Micrometer-Scale Patterning of Multiple Dyes on Hyperbranched Polymer Thin Films Using Photoacid-Based Lithography

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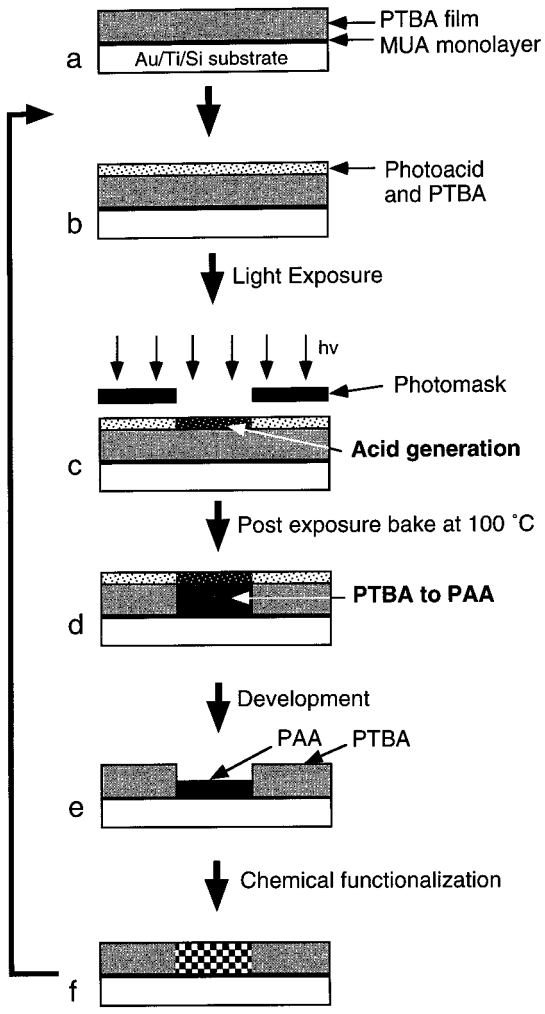
Micrometer-scale patterns of eosin and dansyl dyes on hyperbranched poly(acrylic acid) (PAA) organic thin films have been prepared using a combination of photoacid-based lithography and covalent grafting of dye molecules. Reflection infrared spectroscopy, optical microscopy, and fluorescence microscopy indicate that the dyes were attached within the intended regions. Patterned dye features having a spatial resolution as small as 5 μm were prepared.

Introduction

This paper describes patterning of multiple fluorescent dyes on a hyperbranched poly(acrylic acid) (PAA) thin film using photoacid-based lithography. Our approach consists of three key steps (Scheme 1). First, covalent grafting of the hyperbranched poly(*tert*-butyl acrylate) (PTBA) thin film to a Au substrate followed by overcoating with a layer of photoacid. Second, photolithography, which generates acid in the exposed region and thereby catalyzes the hydrolysis of PTBA to PAA. Third, selective chemical modification of the PAA regions of the film with amine-functionalized dyes.

Photoacids are chemical species, such as the triarylsulfonium hexafluoroantimonate used in this work, that release a proton upon photolysis.[1] Photoacids have been widely used for chemical amplification of photoresists[2] and as photoinitiators for cationic polymerization.[3] Here we show that surface-confined hyperbranched polymers, which have chemical and structural properties that are suitable for a number of technological applications including corrosion inhibition, chemical sensing, and cellular engineering,[4−11] can be patterned using photoacids and that the resulting polymeric design can subsequently be modified with high spatial resolution using two different fluorescent dyes.

Scheme 1



In previous reports we demonstrated that hyperbranched polymer films are remarkably versatile materials. For example, they can be covalently modified with a broad range of functional groups, including fluorophores, electroactive groups, perfluorinated moieties, dyes, and other polymers.[5−8] In addition to this high level of

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synthetic flexibility, hyperbranched polymer films are covalently linked to the surface and thus not prone to delamination, and because of their hyperbranched structure they tend to passivate defects more effectively than linear grafts. Moreover, in an earlier report that complements the results described in this paper,[7] we showed that hyperbranched polymer films can be patterned using a combination of microcontact printing (μ-CP)[12] and subsequent polymer grafting. Such patterns are able to direct the growth of living cells[8] and location of enzymes.[9]

Other photolithographic methods involving photodeprotection[13] and photoactivation[14] have also been reported for patterning functional organic thin films. There are also a few reports describing patterning of multiple layers of oligonucleotides, but the preparation of these materials is typically too complicated for routine applications.[15,16] Finally, in a preliminary account of work that is closely related to that described here, Benoit et al. described a photoacid-based photolithographic approach for preparing patterns of polymer brushes consisting of hydrophobic poly(*tert*-butyl acrylate) regions and hydrophilic poly(acrylic acid) regions.[17] However, to the best of our knowledge there are no previous reports of using a photoacid-based lithographic method as the basis for subsequent covalent grafting of multiple functionalities onto a polymeric pattern. Here, we demonstrate this principle by grafting two different fluorescent dyes, eosin and dansyl, to a photoacid-patterned, hyperbranched PAA film covalently linked to a gold surface.

