

Timothy B. Stachowiak<sup>1</sup>  
Dieudonne A. Mair<sup>1</sup>  
Tyler G. Holden<sup>1</sup>  
L. James Lee<sup>2</sup>  
Frantisek Svec<sup>4</sup>  
Jean M. J. Fréchet<sup>1,3,4</sup>

<sup>1</sup>Department of Chemical Engineering, University of California, Berkeley, CA, USA

<sup>2</sup>Department of Chemical and Biomolecular Engineering, Ohio State University, Columbus, OH, USA

<sup>3</sup>Department of Chemistry, University of California, Berkeley, CA, USA

<sup>4</sup>The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

## Original Paper

# Hydrophilic surface modification of cyclic olefin copolymer microfluidic chips using sequential photografting

The plastic material known as cyclic olefin copolymer (COC) is a useful substrate material for fabricating microfluidic devices due to its low cost, ease of fabrication, excellent optical properties, and resistance to many solvents. However, the hydrophobicity of native COC limits its use in bioanalytical applications. To increase surface hydrophilicity and reduce protein adsorption, COC surfaces were photografted with poly(ethylene glycol) methacrylate (PEGMA) using a two-step sequential approach: covalently-bound surface initiators were formed in the first step and graft polymerization of PEGMA was then carried out from these sites in the second step. Contact angle measurements were used to monitor and quantify the changes in surface hydrophilicity as a function of grafting conditions. As water droplet contact angles decreased from 88° for native COC to 45° for PEGMA-grafted surfaces, protein adsorption was also reduced by 78% for the PEGMA-modified COC microchannels as determined by a fluorescence assay. This photografting technique should enable the use of COC microdevices in a variety of bioanalytical applications that require minimal nonspecific adsorption of biomolecules.

**Keywords:** Cyclic olefin copolymer / Microfluidic device / Photografting / Poly(ethylene glycol) methacrylate / Surface modification

Received: December 12, 2006; revised: February 21, 2007; accepted: February 21, 2007

DOI 10.1002/jssc.200600515

## 1 Introduction

Microfluidic devices made of plastic are an attractive alternative to devices fabricated from glass, quartz, or silicon. Compared with these traditional substrates, plastic chips are inexpensive and more easily produced in large quantities using high-throughput fabrication techniques such as hot embossing or injection molding. Cyclic olefin copolymer (COC) is of particular interest due to its combination of excellent UV transparency, low autofluorescence, low oxygen permeability, high mechanical strength, and compatibility with a broad range of chemicals and solvents [1, 2]. However, like most commodity plastics, COC is naturally hydrophobic and thus microchannels made from COC are likely to adsorb some processed compounds, resulting in a variety of problems such as sample loss, degradation of resolution in separa-

tions, and difficulties with accurate quantitative analyses.

In order to minimize adsorption of analytes such as proteins, it is necessary to chemically modify the walls of the polymer microchannels. The wide variety of polymer surface modification techniques that have been developed are covered in several excellent reviews [3–7]. While a few other methods have been used [8–12], modification of COC surfaces has primarily been accomplished using photografting [13–20]. Photografting is typically initiated by a photoinitiator, such as benzophenone; UV excitation promotes abstraction of hydrogen atoms from the polymer surface, leading to the formation of surface-bound radicals that may then initiate a surface graft polymerization process [21].

UV photografting of polymeric microfluidic devices is usually performed through a single-step photografting process in which the grafting solution contains both the photoinitiator and the monomer. Although convenient, this single-step process usually leads to the formation of significant amounts of ungrafted polymer in solution. This concurrent polymerization in the bulk solution depletes the monomers available for grafting and, in some cases, causes permanent clogging or gel formation

**Correspondence:** Professor Jean M. J. Fréchet, University of California, Department of Chemistry, Berkeley, CA 94720-1460, USA  
E-mail: frechet@berkeley.edu  
Fax: +1 510-643-3079

