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Complex dynamic substrate control: Dual-tone hydrogel photoresists allow double-dissociation of topography and modulus

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Abstract

Hydrogels are widely utilized as artificial extracellular matrices, but current materials are unable to completely recapitulate the geometric, mechanical and dynamic characteristics of natural tissues. Here, we report an approach to dynamically tune both topography and elasticity in a single photoresponsive hydrogel substrate. Upon exposure to spatially controlled doses of light, a topographically and mechanically micropatterned surface forms. Atomic force microscopy was used to investigate changes in topographical feature size and elastic moduli of the hydrogel surface as a function of irradiation time and wavelength. These photodegradable hydrogels can act as both positive and pseudo-negative photoresists, depending on exposure time and wavelength. By carefully controlling the aspect ratio (surface area to depth) of micropatterned features, unique swelling-induced ordered microstructures can be formed on the surface. These dual-tone hydrogel photoresists therefore allow dynamic tunability in both topography and elasticity, enabling the fabrication of complex and anisotropic biomaterials.

Keywords

Photodegradable hydrogels; dual-tone photoresist; complex topographic patterns; elastic pattern; dynamic control

Engineering a dynamic cell microenvironment that mimics the native extracellular matrix (ECM) is of great clinical interest for tissue (or organ) repair and replacement.^[1] Hydrogels are extensively investigated as two- and three-dimensional scaffolds for cells because of their high water content, tunable mechanical and physicochemical properties and their ability to be formed in the presence of biological materials such as cells, proteins and DNA.^[2–9] In order to recapitulate the natural ECM, researchers have developed a wide variety of techniques to produce hydrogel scaffolds from a variety of monomers^[10, 11] and polymers with controlled elasticities,^[12, 13] chemical patterns,^[14, 15] and varying micro- and

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Supporting Information

Supplementary figures S1–S5 are available from the Wiley Online Library or from the author.

nano-topologies.^[16, 17] Among these replicated characteristics, biomaterials/hydrogels with controlled elasticities and topographies have been the subject of intense research effort in the past decade, as researchers have discovered that mechanical and spatial cues provided by ECM are capable of regulating cellular function and fate such as cell proliferation, migration, spreading, morphology and differentiation.^[18–22] In a striking example of the effect of substrate elasticity on cell differentiation, Engler and coworkers demonstrated that multipotent mesenchymal stem cells become neurogenic, myogenic, and osteogenic when the elasticity of cultured substrates is similar to the rigidity of neural, muscle, and bone tissue respectively.^[23] In an example demonstrating the effect of substrate topography on cell fate, Kim et al. reported that myocytes cultured in nanoscopic grooves and ridges are highly sensitive to the nanoscale topographic features and that the features are necessary for monolayers of these cells to form a contractile tissue.^[24]

In vivo, natural tissues are rarely physically or mechanically homogenous, but rather exhibit a range of elasticities and textures/topographies. For instance, the brain alone has collagen fibers with an elastic modulus ranging from 20–100 kPa and with fibrils of 200–500 nm in diameter.^[25] Using natural tissues as inspiration for designing new hydrogel scaffolds, we are not only interested in how the physical environment affects cell behavior, but in particular, how an inhomogeneous physical environment affects cells. Many strategies and materials to produce hydrogels with controllable and patternable elastic moduli and topographic features have been explored. For example, to obtain hydrogels exhibiting a gradient in elastic modulus, researchers commonly polymerize adjacent solutions of differing polymer concentrations through microfluidic devices or patterned molds,^[26–30] or polymerize with different photo-initiation conditions^[31, 32] to obtain a gradient in crosslinking density. To fabricate hydrogels with controlled topographical features, photolithography,^[33, 34] micromolding,^[35, 36] microfluidics, microcontact printing and gel printing^[37] are usually employed. Although these techniques allow fabrication of physically patterned hydrogels, there are still some limitations in the current research. First, the hydrogel patterns fabricated currently allow either topographic patterning^[34, 38–40] or elastic patterning,^[27, 30, 41, 42] but no hydrogel with controlled variations in both topography and elasticity has been reported. Secondly, most of the demonstrated hydrogel patterns are static or predefined, and cannot be altered dynamically, especially once cells have been introduced into the scaffold. Finally, topography control is typically exerted in a “one-way” manner. For example, hydrogels with defined topography can be prefabricated using a mold to produce a pre-defined surface. Alternatively, topography is patterned after hydrogel fabrication by inducing either hydrogel swelling^[43] or hydrogel erosion,^[40] resulting in features that are either raised or lowered with respect to the original hydrogel surface. With few exceptions, each of these approaches produce a static, unchanging environment for cells.

