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Straight SU-8 pins

R Safavieh, M Pla Roca, M A Qasaimeh, M Mirzaei and D Juncker

Biomedical Engineering Department and Genome Quebec Innovation Centre, McGill University, Montreal, Canada
E-mail: david.juncker@mcgill.ca

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Abstract
SU-8 can be patterned with high resolution, is flexible and tough. These characteristics qualify SU-8 as a material for making spotting pins for printing DNA and protein microarrays, and it can potentially replace the commonly used silicon and steel pins that are expensive, brittle in the case of silicon and can damage the substrate during the printing process. SU-8, however, accumulates large internal stress during fabrication and, as a consequence, thin and long SU-8 structures bend and coil up, which precludes using it for long, freestanding structures such as pins. Here we introduce (i) a novel fabrication process that allows the making of 30 mm long, straight spotting pins that feature (ii) a new design and surface chemistry treatments for better capillary flow control and more homogeneous spotting. A key innovation for the fabrication is a post-processing annealing step with slow temperature ramping and mechanical clamping between two identical substrates to minimize stress buildup and render it symmetric, respectively, which together yield a straight SU-8 structure. SU-8 pins fabricated using this process are compliant and resilient and can buckle without damage during printing. The pins comprise a novel flow stop valve for accurate metering of fluids, and their surface was chemically patterned to render the outside of the pin hydrophobic while the inside of the slit is hydrophilic, and the slit thus spontaneously fills when dipped into a solution while preventing droplet attachment on the outside. A single SU-8 pin was used to print 1392 protein spots in one run. SU-8 pins are inexpensive, straightforward to fabricate, robust and may be used as disposable pins for microarray fabrication. These pins serve as an illustration of the potential application of ultralow stress SU-8 for making freestanding microfabricated polymer microstructures.

1. Introduction

The negative tone epoxy photoresist EPON SU-8 can readily be patterned with aspect ratios as high as 20, and with thickness varying from nanometers to 1 mm [1]. SU-8 has been used for making a wide variety of devices, including cantilever-based biosensors [2, 3], low-cost point-of-care diagnostics [4, 5] and microvalves [6]. One of the major drawbacks of SU-8 is its high residual stress, which leads to bending of the microstructures [7, 8] caused by the mismatch between the thermal expansion coefficients of SU-8 and the substrate.

Many approaches have already been developed to reduce the residual stress in SU-8. One efficient way is to use an adaptive layer made of polymers such as polyethylene terephthalate (PET) [8] or polystyrene [9] that have an expansion coefficient similar to SU-8. These adaptive layers also act as release layers, which can be dissolved and allow detaching of the SU-8 structures without resorting to mechanical force [9, 10]. Similarly, SU-8 itself can be used as an adaptive layer by evaporating a protective layer of metal (i.e. gold or chromium) on top. The sacrificial SU-8 layer is shielded from UV light and from being cross-linked by the metal, and can thus be dissolved at the end of the process, leaving freestanding structures [11]. Another approach is to modify the baking parameters [8–10, 12, 13] and specifically reduce the temperature of the pre-bake. A lower pre-bake temperature results in a higher residual solvent content, which leads to increased mobility of monomers that in turn allow lowering the post-bake temperature and thus the overall stress is reduced [13]. A drawback of this method is a high residual solvent content which tends to evaporate subsequently and lead stress buildup, which is what we observed as part of...
our experiments and describe in the results section. Whereas these strategies help reduce the residual stress and bending by orders of magnitude, the distortion still remains significant. Due to these issues, SU-8 has not yet been used for making thin, long freestanding and straight structures, such as pins for microarray spotting, to the best of our knowledge.

