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Cryo-Preservation for the Repeated Use of a PDMS Prepolymer

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ABSTRACT: Leftover uncured polydimethylsiloxane (PDMS) polymer is generally discarded. In an effort to minimize this waste, we have developed a method for cryo-preserving PDMS prepolymer involving a simple storage technique for the preservation and repeated use over 1 month. Aliquots of the uncured PDMS prepolymer were stored at −20 or −80°C, then conveniently and easily thawed at body temperature (37°C). Proposed cryo-preservation was successfully evaluated using diverse biological and physical tests. This method of cryo-preserving PDMS reduces both the material waste and labor of soft lithography process and may enable soft lithography to be environmentally friendly relative to previous methods. The method may be popularly accepted for application to a variety of common microdevice fabrication procedures. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40378.

KEYWORDS: PDMS prepolymer; cryo-preservation; repeated usage

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INTRODUCTION

Polydimethylsiloxane (PDMS, Sylgard 184 Silicone Elastomer KIT, Dow Corning) is a silicon-based organic polymer and is one of most popular materials for creating microfluidic channels, elastic stamps, and flexible electronics.[1–12] PDMS displays several advantages including optical transparency, gas permeability, and biocompatibility, and such properties facilitate its extensive application in the biological and medical fields.[13–20] The typical process to construct the PDMS microfluidic channels is the preparation of master mold, PDMS replication from a master mold and bonding. During the replication process, a significant amount of PDMS prepolymer is wasted. Additionally, the preparation of PDMS solutions, which consists of mixture of PDMS-base and curing agent and deairing, is time-consuming and laborious works. These processes increase the costs and time required for chip fabrication, and they exert a negative effect on the environment. To address these problems, preservation of extra uncured PDMS prepolymer over long periods of time (more than 1 month) and its repeated use may be a crucial requirement for enhancing soft lithography processes. However, excess PDMS prepolymers cannot be routinely stored for reuse because the PDMS prepolymer begins its crosslinking reaction under ambient conditions.

In this article, we describe a simple storage method for preserving uncured PDMS prepolymer over 1 month, which allows for repeated uses of the prepolymer and reduces the costs, time, and effort required for PDMS solution preparation. The PDMS prepolymer crosslinking reaction is usually accelerated by applying heat. Typical curing times are: 48 h at room temperature (RT) (25°C, 77°F), 2 h at 80°C (176°F), 45 min at 100°C (212°F), and 10 min at 150°C (302°F).[21] We, therefore, hypothesized that reducing the temperature by several tens of degrees could significantly extend the crosslinking time. The uncured PDMS prepolymer was cryo-preserved at several temperatures, and the appropriate temperature for long-term preservation and thawing conditions was identified. The cryo-preserved PDMS prepolymer was preserved for 1 month, after which time we evaluated the characteristics of the resulting cured PDMS by comparison with a nonpreserved polymer sample. The properties of cryo-preserved PDMS were evaluated by observing the contact angle before and after oxygen plasma treatment, the cell viability in a device, the viscosity prepolymer solution, bonding properties between PDMS–glass, repeated usability, and replication of diverse shaped microstructures with high fidelity. The proposed method is expected to contribute to the realization of a cost-effective, labor-free, and environmentally friendly soft lithography process.

EXPERIMENTAL

Cryo-Preservation and Thawing Process

Figure 1(A) illustrates the workflow involved in the conventional PDMS replication process and in the cryo-preserved PDMS replication. The conventional PDMS replication process consists of mixing of PDMS base and curing agent, deairing, pouring PDMS prepolymer onto a master mold, thermal curing, and separation

Additional Supporting Information may be found in the online version of this article.

