# Laser-machining of elastomeric microstencils

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We present laser-machining as a unique approach for the rapid fabrication of poly(dimethylsiloxane) (PDMS) elastomeric microstencils. User-defined patterns are easily created in CAD and imported into CAM control software to machine a thin PDMS membrane with an ArF intra-cavity variable aperture excimer laser (λ=193 nm). Laser-machining can be used to quickly and directly create on-demand microstencils by modifying the CAD program, making laser-machining an attractive alternative to indirectly fabricating microstencils with traditional methods. Laser-machining of the PDMS results in discrete patterns created in the membrane. These etched void spaces can be used to pattern protein and/or cells. Our resulting 50 μm thick microstencils include a wide range of etched feature dimensions (~10 μm – 1 cm) and geometries (Cartesian and polar), and can be used multiple times (>15) demonstrating their functionality and relative durability. Laser-machining of elastomeric microstencils is a scalable process and can be used to control the placement of protein and cells for a variety of applications in the biomedical sciences including high-throughput assays for drug discovery and the creation of cellular micro-environments in single or co-culture platforms.

(Received June 30, 2009; accepted October 12, 2009)

*Keywords:* Laser-machining, Microstencils, Microfabrication, Cellular patterning

## 1. Introduction

*In vivo*, cells reside in specific, organized arrangements to create functional tissue. Traditional *in vitro* methods of cell culture do not allow for precise spatial control to conserve the cellular microenvironment. Many patterning techniques have been developed to modify surfaces for the examination of morphology, cell-cell interactions, and migration. The ability to reproducibly control the spatial placement of cells and protein is valuable for the manipulation of various characteristics of the cellular microenvironment. Controlling cell placement can be accomplished by different methods including: microcontact printing [1, 2], microfluidic patterning [3], and microstenciling [4, 5]. These conventional methods often require a photolithographic mask and a silicon master fabricated in a clean room to indirectly create the devices, stamps, or stencils. If changes to the pattern are necessary, a new master must be created which is costly and time-consuming.

Laser-based micromachining has been previously used to pattern a variety of polymers for biomedical applications [6-8]. In this work, we describe the utilization of excimer laser-micromachining for the direct and rapid fabrication of thin (50 µm) poly(dimethylsiloxane) (PDMS) membrane microstencils. User-defined features specified in a CAD file are exported for CAM control of the excimer laser and motorized stage to create on-demand microstencils. If new pattern dimensions are required, the CAD file can be easily modified. With laser-machining, new microstencils with ~10 µm features over centimeter dimensions can be fabricated and assembled in 15 minutes.

Microstenciling is a versatile method that masks specific regions of a substrate for patterning cells and protein. To illustrate functionality, our laser-machined microstencils were used to pattern adult neural stem cells (NSC) in clusters of varying diameter to investigate the effects of NSC-NSC contact on self-renewal [9].

## 2. Experimental

### 2.1 Microstencil fabrication via laser-machining

For on-demand microstencil fabrication, thin (50 µm) PDMS membranes can be created and stored for later use. These membranes are produced by spin-coating PDMS (1:10 ratio) for one minute at 1500 rpm. The PDMS was allowed to cure for 24 hours at room temperature and cut with a scalpel into ~1 $cm^2$ pieces. The pieces are transferred with tweezers onto a clean glass slide which acts as a support during laser-machining. The user can specify a desired pattern in Vellum, a CAD software package. The user-defined file is exported to the laser and motion software system for conversion to code for controlling the custom built TeoSys laser-machining system (TeoSys, Crofton, MD). This system uses an ArF excimer laser (193 nm wavelength, 10 nano-second pulse width with a mean energy of 20 mJ/pulse) with an intra-cavity variable aperture to improve beam quality (i.e. near $TM_{00}$ beam quality) and etching quality as well. The beam delivery optics include an *in situ* beam energy monitor as well as a co-linear camera viewing system. The laser is focused on the surface of the PDMS membrane and the user adjusts the beam diameter and energy before beginning the laser-micromachining process. The control

program must be run a sufficient number of times to etch through the 50 µm thick PDMS membrane. After the membrane laser-micromachining is complete, PDMS support rings are bonded to the thin membrane using a Harrick plasma asher. The microstencil assembly is then sterilized in 70% ethanol for 30 minutes for use in antibiotic-free cell culture.

### 2.2 Microstenciling process

After laser-micromachining was complete, the microstencil was applied to a clean, sterile surface for patterning protein and/or cells. A drop of 70% ethanol was applied to the surface, the microstencil was then applied, and the entire unit was placed under vacuum for 30 minutes until the ethanol was evaporated. The protein solution was added to the microstencil and incubated for 30 minutes under vacuum. Excess protein was removed and the samples were rinsed thrice with phosphate buffered saline (PBS). If only protein patterning is desired, the microstencil can be carefully removed from the surface with tweezers. For cellular patterning, following protein patterning, the microstencil was seeded with cells. After incubating for four hours, the seeding media was removed and the microstencil was gently lifted off the glass surface using sterile tweezers. The cells remained attached to the spatially controlled protein patterns. The samples were gently rinsed with PBS to remove unattached cells and growth media was added. Samples were cultured for 24 hours (37$^{o}$C; 5% $CO_2$) then fixed with paraformaldehyde for 30 minutes at room temperature.

