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Facile and Multiple Replication of Superhydrophilic–Superhydrophobic Patterns Using Adhesive Tape

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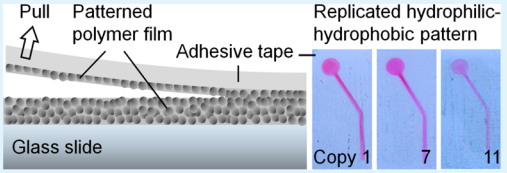
Supporting Information

ABSTRACT: Surfaces patterned with both hydrophilic and hydrophobic regions are useful in a variety of applications. For example, they can be used as surface tension-confined microchannels, in paper-based microfluidics, or for patterning cells. To create a new patterned substrate, usually the entire experimental procedure must be repeated, which can be time-consuming and laborious. In this paper, we present a simple and fast method that allows the transfer of superhydrophilic–superhydrophobic micropatterns in porous polymer films onto adhesive tape. Replicating patterns using adhesive tape is economical, as the fabrication of one patterned substrate can be used to create multiple copies of the micropatterns, which can then be used for several different experiments. We demonstrate that at least twelve consecutive copies can be made from 125 μm-thick patterned polymer films. Since the polymer film is transferred to adhesive tape, which is flexible, the copies can be used on curved surfaces and they can also be cut into different shapes and sizes. We also demonstrate an application of the replicated patterned polymer surfaces as a substrate for reverse cell transfection experiments.



KEYWORDS: *adhesive tape, micropatterns, porous polymer, replication, superhydrophilic, superhydrophobic*

INTRODUCTION

Superhydrophilic and superhydrophobic surfaces have been broadly investigated and there are many applications involved in their use.[1−8] The difference in wettability between hydrophilic and hydrophobic surfaces can be used for patterning liquids[9] or cells[10] on a surface, as surface tension-confined microchannels,[11] in paper-based microfluidics,[12] for studying cell–cell communication,[13] and as channels for peptide separation.[14]

To create a new hydrophilic–hydrophobic patterned surface, usually the entire experimental procedure must be repeated, which can be time-consuming and laborious. Although there are relatively simple methods for creating hydrophilic–hydrophobic patterns using techniques such as microcontact printing[6] or hydrophobic sprays,[11] to our knowledge the possibility of making multiple copies from an already existing superhydrophilic–superhydrophobic micropatterned substrate has not been demonstrated until now. We present a simple method that allows the transfer of superhydrophilic–superhydrophobic micropatterns onto adhesive tape while maintaining the rough surface morphology that is important for the extreme wetting properties of superhydrophilicity and superhydrophobicity. Since multiple copies of a pattern can be made from a single substrate, this results in time optimization and less reagent consumption.

Recently, Zahner et al. published a method for creating superhydrophilic patterns on a superhydrophobic surface by UV-initiated photografting.[15] The micropatterns created using this method are three-dimensional because the superhydrophilic or superhydrophobic surface properties exist through the whole thickness of the porous polymer film. Therefore, separating the porous polymer film horizontally into thin slices should allow for the replication of the superhydrophilic–superhydrophobic micropatterns. In this paper, we show a simple and fast method for the transfer of such patterned surfaces using adhesive tape (Figure 1A). We demonstrate that by applying and then removing a flexible adhesive tape from a porous polymer film patterned with hydrophilic–hydrophobic structures, we can transfer up to twelve copies of the pattern onto the adhesive tape. Each time tape is applied to the surface, a thin layer of the porous polymer film is transferred to the tape from the original polymer surface. The method of replicating patterns using adhesive tape can be applied to most porous materials that are mechanically brittle enough to be delaminated, and can be used in numerous applications where multiple copies of the same pattern are required. In addition, the produced patterned substrates are flexible and the copies can be used on curved surfaces and cut into different shapes and sizes. The method presented here is also convenient for creating multiple copies of either superhydrophilic or superhydrophobic surfaces using adhesive tape.

