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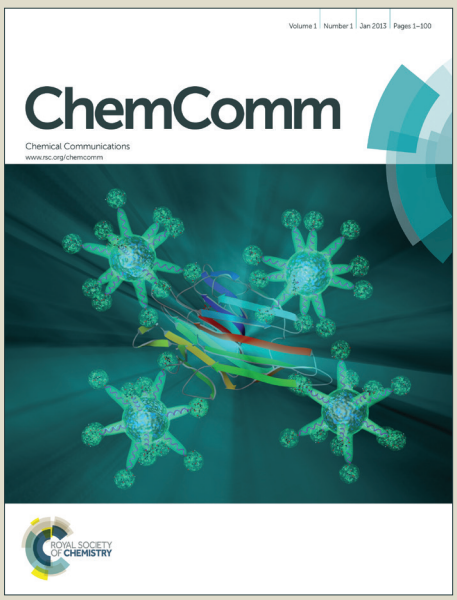

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Near-infrared Light Triggerable Deformation-free Polysaccharide Double Network Hydrogel

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To prepare hydrogel with robust mechanical properties and programmable remotely-controlled releasing ability, we synthesized agarose/alginate double network hydrogel incorporating polypyrrole (PPy) nanoparticles as near-infrared (NIR) laser responsive releasing system. This hydrogel exhibited pulsatile releasing behaviours according to the laser switching while maintaining its morphology and mechanical strength.

Functional hydrogels that are sensitive to light,[1-2] pH,[3] and temperature[4] aroused great interest for drug delivery applications. Specifically, light-responsive hydrogels are attractive because light provide remote, non-invasive switching to control release of therapeutic agents.[5] Hydrogels that could be stimulated by NIR lights were developed for biological applications because NIR light can penetrate body tissue with limited absorbance.[6] Light-induced drug release from hydrogels usually proceeds through sol-gel phase transitions[7] or gel volume contraction-expansion processes.[8] Sol-gel transitions occur through photo-cleavage of photo-responsive moieties linked to hydrogel networks, whereas volume contraction-expansion processes result from photothermal heating of nanoparticles encapsulated in thermal-responsive hydrogels.[7-8]

However, hydrogels dissociate or deform during light-induced drug release processes, causing morphological changes, mechanical failure and uncontrollable release.[9] Therefore, existing light-responsive hydrogels are hardly applied as supporting scaffolds that require stable mechanical strength and shape integrity during the drug releasing process. For example, mechanical failure during light stimulation leads to collapse of drug-eluting hydrogel scaffolds, causing disorganization of seeded cells and unstructured tissue formation.[10] To broaden the applications of light-responsive hydrogels, we developed polysaccharides double network hydrogels as deformation-free NIR-light responsive drug delivery systems through combining agarose/alginate double network hydrogels and PPy nanoparticles.

Agarose is temperature-responsive polysaccharide that exhibits thermo-reversible sol-gel transition behavior.[11] Agarose was used to fabricate various hydrogel constructs with well-controlled shapes, as these constructs can be fabricated simply by lowing temperatures.[12] PPy nanoparticles have strong NIR absorption, enhanced photo-stability and high photothermal conversion efficiency, which makes them an attractive alternative to gold nano-rods as photothermal therapy agents.[13-14] The combination of agarose and PPy nanoparticles could be developed as NIR-light-responsive hydrogels. However, applications of these hydrogels are limited because they dissociate after NIR light irradiation, which leads to mechanical failure as well as morphology changes.

Alginate is polysaccharide composed of β-d-mannuronic acid and α-l-guluronic acid units.[15] Upon addition of multivalent cations such as Ca^{2+}, alginate solution rapidly form hydrogels. However, because it does not show environmental responsive ability, alginate is not used to form light-triggerable materials. In addition, alginate gelation induced by Ca^{2+} occurs rapidly and cannot be controlled due to high water solubility of $CaCl_2$, affecting shape control, uniformity, mechanical strength and drug loading of these hydrogels.[15]

In this study, a two-step sequential gelation process was used to synthesize NIR laser responsive material from agarose/alginate double network hydrogels composited with PPy nanoparticles. The combination of agarose and alginate was synergistic. The agarose/PPy network imparts the hydrogel with ability to respond to light, whereas non-thermal-responsive alginate maintains the mechanical properties of the hydrogel during irradiation. In addition, the agarose hydrogel network was formed as supporting templates for Ca^{2+} diffusion control, which regulates alginate gelation process and form objects with shapes.