Cells in native tissues reside in a dynamic environment in which the physical environment may change spatially and/or temporally (i.e., during tissue remodeling). Therefore, hydrogels in which both topography and elasticity are dynamically tunable undoubtedly represent an important tool for investigating the synergistic effects of inhomogeneities in the physical environment on cell behavior, and better mimic the native microenvironment for

tissue engineering applications. Here we report a photodegradable hydrogel substrate in which the elastic modulus and hydrogel topography are precisely controlled using light. We exploit changes in hydrogel crosslink density induced via photodegradation to create hydrogels exhibiting complex patterns of both elasticity and topography.

Photodegradable hydrogels have garnered significant interest since the seminal report in which Kloxin *et al.* incorporated 2-methoxy-5-nitro-4-(1-hydroxyethyl) phenoxybutanoate (an *ortho*-nitrobenzyl (*o*-NB) linker) into the backbone of a poly(ethylene glycol) (PEG) macromer.^[44] Because the photodegradable linker is incorporated into the backbone of the polymer network, exposing the hydrogel to light results in a decrease in the crosslink density (ρ_x) in the exposed areas. At a critical exposure time, de-gelation occurs, and the sample erodes. Previous studies from our group and others have reported patterns ranging from the macroscale to the microscale and below via photoerosion of hydrogel substrates.^[40, 45] The elastic modulus (E) of a crosslinked material scales directly with crosslink density ($E \propto \rho_x^{6/5}$), so a decrease in ρ_x prior to reaching the de-gelation point results in a softer substrate. Kloxin *et al.* demonstrated this effect through partial degradation of the photodegradable hydrogel to generate a surface with a gradient in elastic modulus.^[32] Because the volumetric swelling ratio (Q) scales inversely with crosslink density ($Q \propto \rho_x^{-3/5}$), patterned partial degradation of a material can also be used to generate features with an increased Q , previously demonstrated by our group.^[45] By carefully controlling either the composition (i.e. fraction of degradable macromer) or exposure time, photodegradable hydrogels can act as positive-tone or pseudo-negative tone photoresists. Careful control of the crosslink density should allow dual-tone behavior in a single photodegradable substrate, which may be utilized to generate complex patterns of both elasticity and topography.

To demonstrate these effects, we prepared photodegradable hydrogels via free-radical copolymerization of varying ratios of the photodegradable macromer, PEG 4000 4-(4-(1-(acryloyloxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate, (PD-PEG_{4K}-DA, $M_n=4672$ g mol⁻¹) and PEG 4000 diacrylate (PEG_{4K}-DA, $M_n=4108$ g mol⁻¹) in phosphate buffered saline (PBS); ammonium persulfate/temtramethylethylene diamine was used as the initiating system. Table 1 summarizes the physical properties of the photodegradable hydrogels after equilibration in PBS. As the ratio of PD-PEG_{4K}-DA:PEG_{4K}-DA increases, the elastic modulus (E) decreases, while the volumetric swelling ratio (Q) and mesh size (ξ) increase. The difference may be due to chain transfer or termination reactions occurring with the photodegradable group, as nitro groups generally have a high chain transfer constant in free radical polymerizations.^[46]

To demonstrate both swelling and erosion in a single composition, a photodegradable hydrogel was prepared from a 4:1 mass ratio of PD-PEG_{4K}-DA to PEG_{4K}-DA. A topographic pattern was created on the gel surface by exposing the hydrogel through a TEM grid with square holes to UV light (365nm) at 7.0 mW cm⁻² for varying amounts of time. With this method, only the square regions that were not shielded by metal bars of the TEM grid were exposed to UV.

