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DNA Hydrogel by Multicomponent Assembly for Encapsulation and Killing of Cells

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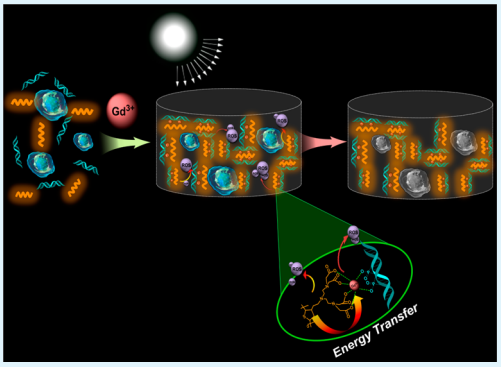
Supporting Information

ABSTRACT: In this work, a new multifunctional assembled hydrogel was prepared by incorporating gadolinium ions (Gd^{3+}) with salmon-sperm DNA and polythiophene derivative (PT-COOH) through chelation interactions. Efficient energy transfer from PT-COOH to Gd^{3+} ions takes place followed by sensitization of oxygen molecule to generate reactive oxygen species (ROS) under light irradiation. Cancer cells can be encapsulated into the hydrogel in situ as the formation of hydrogel followed by killing by the ROS. Integration of imaging modality with therapeutic function within a single assembled hydrogel is therefore anticipated to be a new and challenging design element for new hydrogel materials.



KEYWORDS: *DNA, cationic conjugated polymers, self-assembly, therapeutic hydrogel, cell encapsulation*

Hydrogel, especially DNA hydrogel, has attracted increasing attention because of its diversity and biocompatibility.[1−3] The preparation methods for various hydrogels have been extensively developed, such as cross-link reaction,[4] enzyme catalysis assembly,[5] host−guest interaction,[6] in situ polymerization,[7] radical polymerization,[8] and ionic interaction.[9] The hydrogels have been widely applied to chemistry, material, biology, and biomedical fields.[10−13] Especially, as a kind of biological material, hydrogels can be used to encapsulate and release DNA,[14] proteins,[15] cells,[16] and drugs.[7,17] Very recently, hydrogel modified with aptamer has been developed for sensitive detection of AIV H5N1 virus.[18] In spite of many efforts having been made, multifunctional therapeutic assembled hydrogels with the characteristic of fluorescence imaging, encapsulation, and killing cells simultaneously have rarely been designed and reported. Integration of imaging modality with therapeutic function within a single assembled hydrogel is therefore anticipated to be a new and challenging design element for new hydrogel materials.

Conjugated polymers (CPs) possess excellent light-harvesting ability for amplifying optical signal because of their delocalized electronic structure. Especially, CPs have been designed and synthesized with side-chains modified with charged functional groups such as anionic sulfonic, carboxyl groups or cationic quaternary ammonium groups, which enable them soluble in water medium, and widely used for highly sensitive biosensors and imaging.[19−23] Also, the cationic conjugated polymers can bind to the surface of bacteria or cancer cells and function as reactive oxygen species (ROS) photosensitizers to kill the bacteria or cancer cells under light irradiation.[24−26] Recently, we developed an anionic water-soluble polythiophene derivative used for killing bacteria. In this system, ROS were efficiently generated due to energy transfer from polythiophene to porphyrin.[27] Herein we combined the biocompatibility of DNA with the optical property and ROS generation ability of anionic conjugated polymers to prepare a novel multifunctional DNA hydrogel self-assembled from salmon DNA and anionic polythiophene (PT-COOH) via the chelation of Gd^{3+} ion in situ. PT-COOH with good light harvesting ability could effectively transfer energy to the Gd^{3+} acceptor that improved generation efficiency of ROS under white light irradiation. This assembled hydrogel can be used as multifunctional therapeutic hydrogel with the simultaneous abilities of imaging, encapsulation, and killing of cells.

