

Photoresponsive Hydrogel Microstructure Fabricated by Two-Photon Initiated Polymerization**

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A photoresponsive polymeric hydrogel cantilever that deflects under illumination has been fabricated by using two-photon three-dimensional lithography. The hydrogel was prepared from a comonomer solution containing acryloylacetone, acrylamide, and *N,N'*-methylene bisacrylamide. The photoresponse of the cantilever was activated by photoexcitation of acetylacetone groups at 244 nm. Deflection of the cantilever by $\sim 45^\circ$ was effected upon UV irradiation for 20 min.

1. Introduction

Functional polymeric microstructures are of interest for their potential application to micromachines (microelectromechanical and micro-optomechanical systems), microfluidic systems, microsensor arrays, and biomedical devices.^[1] Polymeric hydrogel materials have been extensively investigated and can respond to a wide variety of stimuli. Responsive hydrogels are interesting active materials because they undergo large chain conformational changes upon swelling or under the influence of external fields. Hydrogel structures can thus retain their responsive properties when scaled down to the very small dimensions involved in microsystems. Recently, thermally active microfluidic channels based on a functional hydrogel material were fabricated by Beebe et al. using lithographic techniques.^[2] One key issue with respect to active polymer hydrogels is their relatively slow response time. It is expected that scaling down hydrogel objects to the micrometer scale should significantly shorten their response times.

A powerful new approach to the fabrication of polymeric microstructures is two-photon 3D lithography using two-photon initiated polymerization (TPIP). Two-photon processes have been used to activate a number of chemical or physical processes and have been utilized in 3D optical data storage,^[3] fluorescence imaging,^[4] optical waveguiding,^[5] and lithographic microfabrication.^[6,7] The 3D resolution in these processes is facilitated by the unique spatial confinement of excitation associated with simultaneous absorption of two photons relative to

single-photon mediated processes. Polymeric microstructures previously fabricated by TPIP have been based on passive polymeric materials. If microstructures comprised of active polymers can be fabricated by TPIP, then a new class of responsive 3D microstructures could be fabricated and investigated for use in various applications. As a first step towards photoactive systems for 3D microfabrication, we have examined hydrogels containing acetylacetone groups that undergo keto–enol tautomerism and can impart photoactivity to the system. In this paper, we report the fabrication of a photoresponsive hydrogel cantilever, containing acetylacetone moieties by two-photon initiated polymerization (TPIP).

2. Results and Discussion

2.1. Photochemical Reaction in Acryloylacetone Hydrogels

Hydrogels containing acetylacetone groups were prepared by polymerization of solutions of acryloylacetone (AA) (1), acrylamide (AAm) (2) and *N,N'*-methylene bisacrylamide (MBAAm) (3). β -diketone compounds, like acetylacetone, are known to exist in the form of enol and diketo structures, which interconvert through a tautomeric equilibrium, as illustrated in

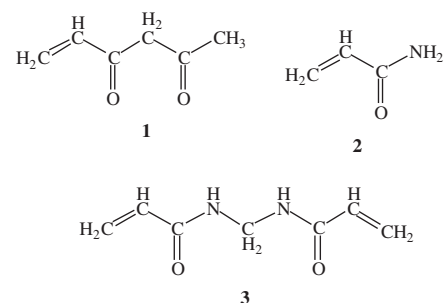


Figure 1.^[8] The equilibrium between the diketo and enol forms can be shifted under illumination with UV light, in favor of the diketo form.^[9] In the case of AA/AAm/MBAAm hydrogels, UV excitation at 244 nm leads to a decrease in the absorption peak at about 280 nm (corresponding to the “chelated” enol form) with irradiation time, as shown in Figure 2.^[10] The

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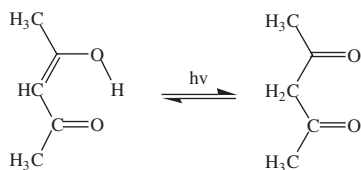


Fig. 1. Scheme for phototautomerization of β -diketone compounds.

