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Microcontact Printing of Macromolecules with Submicrometer Resolution by Means of Polyolefin Stamps

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Microcontact printing (μCP) is a simple and cost-effective method to create micrometer-scale chemical patterns on surfaces. By careful modification of the conventionally used stamping material (poly(dimethylsiloxane) (PDMS)) and the stamping technique (e.g., "thin stamp μCP"), one can create surface chemical structures down to the submicrometer size range. In the present paper we report on the application of a new class of materials—polyolefin plastomers (POPs) for μCP applications. We show that the POP stamps are well suited to print proteins or block copolymers. Comparative studies on reproducibility, homogeneity, and quality of printing between POP and conventional PDMS stamps were also performed. The results show a superior performance of the POP stamps in the nanometer range and an identical performance in the micrometer range compared to PDMS. Further advantages of the POP-based μCP are faster stamp production, the lack of monomeric contamination (typical for PDMS stamps), and the possibility of recycling the POP stamps. We believe that POPs offer a useful alternative to PDMS for μCP and open new possibilities in submicrometer-range printing.

1. Introduction

During the past decade, microcontact printing (μCP) has become one of the most popular laboratory techniques for the fabrication of chemically microstructured surfaces. There are several reasons for this popularity: μCP is fast, is inexpensive, is simple, requires neither cleanroom instrumentation nor absolutely flat surfaces, and offers a way to create complex patterns, albeit with some geometrical constraints.[1] The achievable resolution is also remarkable—30 nm being the current limit (for thiol-based systems).[2] Although μCP was originally used to print self-assembled monolayers of alkanethiolates on gold surfaces,[1,3,4] it was soon extended to the stamping of proteins onto a variety of different surfaces.[5−7] The overwhelming majority of μCP studies have been carried out using poly(dimethylsiloxane) (PDMS) as a stamping material.[1] Although PDMS is well suited for many stamping applications, it has a number of serious drawbacks, which are partially connected to the softness (low mechanical stability) of the material. This softness sets serious geometrical constrains for the realizable structures and limits the achievable resolution of the standard PDMS-based technique.[1,8,9] To overcome these problems, two principal solutions have been introduced (and also combined with each other): (1) A supporting glass/plastic plate was used to increase the mechanical stability of the stamp.[6,10] (2) Special PDMS variants with better mechanical properties for high-resolution μCP were used.[11] Another (often neglected) drawback of PDMS-based μCP is the frequently observed low-molecular-weight ("monomer") PDMS contamination that is present on the stamped surface.[12,13] To solve these problems (mechanics/contamination), instead of creating new PDMS variants we have investigated the possibility of using a new class of materials—polyolefin plastomers (POPs) in μCP applications. In the present paper we describe the use of POPs for printing proteins (Alexa 488-fibrinogen) and block copolymers—fluorescein-poly-L-lysine-*g*-poly(ethylene glycol) (PLL-*g*-PEG-fl*).[13] We compare μCP with POPs to the conventional PDMS-based approach, in terms of both quality and reproducibility.

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(1) Xia, Y. N.; Whitesides, G. M. *Annu. Rev. Mater. Sci.* **1998**, *28*, 153−184.

(2) Biebuyck, H. A.; Larsen, N. B.; Delamarche, E.; Michel, B. *IBM J. Res. Dev.* **1997**, *41*, 159−170.

(3) Kumar, A.; Whitesides, G. M. *Appl. Phys. Lett.* **1993**, *63*, 2002−2004.

(4) Mrksich, M.; Chen, C. S.; Xia, Y. N.; Dike, L. E.; Ingber, D. E.; Whitesides, G. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10775−10778.

(5) Bernard, A.; Delamarche, E.; Schmid, H.; Michel, B.; Bosshard, H. R.; Biebuyck, H. *Langmuir* **1998**, *14*, 2225−2229.

(6) James, C. D.; Davis, R. C.; Kam, L.; Craighead, H. G.; Isaacson, M.; Turner, J. N.; Shain, W. *Langmuir* **1998**, *14*, 741−744.

(7) Bernard, A.; Renault, J. P.; Michel, B.; Bosshard, H. R.; Delamarche, E. *Adv. Mater.* **2000**, *12*, 1067−1070.

(8) Delamarche, E.; Schmid, H.; Michel, B.; Biebuyck, H. *Adv. Mater.* **1997**, *9*, 741−746.

