Fabrication of Poly(ethylene glycol) Hydrogel Microstructures Using Photolithography

Alexander Revzin, Ryan J. Russell, Vamsi K. Yadavalli, Won-Gun Koh, Curt Deister, David D. Hile, Michael B. Mellott, and Michael V. Pishko*

Department of Chemical Engineering, Texas A&M University, 3122 TAMU, College Station, Texas 77843-3122

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The fabrication of hydrogel microstructures based upon poly(ethylene glycol) diacrylates, dimethacrylates, and tetraacrylates patterned photolithographically on silicon or glass substrates is described. A silicon/silicon dioxide surface was treated with 3-(trichlorosilyl)propyl methacrylate to form a self-assembled monolayer (SAM) with pendant acrylate groups. The SAM presence on the surface was verified using ellipsometry and time-of-flight secondary ion mass spectrometry. A solution containing an acrylated or methacrylated poly(ethylene glycol) derivative and a photoinitiator (2,2-dimethoxy-2-phenylacetophenone) was spin-coated onto the treated substrate, exposed to 365 nm ultraviolet light through a photomask, and developed with either toluene, water, or supercritical CO_2. As a result of this process, three-dimensional, cross-linked PEG hydrogel microstructures were immobilized on the surface. Diameters of cylindrical array members were varied from 600 to 7 μm by the use of different photomasks, while height varied from 3 to 12 μm, depending on the molecular weight of the PEG macromer. In the case of 7 μm diameter elements, as many as 400 elements were reproducibly generated in a 1 mm^2 square pattern. The resultant hydrogel patterns were hydrated for as long as 3 weeks without delamination from the substrate. In addition, micropatterning of different molecular weights of PEG was demonstrated. Arrays of hydrogel disks containing an immobilized protein conjugated to a pH sensitive fluorophore were also prepared. The pH sensitivity of the gel-immobilized dye was similar to that in an aqueous buffer, and no leaching of the dye-labeled protein from the hydrogel microstructure was observed over a 1 week period. Changes in fluorescence were also observed for immobilized fluorophore labeled acetylcholine esterase upon the addition of acetyl acholine.

Introduction

Here we describe a photolithographic method for the fabrication of poly(ethylene glycol) (PEG) hydrogel microstructures on silicon or glass substrates. PEG is a nondegradable, hydrophilic polymer that can be cross-linked into hydrogels through various chemistries. In prior research, PEG-based hydrogels were developed by introducing terminal acrylate functional groups which could take part in photopolymerization reactions.[1−3] Similar gels were used to develop highly cross-linked polymer networks capable of protein entrapment for numerous sensing techniques, such as electrochemistry[4] and fluorescence spectroscopy.[5,6] PEG hydrogels have been shown to be both biocompatible[7] and nonfouling in complex environments.[8]

To prevent protein adhesion and control cell attachment, prior research has primarily focused on using PEG or oligioethylene glycol (OEG) thin films to modify surfaces. Soft lithography has been utilized to self-assemble PEG and OEG molecules on silicon, gold, glass, and plastic in order to control protein and cell interactions with these substrates.[9−14] Hammond and co-workers also used SAMs of OEG-terminated alkanethiols as a resist to prevent adsorption of polyion species to the gold substrates.[15] Other researchers covalently grafted PEGs onto various substrates in order to render the substrates more biocompatible.[16−19] In addition, several groups have explored photolithographic patterning of hydrogels.[4,20−24] Sheppard

* To whom correspondence should be addressed. Current address: Department of Chemical Engineering, The Pennsylvania State University, 158 Fenske Laboratory, University Park, PA 16802-4400. Email: mpishko@engr.psu.edu. Phone: (814) 865-2574. Fax: (814) 865−7846.

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and co-workers used alkoxysilane coupling agents to attach microfabricated mesoscale poly(2-hydroxyethyl methacrylate) hydrogel patterns on silicon dioxide surfaces.[20,21] PEG hydrogels containing redox moieties were used to create enzyme electrodes, and photolithography was used to pattern these gels.[4] More recently, Beebe and colleagues photolithographically patterned hydroxyethyl methacrylate hydrogels inside microfluidic channels to serve as pH sensitive flow control elements in MEMS devices. [23,24]

This article describes a simple approach for developing micrometer scale PEG microstructures ranging from 600 to 7 μm in diameter. In addition, hydrogel patterns of PEG molecules of different molecular weights were generated. Silicon or glass surfaces were first acrylated with a trichlorosilane SAM to ensure covalent attachment of the PEG arrays and thereby improve hydrogel adhesion to the substrate. The development of hydrogel microstructures both by a conventional, solvent-based method and with supercritical CO_2 was investigated. The potential of the hydrogel arrays for use as an optical sensor was examined by incorporating a pH-sensitive dye within the array elements. Micrographs of the elements containing fluorescent dye at different pH microenvironments indicated the dye was not destroyed during the photopolymerization process and retained its pH sensitivity. We have also incorporated fluorophore labeled acetylcholine esterase in these gel microstructures and demonstrated that the fluorescence of the gels is sensitive to acetylcholine concentration in a surrounding buffer.

Experimental Section

Materials. Poly(ethylene glycol) diacrylate (PEG-DA) (MW 575), 2,2′-dimethoxy-2-phenylacetophenone (DMPA), anhydrous carbon tetrachloride, anhydrous toluene, *n*-heptane, benzene, acryloyl chloride, triethylamine, and 1-vinyl-2-pyrrolidone were purchased from Aldrich Chemical Co. (Milwaukee, WI). Poly(ethylene glycol) dimethacrylate (PEG-DMA, MW 1000) and tetrahydroxy PEG (MW 18 500) were obtained from Polysciences Inc. (Warrington, PA).

