AIMS Biophysics, 3 (4): 553-562.
DOI: 10.3934/biophy.2016.4.553
Received: 23 September 2016
Accepted: 16 November 2016
Published: 22 November 2016

http://www.aimspress.com/journal/biophysics

Research article

Double casting prototyping with a thermal aging step for fabrication of 3D microstructures in poly(dimethylsiloxane)

Karina Kwapiszewska [1,*], Kamil Żukowski [2], Radosław Kwapiszewski [2] and Zbigniew Brzózka [2]

[1] Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland
[2] Institute of Biotechnology, Faculty of Chemistry, Warsaw University of Technology, Warsaw, Poland

* **Correspondence:** karina.kwapiszewska@gmail.com.

Abstract: The paper describes a cheap and accessible technique of a poly(dimethylsiloxane) (PDMS) master treatment by thermal aging as a step of double casting microfabrication process. Three-dimensional PDMS microstructures could have been achieved using this technique. It was proved, that thermal aging changes nanotopology of a PDMS surface and thus enhances efficiency of double casting prototyping. The thermally aged PDMS master could have been used for multiple and correct replication of over 98% of the fabricated microstructures. Moreover, lack of chemical modification preserved the biocompatibility of PDMS devices. The fabricated microstructures were successfully utilized for 3D cell culture.

Keywords: PDMS; microfabrication; 3D microstructures; biocompatible materials; double casting; thermal aging

1. Introduction

Poly(dimethylsiloxane) (PDMS) is one of the most commonly used materials for fabrication of lab-on-a-chip devices as well as Micro-Electro-Mechanical Systems dedicated to life sciences and biomedical applications [1,2,3]. Due to its mechanical properties, simple fabrication process and design flexibility, PDMS has become a popular polymer for applications ranging from surface micropatterning to the casting of 2D and 3D geometries from stiff master molds with a submicron resolution [4,5]. Using PDMS as a substrate for fabrication of microdevices for laboratory purposes is a relatively cheap solution (approximately fifty times cheaper than using silicon) [6]. Moreover,

PDMS is biocompatible and gas permeable, which renders that it is an attractive material for cell engineering applications [7–13].

There are a number of methods of microfabrication in PDMS, mostly based on the replica molding technique [14]. The step determining cost and time of prototyping of a PDMS microdevice is fabrication of a master, for which photolithography-based techniques are most commonly used [1]. However, there are applications that require more complex techniques of master micromachining. For example, fabrication of 3D masters using photolithography is a toilsome task, as it requires sequencing coating, precise alignment and complicated processing under clean-room conditions [15,16,17], and it cannot provide curved surfaces [13]. Demolding of such masters can also cause their damage. To extend durability of photolithography-made masters a protective coverage is applied [18]. Other, non-photolithographic, microfabrication techniques may be improper for fabrication of a convex master for certain applications due to technical or economic reasons. For example, micromilling is fast and accurate, but micromachining of some types of structures (i.e. sharp corners) requires application of small and fragile tools, which increases time and cost dramatically. For those applications, double casting of PDMS is applied, where first replica is used as a master for second replication [19,20]. However, this is troublesome, because native PDMS layers strongly adhere to each other. Presence of uncured low molecular weight (LMW) chains inside a PDMS mold [21] causes partial cross-linking between a master and a replica. Although chemical modification of a surface of a PDMS master is a widely used solution for this problem [19,20,22,23,24] it has some weak points. Mostly, PDMS surface modification requires homogenous coverage with a hydrophilic material [25]. Therefore, thickness of a cover layer should be set precisely, as it can influence dimensions of a microstructure [19]. The thickness and homogeneity of a coverage should be considered in the context of replication of micrometer-size structures. On the other hand, molecules of the hydrophilic layer can be transferred to the cast surface and remain in a microdevice. It is particularly unwelcome for those applications where the highest purity of the device is required. These reasons motivate efforts to develop other, coverage-free PDMS master treatment methods.