Pin spotting (or printing) is the original method developed for patterning biomolecules such as DNA and proteins as microarrays and is still widely used due to its ease of operation [14]. The idea of robotized pin spotting was introduced as early as 1996 for making DNA microarrays [15]. In protein microarrays, antibodies or purified proteins are microarrayed instead of DNA. Antibody microarrays are used analogously to DNA microarrays to measure the concentration of tens or hundreds of proteins at once from minute amounts of (crude) sample by measuring the fluorescent intensity on each spot [16]. Pin spotting operates by first dipping an array of pins in a 96 or 384 microtiter well plate containing spotting solutions, retracting it along with a small droplet of solution captured by capillary effects on each pin, or inside the slit if there is one, and then printing the pin array onto a surface, thus affecting the transfer of the solution from the pin to the surface. The transfer of liquid is a result of the balance between the capillary retention of the pin and the capillary effects resulting from the dynamic contact between the pin and the substrate; a microscopic gap is formed immediately before and after the contact between the pin and the substrate, and it draws liquid onto the surface. Scaling is easy for contact printing and print heads with 96 pins are common, and heads with up to 192 pins have been produced [17], which is much larger than for bioinkjet printers that are limited to 16 or 32 heads. The scalability stems from the fact that loading and spotting operate using capillary effects so that a single functional pin can be cloned and used as part of an array of pins with identical properties for each one.

Many different pin designs have been developed ranging from simple needles with a blunt tip useful for a few spotting cycles only to pins with slit and reservoirs that can be used to print hundreds of spots following a single dip loading [14]. Pins are traditionally made out of stainless steel, tungsten and titanium, and are manufactured serially using techniques such as sawing, grinding, electric discharge machining (EDM) and laser machining [14, 15]. Ceramic pins and microcapillaries [18] have also been made and shown to be more durable and less susceptible to tapping forces, while improving the spot morphology and consistency [19]. However, both for metal and ceramic pins, the fabrication remains time-consuming and cost intensive.

More recently, Si pins have been introduced. These pins can be fabricated by batch processing using micromachining techniques such as deep reactive ion etching. When using Si, much higher resolution can be achieved, and much smaller pins can be made which can be arrayed at a much higher density, up to one pin every 2.25 mm (which is the same spacing as in a 1536 microtiter well plate) [17]. However, the Si pins remain expensive, because costly deep reactive ion etchers are needed, and they need to be etched through a wafer, further entailing long fabrication time. Another drawback of Si is that it is a brittle material, and consequently, the pins break easily during manipulation and the tips rapidly deteriorate and lose their functionality. Si pins therefore need to be replaced regularly.

In addition, another important issue with these pins is droplets sticking on the outside of the pins, which is more pronounced with rectangular silicon pins compared to the rounded steel pins [20].

One of the major issues with the usage of pins is that the contact between the pin and the substrate can lead to an inhomogeneous spreading of the sample. Spot homogeneity still remains a challenge despite many years of work on pins and is difficult to control, as it depends not only on the geometry of the pin itself, but also on its surface chemistry, on capillary effects and can vary as a function of the number of printing cycles. In particular, the first spots tend to be larger than the later ones as the solution is used up.

Polymer materials have not been used for fabricating pins despite the fact that they are inexpensive, tough and therefore likely to sustain many printing cycles. An important reason is that polymers are difficult to structure at the microscale with the high aspect ratios needed for making spotting pins, with the notable exception of SU-8, but it suffers from high internal stress.

In this paper, we present a novel fabrication process with an annealing step for making long, thin freestanding SU-8 structures that are straight over a length of 30 mm without measurable bending as assessed by optical microscopy. This process is used to make SU-8 pins with an improved design, including a novel capillary stop valve that allows precise metering of the sample. We also introduce surface patterning of pins by microcontact printing of hydrophobic and hydrophilic thiols on the rectangularly shaped pins. We illustrate the resilience of SU-8 pins by reversible buckling, and assess their spotting performance by testing the number of spots that can be produced and by measuring the homogeneity of a microarray of spots.

2. Design of the pins

The pins need to be inserted into a commercial pin holder (see figure S1 in the supplementary materials, available at stacks.iop.org/JMM/20/055001/mmedia) with collimators that serve to align the pins and are made for pins that are 200 μm thick and 1 mm wide, and which impose the width and thickness of the pins [17]. The length of the pins was chosen to be 30 mm, which is needed to reach the bottom of the wells of microtiter plates for loading liquids. The design is shown in figure 1.