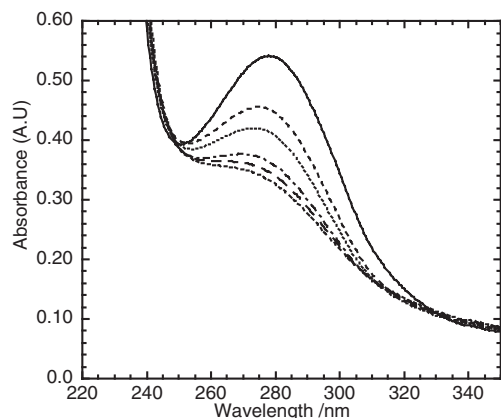


Fig. 2. UV spectra of AA/AAm/MBAAm hydrogel in water measured at different times under UV irradiation. AA content in hydrogel: 9.09 mol-%. From top to bottom: solid line, pristine sample; dashed line, 15 min; dotted line, 30 min; dash-dotted line, 60 min; long-dashed line, 90 min; short dashed line, 120 min.

changes in the absorption spectra under UV illumination are consistent with those observed previously for poly(acryloylacetone) (PAA) in solution and films, which have been attributed to a shift in the tautomeric from the “chelated” enol form to the diketo tautomer.^[8,11] Moreover, consistent with earlier reports, we observed that the keto fraction in the hydrogel increases with an increasing solvent polarity.^[12]

Petkov and co-workers^[8,11] have studied the photoinduced changes in the tautomeric equilibrium of PAA by FT-IR spectroscopy. They observed a decrease with irradiation time in the absorbance at 1605 cm^{-1} , which was attributed to the “chelated” enol CO stretching frequency, and an increase in the absorbance of the band at 1720 cm^{-1} , which was ascribed to the diketo form. We measured the IR spectral changes in the AA/AAm/MBAAm hydrogel before and after UV irradiation. In these samples, we found that both peaks are shifted to higher wavenumbers (1655 and 1728 cm^{-1}) compared with the neat PAA film. Under UV irradiation, the absorbance at 1655 cm^{-1} decreased with irradiation time, while the band at 1728 cm^{-1} appeared and then increased in absorbance. We found no effect of dissolved oxygen on the photoconversion, as indicated by FT-IR spectroscopy. These results support the existence of an enol to diketo form shift in the AA/AAm/MBAAm hydrogel under irradiation.

Petkov and co-workers observed that there was very little reverse reaction (diketo to enol) in the dark after 25 h in PAA films, indicating that the reaction is very slow. We also found that the reverse reaction in the AA/AAm/MBAAm hydrogel is very slow, such that the enol to diketo conversion under irradiation is essentially irreversible.

We noted that irradiation of the AA/AAm/MBAAm hydrogel at 244 nm resulted in an increase in the pH of the water in which the hydrogel was immersed, as shown in Figure 3. The pH change showed little recovery with time after illumination, over a period of about two hours. The slow recovery of the pH change may be related to the irreversibility observed for the reduction in the enol concentration with time.

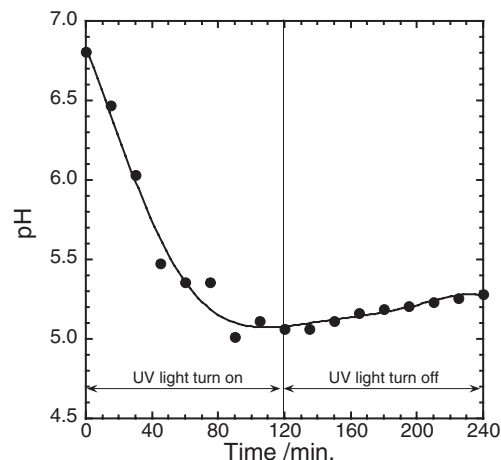


Fig. 3. pH of water in the presence of AA/AAm/MBAAm hydrogel immersed in water measured during UV irradiation and under dark conditions. AA content in hydrogel: 9.09 mol-%.