To characterize the three-dimensional structure of the micropatterns generated on the hydrogels, the surface topography and Sneddon modulus were mapped using AFM. After 30 seconds of UV light exposure, the TEM grid pattern is faithfully reproduced in the squares ($19\text{ }\mu\text{m} \times 19\text{ }\mu\text{m}$), which are swollen relative to the bars by roughly $140 \pm 20\text{ nm}$ in height (Figure 1a). If a longer exposure time is used, ($t=3\text{ min}$), the squares are eroded with a depth of about $1.25 \pm 0.1\text{ }\mu\text{m}$ relative to unexposed areas around them (Figure 1b). The relative elastic modulus map of these same regions reveals that the exposed squares, whether swollen or eroded, are also softer (lower modulus) than the unexposed areas (Figure 1c, 1d) regardless of whether swelling or erosion occurs. This behavior is qualitatively consistent with the scaling relationships between ρ_x , Q and E . That is, as ρ_x decreases (due to light exposure), E decreases and Q increases until the de-gelation point is reached, at which point the network erodes. Although this effect is theoretically possible for any photodegradable hydrogel, it may not be observable or exist in a practical range for all compositions. Nevertheless, under this composition it is easily possible to create both swollen (raised) and eroded (lowered) topographic patterns superimposed on a pattern with decreased modulus by adjusting the extent of cross-link degradation. The range of this double-dissociation could presumably be tuned by adjusting the ratio of PD-PEG_{4K}-DA to PEG_{4K}-DA, and allows more complex patterning of material characteristics than has been previously available in hydrogels, simply by adjusting the exposure conditions.

The transition between swelling and erosion occurs at some critical crosslink density, which will vary with the fabrication conditions and composition of the hydrogel. For a given composition, at a fixed wavelength and intensity of light, this corresponds to a critical exposure time for de-gelation. To determine the critical exposure time for our system, we exposed the photodegradable hydrogels to UV light with the same conditions (365 nm , 7.0 mW cm^{-2}) through a TEM grid for increasing amounts of time and measured the height difference between the exposed and un-exposed regions. When the exposure time is less than 170 s , the exposed areas are swollen relative to the unexposed areas, and the height of exposed areas increases with increasing exposure time (Figure 1e). Beyond 170 s , the exposed areas are eroded relative to the unexposed regions, and the erosion depth increases with increasing exposure time as well. For hydrogels exposed for 170 s , some exposed areas are swollen relative to unexposed regions while others are eroded relative to unexposed regions, as indicated by the positive and negative depths seen at $t=170\text{ s}$ in Figure 1e. This disparity reflects the sensitivity of the swelling/erosion behavior to the local crosslink density; the transition is likely very quick. Small deviations in light intensity or local cross-linking density at 170 s can cause the hydrogel to either swell a great deal, or collapse and erode. While 170 s is the critical de-gelation time for the transition between swelling and erosion under the conditions and gel composition used here, it is also an indirect measure of the critical cross-linking density.

More complex 3D microstructures can be created through sequential UV exposures. To demonstrate this, a hydrogel was exposed to UV light (365 nm , 7.0 mW cm^{-2}) through a TEM grid for 30 s , and the TEM grid was then rotated and translated on the hydrogel surface and exposed for another 30 s (Figure 2a–d). When analyzed by AFM the result is a hydrogel surface with two overlaid square micropatterns in which both sets of squares are swollen (Figure 2e). Areas that were exposed twice (overlapping squares) have a height of

286 ± 29 nm relative to unexposed areas, whereas the height in the regions receiving only one UV dosage (either during the first or second UV exposure) is 135 ± 15 nm. Figure 2e shows the height and modulus profile along the surface through regions experiencing different exposure conditions. The modulus is the greatest in the unexposed areas (red lines), moderate in the areas exposed once (yellow lines) and lowest in the areas exposed twice (green lines). As expected, the modulus decreases with increasing exposure time, and varies inversely with feature height. It is important to note in this experiment the maximum cumulative exposure time is 60 s, well below the critical de-gelation time, thus no erosion occurs. As demonstrated in this experiment, UV modification has a cumulative effect on the photodegradable hydrogel surface even when discretized in time and space.

If the exposure time is increased to 90 s for each sequential exposure, a single exposure time is less than the critical de-gelation time, while the cumulative exposure time exceeds the critical de-gelation time (Figure 3a). Therefore, areas exposed twice should exhibit erosion while areas exposed once should exhibit swelling. This is demonstrated in Figure 3, in which two opposite square micropatterns are observed on the hydrogel surface: one with swollen squares and the other with eroded squares (Figure 3b). Figure 3c is a 3D rendering of the surface, and indicates both erosion (darker pitted regions) and swelling (squares arising from grid pattern). The modulus of the resulting surface can also be mapped (Figure 3d); unexposed areas are the most rigid, twice exposed areas are the softest, and areas exposed once exhibit an intermediate modulus. The decreasing modulus correlates well with increasing exposure time, regardless of whether the exposure induces swelling or erosion. Figure 3e qualitatively summarizes the resultant modulus and topography obtained from sequential 90 s exposures.

