A 3D polydimethylsiloxane microhourglass-shaped channel array made by reflowing photoresist structures for engineering a blood capillary network

Hoon Suk Rho\textsuperscript{a,b,c}, Henk-Willem Veltkamp\textsuperscript{d}, Danielle Baptista\textsuperscript{a}, Han Gardeniers\textsuperscript{b}, Séverine Le Gac\textsuperscript{c,1}, Pamela Habibović\textsuperscript{a,1}.

\textsuperscript{a} Department of Instructive Biomaterials Engineering, MERLIN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, The Netherlands
\textsuperscript{b} Mesoscale Chemical Systems Group, MESA+ Institute for Nanotechnology, University of Twente, The Netherlands
\textsuperscript{c} Applied Microfluidics for BioEngineering Research Group, TechMed Institute, MESA+ Institute for Nanotechnology, University of Twente, The Netherlands
\textsuperscript{d} Integrated Devices and Systems Group, MESA+ Institute for Nanotechnology, University of Twente, The Netherlands

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**ABSTRACT**

This paper describes an innovative yet straightforward fabrication technique to create three-dimensional microstructures with controllable tapered geometries by combining conventional photolithography and thermal reflow of photoresist. Positive photoresist-based microchannel structures with varying width-length ratios were reflowed after their fabrication to generate three-dimensional funnel structures with varying curvatures. A polydimethylsiloxane hourglass-shaped microchannel array was next cast on these photoresist structures, and primary human lung microvascular endothelial cells were cultured in the device to engineer an artificial capillary network. Our work demonstrates that this cost-effective and straightforward fabrication technique has great potential in engineering three-dimensional microstructures for biomedical and biotechnological applications such as blood vessel regeneration strategies, drug screening for vascular diseases, microcolumns for bioseparation, and other fluid dynamic studies at microscale.

1. Introduction

Microstructures with well-defined three-dimensional (3D) profiles are of great interest in a variety of scientific and engineering fields, including optics [1–7], fluid dynamics [8], and biomedical technology [9,10]. To overcome the geometrical limitations existing with conventional channels with a rectangular cross-section, mainly related to varied shear stress and volume at the channels edges, rounded microchannels have been used as a fundamental element in miniaturized devices, e.g., construction of micro-sized membrane valves and peristaltic pumps [11–13], formation of two-phase emulsions [14,15], and smooth flow of particles and cells without clogging [16]. Recently, channels with a circular cross-section geometry were proposed for constructing physiological-like microvessels for the investigation of the structure and function of blood vessels [17–19] and for high-throughput pharmacological screens towards personalized treatment of vascular diseases [20].

One of the most well-known traditional techniques to fabricate round (ed) microchannels is by molding polymers or hydrogels following insertion of tubular components into the matrix [21,22]. Although this method allows facile fabrication and is applicable to a variety of casting materials, the dimensions and surface properties of channels are limited by the size and material properties of the inserts. Moreover, a long channel or channel network design is challenging to achieve due to the required removal of the insert. For the fabrication of complex channel designs, previous studies employed adapted chemical- and gas dry etching [9,23] and laser writing techniques [24] to achieve precise circular cross-section of microchannels. However, these microfabrication techniques required the use of non-biocompatible chemicals, and moreover, the fabrication of complex channel networks remained challenging. Recently, 3D sugar printing has been shown as a promising technique for creating perfusable networks with circular cross-sectional microchannels [25]. Successful creation of microchannel networks by casting PDMS over a 3D printed sugar glass network, followed by easy removal of sugar structures by dissolution, was demonstrated. Nevertheless, the limited resolution of the sugar printing did not allow the fabrication of structures smaller than 100 μm.

Thermal reflow is a process to soften polymers by heating them up

\textsuperscript{a} Corresponding author at: Maastricht University, 6229 ER Maastricht, Universiteitsingel 40, Room C3.577, The Netherlands.
\textit{E-mail address:} p.habibovic@maastrichtuniversity.nl (P. Habibović).
\textsuperscript{1} Equal contributions.

