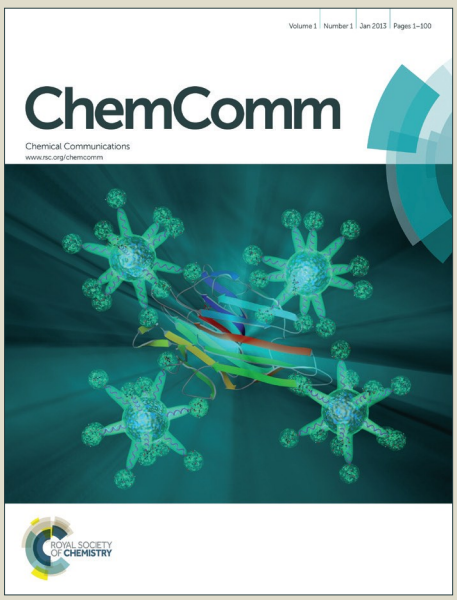
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

ChemComm

COMMUNICATION

Near Infrared Light-responsive and Injectable Supramolecular Hydrogels for On-demand Drug Delivery

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Xinyu Wang, Changping Wang, Qiang Zhang* and Yiyun Cheng*

A near infrared (NIR) light-responsive supramolecular hydrogel consisted of α-cyclodextrin and poly(ethylene glycol)-modified dendrimer-encapsulated platinum nanoparticles was developed. Upon NIR irradiation, this hydrogel underwent a photothermo-sensitive degradation to release the entrapped therapeutic agents in an on-demand and dose-tunable fashion.

Hydrogel-based drug delivery system yields a local high-dose and constant release of therapeutic agents in the pathological tissues and avoids non-specific drug distribution in normal tissues to reduce adverse effects.[1] General hydrogels only allow spatial drug release based on passive diffusion or hydrogel degradation, which cannot satisfy the requirements for clinical drug delivery such as spatially and temporally controlled drug release. To address this deficiency, hydrogels in response to diverse stimuli (e.g., pH,[2] redox potential[3], enzymes,[4] light,[5] magnetic field,[6] electronic field[7] and ultrasound[8]) are extensively explored, among which, light-responsive hydrogels are of great interest due to their advantages including non-invasiveness, spatially and temporally controlled, remote and instant delivery.[5, 9]

Light-responsive hydrogels are generally classified into two major types: photodegradable hydrogels that possess photolabile moieties (e.g., o-nitrobenzyl[10] and azobenzene[11]) in their structures and thermo-sensitive hydrogels (e.g., poly(N-isopropylacrylamine[12]) that embedded with near infrared (NIR)-absorbing nanostructures (e.g., nanorods,[13] nanoshells, [14] and carbon nanotubes[15]) in the matrix. The photodegradable hydrogels release drugs upon light-triggered degradation of their structures. However, the chemical reaction or isomerization for polymer degradation or disassembly require the use of high-energy ultraviolet (UV) or visible light,[16] both of which have phototoxicity concerns and poor ability to penetrate deeply in the tissues. One may use chromophores or upconversion nanoparticles to convert NIR light into UV irradiation,[17] but the low two-photon-absorbing efficiency for chromophores and the high toxicity of the rare elements in upconversion nanoparticles impede their clinical applications. On the other hand, the thermo-sensitive hydrogels entrapped with NIR-absorbing nanostructures undergo a volume expansion/contraction process to release the drugs. However, these hydrogels are generally non-degradable,[18] leading to increased risk of systemic toxicity due to their long-term retention in the body.

