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Force-mediated molecule release from double network hydrogels

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The incorporation of mechanosensitive linkages into polymers has led to materials with dynamic force responsivity. Here we report oxanorbornadiene cross-linked double network hydrogels that release molecules through a force-mediated retro Diels-Alder reaction. The molecular design and tough double network of polyacrylamide and alginate promote significantly higher activation at substantially less force than pure polymer systems. Activation at physiologically relevant forces provides scope for instilling dynamic mechanochemical behavior in soft biological materials.

Mechanochemistry, the study of how applied force facilitates chemical transformations, has gained considerable attention recently in polymer systems.[1, 2] The concept of mechanochemistry was first introduced to polymers by Staudinger in the 1930s.[3] Since then, polymer mechanochemistry advancements have grown into exciting areas such as catalysis, self-healing, drug delivery, and sensory materials.[4] This research has expanded in two primary directions: designing novel mechanophores, and developing new materials incorporating mechanophores.[5, 6] The principle of mechano-responsive materials is based on integrating molecues with force responsive bonds (mechanophores) into polymer backbones or within crosslinkers.[1] For example, spyropyran,[7, 8] 1,2-dioxetane,[9] β-Lactam,[10] cyano-substituted cyclobutene,[11] dithiomaleimide,[12] rotaxane[13] and gem-dihalocyclopropane.[14] have been widely used as mechanophores or multi-mechanophore systems.[15] These mechano-responsive polymers are attractive due to their ability to produce signals for sensing damage, for the improvement of mechanical properties (e.g., self-healing), and for the release of small molecules with a con-current signal or radical/catalyst generation,.[16, 17]

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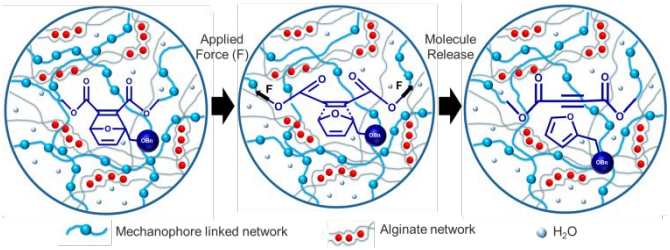

Fig. 1 Synthetic approach for generating the mechanophore crosslinker and scheme for mechanochemical activation and molecule release in double network hydrogels.

All early reports of polymer mechanochemistry employ pure polymer systems or mechanophore immobilization at the interface of hard materials.[18-20] Seminal work by Moore, Sottos, White, and colleagues introduced a polymeric material consisting of a spyropyran ring structure that transformed into the merocyanine form in response to force with an associated color change.[21] Since then, many groups have reported unique mechanochemical systems for mechanochromic and mechanoluminescence force sensors,[9, 22, 23] as well as 3D printing,[24] the activation of mechano-catalysis,[25, 26] materials with self-reinforcing/self-recovery properties,[27] revealing new functional groups,[28, 29] mechanically triggered polymer degradation,[30] and small-molecule release.[31, 32] Despite these advances in the area of polymer mechanochemistry, integration of mechanophores within hydrogels has remained relatively unexplored.[33, 34] This is largely because most mechanophore systems are hydrophobic molecules that cannot be integrated with aqueous systems. Furthermore, hydrogel materials are often brittle and unable to withstand the applied forces necessary for activation.[35, 36] Therefore, studies on hydrogel mechanochemistry have mostly been based on fluoresce/color changes[37] or radical generation.[38]

Here we demonstrate force-activated mechanochemistry in hydrogels for molecular release for the first-time. Key to this advance is the use of a double network hydrogel of polyacrylamide and alginate that imbue the material with high toughness, thereby allowing considerably more deformation to enhance molecular release via the retro Diels–Alder reaction. Boydston and Larsen introduced the concept of flex-mediated release through a retro-Diels-Alder reaction from an oxanorbornadiene mechanophore-based polymer material.[31]

Subsequently, there were several reports exploring this molecular release approach,[39] including cascading reactions[35] and activation in aqueous environments[40, 41] Inspired by these studies, we proposed that mechanophore-liked hydrogels may more readily undergo activation compared to dry polymers.

