

# Synthesis of Degradable Organic Nanotubes by Bottlebrush Molecular Templating

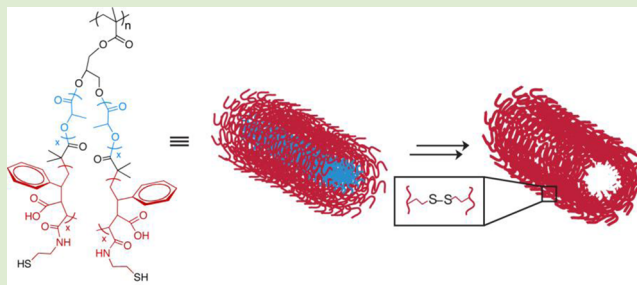
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**S** Supporting Information

**ABSTRACT:** Degradable organic nanotubes were synthesized by a single-molecule templating of core-shell bottlebrush copolymers composed of an etchable inner block (polylactide) and a functional outer block (poly(styrene-co-maleic anhydride)). The pendant mercapto groups generated along the outer block chains by reacting the anhydride groups with cysteamine were oxidized to disulfide groups acting as degradable cross-linking units in the shell layer. Subsequent hydrolytic removal of the polyester inner core provided hollow organic nanotubes held together by disulfide groups as cross-linkers. The cleavage of disulfide linkers by reaction with dithiothreitol resulted in a complete disintegration of nanotube structures into small fragments.



Hollow polymer nanoparticles have been a focus of many research efforts as versatile scaffolds for drug delivery, encapsulation, and imaging.<sup>1–3</sup> Such materials are often required to possess a number of functional attributes that render them biocompatible, biodegradable, and amenable for drug loading and release. Cylindrically shaped nanomaterials have been shown to have prolonged circulation times and potentially effective and unique cell-entry mechanisms.<sup>4,5</sup> As such, polymer or organic nanotubes that combine a favorable nanoparticle shape with easily tunable chemical compositions and accessible cavities can provide a robust platform for therapeutic and diagnostic applications. To date, there have been relatively few reports on the cylindrically shaped polymer nanomaterials capable of undergoing stimuli-responsive degradation. For example, Martin et al. reported fabrication of biodegradable nano test tubes by solid template synthesis.<sup>6</sup> Developing new and easily scalable methods for the preparation of degradable organic nanotubes as effective delivery vehicles remains a challenge.

Bottlebrush copolymers are highly branched macromolecules with a comb-like architecture.<sup>7,8</sup> Steric repulsion between densely grafted polymeric side chains causes the backbone to adopt an extended conformation.<sup>9,10</sup> These round hairbrush-like molecules provide a convenient framework for the preparation of cylindrically shaped nanomaterials. We and others have recently developed a molecular templating approach that utilizes multicomponent bottlebrush copolymers to create structurally and functionally well-defined nanotubes.<sup>11–13</sup> Thus, by using rational molecular design strategies, one can fabricate nanotubes with well-regulated interior and exterior surface chemistries.<sup>14–16</sup> Negatively charged organic

nanotubes with a hydrolyzed poly(styrene-co-maleic anhydride) (PSMA) coating were efficiently internalized by HeLa cells and exhibited low toxicities, thus, establishing their potential utility as nanostructured scaffolds for intracellular delivery of therapeutics.<sup>14</sup> Such nanocapsules, which were constructed from a rigid hydrophobic shell of cross-linked poly(4-(3-butenyl)styrene) and a reactive PSMA outer coating, allowed us to probe surface chemistry effects on nanoparticle-cell interactions but, ultimately, had limited utility in biological applications due to their nondegradability.