(9) Bietsch, A.; Michel, B. *J. Appl. Phys.* **2000**, *88*, 4310−4318.

(10) Rogers, J. A.; Paul, K. E.; Whitesides, G. M. *J. Vac. Sci. Technol., B* **1998**, *16*, 88−97.

(11) Schmid, H.; Michel, B. *Macromolecules* **2000**, *33*, 3042−3049.

(12) Yang, Z. P.; Belu, A. M.; Liebmann-Vinson, A.; Sugg, H.; Chilkoti, A. *Langmuir* **2000**, *16*, 7482−7492.

(13) Csucs, G.; Michel, R.; Lussi, J. W.; Textor, M.; Danuser, G. *Biomaterials* **2003**, *24*, 1713−1720.

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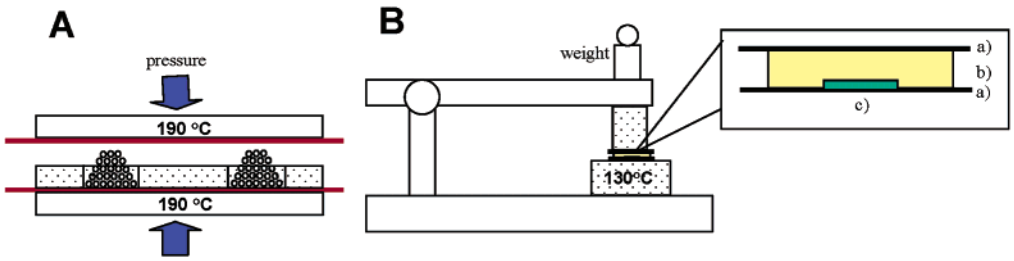

Figure 1. The two main steps of the POP stamp formation. (A) Melting the POP pellets into bars. The red line indicates the two polyimide foils. (B) Replicating the master in the POP bar: (a) silicon wafer (black line); (b) POP bar (yellow); (c) master (green). The sandwich was heated from both sides.

2. Experimental Section

2.1. Substrates. μCP was performed either on tissue-culture polystyrene (TCPS) well plates (Nunclon, NalgeNunc International, Rochester, NY) or on normal glass coverslips (Menzel Glass, Braunschweig, Germany). The precleaned coverslips were washed with ethanol and blown dry by N_2. TCPS plates were used without additional cleaning.

2.2. Stamp Masters. Masters for the micrometer-range structures were produced as previously described.[13]

The master contained lines of 1.5, 5, and 10 μm width and 1 × 1, 3 × 3, 5 × 5 μm squares. The separation distance between the structures was the same as their size.

The masters for the nanometer-range structures were fabricated using electron-beam lithography (EBL) by means of a Raith150 system (Raith GmbH, Dortmund, Germany): After being cleaned, the samples were coated with a 200 nm thick poly(methyl methacrylate) (PMMA) layer (MicroChem Corp., Newton, MA) and hard baked for 60 min at 180 °C. The resist was then exposed with an acceleration voltage of 25 kV and an electron dose of 330 μC/cm^2. The beam current was 13 pA. After exposure, the resist was developed in a methyl isobutyl ketone/isopropyl alcohol (MIBK/IPA) 1:3 solution for 60 s at 22 °C and rinsed with pure 2-propanol. After this lithographic step, the samples were transferred to a dry etching plant (Surface Technology Systems, STS T20, Ulm, Germany). The etching was performed with a mixture of 8 sccm CHF_3 and 20 sccm SF_6 at 41 mTorr, 300 K, and 55 W for 3 min.

2.3. Formation of the Stamps. PDMS (Sylgard 184 from Dow Corning, Midland, MI) stamps were formed as described previously.[13]

