High yield fabrication of multilayer polydimethylsiloxane devices with freestanding micropillar arrays

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A versatile method to fabricate a multilayer polydimethylsiloxane (PDMS) device with micropillar arrays within the inner layer is reported. The method includes an inexpensive but repeatable approach for PDMS lamination at high compressive force to achieve high yield of pillar molding and transfer to a temporary carrier. The process also enables micropillar-containing thin films to be used as the inner layer of PDMS devices integrated with polymer membranes. A microfluidic cell culture device was demonstrated which included multiple vertically stacked flow channels and a pillar array serving as a cage for a collagen hydrogel. The functionality of the multilayer device was demonstrated by culturing collagen-embedded fibroblasts under interstitial flow through the three-dimensional scaffold. The fabrication methods described in this paper can find applications in a variety of devices, particularly for organ-on-chip applications. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4827600>]

I. INTRODUCTION

Microfluidic devices are being extensively investigated for applications such as rapid diagnostics and novel cell cultures for organ-on-chip models.^{1,2} Polydimethylsiloxane (PDMS) is by far the preferred microfluidic material choice and elaborate three-dimensional multi-layer structures in PDMS with complex functionalities have been demonstrated including on-device fluid actuation³ and sophisticated cell separation.⁴ Multilayer PDMS structures have been demonstrated using a variety of approaches, generally based on the stacking of 2D patterned thin layers.^{5,6} For the requirement of fluidic connections between layers (vertical fluidic vias), a variety of approaches have been investigated,⁷ and recently a high-yield batch process has been reported⁸ that overcomes the typical issue of residual PDMS in the via blocking flow.

Pillars are a complementary structure to vias which have importance in multilayer PDMS devices. PDMS micropillars, with dimensions on the order of 100 μm , have been exploited to achieve fluid actuation,³ droplet merging,⁹ cell separation,¹⁰ solid supports for assay,¹¹ as well as structures for containment of hydrogels for 3D cell culture.^{12,13} In this paper, the challenge of high yield fabrication of micropillars within the inner layer in PDMS devices is addressed.

Micropillars can be readily molded in PDMS and used in the top or bottom layers of multilayer structures. However, inclusion of pillars in an inner layer introduces a variety of challenges because these structurally unconnected components will be lost in the assembly process if not properly attached to a carrier or another device layer at all times. High yield processes are required for attachment of the pillars to a carrier film, transfer and de-bonding from the carrier, and attachment to another device layer which may not be PDMS. An approach for transfer and release of PDMS micropillars were reported that used a water-soluble transfer carrier, polyvinyl alcohol, which was dissolved rather than delaminated after transfer.¹⁴ Such an approach raises concerns about polyvinyl alcohol residue in the device and its effect on sensitive assays

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such as primary cell cultures. We have developed a method that does not rely on sacrificial materials and therefore avoids any contamination issues. Here, an inexpensive, high yield process for molding of micropillar arrays on a carrier film and a one-step transfer approach, effective on a variety of polymer substrates including nanoporous membranes, are described.

The test vehicle for this process was a multilayer PDMS device designed with an inner layer including an array of pillars serving as a cage for containment of hydrogels. This configuration emulates a tissue interstitial region for applications such as organ-on-chip technology, which benefit from the integration of miniaturized 3D gels to achieve enhanced cellular functionality and to investigate crucial cell-extracellular matrix interactions.^{12,15} Many microfluidic cell culture devices also include horizontally integrated porous membranes serving as cell supports and mimicking multiple tissue interfaces including vascular and/or lymphatic endothelium, epithelium, as well as stromal and tumor cells.^{16,17} Therefore, the test vehicle included two porous polycarbonate membranes to demonstrate compatibility of the pillar transfer process with membrane-integrated PDMS devices. This configuration enables the development of physiologically relevant cellular models for a variety of tissues, in particular highly vascularized tissues such as liver, pancreas, and lung where functional epithelial cells are separated by a thin layer of extracellular matrix (or stroma) from the endothelial cells lining capillaries. Many physiological and pathophysiological responses involve cell interactions with the perivascular microenvironment which consists of pericytes, smooth muscle cells, and fibroblasts embedded in the extracellular matrix. A hydrogel containment structure in the inner layer of a multilayer PDMS device such as the one of the demonstrated in this work is a platform enabling the development of sophisticated tissue models recapitulating tissue microarchitecture including stroma.