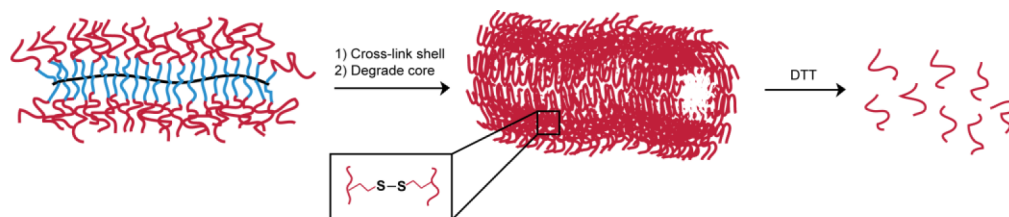
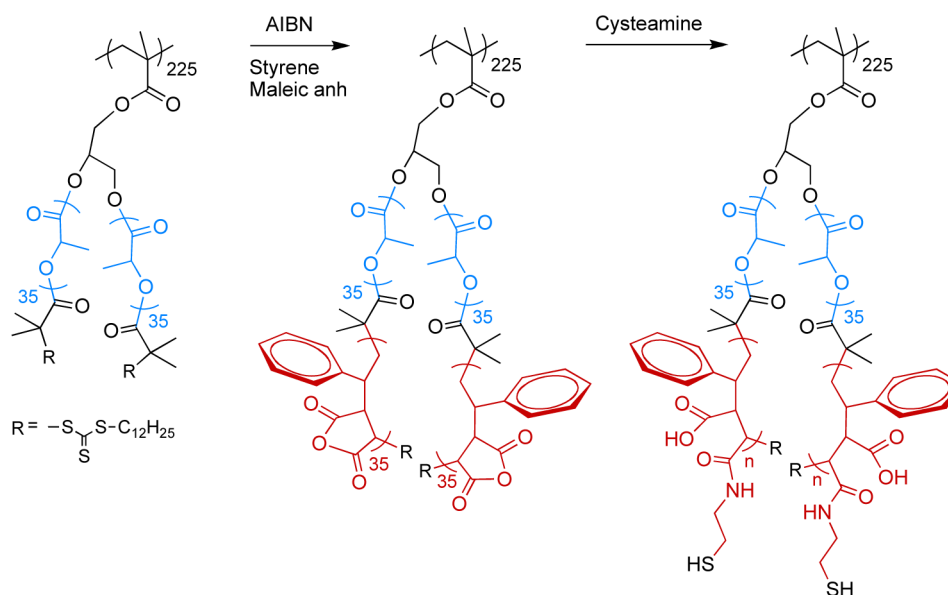
One of the intracellular triggers that can be potentially used for degradation of polymeric delivery vehicles is disulfide bond reduction inside cellular compartments such as endosomes, lysosomes, and cytosol, which is a part of the natural pathway for protein degradation.<sup>17,18</sup> The reduction is often accomplished enzymatically by thioredoxin, or chemically by glutathione, which is present in millimolar concentrations inside cells. Disulfide cross-linked polymer nanoparticles thus can be utilized as stimuli-responsive vehicles that disintegrate upon entry into cells and release their contents.<sup>19</sup> Pursuing this avenue, a number of different disulfide linked polymeric nanostructures have been reported, such as hydrogels,<sup>20–23</sup> cross-linked micelles,<sup>24–26</sup> and star polymers.<sup>27</sup> In this Communication, we disclose a new synthetic strategy for the preparation of degradable organic nanotubes that maintain their 3D structural integrity by use of disulfide linkages (Scheme 1). The nanotubes, stable in an aqueous environment, decompose

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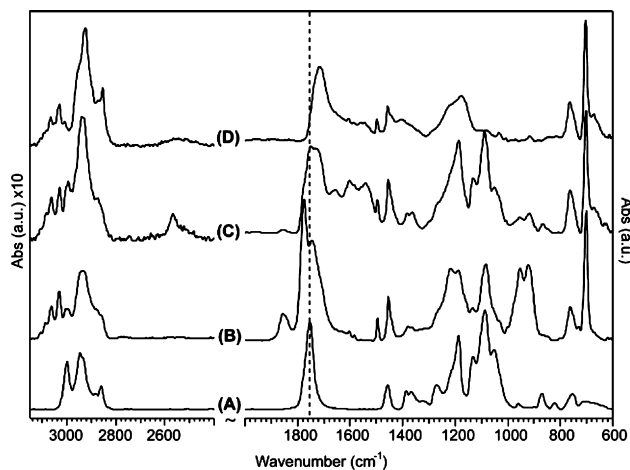
## Scheme 1. Fabrication of Degradable Nanotubes by Molecular Templating of Core-Shell Bottlebrush Copolymers

Scheme 2. Synthesis of Thiol-Functionalized Core-Shell Bottlebrush Copolymers<sup>28</sup>

into small fragments in the presence of reducing agents. More specifically, we demonstrate a dual utility of PSMA brushes, serving both as a cross-linkable component providing structural support for the preparation of hollow organic nanostructures as well as a hydrophilic negatively charged exterior rendering the resulting nanostructures water-soluble. Combined with favorable cellular interactions reported for PSMA-coated nanocapsules earlier,<sup>14</sup> this method of nanotube fabrication will provide new opportunities for the design of effective nanocarriers.