**Abbreviations:** BP, benzophenone; COC, cyclic olefin copolymer; PEGMA, poly(ethylene glycol) methacrylate

[22]. Therefore, several sequential photografting techniques have been developed to address the limitations of conventional single-step grafting [22–29]. For example, Hu and co-workers successfully employed a two-step technique to photograft poly(dimethylsiloxane) (PDMS) microchannels with hydrophilic polymers [22]. In this process, the photoinitiator was first physically adsorbed onto the PDMS surface, then this surface was immersed in a solution containing only monomer and, finally, exposure to UV light was carried out. By localizing the photoinitiator on the PDMS surface, graft polymerization was enhanced and formation of polymer in solution was reduced. However, since the process relies on physical adsorption, the initiator may partially desorb from the surface, especially if it is soluble in the monomer solution used in the subsequent photografting step. To prevent photoinitiator desorption, Ogiwara *et al.* used poly(vinyl acetate) to physically entrap initiator at a polyethylene surface [23], while Ulbricht *et al.* advocated the use of initiator-saturated monomer solutions to inhibit the desorption of adsorbed photoinitiator [24]. With PDMS, the desorption problem is mitigated by the fact that the initiator is not only adsorbed on the outer surface of the polymer but also penetrates a short distance into the swollen and permeable PDMS walls [28]. However, for less permeable substrates, such as COC, initiator is not likely to absorb into the walls and desorption would be a greater concern. We recently utilized an alternative two-step technique for surface modification of high surface area macroporous monolithic polymers [29]. This approach involves the covalent attachment of photoinitiator to the polymer surface before monomer is introduced. In this way, initiator is effectively immobilized onto the surface of the polymer and its desorption is not possible since the initiator is covalently linked to the polymer.

We now report the surface modification of COC surfaces and microchannels in order to increase their surface hydrophilicity and reduce protein adsorption. The COC surfaces were modified using the sequential photografting technique with covalent attachment of the initiator. The photografted surfaces were characterized and evaluated for their ability to resist protein adsorption.

## 2 Experimental

### 2.1 Materials

Benzophenone (99+%, BP) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethoxylated hydroxyethyl methacrylate (poly(ethyleneglycol methacrylate) PEGMA, average  $M_n = 570$  g/mol, containing 10–11 ethylene glycol units) was purchased from Sartomer (Exton, PA, USA). PEGMA was passed through a column contain-

ing inhibitor remover beads of basic alumina (Aldrich). Fluorescein-labeled bovine serum albumin (BSA) was obtained from Invitrogen (Carlsbad, CA, USA). Injection molded microfluidic devices were prepared from COC pellets (Topas 8007 × 10, Ticona, Florence, KY, USA) according to a previously published procedure [20]. The COC microfluidic chips contained a single straight 6.3-cm long channel with a cross section of approximately  $100 \times 100 \mu\text{m}$ .

### 2.2 Instrumentation

An OAI Model 30 deep UV collimated light source (San Jose, CA, USA) fitted with a 500-W HgXe lamp was used for UV exposures. The irradiation power was calibrated to  $15.0 \text{ mW/cm}^2$  using an OAI Model 306 UV power meter with a 260-nm probe head. Solutions were pumped through microfluidic channels using a KDS 210 syringe pump from KD Scientific Inc. (Holliston, MA, USA). Microscopic evaluations were performed using a Nikon TE200 inverted fluorescence microscope (Scientific Instrument Company, Sunnyvale, CA, USA). Images were acquired with a Micropublisher 5.0 RTV CCD camera from QImaging (Burnaby, BC, Canada).

### 2.3 COC surface modification

COC surfaces were modified using a sequential two-step photografting procedure described previously [25, 29]. Briefly, the microchannels were first flushed with a deaerated 5 wt% solution of the photoinitiator (BP), in methanol. Then, the channel was sealed to prevent flow and evaporation, and the chip was exposed to UV irradiation through a photomask. The photomask had five openings of 5 mm width spaced equally 5 mm apart. The exposure times were varied in the different exposed regions by sequentially covering the openings of the mask. After photografting of the initiator alone, the microchannel was rinsed with methanol to remove unbound initiator. Next, a deaerated solution of PEGMA monomer in water was pumped through the activated channel. The channel was then sealed and exposed to UV irradiation through the realigned photomask in the same regions previously exposed in the first step. The microchannel was then rinsed extensively with water to remove the unreacted PEGMA. For contact angle measurements, we performed bulk modification of flat, rectangular pieces of injection-molded COC ( $25 \times 75 \text{ mm}$ ). This bulk modification of the COC plates provided samples with adequate surface area to perform the contact angle measurements. COC substrates were photografted using a modified version of the above protocol. In particular, for UV exposure steps, the solutions of BP or of PEGMA were sealed in the space between two COC plates that were separated by a  $125 \mu\text{m}$ -thick Teflon gasket.

