Micropatterning of biomedical polymer surfaces by novel UV polymerization techniques

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Abstract: The "living" radical polymerization with an iniferter was used to create micropatterned biomedical surfaces. Novel, photosensitive biomedical polymers were created by the incorporation of dithiocarbamate groups from iniferters. A second monomer layer was then irradiated onto the photosensitive polymer substrate created with the iniferter to form a copolymer. Patterns were created on the films by application of modified microfabrication-based photolithographic techniques. The technique was used to create patterns with depths from 5 to 80 μm. In addition, various polymers were incorporated, including polyethylene glycol methacrylates, styrene, and methacrylic acid, to synthesize regions with different physico-chemical properties. Applications include novel surfaces for biosensors and biomaterials for the selective adhesion of cells and proteins. © 2001 John Wiley & Sons, Inc. J Biomed Mater Res 56: 351–360, 2001

Key words: micropatterning; "living" radical polymerization; iniferter; poly(ethylene glycol)

INTRODUCTION

"Living" radical polymerization has been used successfully to create low polydispersity linear polymers from free-radical polymerizations. There are also applications of the "living" radical polymerization in the synthesis of block copolymers.[1–6] Essentially, the technique involves polymerizing a single type of monomer first to create a macromonomer that is capable of acting as an initiator. There is a terminating group at the end of the polymer that may be a thiol group or a halogen and, under the right conditions, will dissociate to form radicals. A second monomer is then added to the system and the polymerization proceeds with the second monomer chemically attached to the polymer of the first monomer.

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In this work, it was desired to create block copolymers using the iniferter radical polymerization technique. The block copolymers would be used in the synthesis of micropatterned polymer films for use in biomaterials and other biomedical applications.

Micropatterning and nanotechnology are becoming increasingly popular for the development of improved biomaterials and devices.[7] Tremendous strides have been made in the micromachining of silicon for numerous applications. Now, nanotechnology and applications of micropatterned surfaces are being considered for other applications (e.g., chemical sensors) for which silicon may not be the first choice because of incompatibility or expense. In particular, numerous researchers are focusing on the development of nanotechnology for biomedical applications.

Some of the biomedical applications include electrochemical sensors used for blood electrolyte and gas analysis or for determination of the glucose concentration of a diabetic patient.[8] Another application is in the development of immunosensors, or bioanalytic sensors to incorporate biological recognition processes such as antigen-antibody, enzyme-substrate, or ligand-receptor to identify and quantify biochemical substances.[9,10] Even though significant advances have been made in this area, several issues must be addressed, including long-term stability of enzymes and

bioreceptors, biocompatibility, nonspecific adsorption of other species, and miniaturization for *in vivo* applications.[8]

Evolving from the development of immunosensors, another application of nanotechnology in the biomedical field is in the area of protein patterning,[11] important for tissue engineering[12,13] and for studies of cell biology.[14–16] Recently, Shi et al.[17,18] described a molecular imprinting technique to create a material surface that is capable of specifically recognizing proteins.

In the development of these intelligent biomaterials and biochips, numerous surface microfabrication techniques have been discovered and developed to create a material for regulating cell functions.[14] Some of the different methods for micropatterning for the localization of biomolecules include formation of self-assembled monolayers[19–21] and photolithography.[22,23] There are several reviews of the different methods for creating the patterns.[11,14,21]

In the self-assembled monolayer technology, microcontact printing is used in conjunction with alkylsilanes or alkanethiol molecules. This technique is fairly easy and can be used for protein immobilization. The disadvantage to this technique is that multiple protein patterning is very difficult.[11] The other technique mentioned, photolithography, uses conventional photoresist technology. Protein patterning is accomplished by using chemical linkers with different pendent groups, typically silane coupling agents. The advantage to this technique and any technique based on photoresist technology is that the technology is well established. In addition, the pendent group can be varied for selective protein adsorption. However, these techniques involve the use of solvents and photoresists, which may denature the proteins.

Patel et al.[24,25] have developed a microfluidic network technique for biomolecular patterning. Although this is an extension of the microcontact printing technique described previously, it can be used for a wider variety of substrates. The technique involved the placement of a poly(dimethyl siloxane) (PDMS) mold in contact with a suitable surface to form capillaries through which the fluids may flow and was very successful for the immobilization of the peptides.

One goal in the development of these micropatterned surfaces is to synthesize regions with different physico-chemical properties. For example, if a material is developed with regions of hydrophobic and hydrophilic surfaces, cell adhesion can be controlled. Various polymeric materials may be ideal for these applications but there exists a need for an easy method to fabricate micropatterned polymer surfaces. In addition, a wide range of thicknesses and dimensions is desired for applications such as microfluidics, "lab-on-a-chip" and controlled drug delivery.