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above their glass transition temperature; as a consequence, polymers naturally rearrange to minimize the surface tension of the system [6,26]. The reflow process is one of the simplest and most effective techniques to obtain 3D photoresist structures with reliable profile and smooth curvature in photolithography. Following the first fabrication of cylindrical microstructures from photoresist [1], the technique has been exploited to create various rounded 3D structures, for example half-cylindrical microchannels [12] and symmetrical and asymmetrical microlenses [2,3,5,6,27–29]. The geometry of the generated photoresist structures has been proven to be affected by various parameters such as spinning speed [7], reflowing time [30], reflowing temperature [30], the surface tension of the resist [7], the aspect ratio of the structures [7,30], and the presence of a residual photoresist layer [31]. Also, polymeric micro-devices cast on the thermally refloowed photoresist structures have shown great potential for use as microfluidic components such as microchannels and valves [12,32,33], and in chemical and biomedical applications including protein analysis [34–36], DNA analysis [37,38], particle/cell trapping [39,40] and microvascular research [10]. However, the shape and dimensions of the cross-section of the microchannels produced in the previous studies were the same over the entire length of the channels [1–7,9–32]. Recently, several fabrication techniques have been introduced to realize 3D tapered structures by the combination of diffuselight exposure lithography and polymer embossing [32], integrating electron-beam grayscale lithography with polymer reflow [41], and photolithography with two photoresists with different melting temperatures [5,29]. Unfortunately, these techniques require repeated processing steps to generate tapered 3D structures, and their surface roughness is often not optimal for cell adhesion and proliferation. Similar, recent advances in 3D printing techniques have enabled the construction of various 3D tapered microstructures [42]; yet, it remains challenging to obtain structures with a size of tens of micrometers.

To address this challenge, here we propose an innovative yet simple method to fabricate half hourglass-shaped 3D structures by combining conventional photolithography with thermal reflowing of photoresists. Various 3D tapered photoresist-based structures were created by varying the dimensions of microstructures, including the ratio of the width of the channels and the length of the tapering between the different widths before the reflow process, and microchannels with different dimensions were fabricated and characterized to optimize the design parameters for obtaining controllable curvature of the 3D tapered structures. Next, these photoresist tapered structures were employed as molds for casting polydimethylsiloxane (PDMS) and generating hourglass-shaped microchannel network. Finally, the resulting microchannel network was seeded with primary human lung microvascular endothelial cells (HLMECs) to yield an artificial vascular network. We believe that our method is advantageous over previously reported methods to create 3D photoresist structures, like diffuser lithography [2,32], stepwise e-beam lithography [4,41], and chemical reflowing [27], paving the way for easy-to-construct hourglass-shaped microchannels and channel networks with varying radii for different biomedical and biotechnological applications.

2. Experimental methods

2.1. General overview of the reflow of photoresist patterns

The proposed microfabrication method for engineering 3D hourglass-shaped microstructures is based on soft lithography with an additional photoresist reflow process during master mold fabrication [26,43]. Photoresist structures were patterned on a silicon wafer by photolithography, followed by an additional heating step to obtain 3D tapered structures. Positive photoresists, e.g., AZ 4500, 9200, and 40XT series (Rohm and Haas Co., Marlborough, USA), and Olin 907 series (HLMECs) to yield an artificial vascular network. We believe that our method is advantageous over previously reported methods to create 3D photoresist structures, like diffuser lithography [2,32], stepwise e-beam lithography [4,41], and chemical reflowing [27], paving the way for easy-to-construct hourglass-shaped microchannels and channel networks with varying radii for different biomedical and biotechnological applications.

