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Indispensable Platforms for Bioimmobilization: Maleimide-Based Thiol Reactive Hydrogels

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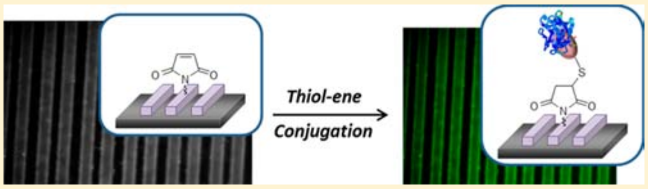
Supporting Information

ABSTRACT: Poly(ethylene glycol)-based hydrogels containing thiol-reactive maleimide functional groups is prepared via a Diels–Alder/retro Diels–Alder reaction sequence using a masked maleimide monomer. Bulk and micropatterned hydrogels containing varying amounts of the thiol-reactive maleimide functional group are fabricated at ambient temperature. During the fabrication, the reactive maleimide functional group in the monomer is masked with a furan moiety and then unmasked to its reactive form via the retro-Diels–Alder reaction. The reactive maleimide groups embedded within the hydrogel are amenable to facile and efficient functionalization with thiol-containing molecules such as fluorescent dyes. Furthermore, these hydrogels are readily biotinylated using the nucleophilic thiol–ene conjugation to enable immobilization of streptavidin onto the hydrogel patterns to achieve facile bioimmobilization. Notably, the extent of functionalization of these hydrogels can be easily tailored by varying the amount of reactive handles incorporated during their fabrication.



1. INTRODUCTION

Design and synthesis of novel reactive polymeric materials have attracted widespread attention in recent years, since they allow fabrication of functional polymeric interfaces that play a crucial role in many areas of material and biomedical sciences.[1] In particular, cross-linked hydrophilic polymeric materials commonly referred to as hydrogels find application in areas such as biomolecular immobilization, tissue engineering, fabrication of biosensors, coatings for implants, and drug delivery.[2] Apart from bulk hydrogels, patterned hydrogels are often designed to mimic natural tissue and are becoming a platform of choice for fundamental study to understand physical, chemical, and biological cues at the cellular level.[3] Fabrication of hydrogels using photoinduced polymerization is widely employed since it offers spatial and temporal control, usually with fast curing rates at room temperature under physiological conditions. Photopolymerized hydrogels have been employed in biomedical applications such as preparation of scaffolds for prevention of thrombosis,[4] postoperative adhesion formation,[5] drug delivery,[6] coatings for biosensors,[7] corneal and orthopedic therapy,[8] and cell transplantation.[9,10]

Applications such as analyte detection and sensing benefit upon employing 3D hydrogel platforms, since 2D slides that are usually employed are often associated with low loading capacities and a weak detection signal.[11] Recently, Spring and co-workers reported PEG-based *N*-hydroxysuccinimide (NHS) containing 3D hydrogel platforms and studied their functionalization using micro contact printing.[12] Patterns on 3D hydrogel layer displayed improved loading capacity, signal sensitivity, and spot morphology when compared with 2D platform. Likewise, a hydrogel microarray that uses various "click" reactions for efficient multifunctionalization was reported by Hawker and co-workers.[13] "Clickable" functional groups such as alkenes and activated esters were incorporated onto the hydrogel surfaces during their fabrication to obtain versatile platforms for facile immobilization of functional molecules such as peptides and dyes.

To date, numerous functional groups have been incorporated into hydrogels to provide a handle for functionalization, attachment, and immobilization of bio(molecules) of interests. Although a number of chemical handles such as *N*-hydroxysuccinimide-based activated esters are available toward this end, the utilization of a thiol-reactive functional group such as maleimide will provide an attractive alternative. Maleimide is an attractive functional group for covalent immobilization of biomolecules and is orthogonal to a large variety of chemical groups that are fast and quantitative, provided a suitable combination of diene and dienophile is selected.[14] In biological science, the maleimide functional group has been long recognized for its selective and facial reactivity toward cysteine residues in protein. The thiol–maleimide conjugation is often utilized for the derivation of biomolecules with small molecules, conjugation of biomolecules, as well as biomolecular immobilization.[15] Although the maleimide group has been extensively exploited in biomolecular immobilization using monolayers on various metallic and glass surfaces,[16] polymeric materials containing maleimide group are not widespread. Synthesis of polymeric materials incorporating an active maleimide group is tricky since the reactive double bond of maleimide participates in radical polymerization. In recent years, protected maleimide-