In addition to using a single photomask (TEM grid) to produce different patterns, combinations of different photomasks may also be used to achieve even more complex surface patterns. For example, we exposed a hydrogel to UV first through a large size grid (200 mesh grid, 90 μm hole width) for 90 s, and then through a small size grid (1000 mesh grid, 19 μm hole width) for another 90 s. The 3D image in Figure 3f shows that small eroded squares sit on a large swollen square. Other topographies can be easily generated by utilizing sequential exposures of different times (i.e. 90s/30s, Figure S1). Overall, varying the exposure time, the number of exposures and the photomasks used allows the facile generation of hydrogels with complex topography and tunable stiffnesses. Carefully manipulating exposure time and utilizing sequential exposures results in dual-tone behavior of these photodegradable hydrogels with doubly dissociated topography and modulus. Exploitation of this and any number of other optical effects possible during photodegradation allows the fabrication of complex, patterned soft materials for studying the synergistic effects of substrate stiffness and microtopography on cell behavior.

The range of tunability for both feature size and modulus is directly relevant to biological studies. For instance, we have demonstrated topographic features from 100nm to 2 μm (seen in Figure 1e) in the Z-direction (3D), and from hundreds of nanometers (diffraction limit) to centimeters in 2D patterns.^[45] Topographical feature sizes ranging from nanometers to micrometers have already been reported to influence cell behavior in static systems.^[20, 40, 47] Additionally, we can adjust the stiffness of a single hydrogel over

significant range (the stiffness of the hydrogel after 30 s/30 s UV exposure at 365 nm, 7.0 mW cm⁻² decreased ~50% from the initial modulus of 55.6 kPa (Table 1) shown in Figure 2e,) and, using different fabrication conditions, can extend this range into both softer and more rigid directions. This is a significant change for cells, and differences of this magnitude have been reported to induce different stem cell differentiation pathways.^[23] Therefore in the current manipulation, the changes of the properties (both modulus and topography) are significant enough and in an appropriate absolute range to be relevant in cell applications. For example, contact guidance on patterned substrates is cell-specific (Figure S2), where human mesenchymal stem cell (hMSC) morphology is guided by surface geometry whereas fibroblast morphology is independent of surface features. Furthermore, hMSCs exhibit feature-size limited contact guidance on substrates with a variety of patterned geometries (Figure S3–S4).

In the literature, a number of groups have reported swelling-induced micropattern formation in hydrogels with patterned regions of varying crosslink density. This effect has been attributed to spatially constrained hydrogel swelling, and was elegantly described to depend on the aspect ratio of the features exhibiting differences in crosslink density (and hence difference in Q).^[48, 49] In a photodegradable system, the crosslink density and Q scale with the concentration of photodegradable groups in the system. At any given time, the concentration of photodegradable groups remaining in the system is given by Equation 1, where ϕ is quantum yield, ϵ is molar absorptivity, I is light irradiance (intensity), λ is wavelength, N_A is Avogadro's number, h is Planck's constant, and c is the speed of light.^[50]

$$PD = [PD]_0 e^{-\left(\frac{\phi \epsilon \lambda I (2.303 \times 10^{-6})}{N_A h c}\right) t} \quad (1)$$

Light attenuation is described by Equation 2, where x is the depth of penetration of light, and I_0 is the irradiance at the surface of the sample:

$$\frac{I_x}{I_0} = e^{-\epsilon [PD] x} \quad (2)$$

If concentration of the photodegradable group is not varied, the patterning depth (and therefore aspect ratio of any features formed) can be modulated either by a) increasing the intensity of light such that a higher intensity of light reaches a specific depth in the material or by b) using a different wavelength of light for which the molar absorptivity of the *o*-NB group is different.

