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A technique to design complex 3D lab on chip involving multilayered fluidics, embedded thick electrodes, and hard packaging - Application to dielectrophoresis and electroporation of cells

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Abstract. Nowadays, Lab On Chips (LOCs) require the development of new technologies in order to integrate complex fluidics, sensors, actuators... Such integration requires overcoming both technological bottlenecks and an increase in term of production cost. We propose a technique to manufacture reusable and complex LOCs made up of SU-8 resist for the fluidic structure, of glass for the hard packaging and are compatible with the integration of thick electrodes. The method is based on the combination of two bonding technologies, both based on a wafer bonder. The first one consists in the bonding of a thin photosensitive SU-8 dry film, which is similar to lamination. The second one is the standard bonding technique which uses a hard substrate covered by a SU-8 layer. The LOC that can be obtained thanks to the combination of these two methods are transparent, can include 3D microfluidic structures, and thick electrodes. Moreover these LOC are reusable, packaged and ready to use. In order to validate the concept we designed a LOC devoted to cell arraying, using dielectrophoresis, as well as to cell electroporation.

KEY WORDS. Bonding SU-8, wafer bonding, thick electrodes, packaging, microfluidics, dielectrophoresis, electroporation, Lab On a Chip.

1 INTRODUCTION

In 1970s Terry *et al* proposed to miniaturize a gas chromatography device [1]. They integrated a miniature valve, a long spiral column and a thermal conductivity detector on a single device. This work is often considered as pioneering in LOC fabrication. Thanks to the advent of microtechnology in the 1990s, fluidic microsystems became more complex. Manz [2] suggested to call those microsystems: “micro total analysis system” (μ TAS). With the evolution and sophistication of the microsystems, the usual term became “Lab-On-a-Chip” (LOC).

Micro and nanotechnologies enabled the development of miniaturization providing hopes and new ideas in this field. Miniaturization provides a better control, interaction and manipulation. At the same time, the complexity of the technology requires more and more energy, time and money. Since most LOCs are developed for a single-use, due to their fragility and to the difficulty or impossibility to clean them, our objective was to make a robust and reusable LOC.

Most LOC have to integrate complex fluidic networks (large size, with any forms, channels at different levels...). Moreover, a large category has to interact with biological materials that require electric or chemical effects to be handled. Finally, LOC should involve sensing functions (electrical, electrochemical or optical) that should be integrated.

A great number of processes were developed to design LOCs. In 1990s the first processes were inherited directly from the microelectronic technologies. These technologies were predominantly based on silicon (Si). Indeed, it is possible to structure it or to dope it to make respectively channels or electrodes. It is also possible to seal these channels using anodic bonding or eutectic bonding. However, the use of silicon brings up several problems: too large electrical resistivity for high current

applications, complexity and time consuming processes as Bosh-process requiring the use of an ICP-RIE. Glass technology is another alternative currently used for chip elaboration [3] which remains complicated and costly. Finally the use of an organic polymer [4] like PDMS (Polydimethylsiloxane) showed to be quite convenient for the fabrication of microchannels [5], three-dimensional (3D) microfluidic chips [6] and even LOCs [7]. PDMS structures can be obtained by micromoulding technologies, but it remains difficult to make an accurate alignment with another layer or with electrodes.

Surprisingly, an advantageous solution has recently emerged from a specific photoresist dedicated to MEMS devices, named SU-8 [8]. SU-8 is a multifunctional epoxy-based negative resist, interesting for its ability to sustain high aspect ratio and 3D structures [9]. Furthermore, it has an excellent chemical resistance, high transparency, biocompatibility [10-12] and a low bonding temperature (<80 °C). All those advantages make SU-8 a good candidate to elaborate LOCs. Today, several distinct techniques based on SU-8 allow the integration of complex microfluidic networks in a microsystem. A first method to make multilevel fluidic structures is based on the patterning of successive SU-8 layers after UV exposure. To isolate each patterned SU-8 layer from following UV exposures a metallic layer is intercalated [13]. In the same way, a second method consists in playing with the exposure dose in order to get a partial insulation [14]. Other alternative consist in using epoxy resists [15] or other materials [16] like sacrificial layers. However, all those methods have an important default: the development of embedded channels remains difficult or even impossible, as the developer penetrates with difficulty long or curved channels.

To overcome this drawback, an alternative approach was suggested, where channels were sealed once structured. This technique relies on the SU-8/SU-8 bonding either by lamination or by wafer bonding. Lamination process allows a SU-8 dry film to be applied on another SU-8 layer already structured. This technique allows reliable fabrication of multilevel fluidic networks [17]. This process has multiple advantages: it compatible with non flat surfaces and it requires a low force during the bonding that helps in limiting the flow of SU-8 in channels and thus the risk of channel clogging. Unfortunately, this technique does not allow the bonding of a hard substrate, highly recommended for the chip packaging (physical protection for the chip and easiness fluidic connection).

