Self-assembly of small molecules affords multifunctional supramolecular hydrogels for topically treating simulated uranium wounds†

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Received (in Cambridge, UK) 26th May 2005, Accepted 5th July 2005
First published as an Advance Article on the web 3rd August 2005
DOI: 10.1039/b507314f

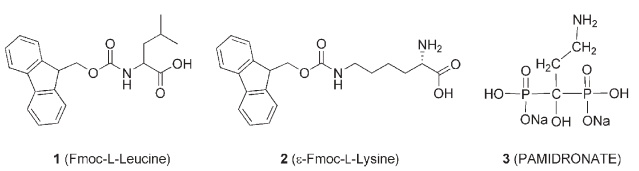
Two types of therapeutic agents, which have discrete yet complementary functions, self-assemble into nanofibers in water to formulate a new supramolecular hydrogel as a self-delivery biomaterial to reduce the toxicity of uranyl oxide at the wound sites.

This communication reports the design and application of a supramolecular hydrogel, whose self-assembled networks of nanofibers consist of antiinflammatory molecules and a uranyl ion chelating ligand, as a biomaterial for topical treatment of simulated uranium wounds. Because of their biocompatibility, biodegradability, and resemblance to the extracellular matrix, hydrogels have attracted intensive research attention in recent years, particularly for tissue engineering and drug delivery. Following the successful applications of polymer-based hydrogels in biomedical engineering[1] and the successful studies on low molecular weight organogels,[2] supramolecular hydrogels, formed by self-assembly of small molecules,[3,4] have recently emerged as a new type of biomaterials that promise important biomedical applications (*e.g.*, hydrogels based on the self-assembly of oligopeptides have been used as scaffolds to grow neurons[5,6]). Inspired by the works reported by Stupp *et al.*[6,7] and Zhang *et al.*[5,8] on oligopeptide-based hydrogels, we have been developing hydrogels as potential biomaterials directly formed by self-assembly of pharmaceutical candidates or agents that are small molecules.[4]

To further explore the *in vivo* activity of supramolecular hydrogels, we designed a multifunctional hydrogel that employs three small molecules as its structural components—two amino acid derivatives that can reduce inflammation[9] and a bisphosphonate that coordinates with UO_2^{2+} and lowers the toxicity of UO_2^{2+}. These molecules self-assemble into networks of nanofibers that form the matrices of the hydrogel. We administered the hydrogel topically on wound sites that had been contaminated with (non-radioactive) uranyl nitrate on the skin of mice. After being treated with the hydrogel, the mice recovered to normal, while the control group of mice (whose wounds were contaminated and untreated) weighed 35% less or expired. Our results indicate that these small molecules maintain their therapeutic effects even when they serve as the structural components of the supramolecular hydrogel. In addition to validating the approach of the direct use of drug molecules to form a hydrogel as a new type of biomaterial, this work, for the first time, demonstrates the *in vivo* activity of supramolecular hydrogels based on small molecules (*i.e.*, molecular weight $<10^3$ g mol^{-1}).

Scheme 1 shows the structures of the three small molecules. *N*-(Fluorenyl-9-methoxycarbonyl)-L-leucine (**1**) and N^{ε}-(fluorenyl-9-methoxycarbonyl)-L-lysine (**2**) belong to a novel class of antiinflammatory agents reported by Burch *et al.*, and **1** displays effective antiinflammatory activity in animal models.[9] Neither **1** nor **2** acts as a hydrogelator in a neutral aqueous solution. Pamidronate was chosen as the third component because, similarly to CO_3^{2-},[4] it could form hydrogen bond networks with **1** and **2**. The addition of pamidronate (**3**) to the suspension of **1** and **2** leads to formation of a hydrogel at pH = 9–10.4 after a heating–cooling cycle, in which **3** probably acts as both a donor and an acceptor of hydrogen bonds to promote hydrogelation since changing the pH value alone (by NaOH and pH >9.0) only results in the solubilization of **1** and **2**.

Fig. 1A shows the optical images of the hydrogels containing 1 equiv. of **1**, 1 equiv. of **2**, and 1, 2, or 4 equiv. of **3**, respectively. Heating the mixtures of **1**, **2**, and **3** to 70 °C and cooling them back to room temperature leads to hydrogelation in three minutes. When 1 or 2 equiv. of **3** are used, the formed hydrogel appears opaque, indicating that it contains insoluble microparticles due to the limited solubility of **1** and **2**, which is confirmed by the electron micrographs.† When 4 equiv. of **3** are used, the resulting hydrogel is transparent, indicating that most of **1** and **2** are utilized to form the matrices of the hydrogel. Fig. 1B displays the linear viscoelastic frequency sweep response of the three hydrogels. All of them exhibit a very weak frequency dependence from 0.1 to 100 rad s^{-1}, with G' dominating G'', suggesting that all samples are highly elastic. The dynamic storage modulus of gel III is two orders of magnitude larger than that of gels I and II, suggesting that the



Scheme 1 Molecular structures of the components of the nanofibers as the matrices of the hydrogel.

