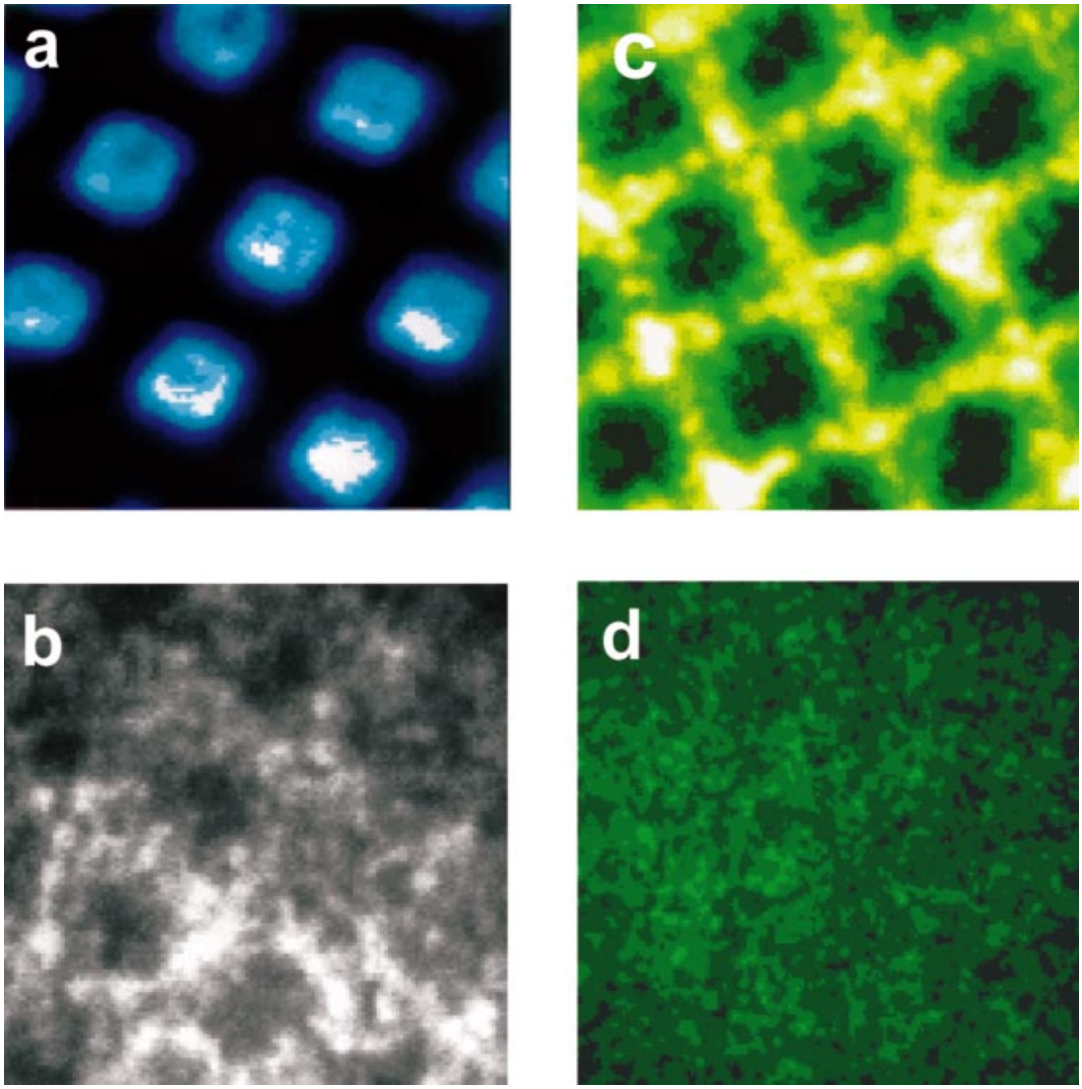

X-ray photoelectron spectroscopic element maps of a patterned, three-layer hyperbranched polymer film (3-PAA) and a 3-PAA film capped with a conformal layer of poly(ethylene glycol) (3-PAA/PEG). a) 3-PAA, Au 4f electrons; b) 3-PAA/PEG, O 1s electrons; c) 3-PAA modified with CsOH, Cs 3d electrons; d) 3-PAA/PEG modified with CsOH, Cs 3d electrons. All images were acquired for 120 s on a Kratos Axis Ultra imaging XPS. We are grateful to Dr. William Lackowski (Texas A & M University) for obtaining these data and preparing the figure.

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Patterning of Hyperbranched Polymer Films

Richard M. Crooks*[a]

*This Review describes new methods for patterning functional hyperbranched poly(acrylic acid) thin polymer films. "Hyperbranched polymer" is a generic term used to describe a wide variety of polymeric materials that contain a high percentage of functional groups, that are highly branched, and that are irregular in structure. Hyperbranched polymer films (HPFs) are prepared by an iterative three-step process: activation of an acid functionalized surface, surface grafting of amine-terminated poly(*tert*-butyl acrylate), and hydrolysis to regenerate the acid surface. The resulting materials have a high density of acid groups, which can be functionalized with moieties that introduce interesting optical, electrochemical, biological, and mechanical properties to the films. HPFs can be patterned with micron-scale resolution using either a template-based approach or photolithography. Templates consist of self-assembled monolayers prepared by microcontact printing, whereas photolithographic patterning relies on selective hydrolysis using photoacids. Biocompatibility can be introduced by grafting a conformal layer of poly(ethylene glycol) atop the HPFs. Such patterns serve as templates for spatially segregating viable mammalian and bacterial cells. In addition to the PAA HPFs, another family of patternable HPFs consisting of dendrimers and an active anhydride copolymer is described.*

KEYWORDS:

biopatterning · hyperbranched polymer · microcontact printing · sensors · surface chemistry

1. Introduction

"Hyperbranched polymer" is a generic term used to describe a wide variety of polymeric materials that contain a high percentage of functional groups, that are highly branched, and that are irregular in structure. Excellent recent reviews of these materials have appeared.[1, 2] Hyperbranched polymers are distinguished from dendrimers[3–6] in that the former have a degree of branching of less than 100%. As a consequence, hyperbranched polymers are easier to prepare than dendrimers and likely to have more commercial value.[1] A rather new area of interest relates to films of hyperbranched polymers covalently linked to surfaces. These interesting thin-film polymers, which were first reported in 1996 by Bergbreiter, Crooks, and their coworkers,[7, 8] are remarkably versatile materials that have found applications in the fields of biosensing,[8–12] chemical sensing,[8, 13, 14] corrosion passivation,[8, 15–18] controlled release,[10, 19] and membrane separations.[20]

Interest in hyperbranched polymer films (HPFs) derives from the finding that they can be grafted to both inorganic[7–11, 14–21] and organic[22–24] substrates, and from their unique combination of chemical and physical properties. For example, the films are tolerant of low-yield grafting reactions and of defects present on the supporting substrate. Moreover, HPFs tend to resist delamination as a consequence of their covalent attachment to surfaces. Because they are prepared by an iterative synthetic approach, they can be prepared in thicknesses ranging from a few nanometers up to hundreds or even thousands of nanometers.[8] Under certain conditions the surfaces of these polymer films can be remarkably smooth.[25] HPFs contain a high density of functional groups, so they lend themselves to further elaboration with dyes, various types of host molecules, electroactive groups, biocompatible moieties, and so forth.[8] Finally, the methodology for preparing HPFs is highly compatible with surface-patterning methods, such as microcontact printing (μCP)[9, 11, 21, 22, 26, 27] and photolithography.[28] The purpose of this Review is to briefly describe the synthesis and properties of HPFs, followed by a discussion of patterning approaches and applications for patterned HPFs.