The preparation based on assembly of the multifunctional therapeutic hydrogel is illustrated in Scheme 1A. In this system, DNA and PT-COOH are both negatively charged and arrange in a random state when mixed together. Upon adding Gd^{3+} ions, they could cross-linkingly assemble to form hydrogel through chelation interactions of coordinating Gd^{3+} ions with phosphate anion in DNA and carboxyl groups in PT-COOH. It has been reported that Gd^{3+} ion could interact with nucleotides to form supramolecular networks.[28] Upon light irradiation, the PT-COOH can sensitize the surrounding oxygen molecules to generate ROS. As shown in Scheme 1B, after chelating with Gd^{3+} ions, the efficient energy transfer from PT-COOH with good light harvesting ability to Gd^{3+} ions occurs.[28,29,31] The energy of Gd^{3+} ions transfers to triplet by intersystem crossing followed by sensitization of oxygen

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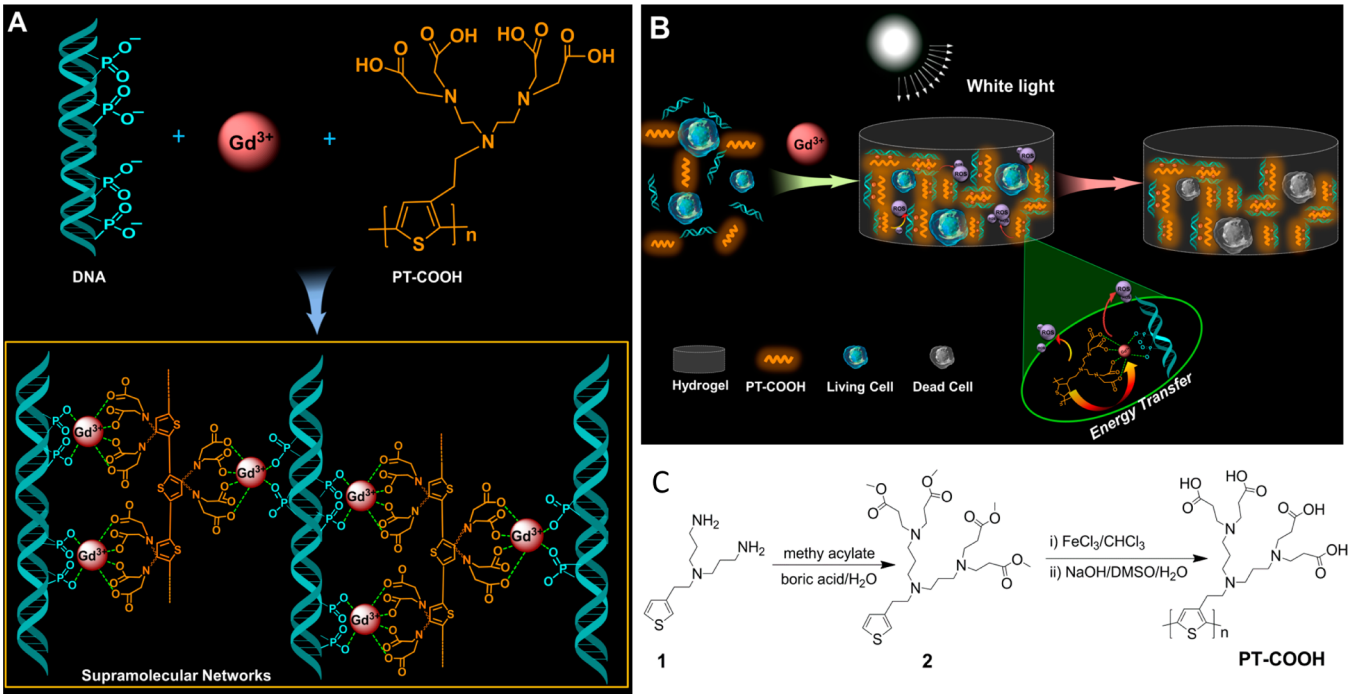
Scheme 1. (A) Illustration of Multifunctional Hydrogel Formation through Chelation Interactions of Gd^{3+} Ions with DNA and PT-COOH; (B) Principle of Cell Encapsulation of DNA Hydrogel Followed by Killing with ROS; (C) Synthetic Route of PT-COOH



molecule to enhance the efficiency of ROS generation. Upon adding Gd^{3+} ions to the mixed solution of DNA, PT-COOH, and cancer cells, the hydrogel forms in situ and the cells are encapsulated into the hydrogel simultaneously. Under white light irradiation, the cancer cells can be killed by the generated ROS from the system.