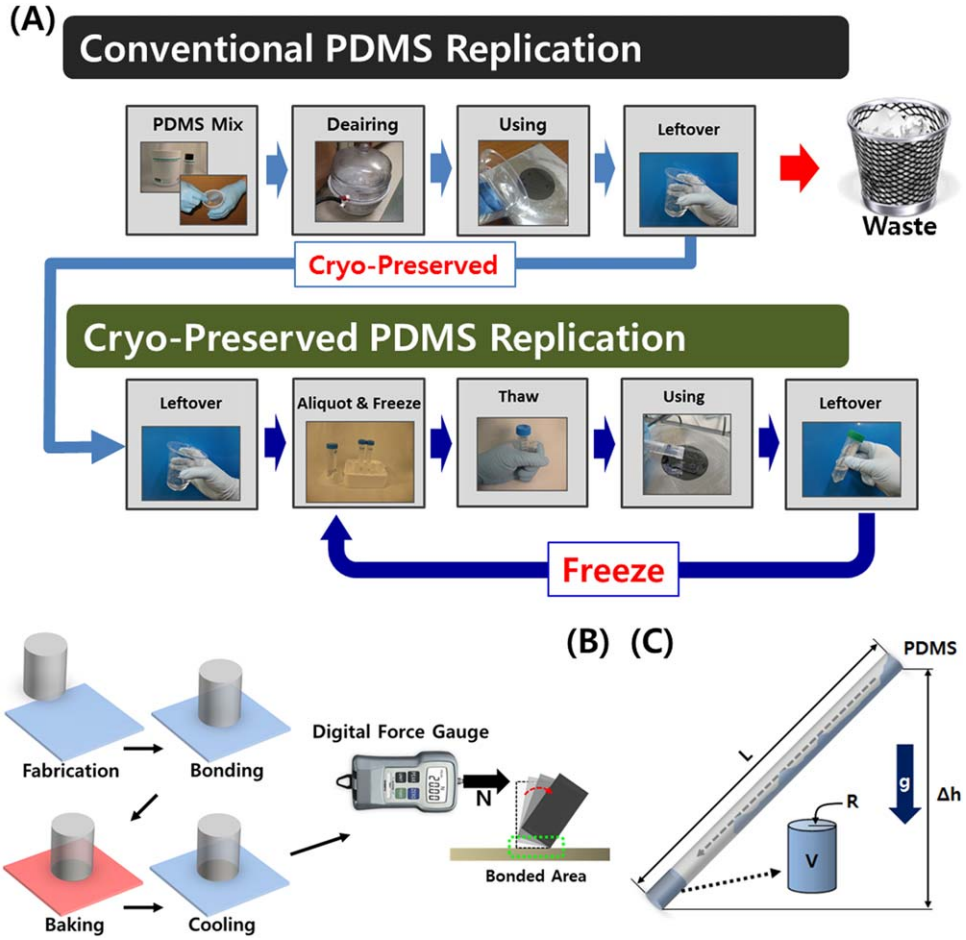


Figure 1. (A) The PDMS replication process workflow: comparison of the conventional method and the cryo-preservation method. (B) Schematic diagram showing the surface treatment and bonding test processes. (C) Viscosity measurements using a home-built viscosity measurement procedure. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of the PDMS from the mold.[3–9] The leftover PDMS prepolymer is generally discarded. By contrast, during the cryo-preserved PDMS replication processes, the leftover PDMS prepolymer could be stored and reused simply by preparing aliquots of the uncured PDMS mixture that are then stored at −20 or −80°C. These low temperatures are generally available in biology, material, and chemistry laboratories, as they are the standard temperatures of freezers (−20°C) and deep freezers (−80°C). Once PDMS prepolymer (10:1 mixture of PDMS base and curing agent) aliquot had been cryo-preserved, the thawing process is very simple. The operator can thaw a frozen PDMS prepolymer aliquot simply by gripping the tube containing frozen aliquot in their hand for 5 min. The frozen PDMS prepolymer aliquot melts at 37°C (normal body temperature). This thawing process is very convenient and easy because it requires no additional treatments or devices. The molten PDMS prepolymer can then be reused by refreezing, and this process can be repeated several times. The repeated use of a PDMS prepolymer aliquot was tested by freezing (at −20°C) and thawing (at 37°C) 10 times.

