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Versatile Functionalization of the Micropatterned Hydrogel of Hyperbranched Poly(ether amine) Based on "Thiol-yne" Chemistry

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The functionalization of a hydrogel with target molecules is one of the key steps in its various applications. Here, a versatile approach is demonstrated to functionalize a micropatterned hydrogel, which is formed by "thiol-yne" photo-click reaction between the yne-ended hyperbranched poly(ether amine) (hPEA-yne) and thiol-containing polyhedral oligomeric silsesquioxane (PEG-POSS-SH). By controlling the molar ratio between hPEA-yne and PEG-POSS-SH, patterned hydrogels containing thiol or yne groups are obtained. A series of thiol-based click chemistry such as "thiol-epoxy", "thiol-halogen", "thiol-ene", and "thiol-isocyanate" are used to functionalize the thiol-containing hydrogel (Gel-1), while the yne-containing hydrogel (Gel-2) is functionalized through a typical copper-catalysed alkyne-azide reaction (CuAAC). FTIR, UV-vis spectra and confocal laser scanning microscopy (CLSM) are used to trace these click reactions. Due to the selective adsorption to the hydrophilic dyes, the obtained patterned hydrogel of hPEA modified with fluorescence dye is further demonstrated in application for the recognition of guest molecules.

1. Introduction

The patterned hydrogel has become of great interest in the past few years because of its wide application in biomedical devices[1–4] and tissue engineering.[5–7] For example, micropatterns on large scales can be used for microarrays of proteins[8] and for the controlled alignment of cells.[9,10] Different techniques such as soft lithography,[11] photolithography,[12–14] nano-imprinting,[15,16] and electron-beam lithography[17,18] have been developed to fabricate micro- and nanopatterned hydrogels. Among these established methods, photolithography was most widely used for the fabrication of patterned hydrogels because of advantages such as large-scale production in indsustry and simple processing. Thomas's group[19] had succeeded in employing interference photolithography to fabricate 3D hydrogel structures with high surface areas on neural prosthetic devices, and demonstrated that the obtained patterned hydrogels could act as a smart drug-delivery system for the delivery of nerve growth factor. As a unique alternative, Hamachi's group[20] exploited a new approach to detect polyamines by using hybrid hydrogels which can satisfy the demands for cancer diagnosis and clinical usage. These pioneering works are of important significance for introducing hydrogels to the applications of biochips,[21,22] microsensors,[23,24] and other novel microdevices.[25–28]

One of the most important issues in this field is to modify patterned hydrogels with target molecules to enable special functions. In cell culture applications, for example, adhesive peptides and growth factors are usually convalently incorporated into the patterned hydrogel to promote adhesion, migration, and proliferation of cells with a gel.[29–32] As for the high-throught technology in lab-on-chip applications, an analyte molecule such as a fluorescent molecule is also neccessary. It is therefore of growing demand to develop robust, fast, and selective chemical reactions for the functionalization of hydrogels under feasible conditions. Due to the heterogeneous reaction between the gel matrix and solution in post-functionlization, however, modification of the patterned hydrogel with a target molecule is challenging, as most traditional chemical reactions require longer reation time, the strict exclusion of water, and vigorous heating/cooling.[32] Fortunately, this issue might be overcome by the advent of "click chemistry", which possesses novel charateristics such as a high reaction speed, high selectivity, and operation under mild conditions.[33–35] It is true that the functionlization of a polymer matrix is dominated by click chemistries. As one of the most studied click chemistries, the Cu(I)-catalyzed alkyne–azide Huisgen 1,3-dipolar cycloaddition (CuAAC) has been widely used for the post-functionalization of hydrogels.[36,37] Occasional problems with the toxicity of a Cu(I) catalyst shifted the attention from this reaction to thiol-based reactions.[38] The reactions between thiols and various groups such as halogens, epoxys, enes, ynes, and isocyanates exhibit the charateristics of click chemistry.[39–44] Additionally, the photogenerating radical-mediated thiol-ene and thiol-yne reactions provide the possibility to realize spatiotemporal control. Thus, thiol-based reactions are now becoming an interesting alternative for functionalizing hydrogels.[39,40,44,45]

