solution is introduced. As the antibody on the beads is specific in its binding to a specific antigen, only the target antigen is immobilized and is thus, *separated* onto the magnetic bead surface due to antibody/antigen reaction. The other, unbound, antigens get washed out with the flow. Next, the enzyme labeled secondary antibody is introduced in the chamber (not shown in figure) and incubated to allow complete binding between the secondary antibody and the antigen. The beads are then thoroughly rinsed to ensure complete removal of unbound secondary antibodies. Now the magnetic beads are released to the sensing chamber and the "substrate" solution is introduced into the sensing chamber. The reaction with the "substrate" produces an electrical measurable product which is detected electrochemically with the inter-digitated electrode array to identify the presence of the target antigen. The reaction product is redox cycled on the inter-digitated array electrode for signal enhancement.

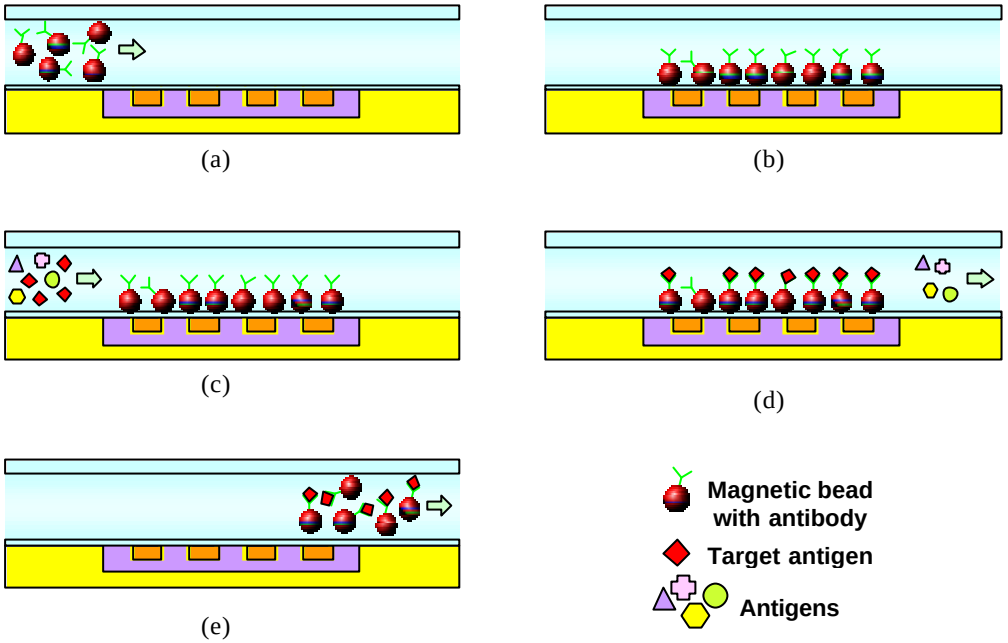


***Figure** 2: Schematic illustration of the immuonoassay procedures. (a) Injection of magnetic beads, (b) Separation and holding of beads, (c) Flowing samples, (d) Immobilization of target antigen, (e) Release to biosensor of bioreactor (after attaching secondary antibodies).*

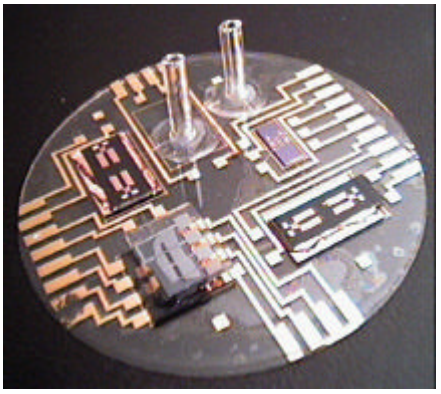


***Figure 3**: Photograph of the 1st generation integrated fluidic system shows the inlet and outlet ports, valve inductors, integrated filterless separator and immuonosensor, and the flow sensor.*

Figure 3 is the photograph of the first generation fluidic motherboard that was developed as a proof of concept device and for the evaluatation of the performance of the MEMS components. Evaluation of the individual MEMS components and devices with the chemistry processes brought compatibility issues, that were capable of compromising the performance of the devices and systems, into focus.