PEG (MW 18 500) was tetra-acrylated according to a published protocol.[25] This polymer consist of two poly(ethylene glycol) chains of molecular weight 9000 linked by a bis-phenol A bisepoxide. Thus, the polymer has two hydroxyl moieties associated with the PEG end groups and two hydroxyl moieties in the center of the polymer associated with the reacted bis-phenol bisepoxide. When modified with acryloyl chloride, the polymer will have four acrylate groups, two at the ends of the polymer and two in the center. In brief, PEG-18 500 (20 g) was dissolved in 200 mL of dry benzene under nitrogen and heated at 40 °C until fully dissolved. The solution was cooled in an ice bath, followed by addition of 0.7 mL of triethylamine and 1.13 mL of acryloyl chloride. The mixture was then heated to reflux for 2 h, followed by stirring overnight at room temperature under nitrogen. The solution was filtered to remove the amine salts formed during the reaction, and then the polymer was precipitated in *n*-heptane. The final product was isolated as a powder by subsequent drying at room temperature in a vacuum oven. FT-IR spectroscopy confirmed that hydroxyl groups on the polymer were acrylated to near completion.

3-(Trichlorosilyl)propyl methacrylate (TPM) was obtained from Fluka Chemicals (Milwaukee, WI). Hydrogen peroxide was purchased from EM Science (Gibbstown, NJ). Sulfuric acid and glass slides were obtained from Fisher Scientific (Fair Lawn, NJ). Glucose oxidase, acetylcholine esterase, acetylcholine chloride, potassium phosphate monobasic, sodium phosphate dibasic heptahydrate, and sodium chloride were purchased from Sigma Chemical Company (St. Louis, MO). Carbon dioxide of 97.7% purity was purchased from Botco (Bryan, TX). The succinimidyl ester variant of 5- (and 6)-carboxy SNAFL-1 was purchased from Molecular Probes (Eugene, OR). All reagents were used as received. Four inch diameter, (111), n-doped silicon wafers with a thickness of approximately 500 μm were purchased from Wafer World, Inc. (West Palm Beach, FL). Phosphate buffered saline (PBS, 0.1 M, pH 7.4) consisted of 1.1 mM potassium phosphate monobasic, 3 mM sodium phosphate dibasic heptahydrate, and 0.15 M NaCl in 18 MΩ·cm deionized water (E-pure, Barnstead, Dubuque, IW).

Equipment. Photopolymerization was performed using an uncollimated, 365 nm, 300 mW/cm^2 light source (EFOS Ultracure 100ss Plus, UV spot lamp, Mississauga, Ontario). Scanning electron microscopy (SEM) was performed with a JEOL T330A at 15 kV (JEOL, Ltd., Peabody, MA). Before SEM characterization, substrates were attached to aluminum mounts with carbon tape and sputter-coated with gold to a thickness of 200 Å. A Veeco Dektak3 (Veeco Instruments, Plainview, NY) profilometer was employed to obtain the heights of individual array members. A Zeiss Axiovert 135 microscope equipped with an integrated CCD camera and fluorescent optical package (Carl Zeiss Inc., Thosrnwood, NY) was used to determine the lateral dimensions and examine the fluorescence of hydrogel microstructures. Fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC) microscope fluorescence filters with excitation and emission wavelengths of 480 ± 30 nm/535 ± 40 nm (FITC) and 540 ± 25 nm/605 ± 50 nm (TRITC) were used when observing the fluorescent nature of the encapsulated dye. Fluorescent spectra of the SNAFL−protein conjugates were recorded using a fluorescence spectrometer (QM-1, Photon Technology International, Monmouth Junction, NJ).

A Gaertner L2W26D ellipsometer (Gaetner Scientific Corp., Chicago, IL) with a He/Ne (632.8 nm) red laser at a 70.00 ± 0.02° angle of incidence was used for thickness measurements of trichlorosilane monolayers. Refractive indexes and film thickness were determined by assuming a homogeneous film model provided by Gaertner software. Attenuated total reflectance/Fourier transform infrared (ATR/FTIR) spectroscopy was performed using a Mattson 5020 spectrophotometer with a ZnSe crystal (Spectra-Tech Inc., Shelton, CT). Time-of-flight secondary ion mass spectroscopy (TOF SIMS) was achieved by bombarding silicon wafers with ^{252}Cf fission fragments. The mass spectrometer used in these experiments is discussed in detail elsewhere.[26,27] Both negative and positive secondary ion (SI) species were analyzed under the same experimental conditions. The bias voltage for the SI species was ±4 kV. The chrome sodalime photomasks used for patterning of the arrays were obtained from Advance Reproductions (Andover, MA). A USAF negative target mask was purchased from Melles Griot (Irvine, CA).

Surface Functionalization. The modification of silicon wafers or borosilicate glass substrates with TPM was performed using standard protocols for silane surface modifications.[28] In brief, substrates were cleaned in "piranha" solution consisting of a 3:1 ratio of 30% w/v aqueous solutions of H_2SO_4 and H_2O_2 (*caution: this mixture reacts violently with organic materials and must be handled with extreme care*), washed with DI H_2O, and dried under nitrogen. Substrates were then treated for 5 min, at room temperature, in a 1 mM solution of TPM in a 4:1 ratio of heptane−carbon tetrachloride in an N_2 atmosphere, followed by washing with hexane and water.

