PDMS-Glass bonded Microfluidic device for Single Cell Analysis: Testing, Alignment, Bonding and Trapping of Polystyrene beads

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Abstract -

Current paper describes the testing, alignment and bonding of a PDMS-glass bonded Lab-on-a-chip device. Single cells can be trapped and studied within the reservoir hosted by the device. In the earlier paper we have described the detailed fabrication of the silicon master. PDMS stamps were prepared out of this master and holes were drilled for fluidic and electrical connections. The upper part of the device is of PDMS (Polydimethyl siloxane) and the lower part is of glass with pre-fabricated gold electrodes. The reservoir is embedded between the main channel (20 µm deep) and three smaller channels (2 µm deep). Electrode configuration is the notable factor which helps in leading one particle/cell in to the reservoir. Pre-sorter electrodes were introduced which will bring far off particles to the trapping range. Particles were trapped by well known di-electrophoresis technique .Once trapped the particle can be kept in the reservoir by a simple electrode pair blocking the outlet of the reservoir. Channels were tested using carboxylate modified fluorescent polystyrene beads for any leakage. Alignment of PDMS and glass was performed using a Karl Suss contact aligner equipped with specific tools from TUDelft. Good bonding was achieved by oxygen plasma treatment of both glass and the PDMS.
Polystyrene beads of 2µm were trapped within the reservoir. Pre-sorter electrodes were facilitated with 3 volts which is good enough to move remote particles to the desired side of the channel. The optimal voltage applied to the trapping electrodes depends on the velocity (in this case its 6 volts). A simple pair of electrodes blocks the particle from coming out of the reservoir once trapped. Presently we are working in collaboration to trap a tumor cell of 4-8 µm and study the responses for changes in PH, temperature, concentration and introduction of new drugs.

Index Terms—- PDMS (polydimethyl siloxane), Di-electrophoresis, Lab-on-a-chip, carboxylate modified fluorescent polystyrene beads

Introduction

In the current work we would like to pen down the testing, alignment and bonding of a fluidic device which can trap a single cell (in the range of 4 µm-8 µm) and with which bio analysis can be performed. The top surface of the device is of PDMS (Polydimethyl siloxane) and that of the bottom surface is of glass. The device is equipped with bigger channels of 20µm depth and smaller channels of 2µm. The cell will be trapped in the reservoir of 20 µm*20 µm embedded in between the bigger and smaller channels. The downstream of the device has a detector which can detect the cell responses. The device is armed with bottom side pre-sorter and main electrodes for the bigger channels to divert the cell in to the reservoir and trap it in. The electrodes equipped to the detector will be used for sensing mechanisms.

Di-electrophoresis is a well known technique for manipulating neutral particles/cells in fluids and it is widely used in micro fluidic systems for forcing particles to desired trajectories. The actuators are simple electrodes. Electrode configuration is the most important variable that has been thoroughly studied before fabrication. Since we have to move the particle in the horizontal plane, the width and the introduction point of the particle are more critical variables. Pre-sorter electrodes were introduced more up stream in the entrance channel which will bring far-off particles to the trapping range and also a minimum distance between pre-sorter and trapping electrodes should be regarded. One more important goal is to keep the cell in the reservoir once it is there. This can be achieved by with a simple electrode pair blocking the outlet of the hole. The particle is repelled from the resulting field. The same repelling force will prevent other particles to enter the hole. The geometry of electrodes was designed so that it is only necessary to modify the applied voltage according to the entrance speed.

The basic device has been fabricated using standard silicon process wafers. Photo resist has been used as a mask to etch the bigger and smaller channels in AMS Bosch. These devices were used as a master to prepare PDMS stamps which in turn were bonded to a glass substrate with pre-fabricated gold electrodes. Electrodes were fabricated using double layer photo resist by lift-off method.

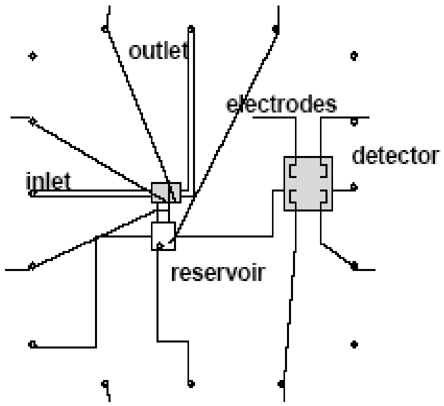

Fig.1 Diagrammatic representation of the device

PDMS stamps preparation

The device fabricated on standard silicon wafer was used as a master to prepare PDMS stamps. PDMS (polydimethyl siloxane)is a silicon based transparent organic polymer. It is a mixture of silicone elastomer and curing agent weighed in the ratio of 10:1. Proper mixing and using a desiccator to remove the air bubbles helps in acquiring clear and transparent viscous PDMS.

