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COMMUNICATION

Highly Responsive and Rapid Hydrogen Peroxide-triggered Degradation of Polycaprolactone Nanoparticles

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We synthesized an oxidation-responsive polycaprolactone (O-PCL) bearing pendant arylboronic esters as H_2O_2-responsive motifs. H_2O_2 induces fast depolymerization of O-PCL within days. Nanoparticles formulated from O-PCL disintegrate and release payload in response to concentrations of H_2O_2 (50 μM) that are relevant to human disease.

Reactive oxygen species (ROS), a group of oxygen-containing radical and non-radical molecules, play a key role in cellular signaling pathways and redox homeostasis.[1,2] Overproduction of ROS contributes to oxidative stress that underlies the progression of many diseases including cancer and neurodegenerative diseases.[3,4] As H_2O_2 is the most abundant ROS, many polymers bearing H_2O_2-responsive motifs, such as arylboronic esters and organochalcogens, have been developed as ROS-responsive nanomaterials for drug-delivery systems and theranostics.[5–8] However, only a limited number of nanoformulations made from such polymers respond to disease relevant levels of H_2O_2 (50–100 μM),[9–13] limiting the translation of these polymers to clinical products. Therefore, it is important to develop a new polymer and nanoformulations incorporating it that show highly sensitive and fast H_2O_2-triggered degradation.

Poly-ϵ-caprolactone (PCL) is a synthetic polyester approved by the U.S. Food and Drug Administration for medical devices. Since the hydrolytic degradation of hydrophobic PCL requires years under *in vivo* conditions, PCL appears as a good candidate polymer backbone for developing drug-delivery systems with low uncontrolled drug release.[14] The Li group prepared PCL functionalized with pendant thioether and selenide groups. Nanoparticles (NPs) formulated from these chalcogen-containing polymers respond to H_2O_2 concentrations greater than 500 μM via a hydrophilicity switch mechanism.[15] On the other hand, the Jeong group reported a PCL-like polymer made by connecting oligocaprolactones via oxalate linkages. The exploitation of oxalate linkages enables faster, in the order of days, hydrolytic degradation.[16] However, sufficient H_2O_2 sensitivity remains elusive.

Intramolecular cyclization by pendant nucleophilic groups is a proven, effective approach to induce polymer disassembly.[17,18] Caging these pendant groups with stimuli-sensitive moieties further enables controlled polymer degradation upon the application of specific stimuli.[13,19–25] In addition, stimuli-responsive polyester was shown to degrade faster and more completely than the corresponding polycarbonate.[25] Herein, we incorporated pendant arylboronic ester motifs on PCL to yield a new H_2O_2-responsive polymer, O-PCL. The design of O-PCL features fast H_2O_2-triggered polyester degradation via hydrolysis of oxidized arylboronic esters, 1,6-rearrangement and intramolecular cyclization. We also demonstrated on-demand degradation and release behaviors of O-PCL NPs in the presence of biologically relevant concentrations of H_2O_2.

To construct PCL with pendant arylboronic ester groups, Baeyer–Villiger oxidation of ketone **1** was performed to give caprolactone **2**. Next, **2** was deprotected using trifluoroacetic acid (TFA) to afford the amine, which was used directly to react with activated arylboronic ester derivative **3** to yield monomer **4**. Finally, ring-opening polymerization of **4** was carried out at 150 °C using stannous octoate as catalyst and dodecanol as initiator to afford O-PCL with weight average molecular weight (M_w) of 22,700 Da and a polydispersity index (PDI) of 2.12 (Scheme 1).

The degradation behavior of O-PCL was firstly investigated by ^{1}H NMR analysis (Figure 1 and S1). To better elucidate the degradation mechanism and observe the degradation products, we treated O-PCL (4 mg/mL, corresponding to 10 mM of arylboronic ester moieties) with 2 equivalents of H_2O_2 (20 mM)

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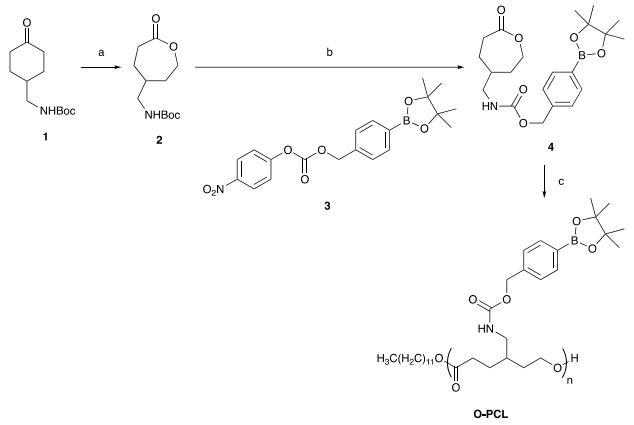

Scheme 1 Synthesis of O-PCL. (a) mCPBA, $NaHCO_3$, $CHCl_3$, rt, 3 h, 57%; (b) (i) TFA/DCM (1:1), rt, 1 h, (ii) **3**, DMAP, Et_3N, DCM, rt, 14 h, 33% over two steps; (c) $Sn(Oct)_2$, dodecanol, 150 °C, 20 h, 44%.

