Surface effects at polymeric interfaces in capillary driving process

Ilenia Viola^{*a}, Anna Zocco^b, Alessandra Fischetti^b, Barbara Foglieni^c, Laura Cremonesi^c,
Maurizio Ferrari^c, Roberto Cingolani^a and Giuseppe Gigli^a

^a NNL, National Nanotechnology Laboratory of INFM, c/o Dip. Ingegneria dell'Innovazione,
Universita' di Lecce, via Arnesano, I-73100 Lecce, Italy;

^bSTMicroelectronics, MFD Division, Application Laboratory Lecce, via Arnesano, I-73100
Lecce, Italy;

^c Laboraf SPA and Unit of Genomics for Diagnosis of Human Pathologies, IRCCS H San
Raffaele, via Olgettina 60, 20132 Milan, Italy.

ABSTRACT

The growing interest in biological micro-array and Lab-on-Chip (LOC) is justified by the possibility of reducing the sample volume, processing time and costs. By means microfluidics seems to be the prevalent tool for the integration of manifold processes in miniaturized devices. Anyway at a sub-millimeter scale the liquid behavior is affected by geometric confinement and wetting properties. In order to confine the liquid sample inside the LOC buried channels we have performed the sealing by poly(dimethylsiloxane) (PDMS), a silicon-based bi-component elastomer. The filling dynamics changes at changing of the polymer mixing ratio of the pre-polymer and the curing agent. In fact, a different concentration of cross-linker modifies the elastic, adhesion and wettability properties of the cover-slip.

Keywords: PDMS, DNA-chip, wetting properties, liquid displacement, confined geometry

1. INTRODUCTION

The state of the art in microelectronics and integrated devices for biological and medical applications finds development in the concept of Biomedical Micro-Electro-Mechanical Systems (BioMEMS). Their growing development is justified by micro and nano-scale fabrication and detection technologies. The main purposes of device integration focus on the reducing of the volumes used and the processing time as well as the enhancing of the output sensitivity¹⁻³.

In the last years, the most successful example of the merging between micro-technologies, chemistry and biology is represented by the DNA micro-array. The growing interest on these miniature systems is justified by using nano-liter size samples and reagents, as well as the possibility to reduce the analysis-time to some minutes rather than the actual hours or days. This realization is allowed by the simultaneously presence and the reduced size of each analysis components in a single chip.⁴ Therefore, microfluidics seems to be a prevalent tool for the integration of manifold processes in a fast way.⁵

Anyway, it is commonly known that in microfluidic systems the sub-millimeter scale significantly affects the liquid behavior, both by geometric confinement and by wetting properties. By means in a micro and nano-environment the imbibition by inlet of a sample liquid, inside the micro-channels, and the consequent filling motion are driven by capillary forces and surface effects. In fact, at the small scale,

*Further author information: (Send correspondence to I.V. and A.Z.); I.V.: E-mail: ilenia.viola@unile.it;
A.Z.: E-mail: anna.zocco@st.com

flow patterns change significantly both with different surface chemical treatments and with the flow rate or driving pressure. Indeed, when the system has a high surface-to-volume ratio (S/V), as micro and nano-devices, the surface interaction of each molecule dominates the diffusive displacement*.⁷

Therefore, the correct design and the choice of the materials involved in the fabrication of lab-on-chip is crucial for the optimization of the analytical processes with reduced sample volume and time.

The main investigation, of this paper, focuses on the liquid behaviour at the solid interface of a DNA lab-on-chip, paying attention both to avoid leak of the sample volume during the thermal and processing cycles and to reduce the cross-contamination risks. The sealing applications have been performed by a silicon-based elastomer slabs at the external connecting device ports (see fig.1) of the STMicroelectronics DNA-chip.^{4,8}

We have investigated the role of surface tension, work of adhesion and elastic properties of different mixing ratio poly(dimethylsiloxane) (PDMS) (*Sylgard* 184, Dow Corning, Midland, MI) used as cover layer.