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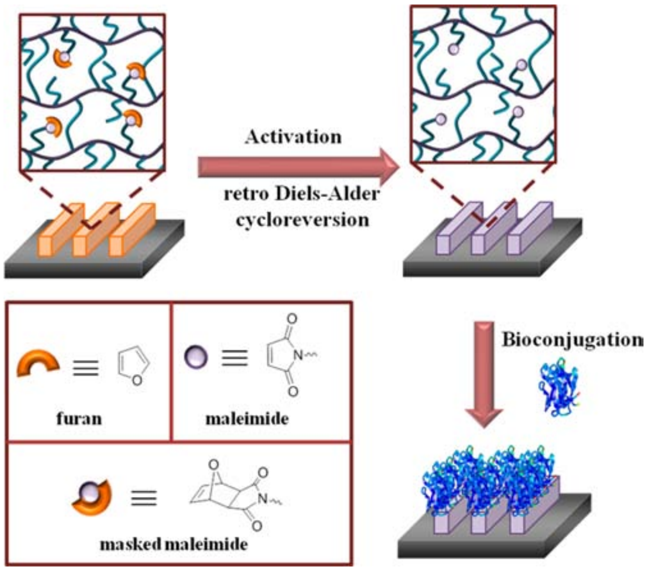
based initiators and monomers were introduced to obtain polymers with maleimide functionality as their end groups and as side chains using a Diels−Alder/retro-Diels−Alder reaction sequence as a protection−deprotection strategy.[17] Recently, we reported the synthesis of maleimide containing thiol reactive bulk hydrogels via a DA/retro-DA-based strategy. PEG-based monomer was polymerized with furan protected maleimide-based methacrylate monomer by using AIBN initiated polymerization at 65 °C.[18] Maleimide side chains from polymers were deprotected by retro-DA reaction during the to generate a cross-linker *in situ* to form hydrogel. Although the method was successful in generating bulk hydrogel, limitations due to hydrogel formation at high temperature curb the extension of this methodology to generate micropatterns and microarrays. A fabrication protocol to obtain reactive hydrogels at ambient temperature would broaden the scope and provide access to maleimide-based thiol reactive hydrogel patterns.

Herein, we outline the fabrication of novel thiol reactive highly functionalizable hydrogels via photopolymerization at ambient temperature. Bulk and patterned hydrogels are prepared by photopolymerization in the presence of PEG-DA cross-linker and a masked maleimide-based methacrylate monomer (Scheme 1). The masked-maleimide groups in the hydrogels are deprotected by retro-DA reaction to render them reactive toward thiol containing molecules via Michael addition under reagent free conditions. Incorporation of tailored amount of the thiol-reactive monomer into the feed allows one to obtain hydrogels with control over the density of functional groups in the matrix. Control over the incorporation of the thiol reactive maleimide groups and their availability for conjugation with thiol containing molecules were demonstrated by attachment of fluorescent dye and immobilization of FITC-streptavidin on the biotinylated hydrogels.

Scheme 1. General Scheme Illustrating the Fabrication and Functionalization of the Maleimide Containing 3D-Hydrogel Patterns Using a Diels−Alder/Retro-Diels−Alder Reaction-Sequence-Based Strategy



2. RESULTS AND DISCUSSION

2.1. Fabrication of Reactive Bulk Hydrogels.

Hydrogels containing varying amounts of masked maleimide monomer were prepared via photopolymerization of the furan-protected maleimide-containing monomer (FuMaMA) along with a comonomer PEGMEMA (M_n = 300 g mol^{-1}), a PEG-diacrylate cross-linker in the presence of DMPA as a photoinitiator (Figure 1). The PEG-based hydrophilic monomer was selected to obtain hydrogels with a bioinert matrix to minimize nonspecific adhesion of biomolecules. The recipe and the reaction parameters investigated in this study are summarized in Table 1, where bulk hydrogels are symbolized by BH-X. Bulk hydrogels BH-1, BH-2, and BH-3 containing 10, 25, and 40 mol % of the furan protected monomer FuMaMA were prepared. For various control experiments, bulk hydrogel BH-4, devoid of any reactive FuMaMA monomer, was prepared. Additionally, bulk hydrogel BH-1-1100 was fabricated using 10% FuMaMA monomer and a high-molecular-weight PEGMEMA (M_n = 1100 g mol^{-1}) monomer in order to probe the effect of hydrophilicity of the monomer on physical characteristics of the hydrogels. An initial survey of photoinitiated gelations was carried out in solvents such as methanol, water−methanol mixture, and dichloromethane. Photopolymerization proceeded with excellent conversions to yield clear and transparent hydrogels. In all cases, gelation proceeded with similar efficiencies and the obtained hydrogels possessed similar microstructure (as inferred from SEM) and water uptake characteristics. This is not surprising since hydrogel formation in this case does not involve any preorganization of the reactants in the solvent, unlike photo-cross-linked hydrogels obtained via cross-linking of ABA triblock copolymers. Due to higher solubility of FuMaMA in organic solvent in this work, we used dichloromethane as the solvent for fabrication of bulk hydrogels and preparation of hydrogel micropatterns.