to completely consume arylboronic ester motifs, and we compared ^{1}H NMR spectra of O-PCL after incubation with or without H_2O_2 for 48 h. In the presence of H_2O_2, arylboronic ester motifs of O-PCL are oxidized and hydrolyzed to reveal phenol. The following 1,6-rearrangement liberates quinone methide **I**, which can be trapped by water to form 4-hydroxybenzyl alcohol **II**, and exposes pendant primary amines on the polymer backbone. These primary amines undergo intramolecular cyclization to break the polymer backbone and generate cyclization product **III**. In addition, primary amines on the side chains could be transformed to secondary amines by reacting with electrophilic quinone methide **I**.[13,22] Although the resulting pendant secondary amine could still undergo cyclization to yield product **IV**, the presence of broad peaks in the ^{1}H NMR spectrum suggests incomplete intramolecular cyclization presumably due to the steric hindrance of the bulky secondary amine. In contrast, we found no difference between the ^{1}H NMR spectra of O-PCL before and after 7 days of incubation at neutral conditions, proving the hydrolytic stability of the O-PCL backbone (Figure S2).

H_2O_2-triggered O-PCL disassembly was also examined by monitoring M_w change using gel permeation chromatography (GPC). The shift of the absorbance peak to a longer retention time indicated O-PCL degraded into shorter fragments after two-day incubation with 20 mM H_2O_2 (Figure 2a). The degradation was fast, within the first 5 h the M_w of O-PCL decreased by 60% (Figure 2b). After 48 h of incubation, oligomers were still present in the reaction mixture, which is consistent with the incomplete cyclization observed in the ^{1}H NMR analysis. Despite the incomplete backbone degradation, the observed quantity of polymer degradation has the potential to induce the disintegration of polymeric NPs for payload delivery.

After validating the response of O-PCL to H_2O_2, we formulated O-PCL NPs by single emulsion and removed emulsifiers using tangential flow filtration. Dynamic light scattering (DLS) analysis indicated successful formation of NPs with Z-average diameter of 148 nm and a PDI of 0.11 (Figure S3). Transmission electron microscopy (TEM) imaging showed the spherical morphology of NPs and confirmed size measurements

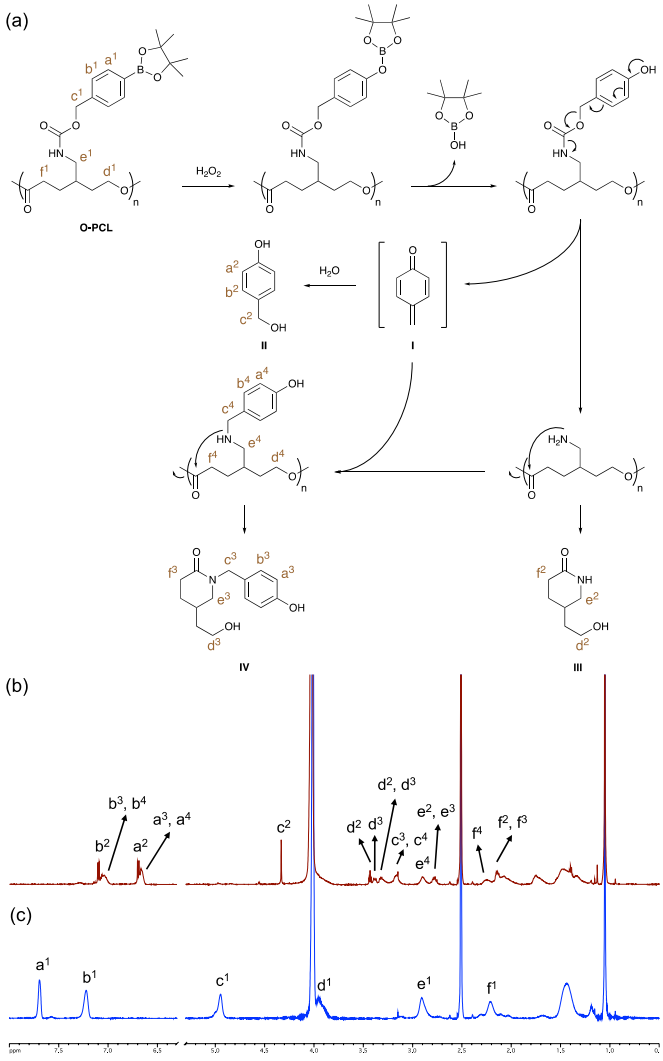

Figure 1 H_2O_2-triggered degradation of O-PCL and ^{1}H NMR spectra of O-PCL incubated in d_6-DMSO/deuterated phosphate buffer (4:1) with 20 mM H_2O_2 (red) and without H_2O_2 (blue) at 37 °C for 48 h.

obtained from DLS analysis (Figure S4). To assess the biocompatibility of O-PCL NPs, NPs were incubated with fibroblasts and macrophages. Based on the cell viability results, O-PCL NPs were well-tolerated by both fibroblasts and macrophages at concentrations up to the highest level dosed, 375 μg/mL (Figure S6).