On the other hand wafer bonding is a commonly used process that enables the bonding of hard substrates, but that requires on the other hand an important external force and a very flat surface. Different reports showed the possibility to use this technique to make channels. Some used auxiliary moats to prevent channels clogging [18], but it makes the design of the system complex and it is not compatible with insertion of electrodes. Others suggested to bond after a soft bake to avoid the flow of SU-8, but the bonding remains very sensitive to the surface roughness [19].

The idea developed in this paper is to combine advantageously two techniques: the photosensitive SU-8 dry films bonding technique and the hard glass substrate bonding technique. Furthermore, we present how thick electrodes (>5µm) can be integrated. This type of electrodes could be essential to obtain strong and uniform electric fields [20]. As a proof of concept a cell biochip, devoted to cell arraying prior to electropermeabilization was made using this new technique.

2 MATERIALS

Single sided polished 2 inch (100) Si wafers (250µm thickness) and 2 inch glass wafers (1mm thickness) were used. AZ5214E and AZ developer from Clariant Corp were involved for the liftoff process. We used the commercial SU-8 2100 or SU-8 3050 negative epoxy photoresist for the spin coating [8]. The SU-8 dry film was from Michrochem Corp.: DF 1000-20, as well as. the propylen-glycol-monoether-acetate (PGMEA) used for the developing and the remover PG used for the removing.

3 CONCEPT AND REALIZATION

The fabrication is based on the use of EVG 501, a commercial wafer bonding machine from EVG (St. Florian am Inn, Austria). This machine allows us to squeeze the different substrate with a required force, temperature and vacuum. During the process we use it twice, the first time to level off a layer of SU-8 and the second time to bond two layers of SU-8.

The first step is to build thin electrodes on a glass wafer by lift-off technique. Then, we form a SU-8 mold on these thin electrodes to make the thick electrodes by electrodeposition (copper electroplating from sulfuric acid solution). When the deposition is finished, the release of the mold by a hot solution of remover PG reveals the thick electrodes. After that, a first layer of SU-8 is deposited to structure the channels. This first layer is strongly irregular due to the presence of the thick electrodes, which explains why a wafer bonder has to be used to make this layer uniform. Then, the bonding of a dry film of SU-8 is performed to close the channels. This step can be repeated to manufacture 3D interconnected channels. Finally the LOC is sealed with a glass substrate (drilled and covered of SU-8), which permits the fluidic interconnections after PDMS blocks are stuck on the glass substrate. The details of the process fabrication are shown in figure 1.