NON-SPECIFIC ADSORPTION

One of the major impediments in ensuring long lifetimes in fabricated systems has been "non-specific adsorption" of the conjugate and the beads to the inner surfaces of the first generation MEMS devices. Whenever any molecule or material binds to a surface not intended, the phenomenon is termed non-specific adsorption. Higher non-specific binding whether, physical or chemical, implies lower bio/chemical compatibility of the materials with the fluids/gases (materials and

liquids/gases are fully compatible with each other, when they do not interfere with the functions of each other). Non-specific adsorption is a common phenomena, routinely encountered in chemistry, particularly in the immunoassay where, generally, the conjugate binds to additional sites (including sensing chamber surfaces) in addition to the specific target, thereby raising the detection limit. Equally significant is the adherence of the magnetic beads to parts of the devices. The latter is particularly undesirable because of multiple reasons: (a) If the bead with captured antigen adheres to the surfaces of the sampling/reaction chamber then the antigen will not be detected. (b) If the same bead is stuck in the sensing chamber then the detection limit for subsequent samples is reduced and (c) the beads stuck on the surface of the devices, being large in size, act as nucleating sites for cluster formation, resulting in fouling and clogging of the micro channels.

A meso-scale system using commercially available conventional microfluidic components was developed to evaluate (a) the MEMS components for performance and compatibility and, (b) for developing passivation schemes for reduced non-specific binding (increased compatibility). Figure 3 is the photograph of the meso-scale system. In the development of this system, it was ensured that the areas of cross-section of the channels in the MEMS components and the Teflon tubes were kept similar in order to facilitate easy comparison. It was experimentally observed that unlike the Si/glass channels, beads rarely stuck to the Teflon channels in the meso-scale system. This result clearly demonstrated that to achieve good results, it is essential to ensure that the working surfaces are made from Teflon or other similarly inert polymers.

To ensure that we continue to benefit from the MEMS fabrication techniques we conformally coated the most offending surfaces of the MEMS components and channels with CYTOP, an amorphous fluorocarbon material which can be spun-on the structures to fabricate conformal coatings. Once the surfaces were coated, the interaction was between the Teflon-like CYTOP surfaces and the fluids, reducing non-specific adsorption and improving bio/chemical compatibility.

To ensure that the functionality of the liquid reagents is not lost after prolonged storage in the micro-reservoirs, these reservoirs were also made with, or lined with the CYTOP membrane [4]. To bond the CYTOP coated components to Si/glass structures, a novel and effective bonding technique has also been developed [9]

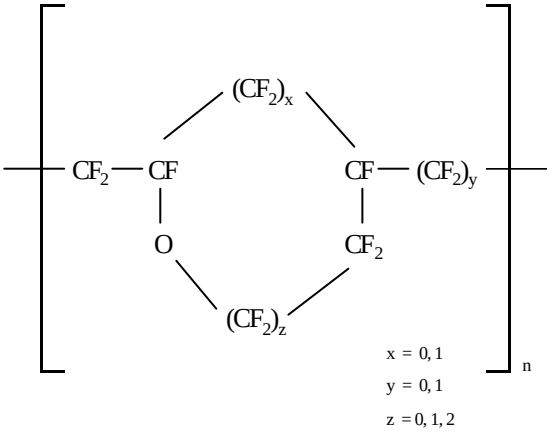


Figure 4*: Chemical structure of CYTOP.*

CYTOP

The CYTOP is a new class of Cyclized Perfluoro Polymer (CPFP) commercialized by the Asahi Glass Company [10]. Unlike solid Teflon (PolyTetraFluoroEthylene-PTFE), this material comes dissolved in solvents and thus can be spun-on surfaces or dip-coated with good adhesion. It is easy to pattern and etch the cured CYTOP films by conventional photolithography and plasma etching. The ring structure of CYTOP is shown in Figure 4. Its morphology is fully amorphous due to the ring structure in the polymer main chain [10]. This ring structure also results in the films being transparent and soluble in some of the fluorine containing solvents. The high optical transparency of the material (>95%) also makes this material very useful for optical applications.