To examine H_2O_2-triggered degradation of NPs, we incubated O-PCL NPs with varying concentrations of H_2O_2 in PBS containing 0.02% Tween 80. The count rate of each incubation sample was monitored by DLS at several time points to track the

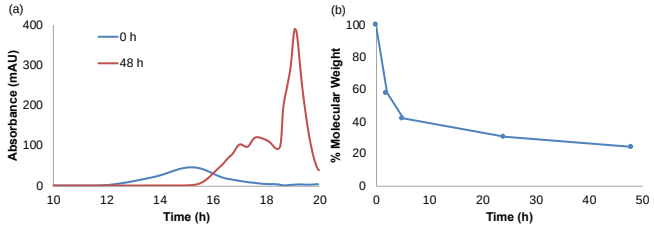

Figure 2 (a) GPC chromatogram of O-PCL prior to the addition of H_2O_2 and after incubation in DMF/phosphate buffer (4:1) with 20 mM H_2O_2 for 48 h. (b) M_w change of O-PCL in the presence of 20 mM H_2O_2 over 48 h.

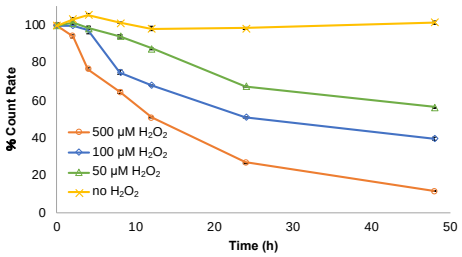

Figure 3 DLS count rate change of O-PCL NPs in the presence of different concentrations of H_2O_2.

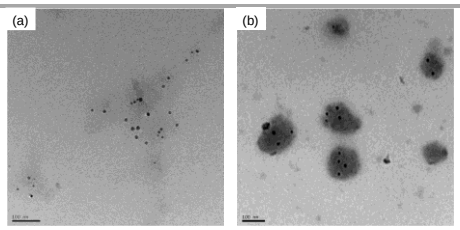

Figure 4 Representative TEM images of O-PCL NPs after incubation (a) with 50 μM H_2O_2 and (b) without H_2O_2 at pH 7.4 for 48 h (scale bars = 100 nm).

change in NP concentration over 2 days (Figure 3). In the presence of 500 μM of H_2O_2, the count rate of the O-PCL NP solution decreased by around 89% after 48 h. Notably, under conditions with 50 and 100 μM of H_2O_2, the count rates dropped to 56% and 39% of the initial value respectively after 2 days. These results clearly indicate disease relevant level of H_2O_2 can trigger the disintegration of O-PCL NPs. The particles also showed long term stability in the absence of H_2O_2. The count rate of the NP solution without H_2O_2 remained constant even after 7 days (Figure S5).

In addition to monitoring the degradation of empty NPs, we also studied H_2O_2-triggered payload release from O-PCL NPs. We encapsulated superparamagnetic iron oxide nanoparticles (SPIONs) into O-PCL NPs (Figure S3 and S4) and used TEM to track the release of SPIONs caused by NP disintegration (Figure 4). After 48 h of incubation with 50 μM H_2O_2, free SPIONs and the faint material resulting from degraded particles were observed in the incubation mixture, indicating the disintegration of O-PCL NPs in response to low, and biologically relevant, levels of H_2O_2. In contrast, intact NPs with distinct boundaries and encapsulated SPIONs were still visible without H_2O_2.

Conclusions

We designed and prepared an oxidation-responsive polyester, O-PCL. H_2O_2-triggered rapid polymer degradation was demonstrated and examined by ^{1}H NMR and GPC analyses. While NPs formulated from this polymer were stable in neutral condition without H_2O_2, they degraded and released payloads in response to H_2O_2 concentrations as low as 50 μM, a biologically relevant concentration. Such high sensitivity to H_2O_2 and general hydrolytic stability gives O-PCL the ability to discriminate between oxidative environments and makes it a promising candidate for future applications as a drug-delivery system specific to diseased tissues characterized by elevated levels of H_2O_2.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

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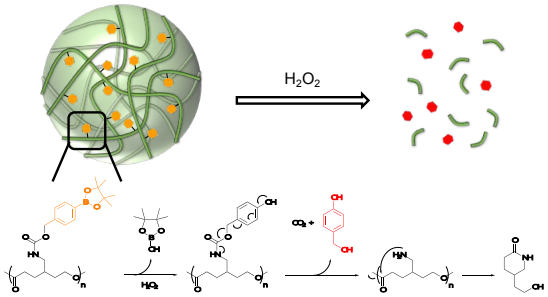

Nanoparticles formulated from fast-degrading oxidation-responsive polycaprolactone are responsive to 50 µM of H_2O_2.