Procedures for the synthesis of PT-COOH are outlined in Scheme 1C. The monomer **2** was obtained by reacting compound **1** with methyl acrylate in the presence of boric acid through Michael addition with a yield of 75%. PT-COOH was prepared through oxidative polymerizing monomer **2** in the presence of $FeCl_3$ as the oxidizing agent in the dried chloroform, followed by hydrolyzation in NaOH aqueous solution and dialysis in water via a membrane with a molecular weight cutoff of 3500 g/mol. The final yield is 13%. The weight-average molecular weight of PT-COOH is about 160 000. Because there are four carboxyl groups per repeat unit, PT-COOH has good water-solubility to ensure its further application in biology. The optical property of PT-COOH was performed in water. As shown in Figure 1a, PT-COOH displayed the maximum absorption at 420 nm and maximum emission at 555 nm with a fluorescence quantum yield of 8% with quinine sulfate as standard. To prove that Gd^{3+} could chelate with carboxyl of PT-COOH, the fluorescence emission spectra of PT-COOH in the presence of Gd^{3+} ions were measured. As shown in Figure 1b, after the addition of Gd^{3+} ions, the fluorescence intensity of PT-COOH was quenched obviously, which indicated that Gd^{3+} ions have chelated with PT-COOH, and energy/electron transfer occurred from PT-COOH to Gd^{3+} ions. The cytotoxicity of PT-COOH was also investigated, where the viability analysis of Jurkat T cells was analyzed by Cell Counting Kit-8 (CCK-8). As shown in Figure 1c, PT-COOH did not demonstrate any cytotoxicity but promoted cell proliferation in some degree during the experimental concentration condition (0−3.2 mM). The result demonstrated that PT-COOH has good biocompatibility. In addition, the biocompatibility of PT-COOH/Gd^{3+} was also studied, and as shown in Figure S4d in the Supporting Information, the PT-COOH/Gd^{3+} complex possesses less cytotoxicity as well.

After verifying the chelation interactions between PT-COOH and Gd^{3+} ions, we prepared the assembled hydrogel by incorporating Gd^{3+} ions with negatively charged DNA and PT-COOH through chelation interactions. We first mixed PT-COOH and salmon sperm DNA in Hepes buffer to obtain a homogenious solution. Then the addition of $GdCl_3$ aqueous solution led to the formation of hydrogel as shown in Figure 2**a**. The formation of hydrogel attributes to chelation interactions of coordinating Gd^{3+} ions with phosphate groups in DNA and carboxyl groups in PT-COOH, resulting in cross-linkingly assembling in situ and forming hydrogel. It is noted that PT-COOH and Gd^{3+} alone could not form hydrogel. The fluorecent photograph of hydrogel was taken upon exposure to UV-light. Bright yellow fluorescence came from the emission of PT-COOH was observed (Figure 2d). Fluorescence microscope and scanning electron microscope (SEM) were utilized to obtain insight into the microstructure of the assembled hydrogel. As shown in Figures 2**b** and 2**e**, three-dimensional cross-linking networks exist in hydrogel. In addition, the intrinsic fluorescence of the hydrogel (yellow emission) from PT-COOH provides more insight into the net structure of the hydrogel. The direct visualization of SEM images further revealed the structure of hydrogel (Figure 2c, f). To get better performance of the hydrogel, its swelling behavior was studied. The wet hydrogel was dried thoroughly in freeze drier and then the swelling degree (Q) of the hydrogel was about eight after swollen in water for 12 h. Besides, the dissolution behavier of the hydrogel was also observed, that is, the inhibition of hydrogel took place at first and then dissolved. The hydrogel was stable for 60 h at least at PBS buffer (see Figure S4a in the Supporting Information).

It is well-known that water-soluble conjugated polymers can sensitize the surrounding oxygen molecules to generate ROS

