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"Pop-slide" patterning: Rapid fabrication of microstructured PDMS gasket slides for biological applications

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ABSTRACT

We describe a "pop-slide" patterning approach to easily produce thin film microstructures on the surface of glass with varying feature sizes (3 μm – 250 μm) and aspect ratios (0.066 – 3) within 45 minutes. This low cost method does not require specialized equipment while allowing us to produce micro structured gasket layer for sandwich assays and could be readily applied to many biological applications.

INTRODUCTION

Glass is widely used as a standard substrate for microfluidic and microchip based applications[1-4] in the form of microscope slides, coverslips and petri dishes. It is cheap, easily available, inert to

many chemicals, and stable over an extended range of temperatures. The transparent nature of glass facilitates imaging of cells and other biological samples, which makes it ideal for many bioanalytical applications. For example, glass slides which are printed with Teflon based epoxy are more commonly used in cell culture and microarray analysis by physically isolating reagents on glass[5, 6]. This prevents cross contamination of substrates allowing multiple analyte detection on the same slide. Recent advances in slide based sandwich assays like the SlipChip[7] and snap chip[8] has further broadened the use of such platforms thereby creating a need for patterning microstructures on the surface of glass.

The primary objective of this work is to allow easy fabrication of microstructured PDMS gaskets on glass. Patterning a layer of thin film microstructures on the surface of glass would not only ease glass-glass bonding for sandwich assays but also facilitate a range of miniaturized biological assays featuring direct imaging.

Soft lithography is commonly used to make polydimethylsiloxane (PDMS) microstructures either on a thick layer of PDMS or a spin coated thin layer of PDMS. PDMS casted from master molds are typically 4-5mm thick. However, working at this thickness will not allow the use of high magnification objectives due to shorter working distances. One could make PDMS slabs to mimic the thickness of a standard microscope glass slide (~1mm) or even thinner by spin coating. However, maintaining the thickness of PDMS accurately can be challenging without the use of additional equipment like a spin coater or an injection molding apparatus. Hence patterning features on commercially available microscope glass slides would more accurately control the thickness of the substrate and make it easier to work within the limits of standard working distances of the microscope objectives.

Current techniques available to pattern microstructures on glass generally require specialized equipment and resources, which limits their widespread application. However, if one were to place glass directly on top of a master mold with spin coated PDMS, it would not be possible to peel a rigid material, such as glass, from the master mold without damaging the microstructural features. Techniques such as micro transfer molding (μTM)[9-11] and micromolding in capillaries (MIMIC)[12, 13], have been developed to produce microstructures on different substrates. However, these techniques suffer from mechanical distortion of edges while peeling away the carrier layer or require reactive ion etching of the thin PDMS layer which blocks access to open microstructural features[14], resulting in increased operating difficulty and the use of expensive equipment. These approaches have been improved for PDMS through-holes fabrication using open capillaries[15] or by modifying the surface polarities of the PDMS prototyping molds[16], but the features produced were in a limited size range (10 μm – 200 μm). Methods of patterning photo-definable PDMS on glass using a photomask[17, 18] or a channel stamping approach using UV curable polymers[19, 20] provide additional options, but these methods require a UV light source and are also limited by the resolution of pattern dimensions achieved on the surface. Thus, there is a need for a simpler and more direct method of producing microstructures on glass that is robust across a range of feature sizes and shapes while accommodating a large pattern area.

To address this need, we present a novel method of producing PDMS microstructures on microscope glass slides that makes use of standard soft lithography techniques. By using a unique combination of PDMS and a releasing agent to ease the separation of the rigid glass slide from the master mold, we are able to directly pattern features onto a glass slide. Our method eliminates the need for a transfer membrane, UV-lamp, plasma cleaner, reactive ion etcher, mask

aligner or a spin coater. We demonstrate that PDMS micropatterns using SU8 master molds produced from low-resolution plastic photomasks (minimum feature size 10 μm) as well as high-resolution chrome photomasks (minimum feature size 3 μm) can be reproduced on a glass slide using our method. We anticipate that our method will facilitate a range of biological assays that would benefit from fabricating thin film microstructures on glass.