Each set of plates was treated with a particular set of photografting conditions.

## 2.4 Contact angle measurement

Static contact angle measurements were taken using a Drop Shape Analysis System G10 (Krüss, Palo Alto, CA, USA). Ten drops of water were placed on the substrate and the contact angle of the left and right sides of each drop were optically measured and averaged. The average of ten data points was used to report the final average value.

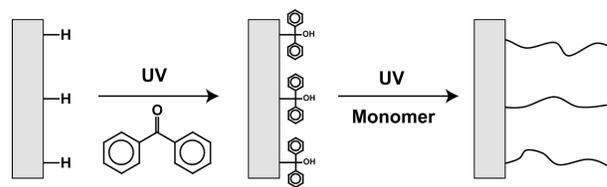
## 2.5 Fluorescence assay of protein adsorption

The extent of protein adsorption on unmodified and PEGMA-photografted COC microchannels was evaluated using a fluorescence assay developed previously [29]. Briefly, the COC channels were flushed with a 0.5 mg/mL solution of fluorescein-labeled BSA for 1 h at a flow rate of 0.25  $\mu\text{L}/\text{min}$ . The microchannels were then rinsed with water for 1 h at 1.0  $\mu\text{L}/\text{min}$  to remove excess BSA. After rinsing with water, micrographs of the channels were taken and the fluorescent intensity was analyzed across the width of the channels using ImageJ software (NIH); the fluorescent intensities were quantified as pixel intensities from the 8-bit images. The reported intensities were taken from the maximum (center) of the intensity profiles. In all experiments, each grafted region was adjacent to non-grafted regions within the same channel. The non-grafted regions served as controls for measuring the relative extent of protein adsorption.

## 3 Results and discussion

Neutral hydrophilic polymers, such as poly(ethylene glycol) and polyacrylamide, are often used to modify surfaces for increased hydrophilicity and to reduce protein adsorption [3]. In a previous study, we evaluated a variety of hydrophilic monomers for the photografting surface modification of macroporous polymer monoliths in order to reduce protein adsorption [29]. We found that poly(ethylene glycol) monomethacrylate (PEGMA) outperformed other hydrophilic monomers, including acrylamide, 2-hydroxyethyl methacrylate, and vinyl pyrrolidinone. Therefore, in this study, we focused on photografting COC surfaces with PEGMA and evaluated the effect of grafting conditions on surface characteristics and protein adsorption resistance.

The two-step photografting technique utilized in this study is illustrated in Fig. 1. In the first step, surface-bound initiators are formed when UV-excited benzophenone molecules abstract hydrogen atoms from the polymer surface. Surface radicals are created, but in the



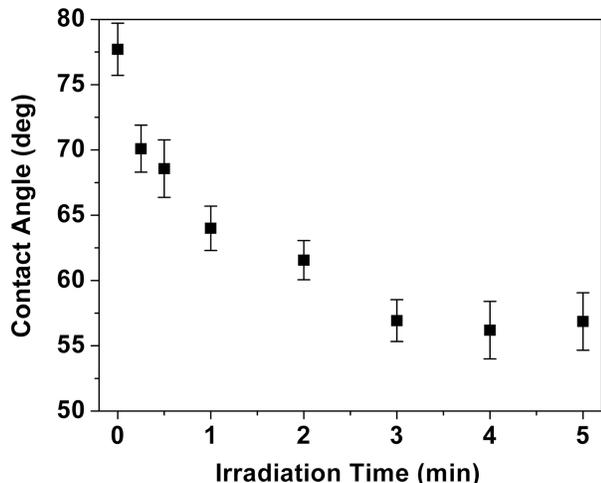
**Figure 1.** Scheme of the two-step sequential photografting process.

absence of monomer, these radicals combine with the newly formed semipinacol radicals to form covalently attached surface initiators. In the second step, graft polymerization from the polymer surface is initiated by UV irradiation of these sites in the presence of a solution containing monomer but no initiator. In addition to reducing the formation of non-grafted polymer in solution, this two-step approach allows many aspects of the grafting process to be tuned and optimized independently, as discussed below.