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† Electronic Supplementary Information (ESI) available: The CD, 2D-NOSEY spectra, emission spectra, and the details of the *in vivo* test (3 pages). See http://dx.doi.org/10.1039/b507314f

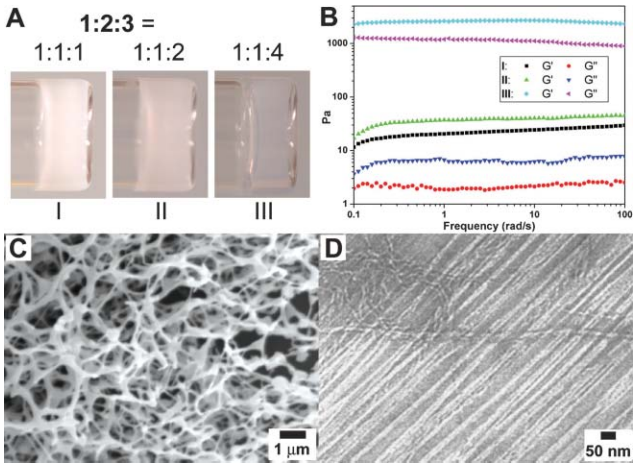


Fig. 1 (A) Optical images of the three hydrogels with different ratios of **1**, **2**, and **3** (The pH values of the resulted hydrogels are ~9.1, 9.2, and 10.4 for I, II, and III, respectively. The minimum concentration of **1** or **2** required for gelation is 5 mM for III). (B) Frequency dependence of the dynamic storage moduli (G') and the loss moduli (G'') of hydrogels I, II, and III at the strain of 1%. (C) Scanning electron micrograph and (D) transmission electron micrograph of the hydrogel III.

density of the nanofibers in gel III is higher than those of gels I and II. The electron micrographs (Fig. 1C and 1D) of gel III also confirm this inference.†

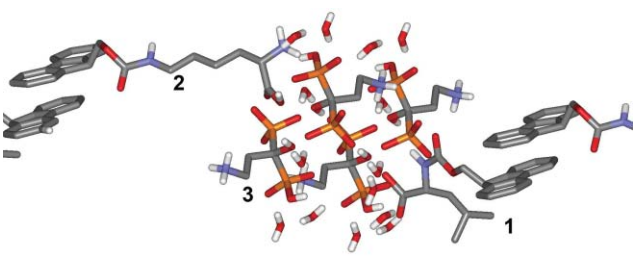
The circular dichroism (CD) and emission spectra of the hydrogels provide some insight into the self-assemblies of **1** and **2**.† Since the mixed solution of L-Leu and L-Lys at 5 mM gives no measurable CD signal, the Cotton effect at about 222 nm indicates the superhelical arrangements of the L-Leu or L-Lys residues, which induces the helical orientation of the fluorenyl groups (the Cotton effect at 260–307 nm).[10] In the solution, **1** and **2** give an emission peak at 309 nm, which shifts to ~330 nm in the hydrogels. This red-shift indicates that two fluorenyl groups overlap in an antiparallel mode,[11] and the weak peak at 378 nm indicates that a small amount of fluorenyl groups dimerize due to a parallel π–π interaction.[11] The broad emission bands at about 452 nm suggest that more than two fluorene moieties stack efficiently in the hydrogels *via* π–π interactions, similar to the case of π-stacked polyfluorenes.[12] To help elucidate the molecular conformation of the three components in the gel III, we recorded the 2D NOESY spectra of gel III at the near-gelation stage.† The cross peaks between the isobutyl group of leucine and the aromatic protons in the spectra indicate that the isobutyl group of **1** has a strong interaction with the aromatic rings, suggesting that the isobutyl group bends toward the fluorene moiety due to hydrophobic interaction. No other long-range interaction was observed from the NOESY spectra, suggesting that **2** and **3** may adopt extended linear conformations. The extended conformation of **2** would permit the stacking of more than two fluorenyl groups, which agrees with the emission spectra of the gel III. The lack of significant intermolecular cross peaks in gel III could result from the formation of the supramolecular polymeric nanofibers. Based on the above information and the crystal structure of disodium pamidronate,[13] we propose that π–π interactions between two fluorenyl groups provide an essential role for forming supramolecular structures, some pamidronate molecules may serve as a linkage to connect Fmoc-leucine and ε-Fmoc-lysine, and the rest of the pamidronate could form extensive hydrogen bonding networks (for the increase of elasticity) with the nanofiber-bonded pamidronate (Scheme 2).