Affinity polyolefin plastomers were obtained as pellets from The Dow Chemical Company (Midland, MI). The plastomers used (Affinity VP8770, Affinity EG8200, Affinity EG8150) are a relatively new class of polymers that emerged from recent developments in metallocene polymerization catalysts. POPs are copolymers of ethylene and an α-olefin such as butene or octene. The metallocene catalyst selectively polymerizes the ethylene and comonomer sequences. Increasing the comonomer content will produce polymers with higher elasticity as the comonomer incorporation disrupts the polyethylene crystallinity. Stamps (from all three Affinity POP variants) were produced as follows (see also Figure 1): The POP pellets were first melted into blocks of 40 × 20 × 5 mm at 190 °C under a pressure of 4 bar using an appropriate metal template. To avoid the sticking of the polymers to the heated metal planes, thin polyimide foils were placed in between. After cooling to room temperature, the solid polymer bars were removed from the template, rinsed with ethanol, and dried under a stream of nitrogen. In the next step the bars were placed over the stamp masters (10 × 10 mm) and placed between two silicon wafers (50 × 30 mm). This "sandwich" was then placed on a heatable plate (heated to 130 °C from both sides). First, a weight of 200 g was put on the top of the sandwich for 5 min, which was afterward increased to 700 g for 4 min. After cooling, the master was easily peeled off from the POP bar, which was then cut to its proper size with a razor blade. Prior to usage, the POP stamps were cleaned with acetone for 5 min in an ultrasonic bath.

2.4. Printing of Proteins and Block Copolymers. Alexa-Green 488 fibrinogen (Molecular Probes, Eugene, OR) was dissolved in phosphate-buffered saline (PBS) (pH 7.4) at a concentration of 40 μg/mL. PLL-*g*-PEG-fluorescein was synthesized as described previously[13] and dissolved in 10 mM 4(2-hydroxyethyl)piperazine-1-ethanosulfonic acid (HEPES-Z1) (pH 7.4) at 1 mg/mL concentration. The clean stamps (both PDMS and POP) were incubated with the appropriate protein/block copolymer solution for 40 min in a laminar-flow hood, protected from light. After the incubation, the inking solution was removed and the stamps were blown dry with N_2. The stamps were then placed on the substrates for about 20 s. To ensure proper contact between the stamps and the surface, a small (stamp-surface-area-dependent) weight of 1 g/mm^2 was applied on top of the stamps. After the stamps were removed, the surfaces were washed with buffer. Optical microscopy was performed under buffer solution.

2.5. Fluorescence Microscopy. The fluorescence microscopy investigation of the printing quality was performed using a 20×/0.4 NA LD-Achroplan objective for the micrometer structures and a 100×/1.4 NA Plan-Apochromat for the nanometer structures (Carl Zeiss AG, Feldbach, Switzerland). Image acquisition was done by using a LSM510 laser-scanning confocal microscope (Carl Zeiss AG). The fluorophores were excited using the 488 nm line of the scanning unit.

2.6. X-ray Photoelectron Spectroscopy (XPS) Measurements. All XPS spectra were recorded on a SAGE 100 (SPECS, Berlin, Germany) using nonmonochromatic Al Kα radiation with an energy of 325 W (13 kV, 25 mA), electron takeoff angle of 90°, and electron-detector pass energies of 50 eV for survey and 14 eV for detailed spectra. During analysis, the base pressure remained below 5 × 10^{-8} mbar. All peaks are referenced to the C1s (hydrocarbon C−C, C−H) contribution set to 285.0 eV.

2.7. Contact Angle Measurements. Advancing contact angle measurements were performed on plane and PDMS/POP stamped surfaces by a Krüss G2 contact-angle-measurement system and the DSA1 drop-shape-analysis system (Krüss GmbH, Hamburg, Germany) using HPLC-quality water (Fluka, Buch, Switzerland). The experiments were performed at 22 °C and 44% relative humidity.

2.8. Scanning Electron Microscopy (SEM) Measurements. The SEM micrographs were acquired with the Raith150 EBL system with a LEO Gemini 1530 column (Raith). The acceleration voltage was 10 kV, and the sample current 100 pA.

2.9. Atomic Force Microscopy (AFM) Measurements. Imaging of the silicon master and the polymer replica stamps in PDMS and POP was performed with a NanoScope IIIa multimode scanning probe microscope (Veeco Instruments, Inc., Woodbury, NY). The silicon master was scanned in contact mode, while the PDMS and POP stamps were imaged in both contact mode and TappingMode. Oxide-sharpened Si_3N_4 probes (Veeco) with a nominal spring constant of 0.06 N/m were employed for scanning in contact mode at zero applied load (load being due to adhesion only) to ensure minimal damage to these low-modulus, highly compliant materials. TappingMode imaging was performed with TappingMode etched silicon probes (Veeco).

