physica status solidi a

phys. stat. sol. (a) **203**, No. 6, 1476–1480 (2006) / **DOI** 10.1002/pssa.200566111

All-polymer microfluidic particle size sorter for biomedical applications

Javier G. Fernandez*, **Christopher A. Mills, Romen Rodríguez, Gabriel Gomila,** and **Josep Samitier**

Laboratory of Nanobioengineering (CREBEC), Barcelona Science Park, Joseph Samitier 1–5, 08028 Barcelona, Spain

Received 20 September 2005, revised 22 February 2006, accepted 22 February 2006
Published online 20 April 2006

PACS 47.55.Kf, 47.56.+r, 85.40.Hp, 87.83.+a

The design and method for the production of an all-polymer microfluidic particle sorter, for use in biomedical applications, is described. The sorter is made from biocompatible materials with properties, such as high optical transparency, that make it useful in a biological laboratory. The method of sorting is designed to be gentle on biological species, using a method of guiding the particles towards the filter, and has been successfully used to separate latex beads depending on their diameters. Preliminary qualitative experiments have been able to separate beads of 45 and 90 μm in diameter from a mixture of the two. These dimensions are on the same scale as those of some eukaryotic cells.

1 Introduction

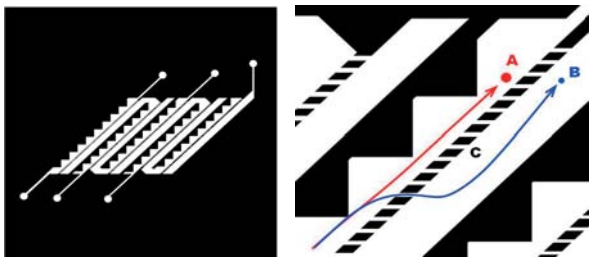
Nowadays microfluidics are an important part of biomedical applications and are used to control and manipulate a broad range of fluids, such as blood samples, bacterial cell suspensions, and protein or antibody solutions. This versatility has promoted a rapid development of different microfluidic devices and their use in a range of techniques [1–6]. They are also used to obtain a variety of measurements [7–9], many of which are already being used for clinical diagnostics [10]. Here, we present the production of a particle sorting device based on a non-aggressive method for sorting micrometric particles in suspension (such as microparticles or cells) according to their size. The main advantages of this design are device scalability, high sorting rates at high concentrations and minimal particle interaction. The last point is particularly important when compared to techniques based on electric fields or laser diffraction. Such interactions can cause large changes in sensitive biological species [11].

2 Methods

The device fabrication method used here is based on a combination of UV lithography and poly(dimethylsiloxane) (PDMS) soft lithography technologies [12]. The UV lithography technique is employed for the construction of a negative mould for PDMS replication. This technique provides a low-cost method for the construction of tall microstructures for MEMS applications [13].

The process for the production of the primary SU-8 mould can be divided into four main tasks: photoresist deposition, selective crosslinking via UV exposure, photoresist development and silanisation.

* Corresponding author: e-mail: jgfernandez@pcb.ub.es



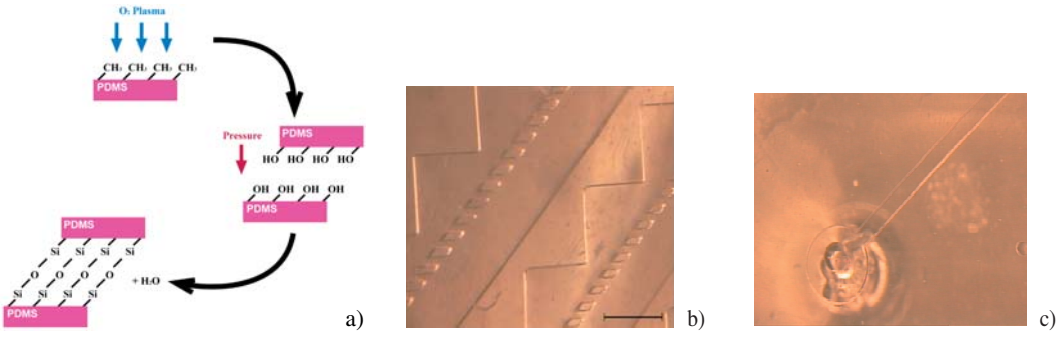
a) b)

Fig. 1 (online colour at: www.pss-a.com) a) General overview of the sorter design and also the basis for the UV lithography mask. The hole at the bottom left hand corner is the sample input; the other five are outputs of the different sized particles. b) Diagram of the operation one of the filters. The diameters (∅) of the particles, A and B, and the distance (d) between filter posts C have the relation ∅(A) > d(C) > ∅(B). Particle B traverses the filter to the secondary cavity and continues on to smaller filters. Particle A is forced to continue to the corresponding output for its size.

SU-8 50 photoresist (Microchem, USA) was spun down onto a 5 × 5 cm^2 glass support at 1200 rpm for 40 seconds to produce a 100 µm thick photoresist layer. A two-step soft bake, at 65 °C for 6 minutes and then at 95 °C for 25 minutes, is completed to prepare the photoresist for the exposure step.