The pins can be divided into three functional parts. The first one is the tip, which comes in contact with the substrate and controls the size of the spots; the second one is the slit, which acts as a microfluidic reservoir and a capillary pump; and the third part is a ‘bubble’ with a stop valve, which is used to precisely control the amount of liquid loaded into the pin. The basis for our design was the use of pins commercially distributed by parallel synthesis [17]. The main changes are the bubble geometry, surface chemistry and material used, all of which contribute to added functionality.
Two layers (figure 1) of the pin and (d) featuring the (two) layers of the pins. (e) Bubble with a capillary stop valve, which was designed to stop the liquid at the end of the microchannel. \( R = 200 \, \mu m \) is the radius of curvature in the top part of the stop valve, \( r = 50 \, \mu m \) is the radius of curvature in the bottom part of the stop valve and \( \beta \) is the angle at the interface between the channel and the bubble, which makes the liquid stop at the end of the channel.

### 2.1. Tip

The size of the tip is determined by the thickness of the tip and its width, which in turn controls the size of the spot deposited on the surface. The pin consists of one or two layers (figure 1(d)), where the first layer controls the ‘height’ of the spots, \( t \), and the two layers together make up \( 200 \, \mu m \) in thickness, which is imposed by the size of the collimator. When making \( 200 \, \mu m \) spots, a single layer is suitable; however, for spot sizes less than \( 200 \, \mu m \), two-layer pins are needed. A tip with a square cross-section of \( 100 \times 100 \, \mu m^2 \) leads to a spot with a rounded square shape having a diameter of \( 140 \, \mu m \).

### 2.2. Slit

For spontaneous filling of the microfluidic channel, there needs to be a free energy gain. The increase of solid–liquid interface during filling (energy gained) needs to compensate for the increase in the liquid–air interface (energy lost) in the slit. Expressed differently, the capillary pressure at the filling front in the slit needs to be negative. For pins with a slit (capillary gap) of width \( w \) and a depth \( t \), and an advancing contact angle between the solid–liquid interface in the microchannel, \( \theta_a \), the governing equation for the total pressure of the liquid in the pin’s slit can be written as follows [21]:

\[
P = \frac{2\gamma}{t} - \frac{2\gamma \cos \theta_a}{w} + \frac{\rho gh}{w} < 0. \tag{1.1}
\]

For a capillary slit with a maximal length of \( 7 \, \text{mm} \), as is the case here, the hydrodynamic pressure difference due to the gravity is \( \Delta P = 70 \, \text{Pa} \) and can be neglected when compared to the capillary pressure. The pressure in the liquid depends on the width of the slit and on the aspect ratio, \( t/w \). By decreasing the aspect ratio, the capillary pressure decreases and when the ratio of the thickness, \( t \), to the width, \( w \), approaches 1, filling becomes conditional on having contact angles very close to 0°, and turns into energetically unfavorable under all circumstances for \( t \geq w \). Using a design similar to the one proposed here, the thickness of the slit is \( t = 200 \, \mu m \), water was assumed as a working medium with \( \gamma = 70 \, \text{mN} \, \text{m}^{-1} \), and \( \theta_a = 45^\circ \) [22]. Under these conditions, \( \Delta P < 0 \) for \( w < 141 \, \mu m \), and the liquid will fill the slit spontaneously. Figure 2 shows the capillary pressure of the liquid as a function of the width \( w \) of the slit. In order to ensure robust filling of the pins and provide a sufficient volume, \( w \) was set to \( 100 \, \mu m \). Close to the tip, where the thickness and the width of the pin are reduced, \( w \) narrows down as well, but since the tip is the last to be drained, it does not affect the capillary pressure during spotting.

In addition, we estimated the elastic deformation of the tip of the pins due to the capillary pressure, which after filling may lead to bending of the tips, if the pins are too soft. For SU-8, which has an elastic modulus of 5.25–6.21 GPa [23], the tip deflection is only about 1 \( \mu m \) for a pin that has \( 7 \, \text{mm} \) long prongs (see figure S2 in the supplementary materials, available at stacks.iop/JMM/20/055001/mmedia). This value is negligible in practice for the slit sizes of \( 100 \, \mu m \) and will not influence the capillary transfer from the pin to the substrate.
2.3. Stop valve