2.2. Photoinduced Swelling of Acetylacetone Hydrogels

UV irradiation of the hydrogels at 244 nm was found to induce significant swelling of the gels. Figure 4 shows the evolution of L/l_0 (L is the length of the gel cylinder at a given time and l_0 is the initial cylinder length) with time for a period of illumination followed by a period with the illumination off, for three gel compositions with different mole percentages of AA. Under UV illumination, the degree of swelling of the hydrogel increases with time, but the dimension of the gel did not change under dark conditions after exposure, indicating that

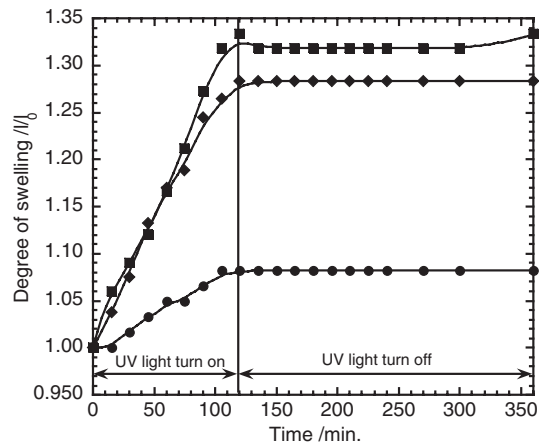


Fig. 4. Degree of swelling versus time for AA/AAm/MBAAm hydrogel in water. AA content in the gel: 1.97 mol-% (●); 4.43 mol-% (■); 9.09 mol-% (◆).

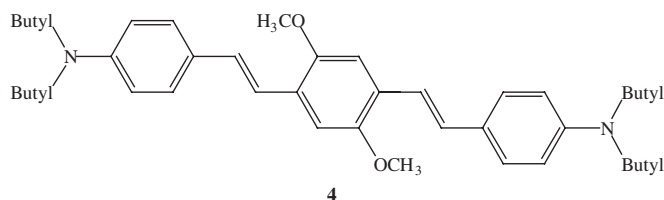
the swelling is essentially irreversible. It is possible that the pH change in the gel results in swelling due to the osmotic pressure between inside and outside of hydrogel.^[13] There may also be a contribution associated with the change in the tautomer concentrations in the gel. The degree of swelling increased with AA content up to 7 mol-%, however at higher AA content the degree of swelling decreased slightly. All gels studied exhibited substantial swelling within 120 min and the swelling appears to be a relatively slow process. Under diffusion limited conditions, the equilibration time, T , of a polymer gel undergoing swelling is given approximately by^[14]

$$T \propto L^2/D \quad (1)$$

where L is the dimension of the gel and D is the solvent diffusion constant in the gel. By reducing the size of the hydrogel structure, the response time can, in principle, be shortened appreciably in comparison with bulk materials under diffusion limited conditions.

2.3. Microfabrication of a Hydrogel Cantilever

Two-photon polymerization initiators and photopolymer resins have been used to fabricate a wide range of microstructures that are of interest in a number of emerging technologies.^[15] These include micro-optical components, such as tapered wave-guides, diffraction gratings, and photonic bandgap type structures, as well as simple micromechanical devices, such as passive cantilever structures. An important parameter that needs to be characterized and ultimately optimized for a two-photon photopolymer system is the sensitivity. Lower power and shorter exposure times can be used to fabricate a desired structure as the resin is made more sensitive. The TPIP sensitivities of a range of semi-solid photopolymer resins based on triacrylate monomers have been characterized under exposure with femtosecond pulses at wavelengths in the range of 730–800 nm. The threshold power for writing (P_{th}) was defined as the power for which a polymeric structure could be fabricated that was adequately cross-linked so as to survive the developing stage. P_{th} values of 0.2 to 0.3 mW were obtained for dye **4** and a related dye as two-photon initiators in a triacrylate based



resin.^[15] Similar threshold measurements were performed for AA/AAm/MBAAm comonomer solutions with dye **4** acting as the two-photon initiator. P_{th} was found to be 0.4 mW at 730 nm with for this hydrogel forming comonomer solution. Optically induced damage was observed for resins with or without **4** at powers greater than 10 mW (damage power, P_D). The dynamic writing power range for polymerization (P_D/P_{th}) is

thus 25 for the gel forming system and is comparable to the results for the triacrylate resin.^[16] This indicates that dye **4** in the presence of AA, AAm, and/or MBAAm monomers has a quantum yield for radical initiation that is comparable to the one in the presence of acrylate monomer, and that this initiator is effective for two-photon initiation of these monomer resins. In the absence of initiator, no polymerization occurred at powers below the P_D .