In this paper, we present our non-chemical PDMS master surface modification for double casting. On the contrary to chemical modifications, we proposed an application of physical treatment of a surface by thermal aging, also known as an extended curing process [21,26]. Thermally aged PDMS has been already presented as a potential substrate for cell culture in a microfluidic device, due to reduced hydrophobic recovery after oxygen plasma treatment [21]. During thermal aging LMW chains remaining in the bulk are gradually cross-linked. Thus, we expected that cross-linking between a thermally aged master and a molded polymer would be limited. This hypothesis was confirmed by our experimental results. We proved better demolding of a PDMS cast on of the PDMS master. On the contrary to the previously published works [21,26,27], we performed low-temperature aging preventing master deformation [27] and applied relatively short aging time [21]. The novel technique was successfully applied and briefly reported by our team in [12,13]. Here, we present a broader study aiming in explanation and optimization of the process. Changes of nanometer-scale PDMS surface topology were detected, and reduction of hydrophobicity of the PDMS surface was observed during the thermal aging process. PDMS masters of different rigidity were used and optimal parameters for PDMS double casting prototyping were set. Therefore at least ten subsequent replications of the thermally aged PDMS master could have been performed. Hydrophobic PDMS microstructures fabricated using the developed technique were applied for a microfluidic device for formation and long-term culture of human tumor spheroids. Presented data confirmed usability and

biocompatibility of microstructures fabricated using proposed technique. To our knowledge, we are the first team applying thermal modification of a PDMS master for double replication.

2. Materials and Methods

2.1. Fabrication

The fabrication method is presented schematically in Figure 1a. 3D microstructure was fabricated in poly(methyl methacrylate) (PMMA) by micromilling using CNC micromilling machine (Minitech Machinery Co.). Poly(dimethylsiloxane) (Dow Corning, Sylgard) was prepared by mixing of pre-polymer with curing agent in a proper weight ratio (see Section 3). Liquid mixture, after degassing, was poured over a PMMA master and left for curing in 70 °C for 3 h (replica molding step). Then, the cured cast was peeled off and the convex master was obtained. Thicknesses of the casts were 4–6 mm. The PDMS master was exposed to thermal aging in a laboratory dryer in 100 °C. Different times of thermal aging were applied (range between 16 and 72 h). Next, replica molding was performed and a concave replica of a PMMA structure was obtained in PDMS. Two PDMS microstructures were bonded using oxygen plasma treatment to form a microfluidic system.

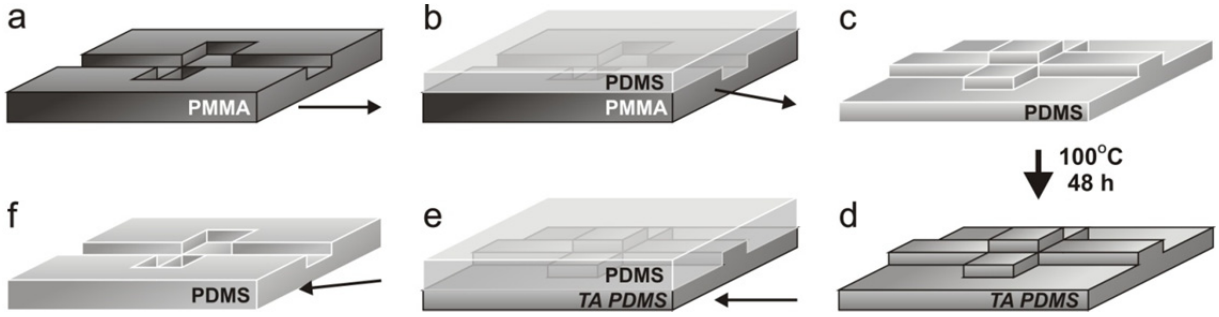

Figure 1. Scheme of the fabrication method: (a) concave initial microstructure of PMMA, (b) first replica molding, (c) convex replica of PDMS, (d) convex PDMS master modified by thermal aging (TA PDMS), (e) second replica molding, (f) concave replica of PDMS.

