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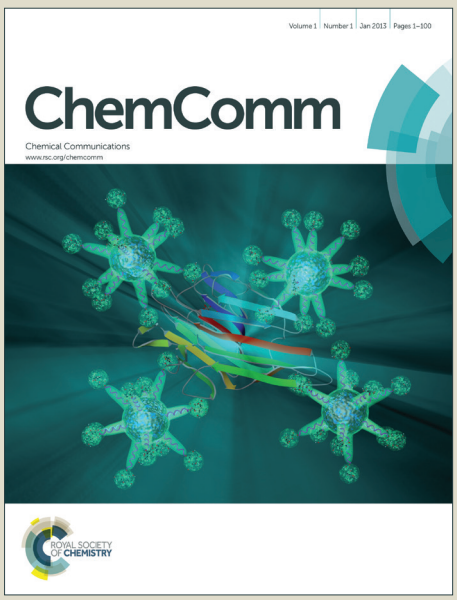

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Ultra-sensitive ROS-responsive tellurium-containing polymers

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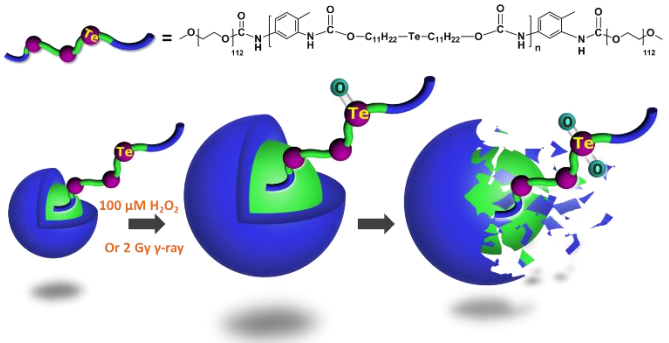
A novel tellurium-containing polymer micelle system is reported to be responsive to 100 μM H_2O_2 and can undergo a series of morphological changes. The polymer micelles also respond to 2 Gy gamma radiation, which is the exact dosage a patient receives one day for clinical radiation therapy.

Reactive oxygen species (ROS), which are highly reactive ions and free radicals that are typically short-lived and tend to react with other molecules to achieve stability, play important roles in regulating biological functions.[1-6] If inadequate anti-oxidant defenses are present and the whole balance is thus disrupted, ROS may contribute to numerous diseases such as cancer, inflammation and other chronic diseases.[7, 8] The elevated ROS levels are considered to be one of the major characteristics of many diseases.[9-11] Methodologies of developing responsive delivery vehicles applicable to the numerous diseases sites with an overproduction of ROS have been proved useful.[5, 12-14] As the ROS typically present in biologically-relevant conditions is as low as 50-100 μM H_2O_2,[15, 16] we suggest using oxidation-responsive materials with ultra-sensitivity that are able to autonomously and precisely respond to sites of excess ROS in human body.

Organotellurium compounds are of vital interest in tuning ROS level.[17, 18] Similar to organoselenium compounds and other organochalcogens, organotellurium compounds are easy to be oxidized from the divalent to the tetravalent state, making tellurides attractive as scavengers of ROS.[9, 19-24] Motivated by their unique biological properties, such as glutathione peroxidase mimic and antioxidant activity, organotellurium compounds have been described as promising pharmacological agents for anticarcinogen or antioxidant therapeutics.[25] However, using tellurium-containing polymer as ROS responsive system is still poorly explored.

Our study described herein showed tellurium-containing polymer micelles are responsive to 100 μM H_2O_2 (scheme 1). Taking advantage of the amphiphilicity switching mechanism of the different oxidized state of tellurium atoms on the polymer backbone, the self-assembled aggregates first swelled dramatically and further evolved into irregular aggregates. We find that it could function as a γ-ray responsive system with dosage as low as 2 Gy, which is the clinically used dosage for a patient per day.[26] These tellurium-containing polymers responsive to ultra-low-concentration ROS are of particular interest because of the promise they hold when it comes to fabricating the next generation of innovative programmable delivery vehicles and ROS eliminating materials under physiological environments.



Scheme 1. Oxidation responsive tellurium-containing polymer micelles that could undergo series of morphological changes triggered by ROS under biologically relevant conditions.

The tellurium-containing polymeric micelles swelled dramatically, in the presence of 100 μM H_2O_2, which is a typical physiologically-relevant concentration.[16] As revealed by the dynamic light scattering (DLS) measurements in Fig 1 a, the hydrodynamic diameter of the micelles was about 35 nm before oxidation, and increased instantly to more than 200 nm upon the treatment of H_2O_2. Interestingly, it was observed with the DLS profile that after about one day, the size of the micelles began to decrease (Fig 1 b). To investigate the aggregation behavior after oxidation, we performed transmission electron microscopy (TEM) observation without stain. The images of the oxidized sample showed spherical micelles with approximately 200 nm in diameter (Fig 1 d), while the original sample was only about 30 nm (Fig 1 c). Since the heavy atom tellurium is mainly located in the micellar core, the larger micelles in the images suggested

the swell in the hydrophobic core. We speculate that the swelling is because of the oxidation of tellurium on the polymer backbone, resulting in the hydration of the micelles. As for the later morphological transformation after one day, we think it is caused by further oxidation of the tellurium on the polymer backbone into higher chemical valence. TEM results indicated that the micelles tend to change into irregular aggregates after longer time (see supporting information Fig S1). Our preliminary results showed that this system also worked under even more diluted hydrogen peroxide, e.g. 50 μM H_2O_2.