Here, we reported an injectable, NIR light-responsive and on-demand degradable supramolecular hydrogel for controlled drug delivery. This gel consists of alpha-cyclodextrin (α-CD) and poly(ethylene glycol) (PEG)–modified dendrimer encapsulated with platinum (Pt) nanoparticles (DEPt-PEG) (Figure 1a). The PEG chains threaded into the cavities of a series of α-CD via the well-known host-guest inclusion to form pseudopolyrotaxane (PPR), and then the strong hydrogen-bond interactions among PPRs provided a physical cross-linking effect that led to the hydrogel formation.[19] Direct mixing of PEG with α-CD did not generate hydrogel but formed microcrystalline aggregates due to uncontrolled host-guest inclusion between PEG and α-CD and subsequently hydrogen-bond interaction among PPRs.[20] In our system, the amine-terminated generation 5 (G5-NH_2) poly(amino amine) (PAMAM) dendrimers were used as a core material to tether high density of PEG chains on their surfaces (Figure S1), like previously reported PEG/α-CD supramolecular hydrogels had the PEG chains anchored on the surface of polymeric cores,[21] gold nanoparticles,[20] and silica nanoparticles,[22] which provides a steric hindrance to partly prevent the host-guest inclusions between PEG and α-CD and the hydrogen-bond interactions among PPRs. Since the hydrogel was degradable in response to heat, dendrimers in the hydrogel were used as a template to synthesize ultrasmall Pt nanoparticles, which could serve as a photothermal agent to trigger the on-demand hydrogel degradation and drug release.

[a] *Shanghai Key Laboratory of Regulatory Biology, School of Life Sciences, East China Normal University, Shanghai, 200241, P.R. China. E-mail: ycheng@mail.ustc.edu.cn; qzhang@bio.ecnu.edu.cn*

Electronic Supplementary Information (ESI) available: Experimental section and ^{1}H NMR data. See DOI: 10.1039/x0xx00000x

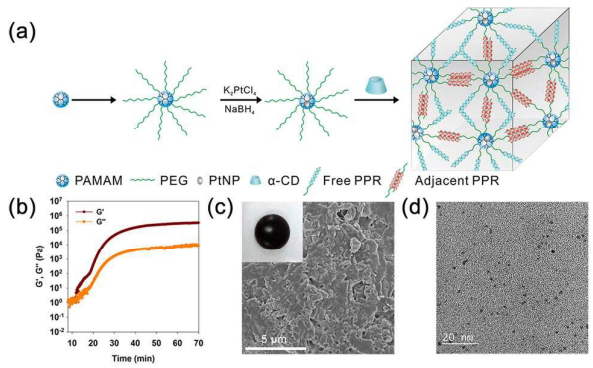


Figure 1. Preparation and characterization of the supramolecular hydrogel consisted of DEPt-PEG and α-CD. (a) Schematic representation of the hydrogel preparation based on the strong hydrogen-bond interaction among PPRs. (b) Hydrogel formation revealed by the dynamic development of G' and G" moduli over time. (c) SEM image and photograph (inset panel) of the hydrogel. (d) HRTEM image of DEPt-PEG.

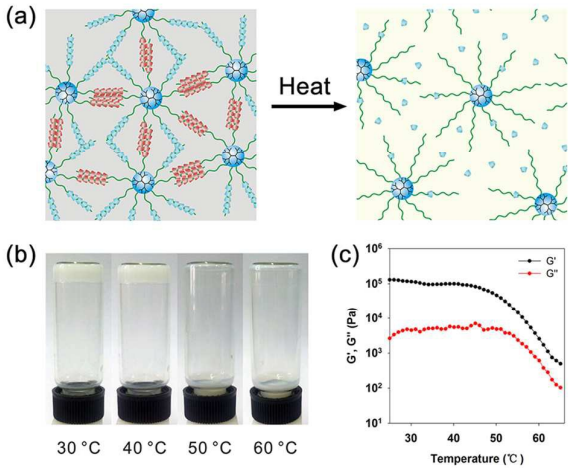


Figure 2. Thermo-responsive hydrogel degradation. (a) Scheme depicts the proposed mechanism for hydrogel degradation. (b) Photographs represent the temperature-dependent translation from hydrogel to sol solution. (c) Hydrogel degradation revealed by the temperature-dependent evolution of G' and G" moduli.