The synthetic strategy for the preparation of bottlebrush copolymer precursors is illustrated in Scheme 2. The core-shell bottlebrushes with a polylactide (PLA) core and a poly(styrene-co-maleic anhydride) shell were synthesized by a grafting-from approach<sup>29–32</sup> with the help of ring-opening and controlled radical polymerizations. First, PLA bottlebrush copolymer was synthesized as previously described from a hydrolyzed poly(glycidyl methacrylate) backbone with an average degree of polymerization of 225 and a polydispersity index (PDI) of 1.13.<sup>11</sup> The average length of PLA branches was calculated to be 35 repeat units by NMR end-group analysis by comparing the main chain PLA methine signal at 5.2 ppm to the end-group methine signal at 4.4 ppm. The PLA bottlebrush copolymers were characterized by size exclusion chromatography (SEC) with polystyrene standards to have  $M_n = 390$  kg/mol and PDI = 1.06. Subsequently, the PSMA shell layer was added by reversible addition-fragmentation chain transfer (RAFT) polymerization from the bottlebrush PLA core.<sup>33,34</sup> The formation of the PSMA layer was confirmed by FTIR spectroscopy ( $\nu = 1855$  and  $1778$   $\text{cm}^{-1}$ ) and by  $^1\text{H}$  NMR

spectroscopy ( $\delta \sim 3.5$  and  $\sim 7.3$  ppm) analyses (Figure 1 and Figure S1). On average, every PSMA branch was 35 repeat



**Figure 1.** FTIR spectra of (A) PGM-g-PLA, (B) PGM-g-(PLA-*b*-PSMA), (C) cysteamine-modified PGM-g-(PLA-*b*-PSMA) bottlebrush copolymer, and (D) the nanotubes.

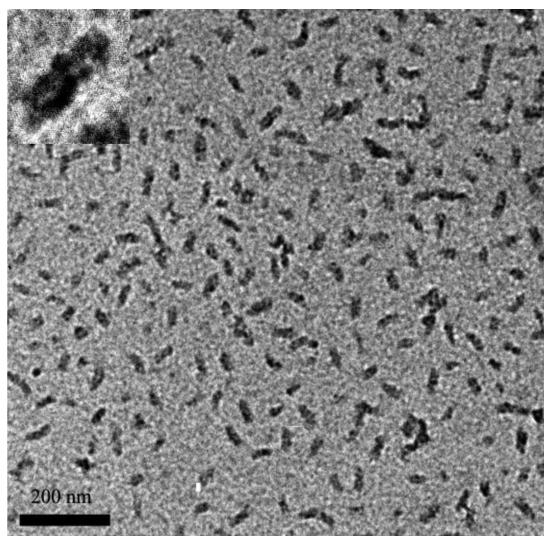
units long, which was calculated by comparing  $^1\text{H}$  NMR signal intensities of the PSMA aromatic protons at 7.3 ppm to the PLA methine protons at 5.2 ppm. SEC analysis of the copolymers exhibited a monomodal molecular weight distribution trace with  $M_n = 590$  kg/mol and PDI = 1.24 (Figure S2), which indicated efficient reinitiation and the formation of well-defined copolymers.

To fabricate degradable organic nanotubes, the mercapto groups were introduced into the shell layer of bottlebrush copolymer precursors. Oxidation of pendant thiols to disulfide linkages was used as a reversible cross-linking strategy for fixating the shell and creating rigid cylindrical polymer nanoparticles. Disulfide cross-linking units can be selectively cleaved back to thiols under mild conditions by using reducing agents, such as dithiothreitol (DTT). In this study, the pendant mercapto groups were arranged onto the outer layer of bottlebrush copolymer by reacting the anhydride groups with cysteamine. In an optimized cross-linking structure, about 30% of the maleic anhydride groups were chemically modified. The rest of the maleic anhydride groups were then hydrolyzed to provide a hydrophilic, carboxylic acid-functionalized shell layer. Such a composition not only offers the nanoparticles enough cross-linking density to maintain the cylindrical shape after the core removal but also ensures a good stability of the nanotube dispersions in an aqueous environment. FTIR spectroscopy analysis confirmed complete disappearance of cyclic anhydride groups ( $1855\text{ cm}^{-1}$ ) as well as the presence of carboxylic acid groups ( $1700$  and  $1727\text{ cm}^{-1}$ ) and amide linkages (ca.  $1650\text{ cm}^{-1}$ ; Figure 1c). In addition, a weak mercapto peak around  $2570\text{ cm}^{-1}$  was also observed (Figure 1), which further confirmed that the pendant mercapto groups were attached to the core-shell bottlebrush copolymer precursor.