The fabrication process used a Kapton film carrier (as proposed by Epshteyn *et al.*¹⁸) which is conveniently commercially available, as opposed to a custom made PDMS carrier.⁵ The Kapton carrier enabled handling and shrinkage-free transfer of delicate thin films of PDMS³ and avoided issues of contamination of water soluble carriers, such as PVA.¹⁴ An inexpensive lamination apparatus was assembled to apply relatively large compression forces. The process parameters which were critical to achieving a >95% yield of pillar molding and transfer bonding are described, and the repeatability is characterized. The utility of the approach was demonstrated in the fabrication of a complex microfluidic structure containing freestanding micropillars within the inner layer and integrated polymer membranes. As a proof-of-concept demonstration of this approach for organ-on chip applications, we cultured normal human lung fibroblast in a collagen hydrogel contained in the inner layer of the fluidic device and under interstitial flow through the three-dimensional collagen scaffold.

II. EXPERIMENTAL METHODS

A. Test structure design

The multilayer PDMS test structure consists of three vertically stacked and aligned channels, separated by two horizontal nanoporous membranes as shown in Fig. 1(a). The middle layer layout includes a central gel cage with two rows of micropillars for hydrogel containment and two parallel microfluidic channels designed to deliver culture media as shown in Fig. 1(b). An auxiliary fluidic channel is included to inject the gel liquid monomer and polymerize the gel *in situ*. Two cage configurations were designed as detailed in Table I. The cage 1 configuration was designed to emulate interstitial flow across the width of the gel. The cage 2 configuration was designed for flowing media along the length of the parallel fluidic channels and is thinner and longer. For both designs, the 52 × 38 mm die include alignment features and round 1 mm diameter posts along the perimeter of the die (Fig. 1(c)), for the purpose of uniform pressure application during molding.

B. Silicon mold fabrication

Silicon masters for molding of PDMS were fabricated by deep reactive ion etching (DRIE) of 6 in. silicon wafers. Etching of silicon was chosen rather than using SU-8 photoresist

FIG. 1. (a) Exploded view of multilayer test structure including three PDMS layers and two nanoporous polycarbonate membranes. The middle PDMS layer contains freestanding micropillars. (b) Illustration of central section of the gel cage formed by micropillars and surrounding flow channels. (c) Die layout for PDMS molds showing top (green), middle (blue) and bottom (red) layers.

because deep molds ($>200\ \mu\text{m}$) were required for the device design. At these thicknesses, it is difficult to obtain straight sidewalls and to resolve small pillars in SU-8, and processing times are lengthy. The silicon wafers were patterned using $16\ \mu\text{m}$ thick photoresist (THB-111N) and mylar photolithography masks. Etch depths ranging from $100\ \mu\text{m}$ to $280\ \mu\text{m}$ were obtained by varying etch times. On selected masters $300\ \text{nm}$ of silicon oxide was deposited by plasma-enhanced CVD (Plasmalabplus 80) to facilitate pillar release. After dicing, the silicon molds were coated with the fluorosilane anti-adhesion coating FDTS, (heptadecafluoro-1,1,2,2-tetrahydrodecyltrichlorosilane, Gelest) by vapor deposition (MVD-100, Applied Microstructures).

C. Design of lamination apparatus

A custom apparatus was designed for lamination of the middle PDMS layer with freestanding pillars to a Kapton film carrier. The design was based on the sandwich system reported by Moraes and colleagues³ but modified for reproducible device fabrication by using standardized materials and controllable force application. The stack shown in Fig. 2 is used to sandwich PDMS between the master and the semi-clear Kapton film carrier during the PDMS cure cycle. A $50 \times 75\ \text{mm}$ FDTS coated glass slide serves as a flat compressive surface and a convenient transport vehicle. A compliant $800\ \mu\text{m}$ thick silicone piece (9010K11, durometer 20 A, McMaster-Carr), cut to the size of the silicon master, ensures even force application across the surface of the silicon master. Because excess uncured PDMS flows out of the mold during compression, an extra Kapton layer is used to facilitate the removal of the master from the glass slide.