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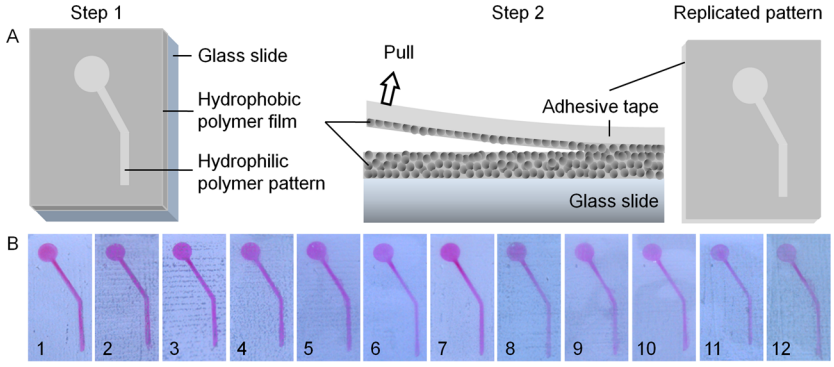

Figure 1. (A) Schematic representation of the transfer of a hydrophilic–hydrophobic pattern in a polymer film onto adhesive tape. Step 1: A thin, porous hydrophobic polymer film is photografted through a photomask to create a hydrophilic pattern. Step 2: Adhesive tape is firmly adhered to the surface of the polymer film and then pulled off in one motion. A thin layer of the hydrophilic–hydrophobic patterned polymer film is transferred onto the adhesive tape. (B) Photos of twelve consecutive copies of a channel pattern that was replicated onto adhesive tape and then stained with a Rhodamine 6G aqueous solution. The diameter of the circle is 2 mm.

EXPERIMENTAL METHODS

Fabricating Porous Poly(butyl methacrylate-*co*-ethylene dimethacrylate) (BMA-EDMA) Films. Hydrophobic, porous BMA-EDMA films were prepared by UV-initiated radical polymerization of a prepolymer mixture containing monomers, cross-linkers, porogens, and a photoinitiator.[16,17] The porogens in the polymerization mixture lead to phase separation once the growing cross-linked polymer chains achieve a critical size. Hence, the formation of these small polymer globules constitutes the porous structure. The mixture consisted of butyl methacrylate (BMA) (24 wt %), ethylene dimethacrylate (EDMA) (16 wt %), 1-decanol (30 wt %), cyclohexanol (30 wt %), and 2,2-dimethoxy-2-phenylacetophenone (DMPAP) (1 wt % with respect to the monomers). The prepolymer mixture was filled between two methacrylated glass plates separated by two strips of 125 μm-thick Teflon film (American Durafilm Co.), and then irradiated with UV light for 15 min. An OAI model 30 deep-UV collimated light source (San Jose, CA) fitted with a 500 W HgXe lamp was used for UV irradiation. The lamp was calibrated to 12 mW cm^{-2} at 260 nm wavelength.

Fabricating Hydrophilic–Hydrophobic Micropatterns in the Porous Polymer Films. To create hydrophilic patterns in the hydrophobic polymer film, the porous polymer film was wetted with a photografting mixture, and then covered with 75 μm-thick Teflon film and a photomask. Then, the polymer film was irradiated with UV light (12 mW cm^{-2} at 260 nm) for 15 min. The photografting mixture consisted of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) (15 wt %) and benzophenone as the initiator (0.25 wt %) in a solution of *tert*-butanol:water (3:1 v/v). All chemical reagents were purchased from Sigma-Aldrich (Germany). Further details about the procedure for fabricating the hydrophilic–hydrophobic patterned polymer films are in the Supporting Information.

Replicating Porous Polymer Films Using Adhesive Tape. Adhesive tape (Tesa, 1.5 cm-wide) based on a stable PVC backing coated with a natural rubber was adhered to the polymer surface with the help of a rubber eraser to remove air bubbles, and then the tape was peeled off. To make multiple copies, the procedure was repeated and the direction of the tape removal was alternated each time.

Scanning Electron Microscopy (SEM). Samples were gold-sputtered using a Cressington sputter coater 108auto. SEM images were obtained with a LEO 1530 scanning electron microscope (Zeiss, Germany). The globule size of the polymer films was determined from the SEM images.

Measuring the Radii of Circular Hydrophilic Patterns on the Copies. The hydrophilic patterns were dyed with Rhodamine 6G and images of the patterns and surfaces were taken with a Keyence BZ-9000 fluorescence microscope (Japan) and also with a digital camera (Nikon). Measurements of the radii of circular hydrophilic patterns and the fluorescent intensity were made with the software ImageJ.