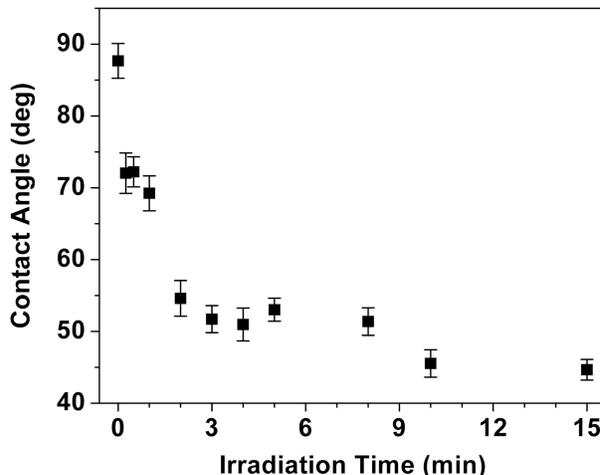
### 3.1 Formation of surface initiators

The first step of the sequential photografting process controls the formation of surface initiator sites. The irradiation time and the concentration of benzophenone both determine the final surface density of sites. Therefore, the effects of these two parameters were investigated. First, BP irradiation time was varied while the other grafting conditions were held constant. The static contact angle of water on the PEGMA-modified COC surfaces was measured to quantify the effectiveness of the surface modification procedure. Figure 2 shows that, as expected, the COC surface becomes more hydrophilic, *i. e.* the contact angle decreases, after photografting PEGMA. The hydrophilicity of the surface improves with increasing BP irradiation time. This trend is predictable since increasing the BP irradiation time should increase the number of resulting surface initiator sites from which grafted poly(PEGMA) chains can be formed in the second step. The decrease in contact angle levels off after approximately 3 min of irradiation, indicating that the surface may become saturated with initiator sites at this point or that the surface density of poly(PEGMA) chains grafted in the second step has reached a limit. Since the poly(PEGMA) chains are much larger than the initiator molecules, the photografted polymer chains most likely limit the final surface density rather than the number of initiator sites. Their bulkiness may prevent grafted polymer chains from growing from each of the available surface initiator sites when the surface density of initiators is high.

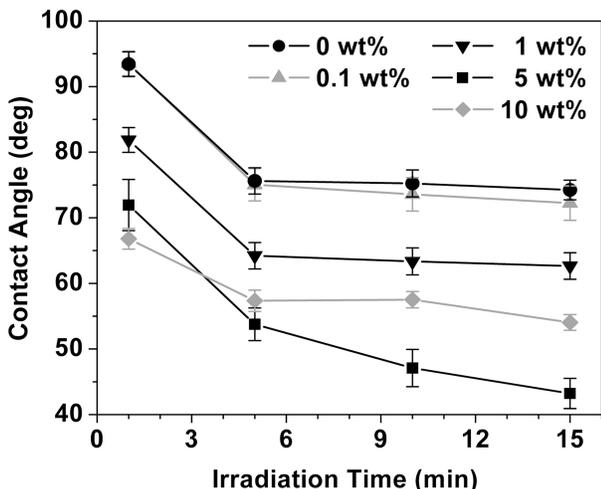
Next, the BP irradiation time was fixed and the effect of BP concentration was investigated. Figure 3 shows that the BP concentration greatly affects the extent of



**Figure 2.** Effect of irradiation time in the first step of the two-step photografting process on average water droplet contact angles ( $n=10$ ) on PEGMA-photografted COC surfaces. Conditions: First step: irradiation for 0–5 min in the presence of a 5 wt% solution of BP in methanol. Second step: irradiation time 2 min; PEGMA monomer concentration 0.1 mol/L in water.



**Figure 4.** Effect of PEGMA irradiation time in the second step of the sequential photografting process on average water droplet contact angles ( $n=10$ ) on PEGMA-grafted COC. Conditions: First step: 4 min irradiation of 5 wt% BP in methanol. Second step: irradiation for 0–15 min; 0.1 mol/L PEGMA in water.



