

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Advanced Drug Delivery Reviews xx (2004) xxx–xxx

 Advanced
 DRUG DELIVERY
 Reviews

www.elsevier.com/locate/addr

Biomimetic materials and micropatterned structures using iniferters

Nicholas A. Peppas^{a,b,*}, Jennifer H. Ward^{a,b}

^aDepartment of Chemical Engineering, Division of Pharmaceutics, The University of Texas at Austin, 1 University Station, C0400, CPE 3.466, Austin, TX 78712-0231, USA

^bDepartment of Biomedical Engineering, Division of Pharmaceutics, The University of Texas at Austin, 1 University Station, C0400, CPE 3.466, Austin, TX 78712-0231, USA

Received 20 August 2003; accepted 10 October 2003

Abstract

In the preparation of biomimetic materials it is often required that efficient methods of polymerization be used, often methods that can lead to biomimetic polymers with relatively narrow molecular weight distribution. Living radical polymerization techniques have successfully been used to create low polydispersity linear polymers by free-radical polymerizations. Although this technique slows down the polymerization of multifunctional monomers, there is little effect on the network structure due to the high concentration of pendent double bonds. There are applications of the living radical polymerization in the synthesis of block copolymers. Essentially, the technique involves polymerizing a single type of monomer first to create a macromonomer that is capable of acting as an initiator because of the reversible bond between the polymer end group and the terminating group. This terminating group may be a thiol 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. We review methods of creating biomimetic 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.

© 2004 Published by Elsevier B.V.

Keywords: Biomimetic materials; Synthesis; Polymerization; Fractional groups

Contents

1. Introduction	0
2. Micropatterning by polymerization methods with iniferters	0
3. Patterned films	0

1. Introduction

Micropatterning and nanotechnology are becoming increasingly popular for the development of

* Corresponding author. Tel.: +1-512-471-6644; fax: +1-512-471-8227.

E-mail address: peppas@che.utexas.edu (N.A. Peppas).

38 improved biomaterials and drug delivery devices
39 [1–7]. Tremendous strides have been made in the
40 micromachining of silicon for numerous biomedical
41 and pharmaceutical applications. Nanotechnology
42 and applications of micropatterned surfaces are
43 being considered for other applications (e.g. chemi-
44 cal sensors) for which silicon may not be the first
45 choice due to incompatibility or expense. In particu-
46 lar, numerous researchers focus on the develop-
47 ment of nanotechnological methods for biomedical
48 applications.

49 For example, there is a significant need for elec-
50 trochemical sensors to be used in *in vitro* and *in vivo*
51 sensing with subsequent therapy. An electrochemical
52 sensor may be used for blood electrolyte and gas
53 analysis or for determination of the glucose concen-
54 tration of a diabetic patient [8] leading to drug release.
55 In these applications, the sensor ideally would be
56 portable, inexpensive, and disposable.

57 The development of immunosensors, or bioanalytic
58 sensors, is also of extreme interest. These sensors
59 incorporate biological recognition processes such as
60 antigen–antibody, enzyme–substrate or ligand–re-
61 ceptor to identify and quantify biochemical substances
62 [9,10]. In nature, cells have the ability to monitor
63 biochemical events.

64 Ideally, researchers would like to develop man-
65 made sensors to mimic cells. Significant advances
66 have been made in this area. However, there are still
67 several issues to be addressed [8]. These include long-
68 term stability of enzymes and bioreceptors, biocom-
69 patibility, nonspecific adsorption of other species and
70 miniaturization for *in vivo* applications.

71 Evolving from the development of immunosen-
72 sors, other applications of nanotechnology in the
73 biomedical field are in the area of protein patterning
74 [11]. For these applications, the goal of micro- and
75 nanopatterned biomaterials is to organize multiple
76 biomolecules on surfaces. This is important for
77 biosensor technology, tissue engineering [12,13]
78 and studies of cell biology [14–16]. In tissue engi-
79 neering, it is essential to organize the cells so that
80 they may create the desired tissue. It is generally
81 assumed that cellular interactions with the artificial
82 material occur through adsorbed proteins. Therefore,
83 much research focuses on the cell–protein–surface
84 interaction [17]. Recently, Shi et al. [18,19] de-
85 scribed a molecular imprinting technique to create

a material surface that is capable of specifically
recognizing proteins.

Research on intelligent biomaterials and biochips,
has made numerous surface microfabrication techni-
ques possible in order to create a material for regu-
lating cell functions [14]. Some of the different
methods for micropatterning for the localization of
biomolecules include formation of self-assembled
monolayers [20–22] and photolithography [23,24].
There are several reviews of the different methods
for creating the patterns [11,14,22].

Self-assembled monolayer (SAM) technology
calls for use of microcontact printing in conjunction
with alkylsilanes or alkanethiol molecules. In one
technique, a stamp of poly(dimethyl siloxane)
(PDMS) is created by conventional photolithography
techniques. It is then inked with an alkanethiol and
placed on a gold surface. The pattern is transferred
in the regions of direct contact due to the formation
of a SAM of the alkanethiol on the gold. The stamp
is then removed and the remaining bare gold
substrate is exposed to a second alkanethiol to
generate a surface pattern with different terminal
groups. 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].

Another technique, photolithography, utilizes con-
ventional 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. [25,26] developed a microfluidic
network technique for biomolecular patterning. Al-
though 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 PDMS mold in contact
with a suitable surface to form capillaries through
which the fluids may flow. They first created a
surface of PLA-PEG-biotin. Next, they placed the
mold over the material to form capillaries. Avidin
was then flowed through the capillaries to create a

86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133

134 patterned avidin surface. Biotinylated peptides were
135 then immobilized on the patterned surface. The
136 technique was very successful for the immobilization
137 of the peptides.