![Fig. 1. Thermal reflow of microstructures made of AZ 40XT-11D. A. The dimensional change of the structures before and after the reflow process was calculated and measured on microscopic images of the photoresist structures (middle) and corresponding PDMS replicas (bottom). B. Scanning electron microscope (SEM) images of AZ photoresist after thermal reflow of the photoresist (top) and corresponding PDMS replica (bottom).](image-url)
illustrated and presented in Fig. 1A (top and middle rows) together with the cross-sectional views of the corresponding PDMS replicas (Fig. 1A, bottom row). In this example, the initial height, width, and length of AZ 40XT-11D photoresist channel were 24 µm, 100 µm, and 2 cm, respectively, and the height of the channel increased to 33 µm after reflowing while the width and length of the structure remained constant. Scanning electron microscope (SEM) images of AZ photoresist and PDMS channels show a reliable rounded profile and smooth surface of the structure, which is advantageous for cell culture applications as compared to rectangular cross-sectional channels [44,45]. The various reflowed microstructure designs with different shapes and sizes further demonstrated the reliability and reproducibility of the process (Supplementary information Fig. S1).

2.2. Design and print of the photomask

A photomask was designed by computer-aided design (CAD) software, CleWin (WieWeb software, Hengelo, The Netherlands), which is comparable with other commercial CAD software, e.g., AutoCAD (Autodesk Inc., CA, USA), CorelCAD (Corel Co., Ottawa, Canada), and SolidWorks (Dassault Systèmes SE, Vélizy-Villacoublay, France). The CAD designs used in this paper are shown in Supplementary Fig. S2. The microfluidic channel designs were printed on a 5 ″ soda-lime glass by LBPG Heidelberg DWL200 (Heidelberg Instruments Mikrotechnik GmbH, Germany). We used a chromium photomask in this work, but emulsion glass mask and film mask could be used for the fabrication of photoresist microstructures larger than 3 µm at a relatively low cost.

2.3. Fabrication of the devices

The PDMS microfluidic devices were fabricated by conventional soft lithography technique on a master mold made by photolithography. The device fabrication consists of two main processes; fabrication of the master mold in a clean room and fabrication of PDMS replicas in a general chemistry laboratory. The schematic overviews on the fabrication steps and materials and equipment required for the chip fabrication and operation are shown in Fig. 2 and Table 1, respectively.

2.4. Fabrication of the master mold

The photoresist microstructures were patterned on a 4 ″ silicon wafer by a photolithography technique [26]. Briefly, hexamethyldisilazane (HMDS, Sigma-Aldrich, Zwijndrecht, The Netherlands) was spun onto a 4 ″ silicon wafer at 3000 rpm for 30 s to increase the photoresist adhesion on the wafer. Next positive photoresist (AZ 40XT-11D, MicroChemicals GmbH, Ulm, Germany) was spin-coated onto the wafer at a spin rate of 3000 rpm for 30 s and baked at 126 °C for 7 min. The wafer was exposed to UV light (12 mW cm⁻²) through the photomask for 35 s (EVG 620 mask aligner, EV Group, Sankt Florian am Inn, Austria), baked at 105 °C for 100 s, and developed in OPD 4262 developer (Arch Chemicals, Inc., Lonza Benelux B.V., Breda, The Netherlands) for 4 min. The resulting AZ 40XT-11D film thickness was 24 ± 1 µm. Thermal reflowing of the microstructures were processed by heating the wafer at 140 °C for 1 min. Next, the mold was exposed to the fume of chlorotrimethylsilane (CTMS, Sigma-Aldrich, Zwijndrecht, The Netherlands) for 10 min in a desiccator for surface hydrophobization of the mold which prevents the adhesion of PDMS during PDMS replica fabrication. A detailed protocol for the fabrication of the master mold is presented in Table 2.
Methods 190 (2021) 63–71

66

secure and tight connection of pins and pipet tips to prevent solution leaking during sample loading, and the bottom PDMS layer with a thickness of 2 mm to minimize the working distance as required for micropipetting. We cast the top PDMS layer with a thickness of 7 mm for flow distribution and the bottom PDMS layer with a thickness of 2 mm to minimize the working distance as required for micropipetting. Two uncured PDMS mixtures (RTV-615, Permacol B.V., Ede, The Netherlands, prepolymer:curing agent = 10:1) were prepared for PDMS-PDMS bonding and pressed, filtered air from the structured bottom side to remove debris in the device. Both PDMS substrates were cleaned with ethanol and blow-dried with compressed, filtered air from the structured bottom side to remove debris in punched holes. The top and bottom layers were aligned under a syringe pump, Harvard Apparatus, Holliston, USA. The scan speed, resolution, and contact force were 2.78 μm, 500 μm, and 24.5 μN, respectively.