Coupled with the need to synthesize regions of different properties, there is also a need to devise techniques to form high aspect ratio structures for microfluidics and BioMEMS applications. Currently, there are two main ways to form such structures. One method is to etch features in silicon with deep reactive ion etching using high-density inductively coupled plasma sources (also possible at cryogenic temperatures).[26] This is a subtractive process, which despite being expensive, has been used to form various microelectro-mechanical structures such as accelerometers and pressure sensors. Another method of forming the high aspect ratio structures includes the use of newly developed polymer resist, SU-8, which can be used in standard lithographic processes.[27,28] Using optical lithography, the material can form high aspect ratio structures, which can be used as molds for electroplating.

Our work detailed herein describes the development of a micropatterning technique, based on free-radical polymerizations, which can be used to form features of controlled surfaces and also be used to form high aspect ratio structures.

Matsuda and coworkers[23,29–31] have successfully fabricated patterned films using ultraviolet (UV) free-radical polymerizations. They created a photosensitive layer by immobilizing an *N,N*-diethyldithiocarbamyl group on the polymer surface and then patterned the surface with various monomers by irradiating through a projection mask. Some of the materials they have worked with include poly(vinyl alcohol), polyethylene glycol (PEG), polystyrene, polyacrylamide, and poly(acrylic acid). This type of polymerization with the photosensitive dithiocarbamyl group is referred to as a "living" radical polymerization or an iniferter (*ini*tiator-trans*fer* agent-*ter*minator) polymerization. Other research groups have in turn expanded on this method for the development of block copolymers and surface-grafted layers for other applications.[32] The work described herein focuses on the initial development of iniferter polymerizations for micropatterning of polymer surfaces with application to biomaterials. Our technique differs from the technique of Matsuda et al. in that the iniferter is directly used to polymerize the base layer and not just immobilized on the surface. It is believed that this makes the polymerization and micropatterning technique easier and transferable to a variety of monomers.

MATERIALS AND METHODS

The iniferter tetraethylthiuram disulfide (TED), the photoinitiator 2,2-dimethoxy-2-phenyl acetophenone (DMPA), and the thermal initiator 2,2′-azobis-(2-methylpropionitrile) were purchased from Aldrich (Milwaukee, WI). The monomers studied, PEG 200 methacrylate (PEGMA) and PEG 200

dimethacrylate (PEGDMA), were obtained from Polysciences, Inc. (Warrington, PA) and used as received. Styrene was also used as a monomer (Aldrich). Concentrations of the initiators and the iniferter in the monomer solution were 1 wt %.

Micropatterning

Patterns are created on the polymer films using a modification of the method to create block copolymers. Figure 1 displays the steps of the block copolymerization process. Monomer A is polymerized in the presence of the iniferter, resulting in polymer chains capped with thiol groups. It has been shown that upon terminating the UV light source, the sulfur radicals will react with all remaining radicals in the system so that there are no trapped radicals in the system.[33] Polymer A is then irradiated in the presence of the second monomer, B. The thiol-terminated polymer chains break down and the propagating polymer chain will react with monomer B resulting in the polymer A-*co*-B.

The micropatterning process is depicted in Figure 2. First, a thin film of polymer A is synthesized in the presence of the iniferter TED. After polymerization, this film is coated with a layer of monomer B. To create patterns, a mask is used for selectively irradiating the polymer film. Upon the second irradiation of polymer A, monomer B is polymerized onto the film in the pattern of the mask.

For the first polymer layer, a sample of monomer and initiator were mixed and bubbled with nitrogen. The monomer mixture was then pipetted between two glass slides separated with 1 mm Teflon® spacers. The sample was irradiated with UV light (EFOS Acticure spot cure system, Mississauga, Ontario) in a nitrogen atmosphere for 8 min at an intensity of approximately 20 mW/cm^2. The polymer sample was then washed with deionized water for 4 h to remove any unreacted monomer and then dried overnight in a vacuum oven. After the sample had dried, it was covered with the second monomer by spin-coating or by pipetting the solution onto the polymer. A mask was then placed atop the polymer and monomer sample with a slight pressure clamp, ensuring contact to prevent oxygen from inhibiting the reaction. The masks used in these studies were chromium-plated glass slides. Next, the sample was irradiated with UV light through a collimating lens for 30 min. Finally, the exposed sample was washed by ethanol to remove the remaining unreacted monomer.

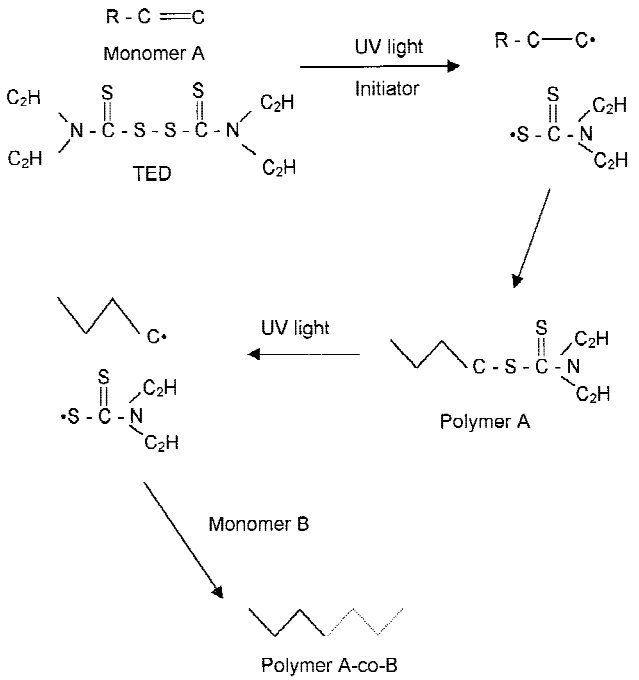


Figure 1. Synthesis of block copolymers in the presence of an iniferter.