138 These micropatterned surfaces exhibit different
139 physicochemical properties. For example, if a material
140 is developed with regions of hydrophobic and hydro-
141 philic surfaces, cell adhesion can be controlled. Var-
142 ious polymeric materials may be ideal for these
143 applications, but there exists a need for an easy
144 method to fabricate micro-patterned polymer surfaces.
145 In addition, a wide range of thicknesses and dimen-
146 sions is desired for applications such as micro-fluid-
147 ics, ‘lab-on-a-chip’ and controlled drug delivery.

148 In addition to the need to synthesize regions of
149 different properties, there is also a need to devise
150 techniques to form high aspect ratio structures for
151 micro-fluidics and BioMEMS applications. Matsuda
152 and coworkers [24,27–29] have successfully fabricat-
153 ed patterned films using UV free-radical polymer-
154 izations. They created a photosensitive layer by
155 immobilizing a N,N-diethyldithiocarbamyl group on
156 the polymer surface and then patterned the surface
157 with various monomers by irradiating through a
158 projection mask. Some of the materials they have
159 worked with include poly(vinyl alcohol), PEG, poly-
160 styrene, polyacrylamide and poly(acrylic acid). This
161 type of polymerization with the photosensitive dithio-
162 carbamyl group is referred to as a living radical
163 polymerization, or an iniferter (initiator-transfer
164 agent-terminator) polymerization.

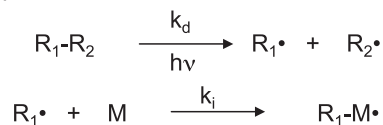
165 Other research groups have, in turn, expanded
166 upon this method for the development of block
167 copolymers and surface-grafted layers for other appli-
168 cations [30]. Peppas and collaborators [31–33] de-
169 scribed the development of a micropatterning
170 technique, based on free-radical polymerizations,
171 which can be used to form features of controlled
172 surfaces and also be used to form high aspect ratio
173 structures. This work focused on the initial develop-
174 ment of iniferter polymerizations for micropatterning
175 of polymer surfaces with application to biomaterials.
176 Our techniques differ from the technique of Matsuda
177 in that the iniferter is directly used to polymerize the
178 base layer instead of just immobilized on the surface.
179 It is believed that this makes the polymerization and
180 micropatterning technique easier and transferable to a
181 variety of monomers.

2. Micropatterning by polymerization methods with iniferters

182
183
184 Patterning is a method that is now considered in
185 the pharmaceutical field to develop new types of
186 microchips for controlled release [33]. In other
187 papers of this issue of this journal, such applications
188 are described. Iniferters (initiator-transfer agent-ter-
189 minator) were originally introduced by Otsu [34,35]
190 for the purpose of simulating a living radical poly-
191 merization and creating block copolymers and more
192 monodisperse polymers. The presence of an iniferter
193 results in a reversible termination reaction, thus
194 allowing for more control of the polymerization.
195 This reversible termination could also be used for
196 creating block copolymers by polymerizing different
197 monomers sequentially.

198 Fig. 1 displays the kinetics of a model reaction in
199 the presence of the iniferter. Ward et al. [31–33] used
200 a combination of the iniferter, tetraethylthiuram disul-
201 fide (TED, chemical structure in Fig. 2) and a con-
202 ventional, photosensitive initiator, 2,2-dimethoxy-2-
203 phenyl acetophenone (DMPA). When the DMPA is
204 irradiated with UV light, carbon radicals are pro-
205 duced. TED irradiation resulted in small, mobile
206 sulfur radicals. The carbon radicals initiated the prop-
207 agation with the functional group of the monomer. It
208 was assumed that, on the time scale of the reaction,
209 the sulfur radical would not react with the functional
210 group of the monomer. However, the sulfur radical
211 would react with other radicals in the system, termi-
212 nating the propagating polymer chain. Upon further

Initiation:



Propagation:



Termination:

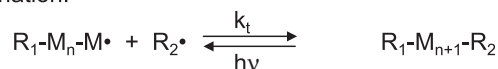


Fig. 1. General mechanism for iniferter kinetics proposed by Otsu and Yoshida.

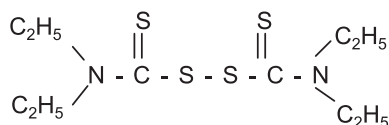


Fig. 2. Chemical structure of TED.

213 irradiation, this termination step reversed, thus rein-
 214 troducing a propagating polymer chain (as shown in
 215 Fig. 1). A considerable amount of research has fo-
 216 cused on the kinetics and the role of iniferters in free-
 217 radical polymerization as compared to conventional
 218 polymerizations [36–38].

219 This reversible termination in the kinetics intro-
 220 duced by the presence of the iniferter can be used to
 221 form block copolymers. Fig. 3 displays the steps of
 222 the block copolymerization. Monomer A is polymer-
 223 ized in the presence of the iniferter, resulting in
 224 polymer chains capped with thiol groups. It has been
 225 shown that upon terminating the UV light source, the
 226 sulfur radicals will react with all remaining radicals in
 227 the system so that there are no trapped radicals in the
 228 system [38]. Polymer A is then irradiated in the
 229 presence of the second monomer, B. The thiol-termi-
 230 nated polymer chains break down and the propagating
 231 polymer chain will react with monomer B resulting in
 232 the polymer A-co-B.

233 3. Patterned films

234 Patterns can be created on the polymer films using
 235 the previously described method of synthesizing block
 236 copolymers. The process is depicted in Fig. 4. First, a
 237 thin film of polymer A is synthesized in the presence
 238 of the iniferter TED. After polymerization, this film is
 239 coated with a layer of monomer B. In order to create
 240 patterns, a mask is used for selectively irradiating the
 241 polymer film. Upon the second irradiation of polymer
 242 A, monomer B is polymerized onto the film in the
 243 pattern of the mask.