Figure 5 shows schematically the procedure for fabrication of the microcantilever. Figure 6a shows a transmission optical microscopy image of micro-cantilever in water. The microcantilever was anchored by using an adhesion promoter on a fused silica substrate.

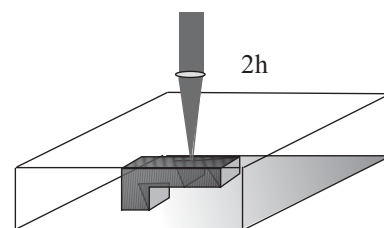


Fig. 5. Schematic diagram of the method for fabrication of a micro-cantilever by two-photon initiated polymerization.

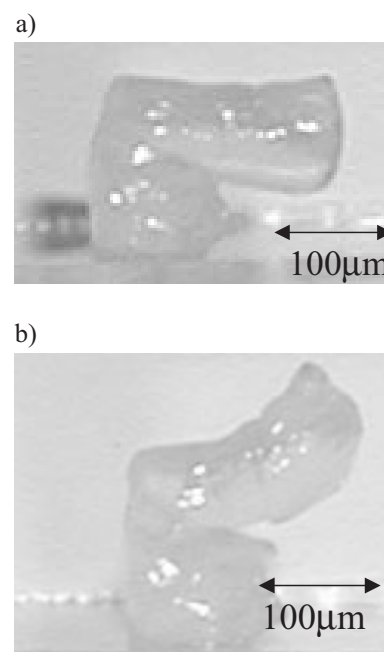


Fig. 6. a) Optical microscopy image of a micro-cantilever in water prior to UV irradiation. The micro-cantilever was fabricated on a fused silica substrate. b) Optical microscopy image of the micro-cantilever in water after UV irradiation. The microcantilever was irradiated with UV light through the fused silica substrate.

2.4. Photoactivation of Hydrogel Cantilever Deflection

Figure 7 illustrates the actuation of the hydrogel microcantilever by illumination. A collimated UV beam was delivered to the underside of the microstructure. The cantilever was observed to deflect upwards by $\sim 45^\circ$ after illumination with an

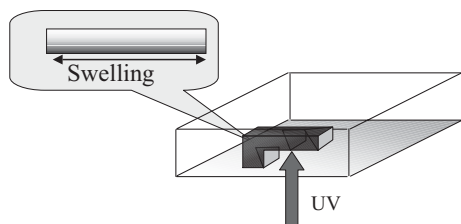


Fig. 7. Schematic illustration of the photoactuation of the hydrogel micro-cantilever. The bottom of the cantilever is swollen by UV excitation due to phototautomerization and associated reactions.

intensity of 3 mW cm^{-2} at 244 nm for 20 min. This is consistent with a swelling of the bottom of cantilever induced by UV excitation. Figure 6b shows an optical microscopy image of the deflected microcantilever in water. The 20 min. response time of deflection is considerably shorter than the time for photoinduced swelling of the bulk specimens described above. However, given the timescales for the photoreaction as described above, the deflection time is likely limited by the kinetics of photoreaction rather than a diffusion process. Through use of suitable hydrogel compositions and photoreactive moieties, the response time of micro-cantilever may be further improved. Although the microcantilever response is irreversible, this work demonstrates that photoresponsive structures can be fabricated by two-photon initiated polymerization. A key advantage of such optically powered microactuators is that they can be controlled remotely without an electrical connection. One can envision the use of reversible photoinduced processes in a suitable host matrix, as a means of achieving reversible photodeflections; for example, the photoisomerization of azobenzene molecules can rapidly induce bulk changes, such as the nematic-to-isotropic phase transition in liquid crystal materials.^[17]