2.2. Measurements

Wetting properties of a thermally aged PDMS surface were determined by water drop contact angle measurements [28] using Olympus MIC-D microscope and Cell-F image analysis software (Olympus). Probes of PDMS were aged for different times, rinsed with 96% ethanol and DI water, dried using compressed nitrogen and used in the experiment. 3 μL DI water drops were placed at 5 different places of each probe and pictures were immediately captured via the microscope. The measurements were performed at room temperature after the end of thermal treatment and repeated 4 days later.

Fabricated microstructures were observed using a tabletop scanning electron microscope (TM-1000, Hitachi) and 3D Laser Measuring Microscope (LEXT, Olympus). The testing microstructure consisted of 60 identical microunits–triangle inserts separating microchannels (Figure 2b).

The accuracy of replication was defined as a percentage of microunits properly replicated (proper replication mean achievement of a structure which is an exact replica of a master within boundaries explained in Section 3. Any changes of the shape, cracks and cavities were considered as failure in Figure 2) to the total number of microunits of the structure.

Surface nanometer-scale topology was measured by atomic force microscopy (AFM) using Ntegra Aura System (NT-MDT, Russia). 9:1 PDMS was cross-linked on a plain PMMA slab, peeled off and bisected. One probe was thermally aged for 48 h, while the other was stored at room temperature. Measurements were carried out one week later at room temperature, 37% humidity. Data analysis was performed using Gwyddion 2.27 software.

2.3. Cell culture

The microdevice was sterilized by UV light and 70% ethyl alcohol. Then the system was filled with cell culture medium (RPMI, supplemented with 20% FBS). A suspension of HT-29 cells (10^6 cells/mL) was introduced into the microchambers. The cells were cultured as Multicellular Spheroids [11,12,13] for two weeks, with medium exchange every 48 h. Life/dead cell viability assay was performed using fluorescent dyes: Calcein-AM and Propidium Iodide (Sigma-Aldrich).

3. Results and Discussion

The goal of our work is to fabricate a 3D biocompatible microstructure of PDMS for 3D culture of human cells. The designed structure (Figure 2) consists of a network of microchannels (100 μm deep, 100 μm wide) and an array of the microchambers of different depths (50–250 μm). The structure was quite difficult to achieve by photolithographic methods, because it would require multi-step exposition and processing in a clean-room. The PMMA master for the first replication was prepared by micromilling, which provided fabrication of the microstructures of various depths. Then, PDMS double casting was chosen to fabricate a PDMS copy of a PMMA structure. PDMS double casting included two replication steps: (1) replica molding of a PMMA master and (2) sequencing replica molding of a PDMS structure fabricated in the first step.

Replica molding of a native PDMS master (cured for 3h in 70 °C) appeared to cause errors. Strong adhesion and partial cross linking between two PDMS surfaces were observed, which resulted in destruction of the master and the replica (Figure 2a, b). Damage of the replica could have been related to the stiffness of a PDMS bulk. The stiffness of PDMS was regulated by a composition of a polymer, which was quantified elsewhere [28]. Liquid pre-polymer was mixed with a curing agent at different weight ratios (10:1, 9:1 and 8:1). Different PDMS types were used for both master and replica preparation, and accuracy of replication was determined (Table 1). The best results were obtained for 9:1 PDMS master. 10:1 PDMS was the most elastic one and it appeared to be too soft and sensitive. On the other hand, 8:1 PDMS was too rigid and could be easily broken during demolding. Therefore, 9:1 PDMS master was selected for further investigations.