244 The advantages of this technique include the ability
 245 to make patterns on polymer films of any type of
 246 monomer that can be polymerized by UV free-radical
 247 polymerization. For example, in a biomaterials appli-
 248 cation, the film may be a polymer to which cells
 249 adhere. Another material to which cells do not adhere
 250 can then be patterned onto the original film. Thus,

251 cells only adhere to the exposed areas. Patterns can be
 252 designed to force the cells to adhere in certain regions
 253 for applications needing precise control of the cell
 254 movement, such as a separation process.

255 In our work, we developed techniques for making
 256 patterned surfaces using UV free-radical polymeriza-
 257 tion. The monomers studied were PEG 200 methacry-
 258 late (PEGMA), PEG 200 dimethacrylate
 259 (PEGDMA) and styrene. The iniferter TED, the
 260 photoinitiator DMPA and the thermal initiator 2,2'-
 261 azobis-(2-methylpropionitrile), AIBN, were pur-
 262 chased from Aldrich (Milwaukee, WI).

263 For the first polymer layer, a sample of monomer
 264 and initiator were mixed and bubbled with nitrogen.
 265 The monomer mixture was then pipetted between two
 266 glass slides separated with 1 mm Teflon® spacers.
 267 The sample was irradiated with UV light in a nitrogen
 268 atmosphere for 8 min at an intensity of approximately
 269 20 mW/cm². The polymer sample was then washed
 270 with deionized water for 4 h to remove any unreacted
 271 monomer and then dried overnight in a vacuum oven.
 272 After the sample had dried, it was covered with the
 273 second monomer by spin coating or by pipetting the
 274 solution onto the polymer. A mask was then placed
 275 atop the polymer and monomer sample, ensuring
 276 contact in order to prevent oxygen from inhibiting
 277 the reaction. Next, the sample was irradiated with UV
 278 light through a collimating lens for 30 min. Finally,
 279 the exposed sample was washed by ethanol to remove
 280 the remaining unreacted monomer.

281 The final sample of the patterned polymer was
 282 examined several ways. Microscopic pictures were

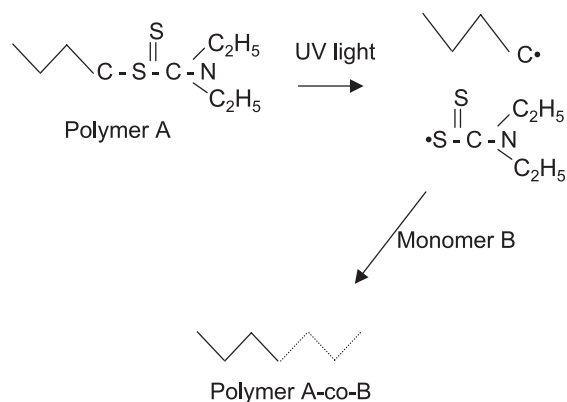


Fig. 3. Synthesis of block copolymers in the presence of an iniferter.

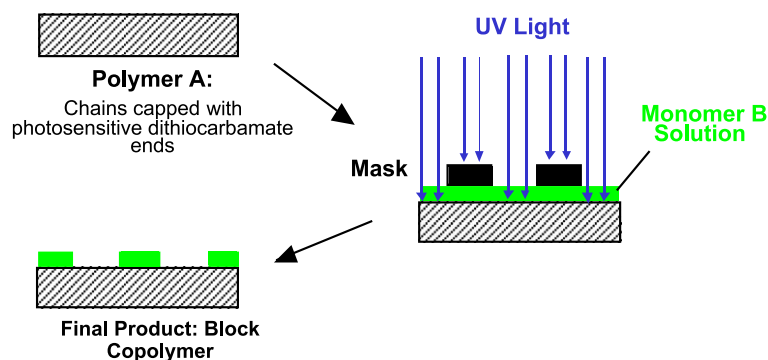


Fig. 4. Synthesis of novel patterned films.

283 taken at $4\times$, $10\times$ and $40\times$ magnification. A
 284 profilometer was also used to determine the height
 285 and profile of the pattern. The instrument runs a
 286 cantilever arm across the surface of the sample.
 287 Finally, a JSM 35 CF scanning electron microscope
 288 was used to examine the patterns formed.

289 Differential photocalorimetry (DPC) experiments
 290 were also conducted to examine the kinetics of the
 291 copolymerization between the two layers.

292 In a typical experiment, the monomer mixture in
 293 the pan was equilibrated at $30\text{ }^{\circ}\text{C}$ for 10 min and then
 294 irradiated with UV light set at the maximum intensity
 295 (approximately 30 mW/cm^2). The heat evolved was
 296 measured as a function of time. The theoretical
 297 enthalpy of the monomer solution was then used to
 298 calculate the rate of polymerization, R_p , in units of
 299 fractional double bond conversion per second. Inte-
 300 gration of the R_p curve versus time provided the
 301 conversion as a function of time. It was assumed that
 302 in the copolymerization of two monomers, the func-
 303 tional groups had equal reactivity. In other words, the
 304 theoretical enthalpy derived for a comonomer mixture
 305 was an average of the enthalpies of the individual
 306 monomers. Methacrylate groups have an enthalpy of
 307 -13.1 kcal/mol [39].

308 4. Micropatterning for biomimesis: peg-based 309 systems

310 We wanted to determine if our micropatterning
 311 method could be used to create micropatterned poly-
 312 mers and to determine the applications and limitations
 313 of the technique. The process could be compared to

the development of a negative resist type process
 since the exposed regions of the monomer B remain
 on the wafer after the polymerization and rinse. In
 addition, the bottom layer (monomer/polymer A) is
 actually used as a substrate and could potentially
 result in low cost micro-devices.

Ward et al. [31] worked with PEGDMA 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 $70\text{ }^{\circ}\text{C}$). Kinetic studies conducted in our labs
 have shown that the polymerization of PEGDMA in
 the presence of the iniferter proceeded more slowly
 than in the presence of DMPA alone. The prominent
 autoacceleration effect typically observed in the po-
 lymerization of multifunctional monomers was not
 observed in the presence of the iniferter. This indicat-
 ed that the thiol radicals were terminating the propa-
 gating chains and thus slowing down the reaction.