The hardened resist is covered with a photomask prepared by printing the required design onto an acetate sheet using a high resolution printer (3700 dots per inch) (Fig. 1a). The mask consists of a negative copy of the required design for the final PDMS device. For SU-8, a negative tone resist, the polymer under the transparent areas of the mask is retained during development. The resist is illuminated using a UV lamp (1 kW, 453 nm) for 8 seconds. The illuminated areas undergo a cross-linking process (Fig. 2a) becoming impervious to the development.

A further two-step hard bake is completed, prior to the development of the resist, at 65 °C for 1 minute and then at 95 °C for 25 minutes respectively. After this baking it is sometimes possible to faintly observe the shape of the device in the photoresist due to the different compositions of the illuminated and non-illuminated areas. Resist development is realised by employing SU-8 Developer (Microchem, USA). The mould is immersed in a petri dish full of developer with the resist uppermost. To ensure complete resist development in the most critical areas of the mould (i.e. the channels between the posts) the solution is sonicated. This drastically accelerates the developing process, and the development time is limited to 3 minutes to completely remove the non-cross-linked resist without compromising the mould structures.



a) b) c)

Fig. 2 (online colour at: www.pss-a.com) a) Schematic diagram of the PDMS to PDMS bonding technique (see text for details). Also shown are optical microscopy images of b) a filter structure within the final bonded device [bar = 1.5 mm] and c) a fluidic connection point between the PDMS device and the PEN tubing.

To ensure that the development process is complete, the master is periodically (every minute) removed from the developer and gently rinsed with propan-2-ol. This reacts with non-cross-linked resist producing a white precipitate. If this occurs, the mould is replaced for another minute. When the precipitate fails to appear we can be certain that the process is complete. Note that excess time in the developer can cause the required structures to be removed; therefore the mould should be checked regularly using the propan-2-ol. After drying, the mould is coated with an anti-adhesion, flourosilane monolayer using a previously described technique [14].

Sylgard 184 silicone elastometer (Dow Corning Co., USA) was used for replication of the SU-8 mould. The elastomer is provided in two parts; a polymer base and a cross-linking agent, which, as the two components are room temperature curable, are mixed just prior to use. A 10 : 1 weight ratio of base to cross-linking agent is used, and the mixture is mixed for ~5 minutes to ensure that the components are well distributed. Unfortunately, this mixing produces bubbles in the PDMS mixture that must be removed to retain the polymers transparency and structural integrity. The mixture is placed in a dessicator at low pressure for ~20 minutes which causes the gas to be removed from the mixture.

The master is then placed in a petri dish, which is resistant to high temperatures, with the resist structures face up. The recipient is filled with liquid PDMS completely covering the master to a depth of ~5 mm. To quickly cure the PDMS it is introduced into an oven at 80 °C for 1.5 hours. After the curing process is complete the PDMS is rigid and can be separated from the mould using a scalpel.

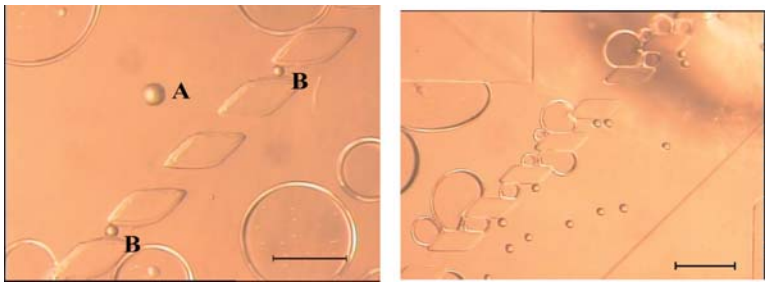
The PDMS replica is sealed using a second, flat piece of PDMS. Both are exposed to oxygen plasma for 10–15 seconds. The action of the plasma can cause 4 processes to occur; cleaning, ablation, cross-linking and chemical modification. The cleaning causes any organic surface contamination to be removed from the polymer, preventing its interference in the adhesion processes. Chemical modification involves the production of new surface functional groups. When the two pieces of polymer are pressed together, these new moieties interact with each other, at points of conformal contact, to produce a direct chemical bond across the interface, effectively turning the two sheets of polymer into one.

In this case, where PDMS is employed, plasma immersion replaces surface methyl groups ($-CH_3$) with hydroxyl groups (–OH). When two hydroxyl groups come into contact they condense and each pair of opposing hydroxyls condense to yield Si–O–Si bonds with the elimination of a water molecule (Fig. 2a). This gives rise to a very strong bond, that when complete allows high pressures to be utilised in the system. The immersion of the (initially hydrophobic) PDMS in the oxygen plasma also makes it temporarily hydrophilic, which assists in the introduction of water-based solutions into the device.

Fluidic connection to the PDMS device is made using 600 μm diameter PEN tubing. To seal these connections, a semi-cured 10 : 1 ratio mixture of PDMS is employed. PDMS in this state has the advantage that it will produce a permanent seal (after curing) without blocking by the entrance to the PDMS channel (Fig. 2c). As high transition temperature polymers are used throughout the device (including the connections), the whole device is suitable for autoclaving. Liquid introduction and flow management is achieved using syringes controlled by microinjectors.