Teflon beakers/plates were used for curing the PDMS. The silicon devices were placed on a weighing paper in the Teflon beaker to avoid sticking of PDMS and which also helps in easy recovery of the PDMS stamps and master after curing. The viscous PDMS, void air bubbles was then poured on top of the silicon devices in the Teflon beaker. Curing can be done for 24hrs at room temperature or in 1 hr at 100^0c. After curing the PDMS, as a whole from the Teflon beaker can be peeled off and the stamps can be cut carefully using a surgical knife. The silicon masters can be reused after standard cleaning methods (with Nitric acid or IPA). Fine holes were drilled in the PDMS for fluidic and electrical connections.

Testing

PDMS being a hydrophobic material does not support the flow of the fluid itself. Oxygen plasma treatment for 20 sec under 50 MT pressure and 50 watts power changes the surface property of PDMS to hydrophilic, which lasts for one to few hours (If the PDMS stamps are stored in water after oxygen plasma treatment the hydrophilic nature retains). PDMS stamps along with the plain glass substrate were treated under oxygen plasma (Europlasma) which works on the principle of diffusion. Applying slight pressure on the PDMS for a minute and leaving the samples for a day increases the adhesion between the PDMS and the glass. Testing of the channels within the PDMS stamps was done using fluorescent polystyrene beads of 2µm in diameter suspended in de ionized water. The beads were injected in to the inlet of the main channel using a syringe. Short movies/pictures of the flow of the polystyrene beads within the channels have been recorded without any leakage using Olympus inverted microscope

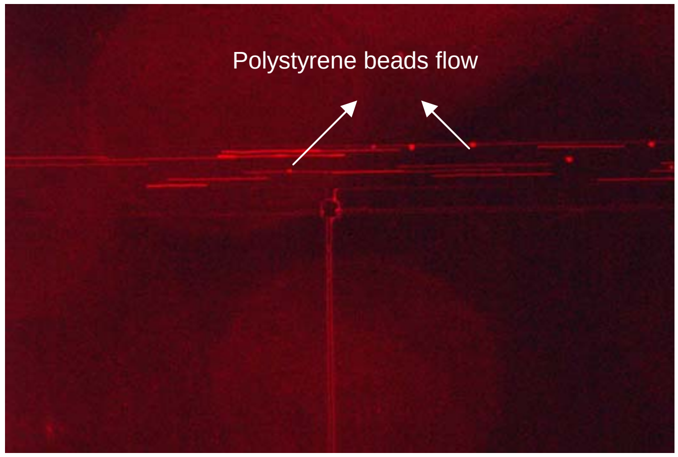

Fig.*2 Photograph of the flow of the polystyrene beads within the main channel. The reservoir embedded in between 3 smaller channels can also be seen*

Alignment and Bonding

Prior to alignment, holes were drilled manually in PDMS for fluidic and electrical connections. Alignment was done using Karl Suss contact aligner with the help of specific external tools developed at TUDelft, which includes a dummy mask plate with a small holder at the centre that can hold small devices (2*2 cm glass substrate in this case) with vacuum and a dummy substrate holder which can hold small devices (PDMS stamps in this case).

Aligning PDMS to glass was one of the critical steps in the whole process as PDMS has good affinity towards glass and this led to several repetitions of the step for good alignment. Once the alignment was done both the samples were left in contact mode for 10-15 min, just to make sure that the adhesion lasts until the whole device was taken for oxygen plasma treatment. Treating under oxygen plasma the bonding between the PDMS and glass gets stronger along with the surface property of PDMS changing to hydrophilic. The device can be stored in water to retain its hydrophilic nature or re-treat under oxygen plasma.