2.5. Fabrication of the PDMS devices

PDMS substrates with microstructures were fabricated by soft lithography technique [12,43]. Hourglass-shaped PDMS microfluidic channels were obtained by combining and aligning two PDMS substrates, which both contained the same half hourglass-shaped structures. Two uncured PDMS mixtures (RTV-615, Permacol B.V., Ede, The Netherlands, prepolymer:curing agent = 10:1) were prepared for PDMS-PDMS bonding [12,36,40,46] and poured onto two wafers exhibiting the same photoresist patterns. We cast the top PDMS layer with a thickness of 7 mm for secure and tight connection of pins and pipet tips to prevent solution leaking during sample loading, and the bottom PDMS layer with a thickness of 2 mm to minimize the working distance as required for high-resolution imaging using an inverted microscope. The PDMS layers were cured at 80 °C for 45 min, peeled off from the wafers, and cut to the device dimensions. Holes for inlets and outlets were punched with a 25-gauge punch (Syneo Co., Angleton, USA) through the top substrate. Both PDMS substrates were cleaned with ethanol and blow-dried with compressed, filtered air from the structured bottom side to remove debris in punched holes. The top and bottom layers were aligned under a syringe pump, Harvard Apparatus, Holliston, USA. The scan speed, resolution, and contact force were 2.78 μm, 500 μm, and 24.5 μN, respectively.

2.7. Cell culture

Primary human lung microvascular endothelial cells (HLMECs, Cell Biologics Inc., Chicago, USA) were cultured in T25 flasks pre-coated with a gelatin-based solution (Cell Biologics Inc., Chicago, USA) and maintained in complete microvascular endothelial cell growth medium (Cell Biologics Inc., Chicago, USA). Medium was refreshed every 2–3 days. Cells were maintained at 37 °C in a 5 % CO₂ humidified atmosphere.

2.8. Cell seeding in the PDMS devices

Prior to seeding, PDMS chips were sterilized with 70 % ethanol for 12 h to enhance adhesion. In this research, PDMS was used for creating replicas from the photoresist structures; however, other polymers and hydrogels can be cast on the master mold. In the case of hydrogel structure fabrication, two hydrogel replicas can be combined by a twice cross-linking method, which brings the same bonding strength as a bulk hydrogel at the interface of the two replicas [47].

2.6. Analysis of the fabricated microstructures

The curvature of the top, parallel to the length of the photoresist structures, was measured with a Bruker Contour GT-I white light interferometer with Vision64 Analysis software (Bruker Nano Surfaces Division, Tucson, USA). Measurements were processed with the vertical scanning interferometry setting at 1× speed with a 20× objective and 0.55× digital magnification. Post-measurement processing was done using modal tilt removal. The curvature perpendicular to the length of the photoresist structures could not be measured with white light interferometry since the reflected light could not be collected by the objective. Instead, a Veeco Dektak model 8 contact profilometer was used. Measurements were performed with a stylus with a tip diameter of 2.5 μm. The scan speed, resolution, and contact force were 2.78 μm s⁻¹, 0.009 μm sample⁻¹, and 24.5 μN, respectively.