Next, the influence of thermal aging of a PDMS slab on its surface properties was investigated. The chemical properties of PDMS remain stable during the aging process at 100 °C, according to [30]. Therefore, submicron changes of the surface topography were expected. Different thermal aging times were applied (16–72 h) and a contact angle of a water drop placed on a polymer surface was

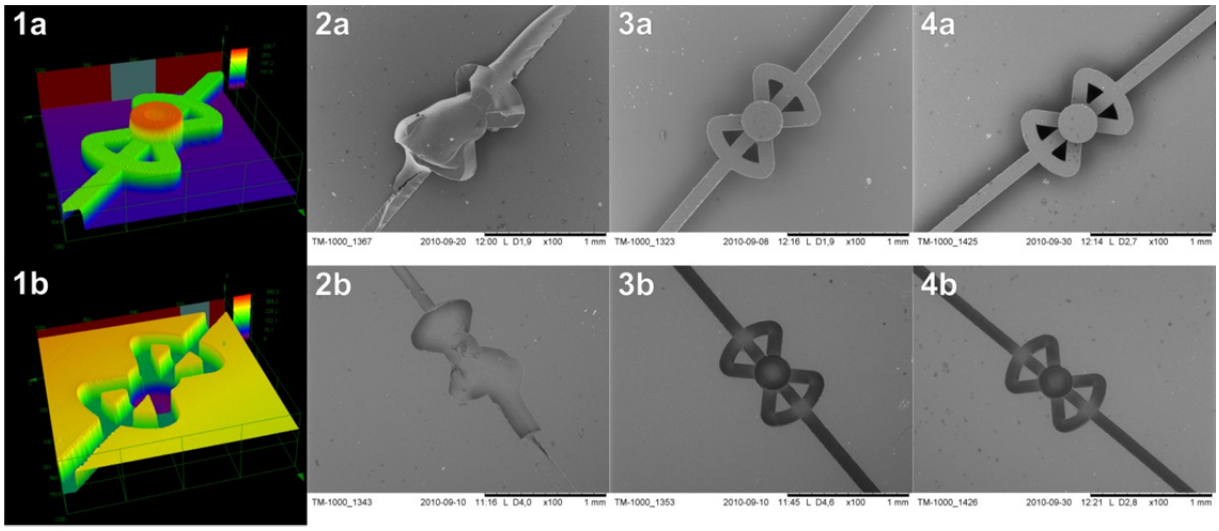

Figure 2. Verification of replication of a microchamber structure (consisting of four triangle microunits): confocal laser microscope profiles of (1a) a convex PDMS master and (1b) concave PDMS replica; SEM micrographs of (2a) native 10:1 PDMS master damaged during demolding, (2b) damaged 10:1 PDMS replica of a native 10:1 master; (3a,3b) 9:1 48h-aged PDMS master and 9:1 PDMS replica (first replication) and (4a,4b) third replication.

Table 1. Results of measurements of accuracy of replication for different types of PDMS masters and replicas. Standard deviations of 4 experiments. Best result for each set of experiments marked with a *.

Native							
Replica		Mold PDMS 8:1		PDMS 9:1		PDMS 10:1	
		Accuracy [%]	SD	Accuracy [%]	SD	Accuracy [%]	SD
PDMS 9:1		-	-	35.9	16	30.3	6.6
PDMS 10:1		16	4	66.7*	4.3	14.7	5.4
PDMS 9:1, Thermally aged							
Replica		Mold 24h aged		48h aged		72h aged	
		Accuracy [%]	SD	Accuracy [%]	SD	Accuracy [%]	SD
PDMS 9:1	1st	68.1	5.4	98.7*	1.5	98.3	1.5
	2nd			94.3	3.7	95.2	2.8
	3rd			88.2	4.6	90.3	4.3
PDMS 10:1				87.3	4		

measured (Figure 3). The contact angle of a water drop defines a hydrophobic/hydrophilic character of the surface. The angle of 90° is a conventional boundary between hydrophobic surface (values > 90°) and hydrophilic surface (values < 90°). In the experiment, the contact angle changed from 97 ± 3°

