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Microarrays by structured substrate swelling

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Abstract

A technique is presented for manufacturing surface microstructures on polymer substrates. It is based on the uptake of solvent vapors by a prestructured polymer, selective swelling, and the flow of the softened polymer. Prestructuring is done by evaporating material, UV illumination, or ion bombardment through a mask. As an application we describe the fabrication of two-dimensional ordered arrays of microvessels which can be of different shape, size, and material. The bottom of the microvessels can be functionalized by several means and it can be transparent to allow for optical analysis. Applications of the microvessel array are seen in combinatorial chemistry, as microreactors, and as confined spaces for controlling crystal growth.

Keywords: Microstructuring; Swelling; Microarray

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1. Introduction

Microstructuring techniques are a prerequisite for number of new technological and scientific developments such microfluidics, lab-on-chip technology, and micrototal-analysis-systems (μTAS) [1,2]. A basic application is the production of arrays of microvessels. Arrays of micrometer sized vessels are useful for the examination of a large number of parallel processes to make statistical analysis, e.g. the nucleation of crystals [3] or the formation of drops of condensed liquids [4]. Microvessel arrays also find application in high-throughput screening of libraries of chemical compounds [5]. Microvessels are usually fabricated in silicon, glass, or polymers using standard micromachining techniques [6,7]. Significant progress has been made during the last years and the number of tools for the fabrication of microstructures has increased significantly. Clark et al. [8] fabricated small arrays of picoliter microvessels (0.4–300 pl; 10–120 μm each side) in polystyrene by embossing techniques, using master structures formed by photolithographic patterning. Pantano and Walt [9] produced densely packed, ordered arrays of circular wells down to 0.125 μm diameter by selectively etching the distal face of a multimode optical fibre (1 mm diameter). Zhao et al. [10] used biaxially oriented polystyrene films which shrink upon heating: surface patterns generated on the substrate using reactive ion etching (RIE) were amplified in height and reduced in planar dimensions upon an asymmetric shrinkage of the material. Whitesides and coworkers [11,12] applied a variety of so-called "soft lithographic techniques" to fabricate arrays of microvessels. Schäffer et al. [13] generated micrometer sized patterns on polymer surfaces via electrostatic lithography. Still, there is a need for new versatile, fast, and low-cost methods. In this report we describe a novel two-step technique for the fabrication of arrays of microscopic vessels in polymer substrates [14].

2. Experimental

To produce microarrays (Fig. 1) a 1×1 cm plate of commercial, extruded [15] polystyrene (PS, Goodfellow

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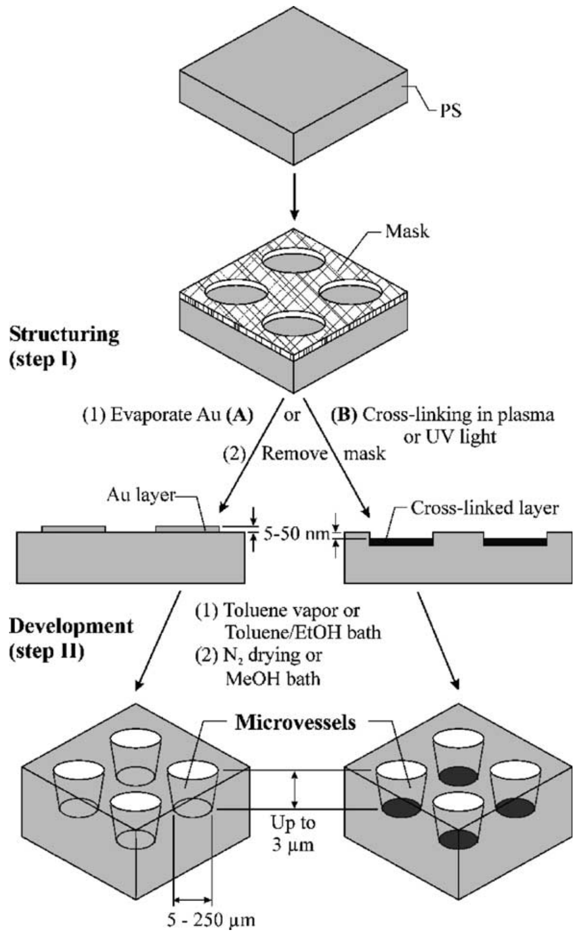

Fig. 1. Strategy to make microvessel arrays by structured substrate swelling.