The second layer (monomer/polymer B) consisted
 of 50 wt.% PEGMA and 50 wt.% PEGDMA (see also
 Fig. 5). Thus, the second layer was not as cross-
 linked, was more flexible, and had a lower glass
 transition temperature. The two layers also swelled
 to different degrees. The masks used in these studies
 were chromium plated glass slides. SEM images
 showed the patterns in the polymer. Interestingly, very
 deep patterns were achieved. However, there was
 some warping of the patterns. This was caused by
 the diffusion of monomers during the reaction and by
 the change in the density going from a monomer to a
 polymer. Profilometry studies were also conducted on
 this sample. The tip of the profilometer was not able

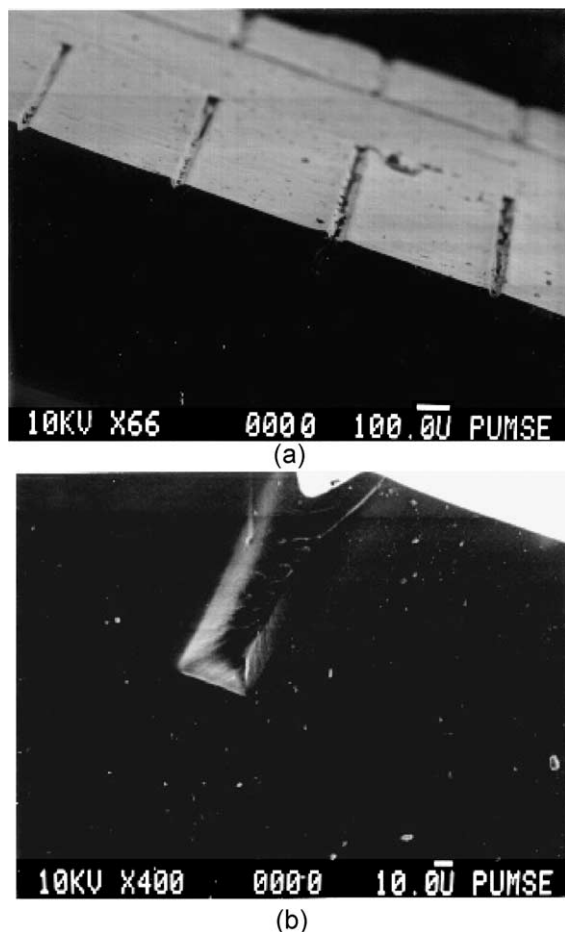


Fig. 5. SEM photographs of patterned polymers. On the top (a) is a tilted view of the top of the polymer. On the bottom (b) is a zoom in of one line in the pattern and the depth is evident.

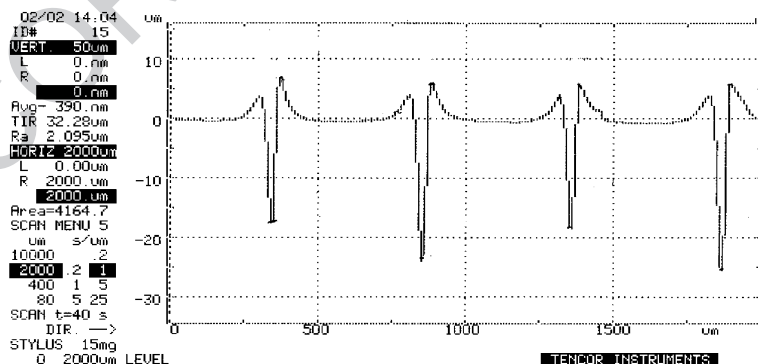


Fig. 6. Profilometer results of the same material as in Fig. 5. The hills before every valley represent a possible buildup of polymer. This is most likely due to diffusion of the monomer into the polymer and subsequent reaction.

to penetrate the trenches in the pattern fully. The profile obtained from the profilometer scan did show slight hills and valleys around the pattern (see Fig. 6), indicating a slight buildup of polymer, evidence of the diffusion of monomer.

One concern with this technique was whether or not the two layers were indeed chemically bonded. To examine this, samples were formed with the patterned layers and then submerged in deionized water for several days. The two layers had different swelling ratios because of the different amounts of crosslinking agent, PEGDMA. The top layer, with only 50 wt.% PEGDMA, swelled more in water than the bottom layer. Even though the two layers swelled to different ratios, the two layers remained bonded to each other throughout the swelling process. If the two layers were not bonded, it is believed that the force of the top layer created due to swelling would lead to detachment of this layer. However, this was not observed, most likely due to the presence of chemical bonds and due to the entanglements of the PEG chains between the two layers.

In another method to verify copolymerization between the two layers, DPC experiments were conducted. A small piece of a layer of PPEGDMA 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/cm²). Fig. 7 displays the conversion profile of the monomer.

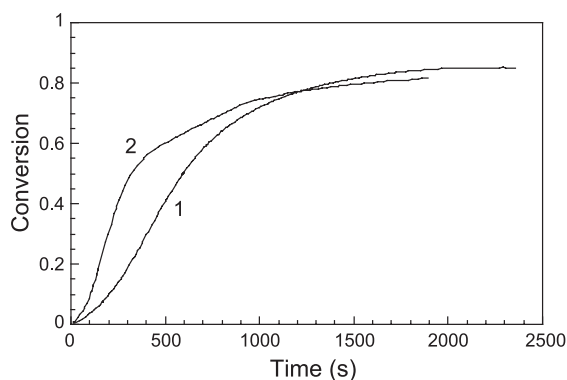


Fig. 7. Conversion profile for the polymerization of 50 wt.% PEG200MA and 50 wt.% PEG200DMA on sample of PPEG200DMA with 1 wt.% TED (1) and without a polymer present (2).