Moreover, the ultrasmall size of DEPt facilitated its clearance out of the body after hydrogel degradation, and the high-density functional groups on dendrimer surface made it feasible to integrate multiple functions such as imaging ligands in the hydrogel. A typical DEPt-PEG/α-CD hydrogel was simply prepared by mixing equal volume of aqueous DEPt-PEG (5 % in weight) and α-CDs (14 % in weight) and then leaving the mixture without disturbance for 60 min. For the preparation of drug-laden hydrogel, the drug was previously dissolved in the aqueous solution with α-CD and then the same gelation procedure was performed. The dynamic rheology measurement revealed that a sol-gel translation took place at a time point of 8 min for DEPt-PEG/α-CD hydrogel indicated by the crosslink of the storage (G') and loss (G") moduli, and a final G' modulus of ~100 kilopascal (kPa) was recorded for the hydrogel (Figure 1b). This final G' moduli was ~3 orders of magnitude higher than that of the PEG/α-CD hydrogels with a polymeric core,[21] which means the existence of DEPt significantly improved the mechanic strength of the hydrogel. The microstructure of the hydrogel was imaged by scanning electron microscope (SEM, Figure 1c). The hydrogel could be prepared with well-designed shape, and the black color of the hydrogel was owing to the homogeneous dispersion of Pt nanoparticles in the matrix. (Figure 1c inset panel). The high-resolution transmission electron microscopy (HRTEM) image reveals that the monodispersed DEPt-PEG had an ultrasmall size of 1.9±0.3 nm (Figure 1d).

Previous investigations suggest that the supramolecular hydrogels derived from PPR assemblies possess the property of thermo-sensitive degradation and reversibly cross-linking due to the de-threading and re-threading of α-CD from PEG chains (Figure 2a).[19c] Likewise, DEPt-PEG/α-CD hydrogel was also supposed to be thermo-sensitive. The as-formed hydrogel in vials were upside down placed in the oven at different temperatures for 30 min. The hydrogels heated at 30 and 40 °C maintained their shape in the bottom of vials, indicating they were solid phase, while the hydrogels heated at 50 and 60 °C melted and flowed down, suggesting they were converted into quasi-sol or sol phase (Figure 2b). The temperature-dependent evolution of the mechanical strength of DEPt-PEG/α-CD hydrogel was further assessed. Both of the G' and G" moduli significantly decreased above a critical temperature of ~ 48 °C (Figure 2c), indicating the collapse of the gel to quasi-liquid phase.

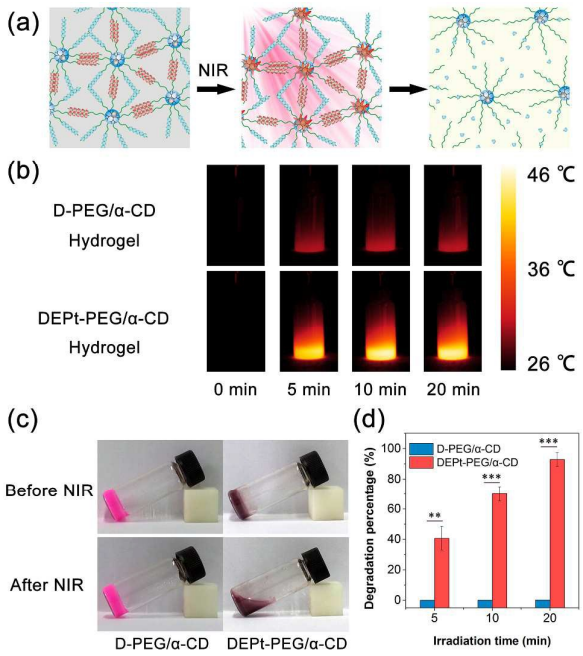


Figure 3. NIR-triggered hydrogel degradation. (a) Schematic of NIR-caused hydrogel degradation. (b) Thermographs of D-PEG/α-CD and DEPt-PEG/α-CD hydrogels irradiated by NIR laser captured at different time points. (c) Digital photographs of D-PEG/α-CD and DEPt-PEG/α-CD hydrogels laden with rhodamine B before and after NIR irradiation. (d) Weight loss of hydrogels after NIR irradiation for different times.