The compression plate consists of $12\ \text{mm}$ thick Delrin block (PDMS does not adhere to Delrin) bolted to a $12\ \text{mm}$ thick aluminum plate that prevents the Delrin from bowing and deforming during the elevated temperature cure of PDMS. The compression plate slides up and down four $0.5\ \text{in.}$ diameter guide posts. A top plate ($12\ \text{mm}$ aluminum) was mounted on top of the guide post and a single brass bolt threaded in the center applies force to the compression plate. A torque wrench ($0\text{--}120\ \text{in-oz}$) is used to tighten the screw to a reproducible force level.

TABLE I. Geometrical parameters of the pillar designs.

Layout	Pillar size (μm)	Pillar pitch (μm)	Number of pillars	Cage dimensions (mm)	Die compression area (mm^2)
Gel cage 1	250×250	400	48	1.2×10	125
Gel cage 2	150×200	350	126	0.4×22	99

FIG. 2. (a) Multilayer stack of rigid and compliant material placed in custom lamination tool. (b) Photograph of the compression apparatus with two press units. A Kapton-laminated PDMS layer attached to the silicon mold is shown in front of the apparatus.

Two compression units were assembled on an 8" $\times$ 8" optics breadboard as shown in Fig. 2(b), and the entire assembly fits conveniently into the PDMS curing oven.

D. Molding process

Sylgard 184 (Dow Corning, from Ellsworth Adhesives) was mixed in 10:1 ratio and poured onto the silicon masters. For the top and bottom layers, the molds were poured with an excess of PDMS extending above the master and cured. For the middle layer, a small amount of PDMS was spread evenly across the master. An oxygen plasma treated 125 μm -thick Kapton film carrier (2271K3, McMaster-Carr) was placed on the PDMS coated master and degassed. The oxygen plasma promoted adhesion of the cured PDMS to the Kapton to allow peeling it off the master. Care was taken when placing the kapton film to avoid trapping air bubbles. The Kapton covered master was placed in the pre-heated lamination apparatus under uniform high compression force during cure at 65 $^{\circ}\text{C}$.

E. Multilayer device assembly

Devices were obtained by bonding PDMS layers and polymer membranes using the sequence shown in Fig. 3. Optically transparent polycarbonate membranes (Cyclopore Thin Clear, 7091-4710, Whatman) with 1 μm pore size and 10 μm thickness were used. All oxygen plasma treatments were performed at 200 W using the MVD-100 tool. Polycarbonate membranes were bonded to the top and bottom PDMS layers according to the process described by Ref. 19. Briefly, the membranes were plasma treated for 300 s and immediately soaked in a 5% aqueous solution of aminopropyltriethoxysilane (APTMS, Gelest) at 80 $^{\circ}\text{C}$ for 20 min. PDMS films were plasma treated for 20 s and the aminosilane treated membrane was contact bonded. The next step was the transfer of the micropillars from the Kapton carrier onto the lower PDMS layer with bonded membrane. The micropillars were treated with oxygen plasma for 20 s followed by thermocompression bonding at 65 $^{\circ}\text{C}$ for a time of at least 10 h with a weight of at least 500 g. The bonding was done under a stereoscope looking through the transparent Kapton film to the bottom channel layer with a custom alignment jig. The jig included a transparent polycarbonate vacuum chuck to hold the samples and a micropositioning translation stage so that bonding alignment accuracy of $\pm 50 \mu\text{m}$ was routinely achieved. After the thermocompression bonding, the Kapton film was released by soaking the sample in an isopropanol bath. The top PDMS layer with bonded membrane was then attached to the middle layer by a second thermocompression bonding step. Fluidic connections were achieved using right angle 22 gauge stainless steel connectors (SC22/15RA, Instech Laboratories) press fit and glued to punched holes in PDMS.

F. Cell culture and hydrogel sample preparation

Normal human lung fibroblast cells (NHLF's), obtained from the UNC Cystic Fibrosis/Pulmonary Research and Treatment Center at the passage P6, were cultured in DMEM

FIG. 3. Process flow for multi-layer device assembly.

