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Reactive Oxygen Species (ROS)-Responsive Tellurium-Containing Hyperbranched Polymer

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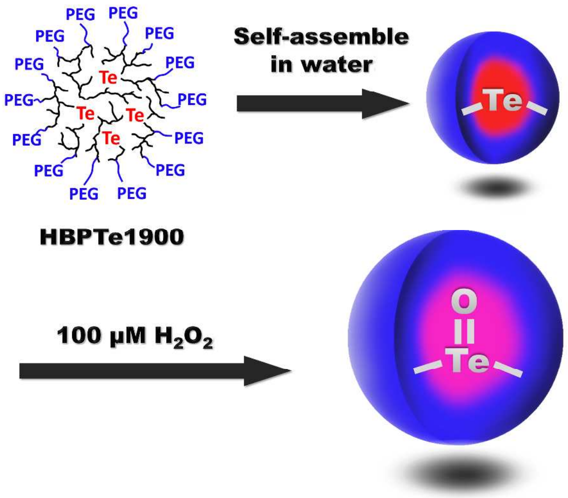
Materials responsive to reactive oxygen species (ROS) at a physiological level are of great significance in diagnostic and therapeutic applications. We have synthesized a water-soluble tellurium-containing hyperbranched polymer and demonstrated that it forms a kind of ROS-responsive aggregates. In aqueous environment, the polymer aggregates can swell under biologically relevant concentration of H_2O_2, which could be expected to find applications in fields such as elimination of excess ROS.

Reactive oxygen species (ROS) include hydrogen peroxide, peroxynitrite (ONOO$^-$), hydroxyl radical (OH$^\cdot$) and so on, which are by-products produced in biochemical reactions existing widely in living organisms. At a moderate concentration, ROS play an important role in metabolism such as cell growth, apoptosis and migration.[1] However, in regions with overproduction of ROS, the oxidative stress induces damage to cell components including DNA, lipids and proteins.[2] Thus great attention has been paid to developing materials responsive to ROS[1][3-7] for their potential applications in fields such as oxidation responsive nanocarriers[8-21], sensing[22][23] and elimination of excess ROS[24][25].

Hyperbranched polymers are macromolecules with highly branched three-dimensional topology.[26] Due to their unique chemical and physical properties such as large numbers of terminal functional groups and low solution viscosities,[26-28] they have received much attention[29-32] with potential applications including biomimetics[33][34], macroscopic molecular self-assembly[35] and coatings[36].

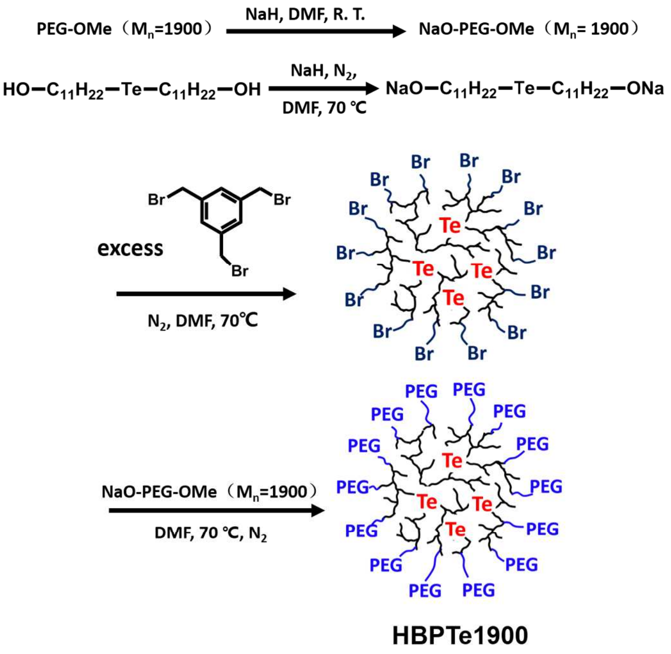
Tellurium-containing organic compounds were seldom studied, but this element gained more attention recently.[22][37-45] While selenium-containing compounds are thought to be easily oxidized by ROS[11][12][46-54], tellurium-containing compounds may possess a higher ROS sensitivity because the electronegativity of tellurium is lower than that of selenium.[55] Moreover, it is reported that tellurium-containing compounds may possess less toxicity than their selenium counterparts.[56] Exploiting these features, several tellurium-containing systems for biological applications involving redox reactions have been introduced. [22][40][57][58] However, tellurium has not been employed to construct a water-soluble hyperbranched polymer with responsiveness to ROS in a biologically relevant concentration.