Measuring Water Contact Angles (WCAs). A UK 1115 digital camera from EHD imaging GmbH (Germany) was used to take images of water droplets on the surfaces under ambient conditions. The static (θ_{st}), advancing (θ_{adv}), and receding (θ_{rec}) water contact angles (WCAs) on copies of a porous BMA-EDMA polymer film were measured in three different places on each sample and the average was calculated. The WCAs were determined using ImageJ software with a DropSnake plugin.

Cell Experiments. To make the transfection mixture, 6 μL of 0.5 μg μL^{-1} plasmid DNA (H2B-RFP) was mixed with 7 μL of Lipofectamine 2000 (Invitrogen) and incubated for 20 min at RT. After being freshly prepared and filtered, 3 μL of 0.4 M sucrose in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen) and 7.25 μL of 0.2% gelatin (Sigma, Germany) in water were added to the mixture. 3.5 μL of the transfection mixture was pipetted into each of 3 hydrophilic spots (AMPS photografted, 2 mm diameter circles) on copies 1, 4, 8, and 12 from each of three BMA-AMPS patterned polymer films. The transfection mixture was allowed to dry and then each taped copy was seeded with HEK 293 cells (20 000 cells cm^{-2}) and incubated for two days. The cells were then fixed in 3.7% formaldehyde in Dulbecco's phosphate-buffered saline (PBS) for 20 min, washed 1× with PBS, incubated with 0.1% Triton X-100 in PBS for 15 min, washed 1× with PBS, incubated with 0.5 μg μL^{-1} 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) for 15 min, washed 2× with PBS, and then mounted and covered with glass coverslips. Images were taken using a Keyence BZ-9000 fluorescence microscope (Japan). ImageJ software was used to count the cells.

RESULTS AND DISCUSSION

In this paper, the fabrication, characterization, and application of replicated porous polymer surfaces are presented. The method of fabrication consists of producing a hydrophobic porous polymer film (BMA-EDMA) on a glass slide, and then modifying it by UV-initiated surface photografting[18,19] through a photomask to create hydrophilic patterns of defined geometry. The average size of the globules in the porous polymer film was measured to be 486 ± 73 nm.

The replication procedure consists of using adhesive tape to make copies of an original hydrophilic–hydrophobic patterned porous polymer film simply by applying the adhesive tape to the polymer surface and then peeling off the tape (Figure 1A). This leads to the transfer of a thin, porous polymer layer (5–10 μm-thick) from the original porous film to the adhesive tape (Supporting Information Figure S1). This method is efficient in that twelve copies of an original 125 μm-thick patterned polymer film can be made with successful transfer of the pattern to all of the copies. Complex patterns, such as channels,

can be transferred to the adhesive tape, which can then be used on flat surfaces as well as on curved surfaces (Figure 1B, Supporting Information Figure S2). The feature size of a pattern that can be replicated using this method will depend on several factors, such as the size of the polymer globules and the quality of the adhesive tape. The globule size depends on the type of porogen used and can be reduced to approximately 50–100 nm.

The reproducibility of the replication procedure was demonstrated by comparing the wettability of the replicated hydrophilic pattern to that of the original patterned surface. The hydrophilic spots of twelve consecutive copies of a BMA-EDMA polymer film photografted with AMPS were filled with Rhodamine 6G aqueous solution, proving that the barriers between the hydrophobic and hydrophilic regions still existed and that the whole pattern was successfully transferred to the tape (Figure 2A). After the thirteenth copy, the original polymer film showed irregularities and the underlying glass slide could be seen.