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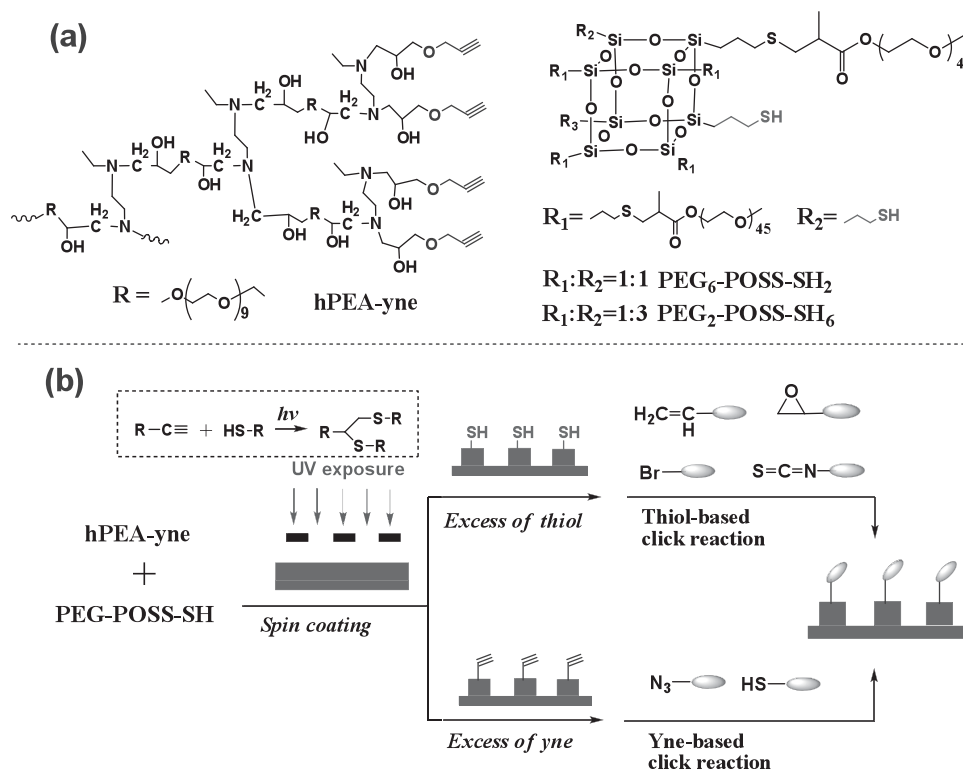
Scheme 1. The general procedure for fabrication and functionalization of the patterned hydrogel: a) Chemical structure of hPEA-yne and PEG-POSS-SH as starting material for preparation of hydrogel; b) the process of photolithography to fabricate the patterned hydrogel by using thiol-yne photo-crosslinking chemistry, and the strategy for the functionalization of the obtained patterned hydrogel through thiol or yne-based click reactions.

To obtain a novel patterned hydrogel with easy preparation and facile functionalization, we here used thiol-yne photoclick chemistry to fabricate a micropatterned hydrogel of hyperbranched poly(ether amine) (hPEA) through photolithography (**Scheme 1**). The combination of photolithography and thiol-yne photocrosslinking chemistry provides two obvious advantages: 1) easy fabrication of the patterned hydrogel; 2) versatile functionalization of the patterned hydrogel through thiol or yne-based click reactions. Poly(ether amine)s (PEAs) developed by our group recently possess a sharp response to temperature, pH, and ionic stength. PEA materials such as polymer brushes and hydrogels exhibit selective adsorption to guest molecules such as biomolecule proteins and hydrophilic dyes, and thus can find potential application in microsensors, separation technologies, and bioassays.[46–49] Here, we demonstrate that the patterned hydrogels with residue thiol or yne groups can be functionalized through a variety of thiol or yne-based click reactions. Finally, the patterned hydrogel functionalized with fluorescence is applied for the recognition of guest molecules.

2. Results and Discussion

2.1. Fabrication of Micropatterned Hydrogels

As to selecting the chemistry and materials to produce the patterned hydrogel, we took a variety of physical and chemical features into consideration (Scheme 1). Hyperbranched poly(ether amine) comprising PEG short chains and amino groups in backbone, was chosen as one of main components of the matrix material due to its novel responsiveness and selective interaction with guest molecules.[46,49] The other component, PEG-POSS-SH is composed of an inorganic polyhedral oligomeric silsesquioxane (POSS) skeleton and poly(ethylene glycol) (PEG) grafted chains. The incorporation of inorganic POSS into the patterned hydrogel might enchance the mechanical strength and thermal stability of the resulting cross-linked network.[50] The grafted PEG chains can increase the compatibility between the two components, hPEA-yne and PEG-POSS-SH, which is important for the fomation of a homogeneous hydrogel. Due to its tolerance to oxyen and water, the use of thiol-yne photocrosslinking chemistry—which performs well under bengin reaction conditions—allows the facile fabrication of the patterned hydrogel through photolithography. Moreover, thiol- and yne-based click chemistry provide a rich selection of methods to functionalize the patterned hydrogel.

The whole strategy to fabricate and functionalize the patterned hydrogel is illustrated in Scheme 1. The chloroform solution of hPEA-yne and PEG-POSS-SH, with a trace of photoinitiator I907, was first spin-coated onto the substrate. After evaporation of the solvent, the polymer layer of hPEA-yne and PEG-POSS-SH with a thickness of about 950 nm was covered with a mask and then irradiated with UV light. The polymer layer of the exposure area was photocrosslinked through the radical-mediated thiol-yne photoclick chemistry. After being developed in ethanol, the polymer in the unexposed area was
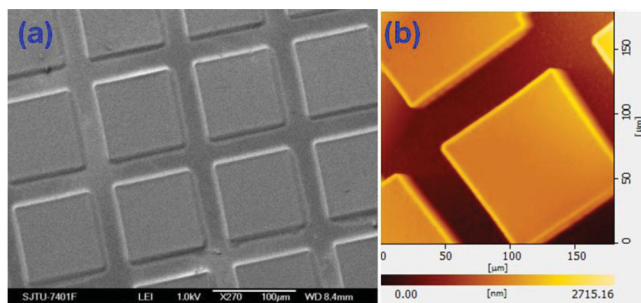
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Figure 1. The morphology of the patterned hPEA hydrogel (Gel-1) on silicon substrate: a) SEM image and b) AFM image.

removed, leaving the cross-linked hydrogel pattern behind. By controlling the ratio of hPEA-yne and PEG-POSS-SH, the excess of unreacted thiol or yne groups will stay in the resulting hydrogel, which allows further functionalization. Through this approach, we fabricated two types of patterned hydrogels for the following functionalization: Gel-1 containing thiol groups and Gel-2 containing yne groups. The topography and morphology of the obtained hydrogel patterns were revealled by microscopy: atomic force microscopy (AFM) and scanning electron micros-copy (SEM) (**Figure 1** and Figure S7). The hydrogel pattern on the surface was found to be neat and smooth, suggesting an excellent pattern quality.

