A three-dimensional graphene oxide supramolecular hydrogel for infrared light-responsive cascade release of two anticancer drugs†

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A three dimensional supramolecular hydrogel consisting of prodrug-modified graphene oxide and β-cyclodextrin was developed. This hydrogel with a well-ordered interior microstructure integrated hydrophobic and hydrophilic anticancer drugs into a single multifunctional platform, and underwent a gel–sol transition leading to cascade release of two drugs in an on-demand fashion upon NIR light irradiation.

Recently, significant progress in co-delivery of different therapeutic agents and sustained release of the loaded drugs has been achieved by using hydrogels as carriers.  However, it is still difficult to achieve cascade release of two or more drugs from a single hydrogel platform. Thus, well-designed co-delivery systems, which could load each drug in different regions of hydrogels and/or utilize different interactions, are urgently needed. Compared with conventional polymer hydrogels, hybrid hydrogels offer not only interior hydrophilic pores but also particle surfaces for loading different therapeutic agents. Such a property is particularly useful for regulating the loading region and controlling the release sequence for different drugs, which is crucial for realizing cascade release of multiple drugs. The controlled release of different therapeutic agents from hybrid hydrogels can be regulated either by internal stimuli offered by polymers (e.g., pH, redox potential and enzymes) or by external stimuli such as light irradiation owing to the intrinsic functionalities of inorganic nanoparticles or nanocarbons. Among hybrid hydrogels, the graphene oxide (GO)-based hydrogel is considered as an ideal multidrug carrier owing to its large pore volume, good biocompatibility, and excellent adsorptive capability for hydrophobic drugs. More importantly, GO has high photo-thermal conversion efficiency after irradiation by near-infrared (NIR) light. NIR light has recently become an attractive stimulus because of its minimal damage to normal tissue, and can efficiently penetrate the relevant tissues with tunable intensity. Once GO is incorporated into thermo-sensitive hydrogels, it will convert NIR light into heat and further trigger an expansion/contraction or gel–sol transition of the hydrogels to release cargos. However, to the best of our knowledge, few works have touched on the cascade release of multiple drugs from GO-based hydrogels with NIR light control. Moreover, the reported GO hydrogels generally have a poorly ordered or chaotic interior structure depending on randomly arranged GO sheets with soft cross-linkers (polymers, small ammonium salts, metal ions and DNA), which cannot satisfy the requirements for precisely controlled multidrug delivery.

