ACS Macro Letters

Letter

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Diels–Alder Click-Based Hydrogels for Direct Spatiotemporal Postpatterning via Photoclick Chemistry

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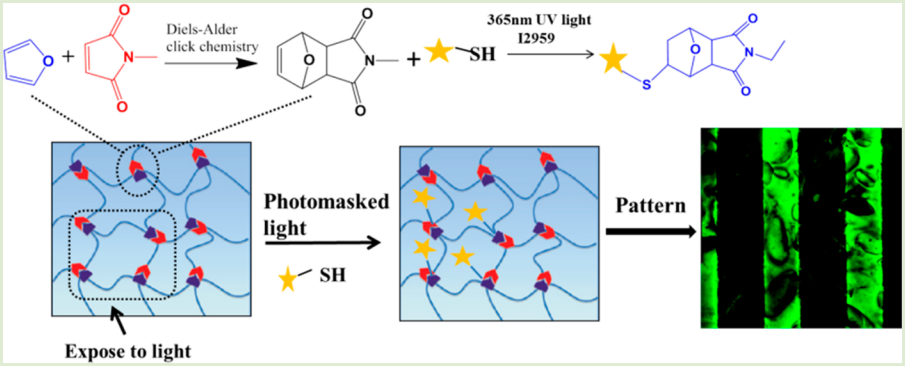
Supporting Information



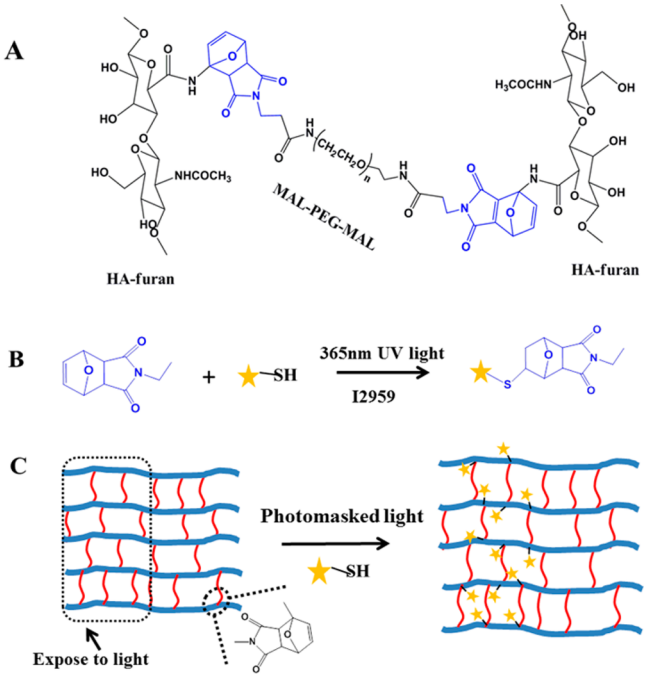
ABSTRACT: Click chemistry not only has been applied to the design of hydrogel scaffolds for 3D cell culture, but also is an efficient way for hydrogel postfunctionalization and spatiotemporal patterning. To the best of our knowledge, only azide–alkyne cycloaddition (SPAAC) has been exploited by combining photoinitiated thiol–ene click reaction to realize the 3D patterning of hydrogels. In this work, the cyclohexene derivative, which "clicked" by functional groups between furyl and maleimide, were successfully functionalized by thiol-modified molecules or peptides through thiol–ene click reaction. It illustrates a hydrogel that formed via Diels–Alder (DA) click chemistry between furyl-modified hyaluronic acid and bimaleimide functional PEG molecule can be allowed for the directly photoactivated thiol–ene chemistry for hydrogel spatiotemporal patterning. Since the cyclohexene derivatives produced by DA reaction can be employed in all subsequent 3D network patterning by using photoclick reactions, it suggests a new way to design and postfunctionalize all of the DA click-based hydrogels with specific regional bioactive cues.

