Fabrication of rigid microstructures with thiol-ene-based soft lithography for continuous-flow cell lysis

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In this work, we introduce a method for the soft-lithography-based fabrication of rigid microstructures and a new, simple bonding technique for use as a continuous-flow cell lysis device. While on-chip cell lysis techniques have been reported previously, these techniques generally require a long on-chip residence time, and thus cannot be performed in a rapid, continuous-flow manner. Microstructured microfluidic devices can perform mechanical lysis of cells, enabling continuous-flow lysis; however, rigid silicon-based devices require complex and expensive fabrication of each device, while polydimethylsiloxane (PDMS), the most common material used for soft lithography fabrication, is not rigid and expands under the pressures required, resulting in poor lysis performance. Here, we demonstrate the fabrication of microfluidic microstructures from off-stoichiometry thiol-ene (OSTE) polymer using soft-lithography replica molding combined with a post-assembly cure for easy bonding. With finite element simulations, we show that the rigid microstructures generate an energy dissipation rate of nearly 10^7 , which is sufficient for continuous-flow cell lysis. Correspondingly, with the OSTE device we achieve lysis of highly deformable MDA-MB-231 breast cancer cells at a rate of 85%, while a comparable PDMS device leads to a lysis rate of only 40%. © 2014 AIP Publishing LLC.

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I. INTRODUCTION

The introduction of soft-lithography fabrication using the elastomer polydimethylsiloxane (PDMS) resulted in a great expansion of research in the field of microfluidics.¹ Two primary advantages of elastomer-based soft lithography drove this explosion of microfluidics research: (i) the low cost and ease of soft-lithography-based fabrication enables rapid prototyping of devices and (ii) the flexibility of the PDMS elastomer can be leveraged to fabricate actuated components, in particular, valves and pumps,² into the microsystems. Thus, PDMS and soft lithography assisted in the evolution of microfluidic devices to a paradigm in which sophisticated and highly functional devices are fabricated through replica molding, which dramatically decreased the cost per device as compared to silicon- or glass-based devices.

While it is clear that soft lithography is a universally valuable technique to the field of microfluidics, PDMS as a material for soft lithography may have disadvantages in many important applications. The vapor porosity of PDMS leads to evaporation of samples, the hydrophobicity of PDMS leads to challenges with wettability, and the deformability causes feature sizes to change under various pressure conditions. In particular, this lack of rigidity prohibits the use of PDMS for a number of applications that require fixed and predictable channel dimensions under high pressure.

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One such application is rapid and continuous-flow cell lysis in a microfluidic channel. Although a number of techniques have been demonstrated for on-chip cell lysis, including chemical,^{3,4} electrical,^{5,6} acoustic,^{7,8} and optical,^{9,10} these methods require a high residence time in the microfluidic channel, and thus cannot be used for continuous-flow processing. Only mechanical shearing of cells offers the lysis speed to enable continuous-flow processing. The use of mechanical shearing for continuous-flow cell lysis has been reported in silicon devices using narrow channels with features on the scale of a few microns.^{11,12} However, as the devices are silicon-based, they are not replica molded and thus are quite expensive to produce. Fabricating the devices with soft-lithography would dramatically improve the cost, but unfortunately soft materials are not compatible with the mechanical lysis concept due to significant channel expansion under the required high pressure.

Here, we demonstrate the *soft-lithography*-based fabrication of *rigid* microsystems for the application of rapid and continuous-flow cell lysis. We accomplish this through the use of an off-stoichiometry thiol-ene (OSTE) polymer¹³ (OSTemer Allyl 30, Mercene Labs, Stockholm, Sweden) in combination with a post-assembly cure bonding procedure. This thiol-ene polymer, as with PDMS, can be replica molded using soft-lithography techniques, but unlike PDMS, is rigid when fully cured. Carlborg *et al.*¹³ demonstrated that by having a large stoichiometric excess of the reactive monomers, the surface and mechanical properties of the thiol-ene substrates could be tuned. We chose a formulation with 30% excess *ene* functionality that yields a rigid polymer when cured. It has a reported Young's modulus of 1740 MPa compared to 0.8 MPa for PDMS.¹³ Fabrication of microstructures using thiol-enes was first demonstrated by Harrison *et al.*¹⁴ Their method used direct photolithography to pattern the thiol-ene; however, soft-lithography of thiol-enes has since been demonstrated in numerous applications including droplet generation,¹⁵ soft-lithography stamps,¹⁶ microchip capillary electrophoresis,¹⁷ cell culture,¹⁸ and optofluidics.^{19–21}