(10–013-CV, Corning Cellgro) supplemented with 10% FBS (vol/vol; Phenix Research) and passaged every 3 to 5 days at a 1:4 ratio. All cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. NHLF's for experiments were used between passages P8-P12. Collagen hydrogels were prepared at concentrations of 2 and 5 mg/ml by neutralizing with NaOH an acidic rat tail collagen I solution (10.76 mg/ml, BD Biosciences). For cellularized gel, the NHLF's were trypsinized, centrifuged at 600 g for 5 min, re-suspended in culture media and counted. Cells and 5X DMEM were added on ice to the neutralized solution for a final concentration of 1×10^6 cells/ml in 5 mg/ml collagen. The cell-collagen mixture was injected, on ice, into the loading channel of the fluidic device until it filled the cage area. The device was then placed in a 37 °C humidified atmosphere containing 5% CO₂ where collagen polymerizes. Devices were left in the incubator overnight and then were connected to a gravity driven flow system using reservoirs with medium at different heights. An average interstitial flow rate of 0.1 μ l/min across the gel cage was established by connecting the reservoirs to flow channels adjacent to the gel cage. Viability of cultured NHLF's was assessed by adding an appropriate concentration (4 μ M Calcein AM and 2 μ M Ethidium homodimer-1 in Live Cell Imaging Solution) of viability assay reagent (LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells, Life Technologies) and incubating for 30 min at 37 °C. The fibroblast containing gel in the device was flushed with fresh Live Cell Imaging Solution and then imaged on an inverted fluorescence microscope.

III. RESULTS AND DISCUSSION

A. Lamination apparatus

Our objective was to design an apparatus providing a more reproducible force than a crude C-clamp solution and more cost-effective than a calibrated motor controlled compression instrument. The molding apparatus with the single central pressure point combined with the Kapton carrier produced consistent and uniform through-hole PDMS layers. Earlier versions of the apparatus utilized four pressure points located at the mold corners which resulted in an uneven application of force and breaking of the silicon masters. The consistency of the force applied by the torque driver was characterized by placing a spring into the mold press. The spring compression length Δx was measured using a digital caliper as function of torque. The spring

elastic constant K was measured separately, using an Instron 5900 series mechanical tester and found to be $K = 3.5 \text{ KgF/mm}$. The applied force was obtained by $F = -K\Delta x$ and it is plotted as function of the torque in Fig. 4(a). Three separate measurements were carried out on each of the two identical presses and the graph shows the reproducibility of the applied force. The apparatus enables molding of patterns requiring large compression forces, which would be impractical to achieve by applying a weight as load. We found that high yield micropillar molding required 140N compression (equivalent to 31 pound-force); this would require an impractically large volume block (e.g., $15 \times 15 \times 8 \text{ cm}$ for stainless steel) to apply the same load.

B. Pillar molding

The lamination apparatus performance was evaluated with both gel cage designs and a test design without pillars. The design without pillars is a continuous film with relatively large features. It was faithfully molded using the custom-lamination process with FDTs-coated silicon masters with thicknesses ranging from $100 \mu\text{m}$ to $280 \mu\text{m}$, and a wide range of processing conditions were acceptable. For the design without pillars, a 100 N compression force was applied and curing at 65°C for any time greater than 45 min was found to be adequate to provide a thin film with no PDMS residue in the through-hole area. The PDMS-Kapton film was then peeled off the master at room temperature and the thin film PDMS, now bonded to the Kapton carrier, was further cured for 5 h. In this process, the time requiring the lamination apparatus is only 45 min per sample, after which time the apparatus is available for use with the next sample.

Molding of the designs with pillars was done using masters with $150 \mu\text{m}$ deep features, a thickness more than adequate for a number of applications and corresponding to an approximate 1:1 ratio of depth to feature size. The thin film molding of the pillar layouts was more challenging than the layout without pillars and required significant process optimization. The conditions described above resulted in pillar yields of 0–50%. The yield was defined as the number of pillars adhered to the Kapton carrier divided by the total number of pillars in the design. Multiple parameters were investigated that did not consistently improve yield including: increasing the curing time under compression from 45 min to overnight, varying the temperature of mold-master separation (-40°C , room temperature and 65°C), and increasing oxygen plasma treatment of the top Kapton layer from 30 s to 300 s. The silicon master FDTs treatment was varied with increased reaction time and decreased vapor pressure with no molding yield improvement.