To test the first effect, a hydrogel was exposed to UV light (365 nm, 22.5 mW cm⁻²) through a TEM grid for 90 s, and the TEM grid was then rotated and translated on the hydrogel surface and exposed for another 90 s. Although the % transmittance of light is unchanged, utilizing a higher intensity results in a higher absolute intensity through the depth of the hydrogel. The translation of the grid between exposures creates several regions with different exposure conditions, including an area that is first patterned with squares, and then uniformly exposed during the second exposure (because the grid no longer masks that area). Rather than a simple uniform erosion of the first patterned squares, as was observed using

lower intensity light (7.0 mW cm^{-2}), a slight basket weave pattern was created upon equilibration (Figure 4a). This basket weave pattern can be attributed to constrained swelling of the degraded regions- that is, the degraded features are still covalently linked to the bulk, undegraded hydrogel, which constrains the swelling at the interface between the two regions. At lower intensity (7 mW cm^{-2}), photodegradation is restricted to the surface of the hydrogel, as the attenuation along with a short exposure time do not allow for extensive photodegradation far below the surface (Figure S5). The interfacial area between the degraded and undegraded regions is greater using high intensity light because the depth of the feature is greater while the 2D area of the pattern remains unchanged. Only with the use of high intensity light was the ratio of exposure depth to 2D area large enough to induce the deformation and constrained swelling necessary for the basket weave pattern. Our results are consistent with previous reports relating buckling to aspect ratio. That is, qualitatively, the aspect ratio of the features increases when a higher intensity is used (the squares penetrate deeper into the hydrogel), and as a result a swelling-induced micropattern is observed. If the same exposure conditions are used, but a lower mesh TEM grid is used, the aspect ratio decreases (the depth of penetration is unchanged but the squares are larger) and the basket weave pattern no longer forms (Figure 4b). This swelling/buckling phenomenon is another tool that can be used to create complex and changing topography and modulus, depending on gel fabrication and exposure conditions.

In addition to increased light intensity, the wavelength of light exposure also affects the depth of penetration due to the change in molar absorptivity of the photodegradable moiety. The molar absorptivity decreases from $3500 \text{ M}^{-1}\text{cm}^{-1}$ at 365 nm to $845 \text{ M}^{-1}\text{cm}^{-1}$ at 405 nm .^[51] The intensity of light through a 1 mm thick gel over the course of a long exposure can be modeled roughly with the Beer-Lambert law (Figure S2), assuming a sufficiently dilute concentration of absorbing species in the range of applicability for Beer-Lambert's Law. In this model, 405 nm light can penetrate roughly 3–4 times deeper than 365 nm light, but takes much longer to degrade the material close to the surface. Because of the difference in molar absorptivity, the total number of degraded moieties could be the same, but their distribution through the gel thickness would be different. When changing the wavelength, in order to achieve an equivalent cumulative flux, the exposure time must be adjusted to reflect the relative ratio of molar absorptivities at the two wavelengths, and, ideally, take into account the absorptivity of the degraded species. We exposed the hydrogels to light at 405 nm with the same high intensity of 22.5 mW cm^{-2} and the equivalent exposure time (335.8 s) as that used with 365 nm light (90 s). Under these equivalent conditions, the basket weave pattern is obtained (Figure 4c). Additionally, many larger pattern sizes (up to 200 mesh size grid) (Figure 4d and 4e) result in the formation of this micropattern, in contrast to the patterns formed when an equivalent dose of 365 nm light is used. Compared to 365 nm light, 405 nm light penetrates deeper into the hydrogel, reinforcing the hypothesis that the aspect ratio of the patterned features is critical in forming swelling-induced micropatterns. Thus the basket weave, or any other derivative pattern can be generated on the surface of a photodegradable hydrogel through the swelling/buckling behavior, and the patterns can be designed and differentially controlled by initial pattern size as well as exposure intensity and/or wavelength.

In summary, we demonstrate an approach to creating complex topographic microstructures with varied modulus profile on photodegradable hydrogel surfaces. By exposing the hydrogel to UV light through a photomask, both swollen and eroded micropatterns with a decreased modulus profile can be created on the photodegradable hydrogel surface under different exposure conditions. Using sequential patterning and the switch between local swelling and erosion of the hydrogels (characterized here as a critical time), multilayer topographical patterns with interlaced swelling and erosion are easily generated. In addition, a derivative basket weave pattern, induced by deformations caused by the interfacial stress between areas of different crosslink density, can be created by various combinations of the light intensity, wavelength, and mask feature size. The optical and photochemical properties of a photodegradable hydrogel enable multiple levels of control over substrate characteristics. This provides great flexibility for fabricating micropatterns on these substrates, and allows in-situ manipulation of the materials. The approach reported here is a powerful and simple method for fabricating diverse topographies with varied modulus profile on a single hydrogel surface simply by manipulating the exposure time, light intensity and wavelength. This report therefore demonstrates the potential of photodegradable hydrogels for the fabrication of complex, anisotropic biomaterials.

