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| Original Article |

Microfluidic Spinning of Grooved Microfiber for Guided Neuronal Cell Culture using Surface Tension Mediated Grooved Round Channel

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Abstract : Microfibers with groove structure enable the guided culture of diverse cells. For the fabrication of grooved microfibers, microchannel with a few microscale groove structure needs to be fabricated using complicated photolithography processes. In this study, a novel method to fabricate a round channel embedded microfluidic device for generating continuous grooved microfibers is introduced. The surface tension of PDMS prepolymer was used to construct the round channels and groove patterned structures in the microfluidic device. The self-organized multi-droplet structures were utilized to engrave the groove on the microfiber without any complex procedure. Using the device, the groove patterned (*ca.*10 μm of pattern size) microfiber was successfully fabricated. Microfibers were continuously produced without clogging and the diameters of the grooved microfibers were varied with the flow rate. For the test of usability for cell alignment, fibers were coated with laminin and the neuro-progenitor cells from prenatal rat were seeded on the prepared grooved fibers and cultured. The guided growth of neuronal cells on the fiber was observed through the immunostaining with neurofilament.

Key words: *Grooved microfiber, Round channel, surface tension, PDMS droplet, neuronal cell guiding*

1. Introduction

Physicochemically functionalized micro- and nano-fibers have been widely used in biomedical engineering fields[1-3] such as cell therapy[4], drug delivery systems[5,6], tissue engineering applications[7,8], and directional water collecting.[9, 10] Especially, microfibers on which diverse topological structure were engraved can be commonly found in nature. For example, the spiders use grooved microfibers to encapsulate their eggs safely[11], because groove patterning on microfiber enhances its mechanical strength. The spindle-knot structure of spider fiber allows the water droplet formation from the vapors in the air.[12] It is well known that the grooved nano- and micro-structure enhances the cell attachment and the guidance of cell growth[13], and we previously reported the alignment of neuron and muscular cells using the flat and round grooved fibers.[8] This technology could be used in the regeneration of the nervous system (*e.g.*: spine and sciatic nerve) and the muscles, as they require well-aligned tissue. However, it is difficult to spin grooved fibers using conventional spinning platform. Aligned nanofibers fabricated by electrospinning could be solution for cell alignment[2], however, aligning process with uniform fibers is still challenging. Furthermore, for the extensive applications as fibrous scaffold, it is critical to produce grooved fibers in large quantity with simple and cost effective process. Recently, several researches have reported methods to fabricate microfibers with chemical encoding or different topology using microfluidic channels and such microfluidic spinning method enables the production of grooved fibers without complicated process and facilities.[7,8, 14] For the microfluidic spinning, the glass micro-capillary channels[15], and the rectangular[9,10,16] and co-axial PDMS microfluidic devices have been broadly used.[7,17,18] Although these approaches successfully generate continuous microfibers, following challenges are still existing; intense labor and skills needed in setting the glass micro-capillary tube[15] and specialized tools needed in fabrication of the co-axial microfluidic devices[7,17], and clogging in the rectangular channel device.[17] For the generation of grooved microfibers, engraving of the grooved structure in the round channels is required. However, the fabrication of the round channel and engraving a few microscale grooved structure in the round channels requires well-

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trained skill and complicated photolithography processes to make the few microscale groove patterned microfiber. Therefore, the fabrication of grooved fibers in general laboratory is still difficult and easier process needs to be developed to address this problem.

In this paper, we introduce a simple method for fabricating a round microfluidic channel with a few microscale grooved structure inside the round channel without any use of specialized tools, complex processes and well-trained skill. The surface tension of viscose PDMS prepolymer is used for the self-forming of the rounded channel and groove patterned structures. The self-organized grooved structures deform the shape of moving sample and sheath flow, which allows the construction of a few microscale groove structure on the microfibers. The microfibers were continuously produced without clogging and the diameters of the grooved microfibers were varied with the flow rate. For the test of usability as scaffold for cell alignment, the neuro-progenitor cells from prenatal rat were seeded on the prepared grooved fibers, and the guided growth of neuronal cells on the fiber was observed.