We tested the effect of compression pressure up to values as high as 275N, which can be conveniently obtained with this lamination apparatus. Increasing compression pressure resulted

FIG. 4. (a) Force calibration results of the two compression press units as function of the torque. Each point shows mean and standard deviation from 3 measurements. (b) Outcome of repeated molding tests for cage 2 designs. The molding yield is defined as the relative number of pillars molded and transferred from the silicon master to the Kapton carrier. The molds were “cleaned” between processes by curing a two mm thick layer of PDMS on them and peeling it off. The arrow indicates the mold was recoated with FDTs.

in only slight increases in yield to approximately 50%, which is a not acceptable value for implementation in a device. The critical change for achieving high transfer yield was coating the silicon master surface with 300 nm SiO₂ prior to the FDTS treatment which improved yield to an average $96.2 \pm 2.0\%$ ($n=5$) for cage 1 and $96.7 \pm 1.8\%$ ($n=5$) for cage 2 design. Scanning electron microscope imaging did not reveal topographical differences on the roughness of the via walls; so we interpret the yield improvement as improved anti-stiction of fluorosilanes on relatively thick oxide surfaces as compared to thin native oxide of the silicon wafer.²⁰ We repeated the evaluation of the compression force dependence with SiO₂-FDTS coated masters and found that the 100N molding force used for design without pillars is still inadequate and that a compression of 140N or larger is required to achieve >95% yield. At either of these forces no residual PDMS was observed between the pillars or in the flow channels. It has been reported that often the sandwich molding technique leaves a thin film of PDMS rather than a clear via.³ We observed this micromembrane formation during early tests, before the stack of soft and hard material for the molding process was optimized to the structure described in Sec. II C. Using the described lamination apparatus and procedure, the thin film PDMS residue was no longer an issue, likely because of the uniformity of the applied force. We note that the PDMS micromembrane formation has also been attributed to uneven height of SU-8 masters.³ Our approach uses silicon etched masters with uniform feature height, therefore eliminating this factor in the formation of thin film residues.

We also characterized the repeatability of the molding process using the same master. For the molds without pillars, the masters were found to be reusable without cleaning or recoating with FDTS. For the designs with pillars, it was necessary to remove any non-transferred pillars from the master prior to re-use. The best approach for removal of these pillars is to mold a thick layer of PDMS with the master which, when peeled off, removes the remaining pillars along with any thin residue. This procedure enables high yield (>80%) for three to four consecutive moldings. Fig. 4(b) illustrates the molding yield from cage 2 oxide-coated molds as a function of the number of uses. When the molding yield decreased below an acceptable level, the master was cleaned in an ozone chamber for 20 min and re-coated with FDTS with full recovery of yield value, including several samples with 100% yield. The same recovery of yield after recoating for consecutive moldings was observed for cage 1 molds (data not shown).

C. Evaluation of gel cage

The gel cage with two adjacent fluid channels was designed to investigate interstitial flow across the collagen gel, in a configuration similar to the one reported by Sudo *et al.*¹³ The collagen cage pillar designs were selected based on a report¹² indicating that gels surrounded by a staggered array of pillars with dimensions larger than 100 μm are able to withstand pressure driven interstitial fluid flow. A simple single-layer PDMS molding of the gel cage layout bonded to a PDMS lid was used to verify that cage design 1 with acellular collagen I is able to withstand pressure driven flow across the gel. Visual observation of a dye solution verified uniform flow across the gel cage without gel delamination or fracture for flow up to 0.5 $\mu\text{l}/\text{min}$ (Fig. 5(a)). To evaluate long-term stability of the gel in the cage designs, the device was placed in a humidified incubator at 37 °C with gravity driven flow. (The gel dries out without flow.) At 5 mg/ml collagen concentration, the cage was stable in the incubator for a period of two weeks under an average flow across the gel of 0.07 $\mu\text{l}/\text{min}$, as measured by change in medium height over time. Such a flow rate refreshes the medium compartment in 20 min and it is therefore adequate to sustain cell culture. For lower collagen 1 concentrations (2 mg/ml), the gel delaminated and flowed away after 3 days. The gel cage design 2, with media flow parallel to the cage, was also adequate to maintain the gel structure for a period of two weeks. A collagen-embedded culture of NHLF's was demonstrated in a cage 1 design with interstitial flow across the gel. A 2.5 μl solution of cells and collagen was injected through the loading channel and polymerized *in situ* at 37 °C. After overnight incubation at 37 °C, the cells exhibited the expected spindle-shape morphology (Fig. 5(b)) and maintained it for up to 4 days, with medium being constantly refreshed by the gravity-based flow.