To minimize the variation of spot size and volume during spotting, it is important that the capillary pressure remains constant during the entire printing process. A pin with a straight conduit that stops at once (i.e. a dead-end channel) would satisfy the capillary pressure condition; however, because the conduit is open, dewetting of the liquid at the ‘dead-end’ is energetically unfavorable and the liquid column would dewet somewhere along the path and the liquid remains trapped in the pin at the dead-end. It is therefore important to include a stop valve in the flow path that stops the liquid from reaching the end of the slit, and design the valve such that it constitutes the location offering the least resistance to the draining of the liquid. Existing pins typically use graded enlargement of the channel width at the end of the capillary, which gradually increases the width to depth aspect ratio until filling becomes unfavorable. Because of the graded change, there is no definite stop, which in turn can lead to initial variability when starting to spot [17]. Stop valves can be formed by a sudden enlargement of a microfluidic conduit. When operating in a closed microchannel, it is difficult to make an efficient stop valve, because although the width can easily be enlarged, it is challenging to enlarge the depth simultaneously [24]. In the case of a pin, however, there are no bottom and top walls, and very robust valves can be produced that reliably stop a solution, even for highly wetting liquids [24–27].

3. Materials and methods

3.1. Microfabrication of the pins

We fabricated one- and two-layer SU-8 pins using standard microfabrication procedures (figure 3 (for one-layer pins, steps (d) and (e) were skipped). First we crushed a polystyrene Petri dish (Fisher Scientific, Canada) and dissolved 10 g of polystyrene chips in 100 ml of toluene (Fisher Scientific, Canada) by stirring the solution at 80 °C for 5 h. We then poured 5 ml of the solution on a 4 in Si wafer and spin-coated the substrate at the rate of 3000 rpm for 30 s to form a 3–4 μm thick polystyrene layer. Subsequently, we placed the silicon wafer on a hotplate at 80 °C for 5 min to remove the solvent. The thin layer of polystyrene fulfills two functions. Firstly, it helps minimize the residual stress in the SU-8, and secondly, it serves as a sacrificial layer that facilitates the release of the pins from the substrate [9]. We patterned two layers of SU-8 using standard photolithography process atop the polystyrene layer. The parameters of the baking steps used to make the SU-8 pins are summarized in table 1. We then developed the microstructures of both layers at once by immersing the wafer in the SU-8 developer (propylene glycol methyl ether acetate, Microchem, USA), which was followed by the release of the SU-8. The wafer was immersed in a toluene bath at 80 °C for 5 min to remove the solvent. The pins were characterized by both optical (LV150 industrial microscope, Nikon, Japan) and scanning electron microscopy (S-3000N variable pressure SEM, Hitachi, Japan).
3.2. Annealing of SU-8 to eliminate the residual stress

We developed an annealing process to remove the residual stress in SU-8. The freestanding pins were clamped between two rigid glass slides (75 × 25 × 1 mm³ microscope slides, Fisher Scientific, Canada) by using paper clips (fold back clips, Staples, Canada; see figure 3(g)). Using a programmable oven (Lindberg Blue M, Fisher Scientific, Canada), we subjected the setup to a heat cycle which consisted of first a temperature ramp from room temperature to 150 °C in 15 h, then keeping it constant at 150 °C for 15 h and finally cooling it down to room temperature in 24 h.

3.3. Surface treatment of the pins

We coated the pins by sputtering 10 nm Ti followed by 50 nm of Au. Then we used a flat stamp of poly(dimethylsiloxane) (PDMS; Sylgard 184, Dow Corning) inked with a solution of 1% n-decanethiol (Fluka, Canada) in ethanol to print on the top- and back-side surface of the pin to coat the outer surfaces with a hydrophobic n-decanethiol [22]. The slit of the pins was then selectively coated with hydrophilic thiols by immersing them for 1 h in a 1 mM solution of PEG-thiol (Rapp Polymere, Germany) in water. Figure S3 in the supplementary materials, available at stacks.iop.org/JMM/20/055001/mmedia, illustrates the entire patterning process.

3.4. Spotting and imaging of proteins

We first placed the pins in a commercial pin-holder (Parallel Synthesis, USA) with two micromachined collimators (see figure S4 in the supplementary materials, available at stacks.iop.org/JMM/20/055001/mmedia, for more details), and spotted the microarray using a customized spotting robot (nano-plotter 2.0, Gesim, Germany). Chicken antigoat IgG, labeled with fluorescein, was diluted at a concentration of 200 μg ml⁻¹ in a solution of carbonate buffer at pH 9 containing 10% glycerol. The solution was spotted with a single pin on an epoxy-coated glass slide (Nexterion, Schott, USA). The slide with the array of droplets forming the protein array was scanned with a laser scanner (LS400, Tecan, NC, USA) using 532 nm laser and a resolution of 10 μm. The images were analyzed using Image J.