Device Fabricability

Three types of master mold were used to test whether the cryo-preserved PDMS prepolymer could faithfully replicate master molds having (1) cylindrical wells 400 μm in diameter and 400 μm in depth; (2) rectangular channels 400 μm in height, 1000 μm in width, and 10 mm in length; and (3) a curved structure (concave). The rectangular and cylindrical master molds were fabricated using a standard SU-8 based patterning process,[5–7,22,23] and the concave master mold was fabricated by deflecting a thin PDMS layer, as described previously.[17,24] Nonpreserved PDMS prepolymer, a cryo-preserved PDMS prepolymer at −20 and −80°C, and a PDMS prepolymer submitted to repeated cryo-preservation cycles (at −20°C) were poured over the three types of master mold, and the replicated PDMS structures were compared. The replicated PDMS structures were imaged using scanning electron microscopy (SEM, JSM-7500F, JEOL, Japan).

Surface Treatment and Bonding Test

The bonding property of a PDMS device is very important for fabricating leakage-free microfluidic channels. Bonding between a glass surface and a PDMS structure having cylindrical post-shape prepared from the cryo-preserved and nonpreserved PDMS prepolymer was tested using a digital force gauge (SHIMPO, FGN-50B, Japan). As shown in Figure 1(B), two types of post with a small diameter (2.5 mm, bottom area: 19.6 mm^2) and a large diameter (4 mm, bottom area: 50.2 mm^2)