2. Synthesis of Hyperbranched Polymer Films

The synthesis of HPFs (Scheme 1) begins with preparation of an acid-functionalized surface. On inorganic surfaces, such as gold, it is convenient to use an adherent monolayer of mercaptoundecanoic acid (MUA),[7] but any bifunctional molecule that can be linked to an inorganic substrate at even submonolayer coverage and which presents a reactive group for subsequent reactions, is sufficient. For organic substrates, such as polyethylene (PE), the situation is even simpler because surface oxidation of PE provides an adequate number of acid groups to promote subsequent grafting.[22–24] Activation of the acid groups through a mixed anhydride followed by reaction with an α,ω-diamino-poly(*tert*-butylacrylate) (H_2NR-PTBA-RNH_2, $R = (CH_2)_2NHCO(CH_2)_2C(CN)(CH_3)$, henceforth denoted as PTBA) yields the first grafted layer, which we refer to as 1-PTBA. Hydrolysis (*p*-TsOH, 50 – 55 °C, 1 h) results in formation of the first grafted poly(acrylic

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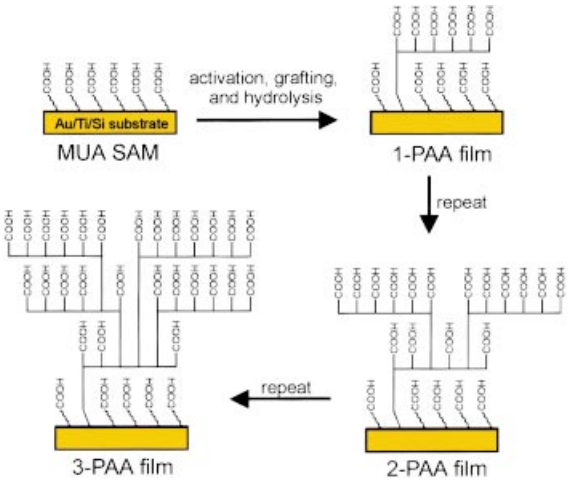


Scheme 1. *Illustration of the growth of a hyperbranched polymer film prepared by three iterations of activation, grafting, and hydrolysis.*

acid) layer (1-PAA). Repetition of these steps produces additional grafting of PAA (or PTBA if the final hydrolysis step is omitted) at multiple sites on each prior graft leading to a layered HPF (namely, 1-PAA, 2-PAA, 3-PAA, and so forth). Infrared data corresponding to the sequence of reactions required to prepare a 1-PAA film are shown in Figure 1.

There are three key aspects of the hyperbranching approach that distinguish it from linear surface grafting methodologies.[29–36] First, as a consequence of nonlinear (hyperbranched) growth, the thickness of HPFs increases nonlinearly during the first four or five grafting stages, and linearly thereafter (Figure 2). Thus, in contrast to linear grafting methods, hyperbranching results in rapid, but highly reproducible, film thickness increases after each grafting iteration. Second, HPFs effectively passivate surface defects. The two reasons for this are illustrated schematically in Figure 3. Specifically, HPFs tolerate grafting errors because of the multiplicity of potential reaction sites (Figure 3 b), while linear grafts result in propagation of grafting errors throughout subsequent grafting iterations (Figure 3 a). Additionally, hyperbranched grafting results in polymer spreading (Figure 3 b), which also tends to passivate defects. Finally, for hyperbranching to occur only a small percentage of the total acid groups used in the previous graft are required to continue film growth. Accordingly, and as discussed in the next Section, HPFs contain a very large number of accessible and reactive acid groups, which can be easily functionalized with both small molecules and polymers of various sorts.[8]

Richard M. Crooks *(born 30 March 1954) received his primary and secondary education in the Chicago area (USA). He attended the University of Illinois (B.S. chemistry), where he studied electrochemistry in the laboratory of Larry R. Faulkner, before receiving a Ph.D. in electrochemistry from the University of Texas (Austin) in 1987. His research, under the guidance of Allen J. Bard, focused on electrochemistry in supercritical fluids. Following a two-year postdoctoral experience at MIT working with Mark S. Wrighton in the field of electronically conducting polymers and microelectrochemical devices, he began his independent academic career in 1989 as an assistant professor in the chemistry department at the University of New Mexico. In 1993 he moved to Texas A&M University where he is currently professor of chemistry and director of the Center for Integrated Microchemical Systems. His interests include chemical and biological sensors, microchemical systems, catalysis, and nanoscale chemistry. He is the recipient of both National Science Foundation and Office of Naval Research Young Investigator Awards, has served on the boards of several professional societies and as an editor and guest editor for several publications, and has published nearly 200 research papers, book chapters, and proceedings.*

3. Functionalization of Hyperbranched Polymer Films

Covalent functionalization of HPFs is straightforward. For example, amidation, esterification, reduction, and alkylation reactions proceed quickly and in high yield.[37] Using these approaches, we have prepared HPFs covalently bound to fluorescent,[8, 28] fluorinated,[8, 15–17, 19] ion-binding,[8] and electroactive functional groups.[8] Based on the disappearance of the acid C=O stretching band (reflection infrared spectroscopy), we estimate that the coupling yield of these types of moieties ranges from 35 – 65 % depending on the monomer and the activation chemistry used.[8, 15, 16] More recent work by Bergbreiter and Tao indicate that conversions > 90 % are possible.[37]

It is also possible to noncovalently modify HPFs using and hydrogen bonding and electrostatic interactions. This type of pH-dependent modification has been used to incorporate metal ions[19] and polycations, such as dendrimers,[10, 19] linear polymers,[19] and glucose oxidase,[10] as well as hydrogen bonding polymers.[38] The magnitude of cationic binding can be very high, which is a consequence of the large changes in the free volume of the film that occur upon electrostatic incorporation of cations and polycations. For example, in situ titrations indicate that incorporation of Na^+ results in a doubling of the thickness of a 3-PAA film. Electrostatic sorption of polycations, such as dendrimers, results in changes in dry-film thickness of up to 230 %.[19] Importantly, these sorption/desorption processes are reversible, which opens up the possibility of using HPFs for applications requiring controlled release.[19]

As mentioned in the previous paragraph, HPFs undergo large volume changes when the pH is changed or when cations are electrostatically incorporated. Importantly, this property can be controlled. For example, the $pK_{1/2}$ of a simple 3-PAA film can be determined by in situ measurement of either the thickness change or carbonyl stretching intensity change as a function of pH. The $pK_{1/2}$ measured by either method yields a value of around 4.3, which reflects the presence of the pendent acid

groups.[19] However, when a 3-PAA film is fluorinated via reaction with a fluorinated hydrocarbon, the interior is rendered hydrophobic. This results in an increase in the $pK_{1/2}$ of the HPF to 6.7 and a large reduction in pH-dependent swelling. Similarly, when such a film is crosslinked with ethylenediamine, swelling is reduced and the $pK_{1/2}$ again shifts to a higher value. This shift in $pK_{1/2}$ is a consequence of the energetic cost of deprotonating acid groups held in close proximity with one another.