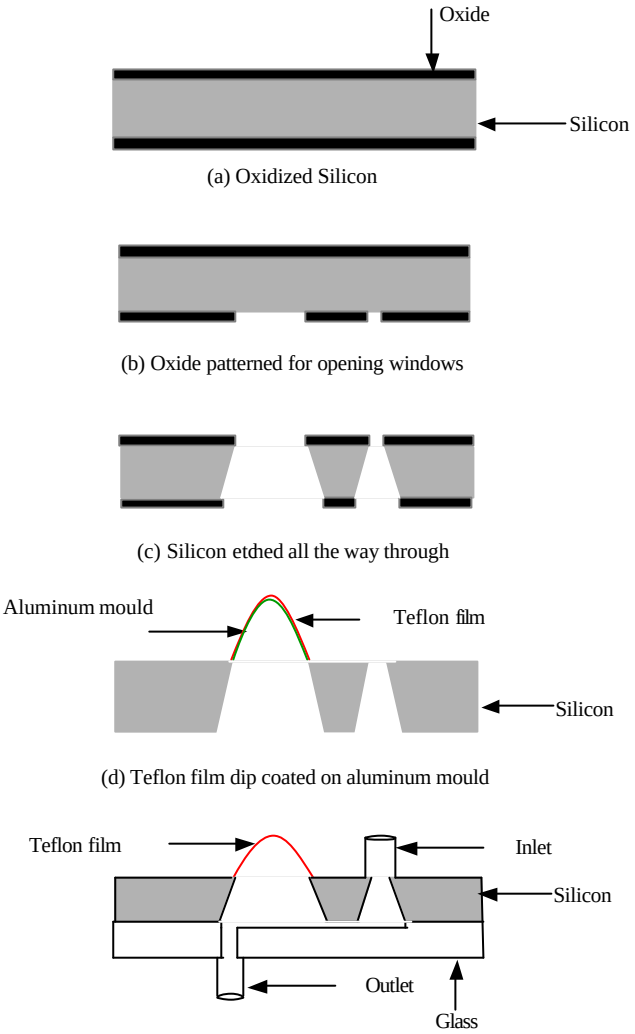


***Figure 5**: Processing sequence for fabrication of the reservoir.*

EXPERIMENTAL

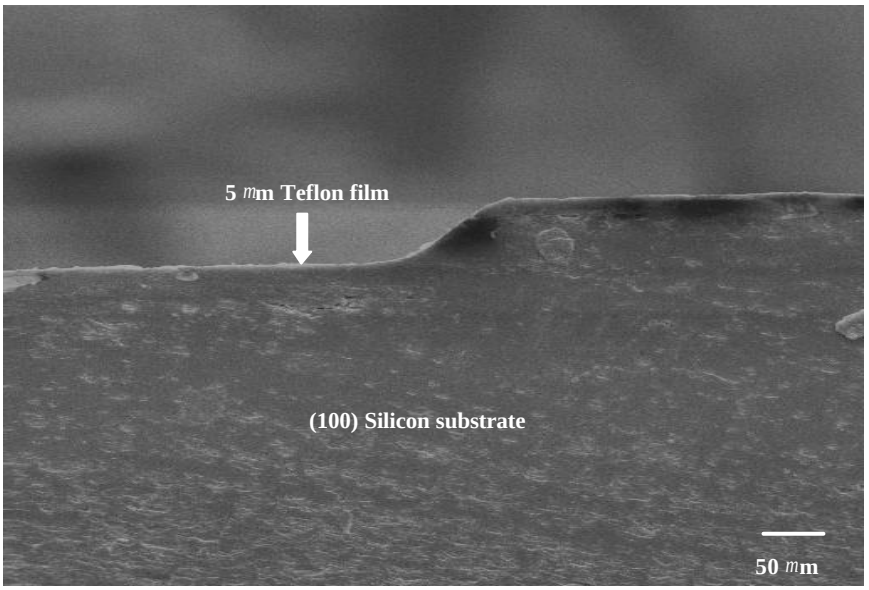
As discussed earlier, CYTOP was used to (a) coat channels in Si and glass, (b) fabricate reservoirs to store liquids and (c) bond coated/uncoated MEMS components. All experiments were carried out on 2 inch substrates. The CTL-809M, grade of CYTOP with high viscosity (~300 cp) was used for all experiments .