Table 1

| Materials and equipment required for the device fabrication and operation. |
|-----------------------------|-----------------------------|
| **Materials**                | **Equipment**               |
| Microfluidic connection and handling | | |
| - Precision stainless steel tip, 23 gauge, Nordson Co., Nordson Benelux B.V., Maastricht, The Netherlands | - Syringe pump, Harvard Apparatus, Harvard Bioscience Inc., Holliston, USA |
| - Tygon tubing, 0.020” ID × 0.060”OD, Cole-Farmer Instrument Co., Metrohm Nederland B.V., Barendrecht, The Netherlands | - Stereo microscope, Leica Microsystems B.V., Amsterdam, The Netherlands |
| - Glass petri dish | - Inverted fluorescence microscope, Nikon Eclipse Ti2, Nikon Instruments Europe B.V., Amsterdam, The Netherlands |
| - Plastic pipette | - Laser scanning confocal microscope, Leica SP8 STED, Leica Microsystems B.V., Amsterdam, The Netherlands |
| - Plastic desiccator | - | |
| - Scalpel | - | |
| - Punch, 25-gauge, Syneo Co., Angleton, USA | - | |

Table 2

| Fabrication protocol for patterning photoresist structures on a silicon wafer. |
|-----------------------------|-----------------------------|
| **Promoting photoresist adhesion on the wafer with HMDS** | | |
| - Deposit a drop of HMDS on the wafer. | - | |
| - Spin coat the wafer with a ramping process at 500–1500 rpm for 5 s, 500 rpm for 5 s, 500–1000 rpm for 5 s, and 1000 rpm for 5 s, respectively. | - A Z40XT-11D coating on the wafer | | |
| - Develop the AZ 40XT-11D coating on the wafer | - | |
| - Spin coat the wafer with a ramping process at 0–500 rpm for 5 s, and 500 rpm for 5 s, 500–1000 rpm for 5 s, and 1000–2000 rpm for 5 s, and 2000 rpm for 30 s. | - | |
| Pre-exposure baking, UV exposure, and post-exposure baking | - | |
| - Bake the AZ 40XT-11D coated wafer on a hot plate at 125 °C for 7 min. | - A Z40XT-11D coating on the wafer | | |
| - Leave the wafer at room temperature for 10 min. | - | |
| - Expose the wafer under a UV light (12 mW cm⁻²) for 35 s. | - | |
| - Leave the wafer at room temperature for 10 min. | - | |
| - Bake the wafer on a hot plate at 105 °C for 100 s. | - | |

Developing AZ 40XT-11D

- Developing AZ 40XT-11D in OPD 4262 developer for 4 min.
- Rinse the wafer with DI water and dry with filtered nitrogen gas.
- Reflowing AZ 40XT-11D

- Bake the wafer on a hot plate at 140 °C for 1 min.
- Hydrophobisation of the wafer

- Place 1 ml of CTMS in a small open container in a desiccator.
- Place the wafer in the desiccator for 10 min.
15 min, washed with medium and coated with the same gelatin-based solution for 5 min. The HLMECs suspension, at a density of $6 \times 10^5$ cells ml$^{-1}$, was injected into the channels, and the chips were incubated in a humidified 5% CO$_2$ incubator at 37$^\circ$C for 2 h for cell attachment. After incubation, HLMECs were seeded again, and the devices were rotated by 180$^\circ$ to ensure homogeneous cell coverage along the channels. Cells were allowed to adhere for 2 h before perfusion of HLMEC complete medium at a flow rate of 20 $\mu$l h$^{-1}$.

2.9. Fixation and immunofluorescent staining of HLMVECs

Cells were fixed after 72 h of culture in the PDMS channels using 4% (w/v) paraformaldehyde solution (PFA, Sigma-Aldrich, Zwijndrecht, The Netherlands) in phosphate buffer saline (PBS, Thermo Fisher Scientific Inc., Landsmeer, The Netherlands) during 30 min at room temperature. After fixation, samples were washed three times with PBS and permeabilized with 0.1% Triton-X 100 (Sigma-Aldrich, Zwijndrecht, The Netherlands) in PBS for 10 min at room temperature. Cells were then blocked in CAS-Block (Thermo Fisher Scientific Inc., Landsmeer, The Netherlands) for 1 h at room temperature. Cell nuclei and filamentous actin (F-actin) were stained with DAPI (Sigma-Aldrich, Zwijndrecht, The Netherlands) for 20 min, and Alexa Fluor 647 Phalloidin (Thermo Fisher Scientific, Eindhoven, The Netherlands) for 30 min, respectively, both at room temperature.

