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A novel H_2O_2 responsive supramolecular hydrogel for controllable drug release†

Chunhua Ren, Liping Chu, Fan Huang, Lijun Yang, Huirong Fan,* Jianfeng Liu and Cuihong Yang*

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Due to the important significance of hydrogen peroxide (H_2O_2) in physiology, aging and disease in living organisms, tremendous effort has been devoted to develop H_2O_2 responsive materials for the detection of its over production or for controlled drug release. However, it is still challenging to develop H_2O_2 responsive supramolecular hydrogels. In this study, we designed and synthesized a novel H_2O_2 responsive peptide hydrogelator bearing the thiazolidinone group. A supramolecular hydrogel based on peptide self-assembly was prepared through a heating–cooling process and its gel–sol phase transition could be triggered by the removal of thiazolidinone groups upon H_2O_2 oxidization. The excellent H_2O_2 responsive property of the supramolecular hydrogel was investigated by LC-MS, rheology and TEM. A drug release study *in vitro* demonstrated that the gel–sol phase transition could be applied for releasing gemcitabine sustainedly and controllably. Our study could provide a new way for the design of H_2O_2 responsive materials and hold great potential in the application of anticancer drug delivery.

Peptide-based supramolecular hydrogels have attracted extensive research interest in the past few decades due to their inherent advantages, such as ease of design and synthesis, good biocompatibility and degradability.[1] Since supramolecular hydrogels are formed by non-covalent interactions (hydrogen bond, π–π, hydrophobic, and charge interactions), they are therefore extremely sensitive to external stimuli, including pH,[2] light,[3] enzymes,[4] ions,[5] and redox agents.[6] Increasing numbers of intelligent responsive supramolecular hydrogels have been developed so far, and they have shown great promise in the applications of drug delivery,[7] cancer cell inhibition,[8] vaccine adjuvants[9] and detection of important analytes.[10]

Hydrogen peroxide (H_2O_2), as a second messenger for intracellular signal transduction,[11] usually results from cellular metabolism of molecular oxygen. In most cases, it is maintained at well-balanced concentration levels and plays crucial roles in cell proliferation, cell differentiation and cell migration.[12] The over-production of H_2O_2 can lead to high oxidative stress and therefore impaired cellular structures.[13] Many reports have demonstrated that a series of pathologies are associated with elevated levels of H_2O_2 including inflammation,[14] cancer,[15] cardiovascular disorders,[16] and neurodegenerative diseases.[17] Consequently, enormous efforts have been made to develop H_2O_2 responsive nano-systems for controlled drug release to treat these diseases or for the detection of its over-production. These nano-systems are mostly based on the conventional peroxalate ester that can react with H_2O_2. For example, Murthy and co-workers have reported on nanoparticles formulated from peroxalate and fluorescent dyes capable of imaging hydrogen peroxide *in vivo* with high specificity and sensitivity.[18] There are also several reports regarding H_2O_2 responsive supramolecular hydrogels. For example, Hamachi and co-workers have recently reported on responsive peptide-based hydrogels containing a H_2O_2-reactive boronoarylmethoxycarbonyl group and capable of retaining the activity of encapsulated enzymes.[19] They have demonstrated that the programmable hybridization of the hydrogel and oxidases enables the resulting materials responding to not only H_2O_2 molecules but also a variety of disease-related biomarkers. These pioneering works highlight the importance of H_2O_2 responsive materials including the supramolecular hydrogels.