Ltd., UK) is selectively covered by a mask, in our case a TEM grid (Agar Scientific, Stansted, UK). Then the polymer is treated in one of the three following ways:

- A 5–50 nm thick gold layer is deposited in an evaporation chamber (Baltec MED 020).
- The polymer is exposed to an air/argon plasma in a plasma reactor (GaLa PlasmaPrep2, power 40 W, pressure ≈0.2 mbar, process time 400 s).
- The polymer is exposed to UV light (Heraeus Noblelight GmbH) from an Excimer light source (wavelength 222 nm, power density 100 mW cm^{-2}, process time 300 s).

Afterwards the mask is removed. Unlike the gold deposition and the plasma processing, the irradiation with UV light does not require vacuum.

In the second step the polymer substrate is exposed to a solvent vapor (toluene in our case) or plunged into a liquid solvent (25 vol.% toluene in ethanol in our case). This ‘‘development step’’ primarily causes an expansion (swelling) of the polymer. It is carried out in a gas-tight, transparent container under optical control by a video microscope. The desired structure is developed within 3–10 min. Finally the polymer substrate is dried under a soft air or nitrogen flow. Alternatively, it is immersed into a liquid (methanol in our case), which does not dissolve the polymer, but into which toluene can rapidly diffuse. The whole process takes 5–10 min. In the following we call the process ‘‘structured substrate swelling’’ (SSS).

3. Results and discussion

As an example, a microvessel array produced by the plasma/solvent treatment process is shown in Fig. 2. The observed three-dimensional topography resembles the pattern of the mask, the bottom of the microvessels corresponding to the holes in the mask, i.e. the plasma treated surface. Polymer samples treated with UV light yielded similar results, which are not shown here.

3.1. Surface modification and polymer swelling

The basic physical process crucial for the microvessel formation is polymer swelling, i.e. the absorption of

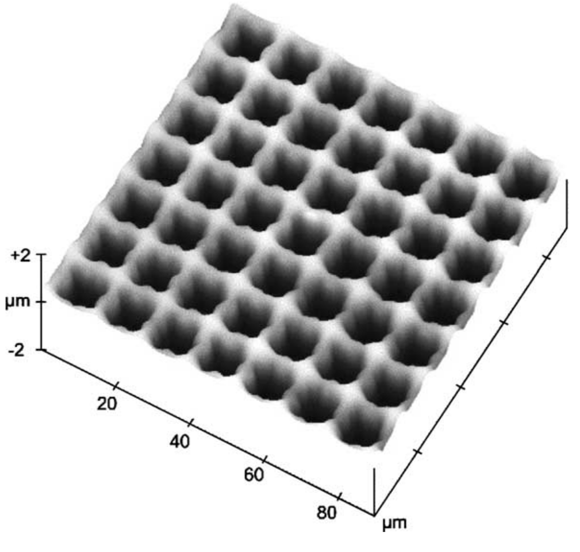

Fig. 2. An atomic force micrograph (BioScope, Veeco, Santa Barbara, CA) of 7×7 cutout of a 100×100 array of microvessels fabricated using a TEM grid with square holes of 7.5 µm edge length and applying the plasma/solvent treatment to the polystyrene substrate. The air/argon plasma etched and cross-linked the polymer surface. An etching depth of the exposed areas of 5 nm was measured with an AFM. The solvent bath (25 vol.% toluene in ethanol, process time 120 s) caused the polymer in between the cross-linked patches to swell. Finally the array was dried under a nitrogen flow for 60 s.

solvent from the vapor or liquid phase [16–19]. At gold covered or previously UV/plasma exposed areas solvent uptake is hindered; UV light and plasma leads to cross-linking of the polymer, which makes it less permeable for the solvent. The uncovered/untreated polymer swells and forms the walls, while the plated/cross-linked polymer forms the bottom of the microvessels.