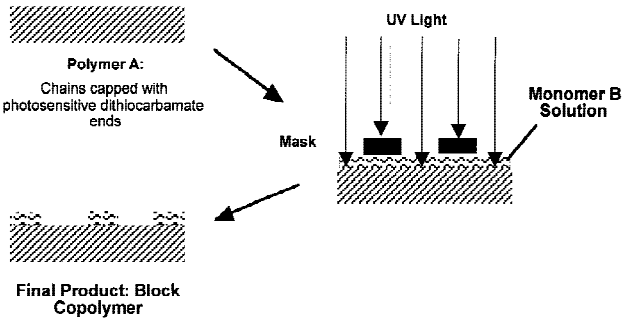


Figure 2. Synthesis of novel patterned films.

One issue in this technique was the exposure to oxygen, which inhibits reaction. It was not possible to perform the entire technique in a nitrogen environment, particularly the spin-coating. Therefore, all monomer solutions were bubbled with nitrogen for 20 min before use. In addition, when the top slide or mask was placed on top of the solution, a clamp was used to apply a small amount of pressure (not enough to displace any monomer) and to develop a seal between the spacers and the glass slides to prevent diffusion of oxygen. This was found to be adequate. In cases in which the monomer solution was not bubbled with nitrogen or the top slide was simply placed atop the material, polymerization did not take place.

The final sample of the patterned polymer was examined several ways. Microscopic pictures were taken at 4×, 10×, and 40× magnification. A profilometer (Alpha-Step 200; Tencor Instruments, San Jose, CA) was also used to determine the height and profile of the pattern. The instrument runs a cantilever arm across the surface of the sample. Finally, a JSM 35 CF scanning electron microscope was used to examine the patterns formed (after a thin layer of gold was evaporated on the sample to prevent charging).

Kinetics of micropattern preparation

Differential photocalorimetry (DPC) (Model DPC930; TA Instruments, New Castle, DE) experiments were also conducted to examine the kinetics of the copolymerization between the two layers. A small sample of the first layer, less than 20 mg, was placed in a small aluminum pan. Next, 2–6 mg of the monomer mixture used for the second layer was

placed on top of the first layer ensuring that the droplet was completely on the substrate layer and not at the bottom of the pan. The pan was covered with a clear disk of polyethylene to prevent evaporation of the monomer. The apparatus was purged with nitrogen to prevent inhibition of the polymerization because of the presence of oxygen. The standard also contained a small amount of the substrate for better accuracy.

In a typical experiment, the monomer mixture in the pan was placed in the DPC, equilibrated at 30°C for 10 min and then irradiated with UV light set at the maximum intensity (approximately 30 mW/cm^2). The heat evolved was measured as a function of time. The theoretical enthalpy of the monomer solution was then used to calculate the rate of polymerization, R_p, in units of fractional double bond conversion per second. Integration of the R_p curve versus time provided the conversion as a function of time. It was assumed that in the copolymerization of two monomers, the functional groups had equal reactivity. In other words, the theoretical enthalpy derived for a comonomer mixture was an average of the enthalpies of the individual monomers. Methacrylate groups have an enthalpy of −13.1 kcal/mol.[34]