In order to obtain thiol-reactive hydrogels, the removal of the furan protecting group was accomplished by subjecting the hydrogels to retro-Diels−Alder cycloreversion reaction by heating them to 110 °C in toluene. In order to probe the amount of incorporated FuMaMa monomer as well as observe the removal of the furan moiety, TGA analysis of bulk hydrogel samples before subjecting them to retro-DA reaction was utilized. The TGA result of hydrogels containing FuMaMA monomer (BH-1, BH-2, and BH-3) indicates a continuous weight loss starting from 60 to 180 °C, associated with the fragmentation of furan protected maleimide group of FuMaMA via the retro-DA reaction (Figure 2).[19] According to TGA, the observed weight losses were 2.0%, 6.1%, and 11.0% for bulk hydrogel BH-1, BH-2, and BH-3, respectively, and 0.73% for bulk hydrogel BH_{1100}-1 (Supporting Information Figure S3). This is in good agreement with the expected weight loss if all FuMaMA monomer was incorporated in the hydrogel during the photopolymerization (Table 1). As expected, no noticeable weight loss was observed for the hydrogel BH-4 since it does not contain the masked maleimide monomer. Additionally, elemental analysis of the hydrogels was undertaken to ascertain the FuMaMA contents. From the elemental analysis, significant increase in total nitrogen content in the gel was observed as the amount of FuMaMA monomer was increased in the feed (see Supporting Information Table S1).

The water uptake profiles of the photopolymerized bulk hydrogels, BH-1, BH-2, BH-3, and BH-4, are depicted in Figure 3. As the concentration of the relatively hydrophobic FuMaMA monomer increases, the final equilibrium swelling of the hydrogels reduces. For example, the swelling of the BH-0 reached 672% after 30 h, while the swelling ratios were 432%, 231%, and 81% for BH-1, BH-2, and BH-3 hydrogels,

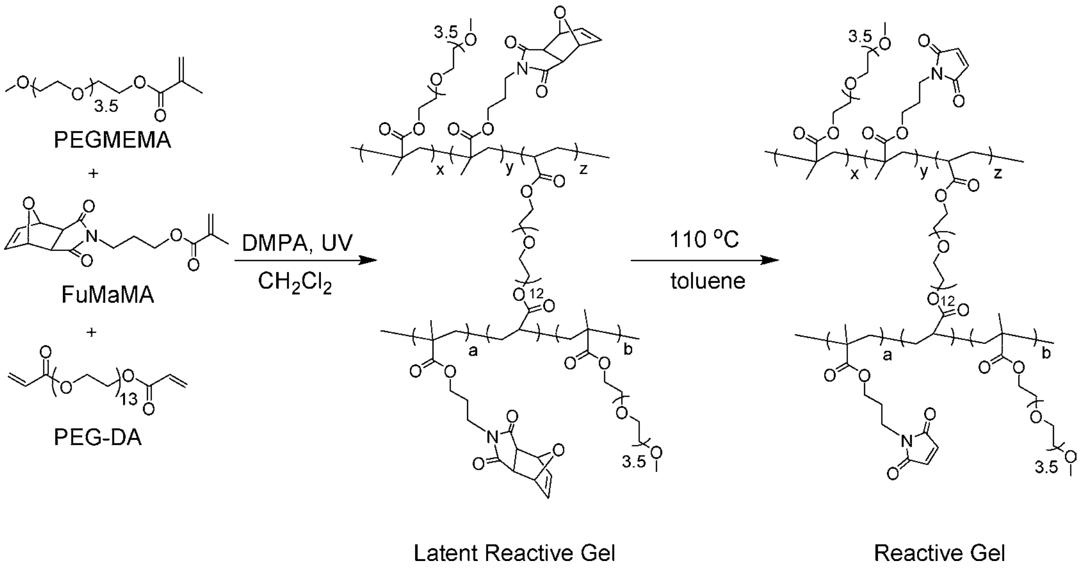


Figure 1. Fabrication of latent-reactive hydrogel via photopolymerization and activation to reactive hydrogel.

Table 1. Photopolymerization of Bulk Hydrogel in the Presence of FuMaMA

Sample	% (mole) of FuMaMA	Furan (weight %) observed[b]	Furan (weight %) theoretical[c]	Gel content (%)[d]
BH-1	10	2.0	2.13	98
BH-2	25	6.1	5.32	97
BH-3	40	11.0	8.52	99
BH-4[a]	0	0	0	97
BH-1-1100[e]	10	0.73	0.68	94

[a] Hydrogel without FuMaMA monomer. [b] Calculated from the amount of furan released as observed in TGA. [c] Calculated from the amounts of monomers and cross-linker which are used for hydrogel synthesis. [d] Conversion = (dry gel weight/total weight of monomer) × 100. [e] PEGMEMA monomer (M_n = 1100 g mol^{-1}) was used instead of PEGMEMA (M_n = 300 g mol^{-1}).