380 Also included in this figure is a control experiment. In
 381 the control experiment, just the monomer mixture was
 382 placed in the sample pan. Even though there was no
 383 initiator in this monomer mixture, polymerization still
 384 occurred because the UV intensity was so high.
 385 Radicals were generated because the UV light was
 386 able to break down the bonds.

387 Fig. 7 shows a faster polymerization for the mono-
 388 mer mixture alone than for the monomer mixture on
 389 top of the polymer sample. The rates of polymeriza-
 390 tion for the two reactions are shown in Fig. 8. From
 391 this figure, it is evident that the rate of polymeriza-
 392 tion on top of the polymer sample was significantly
 393 slower. These results indicate that indeed there was

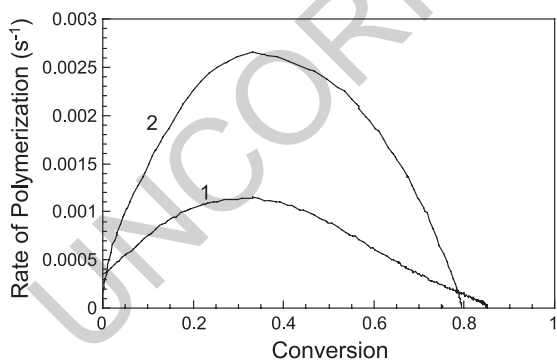


Fig. 8. Rate of polymerization as a function of conversion for the polymerization of 50 wt.% PEG200MA and 50 wt.% PEG200DMA on a sample of PPEG200DMA with 1 wt.% TED (1) without a polymer present (2).

a reaction between the polymer sample and the 394
 monomer mixture. The presence of an iniferter sig- 395
 nificantly slows down the reaction because of the 396
 reversible termination reaction [37]. Therefore, it is 397
 concluded that the sulfur radicals had diffused from 398
 the polymer to the monomer mixture during the 399
 photopolymerization. As the sulfur radical diffused, 400
 carbon radicals were produced on the polymer sample 401
 and the functional groups of the monomer mixture 402
 were able to react onto the surface to form a chemical 403
 bond between the two layers. 404

In the next set of micropatterning experiments, the 405
 same materials were used only a different mask with a 406
 different pattern was implemented. This was a dark- 407
 field mask, thus all of the patterns on the polymer 408
 were protruding up instead of being trenches. One 409
 purpose of this pattern was to determine the precision 410
 that could be attained with this free-radical polymer- 411
 ization synthesis method. Two different methods of 412
 fabricating the patterns on the polymers were used 413
 with this mask. 414

In this first method, monomer B was again 415
 pipetted onto polymer A, resulting in a fairly thick 416
 second layer after polymerization. Fig. 9 shows a 417
 section of the pattern containing crosses of various 418
 sizes, from 5 to 20 μm . Profilometry studies were 419
 also conducted on this material. Fig. 10 shows the 420
 results for scanning across a wide square in the 421
 pattern. The results indicated that very deep patterns 422

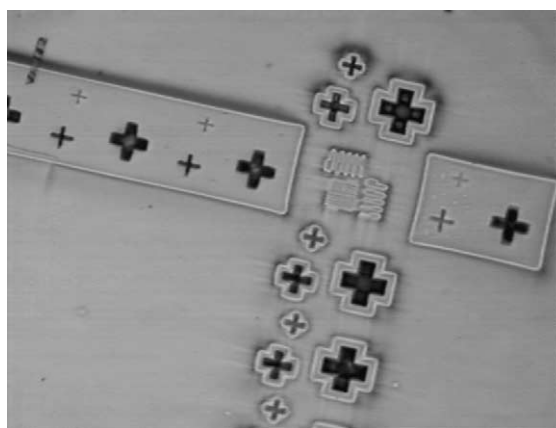


Fig. 9. 10 \times optical top view micrograph of the features polymerized with a dark-field mask. The monomer/polymer B thickness was approximately 80 μm . The sides of the cross features are approximately 20 μm .

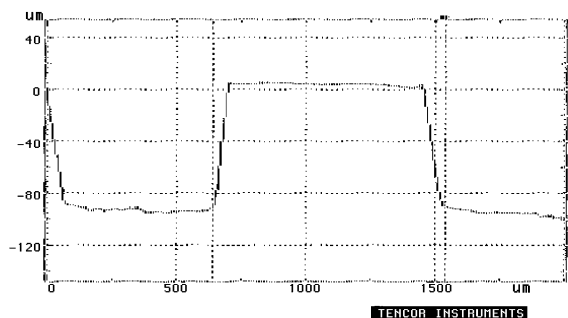


Fig. 10. Profile of a section of the pattern with a wide square. The depth of this square is approximately 90 μm .

of approximately 60–100 μm were attained with this method of synthesis.

Patterns with much smaller features were also produced. Monomer B was spin-coated onto polymer A at 1750 rpm for 20 s. Here spin-coated samples were thinner and resulted in finer features. Fig. 11 demonstrates that patterns with a dimension of 5 μm can be attained with the spin-coated films. These lines were approximately 5 microns high and were narrower at the top than at the bottom, providing more evidence of the diffusion of monomers during the polymerization process. An SEM photo at 700 \times magnification of this surface (Fig. 12) clearly showed the precision which was attained with this method. These spin-coated surfaces were also smoother,

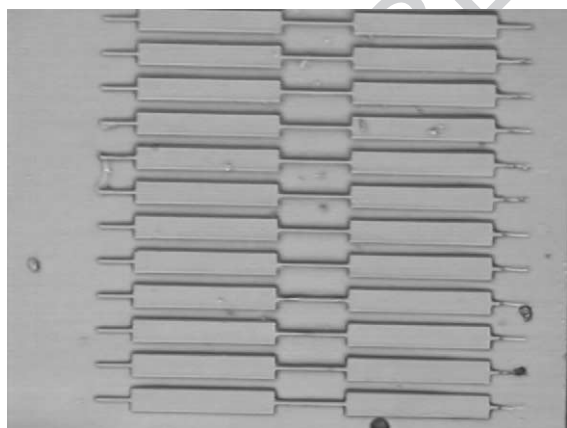


Fig. 11. 10 \times optical top view micrograph of the spin coated features. These patterns are between 5 and 10 μm thick. The larger squares are approximately 30 μm wide while the smaller lines are about 5 μm wide.