Preparation of SNAFL-GOX and SNAFL Labeled Acetylcholine Esterase. Using an established protocol, SNAFL-1 labeled glucose oxidase (SNAFL-GOX) or acetylcholine esterase (SNAFL-AChE) was prepared by reacting the succinimidyl ester activated version of SNAFL-1 (1 mg of dye dissolved in 100 μL of DMSO) with the lysine residues present on the enzyme dissolved in 100 mM PBS (adjusted with NaOH to pH 8.2)

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according to an established protocol.[29] Unreacted dye was separated overnight by dialysis. The final enzyme concentration was approximately 2 mg/mL.

Fabrication of Hydrogel Patterns. PEG hydrogel patterns were prepared from PEG-DA and PEG-DMA as well as PEG-tetraacrylate (PEG-TA). In experiments using PEG-DA as the macromer, a precursor solution containing 10 mg of DMPA dissolved per milliliter of macromer was prepared. When fluorescent hydrogel arrays were to be prepared, 50 μL of the SNAFL labeled enzyme solution per milliliter of precursor solution was also added. This viscous precursor solution (PEG-DA is a liquid at room temperature) was then spin-coated at 3500 rpm onto the surface of an acrylated wafer, forming a uniform polymer layer. As is well-known for spin-coated films such as photoresists, the thickness of the polymer film was dependent on the viscosity of the precursor solution, the angular velocity, and the spin-coating time.[30] The layer was covered with a photomask and exposed to 365 nm, 300 mW/cm^2 UV light for 0.6 s in air.

Arrays based on PEG-DMA were fabricated in a similar fashion. However, PEG-DMA, a waxy solid at room temperature, required melting at 40 °C prior to use. The viscous macromer solution was then applied to the substrate at 40 °C, and the coated substrate was cooled to room temperature prior to photopatterning. Thus, PEG-DMA films were patterned in the solid state with the photomask brought into direct contact with the hardened polymer film.

To prepare patterns with those of PEG-TA, the macromer was dissolved in DI water (30% w/v). DMPA (600 mg) was dissolved in 1 mL of 1-vinyl-2-pyrrolidinone. Finally, 10 μL of the photoinitiator solution was added to 1 mL of the aqueous PEG-TA solution. This viscous precursor solution was spin-coated in the same manner as described above. Because of the lower concentration of the polymer in the precursor solution, the time of UV exposure, however, was extended to $\sim$1 min to permit the diffusion and subsequent reaction of acrylate moieties. The photomask was brought into either close proximity to or contact with the polymer solution layer.

These films behave as negative photoresists. Areas exposed to UV light underwent free radical polymerization and subsequent cross-linking, thus becoming insoluble in the developer. Patterns based on all macromers could be developed by washing away un-cross-linked macromer with anhydrous toluene or DI water.

The development of microstructures based on low molecular weight PEG-DA was also performed using supercritical CO_2 (SC-CO_2) to remove unpolymerized PEG from the surface. After UV lithography, the substrates were placed into a 316-stainless steel pressure cell constructed from a 2-in. by 2-in. long cylinder with a 1.125-in. bore. The pressure cell was pressurized to 80 bar with CO_2 by a miniPump metering pump (model number 870046, from Thermo Separation products, Riviera Beach, FL). The temperature of the pressure cell was maintained at 35 °C by a heating mantle (Rope Heater FGR-030, from Omega, Stamford, CT) equipped with a temperature controller (model CN 76000, from Omega, Stamford, CT). Once the pressure of 80 bar was achieved, isobaric conditions were maintained by a back-pressure regulator (model number 26-1722-24-161, obtained from Tescom Corporation, Elk River, MN). A CO_2 flow rate of 150 mL/h was maintained for 2 h by a LC-5000 syringe pump (Isco, Lincoln, NE). The system was then returned to atmospheric pressure, and the patterned materials were removed.

Fluorescence Detection. The fluorescence of SNAFL-GOX containing microstructures was examined using filtered light on the fluorescence microscope. Filters with excitation/emission wavelength ranges of 480 $\pm$ 30 nm/535 $\pm$ 40 nm and 540 $\pm$ 25 nm/605 $\pm$ 50 nm were used to visualize the emission of the immobilized fluorophore under acidic and basic conditions, respectively. The silicon wafer containing the hydrogel array elements was placed on a glass slide cover slip and examined for fluorescence microscopy. Wafer elements were exposed to solutions of 0.1 M PBS, adjusted to pH 12.5 and 5.5 with NaOH and HCl, respectively, for approximately 5 min to alter hydrogel pH. Elements were first examined in a dehydrated state after development with toluene. They were then hydrated in 0.1 M PBS and exposed to the acidic and basic solutions.

The fluorescence of SNAFL-AChE containing microstructures was examined using filtered light on the fluorescence microscope. Filters with excitation/emission wavelength ranges of 480 $\pm$ 30 nm/535 $\pm$ 40 nm and 540 $\pm$ 25 nm/605 $\pm$ 50 nm were used to visualize the emission of the immobilized fluorophore. The silicon wafer containing the hydrogel array elements was placed on a glass slide cover slip and examined for fluorescence microscopy. Wafer elements were exposed to solutions of 0.01 M PBS with acetylcholine chloride concentration varied from 0 to 2 μM. The fluorescence intensity at each concentration was quantified using the ratio of intensity of emission at 535 nm compared to 605 nm, representing the acidic and basic forms of SNAFL-1.