This communication is aimed to combine tellurium chemistry with hyperbranched polymers for fabricating ROS responsive materials (Scheme 1). Considering the good reactivity of tellurium, we expect that tellurium-containing hyperbranched polymers may form aggregates in water, which can exhibit responsiveness to stimuli of H_2O_2 at a physiological level.



Scheme 1 Illustraion of the formation of HBPTe1900 aggregates and the volume change upon stimuli of ROS at a physiological level.

A hyperbranched tellurium-containing amphiphilic polymer was designed and synthesized. The synthetic route is described in detail (Scheme 2). Briefly, the dialkyl telluride bearing two hydroxyl groups, 11,11'-tellurobis(undecan-1-ol), was deprotonized by NaH, then polymerized with three armed 1,3,5-tris(bromomethyl)benzene to obtain a hyperbranched tellurium-containing polymer with peripheral benzyl bromide groups. Next, poly(ethylene glycol) (PEG) monomethyl ether of 1900 g mol^{-1} was reacted with the benzyl bromide groups on the periphery of the above hyperbranched polymer, thus an amphiphilic hyperbranched polymer with branches containing telluriums and shells of PEG was obtained, denoted as HBPTe1900. The number-average molar mass of HBPTe1900 was 5.0x10^5 g/mol shown by gel permeation chromatography (GPC) (Table S1). The existence of tellurium in HBPTe1900 was confirmed by X-ray photoelectron spectroscopy (XPS) (Fig. S1) and atomic emission spectroscopy (AES) (Table S2). Tellurium content by weight was around 3.7% for HBPTe1900, as calculated according to AES results (Table S2). Shorter chain of PEG-OMe such as PEG 750 was also considered, however, it only produces HBPTe750 with low solubility and low stability, as shown in Fig. S2 (supporting information).



Scheme 2 Synthetic route of HBPTe1900.

Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were employed to study if HBPTe1900 formed aggregates in water. DLS results (Fig. 1a) showed that HBPTe1900 aggregates possessed an average hydrodynamic diameter of about 280 nm in aqueous solution. Because tellurium possesses high atomic number, tellurium-containing materials could be observed by TEM without staining. From TEM experiments (Fig. 1b), it was observed that HBPTe1900 aggregates possessed an average diameter of 28±7 nm. The difference of the data from DLS and TEM could result from that, on the one hand, only the tellurium-containing branched parts were observed for an unstained TEM sample; on the other hand, TEM measured the aggregates in a dry and shrunken condition, while DLS measured in a wet condition which caused the aggregates to swell and endowed them with outside solvent layers.[59][60]

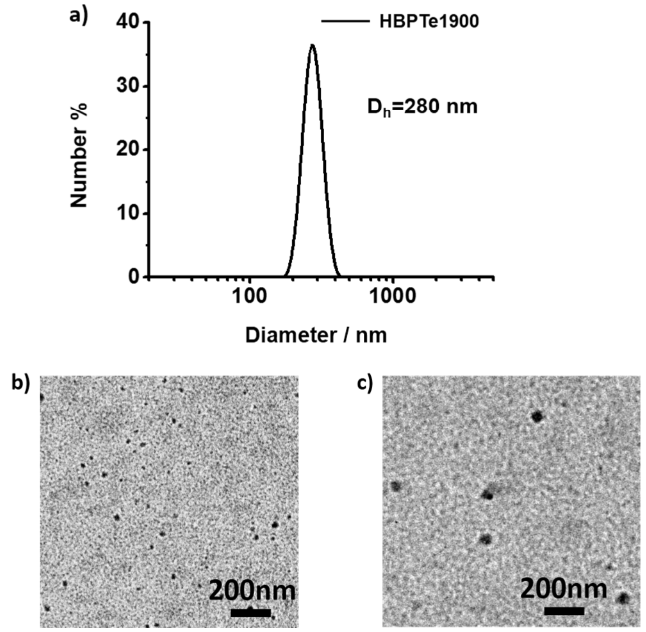


Fig. 1 DLS plots of HBPTe1900 aggregates (a). TEM images of HBPTe1900 aggregates before (b) and after (c) oxidation by 100 μM H_2O_2 for 24 h, the samples were all unstained.