Experimental Section

Materials. α,β-Diamino-terminated poly(*tert*-butyl acrylate) ($H_2NR-PTBA-RNH_2$, R = $C_2H_4NHCOC_2H_4C(CH_3)CN$) was synthesized using a previously described procedure.[4,5] The photoacid, triarylsulfonium hexafluoroantimonate (Aldrich), was recrystallized in ethanol prior to use. 5-((5-Aminopentyl)thioureidyl)eosin and 5-dimethylaminonaphthalene-1-(*N*-(5-aminopentyl)sulfonamide (dansyl) (Molecular Probes) were employed as primary amine-terminated fluorescent dyes and used without further purification.

Photoacid Patterning. The hyperbranched PTBA thin films were prepared using a previously described procedure.[4] Briefly, clean Au substrates were immersed in an ethanolic 1 mM mercaptoundecanoic acid (MUA) solution to form a self-assembled monolayer (SAM). The carboxylic acid groups of the MUA SAM were then activated for 10 min with ethylchloroformate in dry DMF containing *N*-methylmorpholine, rinsed with ethyl acetate, dried in N_2, and then immersed in a solution of $H_2NR-PTBA-RNH_2$ (R = $(CH_2)_2NHCO(CH_2)_2C(CN)(CH_3)$) in DMF for 1 h. Following covalent linking, PTBA was hydrolyzed with methylsulfonic acid to yield the first layer of poly(acrylic acid) (1-PAA). Activation, grafting, and hydrolysis were repeated to yield a 2-PAA film, and a third activation and grafting cycle (but without hydrolysis) resulted in a 3-PTBA film 50 nm thick (part a of Scheme 1).

The PTBA film was coated with the photoacid by dipping the substrate into a CH_2Cl_2 solution containing both the photoacid and PTBA (part b of Scheme 1). The photoacid-coated PTBA film was irradiated through a 600-mesh TEM grid (Electron Microscopy Science, Fort Washington, PA) using UV light from a low-pressure mercury lamp (model 6035, Oriel, Stratford, CT) for 2 min under a N_2 atmosphere (part c of Scheme 1). The patterned PTBA film underwent a postexposure bake (PEB) at 100 °C for 2 min to hydrolyze the *tert*-butyl esters of the PTBA film (via the photogenerated acid) to yield PAA (part d of Scheme 1). The resulting film was developed with ethanol and dried under N_2, which results in a patterned film consisting of spatially segregated PAA (exposed regions) and PTBA (masked regions) (part e of Scheme 1).

The PAA regions of the developed film were activated using ethyl chloroformate,[4,5] and then the film was immersed in a 1 mM DMF solution of the amine-terminated dansyl fluorescent dye for 1 h. This results in formation of an amide linkage between the dye and the activated PAA film (part f of Scheme 1). The resulting film was then sonicated and rinsed with DMF and ethanol and dried with N_2. The cycle of photoacid coating, exposure (in the absence of the mask), and amidation using amine-terminated eosin was repeated to yield patterns of multiple fluorescent dyes on the same hyperbranched polymer thin film. Although a mask was not used during the second cycle in this study, it is clear that multiple mask levels could be used to pattern more than two dyes or other chemical species.

Images of the patterned films were observed using an optical microscope (Nikon Optiphot, Japan) and a fluorescence microscope (Nikon Eclipse E800) equipped with a Photometrics Sensys CCD camera.[18] MethaMorph imaging acquisition software was used to obtain images. Further processing, including false-color imaging, was accomplished using Photoshop software (Adobe, v. 2.5.1). Fluorescent images taken through a 540−580 nm excitation filter, 595 nm beam splitter, and 600−660 nm emission filter were assigned the color red, while images taken with a 330−380 nm excitation filter, 400 nm beam splitter, and 420 nm emission filter were assigned the color blue.