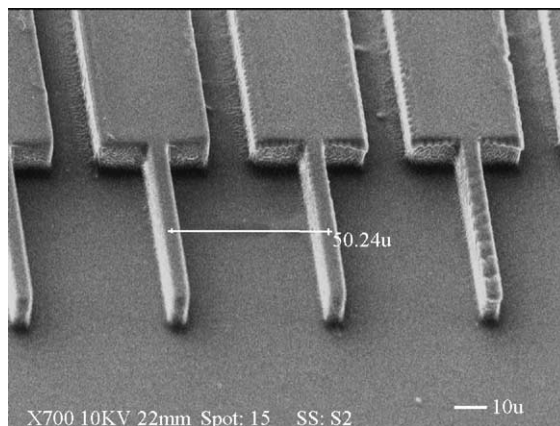


Fig. 12. SEM photo at 700 \times of the spin coated features shown in Fig. 11.

though there was some roughness along the sidewalls of the material.

5. Micropatterning of multiple biomimetic polymers

Patterns of two different polymers for biomedical applications require precise control of cellular movement. Therefore, micropatterning with various other polymeric materials has also been investigated. We were able to successfully polymerize the 50/

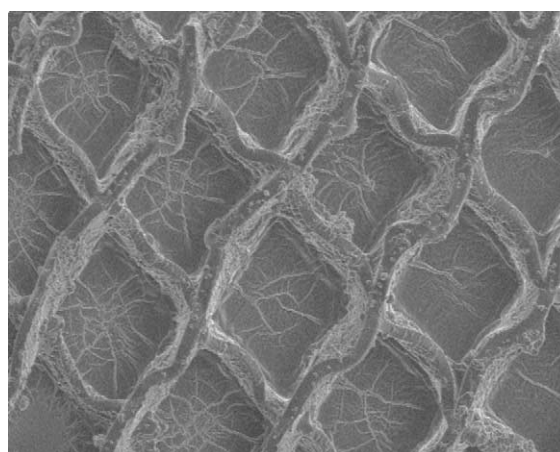


Fig. 13. Polymer sample with multiple patterns. After polymerizing the first pattern, the mask was rotated 90 $^\circ$ and a second pattern was polymerized. Magnification at 200 \times .

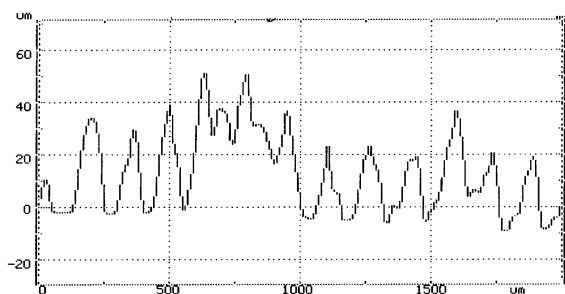


Fig. 14. Profilometer scan of the material shown in Fig. 13.

447 50 mixture of PEGDMA and PEGMA onto a layer
 448 of polystyrene with 5 wt.% divinylbenzene as a
 449 crosslinker. The polystyrene layer was thermally
 450 polymerized because of degradation that occurred
 451 while photopolymerizing a thin layer. The initiators
 452 used in this layer were 1 wt.% TED and 1 wt.%
 453 AIBN. The monomer mixture was placed between
 454 two glass slides and heated in an oven at 70 °C for
 455 12 h.

456 The possibility of building a three-dimensional
 457 structure has been studied. In these polymerizations,
 458 a first micropattern of the P(PEGMA-co-PEGDMA)
 459 material (50 wt.% PEGDMA) was formed. This
 460 micropatterned material was then washed with meth-
 461 anol. Another layer of the PEGMA/PEGDMA mono-
 462 mer mixture was pipetted on top of the patterned
 463 surface. The pattern was placed on top of the surface
 464 rotated at 90° from the previous position. The sample
 465 was irradiated with UV light again. This resulted in a
 466 pattern on top of a pattern.

467 Fig. 13 shows the grid made by patterning lines at
 468 approximately 90° to each other. The crossover of the
 469 second pattern over the first pattern is evident in the
 470 SEM picture. The lines here are very deep, approxi-
 471 mately 50 μm. The high aspect ratio (10 μm wide and
 472 100 μm deep) causes the patterns to become wavy
 473 because the polymeric material is flexible.

474 Fig. 14 shows the profile from a scan of the
 475 material shown in Fig. 13. The multiple peaks in
 476 succession from 600 to 900 μm are from the probe
 477 scanning directly over the crossing of the two pat-
 478 terns. There is a difference of about 10 to 15 μm
 479 between the height of the second pattern and the
 480 height of the first pattern. The larger peak at 1500
 481 μm corresponded to another crossing of the two
 482 patterns. The average height of the first pattern was

about 30 μm while the average height of the second
 pattern was about 45 μm.

6. Conclusions

Micropatterned biomimetic polymer surfaces can be
 synthesized by iniferter-based, free-radical polymeri-
 zation techniques. PEG200DMA and PEG200MA
 were successfully copolymerized onto a surface of
 highly crosslinked PEG200DMA. Very deep patterns
 can be achieved with high aspect ratios. Other
 materials, such as styrene and methacrylic acid, can
 also be incorporated into the patterns. Exploration of
 these polymerizations can lead to the development of
 micropatterned hydrogels, which are water insoluble,
 environmentally sensitive networks [40–45]. Patterns
 of these materials are able to swell or collapse in
 response to a change in the environmental pH,
 temperature, ionic strength or electromagnetic radia-
 tion. Possible applications of this type of material are
 for the sustained release of bioactive agents and for
 biosensors.