4. Results and discussions

4.1. Fabrication

Two variants of the pins were fabricated using either one or two layers of SU-8, but always with a total thickness of 200 μm so as to fit into the collimator (see figure 1). Figure 4 shows micrographs of both types of pins. The single-layer SU-8 pins made using only one lithographic exposure had a slit with a width of 50 μm and a total width of 75 μm at the tip. Two-layer pins were fabricated using two-step lithography process with a tip size of 50 × 75 μm² and therefore lead to much smaller spots.

The volumetric capacity of the pins was adjusted by making channels with different lengths, and by making pins with two channels (see figure S5 in the supplementary materials,
Figure 5. Visualization and quantification of the deflection \( \partial \xi \) of 200 \( \mu \text{m} \) thick SU-8 pins seen lying on their side and that were fabricated using different processes described in the text. \( \partial \xi \) is defined as the deflection of a pin at the tip when fixed by the two anchor points of a collimator marked by an X. (a) Pin fabricated according to the recipe recommended by the manufacturer, which leads to a deflection of \( \partial \xi_1 \approx 6 \text{ mm} \). (b) Pin made using optimized baking parameters and an adaptive polystyrene layer, \( \partial \xi_2 = 500 \pm 200 \mu \text{m} \). (c) Pin fabricated with the same recipe as in (b), and subjected to an annealing step, \( \partial \xi_1 = 0 \pm 30 \mu \text{m} \).

available at stacks.iop.org/JMM/20/055001/mmedia, for additional details). The pins presented here have a transfer capacity of 100–300 nl.

4.2. Annealing process

A critical parameter for pin spotting is the alignment and straightness of the pins. Indeed, high-density arrays have a pitch of only 250 \( \mu \text{m} \), which implies that the pins need to be perfectly straight as a deviation of even a few hundred micrometers (for a length of 30 mm) can lead to an overlap of adjacent spots. Bending of the SU-8 structures is caused by the internal stress, arisen as a consequence of the thermal mismatch and the asymmetric configuration of the SU-8 atop of adjacent spots. When annealing with 175 \( ^\circ \text{C} \), we found that the tips of the pins were stuck to one another after processing. By reducing the annealing temperature to 150 \( ^\circ \text{C} \), the tips remained separate while still minimizing the stress. We also found that slow ramping, and in particular, slow cooling further helped reducing the residual bending. When using the set of optimized parameters presented in section 4.2, the residual bending of SU-8 was reduced within the accuracy of the optical measurements used, \( \partial \xi = 0 \pm 30 \mu \text{m} \) (see figure 5(c)). Here, the value \( \pm 30 \mu \text{m} \) corresponds to the estimated accuracy of our imaging setup. We also found that annealed pins were stable for at least 10 months, figure 5(c), whereas non-annealed pins tend to bend after a few weeks up to \( \partial \xi = 2000 \pm 1400 \mu \text{m} \) (figure is not shown), which we attribute to the evaporation of the residual solvent.

4.3. Toughness and flexibility of the polymer pins

Polymers are tougher and more flexible than Si, which should translate into more reliable pins. Indeed, Si pins regularly break due to the brittleness of Si. To illustrate...
the resilience of the SU-8 pins, they were subjected to a bending cycle (see figure 6, showing how the pin buckles without breaking and flexes back to its original shape (also see the movie S1 in the supplementary materials, available at stacks.iop.org/JMM/20/055001/mmedia). The tip of the pin did not show any visible deterioration (data not shown). Although such high loads are not normally applied during microarray spotting, software glitches and human errors sometimes result in pins being subjected to high strain, which often lead to the destruction of metal or Si pins. In the course of a printing experiment described below, a pin was subjected to 2000 buckling cycles, as shown in figure 6 without observing notable permanent deformation for the pin or the tip.