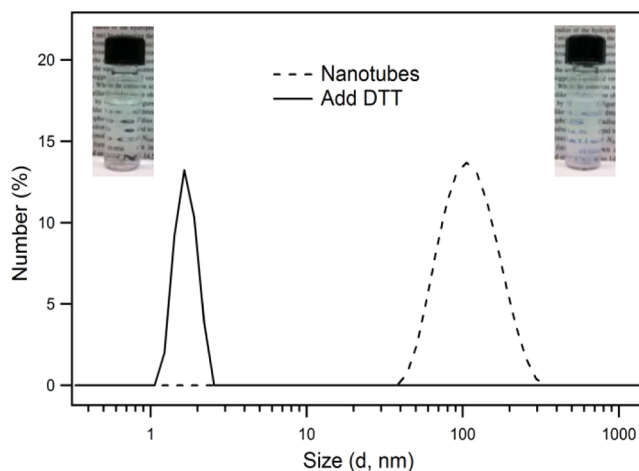
The cross-linked bottlebrushes copolymers were fabricated by treating the cysteamine modified core-shell bottlebrushes with  $\text{I}_2/\text{THF}$  catalyst system, which promoted oxidative coupling of pendant mercapto groups in the shell layer. The cross-linking was carried out in a dilute solution to avoid intermolecular coupling. FTIR analysis showed a complete disappearance of the mercapto peak after oxidation, indicating efficient dimerization of thiol groups and the formation of shell-cross-linked bottlebrush copolymers. While spectroscopically interchain and intrachain cross-linking reactions within a single bottlebrush molecule cannot be distinguished, we believe that a high density of side chains favors interchain reactions, which results in robustly cross-linked shells. Cylindrical polymer nanoparticles obtained by bottlebrush shell cross-linking were easily dispersed in water. As observed by transmission electron microscopy (TEM), the fabricated nanoparticles had an average length of 40 nm and a diameter of 15 nm (Figure S3), comparable in dimensions to those obtained from other bottlebrush copolymers with backbones and branches of similar lengths.<sup>11,15</sup>

Cross-linked polymer nanoparticles were then subjected to alkaline hydrolysis to remove the PLA core and etch a uniform pore along the length of cylindrical nanoparticles. Complete removal of PLA was confirmed by the disappearance of the characteristic PLA carbonyl stretch peak ( $1758\text{ cm}^{-1}$ ) in the FTIR spectrum (Figure 1d). The morphology and formation of tubular nanostructures was further characterized by TEM. As shown in Figure 2, cylindrical morphology was maintained during the core removal process, with tubular structures clearly observed at high magnifications. The as-prepared nanotubes had an average diameter of 17 nm and an average length of 50 nm. Dynamic light scattering (DLS) analysis in water also confirmed the formation of well-dispersed nanoparticles with an average hydrodynamic diameter of 103 nm (Figure 3 and Figure S4).

The nanotubes fabricated by the described method are held together by labile disulfide linkages and therefore are expected to disintegrate upon exposure to a reducing environment. To



**Figure 2.** Transmission electron micrograph of degradable nanotubes (the sample deposited from an aqueous solution, no staining).



**Figure 3.** DLS analysis of disulfide-cross-linked nanotubes before and after degradation in the presence of DTT. Insets show opacity of the aqueous solutions.

evaluate degradability of the prepared organic nanotubes, we used DTT as a model water-soluble reducing agent. Upon addition of DTT to the nanotube aqueous solution and subsequent stirring for 4 h, the solution changed from being lightly cloudy to completely clear. This change in solution opacity was attributed to the decomposition of large nanoparticles responsible for light scattering in the original solution. Moreover, DLS analysis revealed a significant particle size change from one hundred to a few nanometers after DTT addition (Figure 3). At the same time, the nanotube dispersions were stable for days in the absence of DTT. The results indicate that the nanotube shells are stable in an aqueous medium but are rapidly disintegrated in the presence of reducing agents, which could be attributed to the cleavage of disulfide groups responsible for maintaining structural integrity of nanotubes.

In summary, we successfully demonstrated the synthesis of new thiol functionalized bottlebrush copolymers and their transformation to degradable organic nanotubes. The bottlebrush copolymer precursors were composed of a polylactide core and a poly(styrene-co-maleic anhydride) shell. The anhydride groups in the PSMA shell layer were reacted with



cysteamine to generate pendant mercapto groups that could be subsequently oxidized to produce a cross-linked shell based on disulfide linkages. Hydrolytic removal of the PLA core from shell cross-linked bottlebrushes produced hollow organic nanotubes. The resulting nanotubes, whose structure was remarkably maintained by disulfide linkages, can be decomposed into small polymeric fragments in the presence of reducing reagent, such as DTT. Thus, we have generated a new class of biodegradable organic nanotubes by introducing a reversible bottlebrush shell cross-linking chemistry based on cleavable disulfide groups. The combination of a new cross-linking chemistry and a facile functionalization of anhydride-containing bottlebrush precursors<sup>14</sup> can provide a rapid access to a library of nanostructured vehicles tailored for the effective intracellular delivery process.

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental details, including NMR, GPC, TEM, and DLS characterization of polymers (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.

## ■ ACKNOWLEDGMENTS

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