We wondered whether the aggregates of tellurium-containing hyperbranched polymers were responsive to low concentration of H_2O_2. To this end, H_2O_2 was added to the solution of HBPTe1900 aggregates, reached a concentration of 100 μM. DLS results (Fig. 2) showed that after H_2O_2 was added, HBPTe1900 aggregates swelled gradually and reached a diameter of 5 times as large as the original ones in 24 h. TEM observation (Fig.1b and 1c) also showed that, 24 h after H_2O_2 was added, the average diameter of HBPTe1900 aggregates increased to 66±5 nm, which was about 2.4 times as large as the original ones. It is reported that the concentration of ROS in inflammation area is from 50 μM to 100 μM. [15] Our experiments suggest that the aggregates of tellurium-containing hyperbranched polymers may be a proper candidate to be responsive to ROS of such a low concentration.

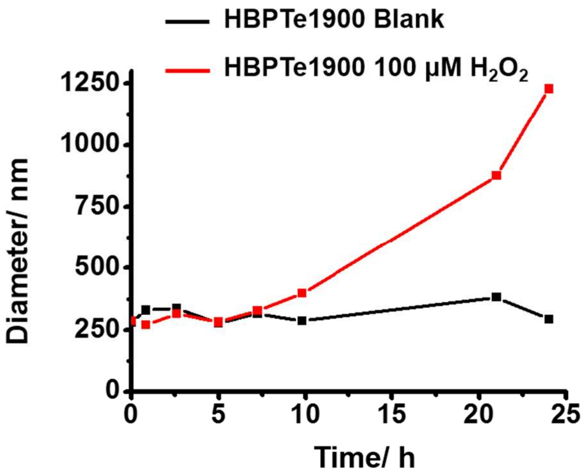


Fig. 2 Change of the diameter of HBPTe1900 aggregates upon 100 μM H_2O_2 over time, measured by DLS.

We assumed that the swelling of HBPTe1900 aggregates could result from the change of the chemical environment of tellurium atoms in the polymer. To confirm this assumption, XPS and Fourier transform infrared spectroscopy (FTIR) were employed to study it. In the XPS results (Fig. 3), 583.6 eV and 573.2 eV were the binding energy of Te $3d_{3/2}$ and Te $3d_{5/2}$ orbitals of unoxidized tellurium, as confirmed by a model compound 11,11'-tellurobis(undecan-1-ol) (Fig. S3), while 586.4 eV and 576.1 eV should be corresponding to Te $3d_{3/2}$ and Te $3d_{5/2}$ orbitals of oxidized tellurium. Thus, the results indicated that 24 h after H_2O_2 was added, nearly all tellurium atoms were oxidized. FTIR (Fig. S4) showed that the peaks at 532 cm^{-1} and 507 cm^{-1} on the spectrogram of unoxidized HBPTe1900, which should be attributed to C-Te stretching vibration, disappeared 24 h after addition of H_2O_2. This can be caused by the chemical environment change of tellurium after oxidation. After the oxidation, oxygen atoms could be connected to tellurium atoms, resulting in an increase of local hydrophilicity, which is responsible for swelling of HBPTe1900 aggregates.