RESULTS AND DISCUSSION

Our goal was to use a master mold to directly pattern PDMS microstructures on glass. However, in contrast to flexible transfer substrates like polyethylene sheets or a layer of PDMS, a microscope glass slide is more rigid and difficult to separate from the master mold once stuck to a PDMS layer. Our process addresses this problem with the addition of hexane (a releasing agent) to the PDMS mixture, as well as a series of optimized heating steps, that allows the PDMS to crosslink and separate from the master mold while remaining attached to the glass slide, because PDMS has more affinity for glass than for a silanized SU8 master mold as explained further (Figure 1A). Treating a Si wafer with perfluorinated organosilane causes the surface, which normally has polar functional groups (-Si-OH), to become non-polar and hydrophobic with a lower surface energy when compared to an untreated, plain glass slide[21-24]. Therefore, PDMS naturally has a higher adhesion affinity to a hydrophilic glass surface than to the hydrophobic surface of the silanized Si wafer. However, the rigid nature of glass makes it difficult to initiate release of the thin film to the glass slide, and so without a protocol modification, it remains stuck to the Si wafer. Therefore, we added a releasing agent, hexane, to provide a means to promote the release. The primary heating steps (from 60°C to 100°C) allow the PDMS monomer to crosslink, while we hypothesize that heating to 145°C (more than twice the boiling point of hexane at 68°C) causes hexane vapor to increase and results in separation of

the PDMS microstructures from the mold. It is possible that evaporation of hexane during the initial heating steps (from 60°C to 100°C) also aids in the transfer process, but we have empirically found that higher temperatures are necessary to enable easy release. This method results in a uniform thin film containing the microstructures of interest patterned onto the glass slide (Figure 1B&C). Thus, without the aid of a spin coater, one can use our approach to produce a thin film PDMS membrane by peeling it off from the glass using tweezers.

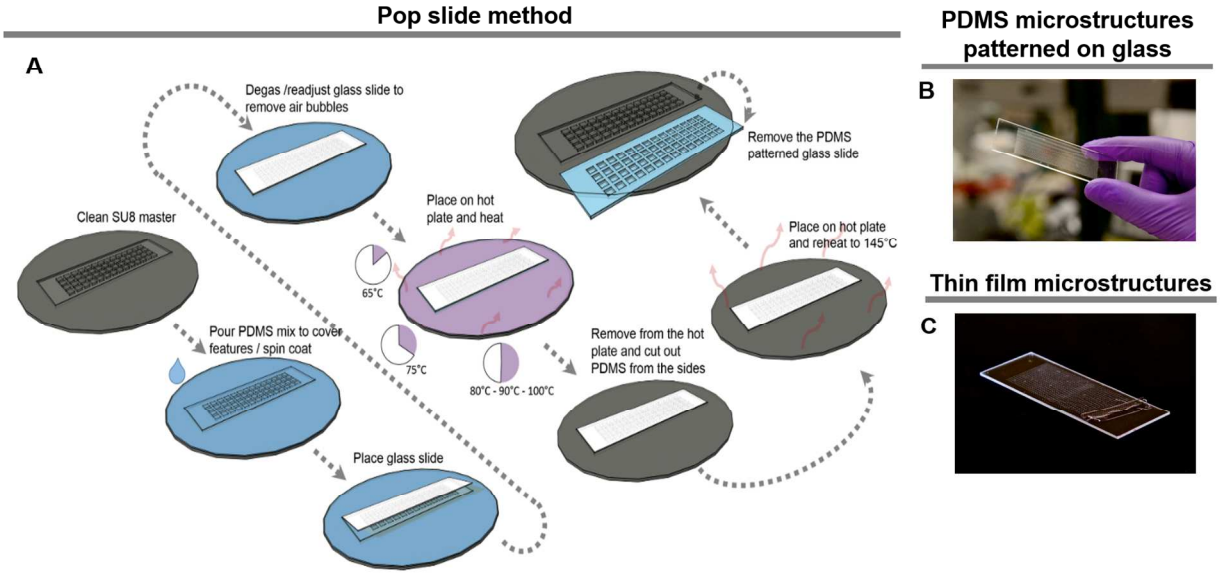

Figure 1. (A) Schematic representation of pop-slide patterning methodology. (B) Photograph of PDMS microwells patterned on a microscope glass slide using this method. (C) Photograph of thin film PDMS microstructures