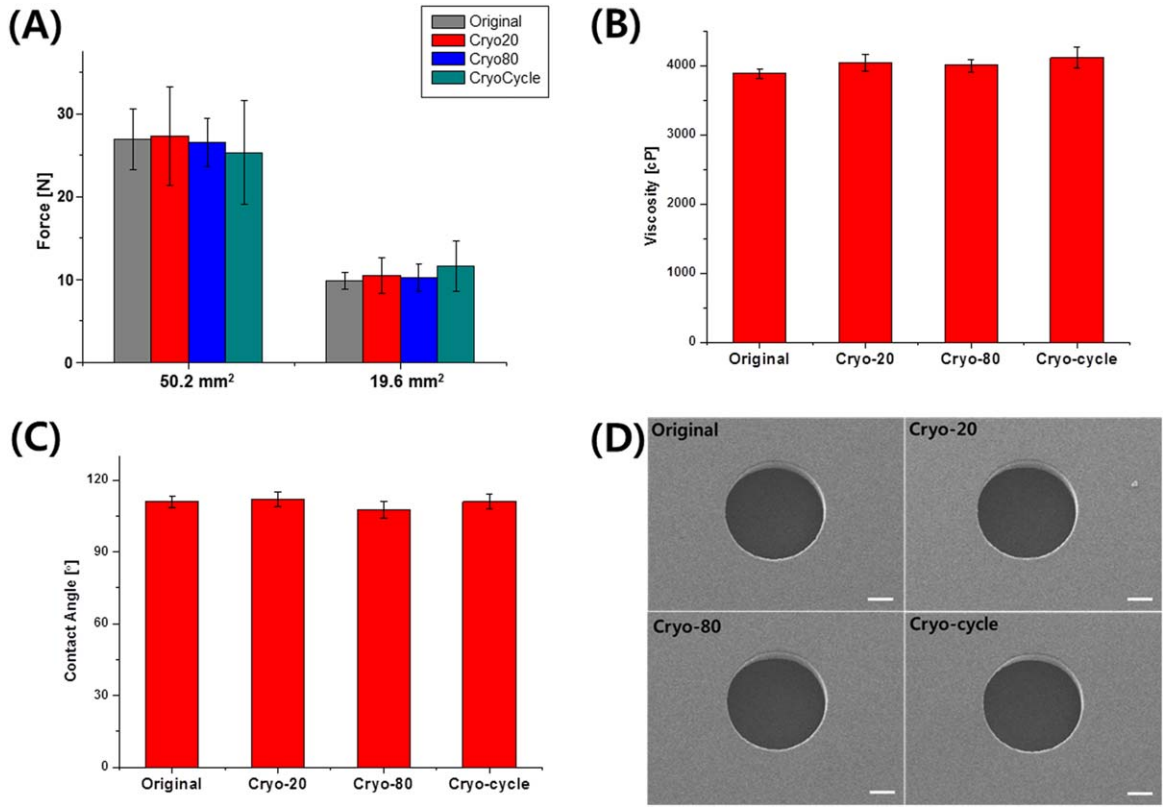


Figure 2. Comparison of the properties between cryo-preserved and nonpreserved PDMS. (A) The attachment forces between a slide glass and cylindrical posts with different bottom areas. ($n = 20$). (B) Viscosity of the uncured PDMS prepolymer determined by a home-built viscometer (based on a principle similar to that underlying the Ostwald viscometer) at RT (20°C) ($n = 10$). (C) Contact angles of DI water droplets on the cured PDMS devices, measured using a goniometer. ($n = 5$). (D) Fabricability of the devices prepared from each type of the uncured PDMS prepolymer. The scale bar indicates 100 μm (Original: an uncured nonpreserved fresh PDMS prepolymer, Cryo-20: a cryo-preserved PDMS at −20°C, Cryo-80: a cryo-preserved PDMS at −80°C and cryo-cycle: a PDMS prepolymer submitted to repeated cryo-preservation cycles) ($n = 5$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

were prepared. The bottom area of each PDMS post was exposed to oxygen plasma for surface treatment and then bonded to a plasma-treated slide glass. The adhesion test was conducted by pushing the head of a bonded PDMS post using a digital force gauge, and the peak force was measured to indicate the force required to detach the adhered posts from the slide glass. The bonding test procedure is illustrated in detail in Supporting Information Figure S1.

Viscosity Measure

The viscosities of the cryo-preserved PDMS prepolymer and the nonpreserved PDMS prepolymer were measured using a home-built viscometer (based on the principle of Ostwald viscometer) at RT (at 20°C). The schematic of viscosity measurements are illustrated in detail in Figure 1(C) and in Supporting Information Figure S2. The viscosity could be estimated using eq. (1)[25]

$$\mu = \frac{\pi R^4 \rho g t \Delta h}{8LV} \quad (1)$$

where R is the radius of the tube, ρ is the density of the PDMS, g is the acceleration due to gravity, t is the time required for the flow to cease, Δh is the distance between the bottom and top of the tube, L is the length of the tube, and V is the volume of PDMS.

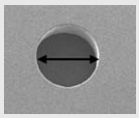
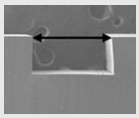
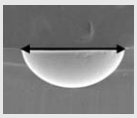
Contact Angle Measurements

The contact angles were measured using deionized (DI) water, a goniometer, and the sessile drop method at RT (at 20°C). The slope of the tangent to the drop at the liquid–solid–vapor interface line was calculated using ImageJ (1.46, NIH). A 10-μL droplet of DI water was placed on the normal and cryo-preserved PDMS surfaces using a micropipette.

Cell Culture and Sample Preparation

All cell manipulations were performed in a biosafety cabinet (Level II), and cell cultures were conducted in a traditional cell culture incubator (SHEL LAB, 37°C, 5% CO_2). The Madin–Darby canine kidney (MDCK) epithelial cell line (Korean cell line bank) was cultured in a cell culture flask with Dulbecco's Modified Eagle's Medium (high glucose DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco), and 1% antibiotics (pen/strep). The cells were washed with phosphate buffered saline (PBS, Gibco), removed from the flask using 0.05% trypsin–EDTA (Gibco), and placed in the culture medium. The cells were then centrifuged (1000 rpm for 5 min), resuspended in the culture medium, and a 1.5×10^6 cells/mL culture media solution was prepared in a 15-mL conical tube (BD science).