Due to the high water content, tissue-like elasticity and in situ encapsulation property of cells or bioactive cues, hydrogels have become a unique platform for three-dimensional cell culture to promote the regeneration of soft tissues.[1] In the past few years, the researchers were interested in intrinsic physical properties of hydrogels, such as cross-linking density, mechanical, degradable property, and environmental stimuli sensitivity.[2] Meanwhile, the influence of these intrinsic physical cues on cell fate was also involved and took serious consideration.[3,4] For instance, it has been found that when MSCs are grown on soft gels that mimic the elasticity of muscle, myogenic markers are upregulated, whereas when MSCs are grown on rigid gels that mimic precalcified bone, the cells appear osteogenic.[5] However, it is a complicated process to control stem cell survival, proliferation, and differentiation within a vivo milieu that needs combining of growth factors, matrices, and forces.[6] In addition to matrices moduli, growth factors (GFs) are robustly required for safe and effective regeneration of functional tissues. Due to its easily deactivation and change of conformation, GF-mimicking short peptides instead of the entire GFs onto biomaterials is a promising alternative.[7,8] The related studies include grafting the peptides to a surface or a scaffold and examining whether the peptides would increase cell responses.[9,10] However, human tissue is a highly specifically assembled with spatial heterogeneity and functionality such as articular cartilage tissue.[11] How to design multifunctional gels in which the bioactive cues could be introduced and be controlled accurately in both time and space has become the key point to realize functional tissue repair.[12] In recent years, a few efforts have focused on development of bioorthogonal hydrogels by click-based reactions to introduced biochemical cues in specific regional space.[13−19] But all the reported hydrogels were cross-linked by azide–alkyne click chemistry.[20,21] In order to subsequential spatiotemporal

Received: November 24, 2014
Accepted: February 13, 2015
Published: February 16, 2015

DOI: 10.1021/mz5007427
ACS Macro Lett. 2015, 4, 289–292

Scheme 1. (A) Furyl-Modified HA Reacts with MAL-PEG-MAL via the Diels-Alder Click to Chemistry Form HA/PEG Hydrogel with Cyclohexene Derivatives; (B) Subsequent Photoinitiated Thiol−Ene Click Chemistry between Cyclohexene Derivatives and Thiol-Modified Bioactive Molecules; (C) Patterning Mechanism in 3D Hydrogel Network by Photomasked Light



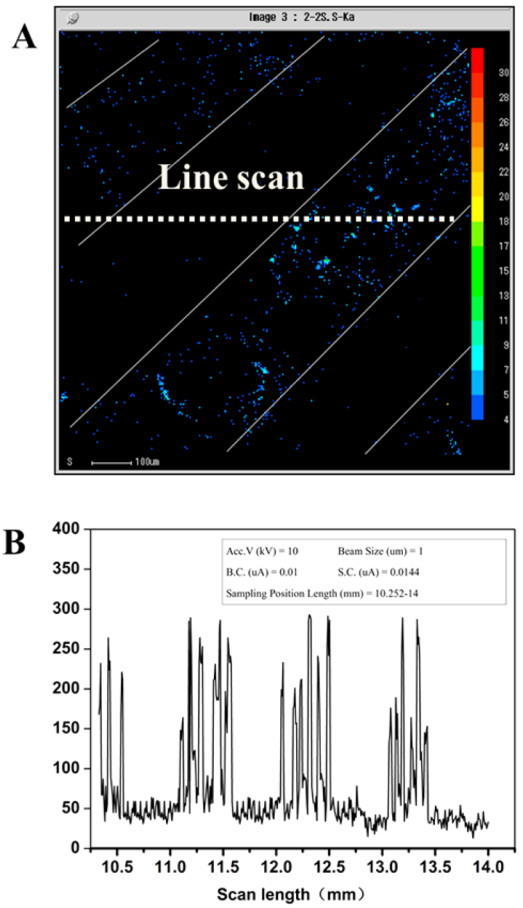


Figure 1. Electronic probe (EPMA) for area scanning and line scanning of elemental sulfur (S). (A) The area scanning of S (scanning area = 1 mm × 1 mm). (B) The line scanning of S (scanning line length = 4 mm). The photomask is with 300 μm lines and 300 μm spacing and only the area that exposed to UV light can detect the S.

patterning via thiol−ene photoclick chemistry, the carbon−carbon double bond or thiol-functional groups must be designed into 3D network in addition.[22,23]

In our previous work, we have synthesized a hydrogel with a fantastic elasticity and resilience which are formed via Diels−Alder (DA) click chemistry between furyl-modified hyaluronic acid and bimaleimide functional PEG molecule. This DA cross-linked hydrogel was first reported by M. Shoichet and co-workers.[24,25] However, the mechanical property and cell encapsulation behavior have not been discussed. Based on their work, we further tuned the hydrogel's modulus from 4.86 to 75.90 kPa, which are comparable with soft tissue elasticity from brain, fat, and muscle to cartilage.[26] Meanwhile, the cell cytocompatibility was also investigated in this hydrogel and the encapsulated cells showed good cell viability. It is worth noting that the hydrogel network is a cyclohexene derivative (marked in blue in Scheme 1A) after DA "click" between furyl and maleimide. Bowman and co-workers have reported a potential photoresist that cross-linked by reversible DA click reaction could become an irreversible network after a further thiol−ene reaction, which implies that the cyclohexene derivative (oxy-norbornene group) can further go through a thiol−ene reaction within a polymer network.[27] Inspired by this work, we utilize DA cross-linked hydrogel in conjunction with thiol-functionalized materials, such as cysteine-terminated peptides to design a complex 3D structure with unique biochemical functionals.