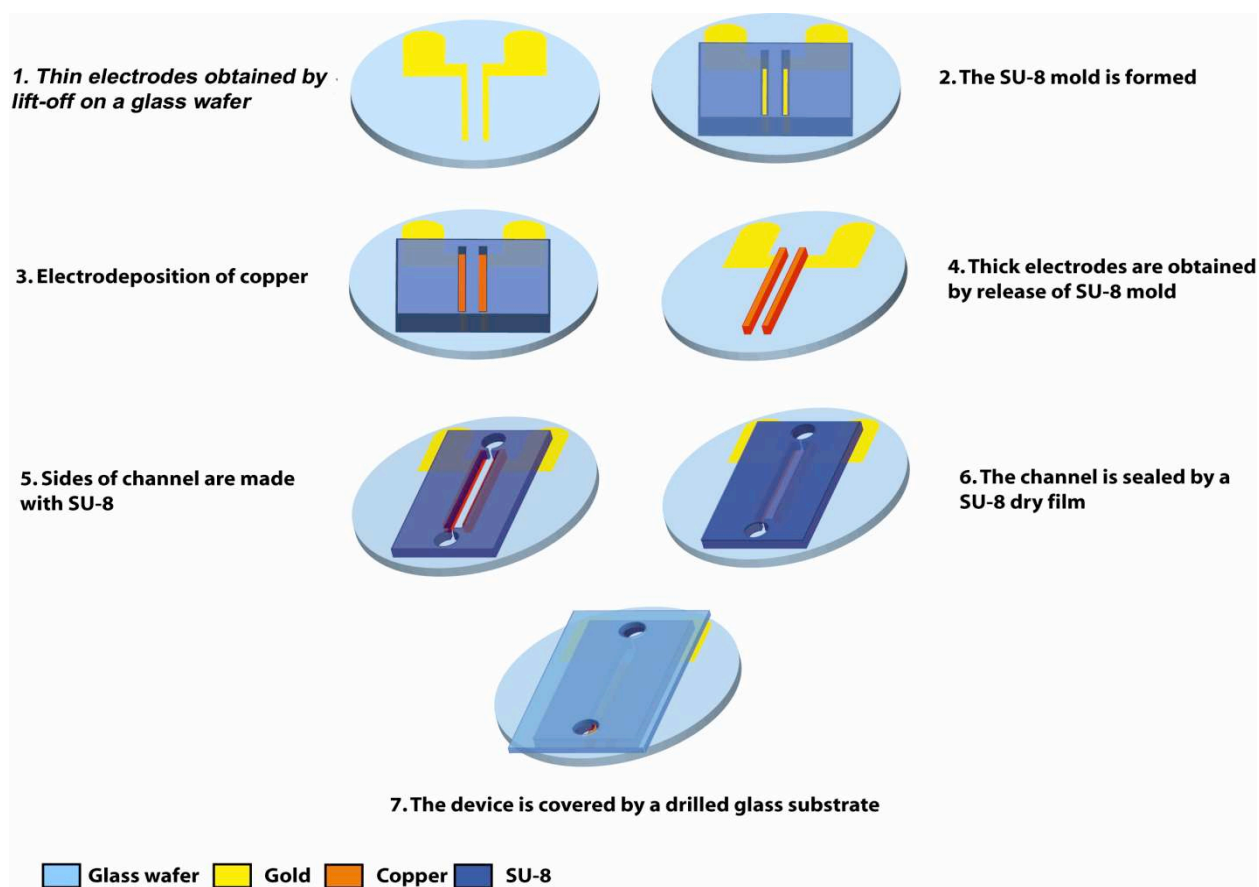


Figure 1. Fabrication process of the fluidic chips.

3.1 Thin electrodes

Thin electrodes are obtained by a liftoff process for the rapidity of the process and in order to avoid metal contamination on non metallic surfaces. The glass wafer is cleaned with a succession of acetone, ethanol and water baths. Then, the reversal photoresist AZ 5214E is spin-coated (1000 rpm during 30 seconds) which gives a layer of 3 μm of thickness. A prebake at 110 $^{\circ}\text{C}$ during 50 seconds is performed to remove the solvent. Then the resist is exposed a first time to UV light with a dose of 2 mJ/cm^2 through an appropriate mask using a conventional EVG 620 lithographic aligner. After that the resist is reversed via a bake at 120 $^{\circ}\text{C}$ during 2 minutes. Next the resist receives a flood exposure of

180 mJ/cm². Finally the resist is developed in a solution of AZ developer mixed with water (1:1) during approximately 90 seconds.

The metal deposition is obtained by a sputtering system from Denton Vacuum. A first layer of 300 nm of titanium is deposited to improve adhesion, then a second layer of 800 nm of gold. The thin electrodes are finally obtained by dipping the chip in acetone solution, thanks to the cap profile of the inverted resist.

3.2 *Thick electrodes*

The objective of our LOC is the generation of an electric field that could interact homogeneously and intensely with the cells. Thereby thick electrodes have to be elaborated. This goal is accomplished in three steps. The first step consists in making the mold for the thick electrodes; the second is the electrodeposition and the third one release of the mold to obtain the desired thick electrodes.

The mold is composed of two layers. The first layer is a thin layer of OmnicoatTM [21] which improves the adhesion of SU-8 on glass and will help to remove the mold afterwards. The second layer made with SU-8 2100 gives the shape of the mold (all parameters used during the fabrication are given in table 1). To ensure that the surface is clean, the sample is exposed to O₂/CF₄ plasma at 200W during 3 minutes. At this level of the process the mold is ready for the electrodeposition.

Electrodeposition of copper was achieved by immersing the sample in an acid copper solution, then by connecting the sample to the cathode and by applying a current of 3.10⁻²A/cm². With this current intensity, approximately 0,8 μm of copper are electroplated per minute. Usually, plating was restricted to 10 or 15μm but it is possible to deposit a much thicker layer (~50μm). To release the mold, the sample is plunged in a hot solution of remover PG (~80°C). Ultrasonic cleaning can be necessary to remove all the SU-8. Finally the microsystem has both thin electrodes and thick electrodes.

3.3 *Buried electrodes and first layer channel*

To obtain a flat layer, in spite of the strong structuration caused by the presence of thick electrodes, we developed a new technique to smooth the surface. This technique, that removes the large roughness (see figure 3.a), consists in a spin of SU-8 3050 and in its squeezing in a commercial wafer bonder (EVG 501).