We used real-time Fourier-transform infrared spectroscopy (FTIR) to trace the thiol-yne photocrosslinking kinetics in the fabrication of the patterned hydrogels because of its ability to distinguish thiol and yne groups independently and simultane-ously. As shown in the FTIR spectra of Gel-1 and Gel-2 upon different exposure time (**Figure 2**), peaks at 2110 and 2560 cm^{-1} can be attributed to C≡CH and S–H streching vibrations, respectively. As to Gel-1, the S–H peak decreases with the increasing time of UV-irradiation first and then remains unchanged after 2 min, while the C≡CH peak almost disap-pears after 2 min of UV irradiation. The obvious signal related to S–H in FTIR suggests the presence of a certain amount of thiol groups in Gel-1, which should be due to the excess of thiol groups to yne groups in the formulation. In contrast, the S–H peak almost disappears in Gel-2, while the C≡CH peak decreases with increasing exposure time first and then remains unchanged. In the formulation of Gel-1 and Gel-2, the molar ratios between S–H and C≡CH are 6/1 and 1/2, respectively. The conversion of thiol and yne in Gel-1 and Gel-2 with respect to exposure time is shown in Figure 2c. The final conversion of yne in Gel-1 and thiol in Gel-2 reached over 95%, while almost 70% of thiol and yne groups are still in Gel-1 and Gel-2, respectively.

2.2. Functionalization of Patterned Hydrogels through Thiol or Yne-based Chemistry

The presence of unreacted thiol groups in Gel-1 and yne groups in Gel-2 allows the functionalization of patterned hydrogels through thiol-based or yne-based reactions, respectively. As for the thiol-based reactions, thiol is an interesting choice as
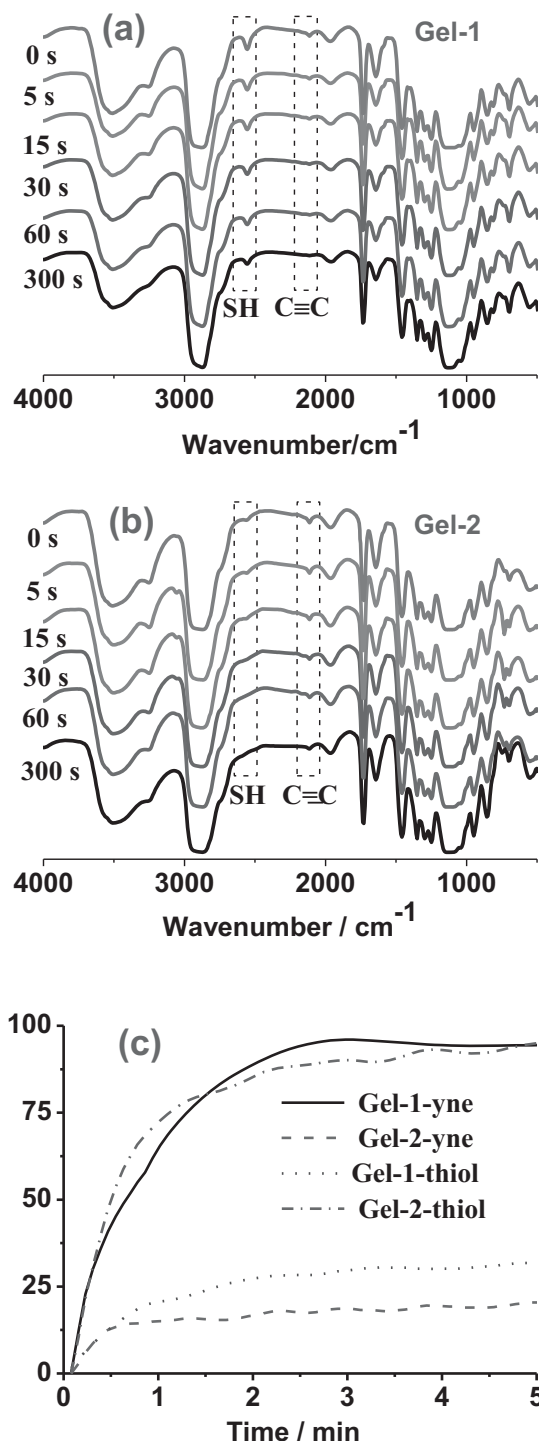

Figure 2. The real-time FTIR spectra of hydrogels with redundant thiol or yne groups exposed for various times: a) Gel-1 with excessive thiol groups and b) Gel-2 with excessive yne groups. c) The yne and thiol conversion curves of Gel-1 and Gel-2. The intensity of 365 nm light is 10 mW/cm^2.