We here first exploited whether thiol−ene photoinitiation reaction could be happened between thiol and cyclohexene derivatives within 3D hydrogel (Scheme 1B). In addition, the FITC-labeled RGDSC peptide was patterned into hydrogel via UV lithography technology (Scheme 1C). At last, the relationship between UV light exposing time and fluorescence intensity and depth in patterned hydrogel was observed by laser confocal fluorescence microscopy. Because of the mild conditions and highly efficiency of "click chemistry", we expect that this hydrogel material can act as a potential tissue engineered scaffold to direct cell function via both tunable mechanics and specific regional biochemical cues within 3D space.

The Scheme 1A shows the cross-linking mechanism of DA reaction. The ^{1}H NMR spectrum of furyl-modified HA is shown in Figure S1. Lyophilized HA-furan was dissolved in PBS at a concentration of 1.5% w/v. The MAL-PEG-MAL was added into the solution for 5 min stirring and the mixed solution was injected into a cylindrical mode to form a gel. The cyclohexene derivative which marked as blue in Scheme 1A was "clicked" by DA reaction between furyl and maleimide groups. For investigating the feasibility of postfunctionalization of DA cross-linked HA/PEG hydrogels with cyclohexene derivative groups using photoinduced radical thiol−ene reactions, a model compound (P-MIN) was prepared according to the steps, as shown in Figure S2. The ^{1}H and ^{13}C NMR spectra of every product are exhibited in Figures S3−S5. From the results, it can be concluded that the DA products (cyclohexene derivatives) could successfully react with thoil functional groups by UV light-induced polymerization.

DOI: 10.1021/mz5007427
ACS Macro Lett. 2015, 4, 289−292

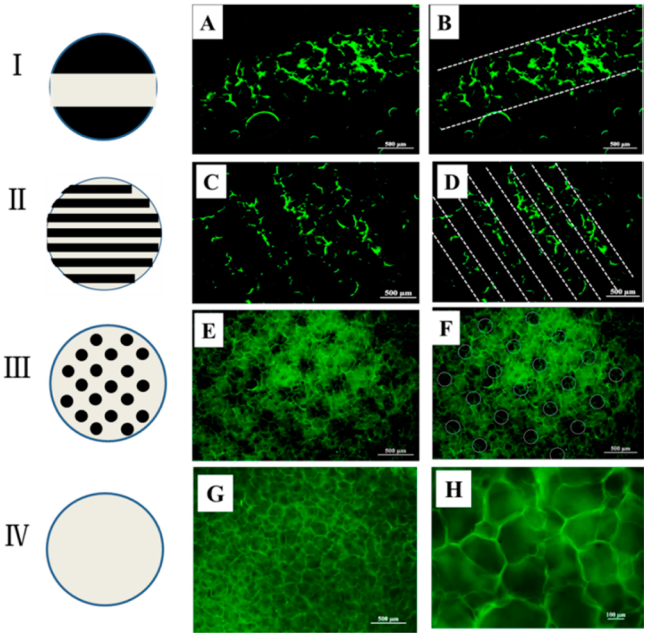


Figure 2. Biochemical patterning in DA-based click gels. I–IV is the photomask pattern: (I) the exposing band of 1 mm; (II) the stripe with 300 μm lines and 300 μm spacing; (III) the diameter of circular region is of 300 μm; (IV) all area is exposing. A–H: The hydrogels with different patterns are labeled by FITC and shown below.

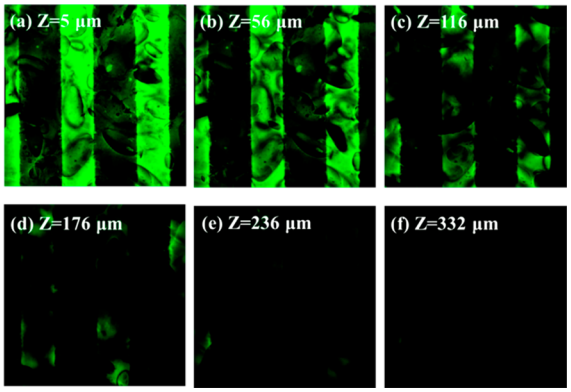


Figure 3. Relationship between fluorescence intensity and pattern depth.