Experimental

Materials

Acetovanillone (Acros, 98%), nitric acid (Fisher, 70%), dimethylformamide (DMF) (Acros, 99%), tetrahydrofuran (THF) (Fisher Scientific, 99.9%), acetone (Fisher Scientific, 99.7%), chloroform (Fisher Scientific, 99.9%), phosphorous pentachloride (Alfa Aesar, 98%), poly(ethylene glycol) (PEG) ($M_n=4000 \text{ g mol}^{-1}$) (Mallinckrodt, 98%), diethyl ether (Fisher Scientific, 99.9%), ammonium persulfate (APS tablets) (Amresco, biotechnology grade), and tetramethylethylenediamine (TEMED) (EMD, 99%) were used as purchased. Triethylamine (TEA) (Fisher, 99%) was distilled under Ar and stored over KOH pellets. Acryloyl chloride (AC) (Alfa Aesar, 96%) and dichloromethane (DCM) (Fisher, 99.9%) was distilled under Ar and stored under Ar in a dry, air-free flask. TEM grids with different mesh sizes were purchased from Electron Microscopy Sciences, and their sizes are as the follows: 75 meshes grid (285 μm hole width; 55 μm bar width), 200 meshes grid (90 μm hole width; 35 μm bar width), 400 meshes grid (37 μm hole width; 25 μm bar width), and 1000 meshes grid (19 μm hole width; 6 μm bar width).

Techniques

All reactions were performed under an N_2 atmosphere using a Schlenk line unless noted otherwise. ^1H NMR spectra (δ ppm) were recorded on a Bruker Biospin Ultrashield 300 MHz NMR Spectrometer. Unless noted otherwise, all spectra were recorded in $(\text{CD}_3)_2\text{SO}$ or CDCl_3 using TMS as an internal standard.

Hydrogel Fabrication

All hydrogels were fabricated by dissolving PEG_{4K}-DA and PD-PEG_{4K}-DA in phosphate buffer at different wt % and composition depending on the experiment. Solutions of APS (0.5M in PBS) and TEMED (0.25M in PBS) were used to radically polymerize the

macromers at final concentrations of 0.1M APS and 0.05M TEMED. The mixtures were vortexed and quickly dispersed between two glass microscope slides separated by two pieces of spacers (thickness = 2mm) on either end, and allowed to polymerize for 20 min, before removing from between the glass slides. Each hydrogel was then cut into 5 mm discs for later analysis and usage.

Fabrication of Hydrogel Patterns

The TEM grid as a photomask was placed directly onto hydrogel surfaces, and the hydrogel disk was surrounded by DI water. An Omnicure 1000 mercury vapor lamp (EXFO) equipped with a 365 nm or 405nm narrow bandpass filter and an Omnicure adjustable collimating adapter was used to deliver light to the hydrogel surface for desired times at 7 or 22.5 mWcm⁻². Light intensity was measured with a spectroradiometer (ILT 950, International Light Technologies). After light exposure, the TEM grid was removed from the hydrogel surface, and its topographic features were examined by phase contrast microscopy (Zeiss Axiovert Observer Z1) and atomic force microscopy (AFM).

Dynamic Mechanical Analysis (DMA)

The bulk static elastic moduli of hydrogels were determined using a Q800 dynamic mechanical analyzer (DMA) (TA Instruments). The measurement was performed in strain rate mode. The hydrogel samples were prepared in disk shape with 5mm diameter and approximately 2.5 mm thickness. The samples were mounted into the bottom of the submersible compression clamp, surrounded by DI water, and then compressed (one dimension, unconfined) with a constant strain rate of 60% strain min⁻¹ to a final strain of 30%. The values of the bulk elastic modulus were calculated from the slope of the stress-strain curve from 10% to 15% strain. Three samples were measured for each hydrogel composition. The study was conducted at room temperature.