Bo and Zang have shown that the rate of mechanochemical activation for the furan/maleimide adduct depends on the polymer arms' relative proximity (proximal vs. distal) to the scissile bond, with proximal positioning demonstrating higher activation[42] We designed a Diels-Alder adduct mechanophore (Oxo-OBn) formed by alkyne/furan Diels-Alder cycloaddition to contain a pendent molecule proximal to the scissile bond (Fig. 1; Fig. S1-S3). We synthesized the Oxo-OBn mechanophore molecule by first reacting bis(6-hydroxyhexyl)but-2-ynedioate and 2-((benzyloxy)methyl)furan Next, we performed Fischer esterification between acetylene dicarboxylate and 1,6-hexanediol. The benzyl furfuryl ether was formed through a reaction between furan-2-yl methanol and (chloromethyl)benzene followed by cycloaddition with the alkyne. Finally, the reaction with methacryloyl anhydride yielded the Oxo-OBn crosslinker. Nuclear magnetic resonance spectroscopy (NMR) confirmed the synthesized molecular structure at each step (Fig. S4-S6).

To incorporate the Oxo-OBn mechanophore into a hydrogel network, we substituted the mechanophore in place of bis-acrylamide as the crosslinking agent with DMSO as solvent and ammonium persulfate as radical initiator (Fig. 2A). After complete washout of DMSO monitored using FTIR (Fig. S7), we performed preliminary mechanical tests of as-prepared single network hydrogel samples. The hydrogels showed brittle failure under tension and compression with no evidence of mechanophore activation and molecule release (data not shown). To combat the low strength and toughness of the mechanophore-linked polyacrylamide gel network, we fabricated double network hydrogels consisting of two interpenetrating polymer networks, where a densely crosslinked brittle network is supported by a flexible network with reversible bonds.[43, 44] In response to stress, the densely crosslinked network will rupture locally, generating internal damage and dissipating energy, while the flexible polymer network remains well crosslinked and keeps the material intact. To test the double network hydrogel concept in our mechanophore-linked network, we incorporated ionically crosslinked alginate as a secondary network within the monomer solution prior to crosslinking to yield a mechanosensitive double-network hydrogel (Fig. 2A).

To characterize the hydrogel assembly, we performed Raman spectroscopy. The peaks from the polyacrylamide and Oxo-OBn spectra are summarized in Supporting Information, Table S1. The spectrum of Oxo-OBn linked polyacrylamide gel features peaks associated with benzene ring breathing (1,000 cm^{-1}) and norbornadiene CH wagging (677 cm^{-1}) (Fig. 2B, Table S2). Next, we acquired the spectra surface mapping at 677 cm^{-1} wavelengths of traditional bis-acrylamide and Oxo-OBn crosslinked polyacrylamide to show the mechanophore distribution, which confirms the presence of mechanophores throughout the hydrogel (Fig. 2C). Moreover, curve fitting of

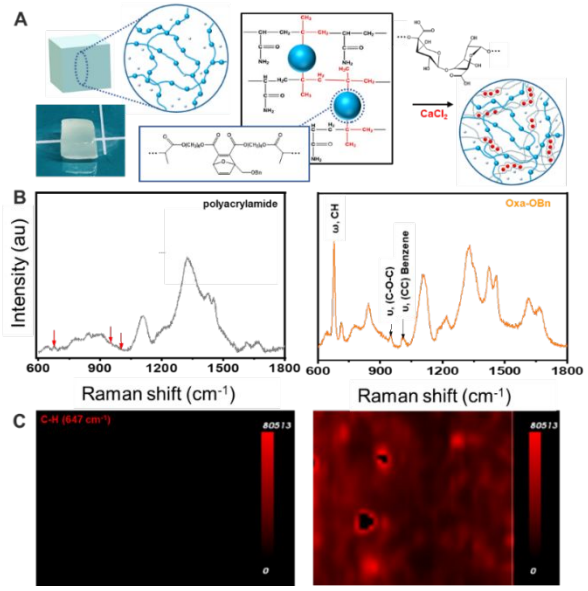

Fig. 2 A. Schematic with accompanying photograph of mechanophores integrated into double network hydrogel. B. Raman spectrum for the bis-acrylamide crosslinked polyacrylamide hydrogel (left) and the oxanorbornadiene crosslinked polyacrylamide hydrogel (right). C. Raman scan of the C-H wagging mode at 647 cm^{-1} for bis-acrylamide (left) and oxanorbornadiene (right) crosslined polyacrylamide.