OSTE polymers can be directly bonded using the reactive groups present on the surface. The standard method involves bonding of two OSTE polymers, one with an excess of thiol groups and the other with an excess of allyl (or *-ene*) groups.^{13,17} The two polymers will react to covalently link themselves under UV exposure. The disadvantage of this method is that it requires two OSTE formulations for bonding. Recently, the bonding of an OSTE polymer to itself was demonstrated.¹⁷ In this case, two pieces of fully cured OSTE with excess allyl were contacted and exposed to UV. Although bonding can be achieved, we found that contacting fully cured OSTE pieces resulted in a bond strength that varied across assembled devices. In principle, full curing of the OSTE will consume all (or most) of the thiol groups, leaving allyl groups at the surface. The allyl groups cannot react with each other to form covalent bonds. For the two fully cured pieces to bond, unreacted thiol groups must be present at the surface. In our experience, the small number of unreacted thiol groups present after fully curing is not sufficient to achieve consistent bonding.

To alleviate this issue, we developed a modified allyl to allyl method. In this protocol, the OSTE is only partially cured on the mold; i.e., it can be removed from the mold without loss of features, though it is not completely rigid. This results in a larger number of unreacted thiol groups at the surface. The OSTE device is then assembled and fully cured by UV exposure. The final cure consistently bonds the two OSTE layers together. With this fabrication technique, we generate microfluidic channels with rigid nozzles as thin as 5 μm and with an aspect ratio of 15.

These nozzle structures are used to achieve cell lysis. The key to continuous-flow shear-based lysis with the nozzle design is the generation of a high energy dissipation rate (EDR).²² The EDR describes the rate of work on the cell. It is an intrinsic property that includes both extensional and shear flows. The EDR can be calculated from^{22,23}

$$EDR = \mu[\nabla U + (\nabla U^T)] : \nabla U, \quad (1)$$

where μ is the viscosity, ∇U is the velocity gradient tensor, and ∇U^T is the transpose of the velocity gradient tensor. Mollet *et al.*²² found that the EDR was largest (indicating greater

cellular damage) at the entrance and exit of a constriction where the extensional and shear flows are greatest.

In this work, we show that the OSTE-based microstructures created with soft-lithography are sufficiently rigid to maintain high EDR, while PDMS-based microstructures expand, leading to a dramatic decrease in EDR. Correspondingly, highly flexible metastatic breast cancer cells are efficiently lysed when forced through the rigid nozzles at high flow rates, while the same cells are not lysed effectively when forced through a PDMS chip fabricated from the same mold due to channel expansion. In our system, cells are lysed at a flow rate of 100 $\mu\text{l}/\text{min}$, which enables the processing of hundreds and thousands of cells in minutes. This simple and rapid cell lysis technique can be employed in future micro total analysis systems to advance the automation of sample preparation in many important applications, including automated sample preparation for genetic tests based on whole blood collection, simplified sample preparation of collected cells in a research lab environment, and genetic analysis of microfluidically recovered tumor cells.

II. METHODS

Previous reports demonstrated continuous cell lysis through parallel channels containing a single constriction.^{11,12} Since the cells were only exposed once to high EDR, constriction widths of 3 μm were necessary to achieve efficient lysis. These narrow constrictions are prone to high backpressure and clogging. Instead, we designed a device to repeatedly subject the cells to high EDR by using a series of constrictions. This enabled the use of larger constriction widths that are less prone to clogging and have lower backpressure. Our design contained 40 parallel channels 52 μm in width with 10 constrictions in each channel, the first five of which measured 6.5 μm across, while the remaining five measured 5 μm across. The design of the device is depicted in Fig. 1.

The master mold was formed by spin coating photoresist (Shipley 1813) onto a silicon wafer to a thickness of 3 μm , pre-baking for 1 min at 95 °C, and exposing to 365 nm UV for 13 s. The photoresist was developed for 75 s in Microposit developer CD-30 (Shipley). The wafer was then etched at a rate of $\sim 2 \mu\text{m}/\text{min}$ to a depth of 70 μm with deep reactive ion etching (DRIE) using the photoresist as the mask. The resist was stripped using Remover PG (MicroChem Corp.).