Atomic Force Microscopy

To examine the surface topography and elastic modulus of hydrogels, AFM measurements were performed in aqueous solution using a Bioscope Catalyst AFM (Bruker Bioscope catalyst atomic force/Zeiss LSM 5 confocal fluorescence microscope) in the Nano & Pico Characterization Laboratory at California Nanosystems Institute (CNSI), UCLA. Images were acquired in peak force tapping mode (PeakForce Quantitatively Nanomechanical Measurement, QNM) with the MLCT probes (Bruker), tip E. For the quantitative measurements of moduli, the spatial sensitivity of the AFM photodetector was first calibrated against a clean glass slide. The spring constant of the cantilever was then measured using the thermal tuning method. The Young's moduli were calculated by using a Sneddon fit with a Poisson's ratio of 0.5 based on soft materials. For each sample, the topology and elastic modulus images were collected at a digital resolution of 256 pixels × 256 pixels. All calibrations and measurements were made in DI water at room temperature.

Statistical Analysis

Data is presented as mean ± standard deviation. At a minimum, three samples were averaged for each data point.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Relative surface topography and elastic modulus maps of hydrogels characterized by atomic force microscopy (AFM) in DI water after exposure to UV light (365 nm) at 7.0 mW cm^{-2} through a 1000 mesh TEM grid ($19 \mu\text{m}$ square holes, $6 \mu\text{m}$ bar width) for 30 seconds (a,c) and 180 seconds (b, d). Scale bar: $10 \mu\text{m}$. The height differences between exposed and unexposed regions of patterns were measured by AFM for a range of exposure times (e).

Figure 2.

Sequential patterning of photodegradable hydrogels. A TEM grid is used as a mask on the photodegradable hydrogel for an initial exposure (a). The grid is lifted and rotated (b), and then placed back on the gel offset from the initial location (c) for the second exposure (d); different regions (1–9) are created with differing sequences of exposure conditions.

Topography (e) of sequentially patterned surfaces after 30s and 30s exposure (365nm, 7 mW cm⁻²); height and modulus profile along regions with different exposures: red (unexposed regions), yellow (regions exposed once), and green (regions exposed twice). Scale bar: 20 μm.

Figure 3.

Topography and modulus map of sequentially patterned hydrogels after 90s/90s exposure (365 nm , 7 mw cm^{-2}). The hydrogel was exposed twice through the same size grid (1000 mesh, $19\text{ }\mu\text{m}$ hole width). a) Depiction of exposure profile for the hydrogel; b) topographic map of the surface obtained via AFM; c) 3D rendering of the surface topography; d) modulus map of the surface obtained via AFM; e) qualitative summary of topography and modulus across a cross-sectional region of the gel as a function of number of exposures; f) pattern generated from sequential exposures through two different TEM grids (200 mesh, $90\text{ }\mu\text{m}$ hole width, then 1000 mesh, $19\text{ }\mu\text{m}$ hole width). Scale bars: $20\text{ }\mu\text{m}$.

Figure 4.

Phase micrographs of photodegradable hydrogels after sequential exposure at high intensity (22.5 mW cm^{-2}) under different wavelengths. All images show the border between regions 3 and 4 (illustrated in Figure 2), where first a pattern was created on the first exposure through the TEM grid and then part of the pattern was covered by the edge of the grid while the other part was exposed to uniform irradiation during the second exposure. a) and b) are hydrogels after sequential exposure to 365 nm for 90 s/90 s through a) 1000 mesh grid, and b) 400 mesh grid. The basket weave pattern is created in region 3 only with the smaller squares (higher mesh size); c), d), e) and f) are hydrogels after sequential exposure to 405 nm equivalently for 335.8 s/335.8 s through c) 1000 mesh grid, and d) 400 mesh grid, e) 200 mesh grid, and f) 75 mesh grid. The basket weave pattern is created using much larger squares than possible with 365nm light. Scale bars: 100 μm .

Table 1

Physical properties (storage modulus (G'), Yong's modulus (E), volumetric swelling ratio (Q) and mesh size (ξ)* of 15wt% hydrogels prepared by different compositions (mass ratio) of PD-PEG_{4K}-DA and PEG_{4K}-DA.

	PD-PEG _{4K} -DA: PEG _{4K} -DA (1:1)	PD-PEG _{4K} -DA: PEG _{4K} -DA (4:1)	PD-PEG _{4K} -DA: PEG _{4K} -DA (9:1)
G' (kPa)	60.2±0.5	48.0±5.3	43.8±1.6
E (kPa)	72.9±6.3	55.5±9.8	50.8±0.4
Q	13.1	13.9	14.1
ξ^* (Å)	65.3	68.6	69.7

* Calculation detailed in supporting information.