A new instrument for automated microcontact printing. Applications to MEMS and biochips fabrication.

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Abstract:

Microcontact printing (μCP) is a versatile tool for the chemical surface modification of substrates based on a mechanical concept. It makes use of an elastomeric stamp with a relief pattern. The stamp is inked with the chemical and the pattern of this chemical is transferred onto the substrate surface by contact. So far this technique has been in general performed manually or with modified expensive microelectronic aligners. We present here a new instrument, called Microcontact Printer, to pattern organic molecules as well as proteins and DNAs with a fully automated process. The mechanical design of this low cost and compact instrument is reviewed. Potential applications are discussed in the field of biochips and MEMS with emphasize on two exemples recently developed in our laboratory: (1) wet etching of glass microfluidic channels patterned by μCP and (2) grafting of biomolecules inside microfluidic channel.

Résumé :

Le microtamponnage (μTP) est un procédé flexible pour la chimie de surface de substrats basé sur un principe mécanique. L'outil de base est un tampon en élastomère comportant des motifs en relief. Le tampon est encré avec un produit chimique, qui est transféré à la surface du substrat par contact. Jusqu'à présent cette technique était mise en œuvre manuellement ou à l'aide d'aligneurs microélectroniques coûteux. Nous présentons un nouvel instrument complètement automatisé, un Microtamponneur, qui permet de créer des motifs de molécules organiques, de protéines et d'ADN. La conception mécanique de cet instrument compact et à bas coût est détaillée. Les applications potentielles pour les biopuces et les MEMS sont passées en revue avec deux exemples récemment développés dans notre laboratoire : (1) la gravure de circuits microfluidiques en verre avec des motifs crées par μTP et (2) le greffage de biomolécules à l'intérieur d'un canal microfluidique.

Key-words: microcontact printing ; MEMS ; biochip

1 Introduction

Microcontact printing (μCP) is a versatile tool for the surface modification of substrates (Xia & Whitesides 1998). It makes use of an elastomeric stamp of PDMS with a relief pattern. As shown in Figure 1, the stamp is inked with the chemical to deposit, so that a pattern of chemical is transferred onto the surface of the substrate by direct contact of the stamp with the substrate. Microcontact printing is in general additive (transfer of material form stamp to substrate), but it can be also subtractive (removal of material form substrate to stamp).

In the case of chemicals like silanes or alkanethiols, it is well known that organic monolayers can be directly formed on silicon or glass substrates (Schreiber 2000). Specifically in the case of alkanethiols, patterns with a thickness of only 2-3 nanometers are good protecting masks for the etching of the substrate, which is under consideration for applications in micro and nanoelectronics (Kumar & Whitesides 1993). Lateral resolution in the 40 nm range has been recently demonstrated (Kang et al 2006).