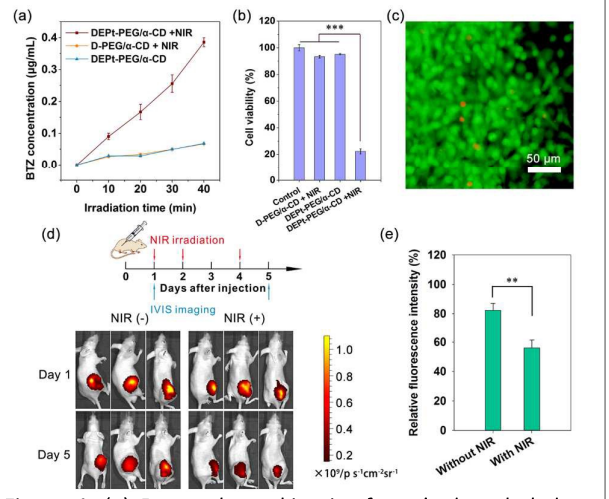


Figure 4. (a) Drug release kinetics from hydrogels laden with BTZ and triggered by NIR irradiation. (b) Cytotoxicity of the released drug revealed by MTT assay on PC9 cells. (c) The biocompatibility of DEPt-PEG/α-CD hydrogel determined by AO/EB staining assay on NIH3T3 cells. (d) *In vivo* NIR-triggered dye release from hydrogel. Cy5.5 was laden in the hydrogel instead of drug, and the hydrogel degradation and drug release was monitored by the IVIS. (e) Relative fluorescence intensity of Cy5.5 remaining in the tumors after three times of NIR irradiations.

Since the supramolecular hydrogel was thermo-sensitive, we synthesized Pt nanoparticles inside the dendrimers, which had been previously proven to have an excellent photothermal effect and good biocompatibility.[23] It was supposed that DEPt-PEG/α-CD hydrogel upon NIR irradiation would undergo a self-heating caused collapse (Figure 3a). Hence, we first evaluated the photothermal effect of the hydrogel. Both of the D-PEG/α-CD (without Pt nanoparticles in dendrimers) and DEPt-PEG/α-CD hydrogels were in situ formed in cuvettes and irradiated by NIR light at a power density of 0.62 W cm^{-2} for 20 min. The thermographs of the two hydrogels were captured at different time points (Figure 3b). The temperature of DEPt-PEG/α-CD hydrogel increased much faster than that of D-PEG/α-CD hydrogel, and finally was increased by ~20 °C. Furthermore, the two kinds of hydrogels laden with a fluorescent dye, Rhodamine B, to visually mimic the drug delivery, were irradiated by NIR laser at a power density of 0.62 W cm^{-2} for different times. As shown in Figure 3c, the DEPt-PEG/α-CD hydrogel was completely degraded after 20 min irradiation, while the D-PEG/α-CD hydrogel was still solid phase. Moreover, DEPt-PEG/α-CD hydrogel could be partially disrupted upon NIR irradiation for different times (Figure 3d), which indicates the drug release kinetics can be precisely controlled by NIR light irradiation.