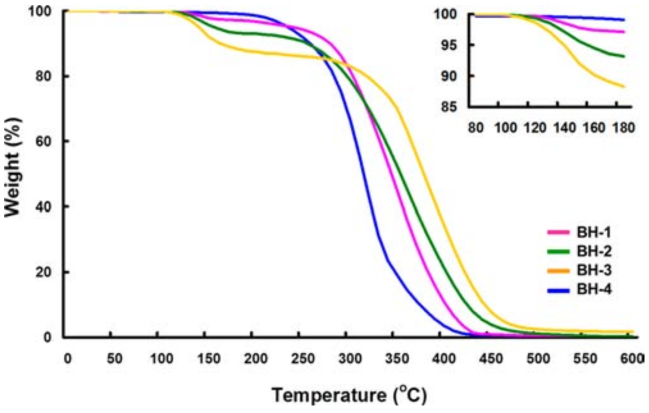


Figure 2. Quantification of furan-protected maleimide in hydrogels using TGA analysis obtained from bulk hydrogels BH-1, BH-2, BH-3, and BH-4.

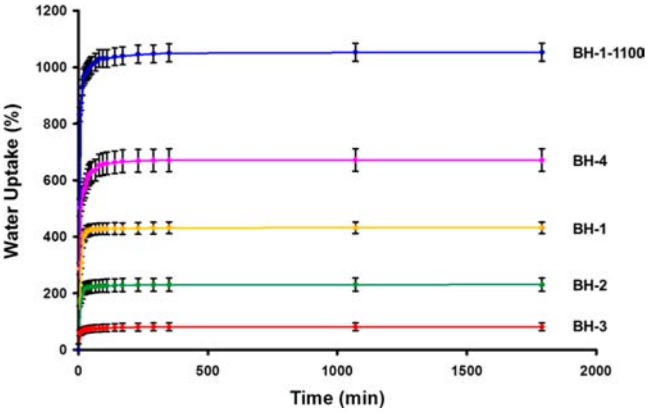


Figure 3. Swelling profiles of photopolymerized bulk hydrogels BH-1, BH-2, BH-3, BH-4, and BH-1-1100.

respectively. One can expect that the swelling will be enhanced by utilizing PEGMEMA monomers with longer hydrophilic chains. Hydrogel BH-1-1100 was synthesized using PEGMEMA (M_n = 1100 g mol^{-1}) along with 10% FuMaMA and compared with the hydrogel BH-1 that contained shorter PEG-based pendant chains. The water uptake of BH-1-1100 was 1053% as compared to 432% for BH-1.

Morphology of the bulk hydrogels was investigated with SEM (Figure 4 and Supporting Information Figure S2). In spite of possessing high water uptake capacity, the microstructure revealed a nonporous rubber-like morphology, although upon utilization of the PEG-based monomer with longer chain length, a more porous sponge like structure of the hydrogel (BH-1-1100) was clearly evident (Figure 4d).

2.2. Fabrication of Hydrogel Micropatterns. The protocol utilized for the fabrication and functionalization of micropatterned hydrogels is illustrated in Figure 5. In order to promote the adhesion of hydrogels onto the silicon surface, interfacial bonding photopolymerization was utilized. For this purpose, the surface of silicon wafer was treated with 3-(trimethoxysilyl) propyl methacrylate (TMSMA). Hydrogel patterns on the silicon wafer were prepared by using the MIMIC technique that involved photopolymerization of the trapped polymeric precursors into channels of PDMS stamp on the silicon surface. The optical microscope and SEM images of hydrogel pattern are shown in Figure 5. It was observed from microscopic imaging that hydrogel patterns were successfully prepared by photopolymerization whose average width of pattern was 30 μm (Figure 5b). The photopolymerized

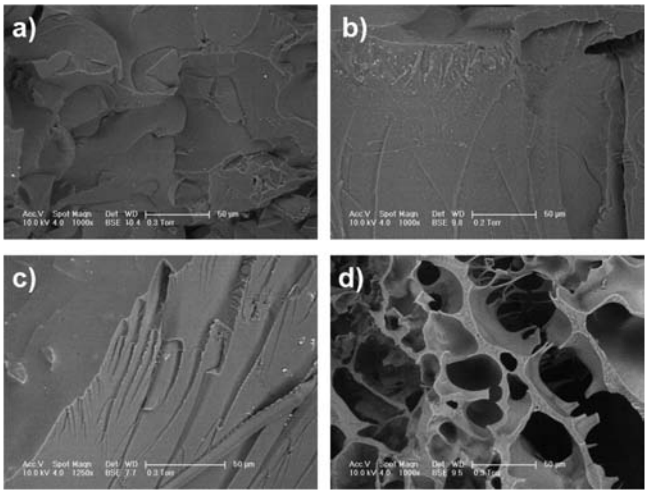


Figure 4. SEM images of bulk hydrogels: (a) BH-1, (b) BH-2, (c) BH-3, and (d) BH-1-1100. Scale bar is 50 μm.