The pop-slide patterning technique allows us to replicate features from the master mold with negligible distortion. Microstructures such as wells and pillars of variable shapes and aspect ratios (height/width) can be readily fabricated using this method. For example, we have produced circular, triangular, and square wells with variable aspect ratios 0.066 to 0.66 (Fig. 2A-C and Fig. S2A-C) and pillars of the same shapes with variable aspect ratios 0.1 to 1 (Fig. 2D-F and Fig. S2D-F).

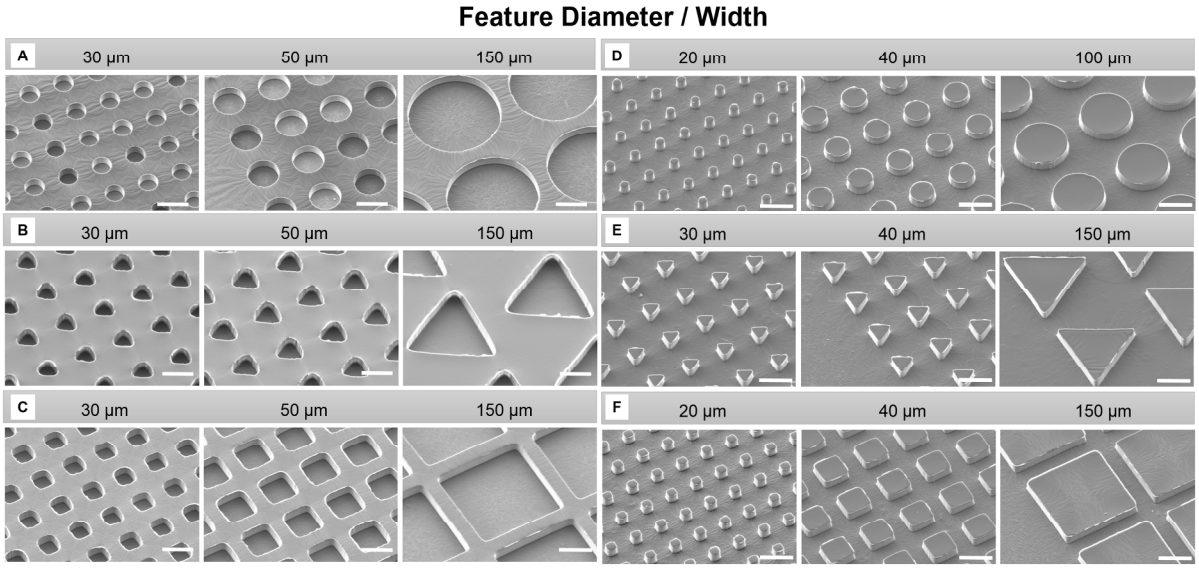

Figure 2. SEM images of PDMS microstructures patterned on a glass slide using pop-slide method. The master mold was prepared using a plastic photomask with 10 µm minimum resolution (see Materials and Methods). (A-C) Circular, triangular and square wells with aspect ratios between 0.066 and 0.33. (D-F) Circular, triangular and square pillars with aspect ratios between 0.066 and 0.5. Scale bar represents 50 µm.

The baking time at different temperatures has been optimized for features with aspect ratios ranging from 0.066 to 3 (Table S1). Features with higher aspect ratios (~ 3.8) can also be patterned on glass using our method with some minor modifications to the protocol. Pillars with aspect ratios > 3.8 tend to bend during the separation process. When PDMS is poured over features with a higher aspect ratio, it gives rise to more bubbles, which require a longer time to degas. It is possible to account for this in the protocol by adding an appropriate amount of the releasing agent in order to avoid excess loss of releasing agent during the degassing step. A 1:1.1 mixture of PDMS (base + curing agent, 10:1) and releasing agent is recommended while working with molds carrying features with higher aspect ratios. The baking time must be

adjusted accordingly to account for increase in the releasing agent, while simultaneously allowing the PDMS to crosslink prior to separating it from the master mold. Uncured PDMS or excess amounts of hexane might give rise to bubbles blemishing the PDMS patterns on the surface of glass.