So far this discussion has focused on homogeneous functionalization of the interior of HPFs. However, it is also possible to selectively modify only the PAA surface with a conformal layer of a different polymer. Perhaps even more intriguingly, it is possible to use small molecules to modify a PAA film, and then subsequently graft additional PAA to the small fraction of acid groups near the polymer film surface that did not react with monomers in the previous step. This latter property is unique to HPFs and it is a direct consequence of hyperbranching, which is itself manifested as a nonlinear increase in the number of acidic functional groups. This important principle is illustrated in Scheme 2, and we have used it to prepare a HPF that has a fluorinated, hydrophobic interior capped with an exterior of unmodified, hydrophilic PAA.[15]

For the purposes of this Review, which focuses on patterning of thin films for placement of biomaterials, the more important finding is that HPFs can be coated with a conformal layer of a different polymer. For example, we have prepared ultrathin "filter layers" atop HPFs containing chemically sensitive moieties within their interior (Scheme 3).[14] Specifically, it is possible to covalently link a cyclodextrin (CD) monomer throughout a 3-PAA HPF, and subsequently graft a thin layer of a polyamine only to the top of the 3-PAA/CD composite film. At high pH the polyamine is neutral and positively charged guest molecules from solution are able to penetrate the polyamine "filter layer" and bind to the cyclodextrin. However, when the pH of the solution is lowered, the polyamine layer becomes positively charged and prevents passage of the positively charged probe molecules and no binding with the underlying cyclodextrin is observed. Nanoscopic filter layers such as these will be useful in applications related to chemical sensors. A similar approach can be used to introduce biocompatibility to HPFs. In this case a layer of poly(ethylene glycol) (PEG) is grafted atop the HPF. As shown in Figure 4, PEG-modified PAA films are important because they resist bioadhesion.[39] This is a key aspect of our studies and will be discussed in more detail later.[9, 11, 25]

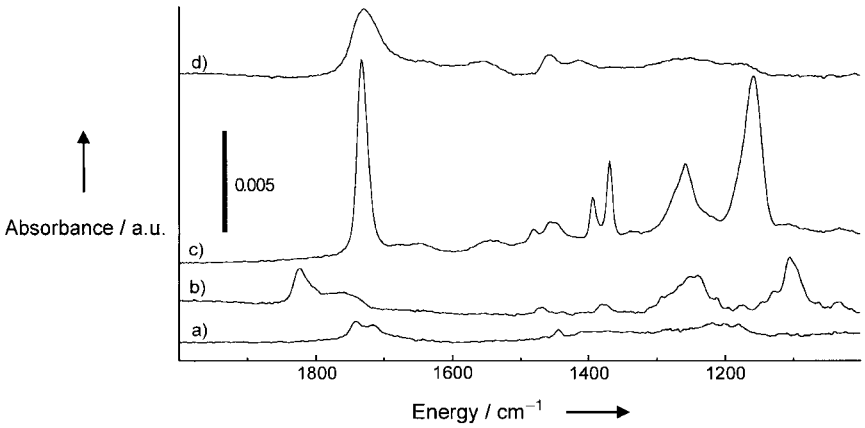


Figure 1. *Fourier transform infrared external reflectance spectrometry (FTIR-ERS) measurements of a) a MUA monolayer, b) the MUA monolayer after activation with isobutyl chloroformate, c) a layer of PTBA grafted on the MUA monolayer, and d) after hydrolysis of PTBA to PAA. Note particularly the region of the spectrum between 1700 – 1800 cm^{-1}. In (a) the double peak is characteristic of MUA, but after activation (b) the two bands shift to higher energy, which is characteristic of the active anhydride. The large carbonyl band in (c) is associated with the ester, and this peak decreases in height and broadens (mainly due to hydrogen bonding) after hydrolysis (d). Adapted, with permission, from ref. [19] (copyright 1998 American Chemical Society).*

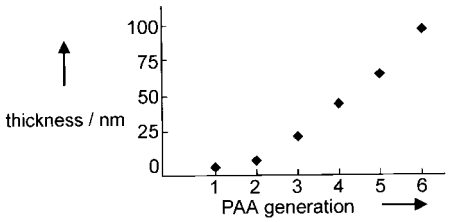


Figure 2. *The measured ellipsometric thickness of hyperbranched polymer films through to the sixth generation. Note that the change in thickness is nonlinear through to the fourth generation, which reflects the hyperbranched growth mechanism. Adapted, with permission, from ref. [7] (copyright 1996 American Chemical Society).*

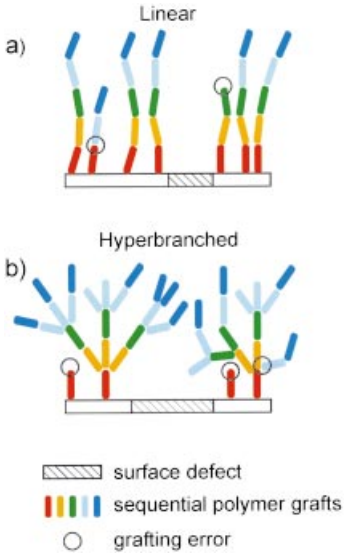
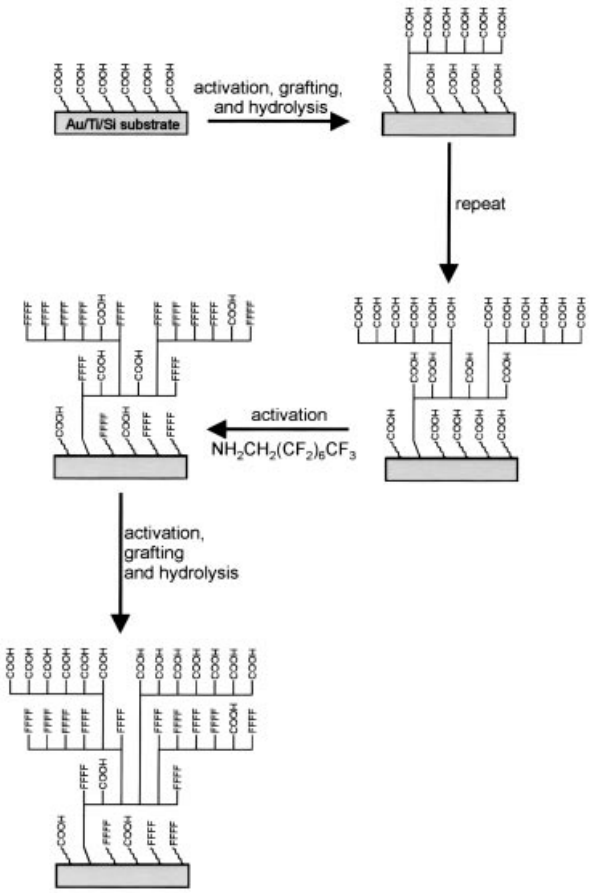