The PTBA film thicknesses were measured by ellipsometry (Gaertner model L2W26D, 632 nm, 70° angle of incidence).[5] Photoacid hydrolysis of the PTBA film to PAA was also confirmed by FTIR external reflectance spectroscopy (FTIR-ERS) (Bio-Rad model FT6000 equipped with a Harrick Seagull reflection accessory).[5]

Results and Discussion

Part a of Figure 1 shows an FTIR-ERS spectrum of a 50 nm thick PTBA film.[4,5] The large peaks at 1734 and 1160 cm^{-1} arise from the *tert*-butyl ester group of PTBA. Likewise, peaks at 2980, 2935, 1394, and 1369 cm^{-1} originate from the methyl groups of PTBA. After dip-coating the PTBA film with the photoacid, exposing to UV light, postexposure baking (PEB), and developing in ethanol, the C−O ester band at 1160 cm^{-1} disappears, the intensity of the carbonyl ester band at 1730 cm^{-1} significantly decreases and broadens, and the *tert*-butyl methyl peaks of PTBA are greatly attenuated. These spectral changes, which are similar to those observed when PTBA undergoes hydrolysis in a $MeSO_3H$ solution,[4,5] can be accounted for by photoacid-induced hydrolysis of PTBA to PAA. Ellipsometric measurements indicate that the thickness of the 3-PTBA film is reduced from 50 ± 2 nm prior to exposure to 25 ± 3 nm after UV irradiation. This finding is in quantitative agreement with our previous results for solution-phase hydrolysis of hyperbranched PTBA films.[4,5] Control experiments confirm that in the absence of the photoacid UV light exposure does not hydrolyze or otherwise photodegrade the PTBA film.

To demonstrate area-selective attachment of two different fluorescent dyes to the same PAA film, a sequence of photoacid patterning and dye functionalization was carried out twice. First, the photoacid-coated PTBA film was exposed to UV light through a 600-mesh TEM grid to generate the 3-PTBA/3-PAA pattern. Second, a dansyl

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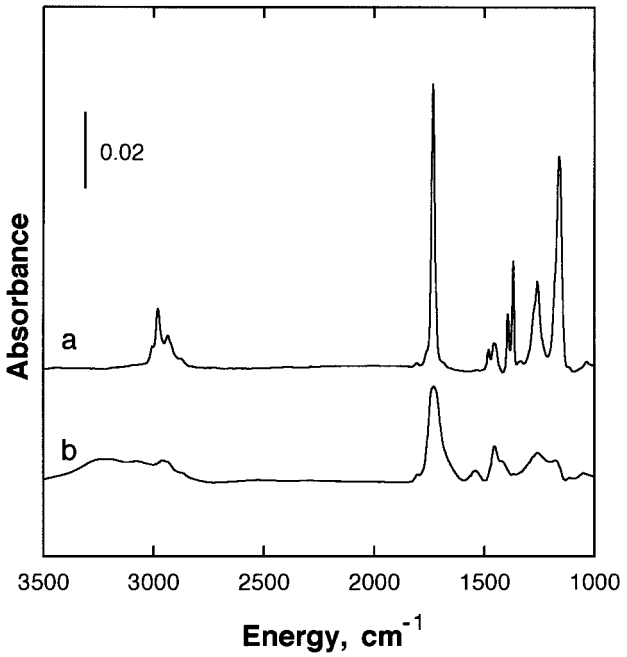

Figure 1. FTIR-ERS spectra of (a) a hyperbranched PTBA film before UV irradiation and (b) after dip-coating the same film with a photoacid, irradiating with UV light, baking, and developing. Note the dramatic decrease in the peak intensity below 1500 cm^{-1} and around 3000 cm^{-1}, which indicate nearly complete hydrolysis of the PTBA film.

dye was covalently linked via amide bonds selectively to only the photopatterned PAA regions of the film. Next, the entire film was recoated with the photoacid and reexposed to UV light, and then the eosin dye was immobilized using the same procedure used to attach the dansyl dye. Part a of Figure 2 shows an optical image of the resulting pattern. The blue color of the dansyl dye is localized within the hexagonal regions of the pattern, and the red color of the eosin dye is present only on the grid lines.