By optimizing our protocol as described, we have patterned a variety of features with higher aspect ratios on glass ranging in size from ~250 µm to as small as 3 µm, including micro-pillars, micro-gratings, single cell traps, rectangular microwells and microchannels (Fig. 3A-C and Fig. S3).

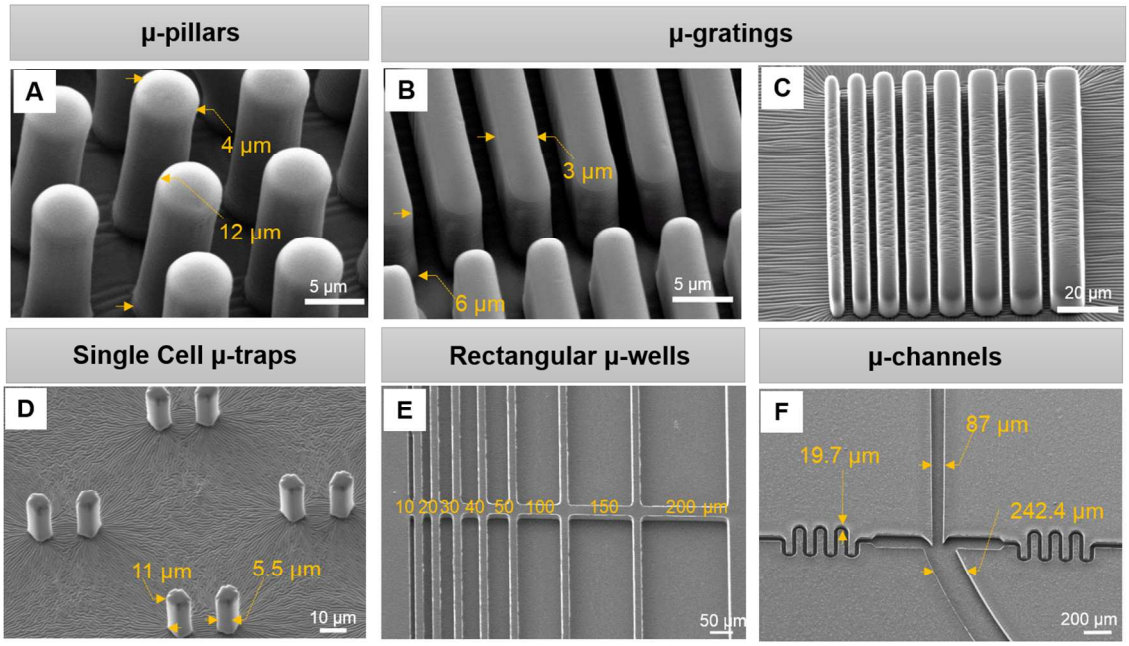

Figure 3. (A-D) SEM images of different PDMS microstructures produced on a glass slide with master mold produced using a chrome mask (minimum feature resolution ~3 µm). Features in (C) range from 3 – 10 µm. (E) SEM image of rectangular wells with variable widths patterned on a glass slide (mold produced using a plastic mask with minimum resolution 10 µm). (F) PDMS microcanals (open microchannels) with serpentine features to help in mixing of fluids are easily micropatterned on the surface of glass.

Our technique produces a very thin layer of PDMS between PDMS microstructures and the surface of the glass slide that is approximately 27 –30 μm when the PDMS patterned slides are fabricated with no additional weight on top of the glass slide. By adding weights (in the form of aluminium blocks weighing around 37g in total) on top of the glass slide during fabrication, we reduced the thickness of the PDMS layer between the glass slide and the patterned PDMS microstructures to 7-8 μm (Figure S4). We believe that this thin layer of PDMS will not affect most biological applications, because cell biology assays are routinely conducted on surface-modified PDMS, and a 7-8 μm thin layer of PDMS would not affect imaging through the glass slide. However if applications require completely hollow structures, then through-glass bottom features produced by other methods like surface micromachining, UV photocurable PDMS or channel stamping may be more appropriate[14, 17].