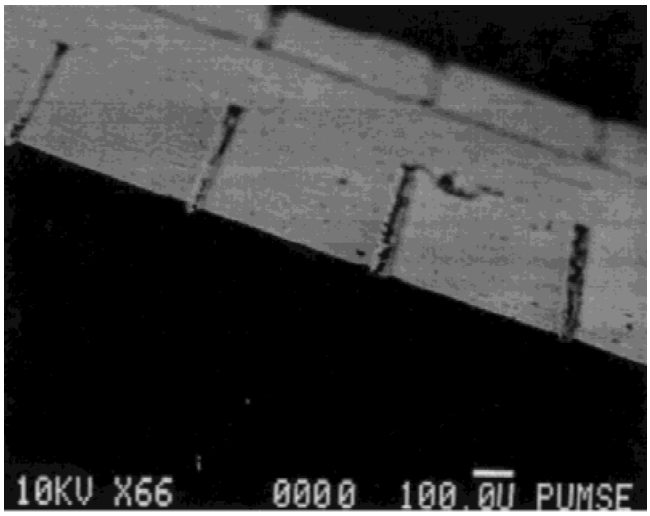
RESULTS

Micropatterning of PEGMA and PEGDMA

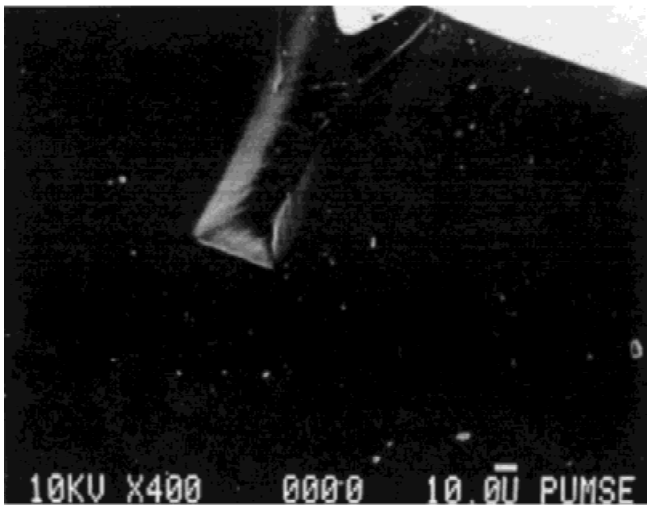
For our initial studies, PEGDMA was used as the bottom layer (referred to as monomer/polymer A). Polymerization of this monomer proceeded rapidly and resulted in a hard, strong, highly crosslinked polymer with a high glass transition temperature (above 70°C). The second layer (referred to as monomer/polymer B) consisted of 50 wt % PEGMA and 50 wt % PEGDMA. Thus, the second layer was not as crosslinked, and a polymer made from this material alone would be more flexible and exhibit a lower glass transition temperature than the substrate layer. If the two layers were polymerized as separate layers, they would also swell to different degrees: the substrate layer will hardly swell whereas the second layer will swell considerably. The objective of this technique is to polymerize materials with different properties and have each layer retain its properties.

In the first set of experiments, monomer B was pipetted onto polymer A and then the mask was clamped onto the sample. This resulted in a reasonably thick (~100 μ) second layer. The mask was a comb-like, light-field pattern with line widths of 57 μ. Microscopic and scanning electron microscope (SEM) images showed the patterns in the polymer (see Fig. 3). Interestingly, very deep patterns were achieved.

In the next set of micropatterning experiments, the same materials were used, but a different mask with a different pattern was implemented. This was a dark-field mask; thus, all of the patterns on the polymer were protruding up instead of being trenches. One purpose of this pattern was to determine the precision that could be attained with this free-radical polymerization synthesis method. Two different methods of fabricating the patterns on the polymers were used with this mask.



(a)



(b)

Figure 3. SEM photographs of patterned polymers. On the top (a), is a tilted view of the top of the polymer. On the bottom (b), is a zoom in of one line in the pattern and the depth is evident.

In this first method, monomer B was again pipetted onto polymer A, resulting in a fairly thick second layer after polymerization. Figure 4 shows a section of the pattern containing crosses of various sizes, from 5 to 20 μm. Profilometry studies were also conducted on this material and indicated that very deep patterns of approximately 60–100 μm were attained with this method of synthesis. The sample was not of com-

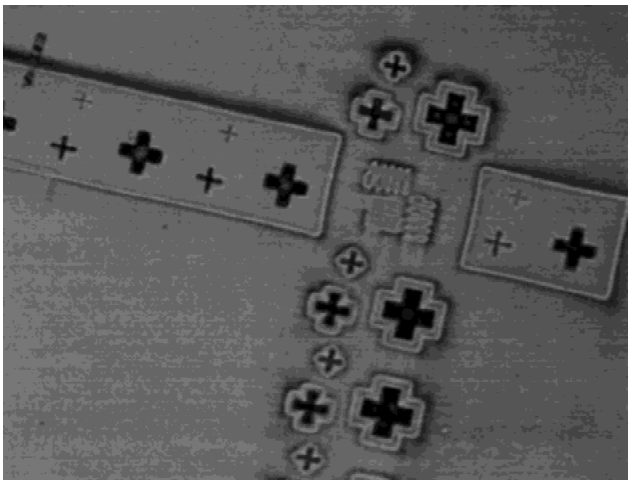


Figure 4. Optical top view micrograph (original magnification ×10) of the features polymerized with a dark-field mask. The top layer was approximately 80 μm. The sides of the cross features are approximately 20 μm.

pletely uniform thickness because the surface of polymer A was not completely flat.

The same technique was also used with a mask containing 10-μm-wide lines that were 120 μm apart. From the SEM photo at 130× magnification of the lines, as shown in Figure 5, it was evident that there was significant depth in the resist. The morphology also indicated a rough surface. There are biomedical applications in which a rough surface is desired, such as tissue engineering. The rough surface can promote cell and protein adhesion.