Table I. Dimenions of the Replicated PDMS Structures (Microwell, Microchannel, and Concave)

	Microwell (diameter)	Microchannel (width)	Microconcave (diameter)
SEM image			
Nonpreserved (μm)	400.5 ± 0.1	1010.0 ± 2.6	960.5 ± 3.2
−20°C Preserved (μm)	400.1 ± 0.4	1004.8 ± 0	962.8 ± 5.4
−80°C Preserved (μm)	400.9 ± 0.5	1007.9 ± 0.65	960.9 ± 5.5
Cryo-preserved cycle (μm)	400.2 ± 0.2	1000.2 ± 1.9	959.8 ± 9.0
Ave. (μm)	400.4 ± 0.3	1005.4 ± 1.3	961.0 ± 5.9
SD (%)	0.07	0.12	0.61

The cell culture chip was fabricated with PDMS and its schematic and dimension is described in Supporting Information Figure S3. After cell loading, the culture chips were placed in a large bioassay dish (Corning) containing filter-sterilized reagent grade water for incubation. The large surface area of water in the bioassay dish helps keep the environment inside and around the cell culture chip saturated with water vapor and thus reduce evaporation.[26] The culture media was then added through the input of culture chip and replaced every other day.

Viability Assay and Immunocytochemistry Staining of the Tight Junctions

The viability of the cultured MDCK cells was assessed 2 days after loading using Calcein AM (2 μM, green dye; live cells) and ethidium homodimer-1 (4 μM, red dye; dead cells) from the LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (Invitrogen). The cells were incubated for 30 min at RT according to the kit protocol, followed by imaging under an inverted fluorescence microscope (EVOS, AMG) after washing twice with PBS. The MDCK cells grown using the microchannel system were immunocytochemically analyzed to determine the distribution of tight cell–cell junctions.[14] The cells cultured for 2 days were fixed for 20 min with 4% paraformaldehyde at RT and were permeabilized using 0.1% triton-X100 in PBS for 20 min at RT, followed by blocking with 3% BSA in PBS for 30 min. The cells were then incubated with a primary antibody (1 μg/mL, ZO-3 rabbit polyclonal antibody, Invitrogen, CA) overnight at 4°C. The primary antibody could be used to characterize the various cell types and was localized to the sites of the tight cell–cell junction. After washing with 0.1% BAS in PBS, the secondary antibody (1:200, Alexa 594-conjugated antirabbit, Invitrogen) was added and incubated for 30 min at RT. The actin was stained with the steps described above, using Alexa Fluor 594 Phalloidin antibody (Invitrogen). Images of the viability assay and the tight junction results were acquired using an inverted fluorescence microscope (EVOS, AMG) after counterstaining with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI, Invitrogen). The ImageJ software (1.46, NIH) was used to quantify the number of live and dead cells in each channel.

RESULTS AND DISCUSSION

The uncured PDMS mixture (or leftover PDMS mixture) was preserved in the refrigerator (4°C), freezer (−20°C), or deep freezer (−80°C). The PDMS prepolymer stored at 4°C was used in a comparison test, however, it completely polymerized within 36 h. By contrast, the PDMS stored at −20°C and −80°C were still useable even after 1 month of preservation. The evaluation results of the cryo-preserved PDMS are described below.