We anticipate that the pop-slide patterning technique could be used for a variety of biological and analytical applications. For example, patterning a series of microwells on the surface of a glass slide facilitates high-content screening of biological analytes while simultaneously performing cell-based assays. Standard microscope glass slides functionalized with PDMS features can be patterned with proteins to aid in the specific adhesion of cells[25] or beads (Fig. 4A). Alternatively, cells can be grown on the glass slide containing micro-well arrays of specific shape and dimensions (Fig. 4B). Live-cell staining with Calcein AM demonstrated that the patterned glass slides are biocompatible. This observation supports our assumption that all residual hexane on the microscope slide is eliminated as a result of the heating process, such that the features are not toxic to the cells.

By combining the ability to both grow cells and pattern proteins on the glass slides, this microstructured PDMS thin film platform could facilitate micro-well-based secretomic analysis[26,

[27]. Such sandwiched glass slides can be readily accommodated in commercial Genepix microarray scanners or any standard microscope stage holders for live cell imaging studies. Capture antibodies or other proteins of interest can be spotted inside the micro-well array, which can then be sandwiched on top of cells or cell islands grown on a glass coverslip. This would serve as independent micro-chambers on which the secreted cytokines can be readily imaged using commercial microarray scanners. Alternatively, micro-compartments can be formed by seeding cells directly inside the microwells (as in Fig. 4B) and then covering the wells with an antibody-patterned glass coverslip. Compared to the first method in which the cells are patterned onto the coverslip, attaching cells to the bottom of the micro-well reduces the amount of stress that acts directly on these cells while sandwiching two glass slides. However, both these methods would be compatible with adherent or surface-immoblized cells, as similar approaches are currently practiced using thick PDMS casts.

In the aforementioned assays that require an isolated cell compartment, if the microwells are patterned onto PDMS, then physical clamps usually hold the thick layer of PDMS to the glass. This often requires customized microscope stage holders to accommodate the clamps. The ability to have thin film microstructures on the surface of glass would act as a gasket and not only allow direct imaging through the glass slide but also allow easy and uniform bonding/sealing of another slide without the requirement of external clamps to hold them for extended assay periods.

Finally, given the ability to pattern smaller microstructures using our technique, we could study the interaction of cells with variable physical microenvironments and substrate rigidities. Patterning micro-pillars and micro-gratings on glass can be very useful to conduct cell mechanistic studies[28, 29]. Such features can also serve to contain ECM patterned on them thus

creating a suitable microenvironment for cells to proliferate and differentiate (Fig. 4 C-D). In addition, cells that are seeded on the thin film microstructures can be peeled off, thus forming a cell sheet[30, 31] that could be further subjected to variable stretching forces for cell differentiation studies[32, 33].