Results and Discussion

Here we present an approach for the microfabrication of PEG hydrogel microstructures onto planar surfaces. The fabrication methods described here use self-assembled monolayers and photolithography to produce spatially defined, densely packed hydrogel microstructures with these elements ranging in diameter from 600 to 7 μm. These microstructures were characterized using SEM, light and fluorescent microscopy, and profilometry. Potential biosensor applications of these hydrogel microstructures were investigated by enclosing pH sensitive, protein-conjugated fluorescent dye (SNAFL) into the elements and altering the microenvironment pH within the gel either directly or through the addition of the enzyme substrate.

Surface Functionalization. In early experiments PEG patterns were generated on silicon surfaces cleaned with piranha solution but not modified with an adhesion promoting monolayer. While patterns could be developed, surface attachment was weak and array elements easily delaminated upon hydration due to swelling of the cross-linked PEG-DA matrixes. To prevent delamination, a self-assembled monolayer of 3-(trichlorosilyl)propyl methacrylate on SiO_2/Si was used to create a reactive surface onto which the gel was covalently affixed during photopolymerization.

The treatment of glass and SiO_2 surfaces with chlorosilanes or alkoxysilanes is a very common and effective way to form dense, self-assembled monolayers on silicon.[31−33] Here we have used a monolayer of TPM as an adhesion layer for the hydrogel microstructures. TPM modified surfaces were found to be highly hydrophobic, which indicated that surface modification took place, since untreated surfaces were hydrophilic. Ellipsometry measurements yielded thickness measurements of 14 $\pm$ 3 Å, pointing to the presence of a TPM monolayer on the silicon surface.

The composition of the monolayer was further characterized with TOF SIMS. A plasma desorption mass spectroscopy technique[34] was used for the analysis of these functionalized silicon surfaces, since this technique allows fragmentation and release of large mass fragments from the substrate surfaces.[35] In this case, clean and TPM modified silicon wafers were bombarded by ^{252}Cf fission fragments, after which mass spectra of positive and

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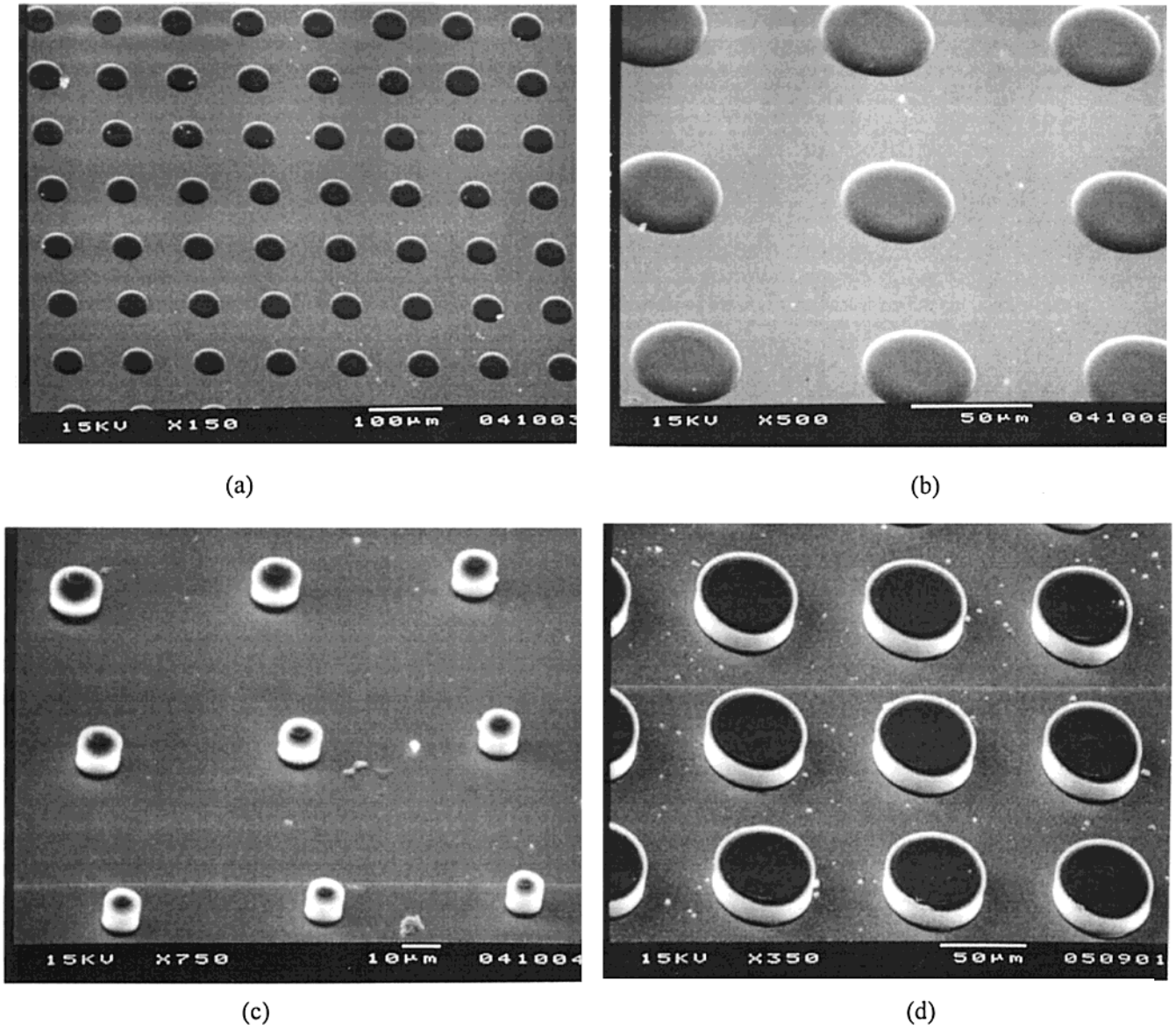