Since **3** is a clinically used drug[14] and forms a stable complex with UO_2^{2+}, its hydrogel can reduce the poisoning caused by the uranyl ions. To evaluate the biological activity of the hydrogels, we used gel III to treat a simulated uranium wound (made by scratching the skin on the back of the mice and externally administering the uranyl nitrate).† Fig. 2 shows the test results. The mice in all groups exhibited initial weight loss the next day because of the wound effects (Fig. 2A). The negative control group recovered quickly from the wound to normal growth on day 2 after the slight initial weight-loss; the positive control group showed continuous weight-loss until expiration in about three to eight days or 35% weight-loss over eight days; when gel III was administered topically to the wounds of the healing group 20 minutes after the wounds were poisoned with uranyl nitrate, there was significant recovery, and none of the toxic effects of uranyl nitrate was observed in the mice's normal daily behaviors, in addition to little weight loss and no death of the mice.

To account for the observed effectiveness of the hydrogels in the *in vivo* test, we suggest that both **1** and **2** migrate into the wound to reduce the inflammation by blocking the recruitment of neutropils into the inflammatory site,[9] and **3** reduces the poisoning by UO_2^{2+} by chelating with UO_2^{2+}. In addition, since the hydrogel is able to "uptake" UO_2^{2+} from a uranyl nitrate solution,† the hydrogel absorbs some of the UO_2^{2+} from the wound site and further reduces the damage caused by UO_2^{2+}. Although its effectiveness for a wound caused with radioactive UO_2^{2+} remains to be tested, the hydrogel should be advantageous in the confinement of the radioactive uranium compared to a liquid-based treatment because the hydrogel absorbs UO_2^{2+} well and has little fluidity. Thus it may be useful as an emergency treatment for uranium wounds.

In summary, we have generated a new supramolecular hydrogel based on multiple components with discrete yet complementary activities and demonstrated the gel's function *in vivo*. This method will allow other combinations of hydrogelators generated from the pool of pharmaceutical molecules and should offer a general and simple alternative to create useful biomaterials.

This work was partially supported by RGC (Hong Kong), HIA (HKUST).



Scheme 2 One of the plausible supramolecular arrangements in the gel III. The molecular mechanics modeling was calculated using Bio+ force field in Hyperchem (partial atomic charges were obtained from the optimized structures of the monomers by the AM1 method).

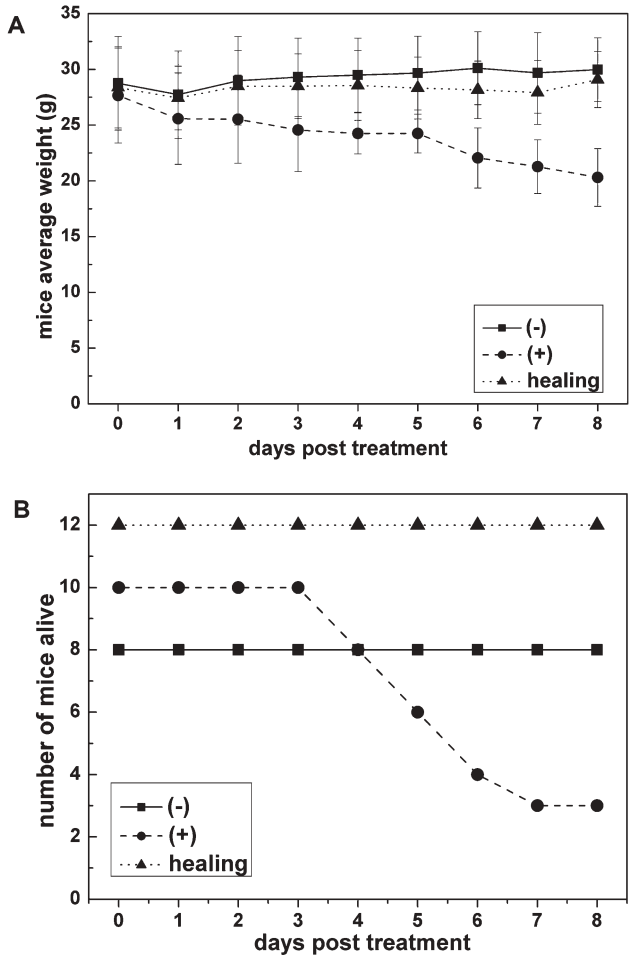


Fig. 2 (A) The change of weight of ICR outbred mice and (B) the number of mice that are alive after being wounded in each group. (−) Wound was made on the back of the mice, but was not contaminated with uranyl nitrate; (+) wound was made on the back of the mice and was contaminated with uranyl nitrate; (healing) the uranyl nitrate contaminated wound was treated with gel III. When compared with mouse weight change in the (−) group, the mouse weight change in healing group was not statistically significant within all 8 days post treatment, while the mouse weight lost in the (+) group was statistically significant ($p < 0.05$) at day 2, and very significant ($p < 0.01$) from day 3 to day 8. 2 mice out of 10 in the (+) group stayed alive 8 days post contamination, while no mice in the (−) or healing group had died by the end of day 8. The weight drop at day 1 was due to the wound effects, with no statistical significance among the three groups.

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