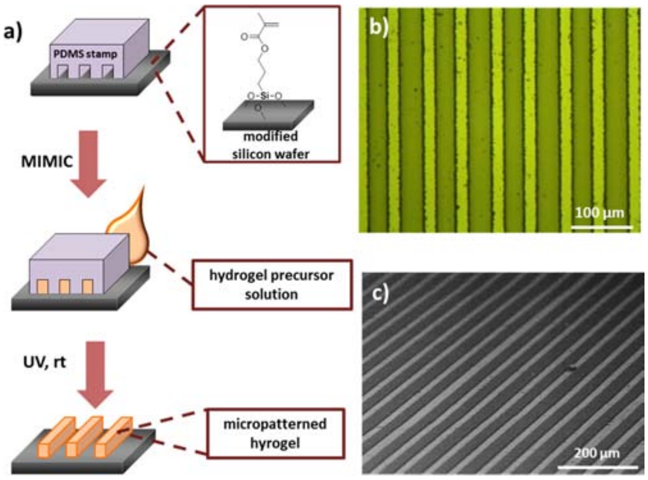


Figure 5. (a) Schematic illustration of micropatterning process and characterization of patterned hydrogel microstructures; (b) optical microscope image; and (c) scanning electron microscopy image.

hydrogel patterns are symbolized in the following sections by HP-X, similar to bulk hydrogels BH-X.

The hydrogel patterns were heated at 110 °C under vacuum for 30 min in order to unmask the FuMaMA groups within the hydrogel via the retro-DA reaction. No deterioration in the quality of the micropatterns was observed during the heating process, and thus-obtained hydrogel patterns adhered well onto the silicon surfaces and did not peel off after immersion and washing with various solvents.

2.3. Functionalization of Hydrogel Micropatterns via Thiol–Ene Conjugation.

The efficiency of functionalization of these hydrogel patterns was investigated by conjugation of a thiol containing fluorescent dye, namely, BODIPY-SH (Figure 6). As a control, the latent hydrogel patterns that were not activated via retro Diels–Alder reaction were exposed to BODIPY-SH. As expected, patterns prior to activation by retro-DA reaction did not exhibit any significant fluorescence. Activated patterns, on the other hand, were fluorescent due to the presence of thiol reactive maleimide groups after activation by retro-DA reaction.

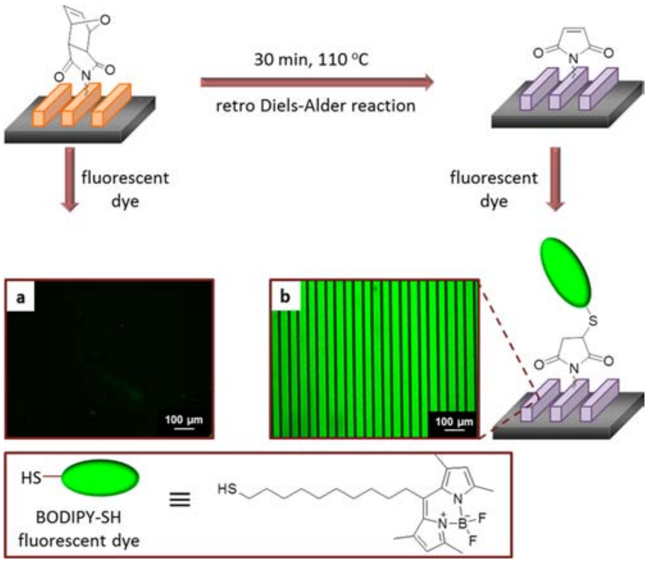


Figure 6. Immobilization of BODIPY-SH on the hydrogel patterns and fluorescence microscope images of BODIPY-SH conjugated hydrogel pattern; HP-1 before (a) and after (b) retro-DA reaction.

Biomolecular immobilization of a fluorescently labeled protein, namely, FITC-streptavidin, was used to demonstrate tuneability of the extent of functionalization by varying the reactive functional group in the hydrogel matrix. Conjugation of biotin-SH onto the patterned hydrogels made them amenable to specific immobilization of streptavidin (Figure 7). All hydrogel patterns (HP-1, HP-2, HP-3, and HP-4) containing active maleimide groups were reacted with excess thiol-containing biotin. Similar experiments of biotinylation were also carried out in bulk hydrogels, and the availability of incorporated biotins was analyzed using a HABA-Streptavidin assay (Supporting Information Figure S4). The assay revealed that the availability of biotin within the hydrogel toward streptavidin varied between 58% and 69% (Supporting Information Table S2). Considering the steric hindrances of the polymer chains and the diffusion within the hydrogels, an appreciable amount of ligand was available toward conjugation in these hydrogels. The biotinylated hydrogel patterns were exposed to a solution of FITC-streptavidin, and after washing off residual unbound streptavidin from the hydrogel pattern, samples were analyzed with fluorescence microscopy. The hydrogel pattern (HP-0) which is not biotinylated due to the absence of maleimide was also exposed to FITC-streptavidin. As expected, no significant fluorescence was observed due to minimal binding of streptavidin due to the bioinert nature of the PEG-based hydrogel matrix (Figure 7a). FITC-streptavidin conjugated hydrogel patterns with different amounts of maleimide were analyzed using fluorescence microscopy, and an increase in the fluorescence intensity was clearly evident with increasing maleimide functionality in the hydrogel, thus indicating facile tunability of extent of biomolecular immobilizations using these hydrogels (Figure 7 and Supporting Information Figure S5).