In the other method, monomer B was spin-coated onto polymer A at 750 rpm for 20 s. The masks used for this study contained much smaller features than the previously mentioned study. The spin-coated samples were thinner and resulted in finer features. Figure 6 demonstrates that patterns with a dimension

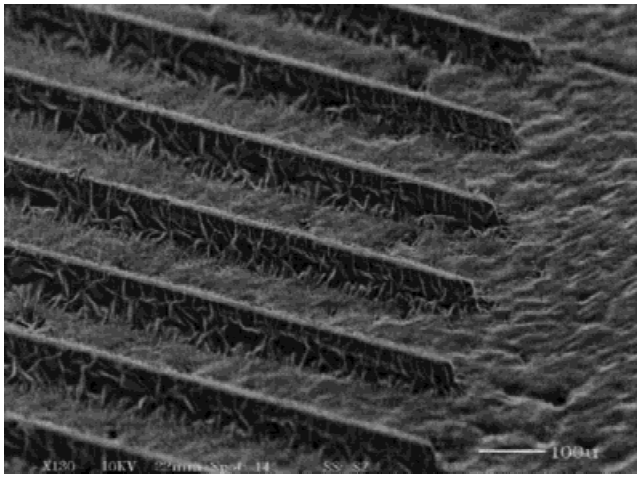
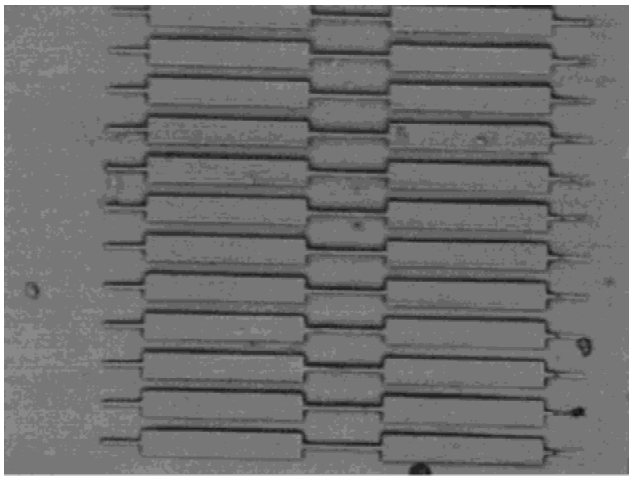
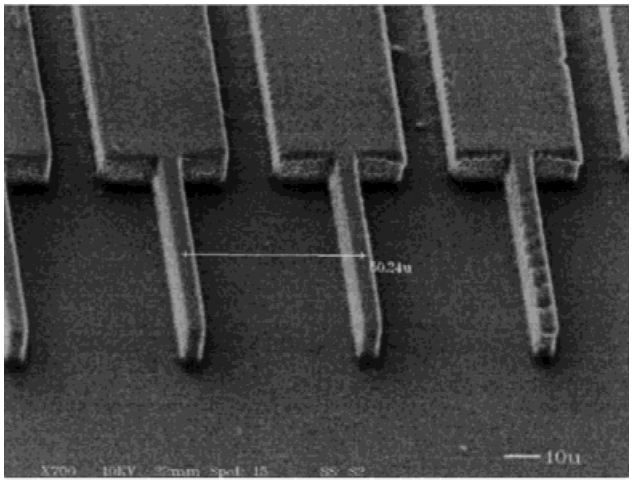


Figure 5. SEM photo at ×130 original magnification of features polymerized with a dark-field mask. The lines in the mask are 10 μm wide and 120 μm apart.



(a)



(b)

Figure 6. Optical top view micrograph (original magnification ×10) (a) and SEM photo at original magnification ×700 (b) of the spin-coated features. These patterns are between 5–10 μm thick. The larger squares are 30 μm wide whereas the smaller lines are 5 μm wide.

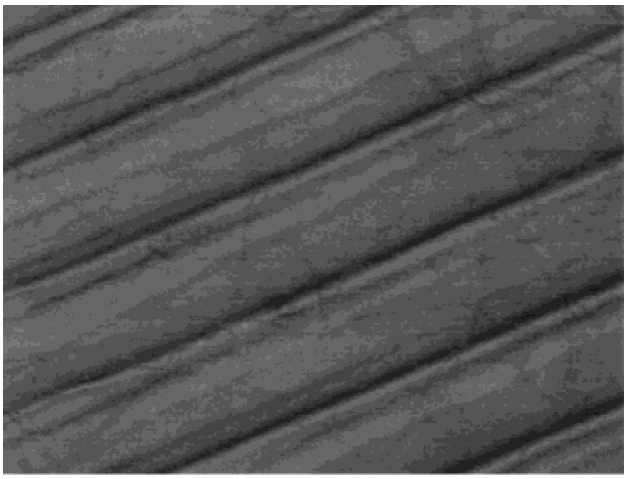
of 5 μm can be attained with the spin-coated films. The SEM photo at 700× magnification shown in Figure 6(b) clearly shows the precision that was attained with this method. Profilometry results across the middle of the pattern shown in Figure 6(a) indicated that the lines were approximately 5 μm high. These spin-coated surfaces were also smoother, though there was some roughness along the sidewalls of the material.