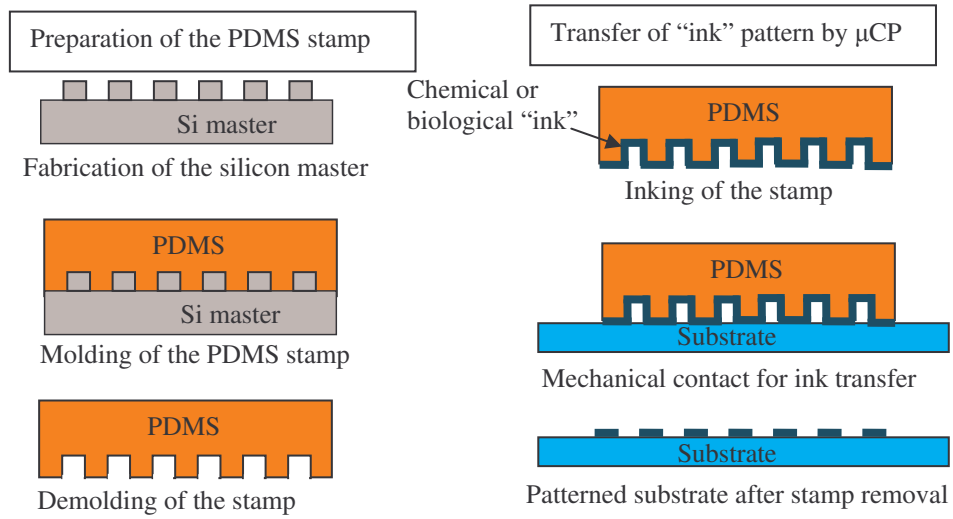

FIG. 1 – The Principle of Microcontact Printing

Microcontact printing is not limited to microelectronics. To our knowledge, µCP is one the most promising way to address organic molecules and chemical on a substrate with such an extreme resolution. This point is illustrated in Figure 2 in the case of DNA and protein chips. This figure shows a comparison between key addressing technologies as regards the lateral resolution of the feature on the chip and the chip integration. The chip integration is defined as the number of different biomolecules probes on the chip (that is to say of different chemical compositions). Potentially, the degree of integration of biochips made by µCP is two orders of magnitude better than the one obtained by photochemistry, which is the actual standard for DNA (Affymetrix technology). This is of course extremely important for biochip sensitivity. Unlike DPN, µCP can pattern large surfaces, so mass production is possible by collective fabrication. As a consequence, different research teams are exploring the potential of µCP for the grafting of DNA or proteins (Delamarche 2005), as well as the direct synthesis of DNA onto biochips (Xiao et al 2002).

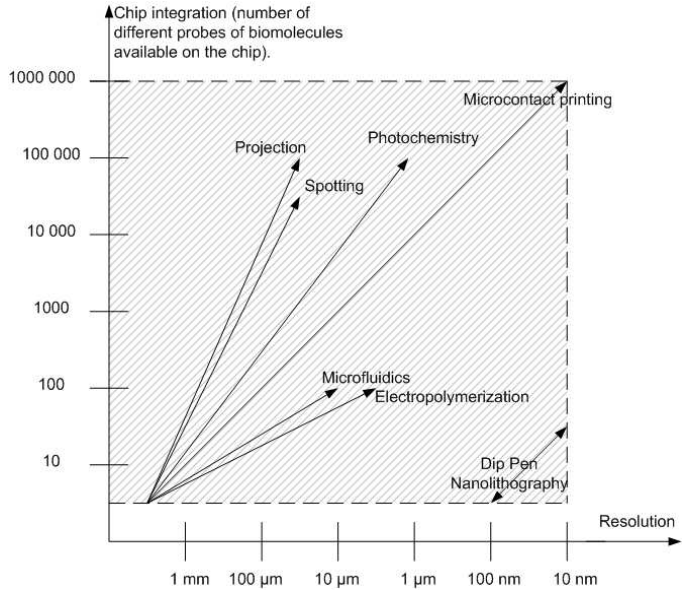

FIG. 2 – Comparison of different technologies for the manufacturing of biochips

To take full advantage of µCP potential, it is essential to perform the stamping with great repeatiblity and accuracy. But so far, µCP has been in general performed manually (Delamarche 2005) or with modified expensive aligners derived from the microelectronic industry (http://www.evgroupcom/). We present here a new instrument, that we call Microcontact Printer, to pattern by µCP organic molecules (silanes, thiols, phosphoramidites for DNA synthesis, Fmoc for peptide synthesis, etc.), as well as proteins and DNAs on silicon or glass substrates with a micrometric resolution. We have developed a low cost and very compact instrument with a process simple and for the first time fully automatic. The mechanical design of the machine is reviewed in the next section. By varying the nature of the chemical and the substrate, it is possible to use this machine in a wide range of applications. To illustrate this point in the second part of this paper, two practical applications recently developed in our laboratory are also discussed: (1) the grafting of biomolecules by µCP inside microfluidic channels and (2) the wet etching of glass microfluidic channels patterned by µCP.

2 Overview of the Microcontact Printer

Our Microcontact Printer aims at patterning microscope slides or small silicon substrates with 50 x 15 cm pattern. For many applications, like the fabrication of protein or DNA chips by grafting of biomolecules, it is absolutely necessary to deposit different chemicals on the same substrate with different stamps. For more demanding applications, like the production of DNA chips by in situ chemical synthesis of oligonucleotides (Xiao et al 2002), it is necessary to sequentially deposit a series of different chemicals exactly at the same place on the substrates So the mechanical repeatability of the movement of the stamp and the possibility to change stamps and substrates are important points. We are looking also for the production of a small series of substrates.

Therefore the Microcontact Printer has been designed to stamp a series of substrates after the initial calibration of the machine with the first substrate and keeping the set up of the machine for all other substrates. The instrument allows full monitoring of the process parameters to carefully control the load on the stamp (see section 4.), use different inks without cross contamination and change easily substrates and the stamps without re-alignment. Process reproducibility, full automation, quality control and use of minute quantity of reactants are also required.