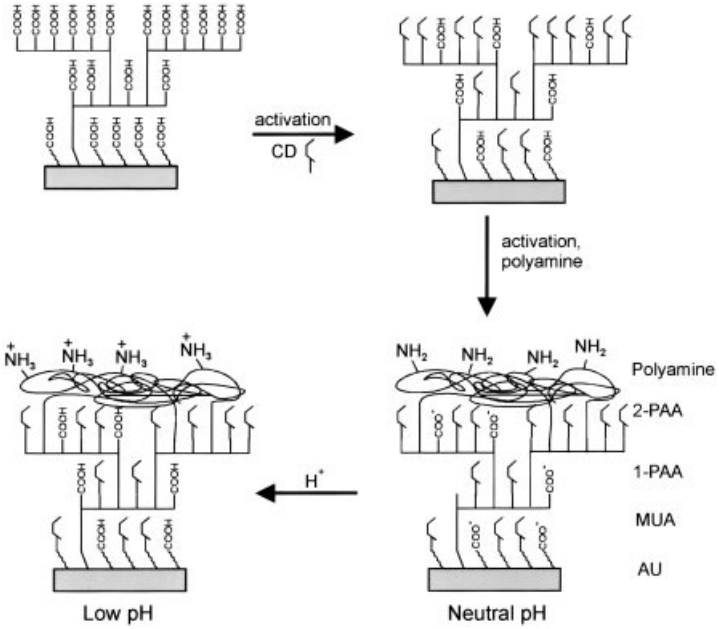
Figure 3. *Comparison of the relationship between polymer growth mechanism and defect coverage for linear and hyperbranched grafting. HPFs tolerate grafting errors because of the multiplicity of potential reaction sites, while linear grafts result in propagation of grafting errors throughout subsequent grafting iterations. Hyperbranched grafting also results in polymer spreading, which also tends to passivate defects.*

4. Patterning of Hyperbranched Polymer Films

Polymer films patterned at micron or even submicron resolution have been of critical technological importance since the 1960s when they were intensively developed as photoresists for the



Scheme 2. *Illustration of the preparation of a hydrophobic HPF capped with a hydrophilic layer. In this case a 2-PAA film was prepared and then the acid groups were functionalized with a fluorinated primary amine (indicated as FFFF in the illustration). However, a small percentage of acid groups remain unreacted, and these can be used in a second step to graft additional PAA to the surface of the now-hydrophobic film. This process can be repeated to further enhance the hydrophilicity of the top surface of the film. The ability to prepare thin organic films having this architecture is a direct consequence of hyperbranching and the consequent nonlinear multiplication of acid groups.*



Scheme 3. *Representation of an approach for preparing chemically sensitive films capped with a nanoscopic filter layer. A 2-PAA film is first modified with a receptor, such as the cyclodextrin (CD) used in this example, and then a capping layer of a polyamine is covalently linked to previously unreacted acid groups on the polymer surface. At high pH the polyamine is uncharged and positively charged substrates are able to penetrate the filter layer and interact with the underlying cyclodextrins. However, at low pH the polyamine layer is protonated and the resulting electrostatic charge repels positively charged substrates. Thus, the polyamine layer acts as a chemically sensitive gate that modulates the ingress and egress of substrates.*

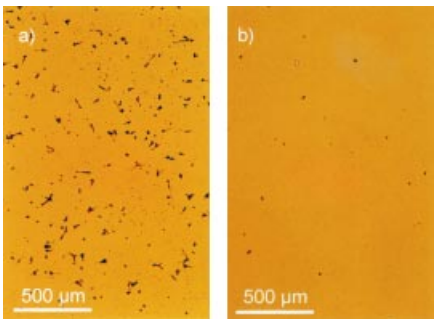


Figure 4. *Optical micrographs of macrophage cells seeded onto polymer surfaces. a) Adhesion and growth on a 3-PAA HPF prepared as in Scheme 1. b) Inhibition of adhesion and growth on a 3-PAA film after covalent grafting a layer of MeO-PEG-NH_2 to the 3-PAA surface. Adapted, with permission, from ref. [11] (copyright 2001 American Chemical Society).*

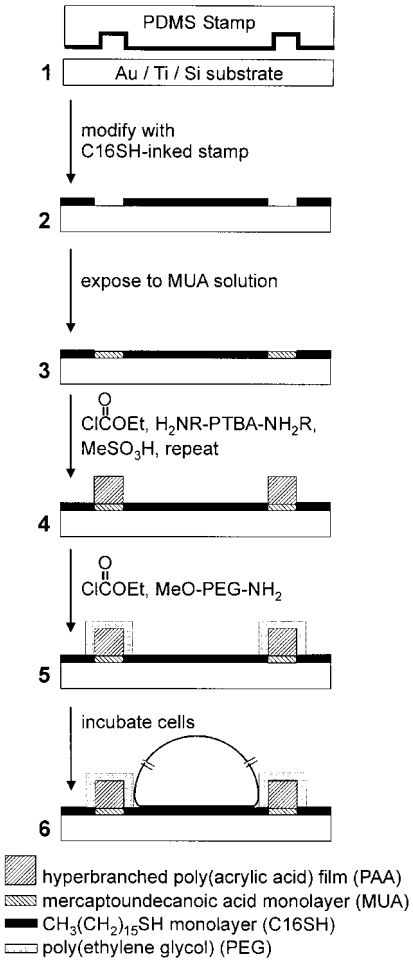
microelectronics industry. More recently, however, a number of new methods that are more chemically versatile and have more interesting three-dimensional architectures have been developed for preparing patterned organic thin films. Here, we restrict most of the discussion to patterns of polymer films prepared using monolayer template approaches, but for the sake of completeness two examples involving the use of photolithography are also included.

Template-based approaches for preparing patterned polymer films require the presence of a surface having spatially segregated reactive regions that restrict polymer growth to specific areas. This can be done in serial by "spotting" the surface using a fast dispensing technology such as ink-jet printing.[40] Such approaches have the virtue of being able to provide multiple, chemically distinct templates on the surface but they are relatively slow. Parallel templating strategies appear to be more viable for most technological applications because they are fast and inexpensive.