### 2.3 Isolation and culture of primary adult murine neural stem cells (NSC)

NSC were used for our cellular patterning experiment. Tissue containing neural stem cells was isolated from the subventricular zone of the adult brain of outbred Swiss-Webster mice ages 8-10 weeks old. Following isolation, the tissue was dissociated using a papain solution (Worthington; 20 units/ml). The dissociated NSC were maintained in suspension as multi-cellular neurospheres in the presence of a defined base medium containing epidermal growth factor (20ng/ml) and basic fibroblast growth factor 2 (20 ng/ml). After 7-10 days larger multi-cellular neurospheres were dissociated with papain (10 units/ml) into a single cell suspension. This single cell suspension was applied to a poly-l-ornithine treated microstencil for micropatterning.

## 3. Results and discussion

Laser-micromachining is an effective method for rapid microstencil fabrication for immediate use in cell culture experiments (Fig. 1). Once the microstencil is laser-micromachined, it can be applied to a surface to spatially pattern a variety of proteins or cells on various materials used in tissue culture. This on-demand fabrication method allows the user to specify dimensions which can be easily modified with CAD software, allowing the new stencils to be fully fabricated in less than 15 minutes. The laser energy can be altered to decrease the fabrication time with some sacrifice to resolution with increasing laser energy. Thicker or thinner membranes can be machined through the optimization of both laser energy and repeated machining in the same region. While 50 µm stencils were used, thicker membranes can be fabricated increasing microstencil durability.

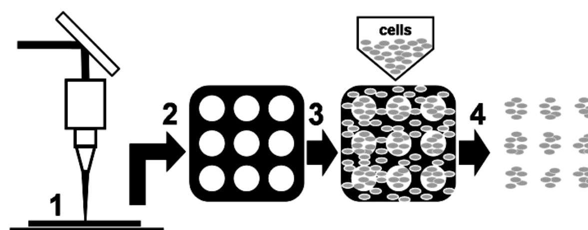


*Fig. 1. Fabrication of microstencils via laser-machining allows microstencils to be created on-demand for immediate use in cell culture experiments. (1) A PDMS membrane is etched by a CAD/CAM controlled excimer laser, (2) leaving the desired patterned microstencil. (3) The microstencil is incubated with cells and following removal (4) leaves the biological material (cells) in a defined pattern.*

A fully fabricated microstencil is comprised of a thin PDMS membrane containing the laser-machined pattern surrounded by a thick PDMS ring (FIG 2). This PDMS ring provides support to the thin membrane containing the stencil features. This assembled microstencil is durable and easy to manipulate with tweezers. The laser-machining process creates some debris and PDMS cracking in the heat affected zone of the microstencil membrane. Caused by sub-threshold irradiation and thermal diffusion, these artifacts are superficial and do not affect the ability of the microstencil to mask the surface.

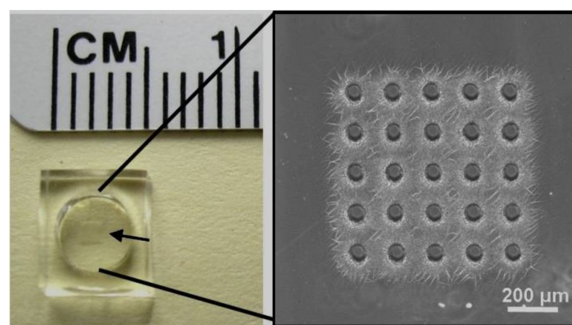


*Fig. 2. A fully assembled laser-machined microstencil has a thin 50µm membrane supported by a ring of thick PDMS. The support ring makes the microstencil robust and can be easily manipulated. The arrow identifies the laser-machined region in the membrane.*

Using the CAD software, a variety of patterns can be generated using this process. Feature patterns fabricated included many shapes, arrays, and text with dimensions ranging from 10-250 µm (FIG 3). The feature dimensions are limited by the beam diameter; a minimum feature size of ~10 µm can be achieved by decreasing the beam diameter. Pattern features can be placed far apart or within ~10 µm of an adjacent feature without compromising the integrity of the microstencil membrane. Feature complexity is a function of laser-micromachining parameters such as beam diameter and laser energy. This feature range is sufficient for cellular patterning as cells are larger than the minimum features generated.