a reactive group due to its good nucleophilicity and ability to participate in radical reactions. Quite a few types of thiol-based reactions exhibit charateristics of click chemistry, and were termed 'click reactions' in the literature.[38,39] To demonstrate the
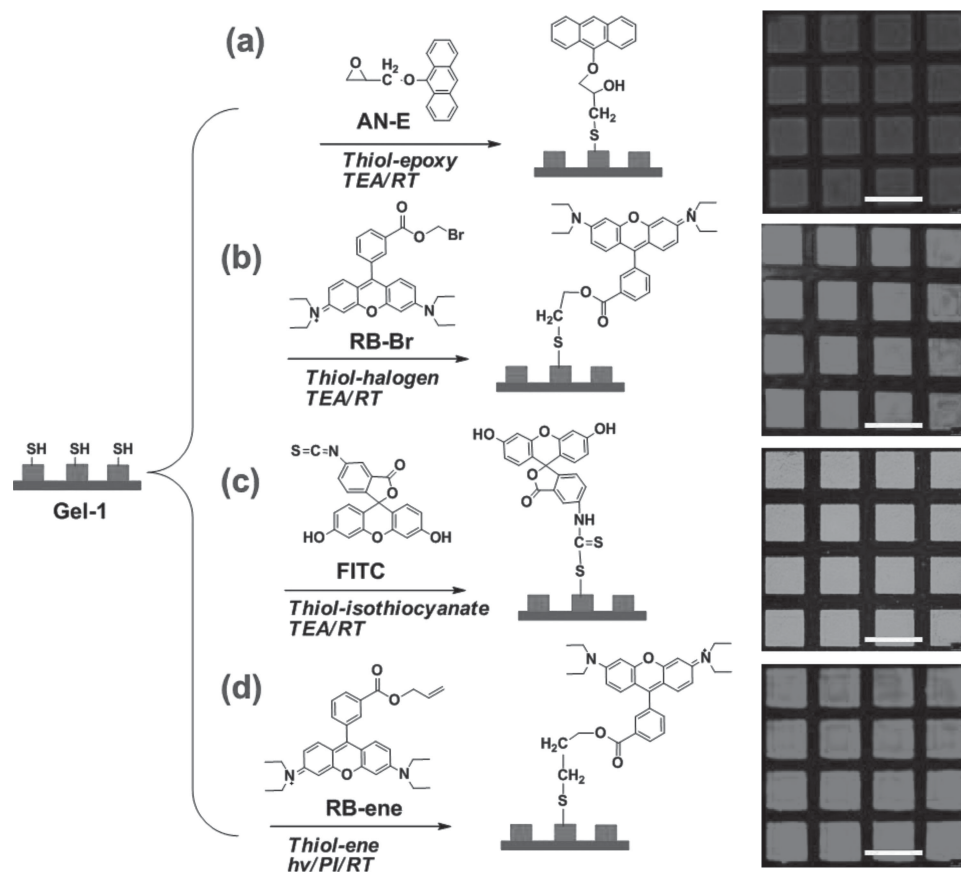
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Figure 3. The strategy to functionalize Gel-1 through four types of thiol-based click reactions and the resulting CLSM images: a) thiol–epoxy, b) thiol–halogeno, c) thiol–isothiocyanate, and d) thiol–ene. All these thiol-based reactions were carried out at room temperature (RT). The reaction time for (a–c) was 6 h, while for (d) it was 15 min. Scale bars correspond to 100 μm.

versatility and feasibility of thiol-based reactions for functionalization of the patterned hydrogels, four types of thiol-based click reactions: thiol-epoxy,[40,41] thiol-halogeno,[42] thiol-isothiocyanate,[43] and thiol-ene[39,44] were used to modify the thiol-containing Gel-1 with different fluorescent dyes (**Figure 3**). Epoxy, halogen, isothiocyanate, and ene groups were selected to react with thiols group, as these four groups are widely attached to different functional molecules. Gel-1 was immersed in ethanol solutions of fluorescent dyes at room temperature (RT) for a certain time. After reaction, Gel-1 was washed rigorously by immersion in ethanol to remove the physically adsorbed dyes. For the reactions thiol–epoxy, thiol–halogeno, and thiol–isothiocyanate (Figures 3a–c), the presence of triethylamine (TEA) can protonate thiol groups, thus increasing their nucleophilicity to carbon atoms in epoxy, alkyl halogen, and isothiocyanate groups. In the presence of trace amounts of photo-initiator, the radical-mediated thiol-ene reaction was triggered by irradiation with UV light (Figure 3d). The conjugation of these fluorescent dyes to Gel-1 was characterized through confocal laser scanning microscopy (CLSM). The homogeneous fluorescence pattern of the modified Gel-1 with the strong blue (Figure 3a), green (Figure 3c) and red emissions (Figures 3b,d), are ascribed to the emission of AN, FITC, and RDB dyes, respectively, indicating

that the functionalization of Gel-1 with fluorescent dyes can be conducted through these four thiol-based reactions.