For coating channels on the substrates, CYTOP was applied using the dip coating technique. The samples were immersed in CYTOP and removed (pulled) manually at a steady rate to ensure a uniform conformal coating. The coated samples were baked at 90 °C for 30 minutes to drive off the solvents. This resulted in a 2-3 µm thick CYTOP film. If the coated channels were to be used directly, the CYTOP was fully cured at 170 °C. If the coated substrate needed to be bonded with another component or substrate, they were bonded by thermal compression technique under a pressure of 0.3 MPa [9]. When using CYTOP as an adhesion layer, CYTOP was spun on the wafers at 2000 rpm to obtain a 2µm thick film. This film was then processed in the manner described above.

250 µm thick (100) Si wafer was used as the substrate and the base plate for the fabrication of the reservoirs. The fabrication process is shown in Figure 5 and described below. The Si wafers were first oxidized. Next, the oxide was patterned for opening of the windows for back etching. The wafers were through-etched anisotropically with KOH to fabricate openings for (a) the reservoir membrane (generally 1 cm X 1 cm) and (b) an inlet port. An aluminum mold, formed in the shape of a filled reservoir, was positioned on the etched reservoir window. The CYTOP was next carefully dispensed over the sandwiched structure and allowed to dry with the aluminum mold in place. After the drying of the first layer, the CYTOP film thickness was built by repeatedly applying CYTOP. The film was dried at 90 °C for 30 minutes and fully cured at 120 °C for 3 hours. The aluminum was then etched away using the aluminum etchant, leaving a freestanding, molded CYTOP reservoir membrane. The structure was then packaged by attaching the glass cap wafer and inlets and outlets.

RESULTS AND DISCUSSION

Initial tests with the CYTOP film showed excellent compatibility with acids, alkalis and most organic solvents. However, the adhesion of the patterned films to the micromachined substrates tended to deteriorate over a period of time. A 10 hour exposure of approximately 3µm thick CYTOP film on Si, to KOH, resulted in the delamination of the film from the substrate. The CYTOP film itself, however was not attacked and did not show any degradation in properties or swelling. The delamination of the film takes place because of the diffusion of chemicals through the film during prolonged exposure, to the substrate/CYTOP interface (10). In this case, the KOH that diffused, both, through and under the CYTOP film and attacked the substrate Si at the CYTOP/Si interface (at a very slow rate) and eventually delaminated the film.



To ensure that the CYTOP/substrate interface did not degrade, the processing was configured such that the CYTOP coated structures were not exposed to etchants that attacked the substrates for prolonged times. Figure 6 is a cross-sectional SEM image of the coated channel. It can be clearly seen that the channel has been conformally coated with CYTOP. These coated structures have resulted in significantly lower non-specific adsorption and improved compatibility.

Figure 7 is the optical photograph of the reservoir made with CYTOP. When integrated on the chip, the reservoir outlets into a valve. The self closing load lock or check valve allows the reservoirs to be refilled. The reservoirs with 5 µm thick film withstood a differential pressure of 2.2 PSI. This pressure is well above the designed operating pressure of the system and hence, is sufficient. The Teflon-like reservoir membrane is not expected to exhibit non-specific binding on its surfaces. This surface is highly compatible with the antigens and beads suspended in liquids.