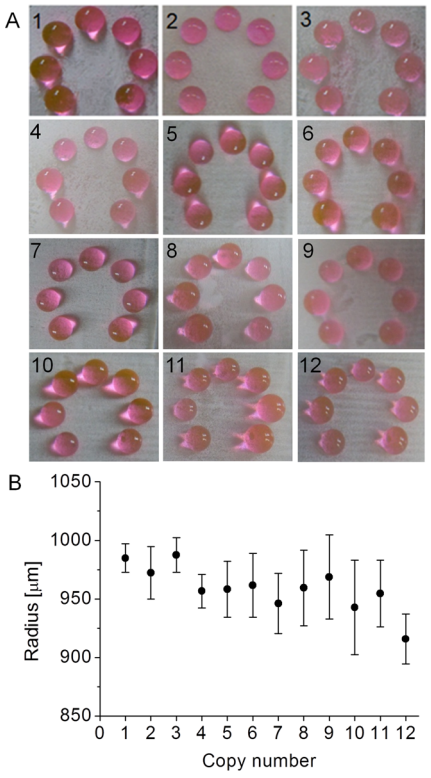

Figure 2. (A) Photographs showing the hydrophilic spots (2 mm diameter circles) of twelve consecutive copies of a patterned polymer film filled with a Rhodamine 6G aqueous solution. (B) Each point in the graph represents the average radius (SD) of the seven circles of the hydrophilic pattern for each copy.

To quantify the reproducibility of the pattern transferred to the adhesive tape, we measured the defined geometry of the pattern with increasing copy number of the replicated patterned polymer film. The seven radii of the circular pattern on each of twelve replicates were measured and the average radius was calculated. As the number of the copies increased, the average radius slightly decreased (Figure 2B). This can be explained by the scattering of UV light during the photografting step, which reduces the photografting efficiency as the light passes through the thickness of the porous polymer film.[17] The polymer layer transferred to the adhesive tape was uniform in the first copy, but as the order of the copies increased, some regions without polymer could be seen on the adhesive tape (Figure 3A).

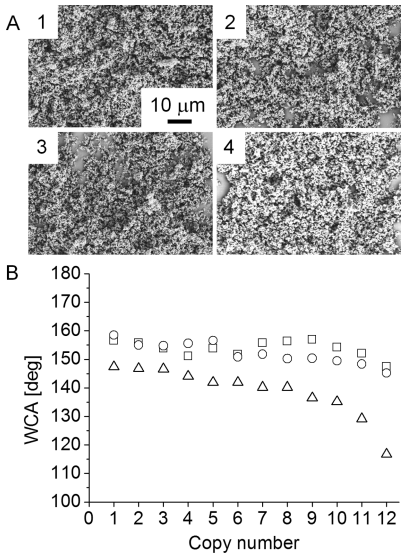

Figure 3. (A) SEM images of the first four copies of an original BMA-EDMA polymer film. (B) Measurements of the average static (□), advancing (○), and receding (Δ) water contact angles of twelve consecutive copies of a 125 μm-thick, porous BMA-EDMA polymer film.

The advantage of porous materials is that their surface properties, such as hydrophobicity, are preserved through the thickness of the porous film.[20] In other words, the hydrophobicity of the porous polymer film is a result of the bulk properties of the material and not just of its surface, so it should also be preserved upon the replication steps. The static (θ_{st}), advancing (θ_{adv}), and receding (θ_{rec}) water contact angles (WCAs) of the copies of a porous BMA-EDMA polymer film were measured. The results show that both θ_{st} and θ_{adv} on the copies virtually do not change after ten consecutive copies (Figure 3B, Supporting Information Table S1). However, θ_{rec} gradually decreased from 147° to 135° from the first to the tenth copy, and then dropped to 117° for the twelfth copy. The increase in WCA hysteresis after the tenth copy can be explained by the nonuniform polymer film coverage on the adhesive tape (Figure 3A). The number of reproducible copies is limited by the thickness of the original polymer film, thus the number of copies can be increased if a thicker polymer film is used. However, the number of reproducible copies is also limited by the mechanical damage to the original polymer film that occurs after each pattern transfer, as well as the scattering of UV light when photografting thicker layers of polymer films.

