

Fabricating Polymeric Curved Structures with Sub-Millimeter Scale for Cell Culture through Interfacial Forces between Liquid and Solid Phases

Chao-Min Cheng^{1#*}, Koji Matsuura^{2,3*}, I-Jan Wang^{1*}, Philip R. LeDuc^{1♦} and
Keiji Naruse^{2♦}

¹Departments of Mechanical and Biomedical Engineering and Biological Sciences,
Carnegie Mellon University, Pittsburgh, PA 15213, U.S.A.

²Cardiovascular Physiology, Graduate School of Medicine, Dentistry and
Pharmaceutical Sciences, Okayama University, Okayama, Japan

³Research Core for Interdisciplinary Sciences, Okayama University, Okayama, Japan

*Equally contributed.

♦Corresponding Authors:

Philip R. LeDuc

Departments of Mechanical and Biomedical Engineering and Biological Sciences,
Carnegie Mellon University, Pittsburgh, PA 15213, U.S.A.

(Email) prleduc@cmu.edu

Keiji Naruse

Cardiovascular Physiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama
University, Okayama, Japan

(Email) knaruse@md.okayama-u.ac.jp

#Current Address:

Whitesides Research Group

Department of Chemistry and Chemical Biology

Harvard University

Cambridge, MA 02138, U.S.A.

*Current Address:

School of Engineering and Applied Sciences

Harvard University

Cambridge, MA 02138, U.S.A.

Abstract

Polymeric curved structures, with their versatility and bio-friendliness, have been widely used in imaging biological systems such as being integrated onto optical fibers and microfluidic channels to stimulate and gather optical information. Herein, we have demonstrated that controlling surface tensions between two polymeric materials at the sub-millimeter scale realizes the fabrication of polydimethylsiloxane curved structures in a simple and efficient manner. The resultant sub-millimeter scale structure has a smooth and symmetric curved surface based on surface tensions and phase transitions. Our system provides a simple, efficient and economic method that does not require high cost fabrication facilities.

With advances in fabrication technology, a number of methods can be used to create small scale structures. Photolithography is one of the important approaches, which plays an important role in integrated circuit manufacturing¹. Alternate technologies have also been developed including soft lithography², nanoimprint lithography³, microcontact printing⁴ and capillary lithography⁵. While these techniques have enabled advances in the field and can provide for the patterning of a range of materials at the small-scale with relatively low cost and high efficiency, the ability to create complex three-dimensional systems in fabrication processes is still challenging since planar limited mask-based processes often dictate the final architecture of a device.

Expanding on this approach in creating gradient patterns provides freedom in studying many processes, which are often limited to binary control in the presence versus absence of a material. Venkateswar *et al.*⁶ previously used electrodes to fabricate a gradient of charged molecules within a liquid. In addition, Bhangale *et al.*⁷ used a metal-transfer process to form arbitrarily shaped surface-conjugated protein gradients. Both of these stamping methods are limited by the gradient patterns, sizes and shapes that they can fabricate. The other approach⁸ that has been used to create patterns include a porous polymer film which was used as a template to guide the patterning of proteins for the creation of a three-dimensional protein pattern formed via the use of a breath-figure method.

In our previous studies, we first introduced a process for creating three-dimensional structures that utilized an inverted microscope with the epi-fluorescence capability. A laser or epi-fluorescent signal was used to activate a photocurable material, SU-8, inducing a shift to a stable solid state^{9,10}. Using this technique and dictating the excitation profile through controlling the x-, y-, and z- distributions with the actuation of the microscope stage and objective, we built three-dimensional microstructures with various configurations on the same substrate. Our related second method was used to make simple and efficient poly(dimethylsiloxane) (PDMS) curved microstructures through controlling surface interactions¹¹. Curved microstructures are created by controlling the solid-liquid interface between a droplet of liquid PDMS and a polymer substrate. By altering the surface characteristics, we control the contact angles of the droplet, which creates defined diameters of these curved microstructures.