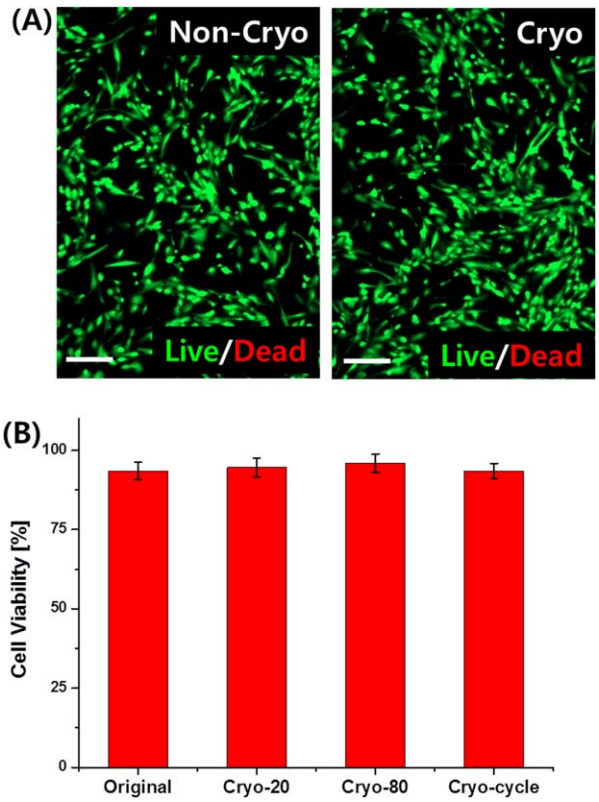


Figure 3. (A) Live/dead staining images of the MDCK cells in a cell culture chip (microchannel system). The noncryo image shows representative images of cells grown on the PDMS devices prepared from prepolymer solutions stored at −20, −80°C and repeatedly cryo-preserved; the Cryo image shows images of cells grown on the PDMS device prepared from a nonpreserved prepolymer solution. (B) MDCK cells viabilities in the different PDMS-based cell culture chip. The scale bar indicates 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