The design of the machine (Figure 3) is based on a pneumatic actuator which pushes the stamp back and forwards. As the movement of this actuator is not accurate enough, the movement of the stamp is guided by a high accuracy miniaturized linear slide. The machine incorporates features allowing the alignments of the stamp with the substrate, the change of substrate and/or stamp. It incorporates a miniaturized and automated inking system by pulverization and also CCD camera for quality control during the inking and the stamp/substrate contact (the inking and the quality systems have been described in a separate paper about the previous generation of machine: Hannes et al. 2005).

The parallelism of the stamp with the substrate during the contact is extremely important to avoid pattern distortion. Therefore the stamp holder is suspended with the two spherical bearings and hold by means of spring. This gives the freedom to align the parallelism with micrometric screws.

The overall mechanical repeatability of the movement of the stamp is in the +/ 0.5 µm range.This machine allows patterning of substrates by µCP with results comparable with conventional mask aligners and UV illuminator for photolithography. The advantages are as follows: (i) the Microcontact Printer is much compact and less expensive than a mask aligner and that µCP, (ii) it allows transferring a greater variety of products than a UV illuminator, which is limited to photopolymers or photochemical products.

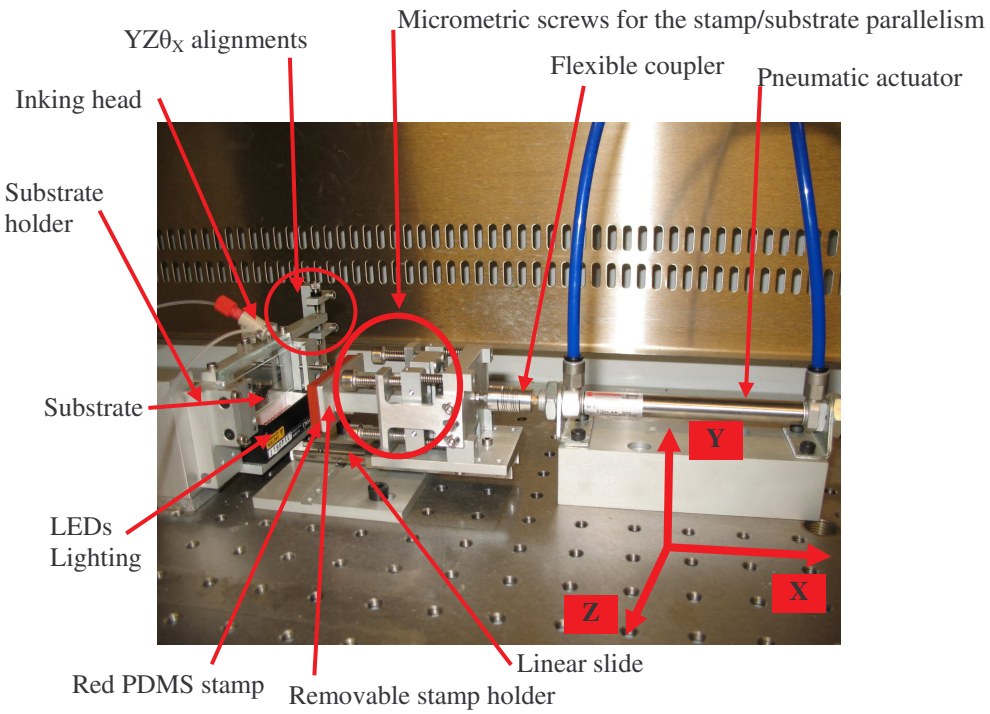

FIG. 3 – Overview of the Microcontact Printer

3 First application for the etching of glass devices for microfluidics

We present here a new process for the fabrication of glass lab-on-chips and plant-on-chips without any photolithographic steps. The etching of glass microfluidic devices, with deep channels is a complex process, which requires so far expensive microelectronic equipment and skilled personal for the etching itself (wet or dry etching) and for the preparation of the masks. These masks made of thick photopolymer, metals, or combination of both, have to withstand the harsh conditions necessary for the glass etching. We report here a flexible, quick and easy process which could be standardized and which could be also of great help for chemists or biologists willing to prepare glass microfluidic devices in their own laboratory. The innovative core of this process (μCP followed by chemical etching) can be performed in a standard chemical fume hood. As a matter of fact, a clean room environment is not needed.