3. Results and Discussion

3.1. Analyzing the Stamping Quality. *Stamp Production.* Three different polyolefin elastomers, Affinity VP8770, Affinity EG8200, and Affinity EG8150, were tested for their suitability for μCP applications. The main difference between these elastomers is their crystallinity,

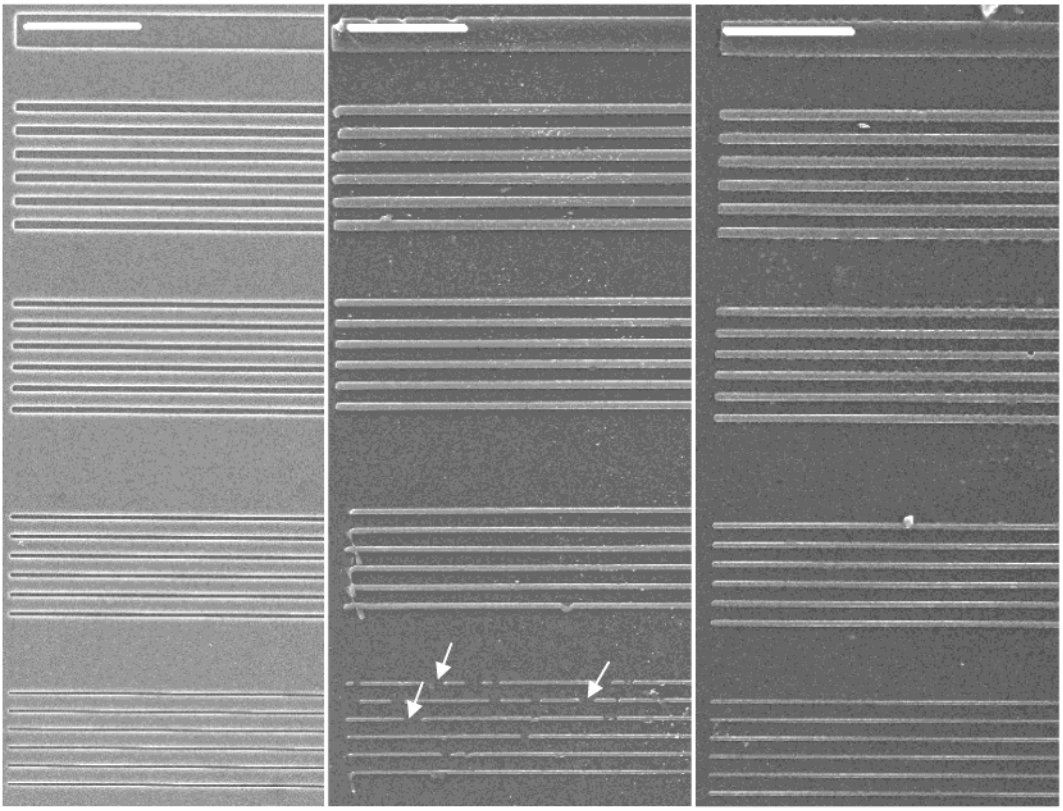

Figure 2. SEM images of the stamp master (left) a PDMS replica (middle), and a POP (VP8770, right) replica. The line thicknesses from the top to the bottom are 5000, 1000, 600, 300, and 100 nm. Scale bars in the corners are 20 μm.