PPy nanoparticles were synthesized with procedures as described previously.[13-14] The resulting PPy nanoparticles were uniform in size with a diameter around 85 nm (Fig. S1a and S1b) and showed absorption band from 700 to 1100 nm (Fig. S1c). PPy nanoparticles solutions at various concentrations were irradiated with 915 nm laser at intensity of 3.5 W/cm^2 for 10 minutes. Temperature of the solutions with 200μg/ml and 400μg/ml of PPy nanoparticles increased from 23 ℃ to 38 ℃ and 49 ℃, respectively, indicating that PPy nanoparticles could be applied as effective photothermal transducers.

Our one-pot strategy for creating agarose/alginate double network composite hydrogel with PPy nanoparticles is shown schematically in figure 1. First, ultra-low gelling point agarose was dissolved in pre-heated (~50 ℃) alginate solution and mixed thoroughly with PPy nanoparticles, which was subsequently injected

into mold (Fig. 1a). Second, the temperature was lowered to 4 ℃ to induce gelation of agarose, forming agarose hydrogel network (Fig. 1b) that served as template to determine the hydrogel morphology and control alginate gelation. Third, free alginate molecules that distributed within agarose network were crosslinked by addition of Ca^{2+} to form alginate hydrogel network, and thus interpenetrating double network composite hydrogel was formed (Fig. 1c). Fourth, NIR laser irradiation was converted into heat by PPy to locally melt agarose network and release loaded compounds (Fig. 1d).

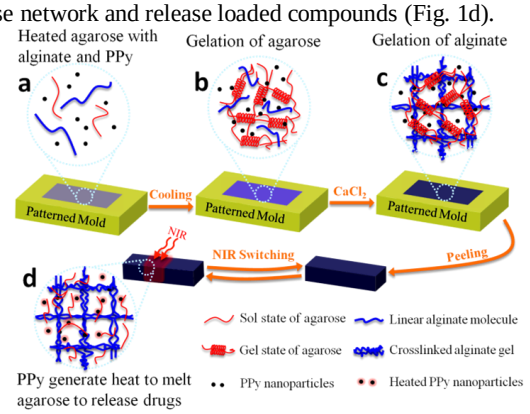

Fig. 1 The process to synthesize NIR laser responsive deformation-free hydrogel (a) pre-heated agarose solution was mixed with alginate and PPy nanoparticles and injected into patterned mold (b) cooling to induce gelation of agarose, determining shape of the hydrogel and served as template for alginate gelation (c) $CaCl_2$ was added to form alginate network, which provided mechanical support during laser irradiation (d) periodic switching of NIR laser to digitally control agarose melting for pulsatile drug release.

To evaluate effects of alginate network, morphology changes of the agarose hydrogel, with and without alginate network crosslinking, were tested after laser irradiation. Without addition of $CaCl_2$ to develop alginate network, NIR laser irradiation caused tremendous changes in the hydrogel shape (Fig. 2a and Movie S1). Before irradiation, the hydrogel was disc-shaped after being peeled from mold. After irradiation (1 W/cm^2 for 23 seconds), rupture occurred in the irradiated area. When irradiated for 42 seconds, the rupture broadened, which led to break-up of hydrogel structure. In contrast, when $CaCl_2$ was added to develop alginate network, the hydrogel maintained its shape during the entire irradiation process. No deformation was observed, even with a higher laser intensity of 4 W/cm^2 and with an irradiation time as long as 120 seconds (Fig. 2b and Movie S2). In this study, we crosslinked the alginate hydrogel with 0.5M $CaCl_2$ for 15 minutes and the alginate hydrogel can remain its integrity during the whole laser irradiation process. Severe heating (100 ℃ boiling for 8 hours) was also applied to investigate the ability of these hydrogels to tolerate heat. No deformation was observed for agarose hydrogel with alginate network support (Fig. S2a), whereas hydrogel with only agarose network dissociated within 2 seconds (Fig. S2b). It was shown that shape of these hydrogels is determined by the agarose concentration (Fig. S3) while the shape integrity of these hydrogels during laser irradiation process is determined by the alginate concentration (Fig. S4).

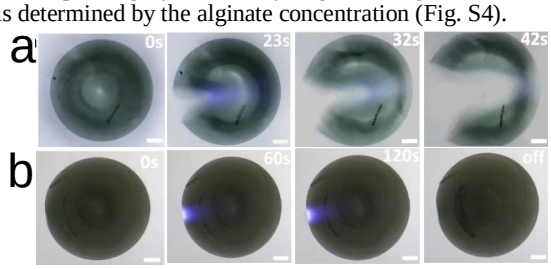

Fig. 2 Microscope observations showing effects of laser irradiation on (a) agarose hydrogel without alginate network (b) agarose hydrogel with alginate network; the scale bar is 200 μm.