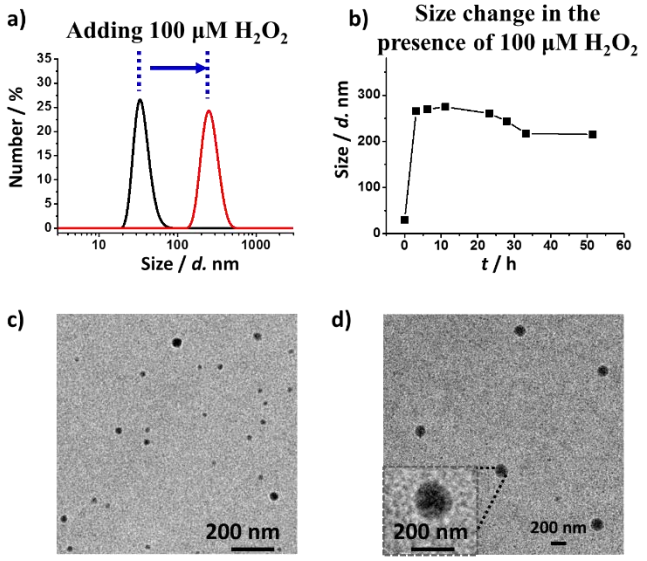


Fig. 1 The ROS responsive behaviours of the tellurium-containing polymer micelles. a) the size increased a lot after treated with 100 μM H_2O_2. b) the profile for the size change over time measured by DLS. c) TEM images of the micelles before oxidation. d) TEM image after 3 h oxidation-treatment. The insert is the zoomed image with the same scale bar as the TEM image in c).

The ultrahigh sensitivity of the tellurium-containing polymer should be ascribed to the oxidation-sensitive nature of tellurium element. Selenium-containing molecules are investigated to be more oxidation responsive than sulfur-containing molecules.[23] According to the periodic law of elements, the telluride compound are expected to be more sensitive to oxidation than the selenide compounds. To investigate whether telluride compounds are better candidates for fabricating ROS-responsive system compared with selenide and sulfide compounds, we synthesized water-soluble telluride, selenide and sulfide dicarboxylic acids as model compounds. Cyclic voltammetry (CV) provided a powerful tool to compare their oxidation behaviors. The CV experiments were carried out in a typical three-electrode glass electrochemical cell at ambient temperature. Fig 2 showed evidently that the telluride compound is much more sensitive to oxidative potential than selenide compound. The oxidative peak was observed at about 0.46 V (vs SSE) for m-$TeC_{10}H_{20}COOH$, while for selenide counterpart, the peak appeared at 0.96 V (vs SSE). The much lower oxidation potential could account for the enhanced sensitivity of tellurium-containing polymer. When it came to sulfide compound, no oxidation occurred even for up to 1.2 V, above which water would decompose (see supporting information Fig S9). Therefore, we conclude that telluride compounds are more sensitive to oxidation than the selenide and sulfide analogues and that tellurium-containing polymer may respond to ultralow concentration ROS. Systematic investigations on the exact oxidation chemistry revealed that in the presence of 1 equivalent H_2O_2, oxidative products with one oxygen atom added were formed (see Fig S10, S11). While with 10 times more H_2O_2, the molecules with 2 oxygen atoms added were identified.