The method of microcontact printing (µCP), pioneered by Whitesides and co-workers,[26, 27] is a simple and effective parallel patterning method that has been used to directly modify surfaces with monolayers, biological materials of various sorts, and polymers.[26, 27] We and others have recently shown that monolayer patterns prepared using the Whitesides approach can serve as templates to direct the growth of organic thin films. For example, Hammond et al. have shown that SAMs patterned by µCP can serve as templates for subsequent layer-by-layer deposition of polycationic and polyanionic polymers.[41, 42] Husemann et al. have shown that µCP of SAMs can also serve to

spatially direct the growth of polymers by surface-initiated ring-opening polymerization of ε-caprolactone.[43] Kratzmüller and co-workers have described a similar approach that results in two-dimensional growth of polypeptides.[44] Shah et al. have demonstrated that surface polymerization can be initiated from patterned monolayers using atom-transfer radical polymerization.[45] Finally, Whitesides and co-workers have shown that surface-initiated polymerization from patterned silicon surfaces is possible,[46, 47] that polymers prepared using this general methodology can serve as etch resists,[48] and that such materials can be released from a surface to yield geometrically well-defined, molecularly thin polymer films.[49] The latter group has also shown that two-dimensional templates prepared by μCP can be used to direct the growth of polymers prepared by chemical vapor deposition.[50]

Templates for HPFs can be prepared on inorganic[9, 11, 21] and organic substrates[22] following the general approach shown in frames 1 – 3 of Scheme 4.[26, 27] For example, to prepare a monolayer pattern on a gold substrate, an elastomeric stamp (poly(dimethylsiloxane), PDMS) is inked with a methyl-terminated *n*-alkylthiol such as *n*-hexadecylthiol (C16SH). Second, the stamp is brought into contact with the substrate, which results in transfer of one monolayer of the thiol from regions of the stamp having positive relief to the gold substrate. Third, unstamped, and therefore not passivated, regions of the surface are modified with a second thiol that presents a reactive functional group to the solution, mercaptoundecanoic acid in this case, by immersing the entire substrate into a dilute ethanolic MUA solution for a short time.



Scheme 4. *Illustration of a method for preparing "cell corrals" using a monolayer template, prepared by microcontact printing, and subsequent grafting of polymers.*

Following deposition of the template monolayer by μCP, HPFs are grafted exclusively to the MUA fraction of the two-component monolayer using the steps shown in Scheme 1. Specifically, the MUA fraction of the monolayer is activated by immersing the entire substrate in an ethylchloroformate solution, next the substrate is exposed to amine-functionalized PTBA, and finally PTBA is hydrolyzed to PAA (Frame 4 of Scheme 4).[9, 21] Additional iterations of activation, grafting, and hydrolysis can be carried out to grow thicker films, with the caveat that the HPF only grows on regions of the surface templated with MUA. Accordingly, the HBF is a negative image of the positive relief of the PDMS stamp used to create the template initially.

Figure 5 shows a tapping-mode atomic force microscopy (TM-AFM) image of a 3-PAA/C16SH patterned gold surface prepared via steps 1 – 4 in Scheme 4. The PDMS stamp was prepared from either a 300 mesh TEM grid or an optical test-mask master.

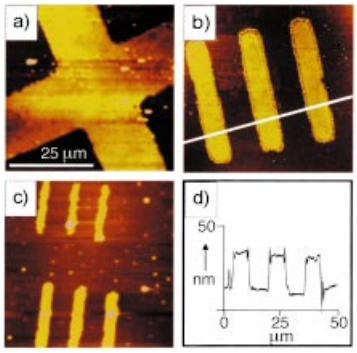
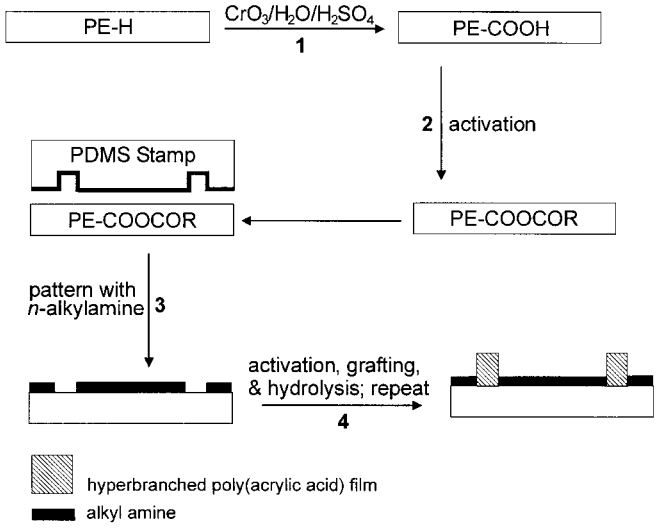


Figure 5. *a – c) 50 × 50 μm² TM-AFM images of patterned 3-PAA HPFs. The pattern in (a) was prepared using a master derived from a TEM grid, while the patterns in (b) and (c) were generated from a commercially available test pattern. d) Cross section of the features identified by the white line in (b). Adapted, with permission, from ref. [21] (copyright 1999 American Chemical Society).*

Figure 5a shows a small region of a pattern fabricated using the TEM-grid master. The critical lateral dimension of the 3-PAA lines is 20 μm and the square C16SH regions are 63 μm across. The height difference between the top of the 3-PAA film and the top of the C16SH monolayer is 25.0 nm, which is in accord with the thickness of the macroscopic, homogeneous films of 3-PAA discussed previously. Figures 5b and 5c are TM-AFM images of 3-PAA films patterned using a PDMS stamp derived from the test mask. Critical lateral dimensions of these features range from 8 μm in 5b to 2 μm in the upper half of 5c. Figure 5d shows a cross section taken from the image in 5b. The width of the interfacial region joining 3-PAA and C16SH is on the order of 500 nm.

Scheme 5 illustrates one method we have used to pattern HPFs on plastics such as polyethylene.[22–24] First, a purified high-



Scheme 5. *Illustration of the methodology used to pattern polyethylene substrates (PE-H) with hyperbranched polymer films. Adapted, with permission, from ref. [22] (copyright 1999 American Chemical Society).*

density polyethylene film is oxidized with a $CrO_3/H_2O/H_2SO_4$ mixture. Second, the resulting acid surface is activated with ethyl chloroformate exactly as for MUA monolayers confined to gold substrates. Next, the anhydride-activated polyethylene substrate can be patterned with *n*-hexadecylamine by μCP to passivate selected regions of the surface. Finally, unpassivated regions of the plastic substrate react with PTBA to yield the amide-grafted polymer layer. Hydrolysis yields the first layer of PAA, while additional cycles of activation, grafting, and hydrolysis yield thicker HPFs.