Figure 1. Scanning electron micrographs of PEG-DA (MW 575) gel microstructures on silicon: (a) 50 μm diameter microstructures; (b) higher magnification view of 50 μm diameter microstructures, (c) 7−10 μm diameter microstructures. The gels in images a, b, and c were developed in toluene. (d) 50 μm diameter microstructures developed in supercritical carbon dioxide.

negative secondary ions were collected and analyzed by means of TOF mass spectrometry. Analysis of the mass spectra of TPM modified surfaces showed that functionalized surfaces contained a greater amount of hydrocarbons in general as well as several distinct fragmentation patterns associated with TPM. Methacrylated trichlorosilane molecules contain carbonyl functionalities that are sites of a characteristic α cleavage. Emission intensities of masses 41 and 69, which are associated with α cleavage of TPM, were present as was a fragment of mass 85, corresponding to the $OC(O)C(CH_3)=CH_2$ moiety of TPM. Another very distinct peak collected in the positive spectrum is mass 85, which corresponds to $C_4H_5O_2$. Hence, evidence collected by mass spectroscopy points to the presence of methacrylated trichlorosilane molecules on the silicon substrates. Altogether, TOF SIMS and ellipsometry indicate that successful surface modification occurred, resulting in formation of a TPM monolayer on the silicon substrate.

Fabrication of Micropatterned PEG Hydrogels. The formation of hydrogel microstructures from PEG-acrylates or -methacrylates was based upon the UV initiated free-radical polymerization of acrylate or methacrylate end groups on the PEG derivatives. DMPA, a photoinitiator used in these studies, dissociates upon exposure to UV radiation, creating highly reactive methyl and benzaldehyde radicals, which then attack unsaturated carbon−carbon double bonds (C=C) of (meth)acrylate functionalities on the macromer, thus initiating free radical polymerization. Since two reactive centers per macromer are created, propagation results in the formation of a poly(meth)acrylate network highly cross-linked with PEG. This network represents a three-dimensional, insoluble structure, capable of entrapping sensing agents such as fluorophore labeled proteins.

The conversion of C=C bonds in these gels was previously monitored using ATR/FTIR spectroscopy. Thin films of PEG-DA were placed on the ZnSe ATR crystal, and an IR spectrum was obtained before and after exposure to 365 nm, 20 mW/cm^2 UV light. Maximum conversion in excess of 90% was achieved within a few seconds of illumination.[36] Since the intensity of the UV source used for actual patterning was an order of magnitude higher (300 mW/cm^2) than that used in the IR studies, a cross-

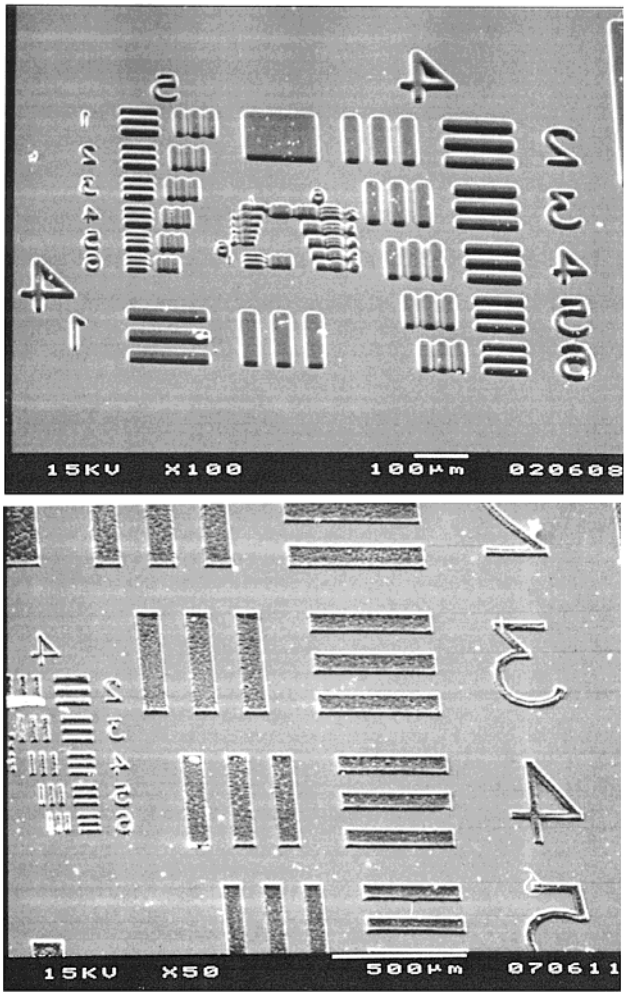

Figure 2. Scanning electron micrographs of patterned hydrogel images of a USAF test target mask: (a, top) gels based on PEG-DA; (b, bottom) gels based on PEG-TA. PEG-TA gels appear significantly rougher because these gels have a high equilibrium water content and thus collapse when dried for SEM analysis.

linked network was created in 0.5 s of exposure to UV light. One should noted, however, that C=C conversion cannot be completely correlated to polymerization in the gels because of the presence of oxygen during illumination. Oxygen can easily react with radicals formed in the gel and effectively terminate propagation. Thus, a fraction of (meth)acrylate groups will be converted to oxygenated species rather than participate in the polymerization process.

The methacrylated surface of the silicon substrate will also take part in the free-radical polymerization reaction because it contains unsaturated C=C functionalities. Methacrylate groups on the surface of the substrate react with radical species present near the surface to effectively anchor the gel to the surface of the silicon. Thus, delamination or detachment of PEG networks from the substrate did not occur, even though extensive swelling did occur in the gels upon exposure to water. Though methacrylates are less reactive that acrylates, no difference in adhesion was seen for gels based on either acrylate or methacrylate terminated macromers.