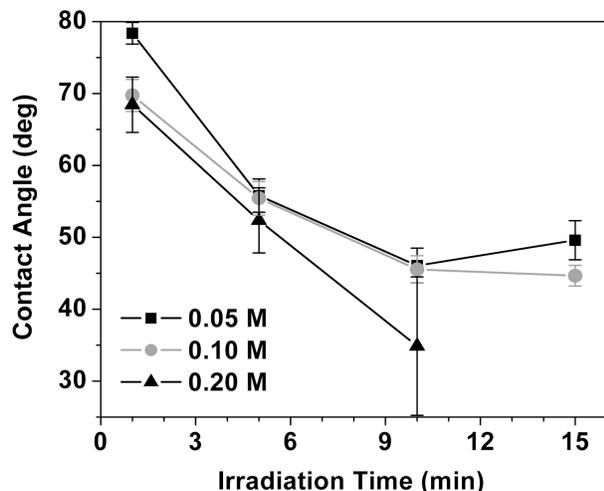
**Figure 3.** Effect of BP concentration on average water droplet contact angles ( $n=10$ ) on PEGMA-grafted COC surfaces. Each curve corresponds to a particular BP concentration used in the first step of photografting. Each column shows the effect of BP concentration at a particular second-step monomer grafting time. Conditions: First step: 4 min irradiation of BP solutions ranging in concentration from 0–10 wt% in methanol. Second step: irradiation for 1, 5, 10, or 15 min; 0.1 mol/L PEGMA in water.

surface modification. In general, increasing the concentration leads to a more hydrophilic surface with a lower contact angle. Again, this trend is understandable since the rate of surface initiator formation is proportional to the concentration of BP. However, at the highest BP concentration, 10 wt%, the contact angles were actually greater than those for the 5 wt% solution. Although the

rate of initiator formation should be proportional to BP concentration, BP strongly absorbs UV light and, at high concentrations, may reduce the transmission of UV light and thus limit the formation of initiator sites. Therefore, an intermediate BP concentration appears best in order to achieve a high rate of formation of surface initiator sites, while maintaining the low UV absorbance needed for transmission of UV light. Finally, it is interesting to note that the contact angle decreases slightly even when no BP is used in the first step (0 wt% BP), indicating that a small amount of grafting occurs in the second step even without using initiator in the first step. Some amount of direct generation of surface radicals is expected whenever a polymer is irradiated with intense UV light, but clearly this is an inefficient process for modifying COC surfaces due to the relative inertness of the polymer itself. Even with 15 min of UV exposure the contact angle decreased very little and it is necessary to use photoinitiator for the successful modification of the COC surfaces.

### 3.2 Graft polymerization

In the second step of the photografting procedure, graft polymerization of the monomer is initiated from initiator sites on the polymer surface. UV irradiation time and monomer concentration both determine the extent of grafting. Figure 4 shows that the contact angle drops significantly after only 2 min of irradiation, but then decreases more slowly with increasing exposure time. We expect that as poly(PEGMA) chains are grafted onto the COC, its surface quickly becomes much more hydrophilic. However, once surface coverage is nearly com-

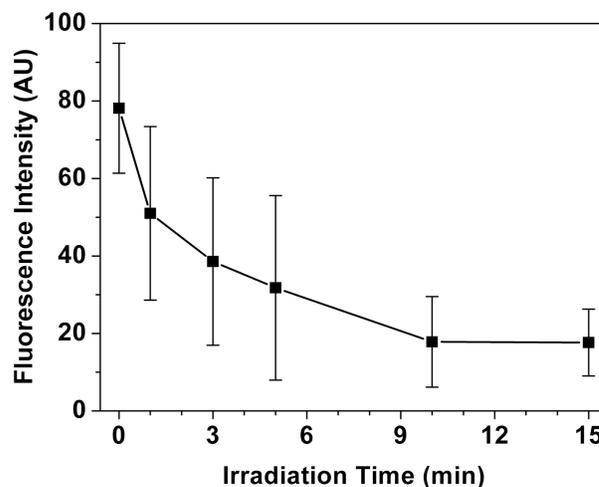


**Figure 5.** Effect of PEGMA concentration and irradiation time on the contact angle of water droplets on PEGMA-grafted COC. Conditions: Same as Fig. 4, except PEGMA concentration was varied from 0.05–0.2 mol/L.

plete, the composition of the surface changes little, even as the grafted chains grow longer. These data presented in Fig. 5 show that increasing monomer concentration yields lower contact angles for each irradiation time. Surprisingly, we observed that the photografting solution partially gelled at irradiation times greater than 10 min for PEGMA concentrations of 0.2 mol/L or more. Since the photoinitiator is localized at the polymer surface, we hypothesize that gel formation is the result of polymerization and transfer processes occurring in the bulk solution as a result of direct initiation by UV irradiation. Highly concentrated PEGMA solutions would be more susceptible to this mechanism than less concentrated ones. Therefore, high monomer concentrations should not be used when photografting microchannel walls in order to prevent the channels from becoming clogged with gel.