3-PAA/C16SH patterns typified by Figure 5 and Scheme 5 can be further elaborated by grafting PEG onto the 3-PAA fraction of the substrate surface. The chemistry required to prepare patterned PEG-capped 3-PAA films (3-PAA/PEG) is identical to that described earlier for unpatterned films (frame 5 of Scheme 4). Figure 6 shows an optical micrograph of a two-component film on a gold substrate prepared using a TEM grid master to fabricate the stamp. The pattern consists of 1.8 nm thick C16SH "corrals" that are 63 μm square, and 54 nm high 3-PAA/PEG walls that are 20 μm wide. The important point is that the fidelity of the underlying 3-PAA pattern is maintained after grafting PEG. X-ray photoelectron spectroscopy (XPS) confirms that patterned 3-PAA/PEG composites are layered, just as they are for the unpatterned films (Figure 4).

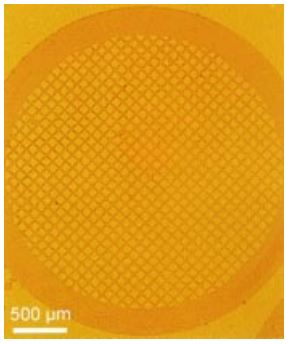


Figure 6. *An optical micrograph of an array of corrals consisting of 3-PAA walls capped with PEG (as shown in Frame 5 of Scheme 4) and C16SH bottoms.*

5. Biopatterning

The ability to direct the growth of cells into predetermined patterns is useful for fundamental studies of cell adhesion, growth, and death, for tissue engineering, and for chemical sensing applications. In a remarkable series of experiments spanning the last several years, Whitesides and co-workers have shown that μCP is a versatile approach that can be used to prepare two-component SAMs that efficiently template the growth of cells.[51] However, there are some potential advantages to using three-dimensional polymer patterns, particularly HPFs, to direct cell growth. First, two-dimensional SAMs have limited stability and are not stable when illuminated with UV light in the presence of oxygen (which might be required for sterilization).[52] In contrast, HPFs are stable and not easily delaminated.[8] Second, defects within SAMs can lead to infidelities in the cell pattern, whereas hyperbranching tends to heal such defects, as shown in Figure 3. HPFs contain a large number of acid groups that can be functionalized with moieties that can influence the adhesion and growth of cells. Finally, HPFs can be used to release chemicals in close proximity to the cells they constrain.

Frame 6 of Scheme 4 indicates that it is possible to pattern both mammalian and bacterial cells using the types of "cell corrals" shown in Figure 6. Figure 7 a is a micrograph of macrophage cells seeded onto a patterned 3-PAA film. That macrophage cells grow on both the methyl-terminated SAM and

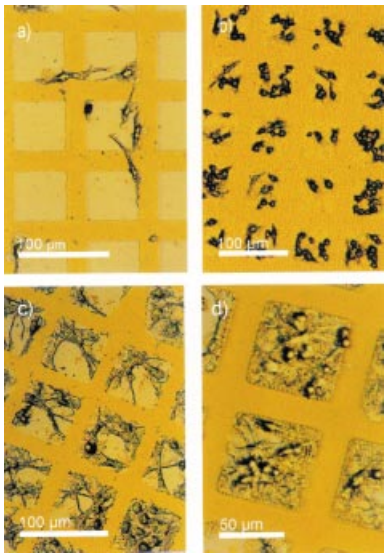


Figure 7. *a) Optical micrographs of cells grown onto a patterned film having 3-PAA walls (no PEG) and C16SH bottoms: Cells adhere to and grow over the 3-PAA walls; b – d) macrophage cells patterned onto the PEG-modified substrate shown in Scheme 6. Note that there is no evidence for cellular interactions between corrals.*

across the 3-PAA corral walls is anticipated by Figure 4. Figure 7 b is an optical micrograph of a patterned surface in which the surfaces of the corral walls have been modified with PEG. It indicates that PEG inhibits cell adhesion and growth, and that the cells are confined exclusively to the methyl-terminated SAM surface. These results clearly demonstrate that simple polymer chemistry can be used to control cell growth; note especially that no part of the cell body escapes the corral. Figures 7 c and 7 d are higher resolution micrographs of macrophage-patterned

regions of the surface. The macrophage cells in these images possess the correct morphology. For example, the peripheral skirt of the cytoplasm and the surface ridges of the cell are evident. Additionally, the cells are well spread as evidenced by the clearly defined extended processes. Figure 7 d clearly shows that the macrophage cells grow only to the boundary of the 3-PAA/PEG corral wall even though the wall is only a small fraction of the cell height. Live/Dead (Molecular Probes) studies indicate that even at this level of crowding the cells are still viable. Macrophage cell growth is anchorage dependent, so as long as there is surface available the cells will continue to grow until the surface is confluent.

In addition to controlling the growth of macrophage cells, these 3-PAA/PEG patterns also template the growth of endothelial cells, hepatocytes, and bacteria.[11] Because mammalian cells are so large each corral generally contains only a single cell. Like the macrophage cells, both these other mammalian cell lines also remain viable for weeks on the patterned surfaces. Figure 8 demonstrates that bacteria can also be patterned onto 3-PAA/PEG substrates.[53] Figure 7 a shows corrals of the same size used to pattern the mammalian cells ($63 \times 63\ \mu m^2$), so it is not surprising that $\sim 10-20$ individual bacteria, which are much smaller than mammalian cells, occupy a single corral. We have been able to reduce the size of the corrals to $10 \times 10\ \mu m^2$ (Figure 8 b) and thereby reduce the number of bacteria per corral to $\sim 1-5$. However, as shown in the Figure, there are defects in these smaller corrals and bacteria are frequently observed to grow on the PEG walls. We are attempting to rectify this problem at the present time.

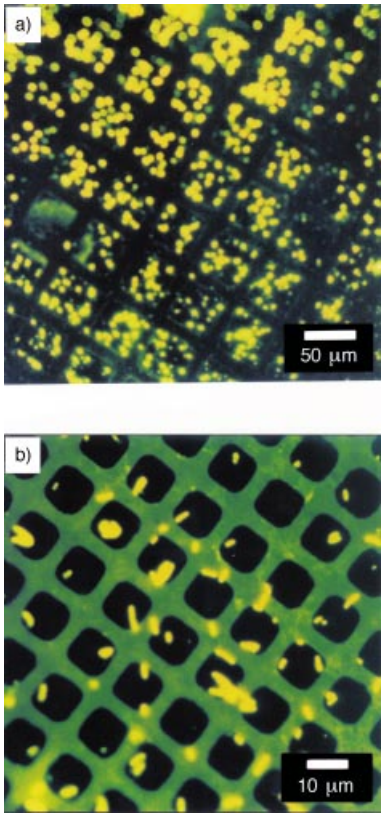
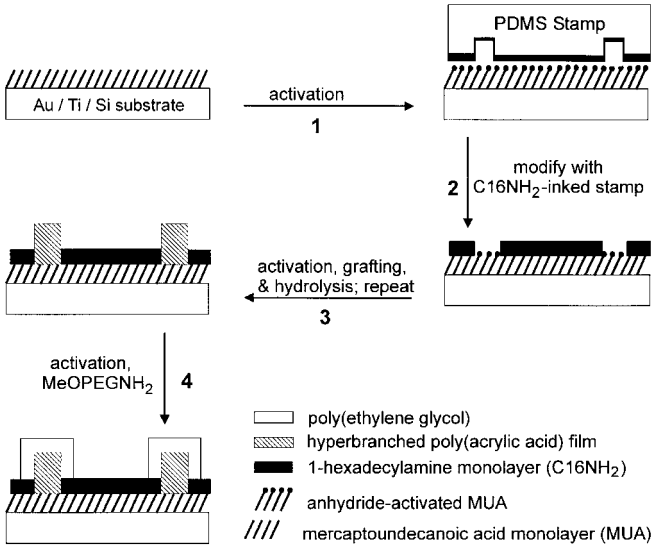