The ability of PEG-DA to gel upon exposure to UV light was used to create negative patterns using photolithography. Such patterns were created by spin-coating the gel precursor onto the substrate and then exposing the coated surface with UV light through a carefully aligned photomask placed on top of the precursor solution layer. Light was projected through the photomask, creating polymerized regions that corresponded to the pattern of the photomask. Unpolymerized PEG was then dissolved in toluene, H_2O, or SC-CO_2 so that only the insoluble, cross-linked patterns remained on the surface. A chrome-coated sodalime photomask used for photolithography contained circles with diameters of 600, 50, and 5 μm arranged into three squares with 20 × 20 elements in each square. When using PEG-DA as the macromer in the precursor solution, these patterns were reproduced on the silicon surface as cylinders with height 8 ± 0.1 μm (in the dry state), as determined by profilometry. Microstructures made using PEG-DMA and PEG-TA were 12 and 3 μm, respectively in height. Proximity lithography was used for patterning 600 μm elements, whereas contact lithography was employed to create arrays of elements 50 μm and smaller in diameter. Contact printing was used because the minimum resolution of a feature is directly proportional to the square root of the gap between a photomask and polymer layer;[30] that is, the gap has to be minimized in order to obtain images at the resolution shown here.

To determine the effect of water-induced swelling on these patterns, PEG-DA, -DMA, and -TA based hydrogel microstructures were generated on glass substrates which underwent treatment analogous to that described for silicon. Array elements immobilized on glass were studied with a transmission light microscope equipped with a video microscopy system. Images of elements before and after hydration were then captured and measured using image analysis software. Hydrogel microstructures were generated on SAM treated and untreated glass surfaces and developed in anhydrous toluene to prevent premature hydration. The diameter of cylindrical elements was then measured before and after hydration. Hydrogel elements based on PEG-DA immobilized on an untreated glass surface showed a 10% increase in diameter upon introduction of water. This result corresponds well to the swelling behavior of PEG-DA spheres characterized in an earlier published study.[36] Upon hydration, hydrogel microstructures easily detached from surfaces not treated with TPM, primarily due to the mechanical forces associated with volume changes in the gel during water induced swelling. On the other hand, hydrogel structures fabricated on TPM treated glass substrates did not show an increase in diameter at the base of the structures because they were covalently fixed to the substrate surface. However, PEG-DA array elements became visibly swollen and increased in height by nearly 30%. Height changes of 55% and 158% were observed for gel microstructures made from PEG-DMA and PEG-TA, respectively. Such behavior is expected because surface anchoring of the gel prevents the hydrogel array elements from swelling laterally at their base but does not inhibit swelling upward.

The morphology of the generated patterns was studied with SEM. Figure 1a and b shows hydrogel patterns with 50 μm diameter elements and a 50 μm distance between the elements. Clearly defined pattern areas with no residual polymer remaining on the substrate were observed. Inspection of an individual array member at higher magnification (not shown here) revealed very smooth surfaces without visible defects in the polymer network. The array shown in Figure 1a contains nearly 300 defect-free elements. The view of the elements presented in Figure 1b emphasizes the fact that hydrogel arrays are three-dimensional matrixes immobilized on a planar surface. This point is further illustrated by Figure 1c,

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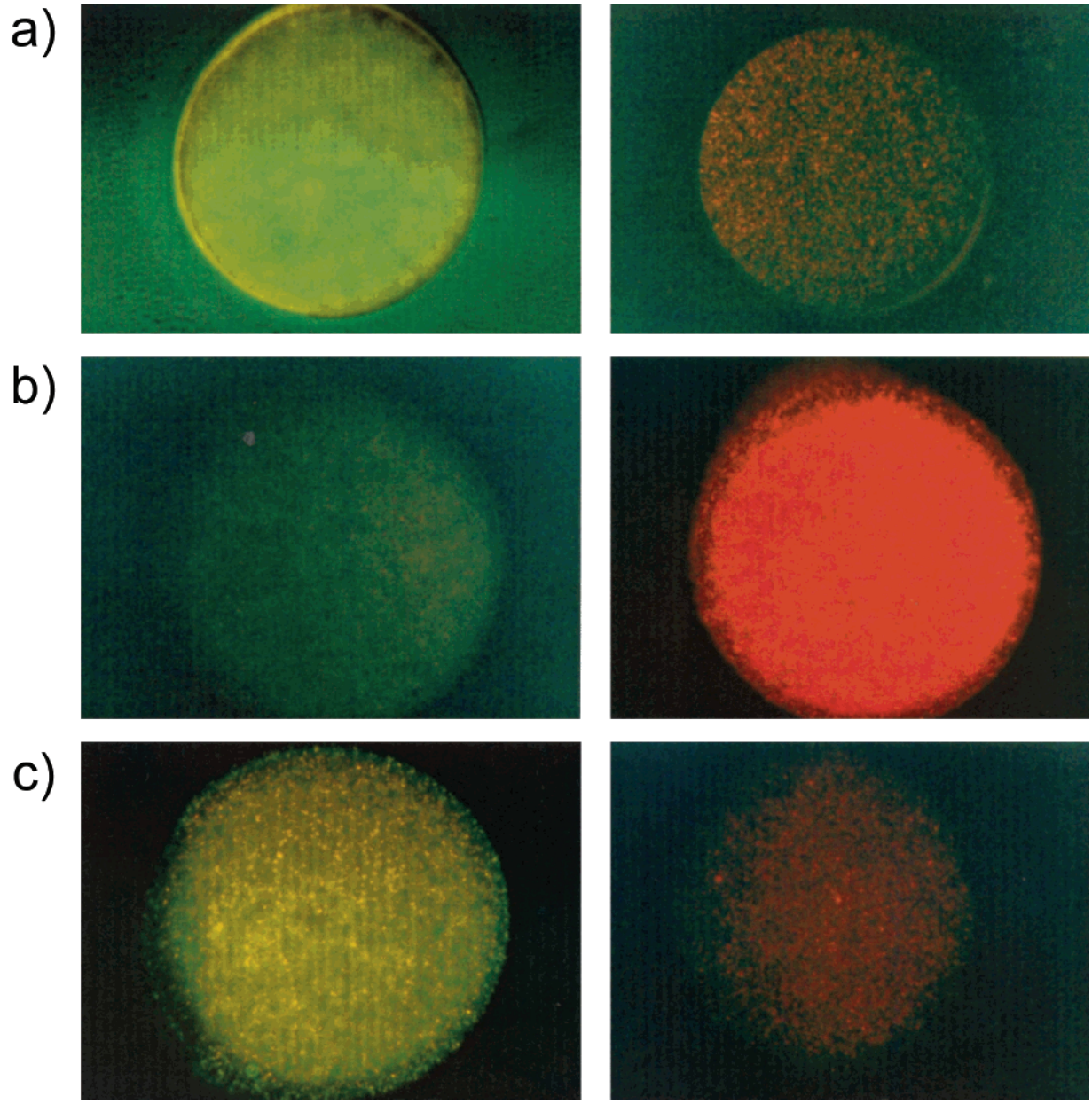