Recently, a novel H_2O_2 responsive group, thiazolidinone modified carboxylic acid, has been reported, and it can release free carboxylic acid upon the activation of H_2O_2.[20] Inspired by this work, we opted to develop a novel H_2O_2 responsive supramolecular hydrogelator bearing the thiazolidinone group. We therefore designed a short peptide derivative Nap-GFFYGD(Thi) (compound **1**) bearing a H_2O_2 responsive thiazolidinone at the C-terminal of the peptide. As shown in Scheme 1, compound **1** was expected to form nanofibers and a hydrogel by supramolecular self-assembly at a given concentration. The removal of thiazolidinone through the oxidation/elimination reaction by H_2O_2 was expected to produce a more hydrophilic peptide Nap-GFFYGD (**2**), resulting the dis-assembly of nanofibres and a gel–

Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Science and Peking Union Medical College, Tianjin, 300192, China. E-mail: yangcuihong@irm-cams.ac.cn

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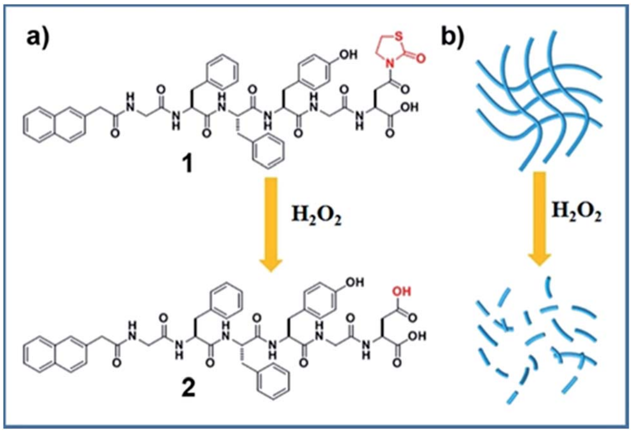

Scheme 1 (a) Chemical structures of Nap-GFFYGD(Thi) (compound **1**) and Nap-GFFYGD (compound **2**) and H_2O_2 triggered conversion. (b) Schematic representation of possible H_2O_2 triggered hydrogel degradation.

sol transition. The synthesis route and purification process of Fmoc-aspartic acid decorated with thiazolidinone (D(Thi)) were described in Scheme S1.† We then prepared compound **1** by standard solid phase peptide synthesis (SPPS) and obtained the pure compound by reverse-phase high performance liquid chromatography (HPLC).

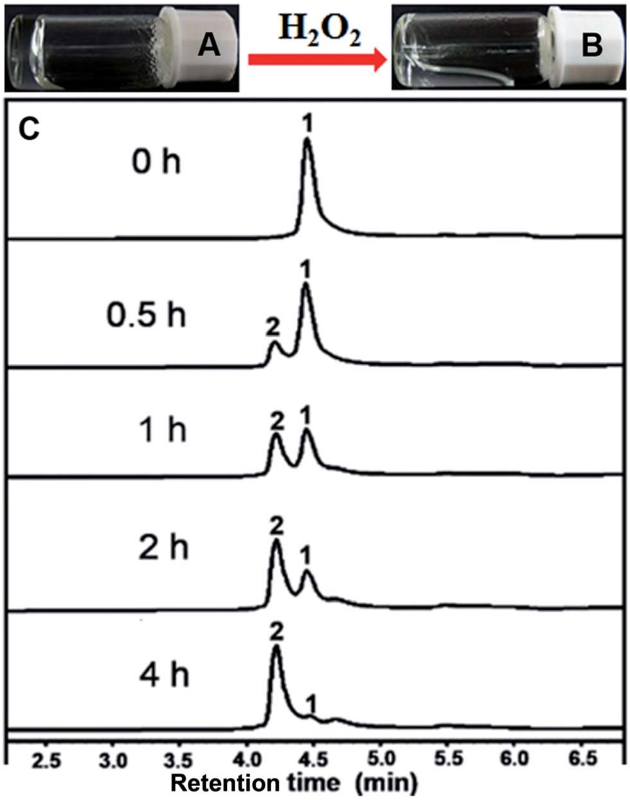

Fig. 1 Optical images of the hydrogel of compound **1** (0.1 wt%) in PBS solution (A) and the resulting solution upon adding 40 mM H_2O_2 to the gel at 37 °C for 4 hours (B) and (C) HPLC traces to demonstrate the transformation triggered by 40 mM H_2O_2 at 37 °C within 4 hours.