### 3.3 Assay of protein adsorption in microchannels

For their use in bioanalytical applications including electrophoretic separations, immunoassays, and proteomic analyses, COC microchannels must exhibit minimal adsorption of analyte biomolecules such as proteins. In the previous experiments, grafting conditions were optimized to increase the hydrophilicity of COC surfaces. However, a surface that is hydrophilic does not necessarily afford resistance to protein adsorption. Therefore, we used a fluorescence-based assay to specifically test the ability of PEGMA-photografted COC microchannels to resist the adsorption of proteins. First, COC microchannels were photografted with PEGMA using the two-step sequential photografting technique and then the channels were flushed with a solution of fluorescein-labeled



**Figure 6.** Fluorescence intensity of adsorbed fluorescein-labeled BSA on regions of a COC microchannel photografted with PEGMA using the two-step sequential method. Average fluorescent intensity ( $n=5$ ) is plotted as a function of PEGMA grafting time. Conditions: First step: 4 min irradiation of 5 wt% BP in methanol. Second step: irradiation for 1–15 min; 0.1 mol/L PEGMA in water.

bovine serum albumin, which served as the model protein in this study. The most abundant protein in blood serum is human serum albumin, which is well known as an adhesive protein that binds to many surfaces. Therefore, its analog, BSA, is often used as a model protein for adsorption studies [30]. After BSA exposure, the microchannels were washed with water and protein adsorption was then observed using a fluorescence microscope. Successful prevention of protein adsorption is indicated by a low intensity of fluorescence. In contrast, high fluorescence intensity indicates substantial adsorption of the fluorescent protein and poor protein adsorption resistance of the surface. The results of these *in-situ* tests are shown in Fig. 6. The monomer exposure time was varied, but other photografting conditions were based upon the optimized conditions determined during the contact angle measurement experiments. Similar to the trend in the contact angle data, the amount of adsorbed protein decreases with increasing exposure time and levels off at longer times. At short monomer exposure times, surface modification is incomplete and measurements of the fluorescent intensities are more prone to variation. However, at longer exposure times, the photografted layer becomes more complete and uniform, yielding a consistent and repeatable reduction of protein adsorption. A significant decrease in protein adsorption is obtained for monomer irradiation times of 10 min or more. Compared with unmodified surfaces, photografting the COC microchannels with PEGMA reduced the amount of protein adsorption by almost 80% under optimum conditions.

## 4 Concluding remarks

The surface modification of cyclic olefin copolymer surfaces and microchannels with poly(ethylene glycol) methacrylate using a two-step sequential photografting technique leads to a substantial increase in surface hydrophilicity and a large reduction in protein adsorption. This photografting technique should allow COC microdevices to be used for a variety of bioanalytical applications that require minimal nonspecific adsorption of biomolecules. In addition, since the photografting technique has previously been shown effective for the hydrophilic modification of macroporous polymer monoliths, it will now be possible to simultaneously modify the walls of a COC microdevice and of the polymer monolith that lies within. This will streamline the process of creating inert, high surface area supports within microchannels for applications such as flow-through enzymatic microreactors.

Although demonstrated with the grafting of COC, the method described in this report is applicable to many other plastic substrates. In addition, this approach may be used with a wide variety of monomers. While this research focused on the prevention of protein adsorption using hydrophilic surface modification, different surface properties, such as chemical reactivity or ionizability, can be photografted for use in different applications. Since our method is rather versatile and the only limiting factor is the UV transparency of the monomer, a broad application of the photografting of plastic chips can be envisioned.