The biochemical pattern of DA-based hydrogels was further confirmed and the scheme is shown in Scheme 1C. The detail methods are showed in Supporting Information. The mercaptopropionic acid patterned hydrogel without fluorescent tag was freeze-dried and characterized by electronic probe EPMA (SHIMADZU, Japan). As shown in Figure 1, the area exposed to UV light has elemental sulfur (S) due to thiol–ene click chemistry, while the unexposed area did not. The area scanning of S (scanning area = 1 mm × 1 mm) is shown in Figure 1A. The 300 μm stripe of S and 300 μm spacing can be obviously found. The line scanning of S is shown in Figure 1B and the actual interval distance (d) is about 400–500 μm. Because the inclined angle between scanning line and stripe was of about 45°, the theoretical interval distance could be calculated by formula below.

$$d = \frac{300}{\sin 45} = 424\ \mu\text{m}$$

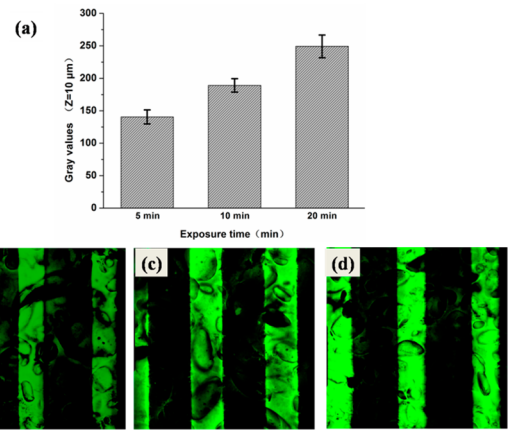


Figure 4. Fluorescence intensity changed along with the exposure time. (a) Relationship of gray values and exposure time. (b–d) Fluorescence images of patterned hydrogel under different exposure time: (b) 5, (c) 10, and (d) 20 min.

The observed value from Figure 1B was consistent with theoretical interval distance.

FITC-labeled RGDSC patterned hydrogel was observed by fluorescence microscope (Eclipsc Ti–U, Japan). The exposed UV light area could conjugate the FITC-labeled RGDSC peptide and four kinds of photomasks (Figure 2I–VI) were used to test the hydrogel biochemical patterning. As shown in Figure 2A,B, the width of fluorescent strip is about 1 mm which was consistent with the exposing area in photomask (width = 1 mm). In Figure 2C,D, the fluorescent areas are stripes with 300 μm lines and 300 μm spacing which are same with photomask II. Similarly, with photomask III, the nonexposing area was a circle and the diameter was of 300 μm, which can be easily found in Figure 2E,F. Figure 2G,H shows the fluorescent images of the hydrogel, which were exposed to UV light without photomask; the fluorescent could be observed in all the hydrogel and the microstructure of hydrogel under swelled state could be also clearly seen. We further studied the swelling and mechanical property of hydrogels before and after postpatterned and found that there is no significant difference between them. The detail results are shown in Figures S6 and S7.

In addition, the relationship between UV light exposing time and fluorescence intensity and depth in patterned hydrogel was observed by laser confocal fluorescence microscopy. From Figure 3, we found that the patterned fluorescence intensity would become weak until disappear when the Z axis value changed from 0 μm (hydrogel surface) to 332 μm (hydrogel inside). Meanwhile, the patterning depth has no significant difference when the exposure time increased from 5 to 20 min. All the results showed that the exposure time have no effect on pattern depth. But the exposure time has a big influence on fluorescence intensity when the Z axis is constant. Figure 4a shows the relationship between gray values and exposure time when Z axis value was kept at 10 μm. Along with the exposure time increasing, the gray value of pattern increased rapidly, which is consistent with the change of fluorescence intensity in Figure 4b–d.

In conclusion, through integrating DA click chemistry and thiol–ene reaction, the biochemical patterned hydrogel was obtained. In addition to RGDSC peptide, any other growth factor mimicking peptides with thiol groups are also expected

DOI: 10.1021/mz5007427
ACS Macro Lett. 2015, 4, 289–292

to be introduced into DA-based hydrogels at specific time and space.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of the furyl-modified HA, functionalization of model compound via sequential Diels–Alder click chemistry (furyl and maleimide), and photoradical thiol–ene click reactions and relevant figures are provided. 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.

■ ACKNOWLEDGMENTS

This work was financially supported by National Basic Research Program of China (973 Program; Grant Nos. 2011CB606204 and 2012CB619100), National Natural Science Foundation of China (Grant No. 51372085, 51202069, and 51172073), Program for New Century Excellent Talents in University (NCET-11-0148), Fundamental Research Funds for the Central Universities (Grant No. 2013ZZ0005), and the Science and Technology Program of Guangdong Province (Grant No. 2012A061100002).

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DOI: 10.1021/mz5007427
ACS Macro Lett. 2015, 4, 289–292