Figure 3. Fluorescent micrographs of a SNAFL-1 labeled protein encapsulated inside of a 600 μm PEG-DA hydrogel microstructure: left, fluorescein filtered light; right, tertramethyl rhodamine filtered light. (a) The gels following development in toluene, (b) hydrogel after exposure to 0.1 M PBS (pH 12.5), and (c) hydrogel after exposure to 0.1 M PBS (pH 5.5).

where 7−10 μm diameter hydrogel elements separated by a distance of 50 μm are presented. The three-dimensional nature of these elements is even more obvious because of an increase in the aspect ratio. It should also be noted that a 5 μm mask feature was used to create the 7−10 μm diameter elements. The increase in feature size on the substrate as compared to the mask is likely due to the mass transfer of free radical species outside the illuminated area and their subsequent reaction, a phenomenon observed with other photopolymerized systems.[37]

The potential for creating microstructures of various geometries from PEGs of different molecular weights was explored by creating hydrogel patterns from an USAF test target mask. Acrylated and methacrylated PEG molecules of three molecular weights (575, 1000, and 18 500) were successfully patterned on silicon surfaces. SEM micrographs of microstructures based on PEG-DA and PEG-TA are shown in Figure 2. The surface morphologies of the two patterns are quite different, with microstructures based on PEG-TA exhibiting higher surface roughness. This is attributed to evaporation of water from the gel prior to metal coating for electron microscopy. Because PEG-TA gels have a high equilibrium water content (in excess of 90%),[38] they will collapse upon drying with the resulting structure having increased surface roughness. Overall, acrylated or methacrylated PEG derivatives of all three molecular weights proved well suited for patterning because hydrogel microstructures consisting of numerous features ranging from several hundred to a few microns were reproducibly obtained.

Supercritical Development. While pattern development using organic solvents as discussed in the previous section yielded satisfactory results, the exposure of the PEG arrays to possibly toxic chemicals, for example,

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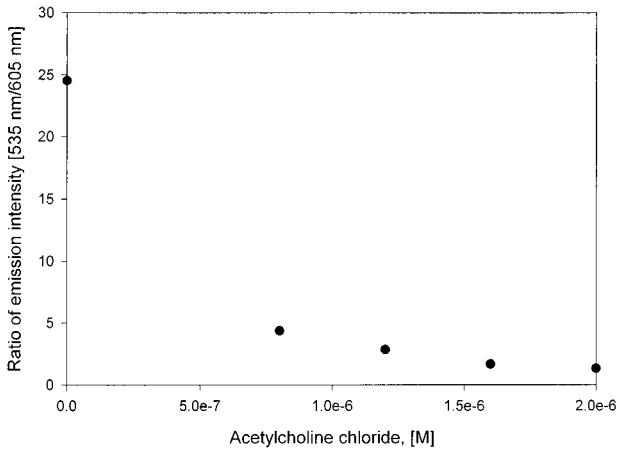

Figure 4. Change in fluorescence emission intensity of SNAFL-AChE immobilized in a PEG microstructure as a function of acetylcholine chloride concentration in 0.01 M PBS. Fluorescence intensity was quantified as the ratio of the emissions at 535 nm compared to 605 nm, representing the acidic and basic forms of the dye.

toluene, may be detrimental to the cell and tissue compatibility of the hydrogel microstructures and the activity of the proteins entrapped in them. Numerous studies have shown that proteins lose some or all of their biological activity when in contact with anhydrous organic solvents[39,40] because of denaturing or loss of the secondary structure. Therefore, an alternative route for development of the hydrogel arrays was explored. As described earlier, water may be used to develop patterns. However, one may wish to keep the gels in the dehydrated state prior to use to preserve the activity of encapsulated biomolecules during storage.