2. Materials and Methods

2.1 Fabrication of Round Channel Embedded Micro fluidic Device

Rounded microchannels were fabricated using the meniscus induced by the surface tension of a PDMS prepolymer.[19-22] Briefly, a base PDMS layer with a rectangular channel structure was prepared by replicating the SU-8 (MicroChem, MA, USA) master mold (Fig 1A (i-ii)). To fabricate the base microfluidic channel, both the base microfluidic channel and a PDMS plate (thickness: 3~5 mm) were treated with oxygen plasma (Fig 1A (iii)) and bonded together. After that, the outlet of the base PDMS device was cut (Fig 1A (iv)). Then, the base PDMS channel with the rectangular-shaped channels was constructed. To fabricate a round channel, the mixture of a PDMS prepolymer (mixture of 10:1 silicon elastomer (Sylgard 184) and curing agent) was injected into the base PDMS channel (Fig 1B). After a few seconds, the PDMS prepolymer was removed from the base PDMS channel by suction with syringe (Fig 1C). Then, due to the meniscus of the viscous PDMS prepolymer filling the rectangular channel, the edges of the channels were rounded.[21] The round PDMS channel was placed in an oven for thermal curing (80°C for 1 h) in vertical position (inlet: upside, outlet: downward) (Fig 1D). By vertically positioning the microfluidic device, the residual PDMS prepolymer in the channel was laced with small prepolymer droplets due to their surface tension forming the groove-like structures (Fig 1E).

2.2 Fabrication of Fibers by The Control of Sample and Sheath Flow

To fabricate the alginate fiber, we prepared the solutions as

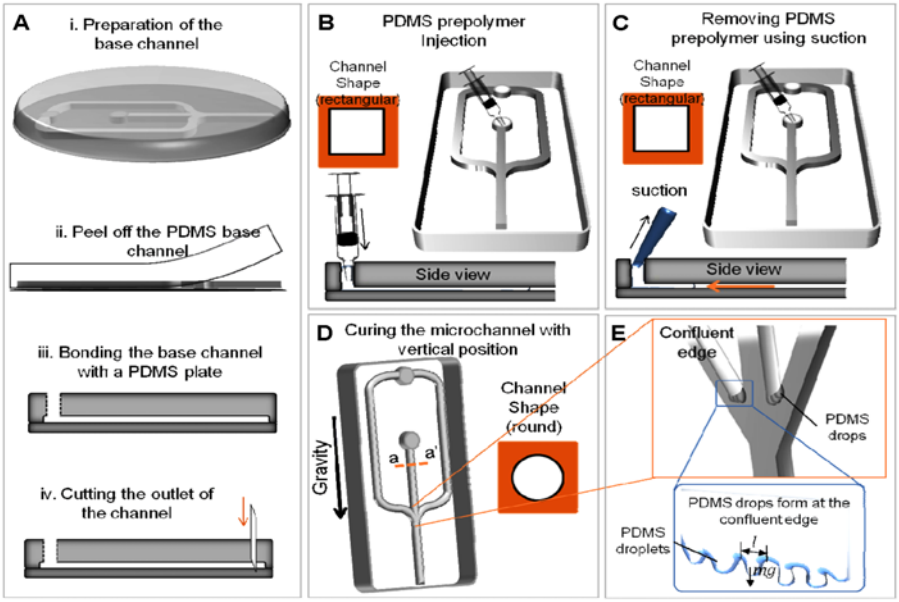

Figure 1. Schematic diagram of the fabrication of a rounded channel microfluidic device and multi-droplet based convex patterns; (A) Preparation of the base PDMS base micro channel, the SU-8 mold was fabricated using the conventional soft lithography. (B) PDMS prepolymer was introduced into the PDMS base channel of a microfluidic chip. (C) The PDMS prepolymer in the microfluidic chip was removed by suction. (D) Liquid PDMS residue in the microfluidic chip spontaneously formed rounded microchannels, and the inset shows a cross section of the channel (round channel). (E) Convex grooved patterns were fabricated using micro droplets formed from PDMS prepolymer.

previously reported.[7,8] Alginate and $CaCl_2$ solutions were prepared as sample fluid (3% w/v sodium alginate, Sigma, St. Louis, MO, USA) and sheath fluid (0.1 g calcium chloride, Sigma, St. Louis, MO USA, dissolved in 10 mL DI water), respectively. Each solution was injected into the inlet of each channel (Fig 1A), and the flow rates of two solutions were controlled using a syringe pump. Sheath flow exerts force to focus sample flow as well as acts as a lubricant for solidified microfibers to be continuously produced without clogging. For the generation of various sized microfibers, sample flow rate was varied from 1.8 to 5.4 mL/h, while the sheath flow rate varied from 20 to 50 mL/h.