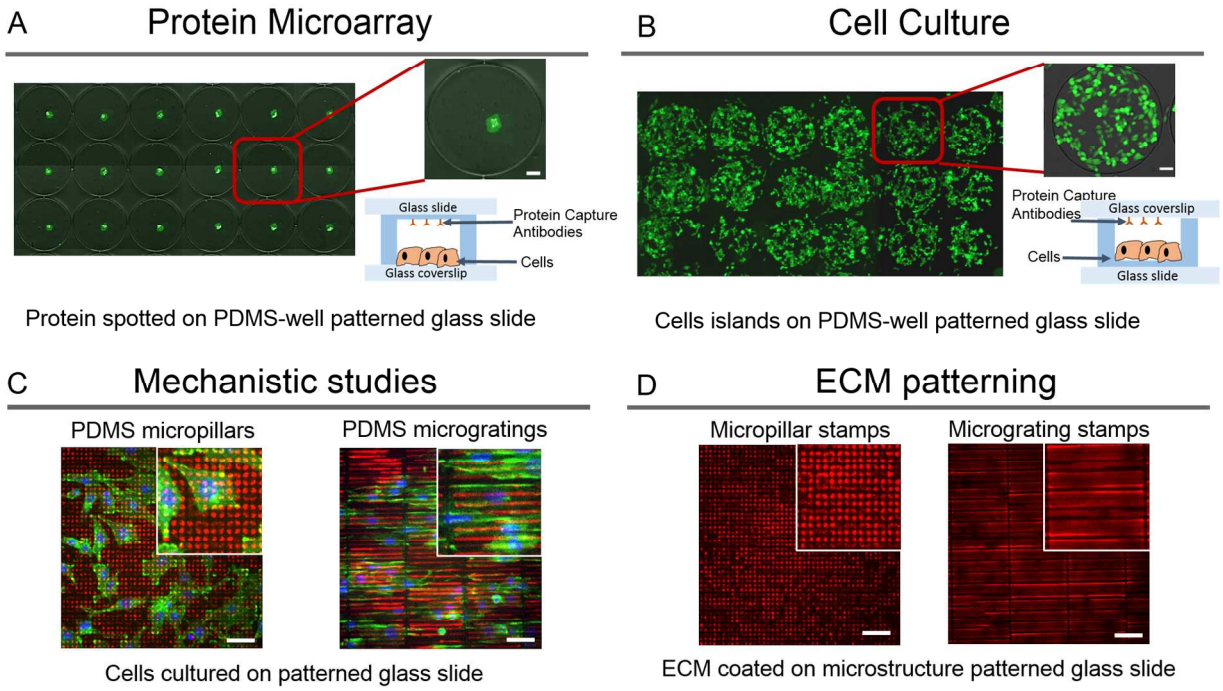

Fig 4: (A) Fluorescent images of protein (FITC-BSA) spotted inside each well of a micro-well array patterned onto a glass slide. Schematic representation of a cell based assay with capture antibodies spotted/coated onto the micro-well array. (B) Fluorescent image showing live cells (stained with Calcein AM) attached inside micro-well array patterned onto a glass slide. Schematic representation of a cell-based assay with cells attached to the micro-well array and sandwiched under a capture antibody-coated slide. (C) 3T3 cells seeded on pop-slide patterned micro-pillars and micro-gratings for cell mechanistic studies. Cells are stained for nuclear and actin localization markers, DAPI and Phalloidin-647 respectively. (D) ECM – rhodamine-

fibronectin coated on micro-pillar and micro-gratings produced by pop-slide patterning. Inserts shown in C and D represent magnified images as shown. Scale bars represent 50 μm.

CONCLUSIONS

We have demonstrated a new approach to pattern thin film microstructures on the surface of glass. The pop-slide patterning technique uses a combination of a releasing agent and optimized heating temperatures that allow us to efficiently produce open PDMS microstructures on glass within 35-45 minutes, which is 2-3 times faster than other methods. We believe that the "pop-slide patterning" technique is most useful for labs that do not have access to a clean room or other microfabrication facilities. Researchers who do not have access to such facilities can have SU-8 molds custom-ordered with any pattern of interest from commercial companies, and then fabricate the micropatterned slides in their own lab with a standard hot plate.

These thin film microstructure patterned glass slides can be readily used for imaging applications due to the transparent nature of PDMS and the glass slide. Potential uses of this technique include, but are not limited to: 1) high-content quantitative imaging of cell- and bead-based assays in a micro-well slide[4, 28, 34]; 2) production of micro-lens arrays on a glass slide[35, 36]; 3) fabrication of multilayer microfluidic devices with an added advantage to support microstructures in the lower layer while imaging through the glass bottom[37-39]; 4) fabrication of open microfluidic canals for easy access to cells in patch clamping analysis[11]; 5) high-throughput screening of cells/stem cell differentiation on PDMS micro-gratings or topographies on glass[40-42]; or 6) membrane stretching and traction force microscopy[29, 41]. With further optimization, we

anticipate it would be possible to pattern microstructures on coverslips to produce, for example, microchannels for single molecule measurements using optical tweezers. We believe that this technique can be widely applied in fields such as biomedical engineering, material science, optics, and imaging.