Usually swelling by solvent uptake causes a reversible expansion of the polymer. When the swollen polymer is dried and the solvent released, it returns to its original size. This reversible swelling of special polymer hydrogels was employed by Hu et al. to generate surface microstructures [20] and by Beebe et al. to control liquid flow in microfluidic devices [21]. In our case, after drying we observe that the shape given to the microvessels is permanent. This unexpected phenomenon of irreversible swelling can have two causes: (i) We used extruded polymer plates. Their surface structure, stress, and density are likely to be different from the bulk. The surface region might be several micrometers deep [15] and its structure is likely to change during the swelling/drying process leading to a lower density/higher volume. (ii) The polymer not only swells by solvent uptake but also becomes more fluid. Thus SSS can be accompanied by a mass flow of polymer.

3.2. *Significance of the surface structure*

To demonstrate that the surface structure of the extruded polymer is important for irreversible polymer swelling we dissolved a sample of the extruded polymer in toluene and cast it again into a plate. In this way the original surface structure is destroyed. After applying SSS the cast plate showed practically no difference in thickness between masked and unmasked areas (Fig. 3a). Close to the rim, however, a protrusion is formed at the outer part of the rim while a depression is observed at the inner rim. This is a clear indication that close to the rim polymer is transported outwards. Mass flow is probably caused by a concentration gradient from underneath the protected areas, where the polymer is closely packed, to the solvent rich intervening regions.

This mass flow is also observed on extruded polystyrene plates (Fig. 3b). It is indicated by a polymer depletion at the rim (a dimple) of the microvessel bottom. Such dimples were observed by atomic force microscopy. Height cross-sections through microvessels showed, that the depth of the dimples increased with the solvent exposure time. As one example Fig. 3a shows three microvessels fabricated using a TEM grid with circular holes of 36 μm diameter and applying the plasma/solvent treatment to extruded polystyrene plates. The polymer substrates were exposed to an air/argon plasma and then to a solvent (25 vol.% toluene in ethanol) for 60, 120, and 180 s. After 60 s the vessel is shallow and the diameter of the bottom plate is ≈36 μm, which is similar to the diameter of the hole in the mask. The polymer around the cross-linked area is swollen by ≈190 nm and material depletion occurs at the border where a tiny dimple is visible. After 120 s exposure to the solvent the dimple becomes more pronounced. The base-plate has a diameter of ≈28 μm and the vessels depth is ≈1.5 μm. Longer solvent treatment leaves the base-plates diameter nearly constant (≈26 μm) but increases further the depth of the microvessel to ≈2.7 μm.