Micropatterning of different materials

It is highly desirable to be able to synthesize patterns of two different polymers for biomedical applications requiring precise control of cellular movement. Therefore, micropatterning with various other poly-

meric materials has also been investigated. We were able to successfully polymerize the 50:50 mixture of PEGDMA and PEGMA onto a layer of polystyrene with 5 wt % divinylbenzene as a crosslinker. The polystyrene layer was thermally polymerized because of degradation that occurred while photopolymerizing a thin layer. The initiators used in this layer were 1 wt % TED and 1 wt % 2,2′-azobis-(2-methylpropionitrile). The monomer mixture was placed between two glass slides and heated in an oven at 70°C for 12 h.

Figure 7(a) displays a sample of polystyrene with poly(PEGMA-*co*-PEGDMA) micropatterned on top. The PEGMA and PEGDMA mixture was spin-coated on top at about 300 rpm and then irradiated for 20 min. The optical photograph in Figure 7(a) was taken after the sample had been immersed in water for several weeks. The fine lines seen in this micrograph are remains of the prolonged immersion and are not believed to be cracks. Even after the immersion, the patterned film is still stable and the pattern is still attached to the substrate surface. A profilometer measurement indicated that the patterns were about 15 μm deep.



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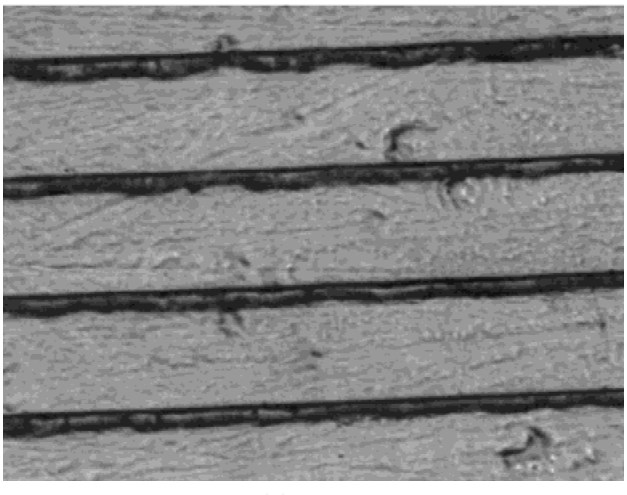


(b)

Figure 7. Optical micrograph at original magnification ×10 of (a) p(PEGMA-*co*-PEGDMA) lines on polystyrene and of (b) p(PEGMA-*co*-MAA) lines on top of p(PEGDMA).

Figure 7(b) shows the optical microscope picture of PEGDMA and methacrylic acid (MAA) polymerized in lines on top of p(PEGDMA). The top layer shown here was pipetted onto the substrate, therefore creating deep patterns. Different depths were encountered in the same sample because the substrate layer was not completely flat. The substrate layer undergoes shrinkage as it polymerizes, which causes the material to curl slightly. To minimize the shrinkage, the substrate layer was kept in a flat position between two glass slides before patterning. The pattern shown in Figure 7(b) is approximately 10 μm deep. Other patterns on the material were much deeper, approximately 50 μm. In the deeper patterns, a waviness, or flexibility in the lines was observed. The polymeric material is flexible and is not rigid enough to hold the shape of the lines with high aspect ratios. To prevent this folding, more crosslinking agent may be added to create a more rigid material.

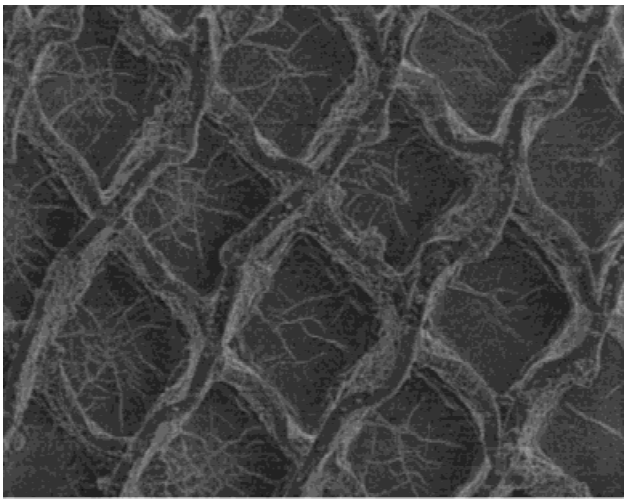
Micropatterning of multiple layers

Finally, the possibility of building a three-dimensional structure has been studied. In these polymerizations, a first micropattern of the p(PEGMA-*co*-PEGDMA) material (50 wt % PEGDMA) was formed. This micropatterned material was then washed with methanol. Another layer of the PEGMA/PEGDMA monomer mixture was pipetted on top of the patterned surface. The pattern was placed on top of the surface rotated at 90° from the previous position. The sample was irradiated with UV light again. This resulted in a pattern on top of a pattern.