2.3 Preparations of Neuro Progenitor Cells

Primary neuronal cells were isolated from the cortical legions of prenatal rat (embryonic day 16) (DBL, Incheon, South Korea), and were purified using centrifugation with 10,000 RPM for 5 min. After purification, the neural progenitor cells were seeded on the grooved microfibers and cultured in nerve growth factor (NGF, 100 ng/mL) supplemented Neurobasal media (Gibco) with B-27 Supplement (Gibco), 0.5 mM L-glutamine, 1% antibiotics containing 10,000 units penicillin (Gibco), and streptomycin. The animal experiments were carried out in the standards of the Institutional Review Board of Korea University.

2.4 Cell Culturing on the Concave Channel Network

For cell seeding on the grooved microfibers, cell suspension solution was prepared with a density of 2.0×10^7 cells/ mL. 0.5 mL of the solution was directly seeded on the grooved microfiber. 3 hours after the cell seeding, the cells were settled on the grooved microfibers, and culture medium was gently applied to remove the cells that were not attached on the microfibers. The media was exchanged to fresh medium every other day. To attach the neuronal progenitor, the grooved alginate microfiber was pretreated with laminin (100 μg/mL) solution. 2 hours after laminin treatment, the microfibers were washed several times using the cell-culturing medium.

2.5 Scanning Electron Microscopy (SEM)

A field emission scanning electron microscopy (FE-SEM, JEOL 4701F, JAPAN) was used to analyze the shape of the round microfluidic device and grooved microfibers. For observation the grooved microfibers, the microfibers were put into a series of graded ethanol (25%, 50%, 75%, 95%, and 100%) for dehydration. The alginate microfibers and the dissected microfluidic device were mounted on a specimen stub with graphite tape, coated with palladium alloy, and observed under the FE-SEM.

3. Results

3.1 Fabrication of Round Microchannel

Figure 2A shows the schematic of the microfluidic chip consisting of round channels for spinning of the microfiber. The self-organized round channels were fabricated using the surface tension of PDMS prepolymer PDMS and their optical or scanning electron microscope (SEM) images are demonstrated in Figure 2B, C, and d. Figure 2B shows the cross sectional optical microscope image of the microfluidic device in which the sample and sheath flow channel have round shapes. The sample flow is introduced through the central cylindrical channel (Fig 2B), and the sheath flow through the adjacent round channels (Fig 2B). Due to the rounded center channel, the cross section of sample flow is introduced into the reaction channel as a circular-shaped flow (Fig 2C). Figure 2D shows

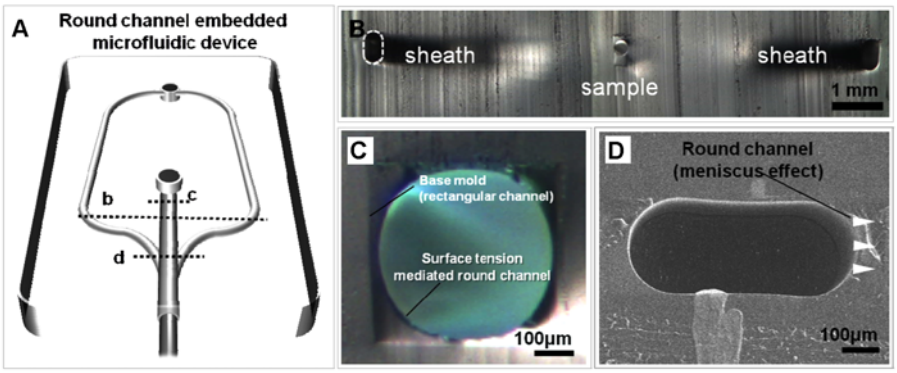

Figure 2. A round channel integrated microfluidic device for fabricating continuous microfiber; (A) Schematic of the surface tension mediated self-organized round channel microfluidic device. (B) An optical microscope image of the cross section of the sample and sheath channels in the microfluidic device. (C) An optical microscope image of a surface tension of The PDMS prepolymer mediated round channel. (D) A SEM image of cross section of the reaction channel. Scale bars indicate (B) 1 mm, (C) 100 μm, and (D) 100 μm.