(native PDMS) to 84 ± 3° (72h-aged PDMS). Another parameter characterizing surface properties is roughness of the surface. AFM measurements of the PDMS surface demonstrated significant changes of nanometer-scale topology during thermal aging (Figure 3). The surface roughness was reduced from R_a = 3.33 nm and R_q = 4.98 nm (native PDMS) to R_a = 0.47 nm and R_q = 0.63 nm after 48 h thermal aging at 100 °C (R_a refers to arithmetic mean surface roughness and R_q refers to root mean square roughness). Surface flattening led to reduction of contact surface between two PDMS molds and thus weakened adhesion and enabled demolding.

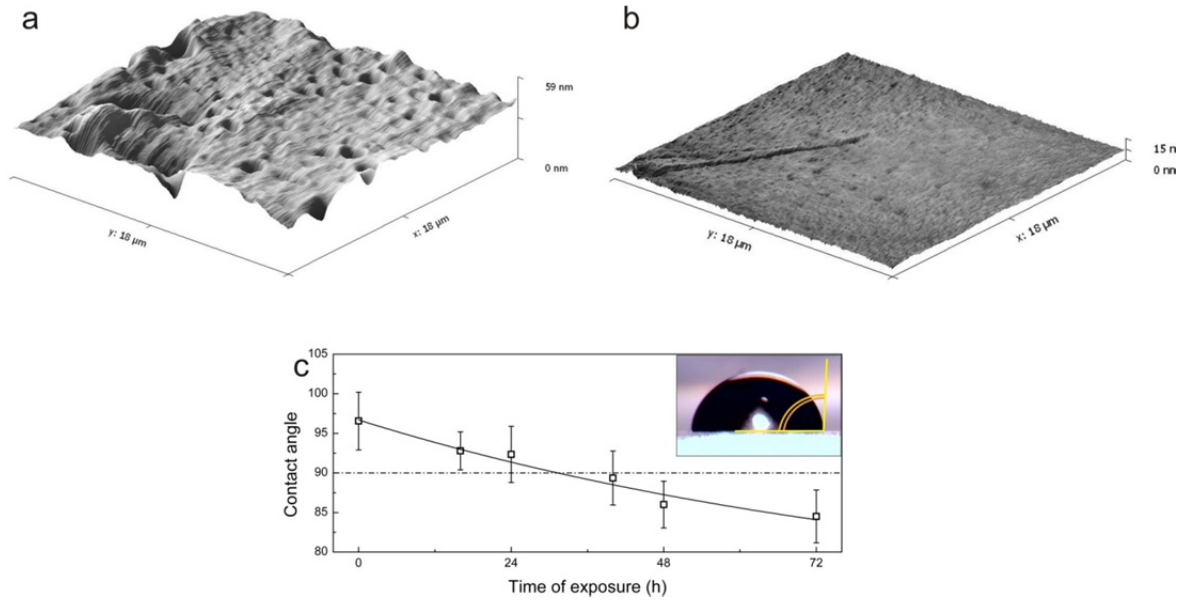

Figure 3. Investigations on changes of the PDMS surface during thermal aging: (a, b) AFM micrographs of (a) native and (b) 48 h-aged PDMS, both graphs of the same scale; (c) results of measurements of contact angle of a water drop on a surface of a PDMS slab exposed to 100°C (error bars correspond to standard deviation of 5 measurements).

To verify, whether change of the topology of the master's surface effects replication and demolding, convex PDMS masters were exposed to 100 °C for 24, 48 and 72 h. Next, a subsequent replica molding was performed using these masters. Accurate replication and lossless demolding were obtained for 48 h- and 72 h-aged PDMS masters (Table 1). Replication of the 24 h-aged master was better than of a native master, but it was not efficient enough. Comparison of these observations and the results of contact angle measurements led to a conclusion, that changing of a PDMS surface character to conventionally hydrophilic (after approximately 40 h of aging) sufficiently enhances subsequent replica molding. Taking into account a compromise between accuracy of replication and time needed for master preparation, 48 h thermal aging was chosen as the optimal period of thermal aging.