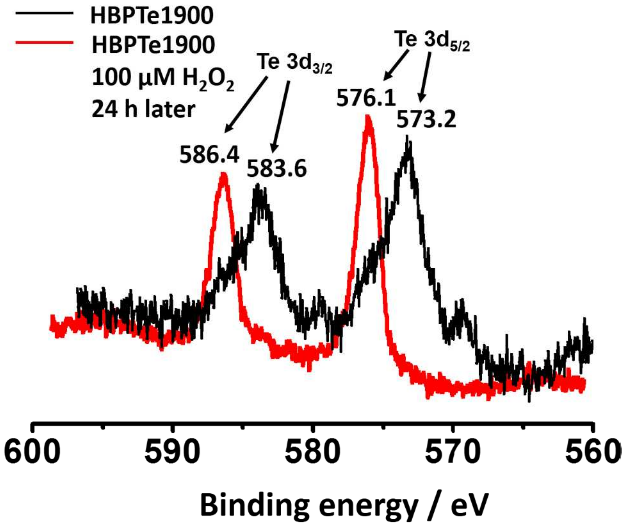


Fig. 3 XPS analysis for the binding energy of Te $3d_{3/2}$ and Te $3d_{5/2}$ orbitals of HBPTe1900 before and after oxidation by 100 μM H_2O_2 for 24 h.

To study if the benzyl groups in HBPTe1900 were oxidized by H_2O_2, we employed benzene-1,3,5-triyltrimethanol (BTMOH) as a model compound, which possesses 3 benzyl groups. As shown in Fig. S5 (supporting information), BTMOH did not change with addition of excess H_2O_2 in 18 h. This indicates that the benzyl groups in the HBPTe1900 were also not oxidized during the experiment.

A question aroused whether the swollen state of HBPTe1900 aggregates could be reversed under reduction. To answer this question, Vitamin C （VC） was chosen as the reductant for its biogenic nature and mild reaction condition. After the aggregates swelled about 5 times under the stimuli of 100 μM H_2O_2, VC was added and reached the same concentration as H_2O_2. DLS results (Fig. S6) showed that, after VC was added, the aggregates shrunk by 4 times during 25 h. This result indicated that HBPTe1900 aggregates possess an oxidation-reduction reversibility.

In order to study the influence of the cross-linking degree on the ROS responsive behavior, we designed and synthesized another hyperbranched tellurium-containing amphiphilic polymer which possessed a higher cross-linking degree. The synthetic route was similar to that of HBPTe1900, and the only difference was that we extended the time for reaction of sodium 11,11'-tellurobis(undecan-1-ol) with 1,3,5-tris(bromomethyl)benzene to obtain a higher cross-linking degree. Thus, this resulting polymer was denoted as HC-HBPTe1900. The number-average molar mass of HC-HBPTe1900 was 9.4x10^5 g/mol shown by GPC (Table S1). From AES (Table S2), the existence of tellurium was confirmed, the contents by weight was around 3.2%. The response behaviour of HC-HBPTe1900 aggregates to a biologically relevant level of H_2O_2 was studied and compared with that of HBPTe1900 aggregates. Before adding H_2O_2, the mean diameter of HC-HBPTe1900 aggregates was about 290 nm measured by DLS (Fig. 4a) and 28±8 nm by TEM (Fig. 4b). The reason of the difference between the results from DLS and TEM was described in previous paragraphs. Then we added H_2O_2 to the solution of HC-HBPTe1900 aggregates, making the concentration of H_2O_2 100 μM. DLS results (Fig. 4d) and TEM (Fig. 4c) results showed that after H_2O_2 was added, the size of HC-HBPTe1900 aggregates remained the same in 24 h, while HBPTe1900 aggregates