Developing curved elements for integration into complex systems is essential for many applications including in biomolecular and cellular manipulation. Due to the size of cells or biological samples, the curved structures at the sub-millimeter scale are desired. To fabricate these elements, leveraging simple surface and material characteristics at a small scale provides novel directions. Herein, we present an alternative approach inspired by inherent properties of materials to fabricate polydimethylsiloxane (PDMS) curved structures at the small scale through a simple and efficient method. The ability to create a curved structure was directly related to the interactions between the liquid PDMS (partially-cured PDMS) and the surface properties of the solid molds such as

hydrophobicity or hydrophilicity. Control over the liquid meniscus prior to solidification was accomplished by varying the interfacial surface tension between the PDMS and a solid mold. According to the Young-Dupré equation^{11,12}

$$\cos \Theta = \frac{\gamma_{SV} - \gamma_{LS}}{\gamma_{LV}} \quad (1)$$

where Θ , γ_{LV} , γ_{LS} and γ_{SV} are contact angle, surface free energies of the liquid-vapor, liquid-solid and solid-vapor interfaces, respectively. The equilibrium contact angle was determined in terms of the surface free energies; however, the normal component was not the only issue with this process from a mechanical perspective.

Before demonstrating this process, we first fabricated a poly(tetrafluoroethylene) (PTFE) (contact angle~110°) and a poly(methylmethacrylate) (PMMA) mold (contact angle~70°) with a microhole array of 0.2-mm diameter and 1-mm and 0.3-mm diameters through microdrilling process, respectively. We then used poly(dimethylsiloxane) (PDMS) (contact angle~110°), which is composed of a silicone T-resin cross-linked by a mixture of vinyl-terminated PDMS (base) and trimethylsiloxy-terminated poly(methylhydroxosiloxane) polymers (curing agent) as the molding material. This is a useful material for fabricating cell arrays because of its advantageous interactions with cells and its low cost. 184-PDMS (Sylgard 184; *Dow Corning* or TSE 3032; *Momentive Performance Materials*) has a highly cross-linked three-dimensional structure and offers high elongation properties with a relatively low modulus. After preparing liquid PDMS, de-bubbles in liquid PDMS through the vacuum pump (around 30 minutes to one hour),

PDMS was cured on a hotplate at a temperature of 65 °C for 10 minutes (partially-cured PDMS). This process then performed through contacting the PTFE or PMMA mold and partially-cured PDMS until the PDMS completely cured; the schematic of this process by controlling the interactions between a solid mold and partially-cured PDMS is as shown in Figure 1. Figure 2 (a) and (b) show that a microlens array and a single microlens can be obtained by controlling surface interactions between two hydrophobic materials from liquid to solid phases. Figure 2 (c) shows the backlight microscope image of a single microlens, with its curved surface facing up against the microscope. It is evident that this configuration resembles a planar convex lens, as can be seen from its light intensity throughput profile (Fig. 2 (d)).

In our previous study¹¹, we demonstrated that liquid PDMS had specific molding characteristics with different substrates to form a PDMS microlens. Herein, this surface tension effect allowed us to form a planar convex lens as well, as shown in Figure 2 (e). Figure 2 (f) shows micro-columns with the aspect ratios of 4. These lenses (as shown in Fig. 2(b)) also were used for the magnification of attached cells on the surface of a PDMS lens in liquid environments (Fig. 2 (g) and (h)). We cultured NIH-3T3 fibroblasts in medium supplemented by 10% calf serum, glutamine, 0.3 mg/ml, penicillin, 100U/ml, streptomycin, 100 µg/ml, and 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid at pH of 7.4, under 5% carbon dioxide on the surface of a PDMS lens. After culturing for 24 hours, fibroblasts were magnified by this microlens (Fig. 2 (h)).

