Title: Supramolecular hydrogels fabricated from supramonomers: a novel wound dressing material

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Severe burn patients frequently suffer from daily repeated wound dressing changes, leading to additional trauma to newly formed tissue and prolonging the healing process. Therefore, wound dressings that are easily removable can have many positive impacts by allowing wounds to heal faster. In this communication, we designed and fabricated a novel wound dressing material, which is capable of easy removal by chemical irrigation. To accomplish this, crosslinkable supramonomers were generated by exploiting the host-guest noncovalent interaction between the tripeptide Phe-Gly-Gly (containing an acrylate polymerizable group) and cucurbit[8]uril. The supramolecular hydrogel was fabricated through the radical copolymerization of acrylamide with the supramonomer acting as a crosslinker. As a result of the dynamic noncovalent nature of the supramonomer, the resultant supramolecular hydrogel is able to dissolve upon exposure to 3,5-dimethyl-1-adamantanamine hydrochloride, an FDA approved drug for Parkinson and Alzheimer's disease. This dissolution allows the material to be easily removed from a wound. Furthermore, the supramolecular hydrogels are able to load and release therapeutic agents to a

system and they are transparent, non-toxic, self-repairable, making it a promising candidate for the new generation of wound dressing.

Wound dressings serve a very important purpose in promoting healthy and timely wound healing by protecting a wound from the environment.[1] Traditional cotton-based wound dressings (bandages, gauzes, etc.) are most commonly employed for covering clean and dry wounds or used as secondary dressing to absorb exudates and protect the wound. Recently, new dressings have been developed that are capable of keeping the wound site moist, since it has been shown that moisture can lead to more rapid and successful wound healing.[2] Classified by the materials from which they are fabricated, these modern wound dressings can be composed of hydrocolloids, alginates, and hydrogels, among which hydrogels possess most of the desirable properties of an "ideal dressing".[3-6] However, most of the hydrogel wound dressings are chemically crosslinked, which usually adhere to the wounds in some degree. Mechanical debridement accompanied with anesthesia is required to remove such dressings, which is time-consuming, has the high risk of damaging newly formed tissue and can cause additional pain to patients physically and emotionally.[7] Gentler and less invasive approaches to remove wound dressing materials are therefore desperately needed for clinical applications.

Supramolecular materials are generated by exploiting noncovalent interactions between components.[8,9] The dynamic nature of noncovalent interactions endows supramolecular materials with reversible, adaptive, stimuli-responsive, self-healing and degradable properties.[10-15] Supramonomers are bifunctional monomers that are fabricated by noncovalent synthesis, but can undergo traditional covalent polymerization.[16,17] Recently, supramolecular polymers have been generated from different types of supramonomers using a variety of polymerization methods.[18-23] Furthermore, supramonomers have been used as supramolecular cross-linkers to

generate supramolecular microgels with stimuli-responsive and degradable properties.[24] In this communication, we show that supramonomers can be used to construct dynamic and degradable supramolecular hydrogels for use as wound dressings that will be capable of dissolution upon the application of a stimulus; this can therefore lead to wound dressings that are capable of promoting fast wound healing. To generate these materials, the host-guest noncovalent interactions between the tripeptide Phe-Gly-Gly ester derivative (FGG-EA) and cucurbit[8]uril (CB[8]) were exploited to yield supramonomers with one acrylate moiety at each end. This is shown in **Scheme 1**. Then supramolecular hydrogels were synthesized via copolymerization of acrylamide (AAm) with the supramolecular cross-linkers. Like traditional chemically crosslinked polyacrylamide (PAAm) hydrogels, the supramolecular hydrogels were able to absorb water. Furthermore, the materials are biocompatible, soft, elastic, and capable of being loaded with therapeutic agents that can be delivered to a system. Due to the fact that these hydrogels are composed of dynamic and reversible supramolecular crosslinks, we propose that the supramolecular hydrogels will be quickly dissolved upon exposure to crosslink disrupting molecules.[25-34] As a result, the stimuli-dissolving supramolecular hydrogel fabricated from supramonomers will present a new generation of wound dressing materials.