FTIR was used to follow these four types of thiol-based reactions to functionalize Gel-1 (**Figure 4**). The reaction with FITC, RDB–ene, RDB–Br and AN–E was confirmed by the obvious decrease of the S–H peak at 2560 cm^{-1} in Gel-1. Most of S–H groups in Gel-1 had reacted with the targeted groups through these four thiol-based reactions. It should be noted that there is still a little amount of unreacted S–H in Gel-1 after these thiol-based reactions, as indicated by the precence of the very weak peak of S–H in the FTIR image. This might be ascribed to the steric hindrance in the heterogeneous functionalization of the gel matrix. We also used UV–vis adsorption spectra to trace the thiol–epoxy and thiol–isothiocyanate functionalizations of Gel-1 (Figure S8). The characteristic adsorptions of AN and FITC increased with the increasing reaction time, and reached a maximum after around 5 h. The appearance of the charateristic adsorptions of AN and FITC indicates that AN–E and FITC were successfully coupled to Gel-1. Based on these characterization results from CLSM, FTIR, and UV–vis specra, it can be concluded that these four types of thiol-based click reactions can be used to funcitonalize the thiol-containing Gel-1 very efficiently.
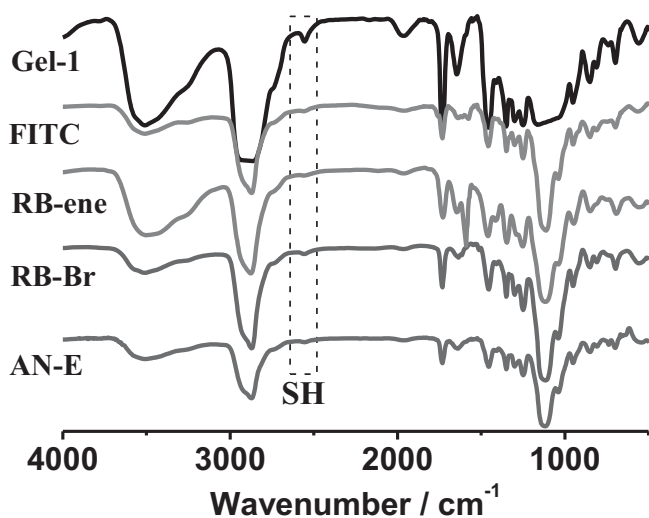
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Figure 4. The FTIR spectra of Gel-1 functionalized through four types of thiol-based reaction. Spectra from top to bottom: pured Gel-1, thiol-based click reaction with FITC, RDB–ene, RDB–Br, and AN–E.

The yne-containing Gel-2 was functionalized through the typical CuAAC click reaction (**Figure 5**). Both hydrophobic thioxathone and hydrophilic rhodamine-B containing azide groups (TX-N$_3$ and RDB-N$_3$) were chosen to be coupled to Gel-2 at room temperature. The homogeneous fluorescent pattern

with blue and red emission of the resulting Gel-2 is indicative of the conjugation of TX-N$_3$ and RDB-N$_3$ to Gel-2 through the CuAAC click reaction (Figure 5). This was also confirmed by FTIR and UV–vis spectra. After incorporation of TX and RDB into Gel-2, the intensity of the signal of C≡C at 2113 cm^{-1} became very weak, providing evidence for the functionalization of Gel-2 (**Figure 6**). The adsorption at 400 nm, which is ascribed to the charateristic adsorption of TX, increased with increasing reaction time in the UV–vis spectra (Figure S9), also suggesting the conjunction of TX to Gel-2. Besides these fluoresecent dyes, we also demonstrate the attachment of the biomolecule GSH to Gel-2 through a thiol-yne click reaction. This approach is useful for biomolecules because many petides are composed of the cysteine amino acid. The attachment of GSH to Gel-2 was confirmed by X-ray photoelectron spectroscopy (XPS) and FTIR. The disappearance of the peak of C≡C at 2113 cm^{-1} in the FTIR (Figure S11) and the increasing intensity of N 1s in XPS (Figure 4c) of Gel-2 after modification can be seen as evidence of the immoblization of GSH in Gel-2.

2.3. Functionalized Patterned Gel-1 for the Recognition of Dyes

The feasible fabrication and versatile functionlization provide our patterned hydrogels as a novel platform on which the target molecules—such as biomolecules, flurescent dyes, and analytes—can be attached through thiol- or yne-based click chemistry. We now further demonstrate that the patterned hydrogel
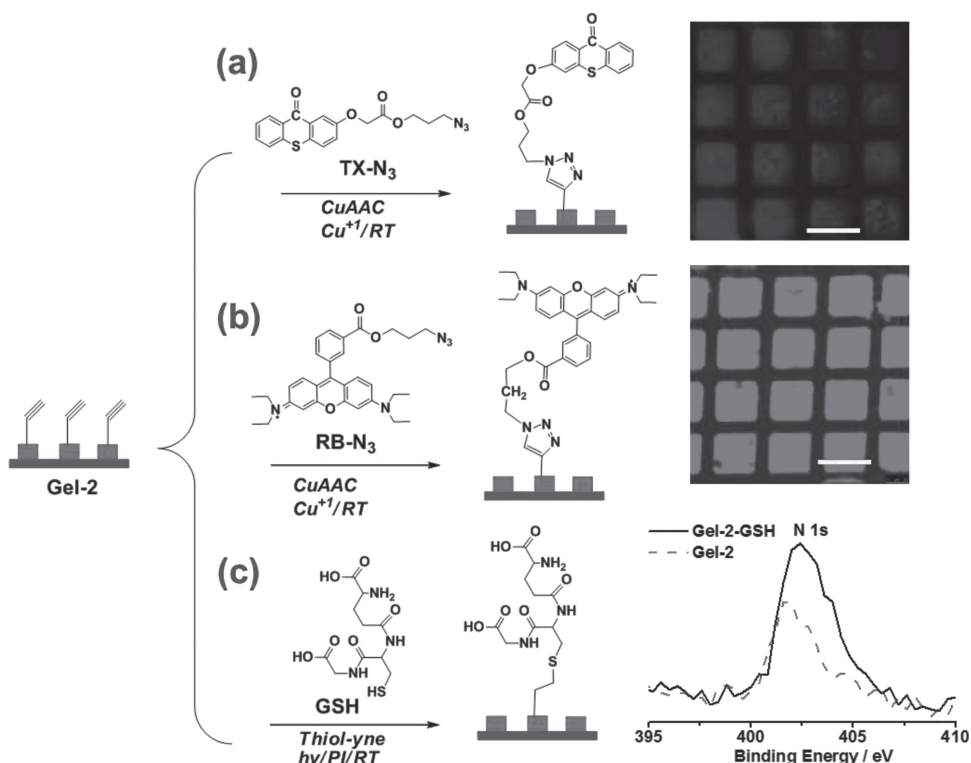