Better resolved images are obtained using fluorescence microscopy (parts b and c of Figure 2). When the patterned film is illuminated with light having a wavelength in the range 330−380 nm, fluorescence is observed at 420 nm in the dansyl-modified regions of the 3-PAA film (Figure 2b). In contrast, when the excitation wavelength is around 540−580 nm, emission is observed around 600−660 nm in the inverse regions of the fluorescence map. That is, only the grid-line regions of the 3-PAA film, which are functionalized with eosin, emit in the range 600−660 nm (Figure 2c). Because only emission from the dansyl derivative is observed in the hexagonal region of the 3-PAA film, we conclude that nearly all of the acid groups in the dansyl-derivatized regions react with dansyl. If they did not, then after the subsequent eosin derivatization reaction significant fluorescence would be observed around 600−660 nm in these regions. Additionally, these results indicate that the dansyl derivative can survive the second iteration of PTBA hydrolysis and dye derivatization.

The maximum spatial resolution demonstrated in this study is 5 μm, which is defined by the dimensions of TEM-grid mask. This is adequate for most biological applications, which is the focus of our work at the present time.[8] However, higher spatial resolution is undoubtedly attainable, because the absorbance wavelength of the photoacid is less than 300 nm.

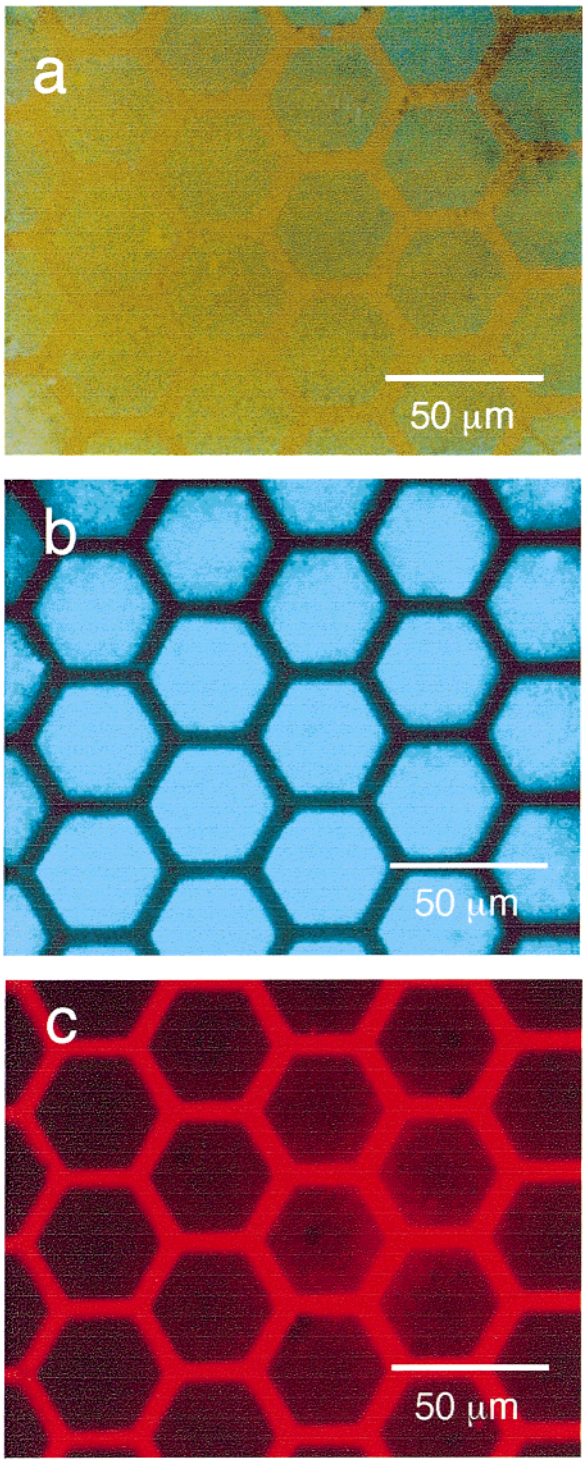

Figure 2. Microscopic images of a hyperbranched PAA film patterned with both eosin and dansyl derivatives: (a) an optical micrograph; (b) a false-color fluorescence image obtained with an excitation wavelength in the range 330−380 nm and an emission wavelength at 420 nm (the blue emission within the hexagonal pattern is characteristic of dansyl); (c) same as (b), except the excitation wavelenth was 540−580 nm and emission was collected around 600−660 nm (the red emission localized within the grid lines is characteristic of eosin).

Summary and Conclusions

In summary, we have shown that hyperbranched PTBA films covalently linked to a solid support can be patterned using a photoacid and that the resulting PAA films can be functionalized with two spatially segregated fluoroscent dyes. Although we have focused this report on dye

modification of the polymer film, the approach should be appropriate for patterning nearly any primary amine-functionalized monomer or polymer. Accordingly, applications to sensor array fabrication using active enzymes, proteins, cells, and DNA are envisioned.

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