In addition, we also used a hydrophilic material PMMA, as a mold to fabricate curved structures. According to equation (1), the dent-type curved structures can be obtained based on our simple method, as shown in Figure 3 (a). Figure 3 (b) shows the sizes of this dent-type structure through scanning the entire sample using laser confocal microscopy. The aspect ratio of the structure was approximately 0.1. We can obtain the similar dent-type structure with 0.3 mm diameter as shown in Figure 3 (c) and (d). The aspect ratio of the structure was approximately 1/3. The aspect ratio of the dent-type structure was increased when the diameter of mold decreased.

We surveyed correlations between diameter and depth of these structures. Figure 4 (a) and (b) show the correlations of the dent-type and convex-type structures, respectively. Interestingly, as increasing the diameter of both molds that we used, the depth of these structures didn't increase. As previously suggested, we found negative correlation between the diameter and the aspect ratio of the structure. We assume that the gravity effect might involve the force balances between partially-cured PDMS and a these molds. The contact force between the mold and the partially-cured PDMS would be reduced in the center of the hole of the mold. When the diameter of the mold is increased, the gravity effect would be increased and the aspect ratio would be reduced. In addition, the characterization and manipulation of individual embryo cells has become a challenging issue in biomedical applications such as cloning, gene expression analysis and cell replacement therapy¹³. We consider that these dent-type structures with sub-millimeter scale diameters would be utilized to identify mammalian embryo

cells with 0.1 mm diameter in the culture. Because this fabrication method is relatively simple, scientists and technicians in biological fields can produce these desired structures readily.

Developing small-sized structures is of essential importance for lab-on-chip systems. Herein, we have demonstrated that with precise control of the curing process and the force exerted on the mold and PDMS, different types of polymeric small structures can be obtained. Without the complex and substantial time consumption of ordinary lithographic process, this fabrication scheme, based on the force balance between a solid mold and the molding material, offers a simple and efficient approach to fabricate polymeric structures. The reusability of mold and structure array itself also implies the reproducibility and parallel nature of this way of fabrication, which is beneficial in the aspect of application.

Acknowledgements

This study was supported in part by the National Science Foundation-CAREER, National Academies Keck Foundation Futures Initiative, Pennsylvania Infrastructure Technology Alliance, the Department of Energy-Genome to Life, the Beckman Young Investigators Program (P.R.L.), and Special Coordination Funds for Promoting Sciences and Technology of MEXT (The Ministry of education, sport, Culture, Science and technology) of Japan (K.M.). C.-M.C. and I-J.W. were supported in part by a Ph.D.

Research Scholarship from Taiwan; C.-M.C. was also awarded by Journal of Cell Science Travelling Fellowship.

References

- ¹J. R. Sheats and B. W. Smith, *Microlithography: Science and Technology* (CRC Press, 1998).
- ²Y. Xia and G. M. Whitesides, *Annu. Rev. Mater. Sci.* **28**, 153 (1998).
- ³L. J. Guo, *J. Phys. D: Appl. Phys.* **37**, R123 (2004).
- ⁴A. P. Quist, E. Pavlovic and S. Oscarsson, *Anal. Bioanal. Chem.* **381**, 591 (2005).
- ⁵K. Y. Suh, Y. S. Kim and H. H. Lee, *Adv. Mater.* **13**, 1386 (2001).
- ⁶R. A. Venkateswar, D. W. Branch and B. C. Wheeler, *Biomed. Microdevices* **2**, 255 (2000).
- ⁷S. M. Bhangale, V. Tjong, L. Wu, N. Yakovlev and P. M. Moran, *Adv. Mater.* **17**, 809 (2005).
- ⁸Y. Zhang, C. Wang, *Adv. Mater.* **19**, 913 (2007).
- ⁹C.-M. Cheng, B. Li and P. R. LeDuc, *Appl. Phys. Lett.* **87**, 164104 (2005).
- ¹⁰C.-M. Cheng and P. R. LeDuc, *SPIE*, **6959**, 69590X (2008).
- ¹¹C.-M. Cheng, J. D. Kubicek and P. R. LeDuc, *Appl. Phys. Lett.* **88**, 053902 (2006).
- ¹²J. Pellicer, J. A. Manzanares and S. Mafé, *Am. J. Phys.* **63**, 542 (1995).