Oscillatory shear rheological measurements were conducted to examine mechanical properties of hydrogels. Strain amplitude sweep tests revealed that the hydrogel samples showed clear plateau of storage modulus. This plateau is commonly referred to as linear viscoelastic (LVE) region, and the end of LVE region represents the maximum shear strain that can be applied to hydrogel before it undergoes irreversible deformation.[16] Without alginate network, agarose hydrogel exhibited low storage modulus (LVE region at 287 ± 17 Pa). In contrast, the storage modulus was substantially greater in the case of agarose hydrogel with $CaCl_2$ added to develop an alginate network (LVE region at 1350 ± 24 Pa). This result indicates that double network hydrogel was stiffer than agarose hydrogel. Rheological properties between alginate hydrogel and double network hydrogel were also compared. These two hydrogels exhibited similar storage modulus; however, the LVE region was longer in the case of double network hydrogel than in the case of alginate hydrogel (Fig. 3a), suggesting that double network hydrogel can resist greater deformations than alginate hydrogel. More importantly, after laser irradiation (4 w/cm^2 for 5 minutes), double network hydrogel maintained an LVE region and a yield strain similar to those of un-irradiated sample (Fig. 3b), indicating that double network hydrogel can withstand the laser-irradiation-induced heating process and maintain its mechanical strength. Effects of stoichiometry between agarose and alginate on the rheological properties of the hydrogel were investigated (Fig. S5), the results indicated that stiffness and yield strain of the agarose/alginate double network hydrogel was depended on the alginate concentrations.

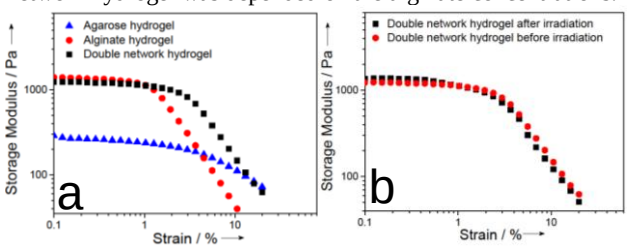

Fig. 3 Storage modulus of (a) agarose hydrogel, alginate hydrogel and agarose/alginate double network hydrogel (b) agarose/alginate double network hydrogel before and after NIR laser irradiation.

The double network hydrogel was treated using two different processes—EDTA chelation (0.1 M) and boiling (100°C for 8 hours) to study the network interactions. After EDTA treatment, the hydrogel maintained its size and shape (Fig. 4a); however, its rheological properties were substantially different (Fig. 4b). Before treatment, storage modulus (G′) of the hydrogel was 1230 ± 150 Pa. After treatment, the G′ was greatly reduced to 418 ± 80 Pa, similar to that of agarose hydrogel. EDTA chelation can dissociate the alginate hydrogel network and thus leaving only agarose hydrogel network to maintain shape and mechanical strength of this material. Consequently, EDTA treatment decreased the modulus of the hydrogel, which became dissoluble by heating (Fig. S6a). Figures 4c and 4d show morphology and rheological properties of double network hydrogel before and after it was boiled. After boiling, its storage modulus was the same as that of an untreated sample (Fig. 4d), indicating that the hydrogel can withstand boiling and maintain its stiffness. Boiling melts the agarose hydrogel, and the alginate hydrogel thus becomes the only network. The alginate network alone exhibits stiff mechanical features that maintain the shape and mechanical strength of this material. Therefore, after the double network composite hydrogel was boiled, its stiffness was unchanged and it becomes soluble by EDTA (Fig. S6b).

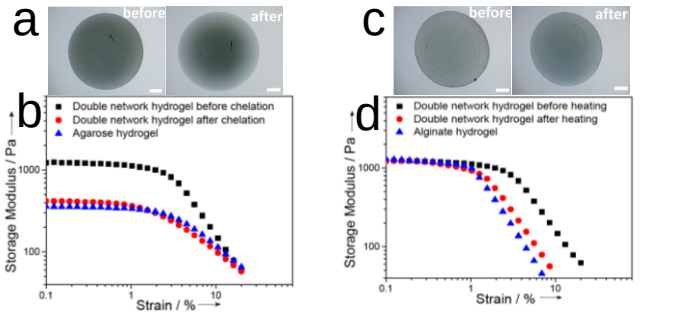

Fig. 4 (a) Morphology of double network hydrogel before and after EDTA treatment and (b) their storage modulus measurements (c) morphology of double network hydrogel before and after boiling treatment and (d) their storage modulus measurements; The scale bar is 200μm.