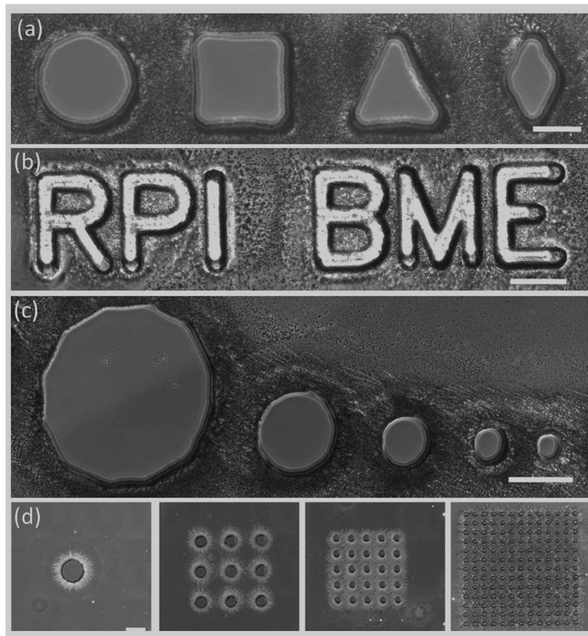


*Fig. 3. Laser-machined PDMS microstencils of varying (a) geometry, (b) text and (c) sizes were fabricated. Microstencils fabricated via laser-machining can have dimensions as small as 10 µm and spaced 10 µm apart. (d). Arrays can be created in a reproducible manner. Scale bar (a-c) = 50 µm; (d) = 250 µm.*

Depending on the pattern complexity, the linear speed of laser-machining is ~14 µm/sec, allowing for a large array (FIG 1d) to be fabricated in less than 15 minutes (90 X $10^6$ pulses), whereas smaller arrays (FIG 1 a-c) can be machined in 2-10 minutes. The user can quickly modify the pattern with CAD software demonstrating the ease of use and direct fabrication of this method. More complicated patterns can be generated automatically using pre-defined algorithms. Laser-micromachining of microstencils is also attractive because it allows for a variety of patterns to be created since the feature dimensions and complexity of these patterns are only a function of the laser beam diameter rather than the master aspect ratio. Production of microstencils with silicon masters may be limited in the production of small features where high aspect-ratio features in the master are fragile or difficult to fabricate. By using laser-machining, small features can be generated in close proximity to adjacent features and are limited only by the laser beam diameter.

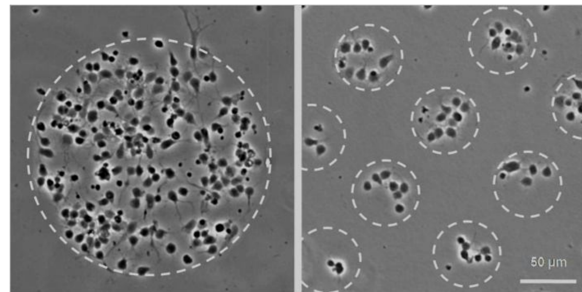


*Fig. 4. Microstencils are used to control neural stem cell placement for cellular interactions studies. Laser-machined microstencils are used to vary the degree cellular interactions by varying the microstencil void geometry. Cells are seeded on the microstencil. Upon removal of the elastomeric membrane, the cells are spatially patterned. The dashed lines represent the void space boundaries of the microstencil.*

Microstencils have been repeatedly used to pattern a wide array of protein without necessitating any harsh chemistries, allowing compatibility for a number of substrates (glass, polymers, and hydrogels). Microstencils can be used to spatially pattern cells or protein by masking regions of the substrate. Cells or protein attach within the unmasked regions. Following the removal of the microstencil, the cells attached to the substrate are spatially patterned. Spatial control of cell placement is important in manipulating cell-cell interactions and engineering microenvironments. In this work, the laser-machined microstencils were used to control homogeneous NSC (NSC:NSC) contact by positioning the cells in regions with increasing feature diameter (FIG 4). The microstencil masks the surface leaving distinct regions of the surface available for cell attachment. By changing the dimensions of the microstencil we are able to control the attachment area, thus the number of cells attaching to void regions can be easily manipulated. Areas with larger diameters supported larger numbers of NSC, while regions with small diameters supported fewer cells per region. We were able to create cellular regions containing different numbers of cells, systematically manipulating the degree of cell-cell contact. These tools can be used to study the effect of cell-cell interactions on NSC fate.

## 4. Conclusions

We have demonstrated a practical method for direct microstencil fabrication using laser-micromachining, a rapid, flexible, direct-write methodology. This is attractive in comparison to master-based soft lithographic approaches because the user can easily specify and modify pattern dimensions with CAD software, allowing new

microstencils with a wide range of features to be directly fabricated in less than 15 minutes. While feature size is limited by the laser beam diameter, adjustments to the laser can be made to obtain features as small as 10 µm within close proximity of one another, which is not easily achieved with masks due to aspect ratio limitations. Additionally, the energy of the laser can be manipulated to reduce etching rates through the PDMS membrane with some sacrifice to feature resolution. Microstenciling is a valuable technique for reproducibly patterning cells and proteins onto many substrates without the use of harsh chemistries or specialized equipment. Rapid fabrication is particularly useful for pilot studies where the necessary stencil features are widely varied. The functionality of our laser-machined microstencils was demonstrated by controlling the placement of NSC to vary the cell number, thus manipulating the degree of cellular contact.

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