¹³M. Andreas, R. Kirchner, H. Müller, P. Hartmann, N. E. Hajj, A. Tresch, U. Zechner, W. Mann, and T. Haaf, Biol. Reprod. **80**, 194 (2009).

Figure Captions

FIG. 1. Illustration of this simple method to fabricate different types of polymeric small structures through controlling the interactions between a solid mold and partially-cured polydimethylsiloxane (PDMS). (a) A convex structure and (b) dent structure can be obtained based on the surface tension effect between surface properties of a molding material and a solid mold.

FIG. 2. Scanning electron microscopy images of (a) a polymeric microlens array and (b) a single microlens on PDMS substrate. Smooth and curved surfaces of PDMS can be obtained by the application of surface tension. The lower row of PDMS pillars in (a) represents a control experiment in which capillary action dominated. (c) CCD image and (d) intensity profile of a single microlens capturing intensity distribution of a parallel beam illuminated from the back on the planar surface of the array (curved surface facing up). The brightest center in (c) shows that this curved structure that we fabricated through the microforce embossing process can be treated as a planar convex lens. (e) Scanning electron microscopy images of PDMS pillars topped with microlenses, achieved by press-and-regression method which precisely controls the force exerted on the structure and (f) micro-columns with a high aspect ratio. A flat surface at the top of

these micro-columns was controlled by the surface tension between the mold and uncured PDMS material. Phase contrast images of NIH 3T3 fibroblasts cultured on the convex structure for (g) one hour and (h) 24 hours. Fibroblasts attached and spread well on the curved structure and sub-cellular structure can be observed through this microlens. Scale bar= 100 μ m.

FIG. 3. (a) and (c) An Optical image of a dent-type structure array through contacting a polymethylmethacrylate mold and partially-cured PDMS. The diameter of a dent structure is around (a) 1 mm and (c) 0.3 mm. (b) and (d) Cross-section laser confocal microscopy images of this dent-type structures with the diameters (b) 1 mm and (d) 0.3 mm. Scale bar= 100 μ m.

FIG. 4. Correlations between the depth and the diameter of (a) dent structures by using PMMA mold and (b) convex structures by using PTFE mold. AR (aspect ratio) means the ratio of depth/diameter.