After obtaining the designed compound, the gelation property of compound **1** was firstly examined. Results in Fig. 1A indicated that it could form a hydrogel at a minimum gelation concentration (MGC) of 0.1 wt% within 5 minutes upon a heating–cooling process. We then tested the H_2O_2 responsive property of the hydrogel. A gel–sol transformation was observed after adding H_2O_2 (40 mM) to the gel at 37 °C for 4 hours (Fig. 1B). H_2O_2-response sensitivity of the hydrogel was evaluated through the gel–sol transition after the addition of 0–100 mM H_2O_2 to the gel. The results showed that at least 4 mM H_2O_2 was required to induce a complete collapse of the gel after 24 hours (Fig. S9†) and the time required for the gel–sol transition ranged from 1.5 to 24 hours depending on the amount of H_2O_2 (Fig. S10†). Liquid chromatography mass spectrometer (LC-MS) was employed to analyse the gel–sol phase transition. As shown in Fig. 1C, compound **1** gradually converted to compound **2** after the addition of H_2O_2. For instance, the conversion percentage was about 50% at one hour time point and reached an equilibrium after about four hours with a conversion rate of about 96% (Fig. S11†). The mass spectra of resulting solution in Fig. 1B (Fig. S7†) indicated the molecular weight peak of Nap-GFFYGD (**2**). These observations clearly demonstrated the success of our design, and the H_2O_2 responsive property of our hydrogel suggested its potential application in controlled drug release.

Rheology was also performed to characterize the mechanical properties of the hydrogels before and after the addition of H_2O_2. The dynamic frequency sweep was carried at a fixed strain value of 0.5% in the frequency range from 0.1 to 100 rad s^{-1}. As shown in Fig. 2A and S12,† before the addition of H_2O_2, the G' value of the hydrogel was an order of magnitude bigger than its

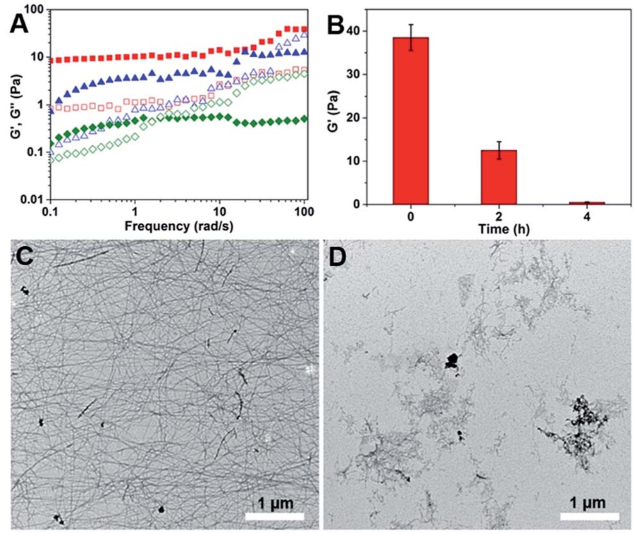

Fig. 2 (A) Dynamic frequency sweep at the strain of 0.5% after the addition of H_2O_2 at different time points (solid symbols, G'; hollow symbols, G''): 0 h (squares), 2 h (triangles) and 4 h (diamond), (B) the G' value at the mode of dynamic frequency sweep at the frequency of 10 rad s^{-1} and strain of 0.5% after the addition of H_2O_2 at different time points. TEM images of the gel without H_2O_2 (C) and with 40 mM H_2O_2 overnight (D).