3 Results and discussion

We have presented a device for the sorting of micrometric particles based on a novel design and employing rapid, inexpensive fabrication techniques. The elastomeric PDMS is used because it has properties – inexpensive, flexible, optical transparency, biological compatibility – which make it a suitable support material for miniaturized biomedical apparatus. Our device is based on a series of micro-gap filters in a serpentine fluidic system. The major advantages of this design come from its simple operating principle. As the main solution flux is in the direction of the posts, the particles travel parallel to them. Due to the dimensions of the device, the liquid is in a laminar flux and therefore needs be deflected in order to interact with the posts. Deflection produced by the corrugated wall forces the particles towards the posts. If their size allows the particles to pass through the filter, they pass into the secondary chamber and continue towards the next filter channel, where the distance between posts becomes smaller. The process is repeated until the particle can not continue and is driven to the corresponding output (Fig. 1b).



a) b)

Fig. 3 (online colour at: www.pss-a.com) Optical microscopy images of a filter in action: a) shows a 90 μm diameter particle, A, proceeding to the output and two 45 μm diameter particles, B, traversing the filter [bar = 300 μm]; b) shows multiple 45 μm diameter particles traversing the filter and proceeding, through the secondary chamber, to the next (smaller) filter [bar = 300 μm].

The main flux, running parallel to the posts, also ensures that large particles do not block the device, but also increases the possibility of small particles continuing without being filtered. However, this probability is reduced by the high number of filter positions (50 size selectors per channel). At the present time, we have attempted to realise particle sorters with 12, 20, 40, 70 and 85 μm and with 60, 80, 100, 120 and 140 μm separation ranges. Successful fabrication results have been obtained for all filter structures with separations between 40 and 100 μm, and we have, as a preliminary experiment, successfully separated 45 μm and 90 μm diameter latex beads from a mixed solution (Fig. 3). However, outside of this range we are unable to filter particles due to the aspect ratios achievable with the SU-8 resist. As the resist is optimised to produce structures ~50 μm tall, problems are encountered, causing the channels to collapse when the aspect ratio of the structures increases above ~2:1.

4 Conclusion

The design and fabrication methods presented here allows for a fast and low cost fabrication (compared to silicon micromachining for example) of a device for the separation of particles depending on their diameter. The biological compatibility of the device means it will be useful for differentiation of the different kinds of cells in a suspension. For example, upon reducing the filter dimensions (to between 5–20 μm), its application to blood analysis could allow the separation of red blood cells depending on mean corpuscular volume (MCV), an indice that is commonly used in medical diagnostics and employed for the classification of anemias [15]. Similarly, the calculation of red blood cell distribution width (RDW) permits the detection of the significant variation in cell size that certain disorders cause. Recent studies [16] point to the reduction of neuronal cell size as an indicator of a possible cause of schizophrenia or bipolar disorder (BPD). The scalability of the design, the improvement of the characteristics of the filter and the modification of the negative master using Focused Ion Beam (FIB) lithography suggests that it may be possible to reach nanometric dimensions with the filter, opening the door to a number of new applications.

Acknowledgements The authors would like to thank the Spanish Ministry of Science and Education for its support for this work via the FPU (JGF) and Ramon y Cajal (CM) grant programs.

References

[1] A. E. Kamholz et al., Anal. Chem. **71**, 5340–5347 (1999).
[2] D. Figeys et al., Anal. Chem. **70**, 3728–3734 (1998).
[3] P. Belgrader et al., Biosens. Bioelectron. **14**, 849–852 (2000).

[4] B. Buchholz et al., Anal. Chem. **73**, 157–164 (2001).
[5] I. K. Glasgow et al., IEEE Trans. Biomed. Eng. **48**, 570–578 (2001).
[6] S. K. W. Dertinger et al., Anal. Chem. **73**, 1240–1246 (2001).
[7] A. E. Kamholz et al., Anal. Chem. **71**, 5340–5347 (1999).
[8] B. H. Weigl and P. Yager, Sens. Actuators B, Chem. **39**, 452–457 (1997).
[9] A. G. Hadd et al., Anal. Chem. **69**, 3407–3412 (1997).
[10] B. H. Weigl and P. Yager, Science **283**, 346–347 (1999).
[11] N. Ben-Dov et al., Biochim. Biophys. Acta – Mol. Cell. Res. **1448**, 372–380 (1999).
[12] Y. Xia and G. M. Whitesides, Angew. Chem., Int. Ed. **37**, 550–575 (1998).
[13] A. S. Holmes and S. M. Saidam, J. Microelectromech. Syst. **7**, 416–422 (1998).
[14] F. Bessueille et al., Biosens. Bioelectron. **21**, 908–916 (2005).
[15] T. A. Cavaliere, Newborn Infant Nursing Rev. **4**, 231–239 (2004).
[16] D. Cotter et al., Cereb. Cortex. **12**, 386–394 (2002).