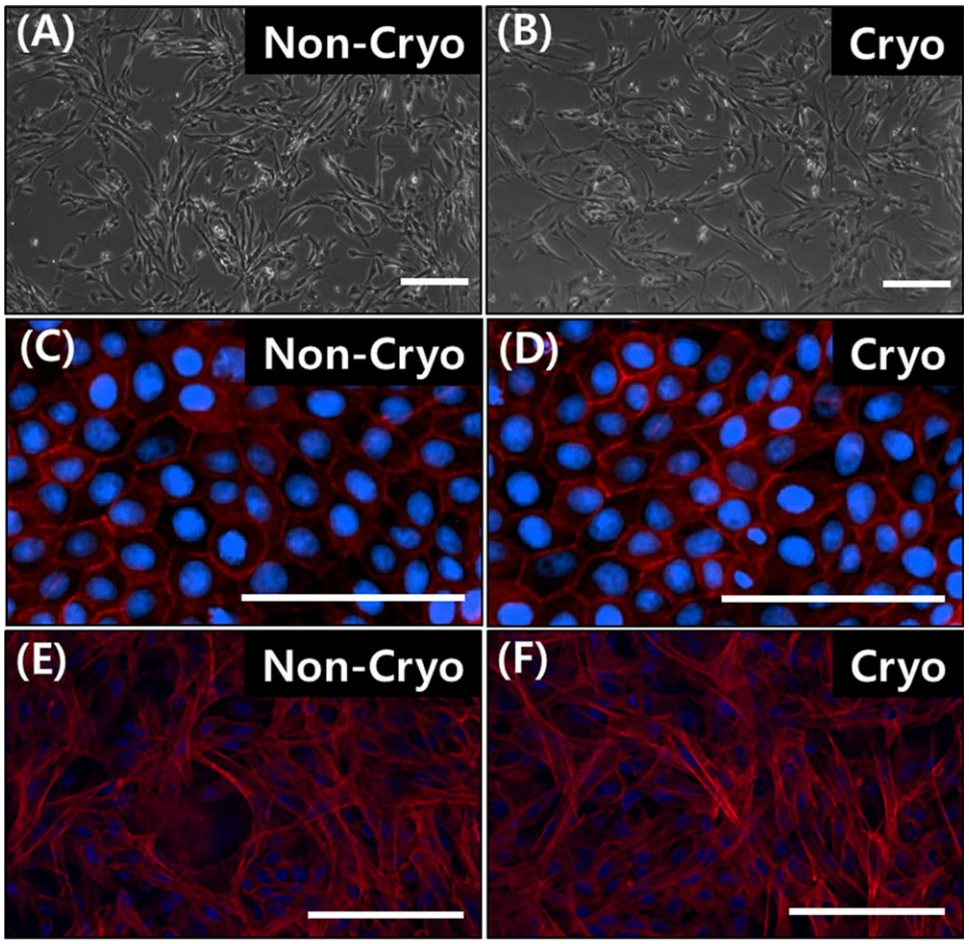


Figure 4. Tests of the cell adhesion and cell–cell interactions to demonstrate compatibility with biological studies: (A) and (B) bright field image of MDCK cells in a cell culture chip. (C) and (D) Staining image of the tight junctions in MDCK cells grown on a PDMS sheet. (E) and (F) Actin staining in MDCK cells grown on the PDMS sheet (red: actin, blue: nuclear). The all Noncryo image shows representative images of cells grown on the PDMS devices prepared from prepolymer solutions stored at −20, −80°C, or repeatedly cryo-preserved; the all cryo image shows images of cells grown on the PDMS device prepared from a nonpreserved prepolymer solution. The scale bar indicates 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Physical Test (Bonding Property, Viscosity, Contact Angle, and Appearance)

The bonding force between a slide glass and a cured PDMS post structure, where the post structures were prepared from nonpreserved or cryo-preserved PDMS prepolymer solutions, were characterized by measuring the peak detachment force. Figure 2(A) shows that the peak detachment force for the large-area posts ranged from 25 to 27 N. The peak detachment force for the small-area posts ranged from 9 to 12 N. These results also showed that the adhesion forces present in the four types of sample did not display significant differences, and the cryo-preserved PDMS prepolymer could be used for microchannel bonding using oxygen plasma surface treatment without displaying a reduced bonding force.

The viscosities of the nonpreserved PDMS and cryo-preserved PDMS prepolymer solutions were measured using a home-built viscometer at RT (20°C). The viscosities of the nonpreserved, −20, −80°C, and repeatedly cryo-preserved PDMS prepolymer solutions were 3887.5 ± 71, 4049 ± 121, 4005 ± 90, and 4120 ± 148 cP, respectively, as shown in Figure 2(B). The contact angles of a water droplet on each PDMS surface were measured using a contact angle goniometer (Ramé–Hart Imaging System, NJ). Figure 2(C) shows the contact angles of sessile droplets on each PDMS surface. The contact angles of the nonpreserved, −20, −80°C, and repeatedly cryo-preserved PDMS prepolymer solutions were $111.0 \pm 2.5°$, $112.0 \pm 3.2°$, $107.6 \pm 3.5°$, and $111.0 \pm 3.0°$, respectively. No significant differences in the contact angles were observed.

The faithful replication of a master mold is very important for the production of precise microchannels. The SEM images of the replicated PDMS structures are shown in Figure 2(D). The cylindrical wells were faithfully replicated without significant differences in shape or dimension. The widths of microchannel, the diameters of the cylindrical wells, and the diameters of concave fabricated using the nonpreserved, −20, −80°C, and repeatedly cryo-preserved PDMS prepolymer solutions were measured, and the results are listed in Table I and Supporting Information Figure S4.