The process begins with a dehydrating treatment on a hot plate at 200°C during approximately 30 min. This step is very important as SU-8 is hydrophobic. Also a thin layer of water in the wafer would increase the stress and consequently decreases the adhesion. After that the SU-8 is deposited and baked to remove the solvent (see table 1). At this time the surface level is varying largely. In order to remove these level variations, the sample is squeezed in the wafer bonder with a force of 700 N at 70 °C. To protect the wafer bonder from a SU-8 flow a PET film is put between the sample and the piston (this film is removed after the squeezing). Finally the sample is exposed to UV (in order to structure the channels) baked and developed. After this step, the microsystem is constituted of the thick electrodes and of the side of the channels. One can note that this technique can be used to limit the edge effects after a spin coating.

3.4 *SU-8 dry film bonding*

In order to close the channels, a technique close to the one developed by Abgrall et al [17] is applied. Its principle is based on the bonding of a SU-8 dry film on a structured SU-8 layer. The SU-8 dry film (20 μm) is sandwiched between two PET protective films. To use it, one PET layer is peeled off to enable the contact between the SU-8 and the SU-8 layer of the device. Then, oxygen plasma is applied on the sample to improve adhesion between SU-8 layers (200 W, 3 minutes). The dry film

(SU-8+one PET layer) is then pressed on the sample thanks to the wafer bonder with a force between 200 N and 300N at 65 °C. After that, the chip is perfectly covered by the SU-8 dry film. To finalize the bonding and to structure the inlets and the outlets, we expose the SU-8 dry film to UV light and we bake it. Until the step of baking it is important to maintain the second PET protective layer because if it was removed before, the SU-8 would not enough stuck to the surface and it could tear up. After baking, the second PET film is peeled off to enable the development of the SU-8 layer added (see table 1). To achieve a channels multilevel network this process has to be repeated as many times as necessary.

3.5 Hard glass substrate bonding

The hard glass substrate bonding is necessary in order to strength the packaging and to create the external fluidic connections (SU8 dry film is too thin and fragile). The glass substrate is drilled for the inlet and outlet, dehydrated and a layer of SU8-3050 is spun on it with the same parameters as those previously used (see table 1). During this step, the fact that SU-8 is a very viscous resist and the holes are very small (1mm diameter) enable to cover well the glass substrate (without problem of uniformity, except at the edges where homogeneity can be simply imposed by using the wafer bonder, see paragraph 3.3). After spinning and baking, we simply remove the SU-8 covering the holes with a drill. At the end, a glass with holes covered by a uniform layer of SU-8 is obtained. An O₂ plasma is performed on the microsystem (200 W, 3 minutes), in order to improve the adhesion between it and the SU-8 layer. Then, the hard glass substrate with SU-8 and the microsystem are put in contact and squeezed together thanks to a wafer bonder (650 N to 750 N at 65 °C). Due to the fact that the substrate is hard, the force necessary is higher than for a SU-8 dry film. Finally, the whole set-up is exposed to UV light and baked to cross-link the last layer.

Table 1st. Omnicoat, SU-8 and SU-8 dry film process parameters used in this study.

Resist	Omnicoat	SU-8 2100	SU-8 3050	SU-8 dry film
1 spin Acceleration (rpm s ⁻¹)	300	300	300	
1 spin Rotation Speed (rpm)	500	500	500	
Time (s)	10	30	30	
2 nd spin Acceleration (rpm s ⁻¹)	300	300	300	
2 nd spin Rotation Speed (rpm)	1000	5000	3000	
Time (s)	60	60	60	
Soft bake (min)	1@200°C	3@65°C, 13@95°C	1@65°C, 10@95°C	
Exposure (mJ/cm ²)		150	200	180
Post-bake (min)		2@65°C, 10@95°C	1@65°C, 8@95°C	2@65°C, 8@95°C
Development in PGMEA (min)		8	5	5
Thickness (μm)	~0,02	~80	~50	20

3.6 Electrical and fluidic connections

To transform the microsystem in a LOC, electric wires are simply welded to the electrodes. For the fluidic connection, PDMS blocks are prepared and drilled to accept the insertion of micro-capillaries. Then the microsystem and the PDMS blocks are exposed to oxygen plasma (30 W, 30 s). The PDMS block and the microsystem are assembled to achieve the bonding. The LOC is now ready to be connected with electric generator, syringes pump...

4 RESULTS AND DISCUSSION

To demonstrate the efficiency of our process two devices were manufactured: the first was a multilevel microfluidic system, and the second a bio-LOC with thick electrodes.