Figure 8. E. coli *confined within 3-PAA/PEG corrals. The viability of the surface-confined bacteria was confirmed by staining with Live/Dead (Molecular Probes) assay kit for bacteria. a) Because bacteria are much smaller than mammalian cells, many bacteria are contained within the $63 \times 63\ \mu m^2$ corrals shown here. b) When the size of the corrals is reduced to $10 \times 10\ \mu m^2$, far fewer bacteria are present within an individual corral. Note, however, that in this case there are a significant number of patterning errors.*

6. Other Microcontact Printing-Based Approaches for Patterning HPFs

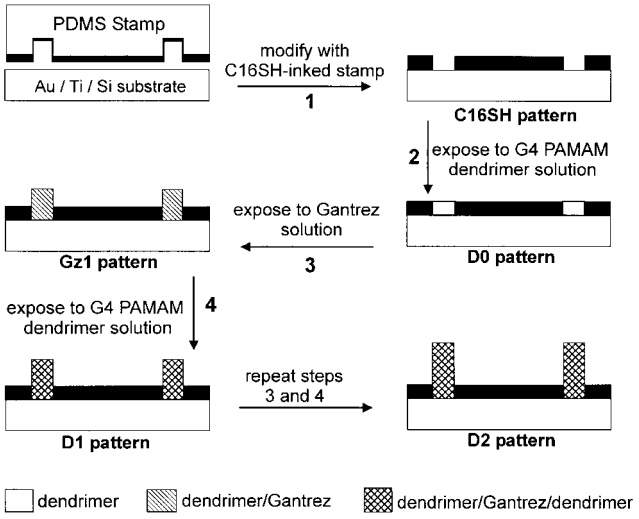
In addition to the approach shown in Scheme 4, there are numerous other methods for patterning HPF using μCP to prepare the template. For example, the approach shown in Scheme 6 is extremely versatile because it does not rely on a specific type of interaction between the substrate and the passivating monolayer (for example between gold and an *n*-alkylthiol as shown in Scheme 4).[54] Instead, any molecular "adhesion layer" that has an affinity for both the substrate of interest and that can be grafted to the HPF is appropriate.[55]



Scheme 6. *Illustration of a method for preparing HPFs using a molecular adhesion layer and selected-area passivation through μCP. Adapted, with permission, from ref. [54] (copyright 2001 American Chemical Society).*

Although it has not been emphasized in this Review, there are other methods for preparing HPFs besides the PAA-grafting approach shown in Scheme 1. Essentially any polymer that provides multiple functional groups is appropriate. Dendrimers,[3–6] for example, are ideally suited for this purpose because their periphery can consist of multiple reactive terminal groups. Scheme 7 illustrates an approach for preparing and patterning hyperbranched polymer films that are based on a two-step synthesis involving sequential reaction of poly(amidoamine) (PAMAM) dendrimers and the active anhydride copolymer Gantrez (poly(maleic anhydride)-*co*-poly(methyl vinyl ether)).[13, 56, 57]

To prepare this type of patterned HPF a fraction of the gold surface is first passivated with an *n*-alkylthiol (C16SH) through μCP. The remainder of the gold surface is then modified with a monolayer of a fourth-generation, amine-terminated PAMAM



Scheme 7. *Illustration showing a method for preparing hyperbranched polymer films based on dendrimers and the active anhydride copolymer Gantrez. Adapted, with permission, from ref. [54] (copyright 2001 American Chemical Society).*

dendrimer (G4-NH_2), which adheres to many different types of materials, including gold, via multidentate amine interactions.[58] The hyperbranched G4-NH_2/Gantrez composite thin film can be selectively deposited onto the dendrimer-modified regions of the surface through a layer-by-layer method we reported previously.[13, 56, 57] Specifically, the active-anhydride Gantrez polymer reacts with primary amines on the dendrimer periphery to yield covalent amic acid functional groups. Similarly, amine-terminated PAMAM dendrimers react with underlying Gantrez layers. This dipping process can be carried out indefinitely to yield ever-thicker films. Although the outermost layer of this sort of film is chemically distinct from the underlying region, the film interior is more or less a homogeneous composite of G4-NH_2 and Gantrez.

Reflection infrared spectroscopy and ellipsometry measurements obtained from unpatterned surfaces initially modified with only C16SH or only G4-NH_2 confirmed that grafting occurs exclusively on the dendrimer-modified gold surfaces.[54] Figure 9 shows a bright-field optical micrograph of a square-grid pattern. The 20 μm wide grid lines are composed of a D2 (dendrimer-terminated) G4-NH_2/Gantrez nanocomposite, and the interior 63 μm wide squares consist of a C16SH SAM. TM-AFM measurements confirm pattern transfer of the G4-NH_2/Gantrez nanocomposite, that no defects are evident (at the resolution of TM-AFM), and that the interface between the SAM and the polymer is remarkably smooth.

7. Photolithographic Approaches for Patterning Hyperbranched Polymer Films

Although μCP is a fast and flexible method for patterning polymer surfaces, it does have some disadvantages compared to photolithography. For example, it is difficult to perform multiple patterning steps on a single substrate because multiple stamps are not easy to align with micron-scale resolution. Additionally, we have found it inconvenient to use μCP to prepare a patterned