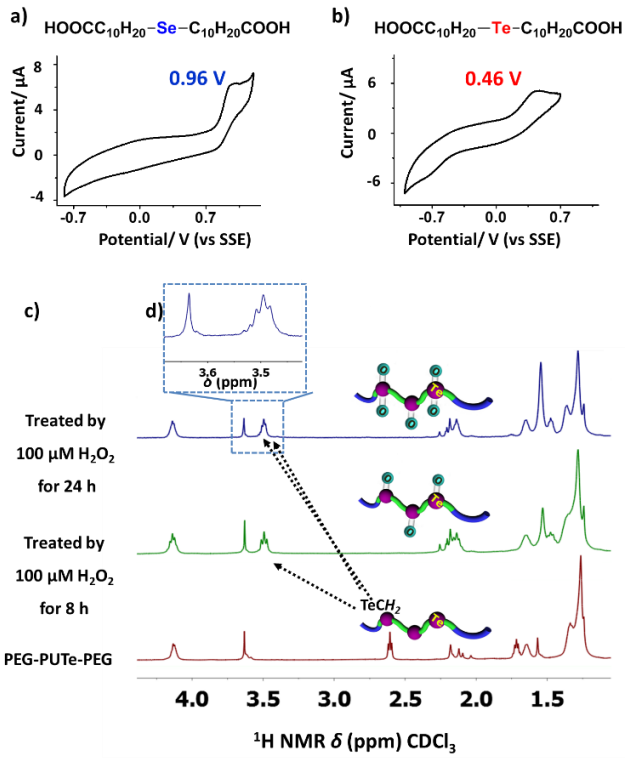


Fig. 2 a) b) Cyclic voltammetry curves of the selenium/tellurium containing model compounds. The solutions were prepared to be 0.4 mM in pH 9.2 buffer to increase the solubility and ensure sufficient conductivity. c) ^1H NMR spectra of the PEG-PUTe-PEG in $CDCl_3$ after oxidized by 100 μM H_2O_2 for different time. d) is the enlarged spectrum of the PEG-PUTe-PEG after oxidation by 100 μM H_2O_2 for 24 h.

To confirm the oxidation-induced chemical transformation on polymer structures, we studied the chemical structure of the tellurium-containing polymers after oxidation. We anticipated that the morphological transformation is due to the stepwise oxidation of the telluride groups. ^1H NMR spectra in chloroform-d showed that after treatment with low concentration H_2O_2 (100 μM), the chemical shifts of tellurium-containing segment changed significantly. As shown in Fig 2c, a new triple peak at 3.50 ppm could be observed, which was ascribed to the α protons of oxidized Te atoms. The chemical shift of the α protons of Te atoms shifted from 2.50 ppm to 3.50 ppm after oxidation. To verify whether it was caused by the oxidation of tellurium atoms, the oxidized model compound m-Te7COOH were transfer to chloroform-d. As shown in the Fig S14, new peaks appeared at 3.49 ppm after oxidation by 1 equivalent H_2O_2. While with increasing amount of H_2O_2, another triple peak appeared at 3.53 ppm (see supporting information Fig S14). The two new peak groups should be ascribed to the two different oxidized states. Peaks for the VI state could totally shift into the lower field if the oxidant concentration increased more. We also found that if the oxidation time of the polymer sample increased to 24 h, small peaks for the VI state could appear on the ^1H NMR spectrum (Fig 2d). The phenomenon could verify the mechanism we proposed before. The micelles first swelled because of the oxidation of the tellurium-containing polymer into the IV states, and then after

one day the transformation to the irregular aggregates was caused by the further appearance of the VI oxidation states.

We also proved that the tellurium-containing polymer may function as a new platform responsive for 2 Gy γ-ray radiation. Radiation chemistry revealed that ROS could be generated if water was irradiated by ionizing radiation. Gamma-ray is one of the most widely used ionizing radiations, because it is penetrating and has strong power. We propose that if the ROS produced by γ-ray radiation could trigger the response of the smart delivery vehicles, and then the responsive morphological changes could be coupled with the radiation therapy. Upon radiation for only 2 Gy, the hydrodynamic diameter increased dramatically to more than 250 nm within 1 h. The size continued to swell a little and then reached a plateau afterwards (Fig 3 b). TEM images (Fig 3 a) could further confirm the obvious size increase of the tellurium-containing polymer micelles (about 80-200 nm). It should be highlighted that 2 Gy is exactly the dosage a patient receives one day for conventional radiation therapy, which would make it highly promising for the future clinical application. Compared with the previously reported research, the γ ray response of this tellurium-containing polymer micelle is indeed much more sensitive and therefore holds the potential to push forward the development of γ-ray-responsive delivery systems.

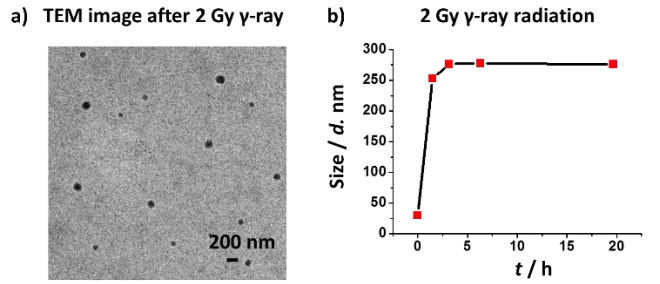


Fig. 3 γ-ray responsive behaviours of the tellurium-containing polymer PEG-PUTe-PEG micelles after 2 Gy radiation. a) TEM image of the micelles 3 h after 2 Gy radiation. b) the size change profile over time after radiation measured by DLS.

Conclusions

In summary, we have designed an ultra-sensitive ROS-responsive tellurium-containing polymer that uniquely takes advantage of the sensitive nature of tellurium element. In the presence of ultra-low concentration H_2O_2, the polymer micelles could evolve dramatically to swelled state and then to irregular aggregates, leading to potential application as platform for the combination of chemo- and radio- therapies. This approach will not only be a major advancement over ROS-responsive materials, but also push forward the development of smart systems applicable to physiological environments.

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Notes and references

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Electronic Supplementary Information (ESI) available: [detailed synthesis and characterization of the compounds; characterizations of oxidation behaviors; stability studies of the PEG-PUTe-PEG micelles]. See DOI: 10.1039/b000000x/.

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