the Raman spectrum across the 1400-800 cm^{-1} wavelength range reveals a peak at 1516 cm^{-1} for C=C stretching[45] corresponding to the double bonds in the oxanorbornadiene ring (Fig. S8)

After radical polymerisation of the Oxo-OBn and acrylamide, the samples were immersed in $CaCl_2$ solution to stabilize the alginate network and stored overnight. Swelling analysis indicates the as-prepared hydrogel swelled 1.4-1.5x after incubation (Figure S9). The sample was removed from the solution, excess water discarded and immediately subjected to compression testing. Compression tests were performed at room temperature to examine the double network hydrogel mechanical properties and mechanochemical reactivity. The original report of tough hydrogels based on polyacrylamide and alginate reported a hydrogel Young's modulus of 29 kPa.[46] Using a similar recipe, double network hydrogels were formed with mechanophore concentrations of 5 wt% and 10 wt%, where the di- methacrylate mechanophore serves as a replacement for bis-acrylamide. We increased the mechanophore concentration up to 30 wt %; however, those materials were exceptionally brittle under compression and were not further studied. The 5% mechanophore loaded double gel can reach a engineering strain of greater that 90% under compression without failing, and it has an elastic modulus of approximately 58 kPa (Fig. 3B and S10). Previous work exploring retro Diels- Alder release of mechanophores from polymers proposed holding times are necessary for the stress field to equilibrate and induce flex activation during compression.[31] The double network hydrogel samples were held under sustained stress for five minutes followed by rinsing with water and immersion in dichloromethane overnight to collect the released furfuryl ether molecules in solution (Fig. 3A). The concentration of small molecules in the eluent was subsequently measured via gas

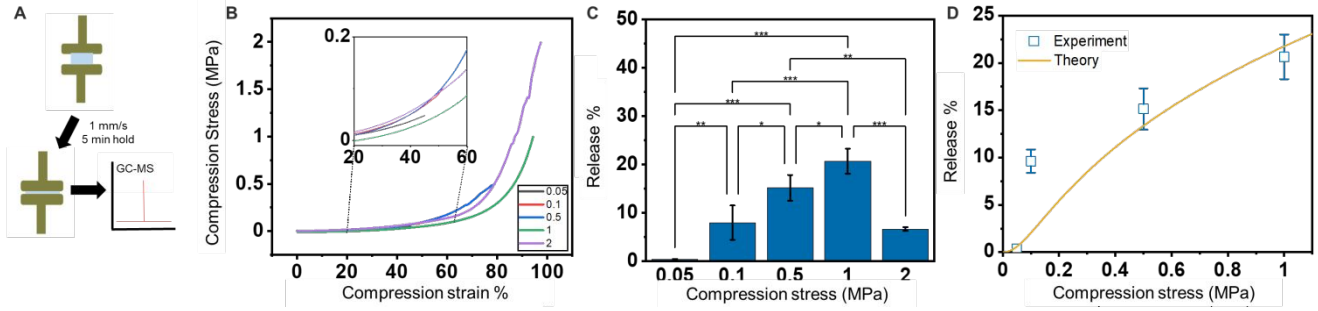

Fig. 3 A. Scheme for compression testing and eluent analysis. B. Engineering stress-engineering strain curves for the mechanophore crosslinked hydrogel (5 wt.%) with a 1 mm/minute compression, 5 min hold at 0.05, 0.1 , 0.5, 1, and 2 MPa. C. Percentage release from mechanophores under various compression stresses along with the results of the statistical tests (*P <= 0.05, **P <= 0.01, ***P <= 0.001 one-way ANOVA analysis). D. Release-strain relationship for observed molecule release vs theoretical prediction.