Figure 5. The strategy to functionalize Gel-2 through an yne-based click reaction and the resulting CLSM images: a) TX–N$_3$, b) RDB–N$_3$, c) GSH. All these yne-based reactions were carried out at room temperature (RT). The reaction time for (a,b) was 6 h, while for c) it was 15 min. Scale bars correspond to 100 μm.
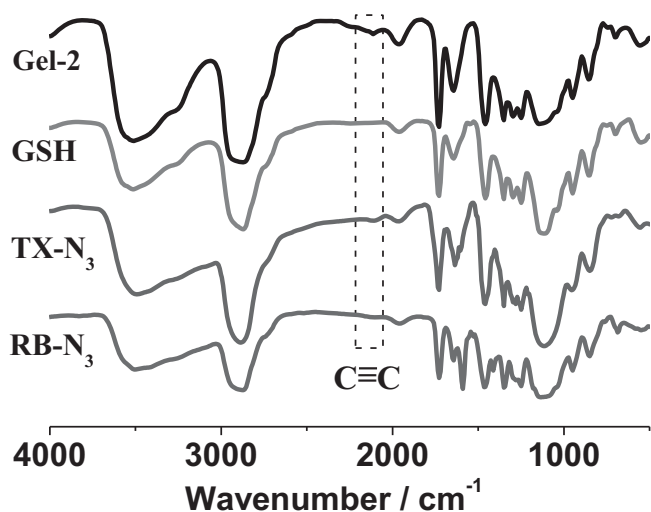
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Figure 6. The FTIR spectra of Gel-2 functionalized through an yne-based click reaction. Spectra from top to bottom: pured Gel-2, yne-based click reaction with GSH, TX–N₃, and RDB–N₃.

functionalized with FITC can be used as an analysis platform for the recognition of hydrophilic red dyes. Our previous research has shown that hydrogel materials of the hyperbranched poly(ether amine) can adsorb the hydrophilic guest dye molecules selectively in aqueous solution.[46–49] It is expected that the selective adsorption of hPEA hydrogels to different guest molecules can be reflected by the fluorescence response of the

patterned hydrogel modified with the fluorescent dye. To probe this idea, we carefully immersed the part of the Gel-1 functionalized with FITC into solutions of three dyes—Ponceau S (PS), Rose Bengal (RB), and Rhodamine 6G (R6G)—for 30 min at room temperature. After washing in pure water for 5 min to remove dye adsorbed on the surface, Gel-1 was then evaluated by CLSM through both green and red channels under a fixed wavelength (488 nm) of blue excitation (**Figure 7**).

As shown in Figures 7a and b, the part of Gel-1 pattern which was immersed in PS and RB solutions was almost dark, whereas the rest of pattern exhibits the strong green fluorescence. The decreased fluorescence intensity of the immersed part of Gel-1 can be explained by the strong adsorption behavior of Gel-1 to PS and RB. In contrast, no obvious difference in the fluorescence pattern between parts immersed and non-immersed in R6G solution was found (Figure 7c), suggesting no adsorption of R6G by Gel-1. To understand the interactions between Gel-1 and these hydrophilic dyes in water, we prepared the Gel-1 on a quartz slide, and recorded its UV–vis spectra after immersion in dye solution. The Gel-1 can adsorb PS and RB, but no R6G (**Figure 8**b). Because PS and RB have a relatively strong visible absorption in the range of 500–600 nm, the green emission of FITC in Gel-1 can be quenched by PS and RB molecules when these molecules are adsorbed into Gel-1. Since no R6G molecules were encapsulated in Gel-1, the fluorescence pattern of Gel-1 did not change after immersion in R6G solution. Fluorescence images taken through the red channel can further distinguish the difference of PS and RB (Figures 7d,e). Upon excitation with the 488 nm laser, the part of Gel-1 immersed in RB exhibited red emission, while the whole Gel-1 was almost dark after immersion in PS solution. The strong red fluorescence emission of the part of Gel-1 immersed in RB might be ascribed to the fluorescence resonance energy transfer (FRET) between the exited FITC and RB. The emission spectrum of FITC overlaps the adsorption of RB very well (Figure 8a), and molecules of FITC and RB in Gel-1 are in close proximity after adsoprtion of RB, both of which favor FRET.[51] This was further confirmed by the fluorescence spectra (Figure 8c), which provide the charateristic fluorescence spectra of the FRET phenomenon. After adsorption of RB, the red emission intensity (582 nm) of the FITC-functionalized Gel-1 was dramatically enchanced in comparison to the non-functionalized Gel-1, while the green emmison intensity (538 nm) of the FITC-functionalized Gel-1 was obviously weakened. In contrast, the adsorped PS can not lead to red fluorescence because PS is not a fluorescent dye and no FRET phenomenon can happen. Based on these fluorescence images of the FITC-functionalized Gel-1, these three hydrophilic red dyes can be identified by the changes in the fluorescence patterns (Figure 8d).
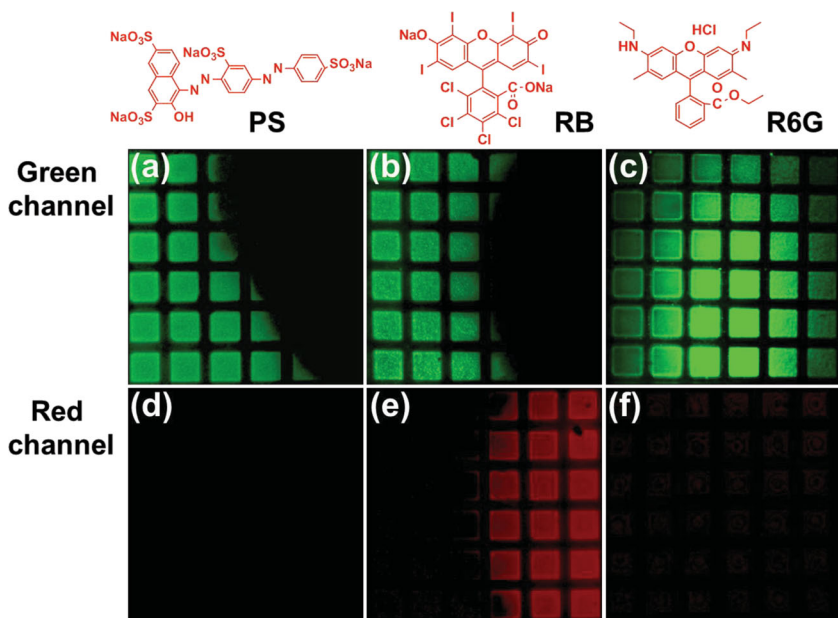