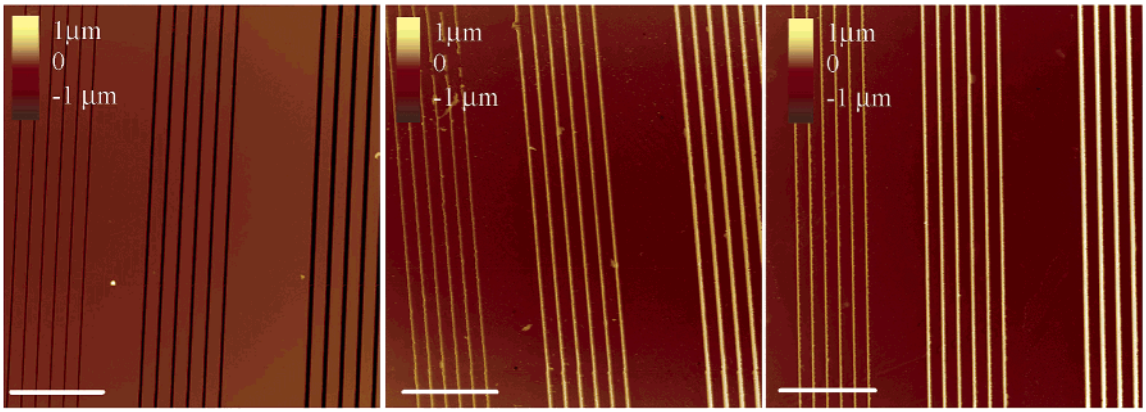

Figure 3. AFM images of the stamp master (left) a PDMS replica (middle), and a POP (VP8770) replica. The line thicknesses from the left to the right are 100, 300, and 600 nm, respectively. Scale bars in the corners are 20 μm.

VP 8770 being the most crystalline (with the highest melting point and mechanical stiffness) and EG 8150 being the most amorphous (with the lowest melting point and highest elasticity). The molding procedure for forming the stamps was the same for all materials. The production time for the stamps amounts to about 1 h—much faster than the approximately 24 h that is reported in the literature for PDMS stamp production.[11] The quality of the stamps was first checked by stamping fluorescently labeled proteins/polymers and using fluorescence microscopy to analyze the transferred structures. In further experiments, SEM and AFM were used to directly test the quality of the nanometer-scale stamps.

Scanning Electron Microscopy. Pattern replication from the master to the PDMS and POP (VP8770 variant) stamps was investigated (besides AFM) by SEM. The examinations concentrated on the nanometer range patterns, because this is the range where the occurrence of replication problems might be expected. Figure 2 shows a typical result of such an investigation showing 1000, 600, 300, and 100 nm thick lines (with 3 μm separations). The replication of the structures both by PDMS and POP seems to be excellent. However, some discontinuities of the 100 nm lines on the PDMS are also observable (see arrows on Figure 2B)—this may be due to wetting and viscoelastic properties of the PDMS, as well as its low bulk elastic modulus.[11]

Atomic Force Microscopy. In addition to SEM, AFM measurements were also performed, to further check the quality of the pattern replication by different stamp materials. Figure 3 illustrates such an experiment. The images nicely demonstrate that, qualitatively, the master patterns were indeed transferred to the stamps. In the case of the PDMS stamp, small defects are visible in the replication of the 100 nm lines. The existence of these discontinuities (similar those observed on the SEM images)