2.10. Confocal imaging

3D imaging of HLMECs on the channel surface was performed using a Leica SP8 STED laser scanning confocal microscope (Leica Microsystems B.V., Amsterdam, The Netherlands). Z-stack images were acquired with a step size of 0.8 $\mu$m. Orthogonal projections and cross-sections were taken using Leica analysis software (Leica Application Suite X, Leica Microsystems B.V., Amsterdam, The Netherlands).

3. Results and discussion

3.1. Fabrication of a half hourglass-shaped 3D microstructure and characterisation of the curvature

To create the desired half hourglass-shaped 3D microstructures, a design was drawn including a neck separating between two tapered structures with two final widths. Fig. 3A presents such a typical design, where the widths of the widest and narrowest parts and the height of the channel are $W_1$, $W_2$, and $H$, respectively. These structures were fabricated from AZ 40XT-11D photoresist, and the initial resist channel had a constant height, which was determined by spin speed for resist coating. Following the reflowing process, the volume of the photoresist in the narrower region was transferred to the wider region at the neck of the hourglass channel due to internal pressure differences (Supplementary information Fig. S3), as depicted in the same Fig. 3A. Since the internal...
Table 3
Dimensions of the AZ photoresist microstructures fabricated to characterize dimension changes as a result of the thermal reflow process. The angle ($\theta$) was calculated from the measured values of the lowest and highest channel heights ($h_1$ and $h_2$) at the tapered region after reflowing the structures. The values of $\theta$ were plotted against the lengths (L), and fitted in a nonlinear regression model.

| $W_1$ [µm] | $W_2$ [µm] | $W_2/W_1$ Ratio | L [µm] | $\theta$ | $\theta_0 + a e^{-bL}$ |
|------------|------------|-----------------|--------|--------|----------------------|
| 50         | 50         | 1.0             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| 0          | 0.1        | 0.1             | 0      | 0.1    | 0                    | 0      | 0     | 0    | 0     | 0     |
| 50         | 40         | 0.8             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 7.5        | 5.5             | 3.2    | 2.8    | 1.8                  | 1.5    | 1.4   | 1.2  | 1.2   | 1.2   |
| 50         | 30         | 0.6             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 20.1       | 12.6            | 8.4    | 5.7    | 5.1                  | 4.1    | 3.4   | 3.0  | 3.0   | 3.0   |
| 50         | 20         | 0.4             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 31.0       | 22.1            | 16.6   | 10.9   | 10.4                 | 9.1    | 7.7   | 6.7  | 6.7   | 6.7   |
| 50         | 10         | 0.2             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 37.6       | 28.7            | 20.5   | 15.4   | 12.9                 | 10.5   | 9.0   | 7.2  | 7.2   | 7.2   |
| 100        | 100        | 1.0             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 0.1        | 0.1             | 0      | 0      | 0                    | 0      | 0     | 0    | 0     | 0.1   |
| 100        | 80         | 0.6             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 4.8        | 3.5             | 2.8    | 2.3    | 1.9                  | 1.5    | 1.2   | 1.1  | 1.1   | 1.1   |
| 100        | 60         | 0.6             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 11.9       | 9.9             | 7.8    | 6.2    | 4.7                  | 3.6    | 2.7   | 2.3  | 2.3   | 2.3   |
| 100        | 40         | 0.4             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 23.4       | 18.1            | 14.6   | 11.1   | 8.8                  | 6.8    | 5.7   | 6.2  | 6.2   | 6.2   |
| 100        | 20         | 0.2             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 32.6       | 25.1            | 20.9   | 15.4   | 12.9                 | 12.3   | 10.4  | 8.8  | 8.8   | 8.8   |
| 150        | 150        | 1.0             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 0          | 0.1             | 0      | 0      | 0                    | 0      | 0     | 0    | 0     | 0     |
| 150        | 120        | 0.8             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 3.6        | 3.1             | 2.6    | 2.2    | 1.8                  | 1.5    | 1.2   | 1.0  | 1.0   | 1.0   |
| 150        | 90         | 0.6             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 9.2        | 7.7             | 6.5    | 5.4    | 4.4                  | 3.6    | 3.0   | 2.4  | 2.4   | 2.4   |
| 150        | 60         | 0.4             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 18.1       | 15.1            | 12.7   | 10.3   | 8.2                  | 6.8    | 5.4   | 4.5  | 4.5   | 4.5   |
| 150        | 30         | 0.2             | 25     | 50     | 75                   | 100    | 125   | 150 | 175   | 200   |
| $\theta$   | 31.3       | 26.9            | 21.5   | 16.0   | 12.9                 | 11.0   | 9.0   | 7.6  | 7.6   | 7.6   |