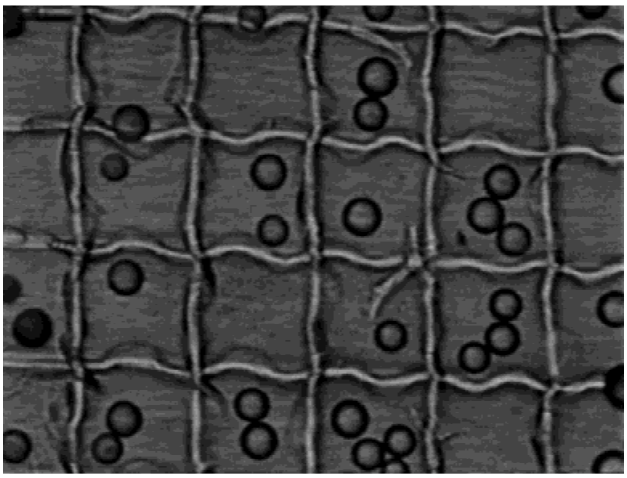
Figure 8(a) shows the grid made by patterning lines at approximately 90° to each other. The crossover of the second pattern over the first pattern is evident in the SEM picture. The lines here are very deep, approximately 50 μm. The high aspect ratio (10 μm wide and 100 μm deep) causes the patterns to become wavy because the polymeric material is flexible. Figure 8(b) shows the same material with latex beads of 40-μm diameters. The beads positioned themselves between the walls of the pattern, demonstrating the depth of the pattern.

DISCUSSION

The main purpose of these experiments was to determine whether the proposed method could be used



(a)



(b)

Figure 8. Polymer sample with multiple patterns at original magnification ×200 (a) and in the presence of 40-μm diameter latex beads at original magnification ×10 (b).

to create micropatterned polymers and to determine the applications and limitations of the technique. The process could be compared with the development of a negative resist type process because the exposed regions of the monomer B remain on the wafer after the polymerization and rinse. In addition, the bottom layer is actually used as a substrate and could potentially result in low-cost microdevices.

The iniferters used in this study were originally introduced by Otsu et al.[35,36] for the purpose of simulating a living radical polymerization and creating block copolymers and more monodisperse polymers. The presence of the iniferter would result in a reversible termination reaction thus allowing for a more controlled polymerization reaction. This reversible termination could also be used for creating block copolymers by polymerizing the different monomers sequentially.

In this work, we used a combination of the iniferter TED and a conventional, photosensitive initiator DMPA. When the DMPA was irradiated with UV light, carbon radicals were produced. TED irradiation resulted in small, mobile sulfur radicals. The carbon radicals initiated the propagation with the functional group of the monomer. It was assumed that on the time scale of the reaction, the sulfur radical would not react with the functional group of the monomer. However, the sulfur radial will react with other radicals in the system, terminating the propagating polymer chain. Upon further irradiation, this termination step will reverse, thus reintroducing a propagating polymer chain. A considerable amount of research has focused on the kinetics and the role of iniferters in free-radical polymerization as compared with conventional polymerizations.[33,37,38]

The reversible termination in the kinetics, introduced by the presence of the iniferter, was used to create block copolymers and the micropatterned surface. Essentially, a photosensitive substrate layer was created by polymerizing in the presence of the iniferter. A second layer could then be polymerized onto the substrate layer upon irradiation. Adapting photolithography techniques and using a chromium-plated mask, micropatterns were created on the substrate surface.

This technique proved valid for synthesizing patterns with high aspect ratios. With the light field mask, very deep trenches were formed. However, there was some warping of the patterns. This was most likely caused by the change in the density going from a monomer to a polymer and the use of a material with less crosslinking agent for the second layer. The second layer may not have been rigid enough for such a high aspect ratio.

The high aspect ratios were attained when the second monomer was simply pipetted onto the substrate surface. If the second monomer was spin-coated onto the substrate layer, much thinner patterns resulted. Better precision was also attained as well as smoother surfaces. Generally, spin-coating could be completed on the substrate surface. However, at times, spin-coating was difficult because of the low viscosity of monomer B and high surface tension. Methods have been developed to curtail these problems, including adding high molecular weight PEG to monomer B or cooling monomer B to make it more viscous.

One concern with this technique was whether or not the two layers were indeed chemically bonded. To examine this, samples were created with the patterned layers and then submerged in deionized water for several days and even weeks in some cases. The two layers had different swelling ratios because of the different amounts of the crosslinking agent PEGDMA. The top layer, with only 50 wt % PEGDMA, swelled more in water than the bottom layer. The two layers swelled

to different extents, as evident by the curling observed of the second layer swelling more than the bottom layer and forcing the bottom layer to bend. The two layers did not separate. If the two layers were not bonded, it is believed that the force of the top layer created because of swelling would lead to detachment of this layer. However, this was not observed, most likely because of the presence of chemical bonds and because of the entanglements of the PEG chains between the two layers.