4.1 Fabrication of the demonstrators

4.1.1 Fabrication process of a multilevel microfluidic system. This device shows the possibility to use SU-8 dry films and wafer bonding to make multilevel microchannels networks. In this process, five SU-8 dry films were used. The first layer enabled to define the shape of the first channel with correspond to the letters “ μ _AS”. The second and the third layers were bonded to seal the first channel and to consolidate it. The fourth layer structured the second channel with the shape of a “T”. Finally the last layer sealed the whole. A drilled glass substrate was bonded to connect it with outside. The figure 2 displays the different fabrication steps. As shown in figure 2.c, glass substrate does not totally cover the device. This is not really a problem because channels were already sealed with SU-8 dry film. A bonding was considered good enough if the inlet and outlet were correctly bonded, which was mostly the case. Lastly, during the sealing of the channel with SU-8 dry film, it could happen that uncross-linked SU-8 flowed at the beginning of the channel. To remove this uncross-linked SU-8, the sample was put in a low power ultrasonic generator during 5 seconds. Normally, if the protocol is followed there is no problem of leakage.

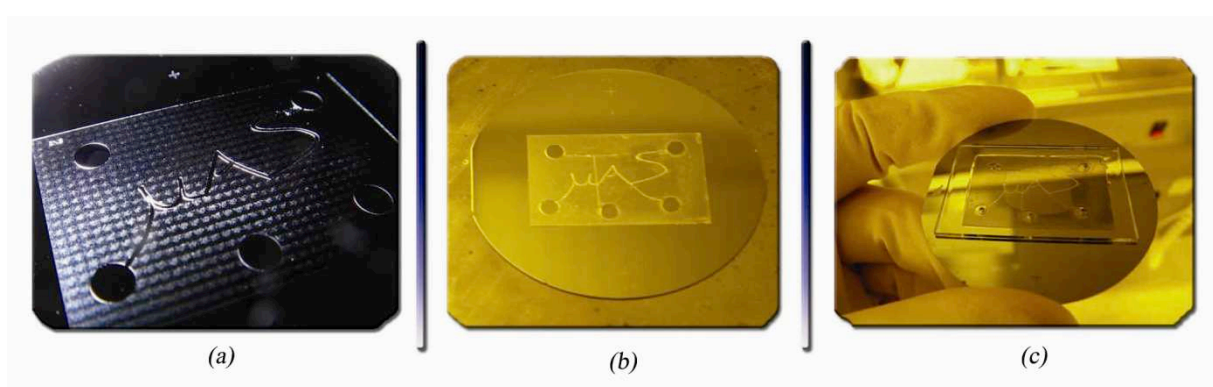


Figure 2. Fabrication steps of the multilevel fluidic system :

- (a) First microfluidic level with the channel “ μ AS”.
- (b) Second level with the channel ‘T’.
- (c) Third bonding of a glass substrate.

4.1.2 *bio-LOC with thick electrodes*. This second demonstrator of our technology targeted a biological application of LOC, namely a biochip devoted to cell placement at precise locations and to cell treatment by means of electric fields. This LOC was constituted of channels, thick electrodes and dielectric structures to focus the electric fields. A bottleneck that could be overcome thanks to the technique presented in this paper was the capability to get thick electrodes buried in thick SU8 (channel), while keeping the capability to seal the whole system with a glass substrate. Indeed, a smooth SU-8 surface (see figure 3.b, 4.a and 4.b) was obtained, allowing a compatibility with further packaging steps (sealing with a SU-8 dry film and a glass substrate). A cross section of the complete device is shown in figure 5.a and an overview in figure 5.b.