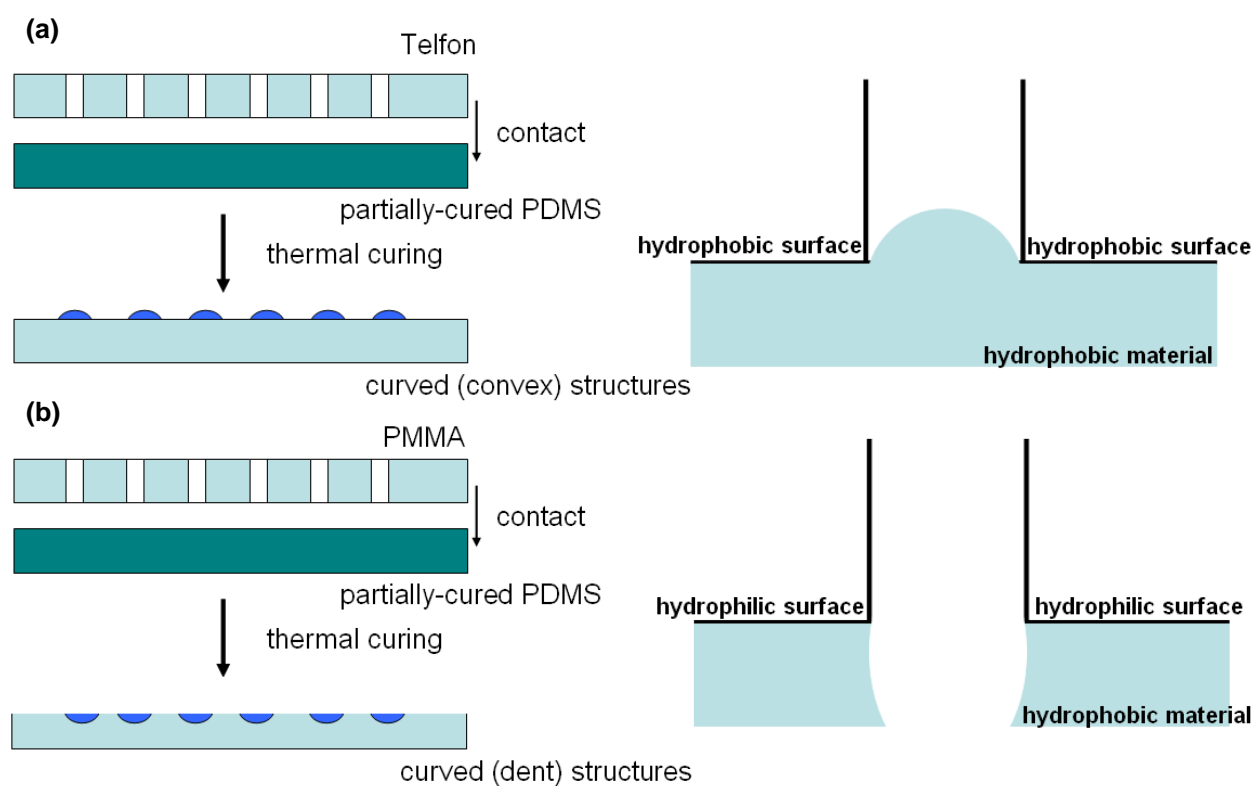


FIG. 1.