EXPERIMENTAL SECTION

Master Fabrication

Standard SU8 photolithography technique was carried out on a 4 in. silicon wafer to produce master molds with patterns of interest. Feature heights in the silicon master were measured using a KLA-Tencor ASIQ profiler. Master molds produced from photoresists that are resistant to organic solvents such as hexane, isopropyl alcohol etc., are recommended for the pop-slide patterning method in order to avoid leaching of features in the master. Alternatively, silicon etched masters can also be used.

Pop-slide patterning

A schematic overview of the method is presented in Figure 1A. Briefly, 1.5 mL of a 1:1 mixture of PDMS (base + curing agent, 10:1) and a releasing agent (hexane) is poured to cover the surface of the feature or spin coated on the silicon master mold (Figure S1A). Organic solvents that are soluble with PDMS and have a low boiling point are suitable releasing agents for this method. Compared to solvents such as isopropyl alcohol, hexane exhibited better release properties with negligible feature distortions. A glass slide is then placed on top of the PDMS mixture covering the silicon master mold without introducing any air bubbles and is degassed (~5-10 minutes) in a vacuum chamber to remove any air bubbles introduced in this step.

Placement of the glass slide on the mold and adjusting it during the process is very simple and is easily repeatable. There is minimal movement of the glass slide (2-5 mm) during the patterning process and it remains in place when the SU-8 mold is heated beyond 70˚C. This movement can be further restricted by placing PDMS/Aluminum blocks on the edges of the slide. Later, the master mold is baked on a hot plate through a series of temperatures (Table S1) to crosslink the PDMS. Excess PDMS on the sides of the glass slide is removed with a scalpel once the PDMS has cured. This is an important step as it reduces the internal stress acting on glass slide attached to the master. The master is then placed back on the hotplate and immediately heated to 145˚C. This sudden heating causes the residual hexane to expand between the PDMS layer, thus causing the glass slide to slightly pop out from the silicon master mold. The glass slide can then be lifted off from its edges using a scalpel. Excess PDMS sticking to the sides of the glass slide can be easily cut using a razor blade. The PDMS patterned glass slide is then rinsed with isopropyl alcohol and taped in order to remove any dust particles. The slides can be spotted with proteins of interest or used for cell culture, either immediately or at a later time. See Supplementary Video 1 for a movie of the pop-slide patterning technique.

Imaging, feature and step height analysis

In addition to observing the patterned features in bright field, a scanning electron microscope, SEM (Hitachi SU-70) was used to inspect the patterned features. The PDMS patterned glass slide was sputter coated (Denton chromium sputtering tool) with 20 nm of chromium prior to imaging. Feature heights of the PDMS microstructures patterned on the glass slide were measured using a KLA-Tencor ASIQ profiler. Multiple replicates of the same master mold showed uniform pattern heights on the glass slide (Fig. S1B). Fluorescent images were obtained

from a Nikon TiE fluorescent microscope, confocal microscope and Evos FL Auto imaging system. All images were processed using ImageJ software.

Protein spotting and cell culture

A commercial microarray spotter, SpotBot 3 (Arrayit Inc., USA) was used to spot FITC-BSA inside the PDMS microwell array patterned on the glass slide. The latest version of the software SpotApp (v5.1.5) and Spocle Generator (v5.2.2) allowed us to spot proteins of interest in any region inside the glass slide. HeLa cells and 3T3 were cultured separately in complete DMEM media (10% FBS) on tissue culture dishes following standard cell culture protocols. Approximately 3 million cells/mL were seeded over a plasma treated glass slide containing the patterned PDMS microwells. A plain glass slide was placed on top of this to allow cells to attach specifically to sites inside the well and later removed and covered with complete media. The cells were allowed to grow overnight within the wells and imaged with a live / dead stain (Life Technologies, USA) 24 hours after cell seeding.

Supporting Information.

A video demonstrating the pop-slide patterning method is presented. In addition, a supplemental table containing the optimized temperatures along with supplemental figures S1-4 as described in the manuscript is presented in this section.

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Author Contributions

R.R. conceived of the project and developed the technique. R.R., A.M-R. and N.K. performed the experiments. R.R. and K.M.J. wrote the manuscript with comments from all authors. All authors have given approval to the final version of the manuscript.

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