PDMS is usually used for biological applications because of the biocompatible, highly inert and optically transparent behaviour^{9,10}. We remind that PDMS is a two-component mixture consisting of a pre-polymer and a curing agent, as cross-linker. The physical and the chemical properties of a bi-component elastomer change with changing the mixing ratio. In particular, higher concentration of the cross-linker gives a more rigid polymer by enhancing the elastic modulus. The curing agent, in fact, acts to determine the extent of cross linking in the polymer¹¹⁻¹³.

2. MICROFLUIDIC APPROACH

The DNA-chip relies on microfluidic technology and is characterized by triangular-shaped buried micro-channels (see fig.2), connected to the outer surface by the inlet and the outlet ports (see fig.3). The channel section is obtained directly by etching the silicon oxide (SiO_2), which determines the typical edge angle of $\alpha \simeq 54.7^\circ$ (figg. 4, 5).

Our microfluidic system can be considered as a horizontal capillary system and a zero-gravity environment can be assumed because $L \gg b, c$, with respect to the figure 5.

Therefore the liquid flow is a thermodynamically driven process, primarily influenced by the surface tension at the different phases (solid-liquid SL , solid-vapor SV and liquid-vapor LV) and thus by the advanced contact angle θ . The pressure drop of the capillary motion is defined by the Laplace expression¹⁴

$$\Delta p = \frac{2\gamma_{LV} \cos \theta}{R} \quad (1)$$

where γ_{LV} is the free energy at the liquid-vapor interface; θ is the contact angle between the liquid and the capillary surface; R is the geometric factor that is a hydraulic radius in the most general case for the capillary shape.⁷

*In a micro-system the time condition for a fast diffusion regime is $T_S \gg T_D$ where T_S is the surface time, namely the average time of each molecule's interaction with the surface $T_S \sim \rho^{-1}(V/S)$, with ρ related to the molecular relaxation rate near the surface⁶; T_D is the molecular diffusion time according to the Einstein equation $T_D \sim D^{-1}(V/S)^2$.

The triangular cross-section of our system channels (fig.6), requires the introduction in eq.1 of the hydraulic radius that is function of the cross-sectional area A_{sec} and of the capillary perimeter P

$$R_h = \frac{2A_{sec}}{P} = \frac{ac \sin \alpha}{(2a + c)} = 58.4 \mu m \quad (2)$$

After loading the chip by a micro-pipette with a micro-liter volume of sample, the filling pressure inside the silicon-oxide triangular micro-channels,^{15,16} is defined according to the eq.1 with the hydraulic radius (eq.2)

$$\Delta p_{int} = \frac{2\gamma_{LV} \cos \theta_{SiO_2}}{R_h} \quad (3)$$

This is the driving pressure that the fluid keeps up through the buried channels from the inlet to the outlet ports (fig.7). After the loading there is the necessity to close the external ports of the chip in order, as we have said, to reduce the cross-contamination risk and the leak of sample during the thermal cycles of the polymerase chain reaction (PCR). The sealing was performed by a layer of PDMS in contact with the SiO_2 surfaces, respectively of inlets and outlets, as in figures 8. The PDMS polymer forms, in fact, a reversible seal with silicon, when it comes in conformal contact with the substrate, but secure enough to avoid the liquid leakage from the micro-channels.