To verify whether the applied thermal treatment affects microstructure's dimensions, the dimensions of the PDMS master were measured using LEXT profilometry and SEM microscopy before and after thermal aging. After 48 h aging, differences of microstructures dimensions detected by profilometry have not exceeded 0.8%. The widths of the structures remained unchanged (changes within error bars of the measurement method) while the heights of the structures decreased of 0.35%

on average.

Another issue of our interest was a durability of a master effecting a number of successive replications possible to perform using one master. Our low-cost, thermally aged PDMS masters were used for multiple replications. Masters made of 9:1 PDMS, aged at 100 °C for 48 h were used for replica molding of 10:1 PDMS, and 9:1 PDMS. It was observed that during demolding of a 10:1 PDMS cast, fragments of the replica remained in the master's hollows disabling its further usage. On the other hand, demolding of a 9:1 PDMS replica was easy and lossless, therefore further replications using the same master were possible. The changes were attributed to mechanical properties of these two types of PDMS, which were discussed above. Finally, optimal conditions of a fabrication process were set as follows: composition of PDMS master and replica: 9:1 PDMS, time of thermal aging: 48 h. Application of these conditions provided at least ten replications using one PDMS master, leading to fully usable elements of a microsystem (see Supplementary Information). In our routine laboratory practice, thermally aged PDMS masters are being used for over ten successful replications (data not shown). The thermally aged masters were useful for second casting step even several months after aging (stored at room temperature). Utilization of a thermally aged PDMS master, with no chemical treatment, suitable for multiple replications significantly reduces the cost of a final device.

The microsystem fabricated using double casting with thermal aging was used for 3D cell culture. HT-29 cells were cultured inside the fabricated microchambers as Multicellular Tumor Spheroids (MCTS) (Figure 4). The 3D microstructure consisted of microchambers with diameter of 300 µm–dimensions corresponding with MCTS diameters–and narrower microchannels for medium in- and outflow. The culture was carried out for two weeks and high viability was maintained, proving that the biocompatibility of the substrate was not affected by the presented method of fabrication [12,13].