3. CONCLUSIONS

Hydrogels as bulk and micropatterns containing various amounts of maleimide functional groups that are reactive toward thiol-containing molecules were fabricated by photopolymerization of a masked maleimide group containing monomer in the presence of a photoinitiator and a PEG-DA-based cross-linker. Efficient conversion of the furan protected

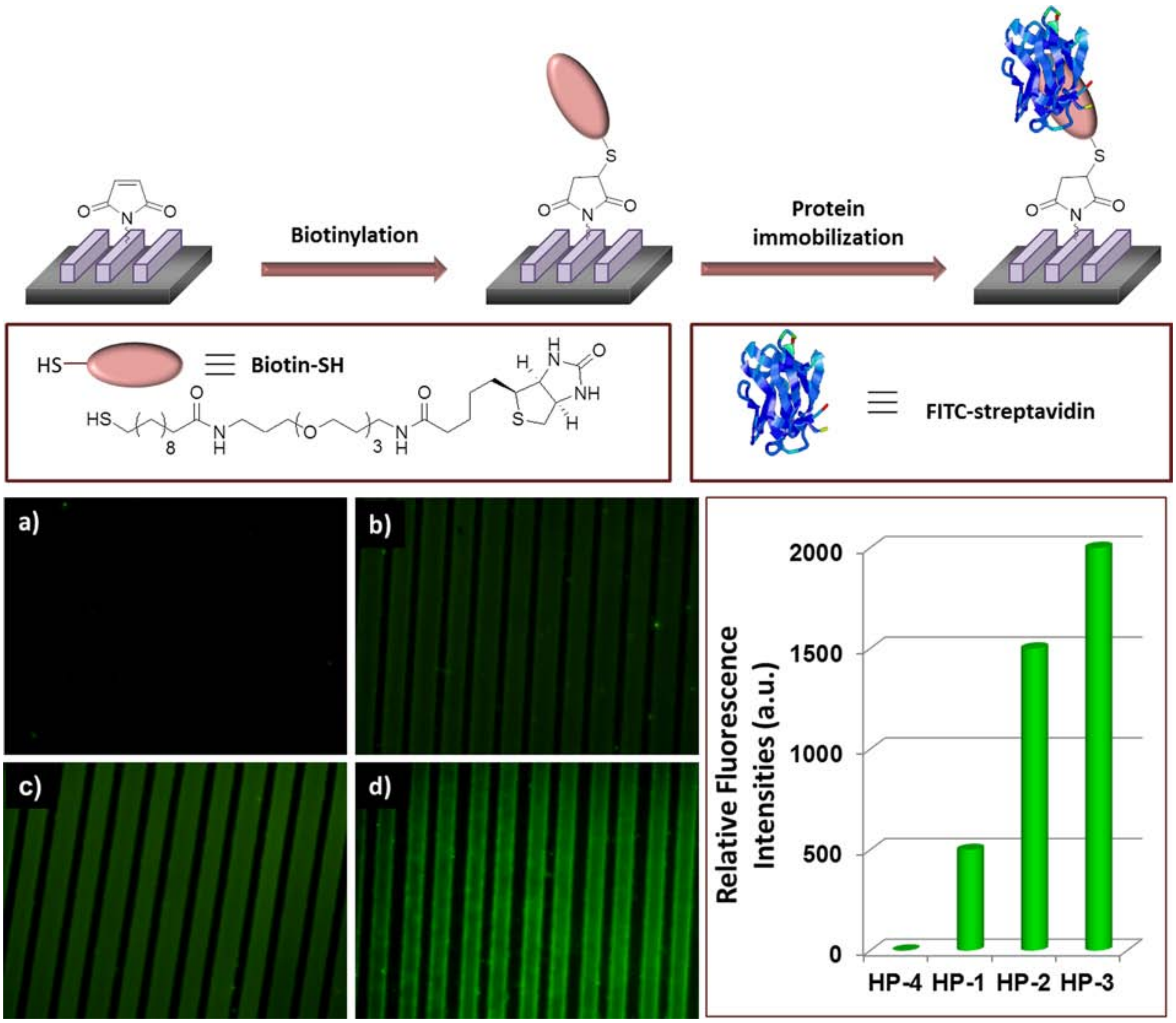


Figure 7. Schematic illustration of biotin attachment and protein immobilization onto hydrogel patterns, and fluorescence microscope images and intensity profiles of FITC-streptavidin bound hydrogel patterns: HP-4 (a), HP-1 (b), HP-2 (c), and HP-3 (d).

maleimide groups into their thiol reactive forms was monitored by TGA analysis of bulk hydrogels. Water uptake of hydrogels was found to be dependent on the mass fraction of hydrophobic FuMaMA in the hydrogel structure. The maleimide-containing hydrogel patterns were efficiently functionalized with fluorescent thiol-containing dye, BODIPY-SH, and a thiol-containing biotin ligand. Efficient immobilization of FITC-streptavidin onto these biotinylated hydrogel patterns could be achieved in a tailored fashion by varying the density of maleimide groups. These easily accessible functional hydrogel patterns can serve as attractive platforms for modulation of cellular attachment and growth on biological interfaces.