Anyway, the physical characteristics of the elastomer slabs placed on the chip were crucial from the point of view of the energetic and thermodynamic conditions, driving the flow. In fact, when the PDMS layer covers the channels ports we can represent the capillary system section with two lateral sides of SiO_2 and top side of PDMS. The interplay of the surface free energies of the two different materials (see table 1) implies to modify the pressure drop in the terminal part of each channels

$$\Delta p_{out} = 2\gamma_{LV} \left(\frac{\cos \theta_{SiO_2}}{a} + \frac{\cos \theta_{PDMS}}{c} \right) \quad (4)$$

The expression (4) shows how the liquid capillary motion inside the micro-channel and the meniscus shape, indicative of the liquid curvature, are strongly influenced by the contact angle, $\cos \theta_{PDMS}$, at the PDMS interface. The contact angle, defined as difference of interfacial free energies[†], is in fact considered as the thermodynamic condition that favors the filling process. Besides, the direction of the fluid displacement, in a capillary environment, complies with an energy minimizing path and thus towards the lower pressure difference $\Delta p = \Delta p_{int} - \Delta p_{out}$.¹⁴

We have observed that the elastic and surface wetting properties of the polymeric cover-slip influence the dynamic mechanism of the fluid motion. In particular, by using a typical PDMS mixing of 10 : 1 we verify a sort of *spring effect*,¹⁷ with the liquid sample wetting the PDMS surface, during the sealing procedure, thus emptying the channel.

The effect could be ascribed to the rigidity of the elastomer layer as well as to the surface tension and wetting properties, resumed in the contact angle measurement.

[†]The contact angle is defined by the Young's equation $\gamma_{LV} \cos \theta = \gamma_{SV} - \gamma_{SL}$

Table 1. Material parameters

Substrate	Contact Angle [θ]	Surface Tension [$\star$] [mN/m]	Young Modulus [MPa]
SiO_2	65.5 ± 0.5	44.45	-
PDMS 5 : 1	109.8 ± 4.2	18.23	4.4
PDMS 10 : 1	112.1 ± 5.6	17.10	3.4
PDMS 20 : 1	124.9 ± 2.1	9.81	2.7
PDMS 30 : 1	131.9 ± 1.2	5.90	2.2
PDMS 9 : 1	96.9 ± 5.2	22.60	5.6

[$\star$] The surface tensions are interpolated by the contact angle goniometer (OCA20, Dataphysics) software with the semi-empirical formula $\gamma_{SV} = \gamma_{LV}[0.5(1 + \cos \theta)]^2$.

3. SURFACE CHARACTERIZATION

PDMS, the two-component silicon-based elastomer is realized by a mixture of pre-polymer and curing agent. The concentration of the curing agent, acting as cross-linker, determines the elastic properties of the final polymer. In fact, the PDMS chains are connected by the cross linker, thus acting on the flexibility defined as the distance between the polymer chains.

Therefore, to solve the spring effect observed in our experiment, we realized polymer slabs with different mixing ratios, thus with different elastic and wetting properties. We realized four PDMS slabs with the mixing ratio of 5 : 1, 10 : 1, 20 : 1, 30 : 1. The elastomers, made with a thickness of about 1 mm, were cured at 65°C for 1 h. Besides, we have tested the effect of the curing temperature and time for the elastomer with a ratio 9 : 1 cured at 140°C for 15 min.¹⁸ We observed that the two softer PDMS, with the lower Young's modulus, 20 : 1, 30 : 1, reduce remarkably the spring effect at the outlets and leave the liquid sample inside the channels.

PDMS is a hydrophobic polymer. Anyway, the different mixing ratios modify also the contact angles and the wetting properties, as shown in table 1. A change of the contact angle value means, in the eq.4, both a different hydrodynamic motion and fluid displacement direction, complying different energy minimizing paths. In particular, we have obtained $\Delta p_{int} = 1 \text{ kPa}$ for the pressure inside the micro-channels. On the contrary, for the PDMS interface we have considered the two extreme situations for the elastic properties, the most rigid and the softest polymers: $\Delta p_{out}(5 : 1) = 0.09 \text{ kPa}$ and $\Delta p_{out}(30 : 1) = -0.12 \text{ kPa}$. Rather than a meaningful quantitative difference, we underline the opposite signs of the pressures. The negative pressure of the 30 : 1 ratio points out a backwards stream that limits the liquid going out from the channels.