Here we successfully developed a strategy for constructing a GO supramolecular hydrogel with a well-defined interior microstructure and NIR light-responsive cascade release behaviour for hydrophilic and hydrophobic drugs. At first, we prepared a GO-CPT-PEG hybrid by mixing a camptothecin-low-MW poly(ethylene glycol) (CPT-PEG) prodrug (Scheme S1, ESI†) with GO water solution; this was possible due to the noncovalent interactions between CPT moieties and GO sheets (Fig. 1A). The contents of GO (37.1 wt%) and CPT-PEG (62.9 wt%) were determined by TG analysis (Fig. S1, ESI†). FT-IR and UV-vis spectra (Fig. S2 and S3A, ESI†) revealed that CPT-PEG peaks superimposing with the absorption curve of GO, suggesting the successful loading of CPT-PEG onto the surface of GO. Under dilute conditions, the fluorescence spectra (Fig. S3B, ESI†) of GO-CPT-PEG hybrids showed drastic fluorescence quenching compared with free CPT-PEG at the same CPT-PEG concentration, further indicating adsorption of CPT-PEG onto the surface of GO. Furthermore, the atomic force microscopy (AFM) image reveals that the GO sheets had a thickness of 1–2 nm with very sharp edges and a flat surface (Fig. S3C, ESI†). In contrast, the thickness of GO-CPT-PEG was increased to ~3–4 nm and the edges and the surface appeared to be relatively coarse, which could be caused by covering and winding of PEG chains on the surface (Fig. S3D, ESI†).
When α-CD was added to GO-CPT-PEG solution (15 mg mL\(^{-1}\)) and treated by sonication, the solution became turbid gradually and then a homogeneous hydrogel was formed after incubation at room temperature. We succeeded in preparing GO hydrogels (G1–G4) from 1 min to 3 h over a broad range of α-CD concentrations, even as low as 50 mg mL\(^{-1}\) (Fig. 1B). The PEG chains can penetrate the cavities of a series of α-CDs from one end far away from the GO sheets to form the well-known pseudopolypyrrole (PPR) structure, and then the strong hydrogen-bond interactions among adjacent PPRs would induce the aggregation of PPRs, thus providing a physical cross-linking effect to promote the hydrogel formation (Fig. 1A).\(^{1b-d,12}\) The typical peak at 19.8° belonging to the rigid crystalline structure of PPR aggregates could be clearly observed in the XRD patterns of G1–G4 (Fig. S4, ESI†), indicating that the introduction of GO has no effect on the PPR forming process. In fact, when α-CD was added to mPEG (\(M_n = 1900\)) solution, a sol rather than a homogeneous hydrogel was formed (Fig. 1B, sol). This is mainly due to the complete cover of α-CD on the low-MW PEG chain and subsequent hydrogen-bond interactions among PPRs. For our system, the CPT-PEG modified GO sheets act as a core material to anchor a high density of PEG chains on its surface, which provides additional supra-cross-links and steric hindrance partly preventing α-CD to be included on the PEG chains completely.\(^{5b,13}\) As shown in Fig. S4 (ESI†), the characteristic peak of CPT-PEG at 23.2° can also be observed for G1 to G4, implying that GO sheets can efficiently prevent the PEG chain from being covered by α-CD completely. The formation of supramolecular hydrogels was confirmed by rheological tests (Fig. S5, ESI†). The storage modulus (\(G’\)) of G1 is much larger than its loss modulus (\(G''\)) over the entire range tested, implying that the elastic response is predominant and the hydrogel has a permanent network. The \(G’\) and \(G''\) of the hydrogel are slightly sensitive to angular frequency, which is a typical feature of supramolecular hydrogels having a high degree of noncovalent cross-linking (Fig. S5A, ESI†). In addition, the hydrogel shows a typical shear-thinning behavior (Fig. S5B, ESI†), which is critical for the injectability of hydrogels.\(^{14}\) Moreover, the low-MW (\(M_n = 1900\)) PEG chains in our system will facilitate their clearance out of the body after hydrogel degradation, whereas the high-MW PEG (\(M_n > 10k\)) chains in other PPR hydrogels have been proven difficult to be eliminated from the body because of their large hydrodynamic radius.\(^{15}\)

The interior structure of the lyophilized GO-CPT-PEG hybrids and hydrogels were investigated using a scanning electron microscopy (SEM) image. As shown in Fig. 2A, the hybrids show randomly distributed GO sheets with a number of threadlike polymers tethered to the surface. However, it is observed that the resulting hydrogels possess well-ordered scaffolds and highly interconnected pores (Fig. 2B–D). The well-ordered scaffolds of GO supramolecular hydrogels with rigid PPR cross-linkers have been confirmed in our previous work which could be mainly ascribed to the self-assembly of GO sheets induced by the strong hydrogen-bond interaction among adjacent rigid PPRs.\(^{1b,2b}\) In addition, the introduction of GO sheets can provide a large number of noncomplexed PEG segments which are beneficial to constructing and stabilizing the scaffolds. Meanwhile, the ice-template can also help to form the porous structure during the freeze-drying process. As the concentration of α-CD increased, the hydrogen-bond interaction among adjacent PPRs was strengthened due to the increase of the number of PPRs, which would enhance the bonding force between GO sheets, thus leading to the formation of more compact scaffolds and highly ordered interior microstructures for the hydrogels (Fig. 2B–D).