Supramolecular hydrogels were prepared by copolymerizing the supramonomers and AAm in aqueous solution under UV irradiation, with 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone added as a photo-initiator. A series of supramolecular hydrogels were generated with different monomer concentrations while keeping the supramonomer concentration constant. To simplify, we named each hydrogel according to the initial monomer concentration. The resultant hydrogels were transparent (**Figure 1**a) while the scanning electron microscope (SEM) images showed that PAAm supramolecular hydrogels had the expected

porous structure (Figure 1b). Rheological measurements were employed to study the dynamic mechanical properties of the supramolecular hydrogels. Samples were subjected to strain sweep tests at 1 Hz to determine the storage modulus G' (describes elasticity) and the loss modulus G'' (describes viscosity), and the linear viscoelastic region (Figure S1). In our case, 1 Hz frequency and 1% strain were chosen to perform the experiments in Figure 1c, 1d. As shown in Figure 1c, all the hydrogels, except for the 0.7 M gel, exhibited properties characteristic of hydrogels, as the measured G' was significantly higher than G''. Both G' and G'' of the supramolecular hydrogels increased as the concentration of AAm increased. In other words, increasing the monomer concentration yielded tougher supramolecular hydrogels. As can be seen in the double log plot in Figure 1d, the complex modulus G* increased linearly as the AAm monomer concentration increased, over the range of 290 Pa to 9320 Pa. This shows that the mechanical properties of the supramolecular hydrogels are tunable over a large range. To further prove the advantage of using the supramonomer strategy to generate supramolecular hydrogels, we synthesized linear polymers with FGG moieties first and then added CB[8] to generate crosslinks, forming "traditional" supramolecular hydrogels. Figure 1d shows that compared to the 1.4 M gel generated from the supramonomer strategy (G'=1200 Pa, G''=610 Pa), the 1.4 M gel generated via the traditional method was significantly less mechanically robust (G'=185 Pa, G''=260 Pa). This may be due to the viscous solution of the polymer with FGG moieties limiting the solubility and diffusion of CB[8] into the polymer to form extensive crosslinks, thus leading to heterogeneous crosslinking points. However, such a problem is successfully avoided using the supramonomer strategy here, where the supramolecular hydrogel is formed by the polymerization between the AAm and supramonomers.

Next, we determined if the prepared supramolecular hydrogels could be dissolved under mild conditions. To answer this question, 3,5-dimethyl-1-adamantanamine hydrochloride (DMADA) was selected as a potential wound irrigant. We point out that the binding constant between FGG-EA and CB[8] is 2.0×10^{11} M^{-2} while DMADA has a much higher binding affinity of 4.33×10^{11} M^{-1} for CB[8].[35] Therefore, we predict that the supramonomer will be destroyed by the competitive replacement of FGG by DMADA, leading to the dissolution of the supramolecular hydrogel. Furthermore, DMADA is water soluble, odorless, and is an FDA-approved drug which is used to treat patients with Parkinson and Alzheimer's disease.[36,37] To investigate this property, the supramolecular hydrogels were exposed to the DMADA, and the initial mass of the hydrogel (W_0) was compared to the mass remaining after dissolution (W_t) and the mass percentage of remaining hydrogel calculated as a function of DMADA irrigation time. As can be seen in **Figure 2**a, exposure to DMADA resulted in the dissolution of the supramolecular hydrogel while exposure to deionized (DI) water, could not destroy the hydrogel network. Moreover, increasing DMADA concentration accelerated the degradation kinetics significantly. For the 1.4 M gel, the 100 mM DMADA solution dissolved the whole hydrogel in less than 2 min, while it took about 7 min upon exposure to 2 mM DMADA. We also observed the relationship between the mechanical strength of the hydrogels and their degradation time. As shown in Figure 2b, at the same concentration of DMADA, the increase of AAm concentration in the supramolecular hydrogel resulted in a higher mechanical strength but much longer degradation times (25 min for 1.4 M gel versus 35 min for 2.1 M gel). To balance the mechanical properties and dissolution behavior of the supramolecular hydrogels, we chose 1.4 M gel which exhibited proper mechanical strength and short dissolution time as the optimized formula for the hydrogel wound dressing. Figure S2 shows the photographs of the 1.4 M gel degradation

process. DMADA-soaked gauze was applied to half of the hydrogel dyed with Rhodamine B. After 1.5 min, the gauze was removed and only the half of the hydrogel covered with DMADA-soaked gauze was dissolved. To the best of our knowledge, this supramolecular hydrogel shows the fastest dissolution time in the wound dressing materials.