Fig. 4. Hourglass-shaped microchannel produced by aligning two half hourglass-shaped structures. A. Photomask design of the half hourglass-shaped structures with various widths ($W_1 = 150$, 100, and 50 µm) but a constant width ratio ($W_2/W_1 = 0.2$). Scale bar, 1 mm. B. Aligned and bonded PDMS layers forming perfectly sealed channels; channels are filled in with food dye for visualization purposes. C. Microscopic images of the channels; top view (top) and cross-sections (bottom). The dashed and dotted lines on the top view indicate the location of cutting of the channels for examination of the channel cross-sections. Scale bars, 100 µm.
pressure was inversely proportional to the width of the channel, the channel height, which was determined by the volume of the photoresist, increased in proportion to the channel width. As a result of these changes, the height of the reflowed resist channel differed depending on the channel width. Fig. 3B presents the height measurements of one specific refloowed channel ($W_1$, $W_2$, and $L = 100, 60, \text{and} 200 \mu m$) with white light interferometry, illustrating that the resist height increased linearly with the channel width from the neck of the structure to the end of the tapered structure. Scanning electron microscopy (SEM) imaging was also performed after the reflowing process, as depicted for a photoresist channel characterized by $W_1$, $W_2$, and $L$, which were 50 $\mu m$, 10 $\mu m$, and 25 $\mu m$, respectively, in Fig. 3C (left). Finally, PDMS was cast on the resulting microstructure, showing a glossy surface and well-defined curvature (Fig. 3C (right)).

To systematically characterize the effect of the two channel widths ($W_1$ and $W_2$) and length ($L$) on the height change of the resist structures after the thermal reflow, we designed various channels with different combinations of $W_1$, $W_2$, and $L$, as summarized in Table 3. The smallest structure in the designs was 10 $\mu m$; however, structures smaller than 1 $\mu m$ can be fabricated by using a high-resolution mask. All designs were patterned in AZ 40XT-11D and reflowed, as before, and the lowest and highest channel heights ($h_1$ and $h_2$) were measured to calculate the angle ($\theta$) as a measure of the curvature of the structure at the tapered region (Fig. 3A, right). The graphs in Fig. 3D show the relationship between the angle ($\theta$), the ratio of channel widths ($W_2$ and $W_1$), and the channel length ($L$). The curvature (estimated here through $\theta$) exponentially increased as the channel length ($L$) decreased at a constant $W_2$-to-$W_1$ ratio, and the channel width $W_2$-to-$W_1$ ratio decreased when the length ($L$) remained constant. Calculating the angle ($\theta$) at three different $W_2$ values (50 $\mu m$ (top), 100 $\mu m$ (middle), and 150 $\mu m$ (bottom)) reveals that the curvature increased with the decrease of $W_2$ where the $W_2$-to-$W_1$ ratio and length are constant. The maximum value of $\theta$ of 37.6 was found at the minimum values of $W_1$, $W_2$, and $L$, which were 50 $\mu m$, 10 $\mu m$, and 25 $\mu m$, respectively. Regression coefficients in the relationship between the angle ($\theta$) and length ($L$) are summarized in Table 3.