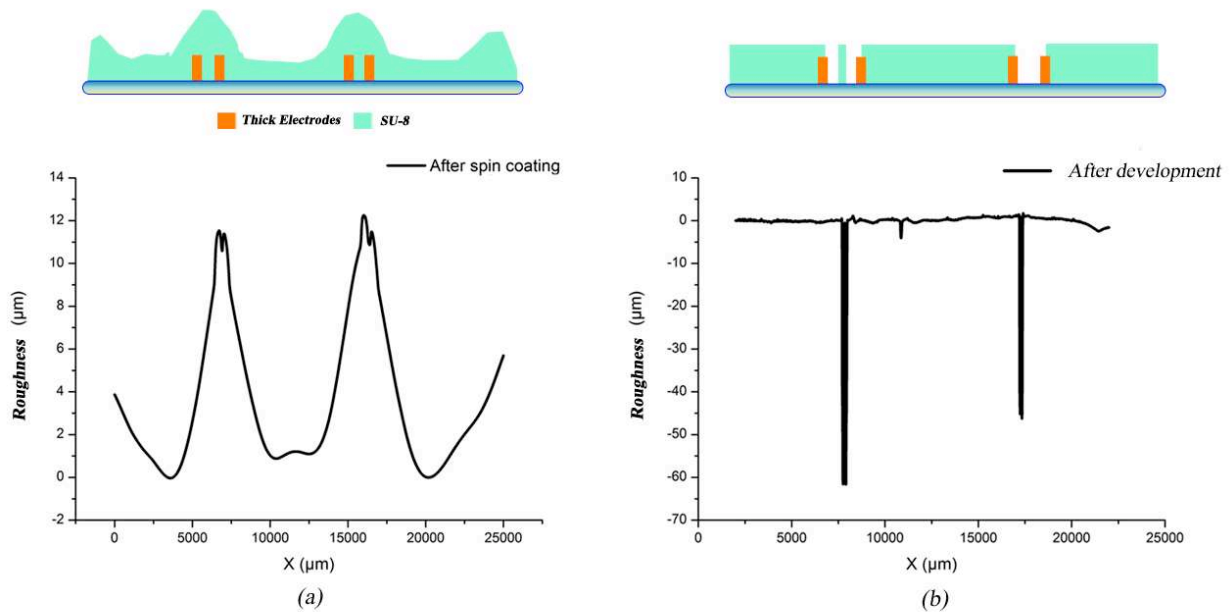


Figure 3. Mechanical profilometer measures of the SU-8 surface just after spinning (a) and at the end of the process (insulation, post-bake and development) (b). At the top, schematic views of the different phenomena.

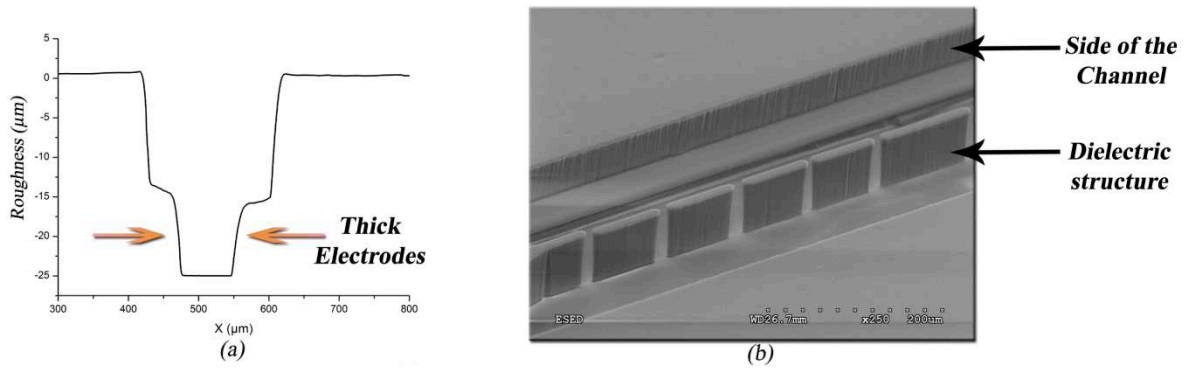


Figure 4. Integration of thick electrodes and dielectrics structures in the channel:
 (a) Mechanical profilometer measures of a channel.
 (b) SEM image of a channel within its center dielectrics structures (SU-8).

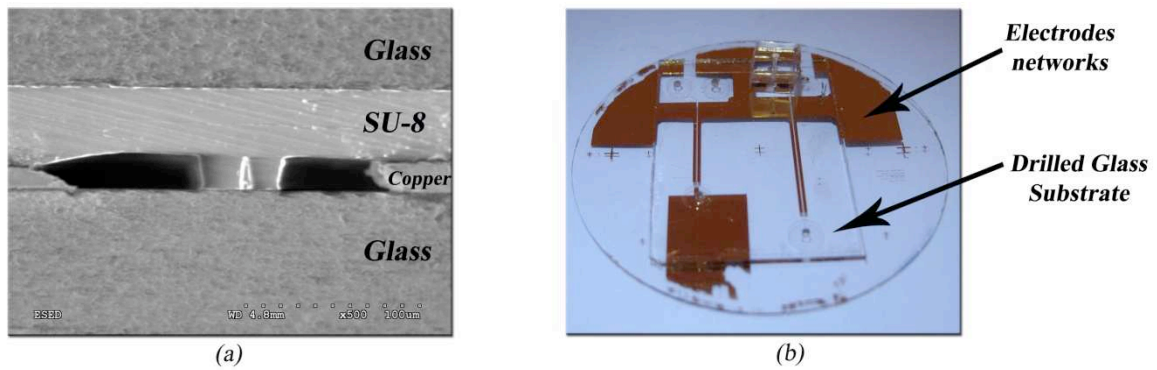


Figure 5. Images of the complete device:
 (a) SEM image of a cross section.
 (b) Overview of the sample.