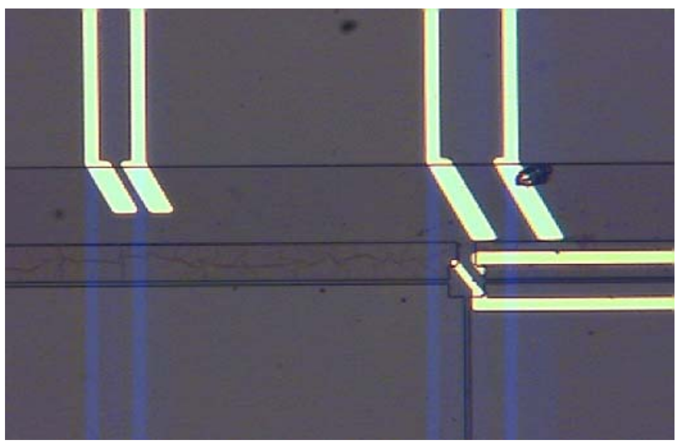

Fig 3.Aligned PDMS stamp and glass with gold electrodes

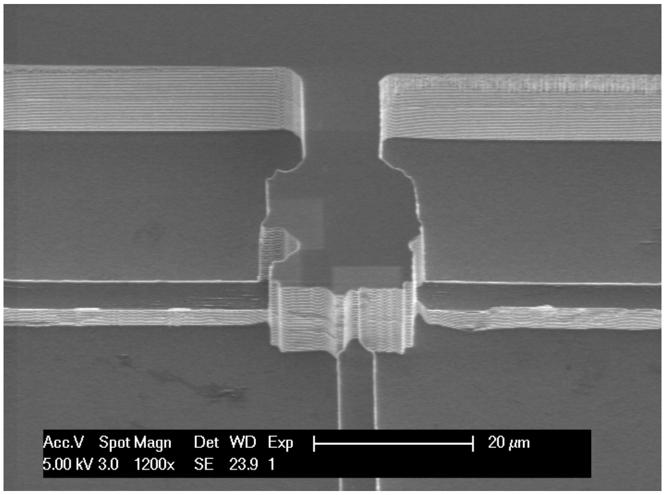

Fig.3 SEM photograph of the reservoir embedded between the main channel and 3 smaller channels

Trapping Polystyrene Beads

After the device was successfully tested and bonded, polystyrene beads were used to study the trapping mechanism.

Carboxylate modified fluorescent polystyrene beads were used which are negatively charged. This modification is to avoid the coagulation of the beads within the fluid. The excitation wavelength of these beads is 580 nm and that of the fluorescence wavelength is 605 nm.

Polystyrene beads of 2µm were suspended in de-ionized water and with the help of a syringe. The fluid was injected through the inlet of the main channel. Pre-sorter electrodes were switched on and 3 volts is good enough to move remote particles to the desired side of the channel. The optimal voltage applied to the trapping electrodes depends on the velocity (in this case its 6 volts). After the injection of the polystyrene beads pre-sorter electrodes were switched on to divert the particle flow towards the reservoir, once the desired particle reaches the reservoir main electrodes were switched on, which diverts the particle in to the reservoir. Once the particle is inside the reservoir there is a simple electrode pair blocking the particle. A set up has been specially developed for these devices with inbuilt electrical and fluidic connections. Thin gold pins were used to connect the gold pads on the device to the electrical connections on the PCB, which are stable. The device is mounted in such a way that the PDMS part faces the fluidic and electrical connections. The whole set up together with the device is mounted on to Olympus inverted microscope and further experiments were conducted.

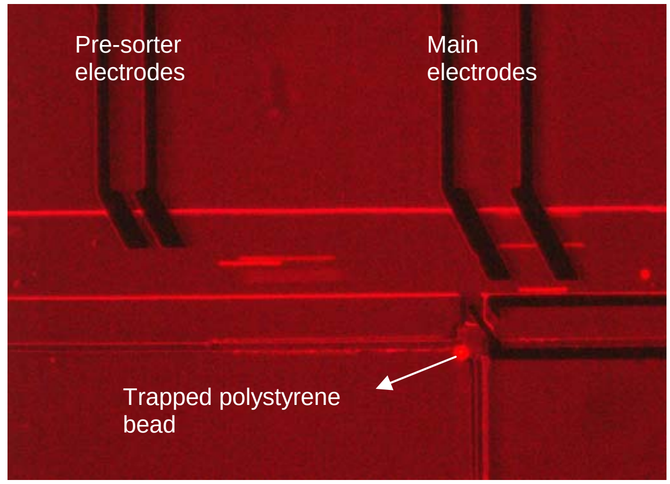

Fig.4 Photograph of the trapped polystyrene bead with in the reservoir, embedded between the main and 3 smaller channels.

Conclusions

The device has been successfully fabricated and PDMS stamps were made out of the master. Etching of channels was done by AMS Bosch etcher and the SEM images of the reservoir, channels and the detector can be viewed in Fig.3. The channels were successfully tested using carboxylate modified fluorescent polystyrene beads for any leakage. PDMS channels were hydrophobic by default, treating both the PDMS and glass with oxygen plasma makes the channels hydrophilic as well as helps for good adhesion between the PDMS and glass. A special setup has been developed on which the device was mounted and polystyrene beads of 2µm were successfully trapped.