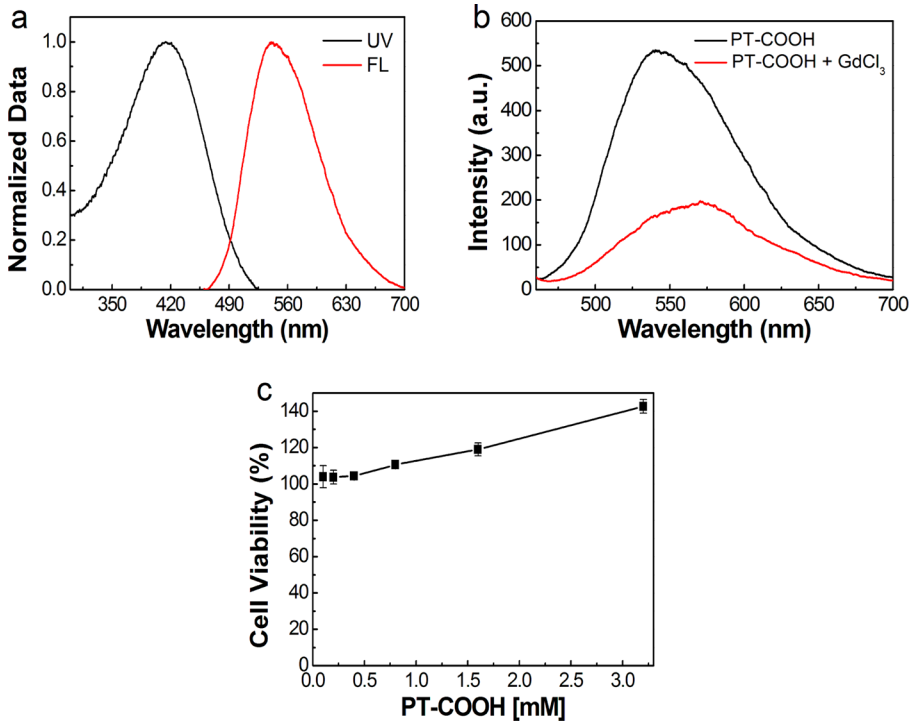


Figure 1. (a) Normalized absorption and emission spectra of PT-COOH in water. The excitation wavelength is 450 nm. (b) Emission spectra of PT-COOH in the absence and presence of $GdCl_3$. [PT-COOH] = 1.0 μM in repeated units (RUs). [$GdCl_3$] = 1.0 μM. The excitation wavelength is 450 nm. (c) Viability of Jurkat T cells incubating with PT-COOH for 12 h. [PT-COOH] = 0−3.2 mM.

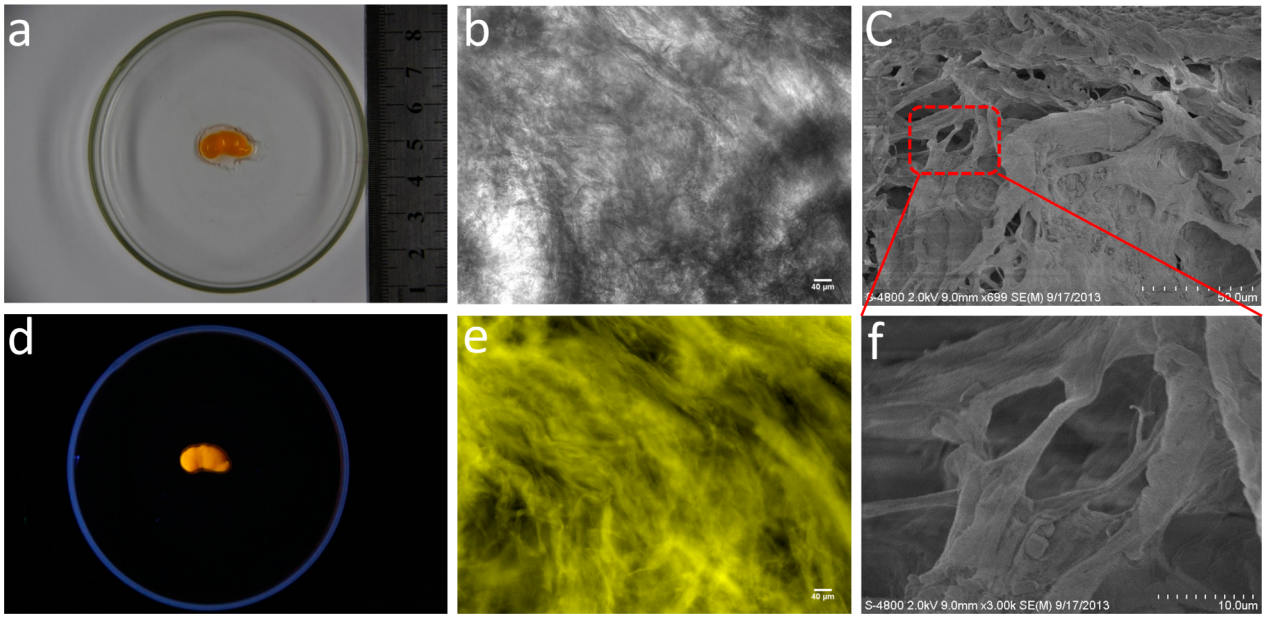


Figure 2. (a, d) Photographs of hydrogel (a) before and (d) after exposure to UV light at 365 nm. (b, e) Fluorescent microscope images under phase contrast bright-field and fluorescence field. (c, f) SEM image of hydrogel and the amplifying image.