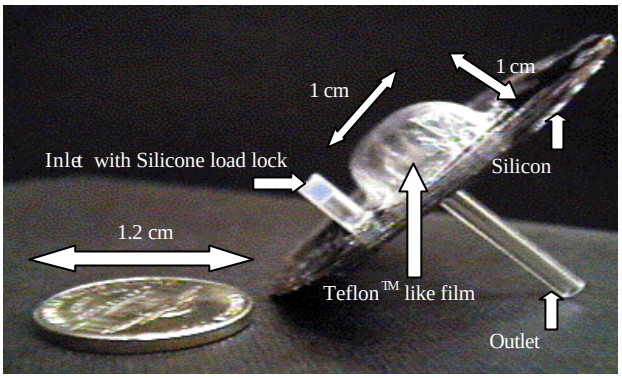


Figure 7: *Photograph of the Reservoir with Stand Alone Teflon-Like Membrane.*

A major challenge in the integration of the coated surfaces with other surface was bonding. The chemical inertness of the CYTOP film, which results in its high bio/chemical compatibility, is a liability when the structures need to be bonded. We have successfully developed a novel thermal compression bonding technique for bonding these Teflon coated structures. In fact this bonding technique has been so effective that we have used it for flip-chip mounting of microfluidic components on the microfluidic motherboards.

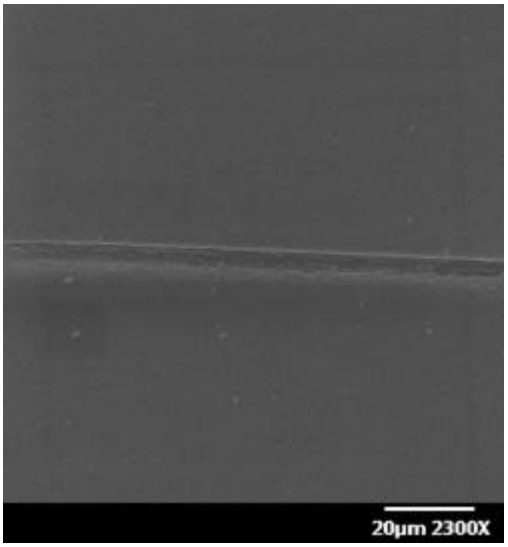
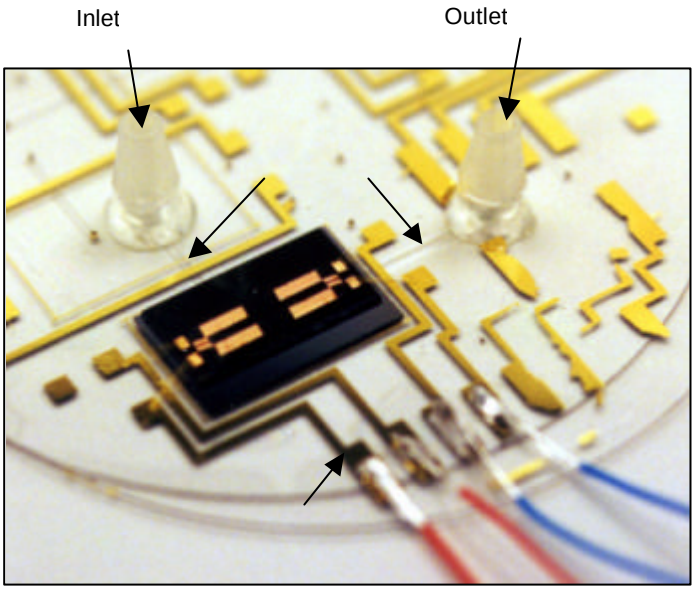


Figure 8: *Photograph of the Reservoir with Stand Alone Teflon-Like Membrane*

Figure 8 is a cross-sectional SEM of the two silicon wafers bonded with CYTOP. Although the CYTOP cross section is very uniform and the top wafer edge is sharp, the bottom edge is blurred. This feature is an artifact of sample preparation techniques. When the cross-sectional specimen is mounted in the mold and polished, the polymer layer, being soft, deforms and flows along the direction of mechanical polishing. As the direction of polishing of this sample was from top to bottom, the bottom edge is blurred because of deformed CYTOP. Figure 9 is an optical micrograph of a micro-valve [5] mounted on the microfluidic board using this technique.