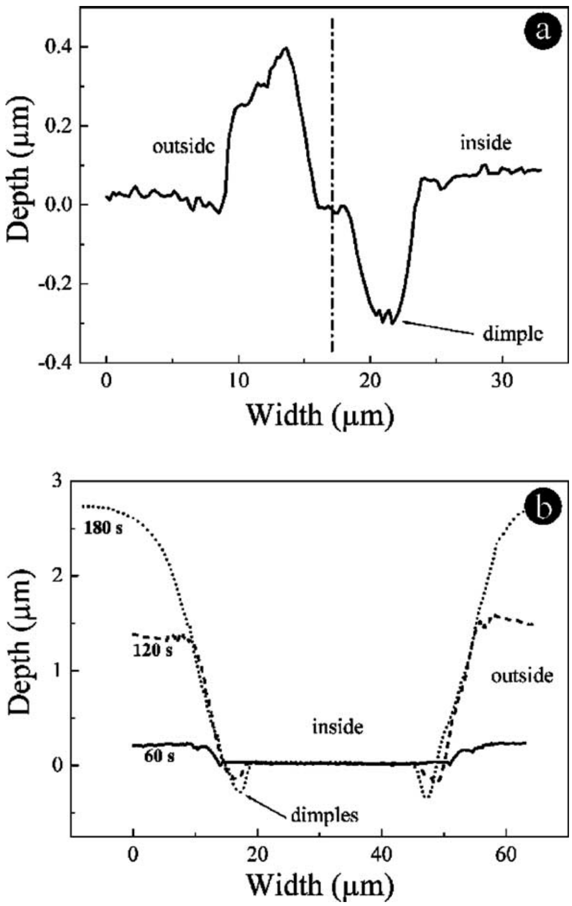

Fig. 3. Half of a height cross-section obtained through a microvessel fabricated on spin-cast PS substrate after applying the plasma/solvent treatment (a). Height cross-sections through three microvessels fabricated using a TEM grid with circular holes of 36 μm diameter and applying the plasma/solvent treatment to extruded PS plates (b). After treating the polymer substrates with an air/argon plasma, they were exposed to a solvent (25 vol.% toluene in ethanol) for 60, 120, and 180 s. All height cross-sections are obtained from atomic force microscope images.

We suggest the following tentative picture of SSS: (i) upon immersion, the solvent diffuses into the polymer at the previously masked regions; (ii) this leads to a swelling and softening of the polymer; (iii) the polymer starts diffusing from regions of higher concentration to regions of lower concentration; (iv) after drying and evaporation of the solvent the microstructures are fixed.

3.3. Functionalisation and use of the microvessels

The nature of the processes involved shows that a large variety of polymer materials with their solvents can be used to fabricate microvessels by SSS. Any metal or other substance that can be deposited (e.g. evaporated or sputtered) may be used during the structuring step represented in the left branch of Fig. 1 (process A). We verified this for gold, chromium, and zinc. Zinc, however, formed clumps, did not adhere well, and flaked off the sample when it was put in a slightly acidic aqueous solution. Gold has the advantage that it can easily be functionalized by thiolisation [22–24]. We demonstrate this with 11-mercapto-1-undecanol to hydrophilize the surface. The advancing and receding contact angles measured with the sessile drop method on a gold-covered polystyrene plate before thiolisation were 88° and 67°. This is due to the well know tendency of gold to adsorb hydrocarbon contamination. In addition, a molecularly thick polystyrene layer might still be adsorbed to the gold. After thiolisation, advancing and receding contact angles were and 47° and 15°, respectively. Fig. 4a and b illustrate the change of hydrophilicity. On a Peltier element we cooled an array of square untreated microvessels down to a few degrees below room temperature at a relative humidity of 65%. Water droplets condensed preferably on the polymer surface. After thiolisation, the water droplets preferentially condensed at the gold bottom of the microvessels.

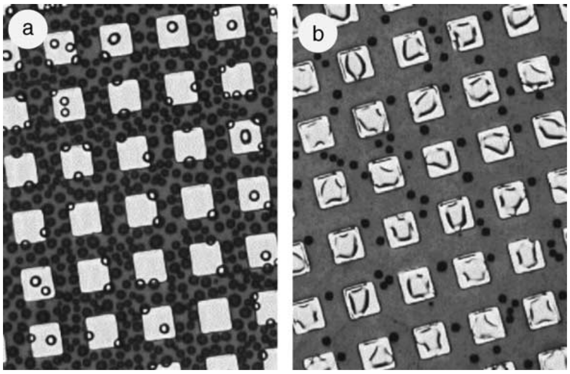

Fig. 4. Condensation of water onto microarrays with gold covered bottoms (side length 45 μm). On microvessels with bare gold water droplets condense on the polystyrene and gold but do not on the hydrophobic gold (a). After thiolisation with 11-mercapto-1-undecanol, water droplets preferentially condense on the gold and spread on the microvessel bottoms (b).

Hydrophilization of the bottom of the microvessels is automatically achieved during plasma or UV light treatment (structuring step in the right branch in Fig. 1, process B), where along with cross-linking an oxidation of the exposed polymer [25–28] results in an advancing contact angle of ≈5°. The bottom of the microvessel remains transparent and can be used for optical analysis. In Table 1 we briefly summarize advantages and disadvantages of the three pre-processing methods.

The microvessels can be filled and kept filled with liquids for any required time. Beside condensation single drops can be deposited onto the array using microdispensers. In addition, dip coating can be used to fill all microvessels at once with the same liquid. The dewetting process is discontinuous filling only the microvessels and leaving the polymer dry [11]. Upon combining discontinuous dewetting and repeated condensation/evaporation cycles regular crystals can be grown within an array of microvessels (Fig. 5).