The supramolecular hydrogel generated here also exhibits other properties that are ideal for wound dressing. First it has good water absorption behavior, guaranteeing its capacity to absorb wound exudate and preserve a moist environment around the wound. As shown in Figure S3, the hydrogels were able to swell to 200% of its original mass within 1 h after immersion in PBS buffer (pH=7.4). Secondly, we demonstrated that the supramolecular hydrogel was non-cytotoxicity. This was done by exposing human keratinocyte cells (HaCaT) to a wide concentration range of the hydrogel solution. As shown in Figure S4, there was little-to-no cytotoxicity for any of the groups, as examined by MTT assay. This is further evidence that these hydrogels could find practical clinical applications as wounding dressings. Finally, we investigated if the supramolecular hydrogels could work as a stable and sterile wound dressing at various environmental conditions, which would minimize the need for frequent wound dressing changes. **Figure 3**a shows that the supramolecular hydrogels preserved their mechanical properties at temperatures ranging from 20 to 80 ℃, even though the crosslinks are noncovalent. In addition, such a supramolecular hydrogel is capable of self-repairing. To demonstrate this, we measured the mechanical properties at an applied frequency of 1 Hz, and showed that the supramolecular hydrogel was stable at 1% strain, while it was destroyed at strain high than 800% (Figure 3b). In the self-healing experiment, at 1 Hz frequency, 1000% strain was applied to destroy the hydrogel network while 1% strain was applied to examine the recovery speed of the hydrogel. As shown in Figure 3c, the supramolecular hydrogel could recover to its original

G'/G'' within 1 min. Therefore, the supramolecular hydrogels exhibit good thermal stability and fast self-repairability, which can meet the needs of a practical wound dressing for clinical application.

Finally, we showed that the materials generated here could load and deliver therapeutic agents, such as antimicrobials, growth factors, vitamins and mineral supplements to wounds. Ofloxacin, a drug for the treatment of bacterial infections, was used to demonstrate that the supramolecular hydrogel could be utilized as a carrier for therapeutic agents. Ofloxacin was added into the pre-gel solution before UV irradiation. A small piece of ofloxacin-loaded hydrogel was immersed into PBS buffer and the release of ofloxacin monitored as a function of time by monitoring the increase in absorbance at 285 nm (the wavelength maximum for ofloxacin, see Figure S5). As can be seen in **Figure 4**, the drug was fully released from the hydrogel network within 1 h. Therefore, in addition to the above advantages, such a supramolecular hydrogel can load and release antimicrobials, which can prevent the infection and promote healing.

Conclusion

Here, for the first time we have employed the concept of supramonomers for the fabrication of supramolecular hydrogels for wound dressings. We showed that the resultant hydrogels were transparent, non-toxic, self-repairable and exhibit desirable mechanical properties that can be tuned over a wide range. We also showed that exposure to the mild chemical irrigant DMADA led to hydrogel dissolution within 2 min, which would alleviate pain and shorten wound-healing time for patients. Considering that the above desired properties of the supramolecular hydrogel fabricated in this way can be tailor-made in a rational manner, we believe that this kind of

supramolecular hydrogel represents a promising candidate for the new generation of hydrogel wound dressing.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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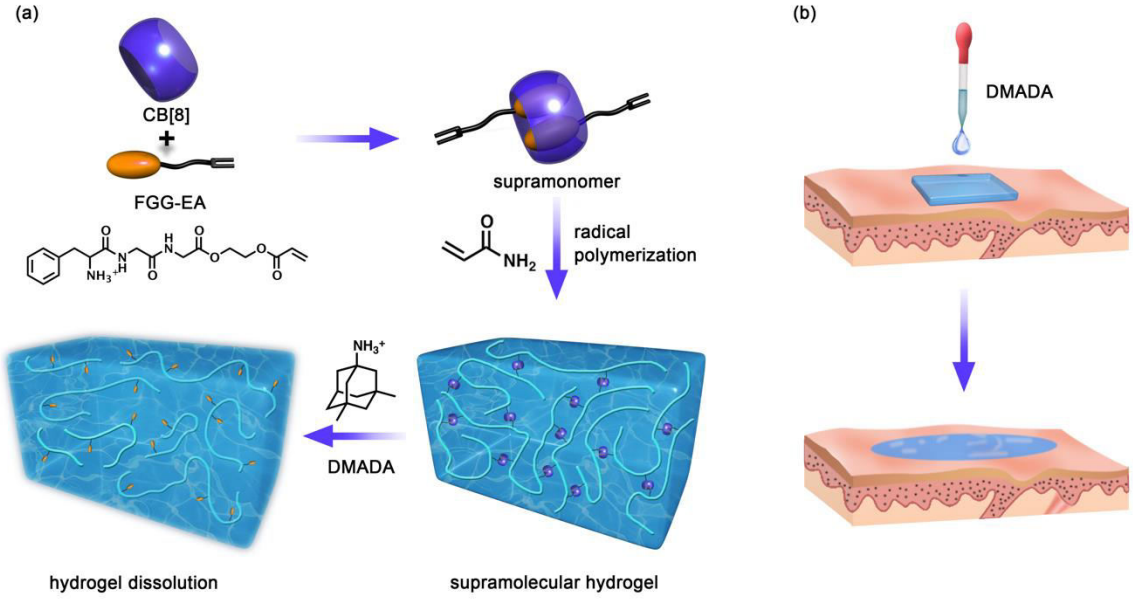
Figures