We have already demonstrated the ease with which multiple copies of a patterned polymer film can be replicated and subsequently used for patterning liquids. To demonstrate that the replicated polymer films are useful for a broad range of applications, including biological experiments, we used the replicates as substrates for reverse cell transfection.[21] Since hydrophilic–hydrophobic patterns are able to confine liquids and cells in arrays of high-density, they can be useful for high-throughput cell and chemical screening applications.[22] We used copies 1, 4, 8, and 12 from BMA-AMPS patterned polymer films, and pipetted a transfection mixture containing histone

2B-red fluorescent protein (H2B-RFP) plasmid DNA and Lipofectamine 2000 onto the hydrophilic spots (2 mm diameter circles) on the copies (Figure 4A). The transfection

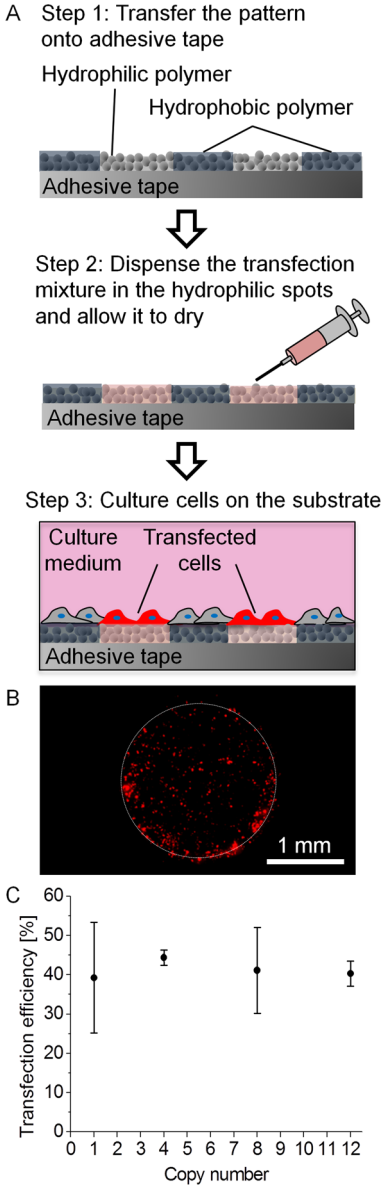

Figure 4. Using the copies of a patterned hydrophobic–hydrophilic (BMA-AMPS) polymer film as substrates for reverse cell transfection. (A) Schematic of the procedure for replicating a hydrophobic–hydrophilic polymer pattern onto adhesive tape, dispensing a transfection mixture in the hydrophilic spots and allowing it to dry, and then culturing cells on the substrate. (B) Image of red fluorescent protein-expressing cells within one hydrophilic spot (outlined in white) after two days of incubation on a copy that was printed with a transfection mixture. (C) The average (SD, n = 3) transfection efficiency of cells cultured on copies 1, 4, 8, and 12 that were printed with a transfection mixture.

mixture was allowed to dry before seeding human embryonic kidney (HEK 293) cells on each taped copy. After two days of incubation, the cell transfection efficiency was quantified by counting the RFP-expressing cells and dividing it by the total number of DAPI-stained cells (Figure 4B, Supporting Information, Figure S5). The average transfection efficiency for all copies was approximately 40% (Figure 4C). This demonstrates that although there were slight changes in the morphology of the polymer film or the water contact angles as the order of the copies increased (Figure 3), there was no significant effect on the cell transfection efficiency. These results show that multiple copies of a patterned polymer film replicated using adhesive tape can be used to pattern bioreactive agents on a surface and perform biological experiments.

CONCLUSION

We introduced a new method for the facile replication of patterned polymer films using adhesive tape. Since the polymer film is transferred to adhesive tape, which is flexible, utilizing the copies is not limited to flat surfaces and they can also be cut into different shapes and sizes. We also demonstrated an application of the replicated patterned polymers as a substrate for reverse cell transfection experiments. This simple method of transferring surface properties using adhesive tape can be applied to a variety of porous polymers or aerogels.[20] We believe that with this novel method it is possible to significantly reduce the time and cost needed to fabricate multiple substrates composed of polymers, and that it will find numerous applications in the biological and chemical fields.

ASSOCIATED CONTENT

Supporting Information

Procedure for fabricating the porous polymer films; SEM image of the cross section of a BMA-EDMA polymer film transferred onto adhesive tape; image of multiple copies of patterns on curved surfaces; fluorescence analysis of the replicated patterned surfaces; water contact angle measurements; details of the cell experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

P.A. and E.U. contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

AMPS, 2-acrylamido-2-methyl-1-propanesulfonic acid
BMA-EDMA, poly(butyl methacrylate-*co*-ethylene dimethacrylate)
SEM, scanning electron microscope
WCA, water contact angle

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