Currently we are working in collaboration, and close towards trapping a tumour cell of 4µm-8µm to study the responses for changes in PH, temperature, concentration, introduction of new drugs, cell to cell signalling etc

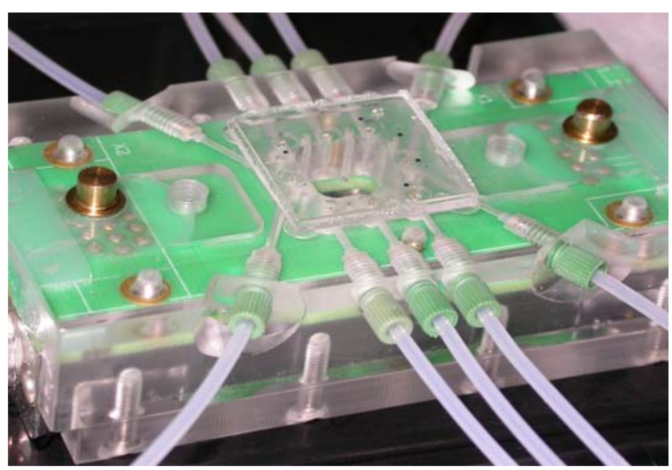

Fig.5 whole set up with fluidic and electrical connections on which the PDMS-glass bonded device is mounted to conduct further experiments

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References

1. J.H. Nieuwenhuis, "Optimization of microfluidic particle sorters based on dielectrophoresis." IEEE Sensors Journal, vol 5, Iss 5, pp 810-816, 2005.
2. Fabrication of nanofluidic devices in glass with polysilicon electrodes.Kutchoukov V.G, Pakula.L, Parikesit G.O.F, Garini.Y, Nanver L.K, Bossche.A, Sensors and Actuators A, Physical 2005, vol.123-4, pp 602-607.
3. Lab-on-a-chip, Nature Insight Vol. 442, issue 7101
4. Hydrophilic surface modification of PDMS using Atmospheric RF plasma.Sung M. Hong, Seong H. Kim, Jeong H. Kim, Hak I. Hwang. Journal of Physics: conference series 34(2006), pp 656-661. International MEMS conference.
5. Method for manipulating and trapping nanoscale objects in the solution. A.E.Cohen, W.E.Moerner. Applied physics letters 86, 093109 (2005).
6. Fabrication of nanofluidic devices using glass-to-glass anodic bonding. V.G. Kutchoukov, F. Laugere, W. Van derv list, L. Pakula, Y. Garini, A. Bossche, Sensors and Actuators A 114 (2004) 521-527.

Biographies

V.R.S.Sarma Mokkapati was born in India on july 9, 1981. In 2001 he received his Bachelor of Science degree in the faculty of Biotechnology, biochemistry and human genetics from Andhra University, India. In 2003 he was awarded M.Sc.degree in the faculty of Biotechnology from Bangalore University, India. The title of his Masters project was " Effect of chlorophyllin (antioxidant) on spleen cells of mice treated with ROS/AAPH" from Bhabha Atomic Research Centre (BARC), Mumbai, India.
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Oana Piciu was born in Romania in the 18th of November 1978. In 2002 she graduated the Faculty of Biology and Geology, section Biology-Chemistry, Cluj, Romania. The title of the Diploma Project was: "The bioreduction of a nitroderivate of 10-propil-5-oxid-phenothiazin using Saccaromyces cerevisiae cells ".
In the next following year she attended the Master Courses at the same Faculty, section Cell Biology and Molecular Biotechnology. The Disertation theme was about: "Clonation of the gene for homoserine dehydrogenase from B.subtillis in E.coli".
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Lujun Zhang Lujun Zhang was born in Shanghai, P.R.China, in 1981. In 2003, he received his B.E. degree in Electronics and Information from Tongji University. In 2005, he earned his M.Sc. degree in System-on-Chip Design from Royal Institute of Technology (KTH), Sweden. He spent the last 6 months of master study doing thesis work at the Electronics Research Laboratory, Delft University of Technology (TUDelft), on the topic of Implementing an Analog Delay for an UWB Autocorrelation Receiver. Since June 2005, he is pursuing a Ph.D. degree at the Electronic Instrumentation laboratory (EI), Delft University of Technology (TUDelft). His research area is Micro-Fluidics Devices for Biochemical Analysis.

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