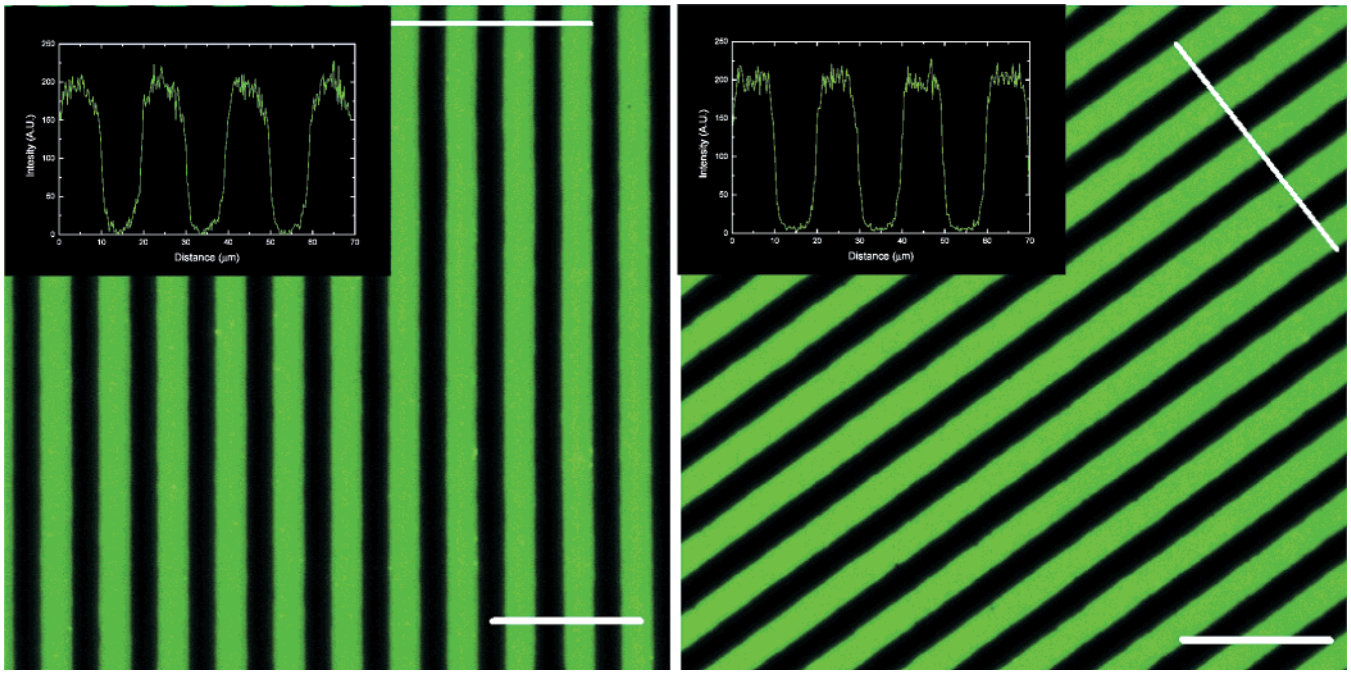

Figure 4. Typical printing assay, by means of fluorescence microscopy, of Alexa488 Fibrinogen using PDMS (left) and EG8150 (right) as stamp material and TCPS as substrate. The line thicknesses were 10 μm with 10 μm separations. The insets show cross sections of the fluorescence intensities measured perpendicular to the line directions. The lines across the stripes indicate the positions at which the cross sections were measured. Scale bars in the corners are 50 μm.

is probably again connected to both chemical and bulk mechanical properties of the PDMS.

Fluorescence Microscopy. The ultimate test for a new stamping material is whether it is possible to ink it and transfer molecules to another substrate, replicating the master structure. We have tested the performance of the POP stamps by printing proteins (Alexa488-fibrinogen) and block copolymers (PLL-*g*-PEG-fl*) onto both glass and tissue-culture polystyrene (TCPS) surfaces using two different masters containing micrometer or submicrometer-sized structures. In each case, the results were compared to those obtained with conventional PDMS stamps.

Figure 4 shows a typical example of a stamping experiment using the micrometer-range structures. There is no observable difference in the homogeneity and the quality of the printed structures between the POP (Affinity EG8150) and the PDMS printed surface. The protein-transfer ratio (from the stamp to the surface), as determined from the fluorescence intensities, was close to 100%—there was no measurable fluorescence left on the stamps as observed by microscopy. The insets on the images show that in both cases the edges are well-defined and there is no fluorescence present between the lines. The different POP variants all gave identical results (data not shown). After being cleaned with ethanol (10 min in ultrasonic bath), both the POP and the PDMS stamps were reusable. In our tests we have reused the POP stamps at least 10 times without observing any changes in the printing quality.

In a previous paper, we have shown that PDMS can be used to stamp the block copolymer PLL-*g*-PEG-fl* onto a variety of surfaces.[13] These observations could be replicated by using POP stamps. Again, there was no observable difference between PDMS- and the POP-stamped surfaces (data not shown). Both with POP and with PDMS stamps the printing quality seemed to be independent of the type of surface (glass or TCPS) or the type of micrometer-sized structure (10, 1.5 μm thick lines and 5 $\times$ 5, 3 $\times$ 3, 1 $\times$ 1 μm^2 squares with 10, 1.5, 5, 3, and 1 μm separations, respectively) (data not shown).