OSTE fabrication followed a soft lithography methodology (Fig. 2). Prior to chip fabrication, OSTE (OSTEmer Allyl 30, Mercene Labs) was brought to room temperature. The silicon mold was sprayed with a light mist of Ease Release 200 twice and allowed to stand for 5 min before pouring a thin layer (0.5 mm) of OSTE (Ease Release 200 was selected over silane treatment because the hydrophobicity of the silane on the mold surface prevented the OSTE from filling the small, high-aspect-ratio features). Air bubbles that formed on the mold were brought to the surface under vacuum. Following degassing for 20 min, more OSTE was poured onto the mold to a total thickness of ~ 1 mm and degassed for 20 min. Air bubbles still present were moved away from the chip features by tilting the mold. OSTE was partially cured under a 100 W handheld UV lamp at a distance of 13 cm for 50 s. The bottom piece of the chip was fabricated by partially curing ~ 1 mm thick OSTE for 30 s under UV onto a glass slide sprayed with the release agent. The partially cured OSTE was removed from the molds and trimmed with scissors. To facilitate inlet/outlet holes the OSTE was softened in an oven at 70 °C (the reported glass transition temperature, T_g , is 68 °C)¹³ for 1 min and then access holes were immediately formed with a 1.2 mm biopsy punch. The top and bottom pieces were bonded together by curing the pieces in contact with each other for 2 min under UV exposure. Devices were placed in the oven for 10 min to finish the curing process. Tubing was inserted into the device and held in place with OSTE cured around it.

PDMS chips were fabricated with the base and curing agent mixed in a 10:1 ratio. It was degassed for 20 min, poured over the silicon mold treated with the release agent to a total thickness of ~ 2 mm, and then degassed for an additional 20 min. The PDMS was cured on a hotplate at 90 °C for 1 h and bonded to cleaned glass slides with oxygen plasma treatment.

FIG. 1. Photomask image for the lysis device illustrating the array of pinch-points used to ensure high lysis efficiency. Inset 1: Zoomed-in image of five parallel channels. Inset 2: Zoomed-in image of the channel and pinch-point with dimensions.

MDA-MB-231 human breast cancer cells were selected for this work because they are a uniquely flexible cancer cell line representative of metastatic cancer cells that may be isolated and analyzed in microfluidic devices. Because of the flexibility of these cells, they are difficult to lyse using microstructures (i.e., they can easily pass through thin microchannels). Cells were grown in DMEM with 4 mM L-glutamine, 4.5 g/l glucose, and reduced sodium pyruvate supplemented with 5 μ l/ml penicillin/streptomycin, 1% non-essential amino-acids, and 10% fetal bovine serum (FBS) in tissue-culture-treated T25 flasks in a 37°C incubator with 5% CO₂. Cells were subcultured every 5–7 days by trypsinization with 0.25%/0.02% trypsin/EDTA.

The cell lysis experiments were performed in three separate devices for each material. First, MDA-MB-231 cells were diluted to 10⁵ cells/ml in media and stained by adding a working concentration of 4 μ M Calcein AM. Before introducing the cells, the lysis devices were primed with 2 g/l pluronic F68 (Sigma Aldrich) to eliminate biofouling. Then the cells were infused through the devices via a syringe pump at a rate of 100 μ l/min for 2 min and collected in a microcentrifuge tube (a video of concatenated fluorescence microscopic images is available in the supplementary material²⁴). Viable cell density was counted using a hemocytometer. Three trials, each on separate devices, were performed for each material.

FIG. 2. OSTE chip fabrication. (a) OSTE is poured onto silicon mold/glass slide and degassed. (b) OSTE is partially cured with UV radiation. (c) Top and bottom pieces are removed from molds and placed in contact with each other. (d) Pieces are bonded together and device is fully cured with UV radiation.

III. RESULTS

A scanning electron microscopy (SEM) image of a lysis device fabricated in OSTE is shown in Fig. 3. Since the material is partially cured prior to bonding, one possible concern would be distortion or closing of the narrow constrictions especially due to the high aspect ratio (depth to width) of 15. However, as shown in Fig. 3, the channels remain open with straight sidewalls. The widths of the channels match those of the silicon master. In addition, the devices are well bonded with almost no noticeable indication of the bonding surface.

To illustrate the advantage of OSTE over PDMS for continuous mechanical lysis, PDMS and OSTE devices were replica molded from the same silicon master. Following fabrication, the devices were imaged and measured to have the same channel dimension when not subjected to flow as shown in Figs. 4(a) and 4(c), respectively. Under high flow rates (100 $\mu\text{l}/\text{min}$), the OSTE chip did not expand (Fig. 4(d)). PDMS, on the other hand, expands, causing an increase in the channel width to $\sim 20\text{ }\mu\text{m}$ (Fig. 4(b)). Such a large expansion in the channel can have a large impact on the cell lysis efficiency.