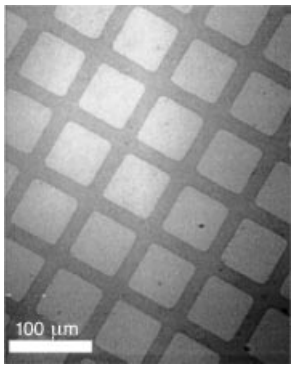
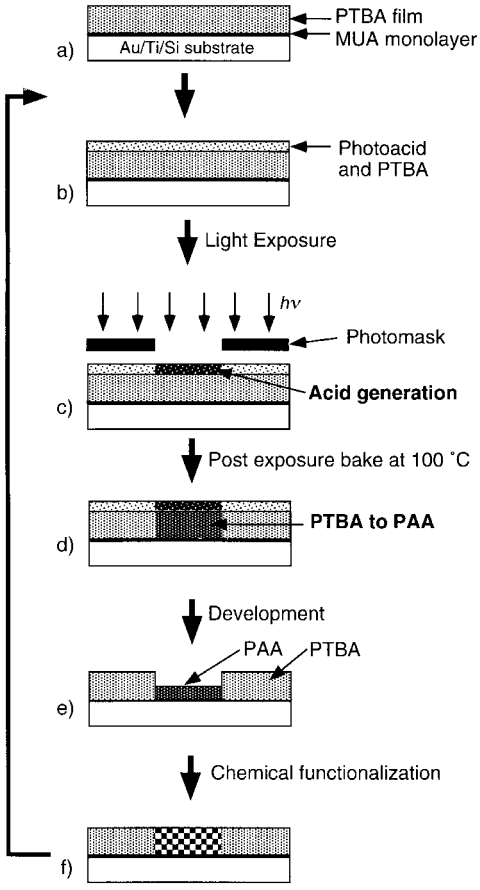


Figure 9. *Optical micrograph of a gold surface patterned using μCP of* n-*hexadecanethiol (C16SH) followed by sequential grafting of G4-NH_2 dendrimers and Gantrez to the unpassivated regions of the surface. The grid is composed of a 20 μm wide, 23 nm thick dendrimer/Gantrez composite, and the squares are 63 μm wide and consist of a 1.8 nm thick C16SH monolayer. The pattern was derived from a 300-mesh TEM grid master. Adapted, with permission, from ref. [54] (copyright 2001 American Chemical Society).*

surface consisting of two different types of polymers (μCP works best when a fraction of the surface consists of a single monolayer, such as the methyl-terminated C16SH described earlier). Accordingly, we developed a method based on photolithography that resolves both of these problems (Scheme 8).[28] Others have reported related photopatterning approaches.[59, 60]



Scheme 8. *Illustration of a photoacid-based approach for preparing photopatterned PAA HPFs. Adapted, with permission, from ref. [28] (copyright 1999 American Chemical Society).*

The approach shown in Scheme 8 consists of two basic steps. First, covalent grafting of the hyperbranched poly(*tert*-butyl acrylate) (PTBA) thin film to a gold substrate followed by overcoating with a layer of photoacid. Photoacids are chemical species, such as the triarylsulfonium hexafluoroantimonate used in this work, that release a proton upon photolysis.[61] Second, photolithography, which generates acid in the exposed region and thereby catalyzes the hydrolysis of PTBA to PAA.

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 dye was covalently linked selectively to only the photopatterned PAA regions of the film via amide bonds. Next, the entire film was recoated with the photoacid and re-exposed to UV light, and then an eosin dye was immobilized using the same procedure used to attach the dansyl dye. Figure 10a 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. 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 10b). 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 10c). 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. This finding helps to prove our earlier contention that the acid groups in HPFs are accessible and reactive. Additionally, these results indicate that the dansyl derivative can survive the second iteration of PTBA hydrolysis and dye derivatization.

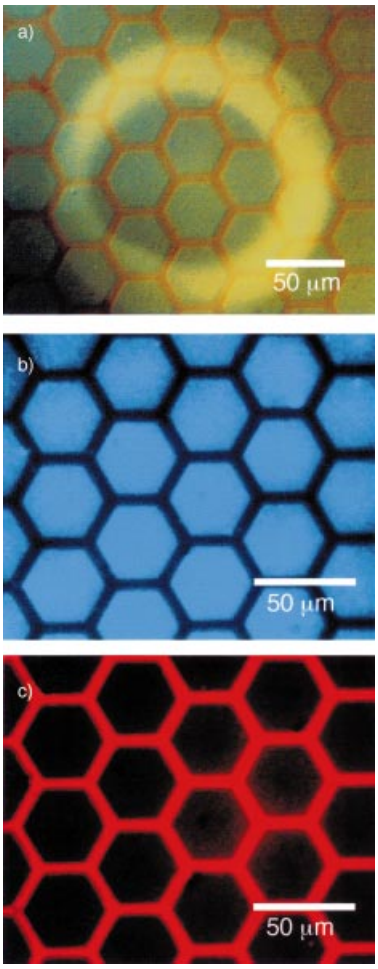


Figure 10. *Micrographs of a hyperbranched PAA film patterned with both eosin and dansyl dye derivatives. a) An optical micrograph (the bright annulus in this optical image is a reflection from the light source, not a defect in the film); b) a false-color fluorescence image obtained with an excitation 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) is the same as (b), except the excitation wavelength was 540 – 580 nm and emission was collected around 600 – 660 nm (the red emission localized within the grid lines is characteristic of eosin). Adapted, with permission, from ref. [28] (copyright 1999 American Chemical Society).*

8. Summary, Conclusions, and Perspectives

This Review has described the synthesis, characterization, patterning, and biomodification of hyperbranched polymer films prepared using a variety of different approaches. As discussed in the introduction, these materials have some very attractive attributes that suggest HPFs might be useful for applications such as bio/chemical sensing, corrosion inhibition, controlled release, and membrane separations. These functions are introduced through electrostatic or covalent attachment of specific small molecules. The template and photolithographic approaches to polymer patterning are especially powerful, because they yield well-defined, chemically active corrals having micron-scale lateral resolution. These corrals may host a wide variety of biological cells that can serve as detectors for sensors. Alternatively, such cellular arrays can be screened for particular biological functions, which is the focus of our current interest.

I am grateful to my colleagues past and present who have contributed to this work. I am especially indebted to Prof. David E. Bergbreiter, the co-discoverer of hyperbranched polymer films, with whom I have shared a productive collaboration for several years. The initial work on these materials was carried out by Prof. Merlin L. Bruening (presently at Michigan State University) and Dr. Yuefen Zhou (now with Ontogen Corp.). The patterning work based on microcontact printing was initiated by Dr. Pradyut Ghosh and Dr. William M. Lackowski, and the photolithographic patterning approach was executed by Dr. Atsushi Aoki. Prof. Michael V. Pishko and his student, Dr. Mary Lee Amirpour, were responsible for most

of the work involving mammalian cells. Brooke L. Rowan was responsible for the work involving bacteria. Others who made key contributions to this work include: Dr. Daniel L. Dermody, Dr. Justine G. Franchina, Dr. Yuelong Liu, Dr. Robert F. Peez, Dr. Kaushik Sirkar, and Dr. Mingqi Zhao. We are also indebted to those who contributed in a variety of less direct ways to the success of this project: Ms. Rachel Agee, Mr. Gaston Aguilar, Mr. Stacy J. Jones, and Mr. Ryan J. Russell. We are also grateful to the agencies who have funded our research. These include the Texas Advanced Technology Program, the National Science Foundation, the Department of Energy via subcontract from Sandia National Laboratories, the National Institutes of Health (for a postdoctoral fellowship to M.L.B.), the Robert A. Welch Foundation, and the Office of Naval Research.

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