It is generally agreed in the literature that a significant challenge for μCP is to print submicrometer-sized structures with rather large (several micrometers) separations. One of the main problems when printing in this size range is the softness (small compression modulus) of the conventional PDMS stamps which results in the inability to replicate a thin line.[11] Different methods (special variants of PDMS and stabilizing solid substrates under the ultrathin PDMS layer) have been introduced to solve this problem. Since the POPs used in this work are much stiffer than PDMS, we hoped that their mechanical stability would be advantageous when printing in the submicrometer range. To test this hypothesis, we have produced a master containing different submicrometer-sized structures and used it to make POP and PDMS stamps. In these experiments we have used only the stiffest (VP7880) POP variant. Again, Alexa488-Fibrinogen was used for inking. Because the requirements of high-resolution optical microscopy, only glass coverslips were used as substrates. A typical result is shown in Figure 5. The stamped structures were 1000, 600, 300, and 100 nm thick lines (Figure 5A left to right), always with 3 μm separations. The separations between the groups of lines were even larger: 12, 17, and 12 μm, from the left to right. Analyzing the quality of the stamping, we can conclude that the thickness of the printed lines corresponds to that of the stamp master. Naturally, because of the limited resolution of optical microscopy, the 100 and 300 nm lines appear to be somewhat wider than their "real" size. It is remarkable that sagging of the stamps does not occur even at the large separations between the line groups. As with the micrometer structures, the stamps were again reusable and the reproducibility of the printing was good.

Figure 5B shows the best stamping result obtained by a "conventional" PDMS stamp using the same master. The difference is clear. Although the quality of the 1000, 600, and, to some extent, the 300 nm lines is still

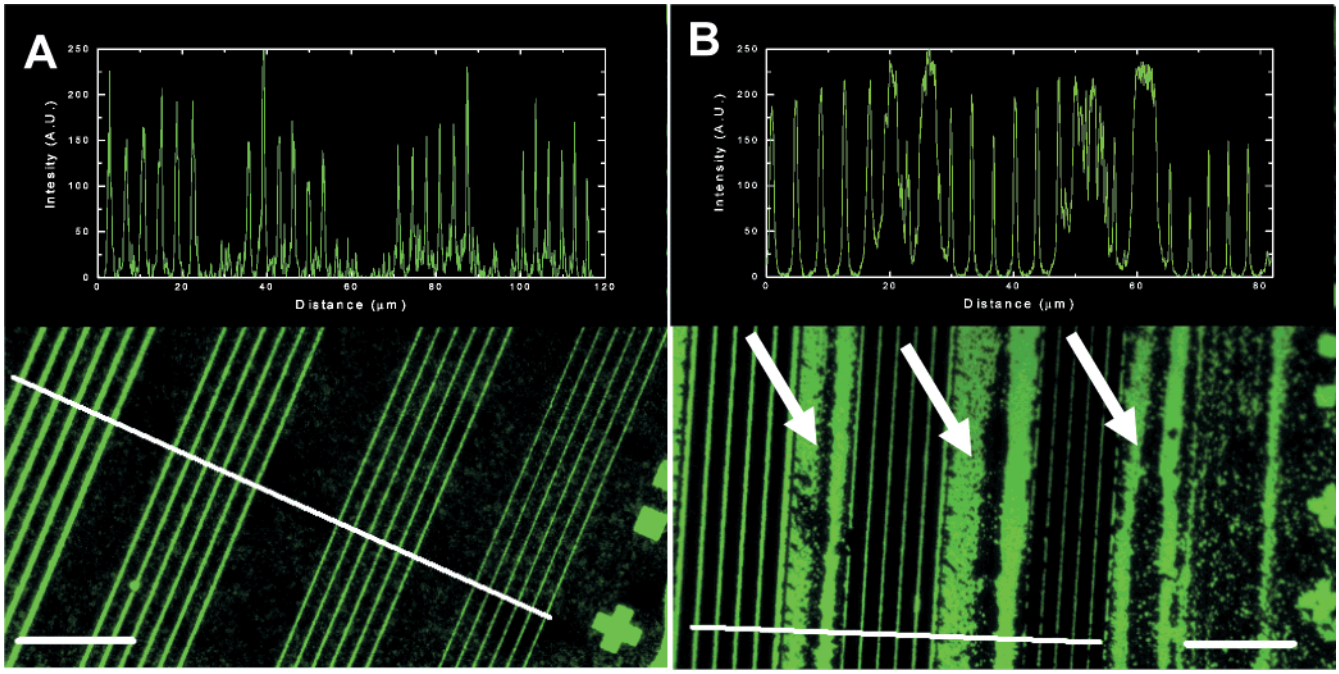

Figure 5. Nanometer-scale printing of Alexa488 Fibrinogen using VP8770 (A) and PDMS (B) as stamp materials, as determined by fluorescence microscopy. The line thicknesses are 1000, 600, 300, and 100 nm with 3 μm separation. The insets show cross sections of the fluorescence intensities measured perpendicular to the line directions. The lines across the stripes indicate the positions at which the cross sections were measured. Arrows on (B) indicate regions where sagging of the stamp occurred. Scale bars in the corners are 20 μm.