CO_2 is an environmentally benign, nontoxic compound which is being used in the pharmaceutical industry as a reaction medium for synthesis of drug compounds[41,42] and as a medium for processing protein powders with retention of activity.[43,44] In addition, SC-CO_2 is being investigated as a possible medium for photoresist image development.[45,46] The motivating factor for the latter studies is the potential of this solvent in achieving high feature resolution as well as the ability to control solvating power by varying temperature or pressure. Therefore, the use of SC-CO_2 for development of patterned biosensor elements may be advantageous because this solvent has been shown to be benign with respect to protein activity while still possessing sufficient solvating power to develop a lithographically patterned organic thin film.

The main concern for the supercritical development of polymer images is the solubility of a given polymer in the supercritical fluid (SCF). Johnston and colleagues reported PEG (MW 600) to be slightly soluble in the supercritical phase, with solubility improving upon addition of surfactants.[47] In the present work, the limited solubility of the polymer in the SCF was overcome due to a continuous supply of CO_2 (150 mL/h) and the facts that only a small amount of un-cross-linked polymer (much less than 0.3 mL) needed to be removed from the surface and that the contact area between SC-CO_2 and the thin film is large. One should note, however, that the development of patterns using SC-CO_2 is unlikely to work for PEG macromers of higher molecular weight because the solubility of PEG in SC-CO_2 will drop substantially as molecular weight increases.

Microstructures based on PEG-DA with diameters of 600, 50, and 5 μm were successfully patterned on silicon surfaces by the method described earlier. A SEM micrograph of an array consisting of 50 μm elements developed in supercritical media presented in Figure 1d compares very favorably to patterns developed in organic solvents. In fact, no differences in morphology of individual array elements of different diameters developed with the two techniques were observed. Hence, SC-CO_2 may prove to be an excellent medium for developing PEG-DA hydrogel patterns.

Fluorescence Sensing. To investigate the potential use of PEG-based microstructures in optical sensors, fluorescent microstructures were fabricated by incorporating a SNAFL-1 labeled protein into the precursor solution. SNAFL-1 is a pH-sensitive dye which exhibits both intensity and emission wavelength changes with changing pH. The dye pK_a is approximately 7.8. In an acidic environment, free dye is optimally excited at 510 nm, with an emission maximum at 545 nm.[48] In a basic environment, the optimum excitation/emission values red shift to 542/645 nm. The conjugated dye had acidic and basic emission maximums of 538 and 620 nm, respectively.

Figure 3 shows a fluorescent micrograph of a 600 μm array element with encapsulated dye labeled protein. Figure 3a shows a microstructure that has undergone development in toluene. When observed on a fluorescence microscope, both 600 and 50 μm array elements initially showed a slightly acidic spectrum, with strong emission through the FITC filter and weak emission through the TRITC filter. These results agree with previous results which showed that the environment inside PEG hydrogels is slightly acidic in unbuffered media.[6] As shown in Figure 3b, after exposure to a buffered solution with a basic pH of 12.5, hydrogel fluorescence underwent a strong red shift. The fluorescent response was reversed by soaking the patterned substrate in a small amount of acidic buffer (pH 5.5), as shown in Figure 3c. Physical encapsulation of SNAFL-GOX inside the PEG hydrogel network was sufficient to prevent dye diffusion out of the gel, over a 1 week period, because the mesh size for a gel of this nature is less than 15 Å. Photobleaching of the dye was not observed during photopolymerization or during repeated excitation of the gels over a 1 week period. As is apparent from the turbidity of the gels in Figure 3, the protein was not soluble in the gel precursor solutions and thus was encapsulated as agglomerates in the gel. This result was also observed previously with proteins entrapped in PEG hydrogel microspheres for use in chemical sensing.[5,6] Proteins entrapped in this manner retained activity, but it is unclear to what extent activity was diminished by agglomeration.

Figure 4 shows the change in fluorescence for SNAFL-labeled acetylcholine esterase immobilized in a 600 μm PEG microstructure. As can be seen, a local pH change

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occurs in the gel, indicating the production of acetic acid from the hydrolysis of acetylcholine. These results indicate that some enzyme activity was retained after the UV polymerization process. We have, in three previous studies, encapsulated proteins such as glucose oxidase, lactate oxidase, concanavalin A, and organophosphate hydrolase.[4−6] In all cases, we have found these proteins to retain activity or binding capacity after the photopolymerization process under nearly identical conditions to those used here. The protein we have studied most thoroughly is glucose oxidase, which we showed in a previous manuscript to lose activity with prolonged exposure to 365 nm UV light. This was attributed to the destruction of the flavin adenine dinucleotide center of glucose oxidase by UV light. There are other potential mechanisms of protein inactivation, including hydrogen abstraction reactions involving the protein during the polymerization process. Obviously, levels of protein inactivation will vary from protein to protein.

Conclusion

We have described the formation of PEG hydrogel microstructures grafted to silicon and glass surfaces. Surface functionalization involving self-assembly of trichlorosilanes on SiO_2/Si was used to ensure attachment of the hydrogel to the substrate surface. After exposure of gel precursor solutions through a photomask, patterns could be developed using solvents such as water or supercritical CO_2. Photolithography was used to obtain hydrogel patterns which could be used for optical sensing, as shown by the incorporation of a pH-sensitive fluorophore into the matrix. This work only utilized one fluorophore and a single spin step as a preliminary study into optical sensing. Multianalyte fluorescence sensor arrays should prove simple to fabricate, however, by repeatedly aligning the wafer and spin-coating additional elements from precursor solutions containing alternate fluorophores. Alternatively, PEG hydrogel precursor solutions could be robotically dispensed on a treated substrate and subsequently photopolymerized to form hydrogel microstructures.

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