The *in vitro* drug release kinetics was evaluated by use the chemical drug, bortezomib (BTZ) as a model, which is used to treat the multiple myeloma and mantle cell lymphoma in clinical trials.[24] DEPt-PEG/α-CD hydrogel laden with BTZ was irradiated by NIR laser, and the drug was continuously released from the hydrogel over time, while there was minimal BTZ released from D-PEG/α-CD hydrogel upon NIR irradiation or DEPt-PEG/α-CD hydrogel without NIR irradiation (Figure 4a). These results first demonstrate the hydrogel could well trap BTZ in the matrix, and then suggests that the photothermal conversion in DEPt-PEG/α-CD hydrogel was high efficient and the as-generated local hyperthermia was critical to trigger the hydrogel degradation and drug release. The anticancer activity of the released BTZ from the hydrogel was further evaluated on PC-9 cancer cells using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The result in Figure 4b reveals that the viability of PC-9 cells was significantly reduced by the released BTZ from DEPt-PEG/α-CD hydrogel upon NIR irradiation (Figure 4b). Taken together, DEPt-PEG/α-CD hydrogel was sensitive in response to NIR irradiation to trigger the drug release in an on-demand and dose-tunable way. The biocompatibility of DEPt-PEG/α-CD hydrogel was further evaluated on NIH3T3 cells, and the result indicates that the hydrogel had minimal toxicity to NIH3T3 cells (Figure 4c). To study the *in vivo* drug release behavior, DEPt-PEG/α-CD hydrogel laden with cyanine 5.5 (Cy5.5) instead of the drug was injected into the tumors in order to visually observe the dye release by using the *in vivo* imaging system (IVIS). In the hydrogel without NIR irradiation, Cy5.5 was minimally released, while in the one treated with NIR irradiation, Cy5.5 was released in large quantities (Figure 4d

and 4e). This result suggests that NIR irradiation could efficiently triggered drug release from the DEPt-PEG/α-CD hydrogel *in vivo*.

Conclusions

In summary, we developed a NIR-responsive supramolecular hydrogel that was composed of DEPt-PEG and α-CD. This hydrogel could be degraded by NIR exposure, and thus was developed as a local delivery system for spatiotemporally controlled drug release. Given the photothermal effect of DEPt-PEG, this hydrogel was also able to be used for combined photothermal and chemotherapy.

Financial supports from the National Natural Science Foundation of China (Grant Nos. 21274044, 21322405 and 21207038), the Basic Research Program of Science and Technology Commission of Shanghai Municipality (14JC1491100), the Shanghai Pujiang Program (Grant No.14PJD016) and the Shanghai Chenguang Program (Grant No. 12CG25) are greatly appreciated. We also would like to acknowledge the electron microscope center of East China Normal University to provide the facilities for sample characterization.