Starting with substrates ready to use, 3"x1" microscope glass slides coated with a 50 nm silver - 150 nm chromium bilayer, this process consists in stamping the pattern of the microfluidic device (e.g. channels, reservoirs, connections, etc.) with the Microcontact Printer. The chemical deposited on top of the silver layer is an alkanethiol, which serves as the first protecting mask. Then the substrate is simply dipped successively in four different beckers (i) to open the silver and chromium layers with acidic solutions, (ii) to remove the silver layer, (iii) to etch the glass through the chromium with fluorhydric acid and (iv) lastly to remove the remaining chromium with aquia regia. This leads to bare glass with 10-70 μm deep channels. Then the substrates can be sealed or covered with glass or other materials (simply by gluing or with an "easy" anodic bonding process under development).

Figure 3 shows an example of a reservoir (diameter 300 μm) and a channel (width 50 μm) etched in a low cost soda-lime glass slide (20 μm etch depth). The overall process is quick: a few seconds for stamping and the different etching steps, except for the etching of the glass which is performed at a rate of ~ 1 μm / minute. For industrial purpose, the process throughput is extremely high, so mass production is conceivable.

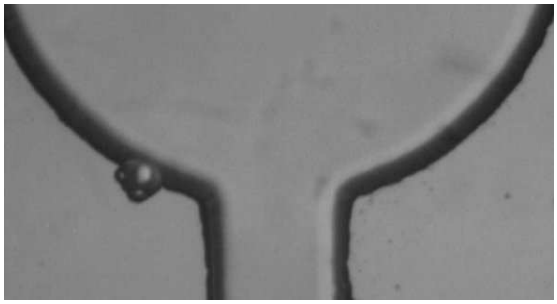

FIG. 4 – View of a reservoir and a channel etched in a microscope slide.

In the field of research, the fabrication of the PDMS stamps can be performed by a rapid prototyping technique (under development) based on ink jet printing. By principle, μCP allows introducing a great flexibility in the design of microfluidic devices, so that modular fabrication is possible. Thanks to the design of the stamp holder of the Microcontact Printer allowing the exchange of stamps, multiple patterning of the same substrate with different stamps are possible. It is thus possible to make a complex microfluidic device by addition (stamping) of different elemental units. The principle is to design a stamp for every elemental chemical function: reservoir, channel, mixer, divider, heat exchanger,… and to dedicate one stamp per elemental function. The modular fabrication and optimization of plant-on-chips is possible with a way of "thinking" close to Chemical Engineering.

4 Second application for the grafting of biomolecules inside microfluidic channels

The Microcontact Printer allows also the grafting of biomolecules not only on top of plane substrates, but also inside (at the bottom) of microfluidic channels. Today, the integration of biochips in lab-on-chips is seen has a powerful solution to perform automatically all the complex and time consuming pre-processing or post-processing steps necessary for the use of biochips. Thanks to the features of our Microcontact Printer, μCP can be used for that purpose.

This is illustrated in Figure 5, which shows on the left side, the principle of the grafting of fluorescent antibodies inside a microfluidic channel without damaging the fragile borders of the glass channel. Key dimensions are listed in the figure. The whole stamp is inked with the antibodies but the PDMS has a raised feature, which is very carefully applied at the bottom of the channel. As a consequence the transfer of antibodies from the stamp to the substrate is limited to the surface contact. This is proven by the scanned fluorescence image of the substrate which shows no significant fluorescence signal outside the channel. A high resolution scanning (not shown) proves that the width of the fluorescence is equal to the width of the PDMS raised feature.

The raised feature is an isolated piece of elastomeric material, a few cm long, with a 30 μm width. The mechanical resolution of the Microcontact Printer allows fine alignment of the feature with the channel. But from the mechanical point of view, the issue was to finely regulate the load of the stamp during the contact, in order to avoid the collapse of the whole stamp. To do so, the solution was not to apply directly the pressure of the pneumatic actuator on the stamp. As show in Fig 5, two micrometric screws with micrometric resolution were used to limit the compression of the stamp (L-L' ~ 2 μ). So the load in excess is applied to the screws and not to the PDMS feature. It was possible to finely regulate the load on the stamp along the 5 cm long raised feature. To the best of our knowledge this is the first time that such a regulation is described for μCP.