4. EXPERIMENTAL SECTION

Materials. The furan-protected maleimide-containing methacrylate monomer (FuMaMA) was synthesized according to a previous report.[17b] Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n = 300 g mol^{-1}) was purchased from Sigma-Aldrich and purified from the inhibitor by passing through an activated aluminum oxide column prior to use. Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n = 1100 g mol^{-1}), poly(ethylene glycol) diacrylate (PEG-DA, M_n = 700 g mol^{-1}), 3-(trimethoxysilyl)propyl methacrylate (TMSMA), 2,2-dimethoxy-2-phenylacetophenone (DMPA), 1-thioglycerol, and triethylamine (TEA) were obtained from Sigma-Aldrich and used without further purification. Methacryloyl chloride was obtained from Alfa Aesar and used as received. Biotinylated (triethylene glycol) undecanethiol (Biotin-SH) was obtained from Nanoscience Instruments (Phoenix, AZ). Fluorescein conjugated streptavidin (FITC-streptavidin) was obtained from Pierce and used as received. 4,4-Difluoro-1,3,5,7-tetramethyl-8-[(10-mercapto)]-4-bora-3*a*,4*a*-diaza-*s*-indacene (BODIPY-SH) was synthesized according to the literature procedure.[20] The HABA/Avidin reagent was obtained from Sigma. Other chemical reagents were obtained from commercial resources and were used as received. Dry solvents such as dichloromethane (DCM), tetrahydrofuran (THF), and toluene were obtained from SciMatCo purification system, and other solvents were dried over molecular sieves. Column chromatography was performed using silica gel 60 (43−60 nm, Merck). Thin layer chromatography was performed using silica gel plates (Kieselgel 60 F254, 0.2 mm, Merck). The plates were viewed under 254 nm UV lamp and/or developed by $KMnO_4$ stain.

Measurement and Characterization. The monomer was characterized using ^{1}H NMR spectroscopy (Varian Mercury-MX 400 Hz) and Fourier transform infrared (FTIR) spectroscopy (Thermo Scientific Nicolet 380 FT-IR spectrometer). A LabConco lyophilizer was used to dry the hydrogels. Thermogravimetric analysis (TGA) was carried out with a TGA Q50 from TA Instruments at a heating sample from ambient temperature to 600 °C at a rate of 10 °C/min under a nitrogen flow (100 mL/min). Elemental analysis data were obtained from Thermo Electron S.p.A. FlashEA 1112 Elemental Analyzer (CHNS separation column, PTFE; 2 m; 6 × 5 mm). To characterize the hydrogel morphology, scanning electron microscopy (SEM) was performed with an ESEM-FEG/EDAX Philips XL-30 (Philips, Eindhoven, The Netherlands) instrument operating at 10 kV. Hydrogels swollen in water were lyophilized and immersed in liquid nitrogen and then broken prior to image acquisition. UV−visible spectra of HABA/Avidin assay were collected on a TU-1880 UV−vis spectrophotometer. Fluorescence microscopy (HBO100 ZEISS Fluorescence Microscopy, Carl Zeiss Canada Ltd., Canada) was used to confirm the attachment of BODIPY-SH and FITC-streptavidin onto hydrogel patterns.

Fabrication of Photopolymerized Bulk Hydrogel. Photopolymerized bulk hydrogels were prepared by UV-initiated free radical polymerization at ambient temperature. Hydrogel precursors were prepared by mixing PEGMEMA (0.2 g, 66.67×10^{-2} mmol), PEG-DA (0.027 g, 3.85×10^{-2} mmol) cross-linker, and DMPA (0.05 g, 19.51×10^{-2} mmol) in CH_2Cl_2 (0.25 mL) with varying feed of the FuMaMA monomer (0.0228 g, 7.84×10^{-2} mmol to obtain the hydrogel BH-1). Precursor was placed under UV source at a distance of 3 cm for 40 min. After photopolymerization, obtained hydrogels were washed with EtOH three times, then washed with water and freeze−dried. BH-2 and BH-3 were synthesized by altering the ratio of FuMaMA monomer. In case of BH-1-1100, PEGMEMA (M_n = 1100 g mol^{-1}) was used instead of PEGMEMA (M_n = 300 g mol^{-1}). In order to check the effect of solvent utilized during photopolymerization on the properties of obtained hydrogel, hydrogels containing 25% maleimide were synthesized in DCM and water/methanol (1:1, v/v) mixture. Morphology analysis via SEM and swelling tests revealed that hydrogels with similar properties were obtained under both conditions (Supporting Information Figure S1 and Figure S2). To determine monomer conversion, the THF wash solutions were dried under reduced pressure and residue was characterized by ^{1}H NMR.The furan-protected maleimide groups in bulk hydrogels were activated to thiol-reactive unprotected maleimide form via the retro-DA reaction. For the activation of gel, 0.2 g of hydrogel was deep into dry toluene and heated at 110 °C under nitrogen for 12 h. Thus, activated hydrogel was dried under vacuum at room temperature overnight. Quantitative characterization of the furan content in the bulk hydrogel was done using thermogravimetric analysis (TGA).