*Support of this research by a grant of the National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health (EB-006133) is gratefully acknowledged. This work was also partially supported by NSF Nanoscale Science and Engineering Center for Affordable Nanoengineering of Polymeric Biomedical Devices. Portions of this work were performed at the Molecular Foundry, Lawrence Berkeley National Laboratory, which is supported by the Office of Science, Office of Basic Energy Sciences, of the US Department of Energy under Contract No. DE-AC02-05CH11231.*

## 5 References

- [1] Lamonte, R. R., McNally, D., *Plast. Eng.* 2000, 56, 51–56.
- [2] Khanarian, G., *Opt. Eng.* 2001, 40, 1024–1029.
- [3] Liu, J. K., Lee, M. L., *Electrophoresis* 2006, 27, 3533–3546.
- [4] Dolnik, V., *Electrophoresis* 2004, 25, 3589–3601.
- [5] Belder, D., Ludwig, M., *Electrophoresis* 2003, 24, 3595–3606.
- [6] Makamba, H., Kim, J. H., Lim, K., Park, N., Hahn, J. H., *Electrophoresis* 2003, 24, 3607–3619.
- [7] Kato, K., Uchida, E., Kang, E. T., Uyama, Y., Ikada, Y., *Progr. Polymer Sci.* 2003, 28, 209–259.
- [8] Gaudioso, J., Craighead, H. G., *J. Chromatogr. A* 2002, 971, 249–253.
- [9] Ahn, C. H., Choi, J. W., Beaucage, G., Nevin, J. H., Lee, J. B., *et al.*, *Proc. IEEE* 2004, 92, 154–173.
- [10] Sohn, Y. S., Kai, J., Ahn, C. H., *Sens. Lett.* 2004, 2, 171–174.
- [11] Lim, Y. T., Kim, S. J., Yang, H., Kim, K., *J. Micromech. Microeng.* 2006, 16, N9–N16.
- [12] Marie, R., Beech, J. P., Voros, J., Tegenfeldt, J. O., Hook, F., *Langmuir* 2006, 22, 10103–10108.
- [13] Rohr, T., Ogletree, D. F., Svec, F., Fréchet, J. M. J., *Adv. Funct. Mater.* 2003, 13, 264–270.
- [14] Stachowiak, T. B., Rohr, T., Hilder, E. F., Peterson, D. S., Yi, M. Q., *et al.*, *Electrophoresis* 2003, 24, 3689–3693.
- [15] Li, C., Yang, Y. N., Craighead, H. G., Lee, K. H., *Electrophoresis* 2005, 26, 1800–1806.
- [16] Yang, Y. N., Li, C., Lee, K. H., Craighead, H. G., *Electrophoresis* 2005, 26, 3622–3630.
- [17] Lin, R., Burns, M. A., *J. Micromech. Microeng.* 2005, 15, 2156–2162.
- [18] Bhattacharyya, A., Klapperich, C. M., *Anal. Chem.* 2006, 78, 788–792.
- [19] Ro, K. W., Liu, H., Knapp, D. R., *J. Chromatogr. A* 2006, 1111, 40–47.
- [20] Mair, D. A., Geiger, E., Pisano, A. P., Fréchet, J. M. J., Svec, F., *Lab-Chip* 2006, 6, 1346–1354.
- [21] Rånby, B., Yang, W. T., Tretinnikov, O., *Nucl. Instrum. Meth. Phys. Res. B* 1999, 151, 301–305.
- [22] Hu, S. W., Ren, X. Q., Bachman, M., Sims, C. E., *et al.*, *Anal. Chem.* 2004, 76, 1865–1870.
- [23] Ogiwara, Y., Kanda, M., Takumi, M., Kubota, H., *J. Polym. Sci. C Polym. Lett.* 1981, 19, 457–462.
- [24] Ulbricht, M., Matuschewski, H., Oechel, A., Hicke, H. G., *J. Membr. Sci.* 1996, 115, 31–47.
- [25] Ma, H. M., Davis, R. H., Bowman, C. N., *Macromolecules* 2000, 33, 331–335.
- [26] Deng, J. P., Yang, W. T., Ranby, B., *J. Appl. Polym. Sci.* 2001, 80, 1426–1433.
- [27] Ulbricht, M., Yang, H., *Chem. Mater.* 2005, 17, 2622–2631.
- [28] Wang, Y. L., Lai, H. H., Bachman, M., Sims, C. E., *et al.*, *Anal. Chem.* 2005, 77, 7539–7546.
- [29] Stachowiak, T. B., Svec, F., Fréchet, J. M. J., *Chem. Mater.* 2006, 18, 5950–5957.
- [30] McClellan, S. J., Franses, E. I., *Coll. Surf. A, Physicochem. Engin. Asp.* 2005, 260, 265–275.