under light irradiation.[24−27,30,32] To prove the ROS generation of the assembled hydrogel, we employed a ROS-sensitive probe, 2′,7′-dichlorofluorescin (DCFH), in which it converted into highly fluorescent 2,7-dichlorofuorescin (DCF, quantum yield: 90%) with an apparent increase in fluorescence intensity at 525 nm in the presence of ROS. Upon irradiating DCFH in the presence of PT-COOH under 10 mW cm^{-2} white light (400−800 nm), the fluorescence intensity at 525 nm increased apparently compared to that of control group without PT-COOH, which confirmed the generation of ROS originated from PT-COOH. It is noted that the addition of Gd^{3+} into solution of PT-COOH led to much more obvious increase of fluorescent intensity of the DCF. Especially when the molar ratio of Gd^{3+}/PT-COOH was one to one (one repeat unit of PT-COOH was chelated with one Gd^{3+} ion), a 7-fold enhancement of the intensity at 525 nm was obtained (see Figure S1a in the Supporting Information). Generally, energy transfer took place between PT-COOH with good light harvesting ability and chelated Gd^{3+},

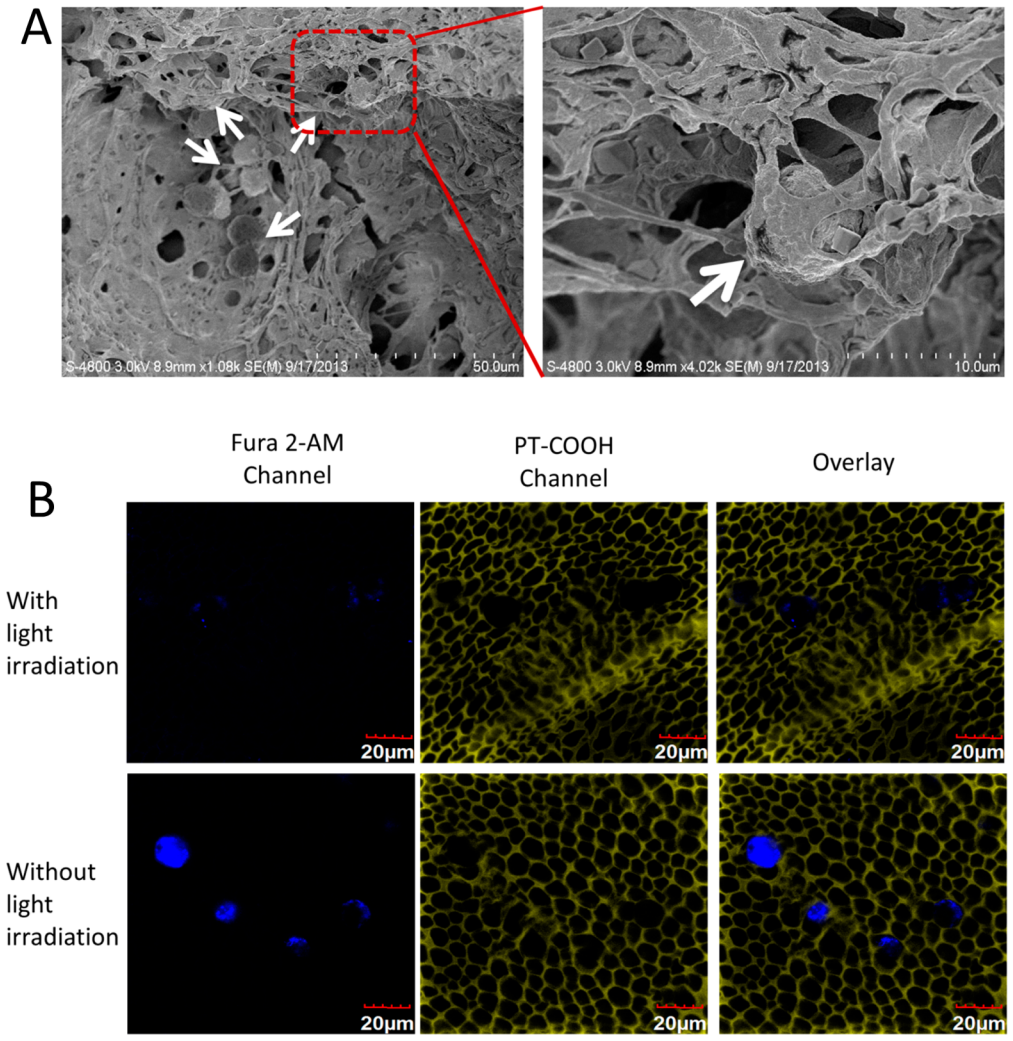


Figure 3. (A) SEM image of the hydrogel encapsulating Jurkat T cells (as denoted by arrows). (B) CLSM images of hydrogels encapsulating Jurkat T cells with and without white light irradiation. The fluorescence of Fura 2-AM is highlighted in blue, and the fluorescence of PT-COOH is highlighted in yellow.