CONCLUSION

We have successfully demonstrated a technique to de-couple the bio/chemical compatibility and fabrication issues in the development of bio/chemical systems. This technique has ensured that the resulting structures exhibit the advantages of both the Si/glass micromachining techniques and the chemical inertness of polymers. We have successfully used these polymers



to fabricate reservoirs for holding fluids and also developed bonding techniques for integrating the CYTOP coated structures with other Si and glass substrates.

The ability to "passivate" surfaces generated using MEMS technology, increasing bio/chemical compatibility, and the ability to bond components at low temperatures (130-170 °C) allow us to develop truly generic microfluidic MEMS components by passivating the working surfaces of our MEMS components with an amorphous fluorocarbon polymer film. The excellent temperature and chemical resistance, optical and electrical properties, and inert surface characteristics allow this material to be used in diverse MEMS applications.

ACKNOWLEDGEMENTS

The bio-chemical development described herein is under the direction of a research team headed by Dr. W.R. Heinemn and Dr. B. Halsall. The authors would also like to thank Dr. K.T. Schlueter for discussions and Mr. Jin-Woo Choi for helping with the schematics.

This research is sponsored by Microsystems Technology Office, DARPA (Microflumes program) under contract AF F30602-97-2-0202 managed by Air Force Rome Laboratories.

REFERENCES

1. D.J. Harrison and A.V.D. Berg (eds.), *Micro Total Systems '98*, Kluwer Academic Press, Dordrecht, 1998.
2. L.P. Lee, S.A. Berger, L. Pruitt and D. Liepmann, "Key elements of a transparent Teflon microfluidic system", *Proceedings of the μTAS '98 Workshop*, Blanff, Canada, Oct. 13-16, pp.245-248, 1998
3. M. Patel, H.T. Henderson, S. Bhansali and C.H. Ahn, "An integrated reservoir for on-chip aqueous storage in microfluidic systems", *Proceedings of the International Mechanical Engineering Conference and Exposition*, Nashville, Tennessee, November, 1999.
4. D.J. Sadler, K.W. Oh, C.H. Ahn, S. Bhansali, and H.T. Henderson, "A new magnetically actuated microvalve for liquid and gas control applications", *Proceedings of Transducers 99*, June 7-11, Sendai, Japan, pp , 1999.
5. N. Okulan, H.T., Henderson, C.H. Ahn,"A new pulsed-mode micromachined flow sensor for an integrated microfluidic system", *Proceedings of the Solid State Sensor and Actuator Workshop*, June 7-11, pp 320-324, 1998.

6. J.-W. Choi, S.Bhansali, C.H. Ahn, and H.T. Henderson, A New Magnetic Bead-Based Filterless Bio-Separator for Integrated Bio-Molecule Detection Systems, *Proceedings of Eurosensors XIII: 13th European Conference on Solid-State Transducers*, Netherlands, September, 1999.
7. C. H. Ahn, H. T. Henderson, W. R. Heineman, and B. Halsall, "Development of a generic microfluidic system for electrochemical immunoassay-based remote bio/chemical sensors", *Proceedings of the mTAS '98 Workshop*, Banff, Canada, Oct. 13-16, pp. 225-230, 1998.
8. W.R. Heinemann, H.B. Halsall, M. Cousino, C.A. Wijayawardhana, S. Purushotama, S. Kradtap, K. Schlueter, J.-W. Choi, C.H. Ahn, H.T. Henderson, "Electrochemical immunoassay with microfluidic systems", *PITTCON' 99*, Orlando, Florida, March 7-12, 1999.
9. K. W. Oh, A. Han, S. Bhansali, C.H. Ahn and H.T. Henderson, "A low temperature bonding technique for assembling microfluidic systems, using fluoropolymers", *Submitted to App. Phys. Lett.*., 1999.
10. CYTOP Technical Report, Asahi Glass Co., Ltd., Japan 1997.