Scheme 1. Schematic depiction of supramolecular hydrogel fabrication from supramonomers and its dissolution process upon DMADA irrigation (a) and its application as wound dressing materials (b).

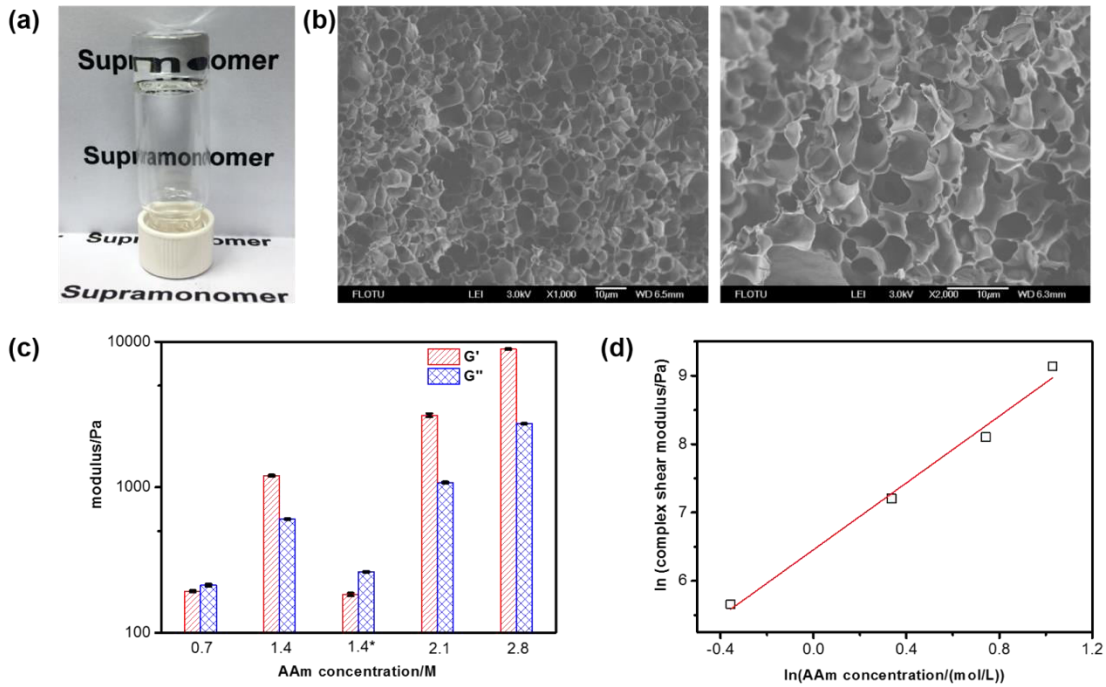


Figure 1. (a) Representative photo of the supramolecular hydrogel; (b) SEM images for 1.4 M gel (c) G' and G'' for the supramolecular hydrogels with different monomer concentration (1.4 M* gel was made from the complexation of the polymer with FGG moieties and CB[8]); (d) Plot on a double logarithmic scale of G* versus AAm monomer concentration (coefficient of determination R^2=0.985).