4. PCR VALIDATION

In order to optimize the PDMS slabs for sealing the inlet and the outlet of a DNA-chip, we should account also for the inertness and compatibility with the biological sample during the analysis procedures.

For this reason we have carried on a polymerase chain reaction putting in the sample solution pieces of PDMS with the five different mixtures, in order to evaluate the influence of polymers on an eventual PCR alteration or inhibition.

For the PCR the genomic DNA, used as control, was isolated from 10 *mL* of EDTA-anticoagulated blood by standard phenol/chloroform methods. Primers were designed to amplify a genomic region of 224 *bp* containing exon 1 of the beta-globin gene. PCR primers were as follows: forward 5' – *GTTACAAGACAGGTTTAAGGAGACC* – 3', reverse 5' – *GCACTTTCTTGCCATGAGCCTTC* – 3'. Polymerase chain reaction was performed on a Hybaid PCR Express thermal cycler (Life Science International, Cergy Pontoise, France), in 50 μ *l* reactions containing 5 – 100 *ng* DNA, 200 μ *M* dNTPs, 10 *mM* Tris-HCl (pH 8.3), 50 *mM* KCl, 1.5 *mM* *MgCl*₂, 1.3 units of thermostable DNA polymerase (AmpliTaq Gold Applied Biosystem, Foster City, CA) and 20 *pmoles* of each primer. Cycling conditions were 95 °C for 10 min (one cycle); 95 °C for 30 sec, 57 °C for 30 sec, 72 °C for 30 sec (35 cycles); 72 °C for 5 min (one cycle). In figure 9 the results of PCR products are reported. The two trends are referred to two different PCR mixtures, with 0.05 % of Bovine Serum Albumin (BSA) and without BSA, respectively.

The more hydrophobic polymer, 30 : 1 that shows the better behavior for the pressure effect, reveals no substantial alteration and a good efficiency of the PCR (see fig.9) and thus the DNA adsorption can be neglected.

5. CONCLUSIONS

Biological micro-array and Lab-on-Chip, based in the concept of BioMEMS, have found a growing development, in the last years, kept up by the interest in reducing the sample volumes, processing times and costs. To this aim, one of the main crucial point is to isolate the integrated device from the outside, in order to avoid the cross-contamination risk and any sample leakage during the thermal cycles. We have performed the sealing of the external ports of a DNA-chip (*STMicronics*) by PDMS slabs.

The chip can be schematized as a microfluidic array. Therefore, we have investigated the surface effects in the terminal part of the buried channels closed with a PDMS cover-slip. We have made elastomer layers with different elastic and wetting properties, by changing the mixing ratio. The effects of different chemical and physical properties have been finally correlated to the pressure conditions in the displacement capillary motion.

Acknowledgments

The authors gratefully acknowledge Dr. A. Frosini and Dr. M. Palmieri (STMicronics), Prof. R. Rinaldi and Dr. F. Calabi (NNL, Univ. Lecce) for useful discussions; finally Dr. G. De Benedetto (Univ. Lecce) for placing the contact angle goniometer to our disposal.