4.2 Hydraulic and biologic tests on the demonstrators

4.2.1 *Hydraulic tests on the multilevel microfluidic system.* Our demonstrator device has two levels: a first level where the channel forms the letters ‘ μ AS’, and a second level with the letter T. Two fluorescent molecules (DCM and Eosine) were injected respectively in these two levels in order to test the functionality of the microfluidic chip. Flowing of the two markers, without any cross-contamination, was clearly demonstrated by fluorescence observations (figure 6.a, 6.b). These tests validated our technology from a hydraulic point of view.

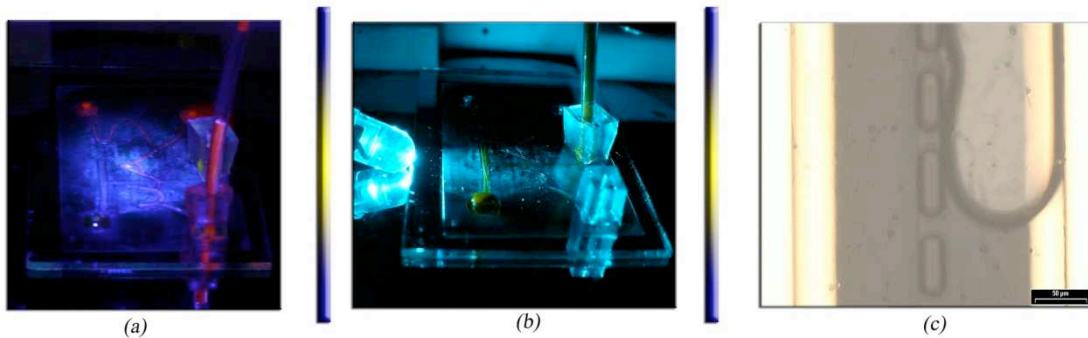


Figure 6. Hydraulic tests:
 (a) Injection of DCM marker (fluorescence emission 620nm) in the channel “ μ AS”.
 (b) Injection of Eosine marker (fluorescence emission at 555nm) in the channel “T”.
 (c) A bubble in the channel of the bio-LOC filled of water.

4.2.2 *Biological tests on the bio-LOC.* The LOC was designed in order to optimize the electric field needed to establish a convenient interaction with the cells. Thick electrodes and dielectric structures were integrated into the device. The bio-LOC had two purposes: cell placement thanks to dielectrophoresis and cell electropermeabilization.

A non-uniform alternating electric field can be used to handle a neutral particle, like a cell, in a liquid. This phenomenon is the consequence of the dielectrophoresis force [22, 23]. Depending of the frequency the particle will be move toward maxima of electric field (positive DEP) or toward minima of electric field (negative DEP). If the cell is approximated to a spherical dielectric with losses, the average dielectrophoresis force is expressed:

$$\langle F_{DEP}(t) \rangle = 2\pi\epsilon_m r^3 \text{Re}[K(w)].\nabla E_{eff}^2$$

Where r is the radius of the cell, E symbolizes the electric field and K is called the criterion of Clausius-Mossotti which is:

$$K(w) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*}$$

ϵ_p^* is the complex permittivity of the particle and ϵ_m^* is the complex permittivity of the surrounding liquid. The orientation of the force depends on the sign of the criterion of Clausius-Mossotti which is linked to the frequency. For our experiments either a high frequency (>100 kHz) which forced the cells to move toward the maxima of the field (figure 7.b), or inversely a low frequency (<10 kHz) to force the cells to move toward the minima of field (figure 7.b) were applied. The consequences of the application of a high and a low frequency signals on the B16 cells are shown in figure 7.

The second objective of the bio-LOC was to electropermeabilize the membrane of the cells which had been previously arrayed thanks to DEP. This point was checked using a fluorescent dye (calcein) introduced inside the cell before the experiment. This molecule can only be externalized from the cell, if the cell membrane is permeabilized. Figure 8 shows that after the application of cell permeabilizing electric pulses, the cells fluorescence decreases due to the leakage of calcein, demonstrating the proper functioning of our bio-LOC. During our tests, the importance of a good cleaning after the end of the experiment to preserve the LOC from obstruction was noticed. Indeed, during biologic experiments a lot of cellular debris could clog the channels. To eliminate existing or potential clogging, the microfluidic system was cleaned with bleach, isopropanol and deionized water. This cleaning with corrosive products allowed us to reuse the microsystem until very big dusts definitively clogged the microchannels.

5 CONCLUSIONS

A new, simple and efficient technique which enables the microfabrication of complex 3D microfluidic structures, involving multilevel fluidics, thin or thick electrodes, and hard packaging with glass is proposed in this paper. In order to prove the feasibility of the method, two demonstrators have been elaborated: a 3D multilevel fluidic demonstrator which showed the functionality of flowing fluids at each level without leakages, and a bio-LOC capable to handle and treat cells thanks to electrical field applied between thick electrodes embedded in the channel.