chromatography-mass spectrometry (GC-MS, Fig. S11) where a non-compressed sample was used as a control. Further details of sample preparation for compression testing can be found in the supplementary information. We tested gels under a broad range of compression stresses from 10 kPa to 2MPa (Fig. 3B). The materials behave elastically under stress <0.5 MPa. However, some plastic deformation occurred in samples exposed to stress ≥0.5 MPa and with repeated loading (Fig. S9B), which poses limitations on their use in high stress applications. Analysis of the eluent from compressed hydrogels indicates no molecule release at 10 kPa with evidence for marginal release at 50 kPa with an increase corresponding to applied force. We observe the highest activation of ~20% released molecules at 1 MPa compression. This in in sharp contrast to previous work, where flex-activation in dry polymer systems showed a maximum of 6-7% release at 35 MPa of force.[31] Above 1 MPa we observe failure of the specimens and network rupture, corresponding to a decrease in molecular release (Fig. 3B and C). A theoretical model for mechanochemically active elastomer and gels was then customized to this material in order to better understand this progression in small molecule release (details in SI).[47] The model shows a similar non-linear release behavior with stress as seen experimentally (Fig. 3D and S12).

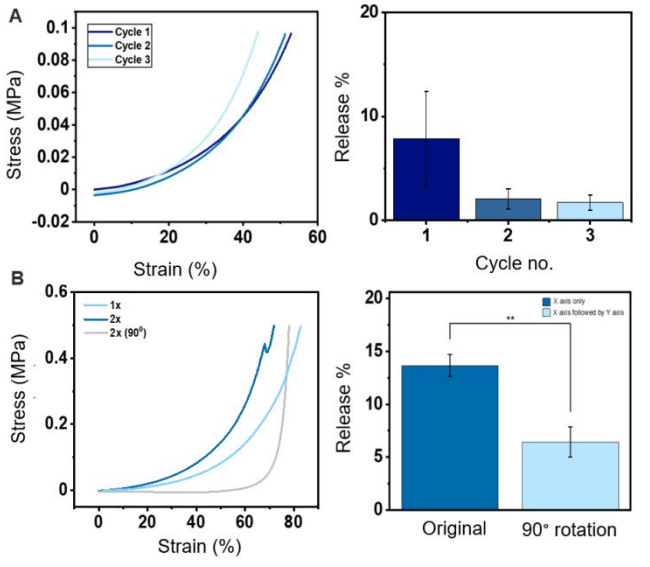

Fig. 4A. Stress-strain relationship for mechanophore crosslinked hydrogels (5 wt.%) with 1 mmmin-1 compression and 5 min hold for successive compressions. Percentage release from mechanophores from the same sample at successive compressions. B. Stress-strain relationship for mechanophore crosslinked hydrogels (5 wt.%) with 1 mm min^{-1} compression and 5 min hold after directional compression. Percentage activation of mechanophores on a sample (5 wt.%) after compression and after 90° rotation along with the results of the statistical tests (*P <= 0.05, **P <= 0.01, ***P <= 0.001 one-way ANOVA analysis).

Next, we asked whether the remaining mechanophores within the double network would be accessible through repeated cycles of loading. To test this, we compressed samples at 0.1 MPa with 1 mm min^{-1} compression rate followed by collection of eluent and analysis by GC-MS between each cycle. There was a diminishing amount of molecule release under successive compressions of the same sample (Fig. 4A). This was expected since the mechanophores that were initially well oriented for release at those stress levels were triggered on the first cycle. In this case, the release upon reloading is thought to be mostly due to a slight rearrangement in the network caused by prior to microdamage. Even after an additional seven cycles of compression (10x total) the quantity of released molecule is <20% total. To further demonstrate this orientation effect, we compressed samples as before, removed the stress, rotated the sample by 90-degs, and reapplied 0.1 MPa compression. As shown in Fig. 4B, the samples show substantially more release after rotation than after repeat cycling in the same direction. The reduction in release of the rotated samples compared to that of initial loading is similar to that predicted by our double network mechanochemical release model (Fig. S13), and this decrease primarily originates from some release occurring along polymer chains transverse to the loading direction under the first compression.

In conclusion, we demonstrate how integrating flex-activated mechanophores into double network hydrogels facilitates molecular release at forces several orders of magnitude lower than previously reported studies in dry polymers. We propose this enhanced mechanochemical response is due to a combination of the aqueous environment and the interpenetrating tough network allowing high degrees of deformation while the samples remain intact. Hydrogels are ubiquitous in society, serving important roles in applications spanning biomedical materials, devices, and biotechnology. Therefore, this work paves the way to mechanosensitive molecule releasing materials such as force-sensitive drug releasing scaffolds, contact lenses, bandages, orthopedic coatings, or device components.

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