3.2. Fabrication of PDMS microhourglass structures

By combining and aligning two symmetric half hourglass-shaped PDMS microstructures, an hourglass-shaped PDMS microchannel was formed. The resulting microfluidic chip included three independent devices consisting each of one channel, with various widths ($W_1$), 150, 100, and 50 $\mu m$, but a constant width ratio ($W_2$/$W_1$) of 0.2 (Fig. 4A). The two PDMS substrates were cast using PDMS mixtures with different prepolymer-to-curing agent ratios, 7:1 for the top layer, and 10:1 for the bottom layer, partially cured, aligned, and cured overnight for PDMS-PDMS bonding [12,36,40,46]. Oxygen plasma treatment could be an alternative method for bonding the two PDMS layers [43]; however, the hydrophilic surface of plasma-treated PDMS substrates limited re-adjusting of the layers during the alignment process. For accurate alignment of the microstructures, the two PDMS substrates were handled under a stereo microscope equipped with a CMOS camera. The smallest feature we designed in this work was of 10 $\mu m$ at the neck of an hourglass-shaped channel having a width of 50 $\mu m$, the resulting microfluidic device being presented in Fig. 4B. Food dye solutions with different colors were introduced in the chip for the visualization of the three independent channels. Fig. 4C shows microscopic images of the aligned channels as top views (top) and cross-sectional views after
3.3. Fabrication of a tubular PDMS microchannel network

A tubular PDMS channel array was fabricated by combining two PDMS parts to create a simple artificial blood vessel network and mimic blood capillaries (Fig. 5A and B). The network consisted of 8 parallel microfluidic channels with a diameter of 50 μm, which allowed easy cell seeding and culture without clogging. These channels were connected to one single inlet and one single outlet through 3 branches having a width of 150 μm. Although the channels of the artificial vessel network are in one plane, unlike in the case vascular networks in vivo, the network design is promising for constructing an in vitro model of a physiological-like network with various sizes and shapes of channels. To demonstrate the suitability of the platform for in vitro cell culture-based studies, HLMECs were introduced into the channel array by a seed-and-rotate method, as described in the experimental section, allowed to attach, and cultured for 72 h. Fig. 5C presents a fluorescence microscopy image, showing HLMEC cells successfully and viably adhered to the microchannel surface, including in the tapered regions. Confocal microscopy was employed to evaluate the formation of blood capillaries in these structures; as presented in Fig. 5D, HLMECs lined the entire channel wall at the center of the narrowest channel after 72 h of culture, demonstrating that the platform can be used for engineering an artificial blood capillary with good control over the “blood vessel” diameter (Fig. 5D).

4. Conclusions

In this work, we established a simple approach to create a 3D microscale hourglass-shaped channels by single-step photolithography in combination with the thermal reflow of photoresist structures. The dimensional design factors, including channel width and length, were validated to achieve controlled curvature of 3D tapered resist structures. A tubular microchannel network with various sizes and shapes was fabricated by combining two PDMS semi hourglass-shaped channels, and HLMECs were seeded and successfully cultured in these channels to provide a proof-of-concept for engineering of a blood vessel-like network. We believe that the technique has great potential in the microfabrication of 3D topographic structures for chemical and biomedical applications including bioseparation, lab-on-a-chip devices, and bio-inspired platforms. In the context of the specific use for creating a physiological blood vessel network as demonstrated here, this technique may be suitable for establishing a high-throughput platform for screening drugs for the treatment of vascular diseases and/or for testing vascular regenerative strategies.

CRediT authorship contribution statement

Hoon Suk Rho: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - original draft. Henk-Willem Veltkamp: Software, Investigation, Writing - review & editing. Danielle Baptista: Investigation, Writing - review & editing. Han Gardeniers: Conceptualization, Writing - review & editing. Sèverine Le Gac: Supervision, Writing - review & editing. Pamela Habibovic: Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymeth.2020.03.007.

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