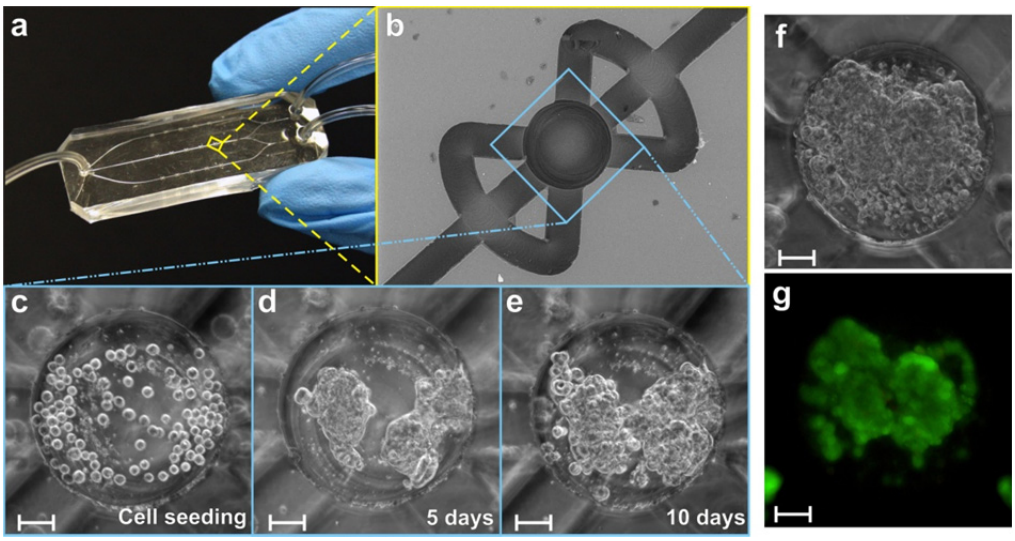

Figure 4. (a) Cell culture microsystem fabricated using double casting technique, (b) single microchamber for 3D cell culture, (c–e) long-term HT-29 spheroid culture inside the microchamber, (e, f) cell viability assay—viable cells demonstrate green fluorescence (Calcein-AM). Scale bars are equal to 50 µm.

4. Conclusion

In this work, a method of enhancing of efficiency of double casting replication was developed and described. The surface of the first PDMS replica, constituting a master for second replication, was modified by the thermal aging process. Thermal aging causes gradual cross-linking of low molecular weight chains in the polymer bulk. Therefore reduced master-to-cast adhesion and cross-linking were observed, and lossless PDMS demolding over the PDMS master was possible. Changes of PDMS surface topology were confirmed by AFM measurements and observations of wetting properties. Different types of PDMS, characterized by different stiffness, were tested. Optimal conditions were found for the PDMS double casting with the thermal aging technique, and several subsequent replications were successfully performed using one thermal-aged PDMS master. Development of a PDMS master not requiring chemical treatment enabled multiple (at least ten) replications of microstructures of strictly defined dimensions. It is also a solution for reduction of costs of 3D PDMS microsystems, because 3D PDMS masters are much cheaper than photolithography-made masters. Structures fabricated using the described technique were used for construction of a fully functional microfluidic cell culture system. The microfluidic system was used for long-term 3D cell culture of HT-29 human colon carcinoma cells.

Acknowledgments

AFM measurements were performed by courtesy of Dr Mariusz Zdrojek and Michał Wąsik of Nanostructures Group (Faculty of Physics, Warsaw University of Technology).

Conflict of Interest

Authors declare no conflict of interest.

References

1. Chudy M, Grabowska I, Ciosek P, et al. (2009) Miniaturized tools and devices for bioanalytical applications: an overview. *Anal Bioanal Chem* 395: 647–668.
2. Kwapiszewski R, Skolimowski M, Ziółkowska K, et al. (2011) A microfluidic device with fluorimetric detection for intracellular components analysis. *Biomed Microdevices* 13: 431–440.
3. Ni M, Tong W, Choudhury D, et al. (2009) Cell culture on MEMS platforms: a review. *Int J Mol Sci* 10: 5411–5441.
4. McDonald J, Whitesides G (2002) Poly(dimethylsiloxane) as a material for fabricating microfluidic devices. *Acc Chem Res* 35: 491–499.
5. Regehr K, Domenech M, Koepsel J, et al. (2009) Biological implications of polydimethylsiloxane-based microfluidic cell culture. *Lab Chip* 9: 2132–2139.
6. Quake S, Scherer A (2000) From micro-to nanofabrication with soft materials. *Science* 290: 1536–1540.
7. Velve-Casquillas G, Le Berre M, Piel M, et al.(2010) Microfluidic tools for cell biological research. *Nano Today* 5: 28–47