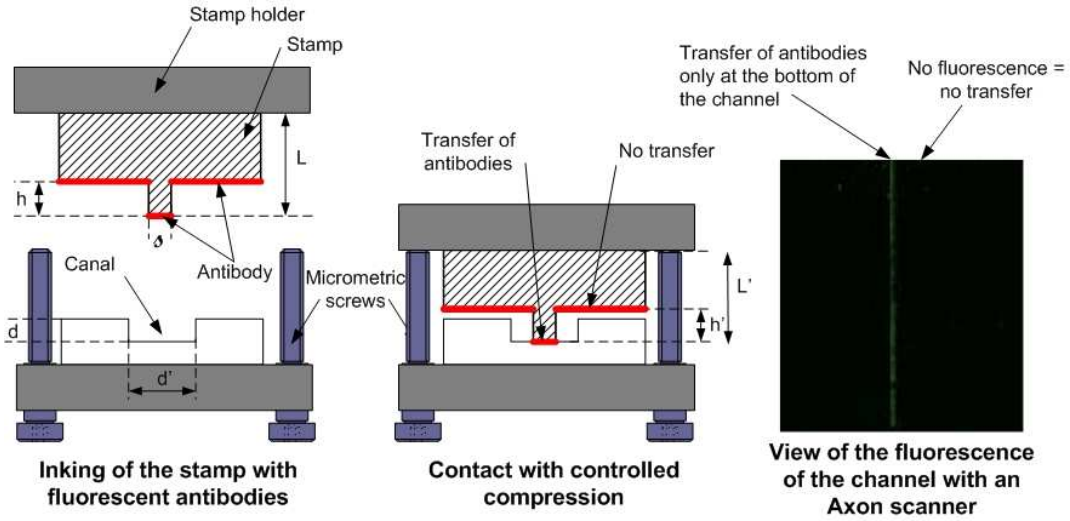

FIG. 5 – Grafting of antibodies inside microfluidic channels by µCP
(d = 20 µm; d'=70 µm; L=1 mm; s'= 30 µm h=25 µm; h'~ 22,5+/-1 µm).

4 Conclusions

In this paper, the design of an advanced instrument for automated µCP is reported. This compact and low cost machine is only made of mechanical part available on the shelves. Its resolution is in the micrometric range and it incorporates many features which should make it useful for chemist and biologist willing to perform specific surface chemistry by µCP. This is illustrated with two applications for the fabrication of microfluidic glass devices and for the integration of biochips in lab-on-chips. It is known also that the stamp deformation during the contact is one of the limiting issue of µCP at very high resolution (Huang et al 2005). We have reported here a new solution to control the load on the stamp with fine regulation. The potential of this solution to reduce stamp deformation at submicrometic resolution is under investigation.

References

Delamarche E. 2005. Microcontact Printing of Proteins. In *Nanobiotechnology*, ed. CMNaCA Mirkin, pp. 31-52. Weinheim: Wiley-Vch Verlag GmbH

Hannes B, Dilosquer G, Bou Chakra E, Bessueille F, Martin J, Cabrera M. 2005. New method for repeatable patterning of substrates for biodevices by automated microcontact printing. *Eurosensors XIX*. 11th-14th September 2005, Barcelona, Spain

Huang YY, Zhou W, Hsia KJ, Menard E, Park JU, et al. 2005. Stamp Collapse in Soft Lithography. *Langmuir* 21:8058-68

Kang H, Lee J, Park J, Lee HH. 2006. An improved method of preparing composite poly(dimethylsiloxane) moulds. *Nanotechnology* 17:197-200

Kumar A, Whitesides GM. 1993. Features of gold having micrometer to centimeter dimensions can be formed through a combination of stamping with an elastomeric stamp and an alkanethiol ``ink" followed by chemical etching. *Applied Physics Letters* 63:2002-4

Schreiber F. 2000. Structure and growth of self-assembling monolayers. *Progress in Surface Science* 65:151-257

Xia Y, Whitesides GM. 1998. Soft Lithography. *Angewandte Chemie International Edition* 37:550-75

Xiao PF, He NY, Liu ZC, He QG, Sun X, Lu ZH. 2002. In situ synthesis of oligonucleotide arrays by using soft lithography. *Nanotechnology*:756-62