Notes and references

1. (a) G. W. Ashley, J. Henise, R. Reid and D. V. Santi, *Proc. Natl. Acad. Sci. U S A*, 2013, **110**, 2318-2323; (b) R. F. Donnelly, T. R. R. Singh, M. J. Garland, K. Migalska, R. Majithiya, C. M. McCrudden, P. L. Kole, T. M. T. Mahmood, H. O. McCarthy and A. D. Woolfson, *Adv. Funct. Mater.*, 2012, **22**, 4879-4890; (c) F. Zhao, M. L. Ma and B. Xu, *Chem. Soc. Rev.*, 2009, **38**, 883-891.
2. P. Gupta, K. Vermani and S. Garg, *Drug Discov. Today*, 2002, **7**, 569-579.
3. M. Nakahata, Y. Takashima, H. Yamaguchi and A. Harada, *Nat. Comm.*, 2011, **2**.
4. P. D. Thornton, R. J. Mart and R. V. Ulijn, *Adv. Mater.*, 2007, **19**, 1252-+.
5. K. Peng, I. Tomatsu and A. Kros, *Chem. Commun.*, 2010, **46**, 4094-4096.
6. N. S. Satarkar and J. Z. Hilt, *J. Controlled Release*, 2008, **130**, 246-251.
7. S. Murdan, *J. Controlled Release*, 2003, **92**, 1-17.
8. N. Huebsch, C. J. Kearney, X. Zhao, J. Kim, C. A. Cezar, Z. Suo and D. J. Mooney, *Proc. Natl. Acad. Sci. U S A*, 2014, **111**, 9762-9767.
9. (a) C. Park, K. Lee and C. Kim, *Angew. Chem., Int. Ed.*, 2009, **48**, 1275-1278; (b) H. Komatsu, S. Matsumoto, S. Tamaru, K. Kaneko, M. Ikeda and I. Hamachi, *J. Am. Chem. Soc.*, 2009, **131**, 5580-5585; (c) I. Tomatsu, K. Peng and A. Kros, *Adv. Drug Deliv. Rev.*, 2011, **63**, 1257-1266.
10. C. A. DeForest and K. S. Anseth, *Angew. Chem., Int. Ed.*, 2012, **51**, 1816-1819.
11. S. Tamesue, Y. Takashima, H. Yamaguchi, S. Shinkai and A. Harada, *Angew. Chem., Int. Ed.*, 2010, **49**, 7461-7464.
12. X. Q. Zhao, T. X. Wang, W. Liu, C. D. Wang, D. Wang, T. Shang, L. H. Shen and L. Ren, *J. Mater. Chem.*, 2011, **21**, 7240-7247.
13. A. Shiotani, T. Mori, T. Niidome, Y. Niidome and Y. Katayama, *Langmuir*, 2007, **23**, 4012-4018.
14. M. Bikram, A. M. Gobin, R. E. Whitmire and J. L. West, *J. Controlled Release*, 2007, **123**, 219-227.
15. A. C. Estrada, A. L. Daniel-da-Silva and T. Trindade, *RSC Adv.*, 2013, **3**, 10828-10836.
16. (a) L. Peng, M. You, Q. Yuan, C. Wu, D. Han, Y. Chen, Z. Zhong, J. Xue and W. Tan, *J. Am. Chem. Soc.*, 2012, **134**, 12302-12307; (b) M. He, J. Li, S. Tan, R. Wang and Y. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 18718-18721.
17. (a) B. Yan, J.-C. Boyer, D. Habault, N. R. Branda and Y. Zhao, *J. Am. Chem. Soc.*, 2012, **134**, 16558-16561; (b) G. C. R. Ellis-Davies, *Nat. Methods*, 2007, **4**, 619-628.
18. (a) W. Li, J. Wang, J. Ren and X. Qu, *Adv. Mater.*, 2013, **25**, 6737-6743; (b) M. A. Azagarsamy and K. S. Anseth, *Angew. Chem., Int. Ed.*, 2013, **52**, 13803-13807.
19. (a) X. Liao, G. Chen, X. Liu, W. Chen, F. Chen and M. Jiang, *Angew. Chem., Int. Ed.*, 2010, **49**, 4409-4413; (b) J. Li, *NPG Asia Mater.*, 2010, **2**, 112-118; (c) W. Ha, J. Yu, X. Y. Song, J. Chen and Y. P. Shi, *ACS Appl. Mater. Inter.*, 2014, **6**, 10623-10630.
20. J. Yu, W. Ha, J. N. Sun and Y. P. Shi, *ACS Appl. Mater. Inter.*, 2014, **6**, 19544-19551.
21. L. Ren, L. He, T. Sun, X. Dong, Y. Chen, J. Huang and C. Wang, *Macromol. Biosci.*, 2009, **9**, 902-910.
22. M. Guo, M. Jiang, S. Pispas, W. Yu and C. Zhou, *Macromolecules*, 2008, **41**, 9744-9749.
23. (a) C. Wang, X. Cai, J. Zhang, X. Wang, Y. Wang, H. Ge, W. Yan, Q. Huang, J. Xiao, Q. Zhang and Y. Cheng, *Small*, 2015, **11**, 2080-2086; (b) X. Wang, Y. Zhang, T. Li, W. Tian, Q. Zhang and Y. Cheng, *Langmuir*, 2013, **29**, 5262-5270.
24. J. Adams and M. Kauffman, *Cancer Invest.*, 2004, **22**, 304-311.