REFERENCES

1. H. Andersson, A. Van der Berg, Microfluidic devices for cellomics: a review, *Sensors and Actuators B*, **92**, pp.315-325, 2003.
2. M.S. Munson, P. Yager, Simple quantitative optical method for monitoring the extent of mixing applied to a novel microfluidic mixer, *Analytica Chimica Acta*, **507**, pp. 63-71, 2004.
3. R. Bashir, BioMEMS: state of the art in detection, opportunities and prospects, *Advanced Drug Delivery Review*, **56**, pp. 1565-1586, 2004.
4. M. Palmieri, DNA lab-on-chip rests on MEMS foundation, *EDN*, 8 January, pp. 24-28, 2004.
5. M.A. Unger, H. Chou, T. Torsen, A. Scherer, S.R. Quake, Monolithic microfabricated valves and pumps by multilayer soft lithography, *Science*, **288**, pp. 113-116, 2000.
6. G.C. Borgia, R.J.S. Brown, P. Fantazzini, E. Mesini, G. Valdre, Diffusion-weighted spatial information from h-1 relaxation in restricted geometries, *Nuovo Cimento*, **14D (7)**, pp. 745-759, 1992.
7. I. Viola, D. Pisignano, R. Cingolani, G. Gigli, Microfluidic for a direct investigation of the structural dynamics of glass-forming liquids, *Anal. Chem*, **77(2)**, pp. 591-595, 2005.
8. Products in <http://www.st.com/>.
9. A. Mata, C. Boehm, A.J. Fleischman, G. Muschler, S. Roy, Analysis of connective tissue progenitor cell behaviour on Polydimethylsiloxane smooth and channel micro-textures, *Biomed. Microdev.*, **4**, pp. 267-275, 2002.
10. E.T. Dimalanta et al., A Microfluidic system for large DNA molecule arrays, *Anal. Chem.*, **76**, pp. 5293-5301, 2004
11. J.A. Rogers, K.E. Paul, G.M. Whitesides, Quantifying distortions in soft lithography, *J.Vac.Sci.Technol. B*, **16(1)**, pp. 88-97, 1998.
12. W. Chang, D. Akin, M. Sedlak, M.R. Ladisch, R. Bashir, Poly(dimethylsiloxane) (PDMS) and silicon hybrid biochip for bacterial culture, *Biomed. Microdev.*, **5**, pp. 281-290, 2003.
13. A. Bietsch, B. Michel, Conformal contact and pattern stability of stamps used for soft lithography, *J.Appl.Phys.*, **88(7)**, pp. 4310-4317, 2000.
14. D. Myers, *Surfaces, Interfaces and Colloids: principles and applications*, Wiley-VCH, NY, 1999.
15. E. Kim, G.M. Whitesides, Imbibition and Flow of Wetting Liquids in Noncircular Capillaries, *J. Phys. Chem. B*, **101**, pp. 855-863, 1997.
16. E. Delamarche, A. Bernard, H. Schmid, A. Bietsch, B. Michel, H. Biebuyck, Microfluidic Networks for Chemical Patterning of Substrates: Design and Application to Bioassays, *J. Am. Chem. Soc.*, **120**, pp. 500-508, 1998.
17. A.V. Bazilevsky, K.G. Kornev, A.N. Rozhkov, A.V. Neimark, Spontaneous absorption of viscous and viscoelastic fluids by capillaries and porous substrates, *J. Coll. Int. Sci.*, **262**, pp. 16-24, 2003.
18. D. Pisignano, E. Sariconi, M. Mazzeo, G. Gigli, R. Cingolani, High-Temperature Microfluidic Lithography, *Adv. Mater.*, **14(21)**, pp. 1565-1567, 2002.

Figure 1. *Sketch of the ST DNA-chip. The inlet and outlet ports are reported as well as the micro-channels.*

Figure 2. *SEM micrograph of the buried channels.*

Figure 3. SEM micrograph showing a detail of the top view of inlets.

Figure 4. SEM micrograph of the inner buried micro-channel surface with a triangular shape.

Figure 5. *The schematic geometry of a micro-channel.*

Figure 6. *A 3D sketch of three micro-channels, connected by the inlet ports.*

Figure 7. *The longitudinal section of the DNA-chip. Features are not in scale.*

Figure 8. The sealing of the chip's inlets and outlets by a PDMS slabs.

Figure 9. The experimental data of the PCR validation carried on putting in the sample solution the different PDMS mixtures, in order to evaluate the influence of polymers. Two different PCR have been performed with 0.05 % of BSA ($\triangle$) and without BSA ($\bullet$). The experimental error bars are reported only for the meaningful data, which are obtained by experimental replications. For PDMS 30 : 1, with the better behavior for capillary effects, no substantial alteration and a good efficiency of PCR are detected.