FIG. 5. (a) Dye solution flowing across a collagen hydrogel in a cage 1 configuration bonded to a blank PDMS lid for a single layer device. The uniform filling of the cage indicates no gel delamination or fracture. (b) Phase contrast image of the single layer device with collagen-embedded fibroblast in the region of the dotted square from (a).

D. Bonding multilayer structures

After cure, the molded PDMS micropillars were transferred from the Kapton film carrier (Fig. 6(a)) to a PDMS substrate by oxygen-plasma treating both surfaces followed by contact bonding at room temperature. Pillars were routinely transferred to a PDMS layer with 100% yield. Transfer onto polymer membranes required an aminosilanization and thermo-compression bonding process. The transfer and bonding yield for pillars on a polycarbonate membrane was evaluated. The polycarbonate membrane was attached to a blank PDMS layer prior to the pillar transfer to simulate the full device process. Using the aminosilanization and thermocompression process described in the methods section, the pillar transfer yield on polycarbonate membrane test structures was 100% in five trials. The transfer yield was significantly lower if a weight of less than 500 g or a bonding time shorter than 10 h was used. The release in isopropanol bath was also found to be critical to achieve high yield, with a soaking time of no less than 5 min. This solvent treatment did not cause any observable swelling nor change of bonding properties of the PDMS, which then received the same oxygen plasma treatment as the top PDMS layer for bonding.

The optimized bonding process was used to fabricate devices with three vertically stacked independent flows, two polycarbonate membranes and a pillar layout in the inner layer. Bonding of the devices required alignment during assembly which was enabled by the transparency of the Kapton film carrier. A microscope image of a fully assembled device (Fig. 6(b)) shows the good alignment between the inner layer and the lower and upper flow channels. A full device with 100% pillar yield was successfully fabricated, and formation of three, independent fluidic paths was verified using three colored dyes as shown in Fig. 6(c). The device

FIG. 6. (a) Photograph of 150 μm thick molded PDMS cage 1 on a Kapton carrier prior to assembly into multilayer device. (b) Microscope image showing alignment of the micropillar array with the upper and lower flow channels. (c) Photograph of a fully assembled three layer device with cage 1 inner layer with colored dyes showing each leak free layer (top = red, middle = green, bottom = blue) (d) Cell viability staining of collagen-embedded fibroblasts at day 4 of culture in the inner layer of a cage 1 device (live cells, green; dead cells, red). Scale bar is 200 μm .

was tested with a syringe pump at flow rates up to 500 $\mu\text{l}/\text{min}$ without leaking. Collagen-embedded fibroblasts were seeded in the inner layer and cultured for up to 4 days when the cells were assessed for viability by morphological observation as well as fluorescence live/dead staining, with an average viability of 80% (Fig. 6(d)).

IV. CONCLUSION

In this paper, we illustrate a process to reliably fabricate multilayer PDMS devices with micropillar structures contained within the inner layers. A cost-effective lamination apparatus that enables repeatable high compression forces is described. A versatile process for molding and transferring PDMS thin films including micropillars is described, which relied on a standard oxide coating of a silicon master prior to fluorosilanization. The high yield (>95%) of the molding process and its repeatability were characterized and the results demonstrate that this is a practical solution to assembly of multilayer devices. We also demonstrated that the micropillar thin film layer can be bonded to non-PDMS components of the devices, such as a polymeric nanoporous membrane. A functional multilayer PDMS device with micropillar arrays in the middle layer serving as hydrogel cage and three independent vertically stacked fluid flows was demonstrated. A collagen-embedded fibroblast culture maintained under interstitial flow across the gel was demonstrated using the micropillar cage structure within the inner layer of a multilayer device; this configuration emulates the physiological arrangement of the stromal tissue sandwiched between different cell layers. The fabrication methods described in this paper can find applications in a variety of devices for biological applications, particularly for cellular disease models and organ-on-chip investigations.

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