3. Conclusions

We have shown that the acryloylacetone, acrylamide, and *N,N'*-methylene bisacrylamide comonomer system can be used as a precursor for TPIP of hydrogel microstructures. We have utilized this system for TPIP in order to fabricate a photoresponsive cantilever structure and have demonstrated the irreversible actuation of a deflection of the cantilever. This work represents a first step toward the fabrication optically controllable and movable 3D micromachine components and microfluidic systems based on photoactive polymer systems. We believe that developing higher performance and reversible photoresponsive hydrogels can both expand the applications of TPIP and offer new capabilities in a number of microtechnologies.

4. Experimental

Acryloylacetone (AA) (1) was prepared according to the procedures of Stork and Ponticello [18,19]. Other reagents (comonomers and solvents) were obtained commercially and purified by standard methods prior to use. Dye 4 was synthesized according to the procedure described in the literature [20].

Photoresponsive copolymer gels consisting of acryloylacetone, acrylamide (AAm) (2), and *N,N'*-methylenebisacrylamide (MBAAm) (3) were prepared in dimethyl sulfoxide (DMSO) solution. AA (in amounts ranging from 1 to 10 mol-%), AAm (710.8 mg, 10 mmol), MBAAm (18.5 mg, 0.12 mmol), and 2,2'-azobisisobutyronitrile (AIBN) (10 mg) were dissolved in a 3 mL portion of DMSO. The solutions were degassed with N_2 . The polymerization was carried out for 22 h at 60 °C in a glass vial in which glass capillaries with inner diameter of 1.5 mm were immersed. The gels were removed from the capillaries, washed with water, and then kept in water for 7 days to exchange absorbed DMSO with water. The dimensional change of the purified gels was determined by using an optical microscope and was expressed by the linear swelling ratios of the gels (L/l_0), where L is the length of the gels under a given condition, and l_0 is the initial length of the gels.

For the microfabrication by TPIP, 0.2 wt.-% photoinitiator (4) (two-photon absorption cross section $\delta = 900 \times 10^{-50} \text{ cm}^4 \text{ s photon}^{-1} \text{ molecule}^{-1}$ [20]) was used in the above comonomer solution instead of AIBN. The micro-cantilevers were fabricated on fused silica cover glass slips. The comonomer solution was contained between a pair of cover slips that were spaced by a 200 μm thick gasket that formed a seal. The fused silica surface was treated with an adhesion promoter, 3-aminopropyl-triethoxysilane, to prevent the detachment of the microstructure. A femtosecond Ti:sapphire laser (Spectra Physics Tsunami, ~ 80 fs pulses at a repetition rate of 82 MHz) was used as the excitation source for TPIP. The laser output was tuned into the two-photon absorption band of photoinitiator at a wavelength of 730 nm. The laser was tightly focused into the films using a high numerical aperture lens (NA=1.4, $\sim 0.35 \mu\text{m}$ radial spot size). The hydrogel microstructure was fabricated by moving the sample at a scan speed of $50 \mu\text{m s}^{-1}$ using a three-axis micropositioner (Sutter MP-285) under computer control. The microstructure was developed by washing with water after exposure to remove the unexposed comonomer solution.

A high-pressure mercury lamp (Ushio, Spot Cure SP-III, 244 nm) was used to induce phototautomerization and photoactuate the hydrogel cantilever. The power of the incident light was measured with a power meter (Ushio, UIT-150 A) and the typical irradiation intensity was 3.32 mW cm^{-2} . The light was delivered to the samples by means of an optical fiber bundle, whose output was collimated. The light was filtered using a 5 cm thick fused silica cell containing water to minimize thermal effects during the photoreaction. The phototautomerization reaction was monitored by using UV-VIS, FT-IR, and $^1\text{H-NMR}$ spectroscopy of the polymer gels in deuterated chloroform.

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