Swelling Study of Bulk Hydrogel. The lyophilized activated hydrogel for 12 h was cut into small pieces and weighed; mean weight was 35 ± 0.5 mg. Each hydrogel was placed into a 25 mL beaker containing 10 mL deionized water at room temperature. At regular intervals the hydrogel was taken out of the beaker, surface moisture was removed by tissue paper, and then it was weighed. After this, the hydrogel was returned to the beaker and the water uptake was measured until the maximum mass was obtained. The percentage amount of water uptake (W_{up}) was calculated using the following equation:

$$W_{up}(\%) = (W_{max} - W_{dry})/W_{dry} \times 100$$

where W_{max} = maximum weight of the swollen hydrogel and W_{dry} = weight of the dry hydrogel.

All swelling studies were carried out in triplicate with different batches of similar hydrogels.

Modification of Silicon Wafer Substrate with TMSMA. Silicon wafer was cleaved into pieces that were approximately 1 cm × 1 cm, and then soaked in "piranha" solution consisting of 3:1 ratio of H_2SO_4 and 30% H_2O_2 solution for 20 min, quickly rinsed with deionized water several times, followed by isopropyl alcohol, and finally dried under a gentle stream of nitrogen at room temperature (*Caution! Piranha solution is highly corrosive and reacts violently with organic substances*). Silicon wafers were soaked in a 10 wt % solution of TMSMA in dry toluene for 12 h at room temperature. Thus, surfaces were washed several times with toluene and methanol and then dried under a gentle stream of nitrogen.

Preparation and Activation of Photopolymerized Hydrogel Micropatterns. A PDMS stamp with a micro-channel relief was carefully placed on the surface of the TMSMA modified silicon wafer. Hydrogel precursor was dropped at the one open end of channels of the PDMS stamp on the silicon wafer to fill them by capillary action. The hydrogel precursor filled PDMS was irradiated using a UV lamp (254 nm) from a distance of 3 cm for 40 min. After exposure to UV-light, PDMS stamp was gently peeled off from the silicon surface and hydrogel patterns were washed with THF to remove any unreacted reactants. The deprotection of the FuMaMA moieties on the hydrogel pattern was carried out by heating them on a hot plate under vacuum at 110 °C for 30 min and then cooling them to ambient temperature under nitrogen.

Functionalization of Hydrogels with BODIPY-SH. Surfaces with hydrogel pattern (HP) were soaked in a solution of BODIPY-SH (0.5 mg/mL in THF) for 12 h at room temperature. Thereafter, the surfaces were washed several times with MeOH to remove any unbound BODIPY-SH. For control experiment, green-fluorescent BODIPY-SH dye was attached to activated HP which contains maleimide and samples without maleimide to identify absence of thiol-reactive maleimide and physical entrapment of dye in the gel. The act-HP samples were incubated with a BODIPY-SH (0.5 mg/mL in THF) solution for 12 h and washed several times with THF. Streptavidin immobilized HP and BODIPY-SH attached HP were analyzed by fluorescence microscopy.

Immobilization of Streptavidin onto the Biotinylated Micropatterns. The biotinylated hydrogel pattern was prepared by soaking the surfaces with maleimide containing hydrogel patterns in solution of Biotin-SH (1.5 mg/mL) MeOH solution for 12 h under nitrogen at room temperature. Thereafter, the hydrogel pattern was washed several times with MeOH to remove any unbound biotin-SH. A solution of FITC-Streptavidin (0.2 mg/mL in PBS) was added directly onto the hydrogel patterns. After immobilization of streptavidin for 30 min, hydrogel pattern was rinsed with water several times and then analyzed using fluorescence microscopy.

HABA/Avidin Assay of Biotinylated Bulk Hydrogel. HABA/Avidin assay was performed to determine the amount of biotin in the hydrogel that is available for binding to streptavidin.[21] HABA/Avidin assay was monitored using UV−vis spectrophotometer absorbance at 500 nm for the HABA/

Avidin reagent and for the solution after addition of biotinylated bulk hydrogel. The calibration curve was obtained by measuring the change in UV absorbance upon the addition of free biotin of known concentration sequentially into the HABA/Avidin reagent. The equivalent amount of biotin available for binding on the biotinylated bulk hydrogel was calculated by comparing the absorbance changes to the calibration curve.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis and characterization data. SEM imaging. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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