One proposed method for characterizing cellular damage (and lysis) is to determine the EDR.²² We verified this with a numerical simulation performed in COMSOL Multiphysics. For this simulation Eq. (1) was simplified to the 2-dimensional case

$$EDR = 2\mu \left[\frac{1}{2} \left(\frac{\partial U_x}{\partial x} + \frac{\partial U_y}{\partial y} \right)^2 + \left(\frac{\partial U_x}{\partial x} \right)^2 + \left(\frac{\partial U_y}{\partial y} \right)^2 \right]. \quad (2)$$

The results for a 5 μm (OSTE) and 20 μm (PDMS after expansion) constriction are shown in Fig. 5. As expected the greatest EDR was at the entrance and exit to the constriction. However, for the 5 μm constriction, the calculated EDR was two orders of magnitude greater than for the 20 μm case. This is better illustrated in Fig. 6 where the EDR along the center line of the channel is plotted. The 5 μm constriction results in a maximum EDR of $4.5 \times 10^6 \text{ W}/\text{m}^3$. This is comparable to previous reports that have shown catastrophic cellular damage under exposure to an EDR of $>10^7 \text{ W}/\text{m}^3$.²² However, for the 20 μm constriction, the maximum EDR

FIG. 3. SEM of OSTE chip cross section. Bonding between the top and bottom piece is excellent. The channel narrows from 52 μm to 5 μm while maintaining high aspect ratio (15:1).

was only $2 \times 10^4 \text{ W/m}^3$ (Fig. 6, inset). This value is well below the EDR shown to result in efficient cell lysis.

The impact of the nozzle expansion on cell lysis was validated experimentally. The devices were designed with an array of constrictions (Fig. 1) to repeatedly expose the cells to high

FIG. 4. Lysis features in PDMS and OSTE with and without pressure. Without pressure, the PDMS nozzle has a width of 5 μm (a); under pressure the 5 μm gap expands to 20 μm (b). The OSTE nozzle is 5 μm as well without pressure (c); the 5 μm gap does not expand under pressure (d).

FIG. 5. Simulation results showing the velocity (a) and EDR (b) for 5 μm constriction and the velocity (c) and EDR (d) for a 20 μm constriction (representing PDMS after expansion).

FIG. 6. Plot of the EDR along the center line for the 5 μm (blue) and 20 μm (red) constrictions. The two peaks represent the high EDR present at the channel entrance and exit. The inset is zoomed-in to allow visualization of the EDR peaks for the 20 μm constrictions.

EDR, ensuring high lysis efficiency. Lysis was performed by flowing the cells through the device at 100 $\mu\text{l}/\text{min}$. Quantified lysis rates are presented in Fig. 7. As mentioned above, PDMS expands under these high flow rates, producing a constriction of $\sim 20 \mu\text{m}$ width (Fig. 4(b)). The MDA-MB-231 cells have nominal diameter of $\sim 20 \mu\text{m}$, but become elongated due to their flexibility. The cells pass through the wider PDMS constriction without experiencing an EDR large enough to ensure lysis, even under repeated exposure to the constrictions. The lower EDR in the wider PDMS channel translates to cell lysis of only 40%. Since the channels in the OSTE chip did not expand under pressure, the cells were exposed to higher EDR (two orders of magnitude greater), yielding 85% lysis of cells. A video of MDA-MB-231 cells undergoing lysis is available in the supplementary material.²⁴

In addition, for the volumes processed, clogging of the channels was not observed, even when a concentration as high as 6×10^5 cells/ml was used (close to 0.25% volume fraction). Since we used a series of constrictions and expansions, we were able to achieve high lysis efficiency, yet relax the requirement for exceedingly narrow channels, which are prone to high backpressure and clogging, to instead take advantage of the larger EDR at the constriction entrances and exits.

FIG. 7. Percent cells lysed in OSTE and PDMS chips. Cells were diluted down to 10^5 cells/ml and infused at a rate of $100 \mu\text{l}/\text{min}$ for 2 min. The OSTE chip lysed 85% of the cells (standard deviation of 8%, error bar), while the PDMS chip only lysed 40% of the cells (standard deviation of 9%, error bar).

IV. CONCLUSIONS

OSTE is an attractive option for applications that require rigid microfluidic channels. It can easily be replica molded and bonded to itself using a partial curing technique. In this report, we demonstrated replica molding of a mechanical lysis microfluidic device with features down to $5 \mu\text{m}$ (aspect ratio of 15) in an OSTE polymer. Compared to PDMS, OSTE does not expand under pressure, enabling it to maintain a high energy dissipation rate, which yielded $85\% \pm 8\%$ cell lysis, while soft PDMS devices generated only $40\% \pm 9\%$ cell lysis.

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²⁴See supplementary material at <http://dx.doi.org/10.1063/1.4897135> for a video of fluorescently-stained MDA-MB-231 cells undergoing lysis within the cell lysis chip.