References

- [1] S.R. Turner, R.W. Blevins, Photoinitiated block copolymer formation using dithiocarbamate free radical chemistry, *Macromolecules* 23 (1990) 1856–1859.
- [2] A. Sebenik, Living free-radical block copolymerization using thio-iniferters, *Prog. Polym. Sci.* 23 (1998) 875–917.
- [3] D.A. Shipp, J.-L. Wang, K. Matyjaszewski, Synthesis of acrylate and methacrylate block copolymers using atom transfer radical polymerization, *Macromolecules* 31 (1998) 8005–8008.
- [4] K. Tharanikkarasu, G. Radhakrishnan, Tetraphenylethane iniferters: polyurethane–polystyrene multiblock copolymer through living radical polymerization, *J. Appl. Polym. Sci.* 66 (1997) 1551–1560.
- [5] K. Matyjaszewski, P.J. Miller, J. Pyun, G. Kickelbick, S. Diamanti, Synthesis and characterization of star polymers with varying arm number, length and composition from organic and hybrid inorganic/organic multifunction initiators, *Macromolecules* 32 (1999) 6526–6535.
- [6] X. Zhang, J. Zia, K. Matyjaszewski, End-functional poly(tert-butyl acrylate) star polymers by controlled radical polymerization, *Macromolecules* 33 (2000) 2340–2345.
- [7] C. Macilwain, US plans large funding boost to support nanotechnology boom, *Nature* 400 (1999) 95–95.
- [8] M. Madou, *Fundamentals of Microfabrication*, CRC Press, Boca Raton, FL, 1997.