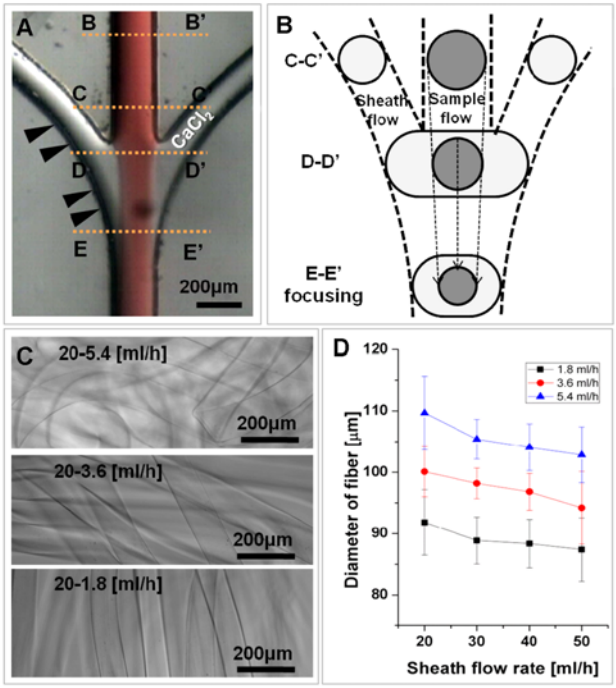

Figure 3. Feature of the developed microfluidic devices (A) An optical microscope image of the round channel microfluidic device. (B) The schematic of the fabrication procedure of the microfiber. (C) Optical microscope images of the alginate microfibers with various sample flow conditions (1.8, 3.6, and 5.4 mL/h) at 20 mL/h of sheath flow. (D) Diameter of microfibers with various sample and sheath flow conditions. All scale bars indicate 200 μm.

the SEM image at the confluent area, and the image reveals that the surface tension-mediated meniscus effect can fabricate a well-organized ellipsoidal channel.

3.2 Characteristics of the Microfluidic Chip

Figure 3A demonstrates the optical microscopic image of fiber spinning. The size of spinning chip is 20 mm × 10 mm and with the thickness of 5 mm. Figure 3B shows the schematics of flow's cross-section at the line BB', CC', DD' and EE'. Using the proposed microfluidic chip, the fibers were continuously created without breakage, and this may be because the channels were rounded. Even though we did not use complicated processes to construct the round channels as reported, the meniscus, driven by surface tension, created the round channels successfully. The fibers were successfully extruded outside the channel indicating that the sheath flow plays a key role as a lubricant and a solidifying agent. Figure 3C shows optical images of the alginate microfibers, and the diameter of the fibers was controlled by changing both the sample, and sheath flow rates. By controlling the sample flow rate (1.8, 3.6 and 5.4 mL/h), the diameters of the alginate microfibers were decreased approximately 10 μm under given sheath flow rate conditions (Fig 3C and D). Figure 3D demonstrates the diameter of the fiber as a function of sample and sheath flow rates. The fibers' diameters ranged from 80 to 115 μm. In this study, clogging was common in the sample (3% of alginate) at the flowrate lower than 1.8 mL/h. During the fiber generation procedure using the round channel embedded microfluidic chip, a higher sample flow rate is needed compared to that needed for the coaxial confluent chip for fabricate microfiber.[17]

3.3 Fabrication of Grooved Microfiber and Neuronal Cell Outgrowth Guiding

Figure 4A illustrates a SEM image of a cross section along the sample channel (the channel was cut along the red dotted line in the Figure 4B), and solidified multiple-droplets of PDMS prepolymer were clearly observed. These structures deformed the sample flow, and the microfibers, on which the grooved structures were engraved, were fabricated by rapidly