NIR laser irradiation induced release of TRITC-dextran from round- (Fig. 5a) and rod-shaped (Fig. 5b) double network composite hydrogel. Before laser was switched on, limited leaching of TRITC-dextran was observed. When the hydrogel was irradiated, streams of TRITC-dextran from the irradiated area were observed, indicating the laser-induced site-specific reagent release. After the laser was switched off, the TRITC-dextran release stopped. Digital control of TRITC-dextran release was accomplished by switching the laser on/off in multiple cycles (Movies S3 and S4). Laser-directed TRITC-dextran release from agarose hydrogel and double network hydrogel was compared (Fig. 5c). To investigate the TRITC-dextran release controlled through laser switching, we exposed the hydrogels to NIR laser for 10 minutes (laser ON cycle) and subsequently incubated the hydrogel at 25℃ for 10 minutes (laser OFF cycle). These cycles were repeated until the TRITC-dextran release reached equilibrium. The agarose hydrogel exhibited massive TRITC-dextran release (more than 80%) after only one exposure to NIR irradiation for 10 minutes. All reagents were released after two cycles of NIR laser irradiation. For the double network hydrogel, 25% of the TRITC-dextran was released after 10 minutes of laser irradiation. In the second 10 minute irradiation cycle, another 25% of the TRITC-dextran was released from the hydrogel, demonstrating precise control of the reagent release profile. In addition, it was proved that both PPy nanoparticles and agarose were not released out during laser-triggered TRITC-dextran releasing process (Fig. S7, S8), ensuring the long-term utility of this hydrogel in applications.

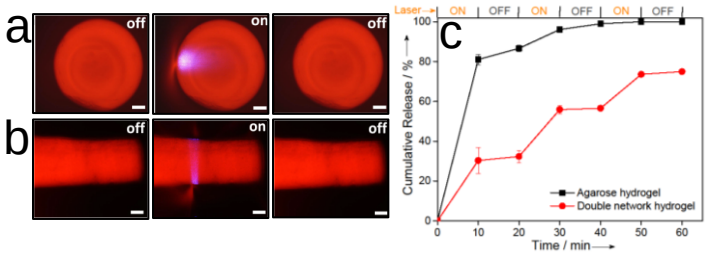

Fig. 5 TRITC-dextran release process from (a) round and (b) rod-shaped double network hydrogels controlled by laser switching. The reagent release was observed around the area that was irradiated (c) comparisons of TRITC-dextran release profiles for an agarose hydrogel and a double network hydrogel in response to periodic laser switching; the scale bar is 200μm.

The NIR laser induced TRITC-dextran release is explained by the sol-gel transitions of the thermal reversible agarose network. Before irradiation, agarose exists as a three-dimensional physically-crosslinked hydrogel network, in which TRITC-dextran exhibited less mobility. Upon laser irradiation, PPy absorbed and converted the irradiation into heat to induce melting of agarose; the TRITC-dextran thereby gained mobility to flow out. When turning off laser, heat generated by PPy nanoparticles dissipated and agarose gelation recurred, TRITC-dextran was trapped and release was discontinued. Thermo-responsiveness of the double network hydrogel is greatly associated with the melting-gelling temperature of agarose. In this study, we chose ultralow melting-gelling temperature agarose, which has melting temperature of 40℃ and gelation temperature of 25℃. Pulsatile release of reagents can be effectively attained through sol-gel transition of this agarose induced by NIR laser switching.

In conclusion, synergistic effects of forming agarose/alginate double network hydrogel composited with PPy nanoparticles were applied to form a light-triggerable, non-deformable hydrogel. By applying multiple cycles of laser stimulation, pulsatile release of TRITC-dextran was approached. The unique advantages of this double network hydrogel in a deformation-free light-triggered release process with precise reagent release control demonstrate the opportunity to design novel light-addressable drug-eluting scaffolds for modular tissue engineering and localized depot releasing systems.

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Notes and references

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Electronic Supplementary Information (ESI) available: Experimental details, Fig. S1-S8 and Movie S1-S4. See DOI: 10.1039/c000000x/

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