Figure 7. CLSM fluorescent images of Gel-1 pattern modified with FITC taken through both green and red channels after immersed partially in PS (a & d), RB (b & e) and R6G (c & f) aqueous solution (the right half). The excitation wavelength of blue light for CLSM is fixed at 488 nm. Scale bars correspond to 100 μm.
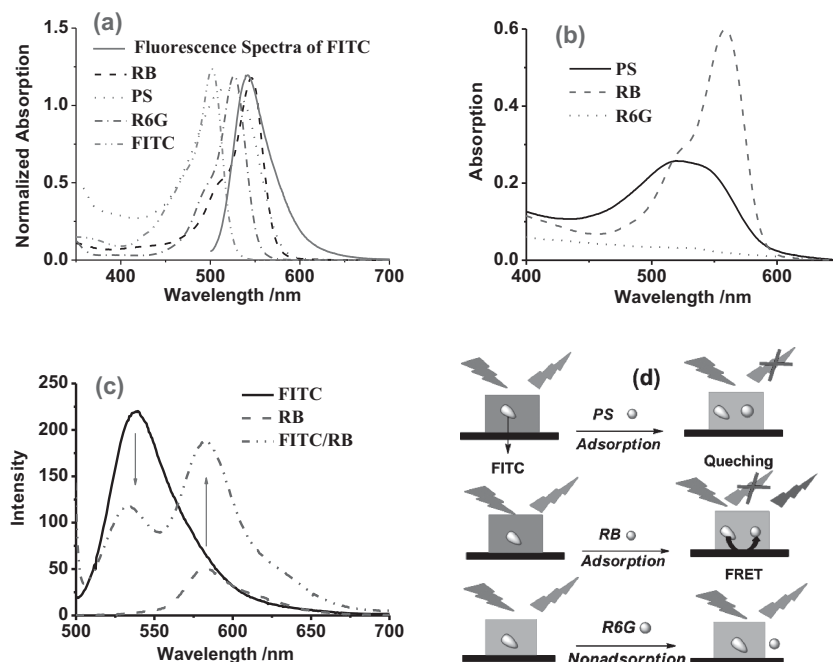
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Figure 8. a) UV–vis spectra of FITC, PS, RB, R6G and the fluorescence emission spectrum of FITC in aqueous solution (Normalized); b) UV–vis spectra of Gel-1 after immesion in PS, RB, and R6G solution; c) fluorescence emission spectra of the Gel-1 functionalized with FITC before and after adsorption of RB, and unfunctionalized Gel-1 after adsorption of RB. The excitation wavelength is 480 nm. d) The proposed mechanism of Gel-1 functionalized with FITC for recognition of three red dyes PS, RB, and R6G through the fluorescence response.

3. Conclusion

We have developed a novel micropatterned hydrogel of hyperbranched poly(ether amine) by combining photolithography and thiol-yne photo-crosslinking. The residue thiol or yne groups allow a robust, efficient, and general approach for the functionalization of the resulting hydrogel. The target molecules with different substitutes can be incorporated to the thiol-containing patterned hydrogel through thiol–epoxy, thiol–halogen, thiol–ene, and thiol–isocyanate click reactions. The patterned hydrogels comprising yne groups can be functionalized through yne-based click reactions such as CuAAC and thiol–yne chemistry. The patterned hydrogel labeled with FITC exhibits selective interactions with three red dyes PS, RB, and R6G, with respect to the fluorescence response, which gives it potential for use in the recognition of guest molecules. The feasible fabrication and versatile functionlization of the patterned hydrogels based on thiol–yne photo-crosslinking provides an important alternative to design novel platforms of hydrogels for different applications.