A disadvantage of conventional microarrays on a planar substrate is that liquid drops have a curved, almost spherical shape. They evaporate fast because the curvature increases their vapor pressure. The smaller they get the higher their vapor pressure becomes. In microvessel arrays the liquid surfaces can be planar. As a consequence, evaporation can be eliminated or better controlled.

SSS can be extended to other combinations of polymer/solvent. For example, we generated surface struc-

Table 1
Advantages and disadvantages of the discussed pre-processing techniques

Pre-processing method	Advantage(s)	Disadvantage(s)
Metal evaporation or sputtering	Bottom can be functionalized Polymer surface structure remains unchanged	Requires vacuum Bottom is not transparent
Plasma treatment	Bottom is transparent	Requires vacuum Sample is slightly etched Changes polymer surface structure
UV treatment	Does not require vacuum Bottom is transparent	After longer process time sample turns yellow Changes polymer surface and bulk structure

Fig. 5. Regular K_2SO_4 crystals in an array of microvessels with hydrophilized bottoms. They were obtained after dip coating the plate into 10 wt.% aqueous K_2SO_4 solution. Their quality was improved by three cycles of condensation and slow evaporation of the water using a Peltier element to cool the microarray slightly below room temperature. The image was taken with a light microscope. Each microvessel is 50 μm wide.

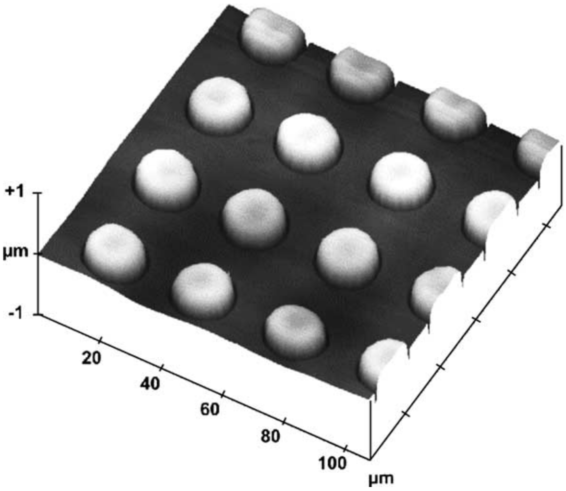

Fig. 6. Atomic force microscope image of a microarray of regular, circular protrusions fabricated using a TEM grid with circular holes of ≈20 m diameter and applying a gold/plasma/solvent treatment to an extruded polystyrene plate. 20 nm thick gold patches were evaporated onto the polymer surface, which was then exposed to an air/argon plasma before removing the gold in aqua regia (33 vol.% HNO_3 in HCl, process time 20 s). This way all the surface was cross-linked, except for the areas protected by the gold. The solvent bath (25 vol.% toluene in ethanol, process time 100 s) caused the previously gold covered parts of the polymer to swell. Finally the array was dried under a nitrogen flow for 60 s.

tures on polymethylmethacrylate (PMMA) using toluene and acetone as solvents, and on polyethylene (PE) using toluene as solvent. The lateral resolution of the structures can be as small as 5 μm. We fabricated arrays of microvessles with lateral dimensions down to 5 μm and depths up to 3 μm.

In addition to concave structures, we are also able to generate convex structures (Fig. 6) with dimensions similar to the concave ones. Here, gold is sputtered through a mask, the free surface is exposed to plasma or UV light, the gold patches are chemically removed by immersing the sample into aqua regia, and at last the areas, which were covered by the gold during the plasma treatment are swelled.

4. Conclusion

Concave and convex surface microstructures can be obtained by means of selectively modifying, structuring, and irreversibly swelling polymer substrates. Two-dimensional ordered arrays of microvessels can be fabricated by this process. Microvessels can be hydrophilized or hydrophobized, functionalized with mono- or multilayers of receptors and ligands, the bottom can be transparent and of optical grade for microscopical and fluorescent analysis.

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