Cell Viability and Cell–Cell Interactions on the PDMS Surface

The cell viability and cell–cell interactions were investigated in the cell culture chip. The MDCK cells were seeded and cultured, and their viability was examined using the live/dead cell staining assay. Figure 3(A) shows a fluorescence image of the live/dead cells (green: live cells, red: dead cells). The cell viability on each PDMS surface was measured, and the results are plotted in Figure 3(B). The viabilities of the nonpreserved, −20, −80°C, and repeatedly cryo-preserved PDMS prepolymer solutions were 93.5 ± 2.7, 94.6 ± 3.0, 95.9 ± 3.0, and 93.5 ± 2.4%, respectively. The cell viabilities were indistinguishable within the tolerance of the measurement. The tight junctions between cells were observed by immunocytochemistry staining. After culturing the MDCK cells in microchannels, the actin filament, nucleus, and cell–cell junction were stained. Figure 4(A) and (B) shows an optical image of the MDCK cells on the surfaces of the nonpreserved and cryo-preserved PDMS surfaces. Figure 4(C) and (D) shows images of the stained cell–cell junctions in the MDCK cells, and Figure 4(E) and (F) also shows images of the stained the actin filaments in the MDCK cells.

The PDMS characterization studies demonstrate that an uncured PDMS prepolymer solution may be stored for at least 1 month without degrading its properties. The solution may also be used repeatedly using this method. Temperatures between −20 and −80°C were sufficient to prevent the uncured PDMS prepolymer from crosslinking during storage. These temperatures are readily available in general biology or material laboratories, which facilitates the broad application of the proposed cryo-preservation method. The proposed method can dramatically reduce the preparation time (mixing the PDMS prepolymer with the curing agent and deairing the solution) and the amount of materials consumed during the sample preparation process. To this purpose, a large amount of the PDMS prepolymer solution may be prepared, divided into aliquots, and cryo-preserved. The microfluidic channels may then be fabricated simply by thawing one aliquot without the need for laborious and time-consuming preparation processes. Several evaluation measurements indicated that the properties of the preserved PDMS prepolymer did not display significant changes or degradation. The solution viscosity is very important for the faithful replication of a microstructure mold. Although, the viscosities differed slightly (3887.5 ± 71 cP for nonpreserved, 4120 ± 148 cP for repeated cryo-preserved), all prepolymers displayed viscosities similar to that of the fresh PDMS (3900 cP, as reported in the Dow Corning data manual). Solutions having this viscosity are expected to replicate a given microstructure with high fidelity, regardless of the presence of rectangular or curved features, and the experimental results show such replication capability. The storing of an uncured PDMS prepolymer aliquot is advantageous for the handling and thawing of a specified quantity of the PDMS prepolymer. The thawing process involving gripping the aliquot in the hand saves thawing time and does not require any devices or extra process. The cell viability and cell–cell interaction tests revealed that cells cultured on the cryo-preserved PDMS were indistinguishable from those cultured on the nonpreserved PDMS. The MDCK cells showed tight cell–cell interactions on both the nonpreserved and cryo-preserved PDMS. These results suggest that cryo-preserved PDMS may be broadly applicable to cell-based studies. Approximately 2 h is required for the preparation of a prepolymer solution, from mixing the PDMS prepolymer with the curing agent to the deairing step. The reuse of a cryo-preserved PDMS prepolymer may minimize the process time and labor (5 min only for the thawing process). We performed a 10 cycle test in which a PDMS prepolymer solution was repeatedly frozen (−20°C) and thawed (37°C). No significant differences or degradation of the PDMS properties were observed.

CONCLUSIONS

We successfully demonstrated that the uncured PDMS prepolymer may be stored for at least 1 month, and the solution may be repeatedly frozen and thawed without degradation. To our knowledge, this is the first attempt to store an uncured PDMS solution over a period of 1 month. This method may realize a cost-effective, labor-minimizing, and environmentally friendly soft lithography process. Evaluation studies demonstrated that the properties of cryo-preserved PDMS were indistinguishable from those of nonpreserved fresh PDMS, and diverse microstructure shapes and channels could be replicated with high fidelity. The cell-based tests demonstrated that the cryo-preserved PDMS is almost similar performance to nonpreserved PDMS in cell culturing. Cryo-preservation of PDMS prepolymer solutions may play a pivotal role in enhancing soft lithography processes in the future.

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