In another method to verify a copolymerization between the two layers, DPC experiments were conducted. A small piece of a layer of poly(PEGDMA) that was polymerized with 1 wt % DMPA and 1wt % TED was placed in the sample pan. Next, a mixture of the top layer monomer, 50 wt % PEG200MA and 50 wt % PEG200DMA, was placed on top of the polymer. This sample was then irradiated with a high-intensity UV light (approximately 30 mW/cm^2). Figure 9(a) displays the conversion profile of the monomer. Also included in Figure 9(a) is a control experiment. In the control experiment, just the monomer mixture was placed in the sample pan. Even though there was no initiator in this monomer mixture, polymerization still occurred because the UV intensity was so high. Radicals were generated because the UV light was able to break down the bonds.

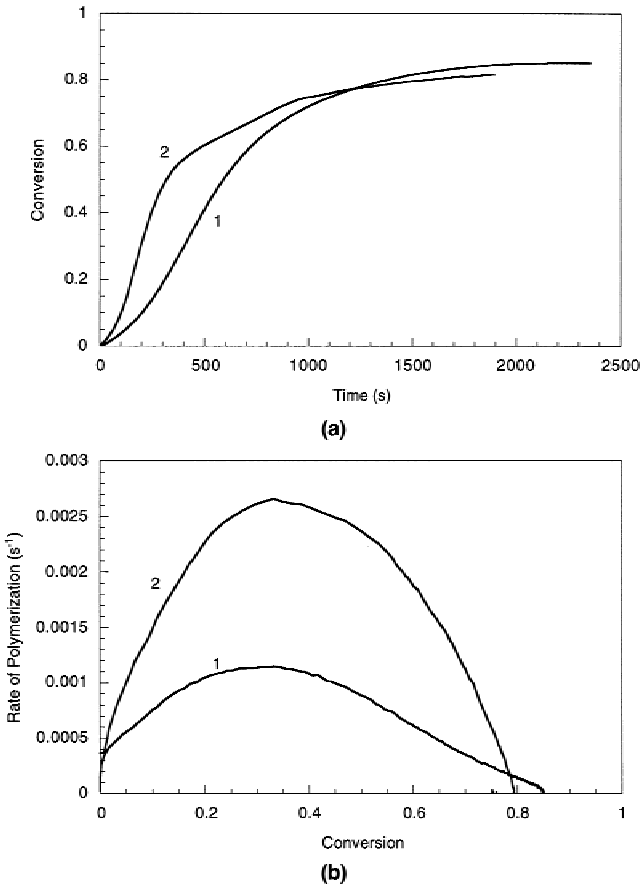


Figure 9. Conversion profile (a) and rate of polymerization (b) for the polymerization of 50 wt % PEG200MA and 50 wt % PEG200DMA on a sample of p(PEG200DMA) with 1 wt % TED (1) and without a polymer present (2).

Figure 9(a) shows a faster polymerization for the monomer mixture alone than for the monomer mixture on top of the polymer sample. The rates of polymerization for the two reactions are shown in Figure 9(b). From this figure, it is evident that the rate of polymerization on top of the polymer sample was significantly slower. These results indicate that indeed there was a reaction between the polymer sample and the monomer mixture. The presence of an iniferter significantly slows down the reaction because of the reversible termination reaction.[38] Therefore, it is concluded that the sulfur radicals had diffused from the polymer to the monomer mixture during the photopolymerization. As the sulfur radical diffused, carbon radicals were produced on the polymer sample and the functional groups of the monomer mixture were able to react onto the surface to form a chemical bond between the two layers.

The advantages of this novel technique for fabricating micropatterned biomedical polymer surfaces include the ability to make patterns on polymer films of any type of monomer that can be polymerized by UV free-radical polymerization. For example, in a biomaterials application, the film may be a polymer to which cells adhere. Another material to which cells do not adhere can then be patterned onto the original film. Thus, cells only adhere to the exposed areas. Patterns can be designed to force the cells to adhere in certain regions for applications needing precise control of the cell movement, such as a separation process.

Exploring these types of polymerizations leads to the development of micropatterned hydrogels, which are water insoluble, environmentally sensitive networks.[39,40] Patterns of these materials would be able to swell or collapse in response to a change in the environmental pH, temperature, ionic strength, or electromagnetic radiation. Possible applications of this type of material are for the sustained release of bioactive agents and for biosensors. Beebe and coworkers[41] were able to micropattern hydrogel posts of acrylic acid and 2-hydroxyethyl methacrylate in a channel and use the hydrogels to control fluid flow in a microfluidic device. As the pH of the surrounding environment was changed, the posts either swelled or contracted. In the swollen state, flow was restricted, whereas in the collapsed state, the fluid could flow around the posts.

CONCLUSIONS

Micropatterned polymer surfaces were synthesized by free-radical polymerization techniques. A mono-

mer mixture of 50 wt % PEG200DMA and 50 wt % PEG200MA was successfully polymerized in various patterns onto a surface of highly crosslinked PEG200DMA. Various patterns were examined to determine limits of the technique. Interestingly, very deep patterns of 60–100 μm were synthesized for high aspect ratios. Finer patterned features were achieved with patterns of only 5 μm deep. Different materials, such as styrene and MAA, could also be incorporated into the patterns.

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