G'' value in the tested frequency range, indicating a true hydrogel formation.[21] After incubation with H_2O_2 for two hours and four hours, the G'' value of the samples become bigger than their corresponding G' value at frequency value of 50 and 2 rad s^{-1}, respectively. At the same time, the G' value decreased dramatically from 40 Pa to 0.4 Pa after four hours' incubation with H_2O_2 (Fig. 2B). These observations suggested the formation of viscous solutions after the addition of H_2O_2. Transmission electron microscopy (TEM) was then used to characterize the morphology of nanostructures in the hydrogel and the obtained solution. As shown in Fig. 2C, uniform nanofibers were observed in the hydrogel with the diameter of about 25 nm and the length of up to several microns. They entangled with each other to form dense networks for the hydrogel formations. Upon the addition of H_2O_2 overnight, the long nanofibers disappeared and only few short nanofibers could be observed (Fig. 2D).

We therefore tested the possible application of our responsive hydrogel in controlled drug release. We choose gemcitabine as a drug molecule to investigate its controlled release property from our hydrogel at 37 °C. A 0.25 mL of PBS solution with different amounts of H_2O_2 was placed on top of the gel (0.2 mL). The upper solution was totally taken out at different time intervals following by adding another 0.25 mL fresh PBS solution containing corresponding H_2O_2. The accumulation percentage of released gemcitabine from the hydrogel were quantified by LC-MS based on a standard curve. Results in Fig. 3 demonstrated that the release speed of gemcitabine depended on the amount of H_2O_2 in PBS solution. That was, the more H_2O_2 in PBS solution, the faster gemcitabine released from the hydrogel. In the absence of H_2O_2, the accumulative release percentage of gemcitabine was only about 10% in 12 hours. While in the presence of 4 and 10 mM H_2O_2, the accumulative release percentage of gemcitabine from the gel was about 30% and 50%, respectively in 12 hours. Since cancer cells exhibit elevated levels of H_2O_2 compared with normal cells,[22] our H_2O_2 responsive peptide hydrogel might be used as a potential delivery system for anticancer drug delivery to tumor sites.

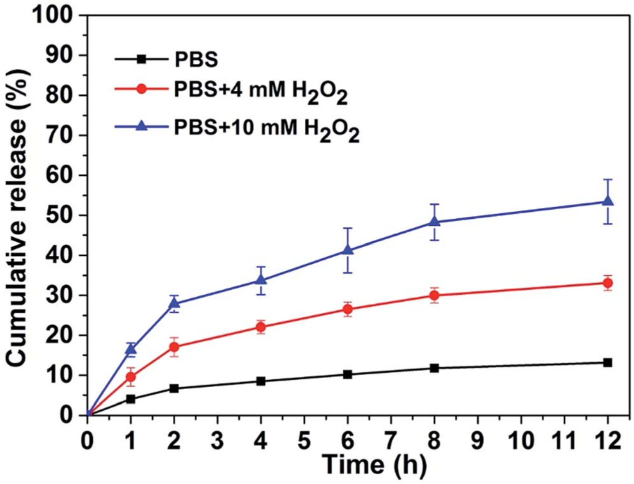

Fig. 3 Release profile of gemcitabine from the hydrogel with different amounts of H_2O_2.

In summary, we have developed a novel supramolecular hydrogel based on peptide self-assembly with a gel–sol phase transition triggered by H_2O_2. The hydrogel showed an excellent H_2O_2 responsive property and could release gemcitabine sustainedly and controllably. Compared with conventional H_2O_2 responsive materials involving peroxalate ester or boronoaryl groups, the synthesis of thiazolidinone modified hydrogelator were relatively easier and more straightforward, and the gelator could avoid being cleaved by ubiquitous esterases *in vivo*. However, due to the limited structure change of thiazolidinone modified hydrogelator upon H_2O_2 oxidization, the H_2O_2-response sensitivity of the hydrogelator developed by us was not as high as that of peroxalate ester and boronoaryl groups and should be further improved in the future study. As the most important marker for reactive oxygen species (ROS), H_2O_2 is increasingly investigated in physiology, aging and disease in living organisms. Our supramolecular hydrogel system could provide a new way for the detection of the overproduced H_2O_2 and hold great potential in the application of anticancer drug delivery.

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