4. Experimental Section

Fabrication of the Micropatterned Hydrogels by Photolithography: The substrate silicon wafer or quartz slide was cut into sqaure chips of 1.5 cm × 1.5 cm in size and the small piece of substrates were first soaked in a 1:2:6 mixed solution (10 mL HF, 20 mL H_2O_2, 60 mL ultrapure water, respectively) for 5 min to remove the possible metal

pollutants, and then washed in ultrapure water. Following that, the silicon substrates were cleaned in another 1:3 mixed solution (30 mL H_2O_2:70mL H_2SO_4) at 150 °C for 3 h to remove the organic pollutants, and then washed with ultrapure water and dried with N_2. The substrates were then soaked in 0.2 wt% (3-mercaptopropyl)trimethoxysilane in toluene for 8 h and then cleaned with fresh toluene and continually dried in nitrogen gas.

A dichloromethane solution of hPEA-yne, PEG-POSS-SH (15% w/w), and photo-initiator I907 (0.5% (w/w) for the solid content), was filtered through a 0.2-μm filter in advance, and then spin-coated onto the modified silicon wafers to obtain Gel-1 (hPEA-yne/PEG2-POSS-SH6) (the mole ratio of SH:yne is 6:1) and Gel-2 (the mole ratio of hPEA-yne and PEG6-POSS-SH2 yne:SH is 2:1). The substrates were spun at speeds of 500 rpm for 10 s and then 3500 rpm for 30 s. After heating at 60 °C for 30 min, the gel layer was covered by a photomask and irradiated with 365 nm UV light for 5 min, then developed in ethanol for 5 min to remove the uncross-linked polymers to get the patterned Gel-1 or Gel-2. The thickness of the gel layer was about 950 nm.

Functionalization of the Patterned Hydrogels through Thiol (Gel-1) or Yne (Gel-2) Click-Chemistry: The patterned Gel-1 was immersed in ethanol solutions of AN–E (2 mg/mL), FITC (2 mg/mL), RDB–Br (2 mg/mL), or RDB–ene (2 mg/mL). To the first three was added 0.1 mL triethylamine (TEA) and they were kept for 6 h at room temperature in the dark. The latter was irradiated by 365 nm UV light for 15 min in the presence of photo-initiator I907.

The patterned Gel-2 was immersed in ethanol solutions of RDB–N_3 (2 mg/mL), TX–N_3 (2 mg/mL), and ethanol aqueous solution containing GSH (2 mg/mL). The first two were reacted in the presence of sodium ascorbate (300 μL, 2 mM, in pure water) and $CuSO_4 \cdot 5H_2O$ (250 μL, 2 mM, in pure water) for 6 h at room temperature. The third solution was irradiated with 365 nm UV light for 15 min with the help of photo-initiator I907. After reaction, the patterned gels modified by GSH were washed by pure water; other functionalized patterned gels were soaked in fresh ethanol for half an hour to remove unreacted monomers.

UV–vis Spectra to Trace the Kinetics of Hydrogel Functionalization: Gel-1 and Gel-2 were prepared on the quartz slides for UV–vis spectra. The quartz plates with unpatterned Gel-1 and Gel-2 were prepared the same way as mentioned above. After curing, the quartz plates with unpatterned Gel-1 were dipped into AN–E ethanol solution (2 mg/mL) and FITC ethanol solution (2 mg/mL), and one drop of triethylamine was added as catalyst. In the presence of sodium ascorbate and $CuSO_4 \cdot 5H_2O$ aqueous solution, slices with unpatterned Gel-2 were immersed in TX–N_3 alcohol solution (2 mg/mL). The absorption intensity was tested via UV–visible spectra every 30 min.

Selective Adsorption and Recognition of Dyes: The patterned Gel-1 samples functionalized with FITC were immersed partially in R6G, PS, and RB aqueous solution (0.5 mg/mL) for 30 min at room temperature. After washing with fresh water to remove excess dye, the patterned Gel-1 samples (parts of which absorbed the dyes) were observed by CLSM.

Characterization Methods: All FTIR measurements were carried out with a Spectrum 100 Fourier-transformation infrared absorption spectrometer (Nicolet IS10). The samples were prepared by dropping dichloromethane solution containing monomers onto a KBr plate or on IR-transmitting silicon wafers and dried at room temperature. The hydrogel's cure kinetics were determined on IR-transmitting silicon wafers, which experienced the same treatments as to form unpatterned Gel-1 and Gel-2 via real-time Fourier-transformation infrared absorption

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spectrometry with an intensity of 10 mW/cm^2. The photocuring dynamics at different curing times were recorded from 4000 to 400 cm^{-1} with a 4 cm^{-1} resolution over 32 scans.

The ^{1}H-NMR spectra in DMSO (D$_6$) and CDCl3 were acquired with a Varian Mercury Plus 400 MHz spectrometer equipped with a temperature control unit. The samples in DMSO or CDCl$_3$ were measured at room temperature. The atomic force microscopy (AFM) images were obtained using a scanning probe microscope (Nanoscope III, Digital instruments) operated in tapping mode. The sample was patterned hydrogel on silicon wafers. Scanning electron microscopy (SEM) was performed on a Sirion-200 electron microscope (FEI Company) at 5 kV. The samples for SEM were sputter-coated with gold to minimize charging. The functionalized patterned gel morphology and luminescence properties were studied in detail by confocal laser scanning microscopy (CLSM, Leica TCS-SP5, Leica, Wetzlar, Germany). X-ray photoelectron (XPS) spectra were recorded on an ESCA LAB 250 spectrometer (VG Scientific) with Al K$_\alpha$ radiation (hν = 1486.6 eV).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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