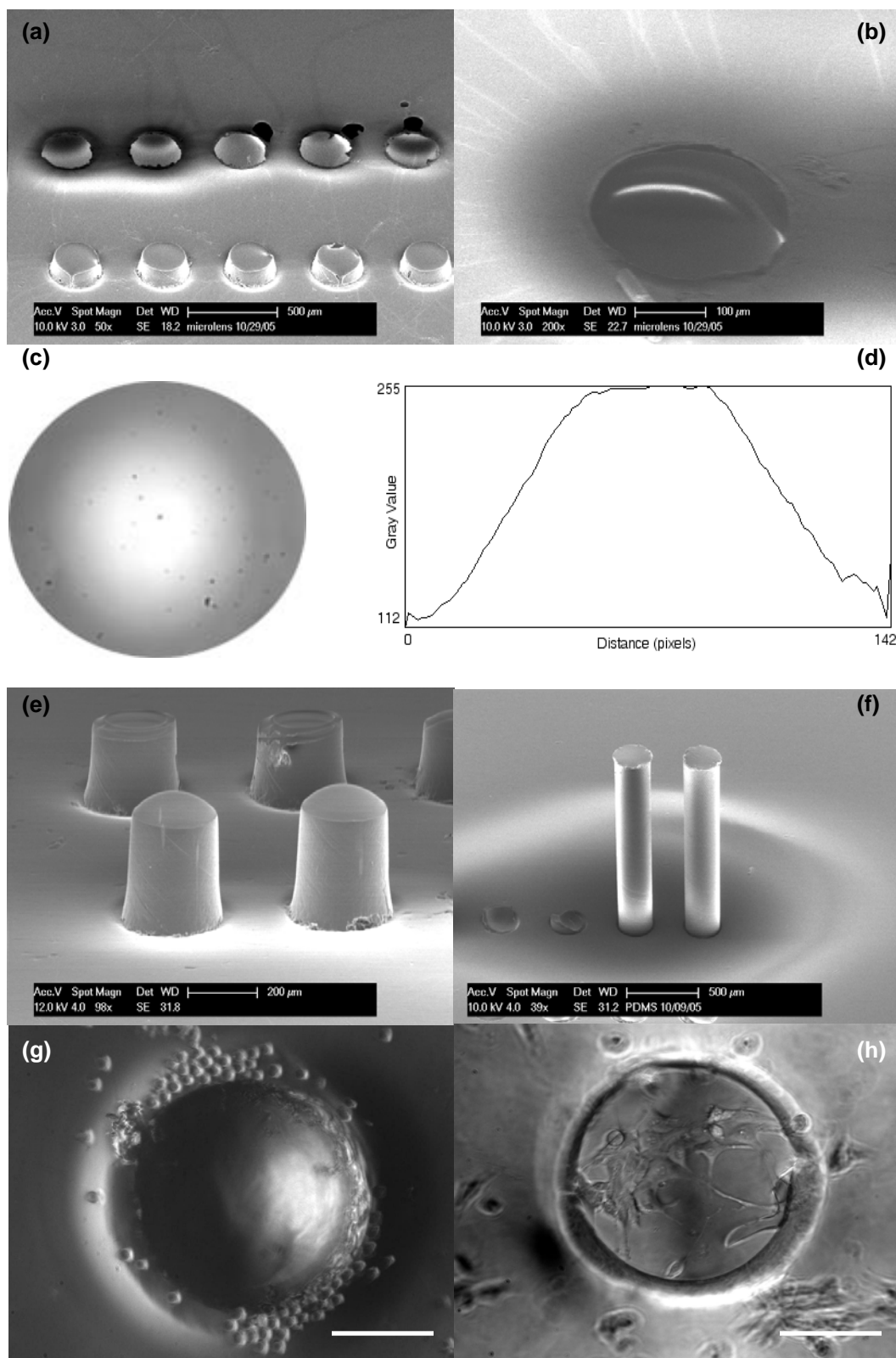


FIG. 2.

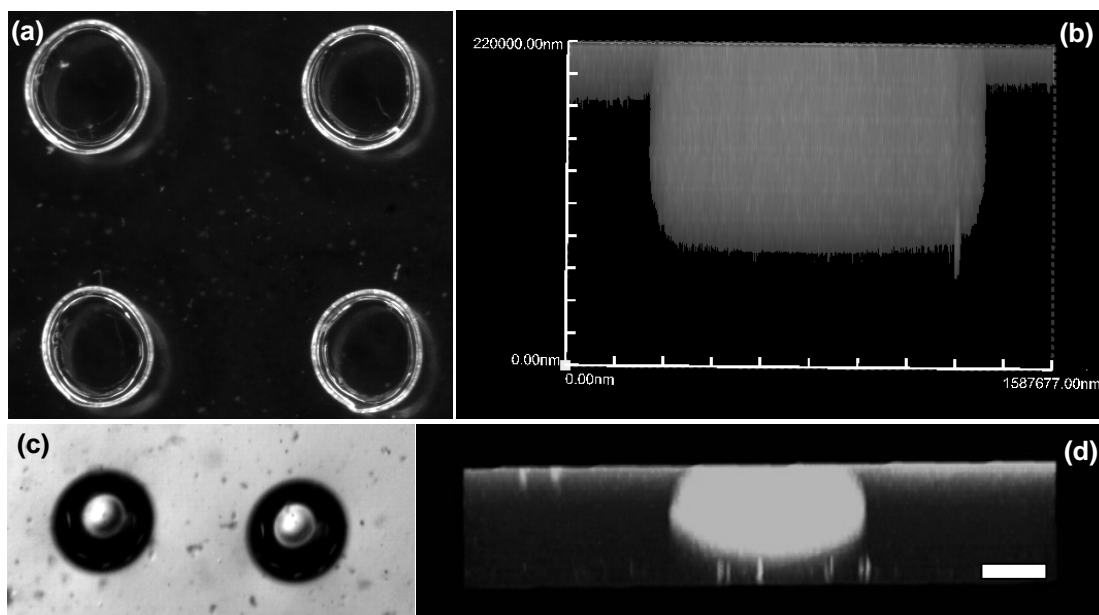


FIG. 3.