8. Leclerc E, Sakai Y, Fujii T (2003) Cell Culture in 3-Dimensional Microfluidic Structure of PDMS (polydimethylsiloxane). *Biomed Microdevices* 5: 109–114.
9. Ziółkowska K, Jędrych E, Kwapiszewski R, et al. (2010) PDMS/glass microfluidic cell culture system for cytotoxicity tests and cells passage. *Sensor Actuat B* 145: 533–542
10. Ziółkowska K, Kwapiszewski R, Brzózka Z (2011) Microfluidic devices as tools for mimicking the in vivo environment. *New J Chem* 35: 979–990.
11. Ziółkowska K, Kwapiszewski R, Stelmachowska A, et al. (2012) Development of a three-dimensional microfluidic system for long-term tumor spheroid culture. *Sensor Actuat B* 173: 908–913.
12. Ziółkowska K, Stelmachowska A, Kwapiszewski R, et al. (2013) Long-term three-dimensional cell culture and anticancer drug activity evaluation in a microfluidic chip. *Biosensor Bioelectron* 40: 68–74.
13. Kwapiszewska K, Michalczuk A, Rybka M, et al. (2014) A microfluidic-based platform for tumour spheroid culture, monitoring and drug screening. *Lab Chip* 14: 2096–2104.
14. McDonald J, Duffy D, Anderson J, et al. (2000) Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* 21: 27–40.
15. Kim J, Heo J, Crooks R (2006) Hybridization of DNA to bead-immobilized probes confined within a microfluidic channel. *Langmuir* 22: 10130–10134.
16. Adams M, Johnston M, Scherer A, et al. (2005) Polydimethylsiloxane based microfluidic diode. *J Micromech Microeng* 15: 1517–1521.
17. Lim C, Low H, Ng J, et al. (2009) Fabrication of three-dimensional hemispherical structures using photolithography. *Microfluid Nanofluid* 7: 721–726.
18. Huikko K, Ostman P, Grigoras K, et al. (2003) Poly(dimethylsiloxane) electrospray devices fabricated with diamond-like carbon–poly(dimethylsiloxane) coated SU-8 masters. *Lab Chip* 3: 67–72.
19. Gitlin L, Schulze P, Belder D (2009) Rapid replication of master structures by double casting with PDMS. *Lab Chip* 9: 3000–3002.
20. Zhuang G, Kutter J (2011) Anti-stiction coating of PDMS moulds for rapid microchannel fabrication by double replica moulding. *J Micromech Microeng* 21: 105020.
21. Eddington D, Puccinelli J, Beebe D (2006) Thermal aging and reduced hydrophobic recovery of polydimethylsiloxane. *Sensor Actuat B* 114: 170–172.
22. Briones M, Honda T, Yamaguchi Y, et al. (2006) A Practical Method for Rapid Microchannel Fabrication in Polydimethylsiloxane by Replica Molding without Using Silicon Photoresist. *J Chem Eng Jpn* 39: 1108–1114.
23. Koerner T, Brown L, Xie R, et al. (2005) Epoxy resins as stamps for hot embossing of microstructures and microfluidic channels. *Sensor Actuat B* 107: 632–639.
24. Wong I, Ho C (2009) Surface molecular property modifications for poly(dimethylsiloxane) (PDMS) based microfluidic devices. *Microfluid Nanofluid* 7: 291–306.
25. Zhou J, Ellis A, Voelcker N (2010) Recent developments in PDMS surface modification for microfluidic devices. *Electrophoresis* 31: 2–16.
26. Leite C, Soares R, Goncalves M, et al. (1994) Surface dynamics of polydimethylsiloxane rubber. *Polymer* 35: 3173–3177.
27. Jeong O, Konishi S (2011) Controlling the size of replicable polydimethylsiloxane (PDMS) molds/stamps using a stepwise thermal shrinkage process. *Microelectron Eng* 88: 2286–2289.

28. Jokinen V, Suvato P, Franssila S (2012) Oxygen and nitrogen plasma hydrophilization and hydrophobic recovery of polymers. *Biomicrofluidics* 6: 016501–016510.
29. Liu M, Chen Q (2007) Characterization study of bonded and unbonded polydimethylsiloxane aimed for bio-microelectromechanical systems-related applications. *J Micro/Nanolith MEMS MOEMS* 6: 023008.
30. Liu M, Sun J, Chen Q (2009) Influences of heating temperature on mechanical properties of polydimethylsiloxane. *Sensor Actuat A* 151: 42–45.

AIMS Press