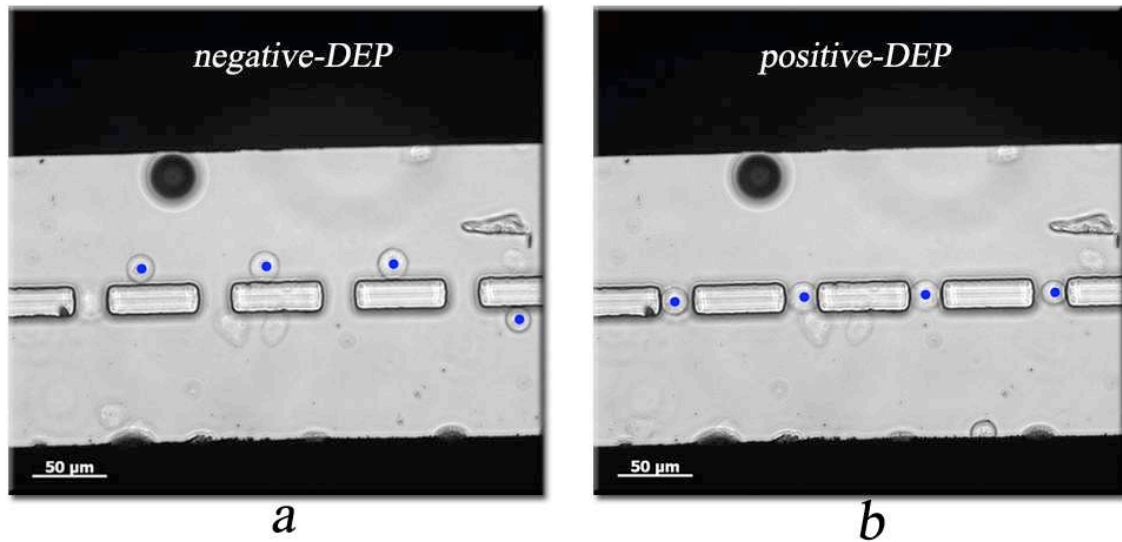


Figure 7. Dielectrophoresis experiment on B16 cells (we put blue points on cells to make easier their visibility):

- (a) Negative dielectrophoresis on 4 cells (10 kHz, 10Vpp).
- (b) Positive dielectrophoresis on the 4 same cells (1 MHz, 10Vpp).

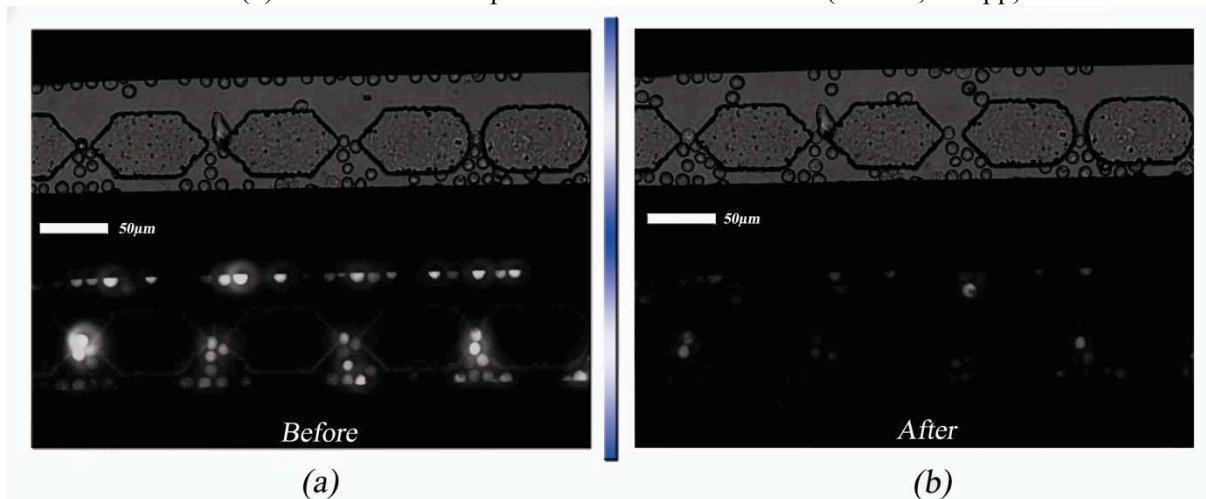


Figure 8. Electroporation experiment on DC3F cells
 (a) Just before the electroporation (high: image in visible, low: fluorescence image).
 (b) 5 minutes after two pulses (25 V, 80 μ s), the fluorescence has drastically decrease.

6 ACKNOWLEDGEMENTS

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