We have previously reported that the PPR-based supramolecular hydrogel possesses a unique thermo-sensitive gel–sol transition property owing to the temperature responsive threading–dethreading process of PPR.\(^{16}\) For this system, as shown in Fig. 3B, when the GO hydrogel was heated at 30 and 40 °C, it maintained the opaque gel phase, while it became a

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**Fig. 1** (A) Schematic representation of the hybrid hydrogel preparation based on the host–guest interactions between prodrug modified GO and α-CD. (B) Optical photos of the sol made of mPEG (20 mg mL\(^{-1}\)) and α-CD (100 mg mL\(^{-1}\)), and hydrogels made of GO–CPT–PEG hybrids (15 mg mL\(^{-1}\)) and different concentrations of α-CD, G1 (50 mg mL\(^{-1}\)), G2 (80 mg mL\(^{-1}\)), G3 (110 mg mL\(^{-1}\)) and G4 (140 mg mL\(^{-1}\)).

**Fig. 2** SEM images of freeze-dried GO-CPT-PEG hybrids (A) and GO-CPT-PEG/α-CD hydrogels G1 (B), G2 (C) and G3 (D). Scale bars: 50 μm for each image.
mobile sol phase at 50 and 60 °C, suggesting that the hydrogel was also thermo-sensitive. It was proposed that upon NIR light irradiation, the GO hydrogel would undergo a unique gel−sol transition due to the self-heating process (Fig. 3A). As shown in Fig. 3C, after irradiation by NIR light for 20 min, the temperature of the CPT-PEG/α-CD hydrogel showed almost no changes, while that of the GO hydrogel was increased above 20 °C, suggesting that the GO hydrogel has high self-heating efficiency. Furthermore, the gel−sol transition behaviour of the two kinds of hydrogels was investigated by NIR light irradiation for different time periods. The GO hydrogel almost completely turned into a mobile sol phase after 20 min of irradiation, while the CPT-PEG/α-CD hydrogel was still in the opaque gel phase (Fig. 3D), suggesting that the temperature of the GO hydrogel could reach the gel−sol transition temperature after irradiation by NIR. Moreover, the solid gel phase of the GO hydrogel could be selectively disrupted upon NIR light irradiation at different positions and times (Fig. 3E), which indicates that the spatially and temporally controlled drug release can be precisely triggered by NIR light irradiation.

Having a highly hydrated and well-ordered microstructure, the GO hydrogel could be further utilized as a carrier for encapsulating another water-soluble anti-cancer drug, 5-FU, which is always combined with CPT drugs to enhance their anticancer activity (Fig. 4A).6 The 5-FU loaded GO hydrogel exhibited a typical dual phase behavior for co-delivery of CPT and 5-FU (Fig. 4A and B). In the first stage, the 5-FU was released from the hydrogel during 24 h at an almost constant rate, while the release of CPT-PEG was sustained for more than 6 days. The 5-FU was released in a diffusion-controlled manner, and such a constant release behavior might be ascribed to its homogeneous distribution in the regular pores of the hydrogel, which is beneficial to the precise control of releasing dose. The CPT-PEG release behavior could be due to the breakup of the hydrogel caused by the dethreading of rigid PPR in the dilute conditions. In the second stage, the CPT-PEG also possessed the ability of releasing CPT by hydrolysis of CPT-PEG (Fig. 4A) in the presence of esterase, which is abundant in the cytoplasm.17 Furthermore, the release kinetics of 5-FU and CPT-PEG showed significant structure-dependent properties. The release rates of 5-FU and CPT-PEG are remarkably decreased from G1 to G3 (Fig. S6, ESI†). This could be ascribed to the fact that more compact scaffolds and smaller pores of the hydrogel would lead to the decrease of the diffusion rate and the breakup of supra-cross-links. Both the GO-CPT-PEG/α-CD and CPT-PEG/α-CD hydrogels laden with 5-FU were further treated by NIR light irradiation. As shown in Fig. 4C, 5-FU and CPT-PEG were released from the CPT-PEG/α-CD hydrogel slowly upon NIR light irradiation, while they were continuously released from the GO-CPT-PEG/α-CD hydrogel at a high speed upon NIR light irradiation. In addition, the dependence of drug release kinetics on the power density of NIR light