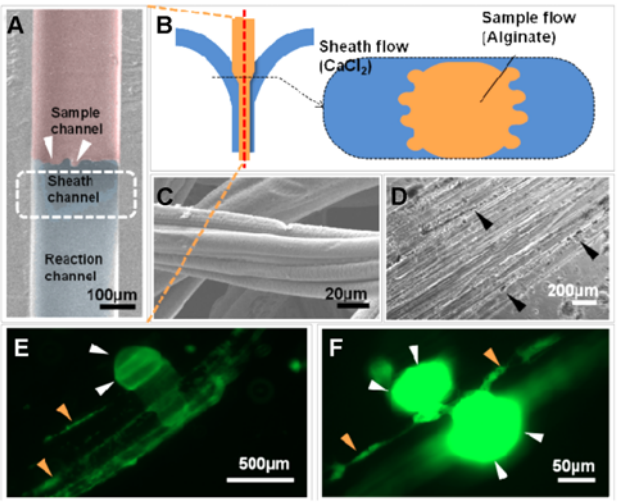

Figure 4. Groove patterned microfluidic devices and microfiber. (A) A SEM image of a cross section along the sample channel (red dotted line in Figure 4B) in the confluence area of the microfluidic chip. Red and blue colored areas indicate sample and reaction channels, respectively, and the white dotted box indicates the sheath channel. (B) The schematic of the grooved microfiber generation using multi-droplet based structures at the edge of the converging area between sample and sheath channel. (C) A SEM image of grooved microfiber. The pattern sizes of the grooved microfiber were revealed to be *ca.* 10 μm. (D) The alignment of differentiated neuronal cells on the groove microfiber at 7days after seeding. (E and F) Aligned neuronal cells on the groove microfiber. Scale bars indicate (A) 100 μm, (C) 20 μm, (d) 200 μm, (E) 500 μm, and (F) 50 μm.

solidifying the deformed flow (Figure 4B). Figure 4C shows the fabricated microfiber with grooved structures, and the grooves, whose size is about a few microns, were clearly observed. This size is similar to the size of previously spun grooved fibers for neuronal cell guidance.[7,8] Figure 4D shows the optical microscope image of grooved microfiber on which neuro-progenitor cells were seeded and cultured for 7days. As shown in this figure, adhered neuro-progenitor cells were well-aligned along the fiber. About 10 days after seeding, the seeded cells were observed using the confocal microscope after fluorescent staining (anti-neurofilament antibodies). As shown in Figure 4E and F (white arrowheads), some aggregation of neurons were observed on the grooved microfibers. The growing neurites were aligned along the grooved structure (Fig 4E and F, orange arrowheads).

4. Discussion

Guided cell alignment is important in engineering of tissues and has been one of the greatest interests in organ regeneration. Nano- and micro-patterned surface has been extensively used for 2D cell alignment, but it is challenging to prepare such surface in laboratory in a simple and cost-effective way. In addition, this approach is limited to cell-behavior study and to apply it to organ regeneration is still one of challenges. Aligned fibrous structure has played an important role in aligned culture of cells and recent progress in microfluidic spinning technology has enabled the spinning of grooved fibers for the aligned cell culture. However, it is difficult to prepare the microfluidic chip to fabricate grooved fibers. For the spinning of grooved fibers, the cylindrical channel on which grooved structure is engraved is required, and a complicated process to construct such channel is necessary. Here, we proposed a simple and cost-effective method to fabricate round microchannel using the meniscus derived by the surface tension of viscous PDMS prepolymer. The autonomously created droplet of PDMS prepolymer was used for the successful creation of groove-like structure inside the round channel after thermal curing. With the prepared microchannel, we have demonstrated spinning of grooved fibers successfully, and the diameter of fibers could be controlled by changing the sample and sheath flow rate. This result indicates that once the mold for microfluidic spinning channel is prepared, the round channel with groove-like structure could be fabricated even in the general laboratory without complicated processes, well-trained skill and facilities. Due to the simplicity and cost-effectiveness, the proposed method could be extensively employed in the fabrication of the fibrous scaffold having cell-alignment capability. To prove the potentiality of the cell-guiding scaffold, we seeded neuro-progenitor cells harvested from the prenatal rat on the grooved fibers, and we observed that they were differentiated to neurons, successfully forming aligned neurites. Using the non-grooved microfiber, we have seeded and cultured neuronal cell alignment, but neuronal-cell attachment to the non-grooved fibers, which indicates that the grooved structure enhances the cell attachment, was rarely observed. Although the grooves on the fibers are not so uniform compared to the reported photolithographic process[7], and the alginate has no cell binding sites the proposed microfluidic chip produced grooved fibers enough to be used for the aligned cell culture with laminin coating.

5. Conclusion

In summary, a round microchannel with groove-like structure was successfully fabricated using the meniscus mediated by the surface tension of PDMS prepolymer without any use of complicated process and expensive equipment. Large amount of grooved fibers could be produced using the microchannel in which grooved structures were autonomously created by the droplet of PDMS prepolymer. Through the cell experiments, it was proven that the groove structures formed by the droplet played a critical role in aligning the neuronal cells. By the alignment of these fibers, we expect that a well-defined organ structure could be created, and the proposed method could contribute to the organ regeneration in the future.