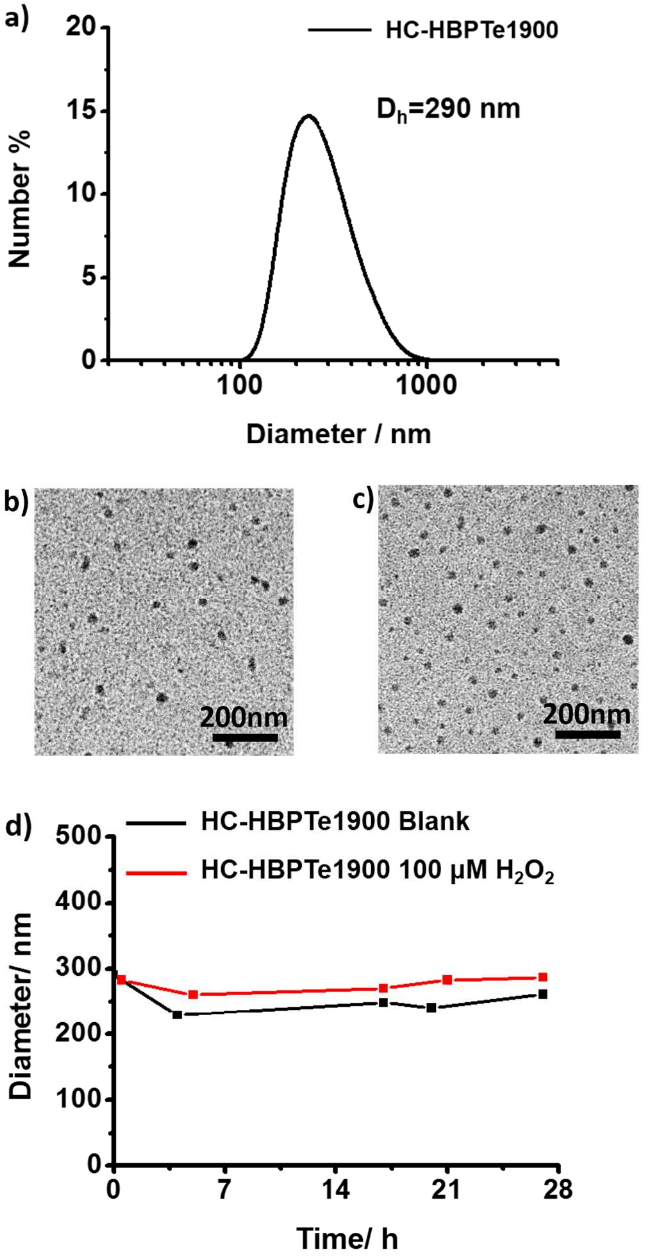


Fig. 4 DLS plots of HC-HBPTe1900 aggregates (a). TEM images of HC-HBPTe1900 before (b) and 24 h after (c) the addition of 100 μM H_2O_2. The samples were all unstained. The diameter of HC-HBPTe1900 aggregates didn't change upon 100 μM H_2O_2 over time, measured by DLS (d).

swelled (Fig. 1b, 1c and 2). Therefore, ROS responsive behaviour can be influenced by cross-linking degree of the tellurium-containing hyperbranched polymer.

We employed XPS measurements to study the mechanism of the difference between behaviors of HBPTe1900 aggregates and HC-HBPTe1900 aggregates under the stimuli of the biologically relevant level of H_2O_2. In the XPS results (Fig. S7a, S7b), as identified in the previous paragraph, 583.7 eV and 573.2 eV were the binding energy of Te $3d_{3/2}$ and Te $3d_{5/2}$ orbitals of unoxidized tellurium, while the peaks around 586.0 eV and 576.0 eV were corresponding to Te $3d_{3/2}$ and Te $3d_{5/2}$ orbitals of oxidized tellurium. We calculated the integral area of each peak, the results indicated that 24 h after H_2O_2 was added, the percentage of oxidized tellurium atoms in HC-HBPTe1900 was much lower than that in HBPTe1900, resulting in smaller change of hydrophilicity, and then no responsiveness to ROS. This phenomenon could result from that the higher cross-linking degree of HC-HBPTe1900 allowed less H_2O_2 to get into and oxidize the branched parts of aggregates. Moreover, the higher cross-linking degree could help to fix the structure of the polymer mechanically to a certain extent. The two factors are effective jointly which are responsible for the fixed size of the aggregates formed by HC-HBPTe1900 before and after oxidation.

In summary, we have designed and synthesized an ROS responsive tellurium-containing hyperbranched polymer. The aggregates formed by tellurium-containing hyperbranched polymer can display responsiveness to the stimuli of biologically relevant concentration of H_2O_2. This study may enrich the field of hyperbranched polymers and provide a new kind of ROS responsive materials. This kind of material could find application in site-specific elimination of excess ROS in living organisms.

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Notes and references

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