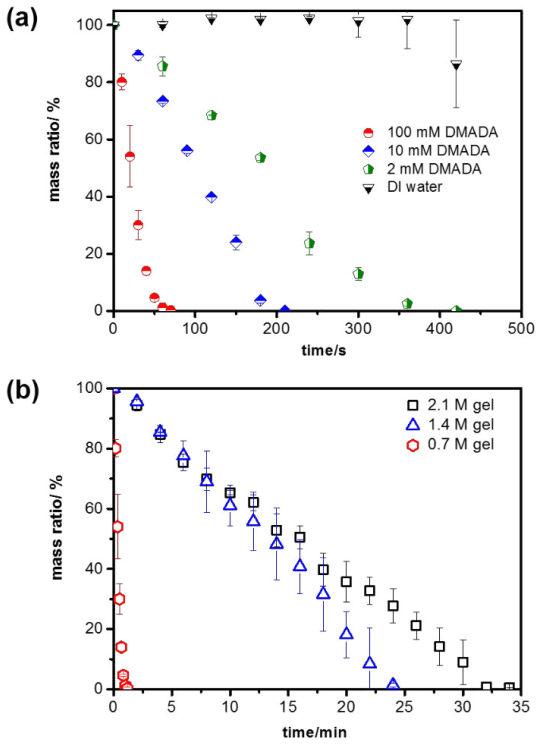


Figure 2. (a) 1.4 M gel's dissolution rate upon exposure to different DMADA concentration as well as DI water; (b) Dissolution rate of hydrogel with different AAm concentration in 100 mM DMADA solution.

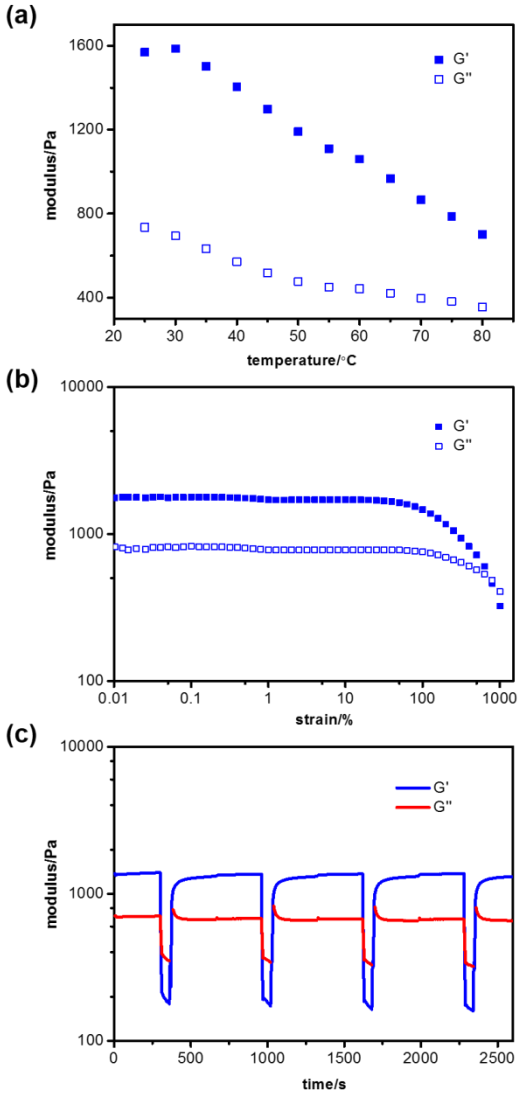


Figure 3. (a) G' and G'' of 1.4 M gel at different temperatures ranging from 25 °C to 80 °C; (b) Strain-dependent oscillatory shear measurement of 1.4 M gel at 1 Hz frequency; (c) Step-rate time-sweep measurements displaying the ability of the 1.4 M hydrogel to self-repair (frequency constant at 1 Hz, 1.4 M gel was subjected to 1% strain for 300 s, then 1000% strain was applied to damage the hydrogel for 30 s and later strain went back to 1% for recovery for another 300 s. This continuous measurement was repeated 4 times).

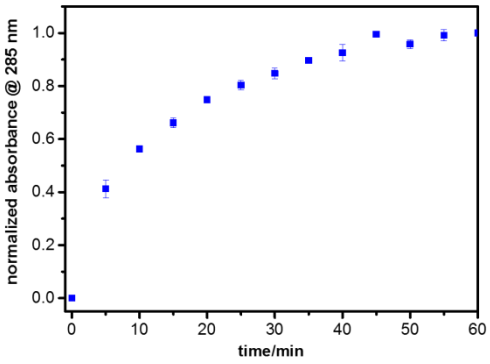


Figure 4. Release profile of ofloxacin from 1.4 M gel when immersed into PBS buffer by monitoring the absorption band of ofloxacin peaked at 285 nm upon time.

TOC

A novel kind of supramolecular hydrogel was fabricated through the radical copolymerization of acrylamide with the supramonomer, which can be quickly dissolved under 3,5-dimethyl-1-adamantanamine hydrochloride irrigation. It provides a new way to remove wound dressing mildly and could alleviate patients pain during dressing change.

Keyword: supramonomer, hydrogel, supramolecular material, wound dressing, host-guest interaction

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