![Fig. 3](image-url)  
**Fig. 3** NIR light-triggered gel−sol transition of the hydrogel. (A) Schematic of NIR-caused gel−sol transition of the GO hybrid hydrogel. (B) Photographs represent the temperature-dependent gel−sol transition. (C) Thermographs of CPT-PEG/α-CD and GO-CPT-PEG/α-CD hydrogels irradiated by NIR laser light captured at different time points. (D) Photographs of CPT-PEG/α-CD and GO-CPT-PEG/α-CD hydrogels before and after NIR light irradiation. (E) Weight loss of hydrogels after NIR light irradiation for different times.
was also investigated. As shown in Fig. 4C and Fig. S7 (ESI†), when the laser power density was higher than 2.5 W cm\(^{-2}\), the release rate of 5-FU and CPT-PEG significantly increased with the increase of power density. By comparison, no obvious rate changes occurred when the power density was lower than or equal to 2.5 W cm\(^{-2}\). These results indicate that the hydrogel could efficiently load 5-FU and CPT in the same matrix with different controlled release profiles, and suitable NIR light irradiation can efficiently control the dual drug release in an on-demand mode. To investigate the NIR-triggered drug release behavior in vivo, the CyN-PEG composed of a near-infrared fluorescent IR-780 dye and a PEG chain was synthesized (Scheme S2, ESI†) and further encapsulated into the hydrogel instead of 5-FU. Chinese KunMing mice bearing H22 ascites sarcoma were intratumorally injected with the CyN-PEG loaded GO-CPT-PEG/α-CD hydrogel and in vivo whole-body fluorescence images were taken on an in vivo imaging system (IVIS). As shown in Fig. 4D, the fluorescence area in the tumor position increased obviously after 20 min irradiation (right panel), while it almost remained unchanged in the case of no NIR light irradiation (left panel). Such a phenomenon could be attributed to the fact that during the entire imaging acquisition period (20 min), under the circumstance of no NIR light irradiation, CyN-PEG was minimally released from the hydrogel, but once NIR light irradiation was administered, its release quantity increased largely. These results suggest that NIR light irradiation could efficiently trigger the gel–sol transition of the GO-CPT-PEG/α-CD hydrogel, thus leading to drug release from the hydrogel in vivo.

Using MTT assays in A549 lung cancer cell lines, the overall cytotoxicities of the CPT-PEG and CPT-PEG/α-CD hydrogels were first evaluated and compared with CPT as a free drug. The results indicate that both the CPT-PEG and CPT-PEG/α-CD hydrogels have a minimal effect on the cytotoxicity of CPT at equal CPT dose (Fig. S8A, ESI†), proving that the CPT-PEG prodrug in the hydrogel still possesses the ability to release CPT in the second release stage to exert its activity. For cancer combination therapy, the dose ratio of dual drugs was expected to play an important role in the therapeutic effect. However, strategies to precisely control the dose ratio of different therapeutic payloads from a single multi delivery platform are still limited. In our system, it is convenient to control the ratio of 5-FU to CPT due to their different locations and loading mechanisms in the hybrid hydrogel microstructure. As shown in Fig. S8B (ESI†), the dose ratio of 5-FU to CPT-PEG can be precisely controlled over a broad range from 0:1 to 4:1. Moreover, an in vitro study on the 5-FU loaded hydrogel showed an enhanced cytotoxicity against cancer cells in comparison with the native hydrogel, and the cytotoxicity increased obviously with the increase of 5-FU dose in the hydrogel. Therefore, the 5-FU loaded GO-CPT-PEG/α-CD hydrogel would show significant potential for combination therapy, serving as an effective dual-drug carrier to conveniently adjust appropriate proportions of CPT and 5-FU.

In summary, we have successfully fabricated a 3D graphene oxide hybrid hydrogel with a well-ordered interior structure via introducing necklace-like PPR as a rigid cross-link instead of traditional soft cross-links. The proposed hydrogel can not only efficiently load two drugs at various proportions in different positions and via different loading mechanisms, but also release the drugs in an on-demand and dual phase fashion upon an NIR light stimulus, which can realize better dynamic control on drugs than the traditional diffusion-based mode. This work may promote design and fabrication of NIR light-controlled reversible platforms for co-delivery of various therapeutic biological agents, cell culture and tissue engineering.

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