and the energy of Gd^{3+} ions transfers to triplet by intersystem crossing followed by sensitization of oxygen molecule to enhance the efficiency of ROS generation.[32] Subsequently, to prove the ability of the assembled hydrogel to produce ROS, the hydrogel was immerged into DCFH aqueous solution followed by white-light irradiation, and then the emission intensity of the solution at 525 nm was measured. Because of the three-dimensional networks of the hydrogel, DCFH could get access into the hydrogel, and the formed DCF from the oxidation of DCFH by ROS under white light irradiation was able to diffuse into the solution medium. In this experiment, after equilibrium for 20 min allowing the diffusion of DCFH, the system was exposed to 35 $mW{\cdot}cm^{-2}$ white light for 0−6 min. As shown in Figure S1b and S1c in the Supporting Information, the fluorescent intensity of the solution at 525 nm increases dramatically as light irradiation time increases, which exhibits the generation of ROS sensitized by PT-COOH/Gd^{3+} complex in the hydrogel.

We investigated the encapsulation of cancer cells into the hydrogel in situ as the assembled hydrogel forms, as well as their killing by the ROS. Jurkat T cell line was used as the model cell in this study. The encapsulation of Jurkat T cells was realized via mixing cell suspension with DNA/PT-COOH mixture followed by injecting the mixture into $GdCl_3$ aqueous solution. SEM images showed that the encapsulation of cells did not influence the networks of the hydrogel, and the cells existed in the networks of the hydrogel (Figure 3A). We next investigated the cell killing by the hydrogel. Cell-permeable Fura 2-AM, the acetoxymethyl ester of the fluorescent calcium probe Fura 2, was chosen as fluorescent probe (maximum emission: 480 nm) to label live cells and distinguish live cells from dead cells. The probe can be loaded by membrane fusion, which is an active transport process and could not occur in dead cells, thus only live cells can be stained.[33,34] To confirm its specific imaging to live cells, we incubated the dead cells and live cells with Fura 2-AM (5.0 μM) away from light for 1 h, respectively. As show in Figure S2 in the Supporting Information, only the live cells could be stained by Fura 2-AM. Besides, Fura 2-AM, with a molecular weight of 1001, is expected to diffuse freely in the hydrogel to track the cell killing. The confocal laser scanning microscopy (CLSM) was used to investigate the cell killing ability of the hydrogel. After encapsulating Jurkat T cells, the hydrogel was irradiated with white light at a dose of 35 mW cm^{-2} for 10 min, and the control group was in dark at the same condition. And then the hydrogels were soaked in Fura 2-AM PBS buffer for 1 h followed by imaging with CLSM. As displayed in Figure 3B, for the group exposed to light, little fluorescence was detected, which

indicated the cell was killed by the generated ROS in the hydrogel. For the case of control group without light irradiation, Jurkat T cells showed strong fluorescence (blue color) stained by Fura 2-AM. A negative control group under light irradiation without ROS generation was set as well, which supported that the ROS induced cell damage (see Figure S3 in the Supporting Information).These results demonstrated that the cancer cells can be encapsulated into the hydrogel in situ as the assembled hydrogel forms, and then be killed by the generated ROS in the hydrogel under white light irradiation.

In conclusion, we developed a multifunctional hydrogel that self-assembles from DNA, Gd^{3+}, and anionic polythiophene (PT-COOH) through chelation interactions. The hydrogel can be used to encapsulate cells in situ and regulate light-mediated cell death. This hydrogel has several unique features. First, the preparation procedure is simple and fast. The hydrogel forms in situ through chelation interactions upon mixing DNA, PT-COOH, and Gd^{3+} ions. Second, the hydrogel has intrinsic fluorescence, which enables it to realize the function of imaging. Third, PT-COOH with good light harvesting ability could effectively transfer energy to the Gd^{3+} acceptor that improves generation efficiency of ROS under white light irradiation. Finally, the cancer cells can be encapsulated into the hydrogel in situ as the assembled hydrogel forms followed by killing by the ROS, offering the hydrogel therapeutic function. This assembled hydrogel can be used as multifunctional therapeutic hydrogel, which broadens the function of hydrogel and exhibits promising application to a wide range of biological and medical fields.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and Figures S1−S4. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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