Table 1. Advancing Water Contact Angles Measured on the Surfaces of Stamp Materials and Glass Coverslips before and after Flat-Stamp Printing

	advancing water contact angle/deg		
substrate or stamp surface	surfaces before stamping	PDMS stamped	POE stamped
coverslip (plasma cleaned)	15 ± 5	87 ± 2	16 ± 5
coverslip (no plasma cleaning)	54 ± 2	76 ± 1	54 ± 2
PDMS	109 ± 2		
POP (Affinity EG8150)	105 ± 2		

acceptable, the 100 nm lines are totally distorted. Furthermore the thickness of the lines varies and does not always correspond to the master. Sagging occurred at all the larger separations (arrows).

3.2. Analyzing the Surface Contamination. *Contact-Angle Measurements.* To test whether stamping modifies the properties (hydrophilicity/hydrophobicity) of the stamped surface, we have measured the advancing contact angles on the stamps and on the stamped surfaces. Nonprinted substrates served as the reference. The stamps were in this case not inked, and nonstructured (flat) stamps were used, but otherwise the printing was performed as described for the proteins, on normal glass coverslips, cleaned either only with ethanol/distilled water washing or by (in some cases) a subsequent oxygen plasma treatment. The results are summarized in Table 1.

The values in the table show that both stamp materials are rather hydrophobic. Upon contacting the surface, however, POP (Affinity EG8150) has no measurable effect on the contact angle, as compared to the control values. PDMS, on the other hand, significantly modifies the properties of the surface, making it hydrophobic. This indicates a contamination of the substrate by the stamp. The effect is stronger on the highly reactive (clean) plasma-treated surface and less pronounced (but still present) on the slightly contaminated ethanol-cleaned surface. A possible explanation for this fact could be that the residual organic contamination present on the glass surface reduces the PDMS contamination.

Table 2. XPS Relative Detected Elemental Atomic Concentrations for Cleaned and POP and PDMS Printed TiO_2 Surfaces (n.d. = not detected)

	% Ti	% O	% C	% Si
TiO_2 clean	22	70	8	n.d.
TiO_2, POP printed	21	71	8	n.d.
TiO_2, PDMS printed	20	65	11	4

XPS Measurements. To obtain more information about possible surface contamination caused by the stamps, XPS measurements were also performed. The samples were prepared in a similar way to those used in contact angle measurements. Printing was again performed with flat/noninked POP (Affinity EG8150) and PDMS, but in this case sputter coated (20 nm) TiO_2 surfaces were employed as substrates. Prior to printing, all substrates were cleaned by oxygen plasma treatment. The cleaning time was 2 min in the case of TiO_2 surfaces and 10 s in the case of stamps. The results of the XPS measurements of stamped TiO_2 substrates were compared to those recorded on a nonstamped (clean) surface. Table 2 summarizes the results.

By analyzing the values, we can conclude the following: the POP printing has no detectable effect on the surface composition of the substrate. In the event of contamination by the POP one would expect an increased amount of carbon on the surface, but this was not observable. Si (as expected) is only detected in the case of PDMS stamping. This is a clear sign of monomeric PDMS contamination and has been previously reported.[12,13] The increased carbon content may be also explained by the monomeric contamination.

Conclusions

In the present paper we have demonstrated the feasibility of using polyolefin elastomers as stamp ma-

terials for microcontact printing of proteins and block copolymers. As shown by SEM and AFM investigations, the replication of the stamp master is effective with both POP (Affinity) and standard (Sylgard 184) PDMS materials, down to the nanometer range. Comparing the performance of POP stamps to those made from PDMS, we can conclude the following: (1) When printing micrometer-range structures, their performance is nearly identical. (2) In the submicrometer range (submicrometer structures with micrometer separations), much higher printing quality is achievable with the POP stamps. This fact is probably due to the higher bulk modulus of the POP stamps.

Comparing the possible surface contamination by the stamps using contact angle and XPS measurements, POP barely modifies the surface, while PDMS displays considerable contamination, presumably by low molecular weight material. The high processing speed and the recycling capability of the POP could be useful properties, when considering the technological applications of this method.

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