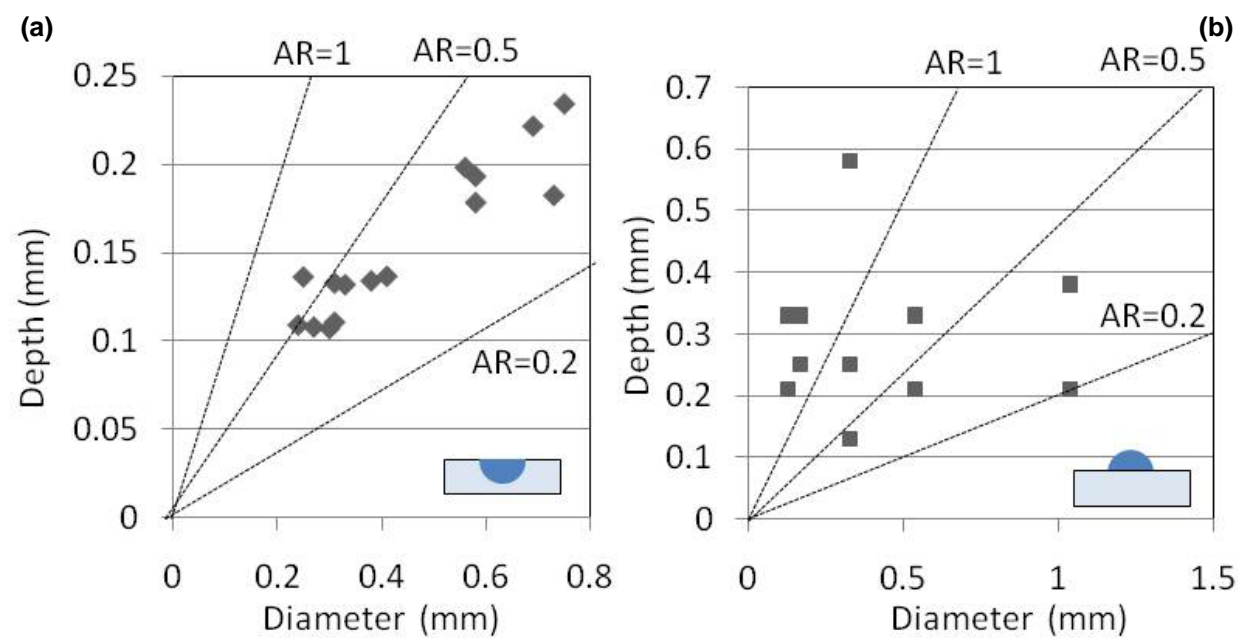


FIG. 4.

<Cover letter page 1>

Applied Physics Letters Editors

April 15th, 2009

Dear Editors:

Please find our electronically submitted manuscript entitled “Fabricating Polymeric Curved Structures with Sub-Millimeter Scale for Cell Culture through Interfacial Forces between Liquid and Solid Phases” by Chao-Min Cheng, Koji Matsuura, I-Jan Wang, Philip R. LeDuc, and Keiji Naruse. We wish to submit the manuscript as a communication in *Applied Physics Letters*. We would be grateful if the manuscript is reviewed and considered for the publication.

To produce curved microstructures is a challenging issue, since there are many steps to produce them by planar limited mask-based processes such as photolithography. In this paper, we have demonstrated that controlling surface tensions between two polymeric materials realizes the fabrication of poly(dimethylsiloxane) (PDMS) curved structures in a simple and efficient manner. The curved structures were produced by utilizing the interactions between the liquid PDMS (partially-cured PDMS) and the surface properties of the solid molds such as hydrophobicity or hydrophilicity.

After the contact of hydrophobic poly(tetrafluoroethylene) (PTFE) molds and partially-cured PDMS, the completely cured PDMS structures were micro-lens and columns. When we used hydrophilic mold poly(methylmethacrylate) (PMMA), the dent-type curved structures were obtained. We also found negative correlation between the diameter and the aspect ratio of the structure, and assumed that the gravity effect might involve the force balances between partially-cured PDMS and these molds. Our system provides a simple, efficient and economic method that does not require high cost fabrication facilities. These fabricated structures can be adapted for cell culture and cellular imaging.