- 529 [9] M.R. Neuman, Biomedical sensors, in: J.D. Bronzino (Ed.),
530 The Biomedical Engineering Handbook, CRC Press, Boca
531 Raton, FL, 1995, pp. 725–727.
- 532 [10] R.P. Buck, Bioanalytic sensors, in: J.D. Bronzino (Ed.), The
533 Biomedical Engineering Handbook, CRC Press, Boca Raton,
534 FL, 1995, pp. 779–787.
- 535 [11] A.S. Blawas, Protein patterning, *Biomaterials* 19 (1998)
536 595–609.
- 537 [12] J. Mayer, E. Karamuk, T. Akaike, E. Wintermantel, Matrices
538 for tissue engineering-scaffold structure for a bioartificial liver
539 support system, *J. Control. Release* 64 (2000) 81–90.
- 540 [13] J. Temenoff, M. AG, Tissue engineering for regeneration of
541 articular cartilage, *Biomaterials* 21 (2000) 431–440.
- 542 [14] Y. Ito, Surface micropatterning to regulate cell functions, *Bio-*
543 *materials* 20 (1999) 2333–2342.
- 544 [15] S. Zhang, L. Yan, M. Altman, M. Lassle, H. Nugent, F.
545 Frankel, D.A. Lauffenburger, G.M. Whitesides, A. Rich, Bio-
546 logical surface engineering: a simple system for cell pattern
547 formation, *Biomaterials* 20 (1999) 1213–1220.
- 548 [16] J. Deutsch, D. Motlagh, B. Russell, T.A. Desai, Fabrication of
549 microtextured membranes for cardiac myocyte attachment and
550 orientation, *J. Biomed. Mater. Res.* 53 (2000) 267–275.
- 551 [17] J.A. Chinn, Biomaterials: protein–surface interactions, in:
552 J.D. Bronzino (Ed.), The Biomedical Engineering Handbook,
553 CRC Press, Boca Raton, FL, 1995.
- 554 [18] H. Shi, W.-B. Tsai, M.D. Garrison, S. Ferrari, B.D. Ratner,
555 Template-imprinted nanostructured surfaces for protein recog-
556 nition, *Nature* 398 (1999) 593–597.
- 557 [19] H. Shi, B.D. Ratner, Template recognition of protein-
558 imprinted polymer surfaces, *J. Biomed. Mater. Res.* 49
559 (2000) 1–11.
- 560 [20] R. Shingvi, A. Kumar, G.P. Lopez, G.N. Stephanopoulos,
561 D.I.C. Wang, G.M. Whitesides, D.E. Ingber, Engineering cell
562 shape and function, *Science* 264 (1994) 296–298.
- 563 [21] C.A. Scotchford, E. Cooper, G.J. Leggett, S. Downes, Growth
564 of human osteoblast-like cells on alkanethiol on gold self-
565 assembled monolayers, *J. Biomed. Mater. Res.* 41 (1998)
566 431–442.
- 567 [22] R.S. Kane, S. Takayama, E. Ostuni, D.E. Ingber, G.M.
568 Whitesides, Patterning proteins and cells using soft lithogra-
569 phy, *Biomaterials* 20 (1999) 2362–2376.
- 570 [23] C.H. Thomas, C.D. McFarland, M.L. Jenkins, A. Rezanian,
571 J.G. Steele, K.E. Healy, The role of vitronectin in the attach-
572 ment and spatial distribution of bone-derived cells on materi-
573 als with patterned surface chemistry, *J. Biomed. Mater. Res.*
574 37 (1997) 81–93.
- 575 [24] Y. Nakayama, T. Matsuda, Surface macromolecular architec-
576 tural designs using photo-graft copolymerization based on
577 photochemistry of benzyl *N,N*-diethylthiocarbamate, *Macro-*
578 *molecules* 29 (1996) 8622–8630.
- 579 [25] N. Patel, R. Padera, G.H.W. Sanders, S.M. Cannizzaro, M.C.
580 Davies, R. Langer, C.J. Roberts, S.J.B. Tendler, P.M.
581 Williams, K.M. Shakesheff, Spatially controlled cell engineer-
582 ing on biodegradable polymer surfaces, *FASEB J.* 12 (1998)
583 1447–1454.
- 584 [26] N. Patel, G.H.W. Sanders, K.M. Shakesheff, S.M. Cannizzaro,
585 M.C. Davies, R. Langer, C.J. Roberts, S.J.B. Tendler, P.M.
Williams, Atomic force microscopic analysis of highly de-
586 fined protein patterns formed by microfluidic networks, *Lang-*
587 *muir* 15 (1999) 7252–7257.
- 588 [27] T. Matsuda, T. Sugawara, Development of surface photochem-
589 ical modification method for micropatterning of cultured cells,
590 *J. Biomed. Mater. Res.* 29 (1995) 749–756.
- 591 [28] J. Higashi, Y. Nakayama, R.E. Marchant, T. Matsuda, High-
592 spatioresolved microarchitectural surface prepared by photo-
593 graft copolymerization using dithiocarbamate: surface prepara-
594 tion and cellular responses, *Langmuir* 15 (1999) 2080–2088.
- 595 [29] K.M. DeFife, E. Colton, Y. Nakayama, T. Matsuda, J.M.
596 Anderson, Spatial regulation and surface chemistry control
597 of monocyte/macrophage adhesion and foreign body giant
598 cell formation by photochemically micropatterned surfaces,
599 *J. Biomed. Mater. Res.* 45 (1999) 148–154.
- 600 [30] B. de Boer, H.K. Simon, M.P.L. Werts, E.W. van der Vegte, G.
601 Hadziioannou, Living free radical photopolymerization initi-
602 ated from surface-grafted iniferter monolayers, *Macromole-*
603 *cules* 33 (2000) 349–356.
- 604 [31] J.H. Ward, R. Bashir, N.A. Peppas, Micropatterning of bio-
605 medical polymer surfaces by Novel UV polymerization tech-
606 niques, *J. Biomed. Mater. Res.* 56 (2001) 351–360.
- 607 [32] J.H. Ward, A. Shahar, N.A. Peppas, Kinetics of living radical
608 polymerizations of multifunctional monomers, *Polymer* 43
609 (2002) 1745–1752.
- 610 [33] M.E. Byrne, E. Oral, J.Z. Hilt, N.A. Peppas, Networks for
611 recognition of biomolecules: molecular imprinting and micro-
612 patterning poly(ethylene glycol)-containing films, *Polym.*
613 *Adv. Technol.* 13 (2002) 798–816.
- 614 [34] T. Otsu, M. Yoshida, Role of initiator-transfer agent-termina-
615 tor (iniferter) in radical polymerizations: polymer design by
616 organic disulfides as iniferters, *Makromol. Chem. Rapid Com-*
617 *mun.* 3 (1982) 127–132.
- 618 [35] T. Otsu, M. Yoshida, T. Tazaki, A model for living radical
619 polymerization, *Makromol. Chem. Rapid Commun.* 3 (1982)
620 133–140.
- 621 [36] J.H. Ward, N.A. Peppas, Kinetic gelation modeling of living
622 radical polymerizations, *Macromolecules*, in press.
- 623 [37] A.R. Kannurpatti, S. Lu, G.M. Bunker, C.N. Bowman, Kinetic
624 and mechanistic studies of iniferter photopolymerizations,
625 *Macromolecules* 29 (1996) 7310–7315.
- 626 [38] A.R. Kannurpatti, K.J. Anderson, J.W. Anseth, C.N. Bowman,
627 Use of living radical polymerizations to study the structural
628 evolution and properties of highly crosslinked polymer net-
629 works, *J. Polym. Sci.-Polym. Phys.* 35 (1997) 2297–2307.
- 630 [39] J.E. Moore, Photopolymerization of multifunctional acrylates
631 and methacrylates, in: S.S. Labana (Ed.), *Chemistry and Prop-*
632 *erties of Crosslinked Polymers*, Academic Press, New York,
633 1977, pp. 535–558.
- 634 [40] N.A. Peppas, Hydrogels and drug delivery, *Curr. Opin. Col-*
635 *loid Interf. Sci.* 2 (1997) 531–537.
- 636 [41] A.M. Lowman, N.A. Peppas, Hydrogels, in: E. Mathiowitz
637 (Ed.), *Encyclopedia of Controlled Drug Delivery*, Wiley,
638 New York, 1999.
- 639 [42] N.A. Peppas, E. Oral, J.H. Ward, Micropatterning and molec-
640 ular imprinting for medical applications, *Proc. Eur. Symp.*
641 *Control. Drug Deliv.* 6 (2000) 21–22.
- 642

- 643 [43] R. Scott, J.H. Ward, N.A. Peppas, Development of acrylate
644 and methacrylate polymer networks for controlled release by
645 photopolymerization technology, in: D.L. Wise, L. Brannon-
646 Peppas, A.M. Klibanov, R. Langer, A.G. Mikos, N.A. Peppas,
647 D.J. Trantolo, G.E. Wnek, M.J. Yaszemski (Eds.), *Handbook*
648 *of Pharmaceutical Controlled Release Technology*, Dekker,
649 New York, NY, 2000, pp. 47–64.
- 650 [44] J.H. Ward, R. Gomez, R. Bashir, N.A. Peppas, UV free-radical
polymerization for micropatterning poly(ethylene glycol)-con-
taining films, *Proc. SPIE* 4097 (2000) 221–228.
- [45] J.Z. Hilt, A.K. Gupta, R. Bashir, N.A. Peppas, A microsen-
sor based on a microcantilever patterned with an environ-
mentally sensitive hydrogel, in: L.P. Lee, J.T. Borenstein,
R.P. Manginelli, M. Okandan, P.J. Hesketh (Eds.), *Bio-*
MEMS and Bionanotechnology, MRS, Pittsburgh, PA,
2002, pp. 173–178.

UNCORRECTED PROOF