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References

1. MS Bae, SI Jeong, SE Kim, *et al.*, Nanofiber scaffold for tissue regeneration using electrospinning method, *Tissue Eng Regen Med*, **5**, 2 (2008).
2. SH Yun, Y-J Kim, OK Kwon, *et al.*, Fabrication and characterization of biodegradable nanofiber containing gelatin. *Tissue Eng Regen Med*, **5**, 4-6 (2008).
3. YK Seo, JK Park, and SY Kwon, Effect of Collagen-GAG on the Anterior Cruciate Ligament Regeneration of Rabbit, *Tissue Eng Regen Med*, **7**, 5 (2010).
4. G Orive, SK Tam, JL Pedraz, *et al.*, Biocompatibility of alginate-poly-l-lysine microcapsules for cell therapy.*Biomaterials*, **27**, 20

(2006).
5. R Langer, New methods of drug delivery,*Science*, **249**, 4976 (1990).
6. AS Hoffman, Hydrogels for biomedical applications, *Advanced drug delivery reviews*, **54**, 1 (2002).
7. E Kang, GS Jeong, YY Choi, *et al.*, Digitally tunable physicochemical coding of material composition and topography in continuous microfibres, *Nature Materials*, **10**, 11 (2011).
8. E Kang, YY Choi, SK Chae, *et al.*, Microfluidic Spinning of Flat Alginate Fibers with Grooves for Cell-Aligning Scaffolds, *Advanced Materials*, **24**, 31 (2012).
9. Y Zheng, H Bai, Z Huang, *et al.*, Directional water collection on wetted spider silk.*Nature*, **463**, 7281 (2010).
10. H Bai, J Ju, Y Zheng, *et al.*, Functional Fibers with Unique Wettability Inspired by Spider Silks, *Advanced Materials*, **24**, 20 (2012).
11. TA Blackledge and CY Hayashi, Silken toolkits: biomechanics of silk fibers spun by the orb web spider Argiope argentata (Fabricius 1775), *Journal of Experimental Biology*, **209**, 13 (2006).
12. Y Zheng, H Bai, Z Huang, *et al.*, Directional water collection on wetted spider silk, *Nature*, **463**, 7281 (2010).
13. GA Silva, C Czeisler, KL Niece, *et al.*, Selective differentiation of neural progenitor cells by high-epitope density nanofibers, *Science*, **303**, 5662 (2004).
14. S-K Chae, E Kang, A Khademhosseini, *et al.*, Micro/Nanometer-scale Fiber with Highly Ordered Structures by Mimicking the Spinning Process of Silkworm, *Advanced Materials*, **25**, 22 (2013).
15. WJ Jeong, JY Kim, J Choo, *et al.*, Continuous fabrication of biocatalyst immobilized microparticles using photopolymerization and immiscible liquids in microfluidic systems, *Langmuir*, **21**, 9 (2005).
16. JK Nunes, K Sadlej, JI Tam, *et al.*, Control of the length of microfibers, *Lab on a Chip*, **12**, 13 (2012).
17. E Kang, SJ Shin, KH Lee, *et al.*, Novel PDMS cylindrical channels that generate coaxial flow, and application to fabrication of microfibers and particles, *Lab Chip*, **10**, 14 (2010).
18. Y Jun, MJ Kim, YH Hwang, *et al.*, Microfluidics-generated pancreatic islet microfibers for enhanced immunoprotection, *Biomaterials*, **34**, 33 (2013).
19. K Lee, C Kim, KS Shin, *et al.*, Fabrication of round channels using the surface tension of PDMS and its application to a 3D serpentine mixer, *Journal of Micromechanics and Microengineering*, **17**, 8 (2007).
20. SH Lee, HN Kim, and KY Suh, Use of directly molded poly (methyl methacrylate) channels for microfluidic applications, *Lab Chip*, **10**, 23 (2010).
21. GS Jeong, Y Jun, JH Song, *et al.*, Meniscus induced self organization of multiple deep concave wells in a microchannel for embryoid bodies generation, *Lab Chip*, **12**, 1 (2012).
22. GS Jeong, JH Song, AR Kang, *et al.*, Surface Tension-Mediated, Concave-Microwell Arrays for Large-Scale, Simultaneous Production of Homogeneously Sized Embryoid Bodies, *Advanced Healthcare Materials*, **2**, 1 (2012).