Figure 6. Images extracted from a movie showing the elastic buckling of an SU-8 pin as it is being pressed against a substrate, followed by its unbending. (a) The SU-8 pin before elastic deformation, (b) and (c) during elastic buckling, and (d) after buckling.

4.4. Surface treatment

Native SU-8 is hydrophobic with $\theta_{\text{water-SU-8}} = 90^\circ$ [31] and therefore in the absence of surface treatment, aqueous solutions do not spontaneously fill the slit of the pin. Figure 7(a) shows a native SU-8 pin that was dipped into an aqueous solution containing fluorescent rhodamine dye. Whereas the slit was not filled, a drop was attached to the tip of the pin. To ensure spontaneous filling of the slit with water, the inside of the slit was selectively made hydrophilic using a self-assembled PEG-thiol monolayer. Following this treatment, the liquid spontaneously filled the slit and no drops attached to the outside of the pin (figure 7(b)–(e)) (see the movies S2 and S3 in the supplementary materials, available at stacks.iop.org/JMM/20/055001/mmedia). The surface treatment is critical to obtain functional pins and prevent droplets from attaching to the outer surfaces of the pins.

4.5. Spotting of proteins

Pins were used to spot a solution with a fluorescently labeled protein as a microarray. Slides are commonly scanned after

Figure 7. Capillary filling of pins. (a) Fluorescence micrograph of an SU-8 pin without surface treatment that was dipped into a solution containing fluorescent rhodamine. (b) Pin coated with a hydrophobic thiol on the outside and a hydrophilic PEG-thiol on the inside. (c) Upon contacting a droplet, (d) the liquid spontaneously fills the slit. (e) The bubble with the stop valve stops the filling and ensures precise metering of the solution within the pin.

Figure 8. Fluorescence images of a microarray of 1392 spots of a fluorescent protein that were spotted using an SU-8 pin in one run with a pitch of 200 μm. (a) Overview of the array with 4 sub-arrays of 20 × 20 spots. The last visible well-printed spot is at the 12th row on the 10th line. (b) Close-up view of 8 × 9 spots and (c) four spots of the microarray.
wiping only, the size and homogeneity of the spot can vary due to inhomogeneity of the surface chemistry. To avoid this problem, we scanned the slides immediately after spotting with an SU-8 pin 2000 times, while the droplets were still present on the glass slide. For the protein spotting, a two-layer pin with a tip size of 50 × 75 μm², and a 7 mm long slit with a capacity of 140 nl was used. The humidity was set at 70% and the solution contained 10% glycerol to further reduce evaporation, which allowed printing 1392 spots in a single run (see figure 8). The spots produced by this pin are 90 μm along the diagonal. The subsequent spots are barely visible, indicating that the sample was used up. Neglecting evaporation, the volume of each deposited spot is 100 pl, which is comparable to the volumes of liquid deposited by conventional pins or inkjet spotting. We found that the maximum size variation falls within the 10 μm resolution of the scanner for the first 1392 spots of the array. Further studies are needed to determine whether the attachment of proteins to the slide surface follows the same trends, which will be tested in future studies.

5. Conclusion

We have introduced SU-8 fabrication process that allows making stress-free, straight structures. The key to the successful fabrication of these pins was the development of an annealing step, which allows making 200 μm thin polymer structures that are 30 mm long and straight within 30 μm. Polymer pins with a novel stop valve design and surface chemical treatment served to illustrate some of the possibilities of this process. The functionality of the pins was demonstrated by mechanically loading and buckling them, and by printing 1392 spots with a constant diameter of 90 μm using a single filling. These pins are made by a two-step standard lithography process without need for complicated equipment beyond a mask aligner, hot plates and an oven for annealing. The capital investment needed for microfabricating SU-8 pins is much smaller when compared to Si pins, for example, which require a deep reactive ion etcher. In addition, the material costs are low as well, since no more than 10 ml of SU-8 are used up. Neglecting evaporation, the volume of each subsequent spots are barely visible, indicating that the sample of liquid deposited by conventional pins or inkjet spotting. We found that the maximum size variation falls within the 10 μm resolution of the scanner for the first 1392 spots of the array. Further studies are needed to determine whether the attachment of proteins to the slide surface follows the same trends, which will be tested in future studies.

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