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Modular Synthesis of Biodegradable Diblock Copolymers for Designing Functional Polymersomes

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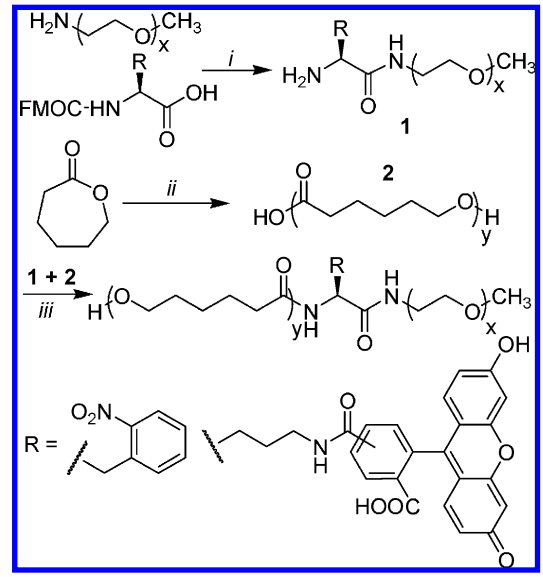
Polymeric vesicles, or polymersomes, constitute a relatively new class of materials based on the self-assembly of amphiphilic block copolymers.[1] Polymersomes offer several distinct advantages over liposomes, including increased mechanical robustness, the ability to solubilize large quantities of hydrophobic and hydrophilic molecules, and typically a complete poly(ethylene glycol) (PEG) surface functionality, offering a stealth character in vivo. Polymersomes are useful in a range of applications, including drug delivery, in vivo imaging, and use as cell mimetics.[2−6] The majority of application-based research has focused on systems using existing diblock polymers. However, new synthetic routes that introduce additional functionality may be beneficial for other applications or increasing the polymersome efficacy.[7] We present here a novel synthetic route that utilizes modular construction of biodegradable block polymers, allowing for the incorporation of a wide variety of chemical groups in the membrane. As two examples, we developed polymersomes that are susceptible to UV-induced degradation or include fluorescence directly in the hydrophobic core of the membrane.

This method for diblock synthesis was inspired by chemistry commonly used for solid-phase peptide synthesis and utilizes amino acids with any desired side groups.[8] In the first step, an FMOC-protected amino acid is conjugated to an amine-terminated PEG through an amidation reaction (Scheme 1; for complete Materials and Methods, see the Supporting Information). Following FMOC removal and purification, the functional PEG (still with an amine terminus) is coupled in excess to a carboxy-terminated poly(caprolactone) (PCL) through a second amidation reaction. Precipitation of the resulting polymer into methanol selectively precipitates the diblock copolymer, yielding the desired product.

As a first example, we incorporated photolabile 2-nitrophenylalanine (2NPA) as the amino acid joining the two blocks. Incorporation of this amino acid into the backbones of polypeptides has enabled site-specific cleavage of the peptide bond between the 2NPA and the next amino acid toward the N-terminus.[9] Initial characterization of the polymer via NMR spectroscopy confirmed the coupling of the 2NPA to PEG and subsequent coupling to the PCL (Figure S1 in the Supporting Information). To rule out the possibility that PEG−2NPA coprecipitated with the PCL but was not actually coupled, gel-permeation chromatography (GPC) was performed on the resulting diblock copolymer (Figure S2). A shift to higher molecular weights was observed for the PCL peak as a result of the PEG coupling, and a second peak corresponding to free PEG was notably absent. Exposure of the GPC sample containing a trace amount of water as a proton source to 365 nm light for 2 h induced a shift back toward the lower-molecular-weight PCL, and the evolution of a second peak corresponding to liberated PEG was observed.

Scheme 1. Modular Synthesis of Diblock Copolymers[a]



[a] (*i*) 1: HBTU, methyl morpholine; 2: Piperidine. (*ii*) H_2O, $SnOct_2$. (*iii*) HBTU, methyl morpholine.

Assembly of polymersomes was accomplished through film rehydration, sonication, and extrusion to remove any aggregated material. The polymersomes were exposed to UV light for up to 6 h. A visible precipitate became apparent after ∼3 h of exposure. The high-resolution NMR spectrum of the polymersomes after exposure showed a complete disappearance of the four characteristic aromatic peaks corresponding to the 2NPA moiety (Figure S3). GPC of exposed polymersomes reconstituted in THF following lyophilization showed a steady increase in the normalized area of the PEG peak (Figure 1a). Interestingly, a shoulder corresponding to higher-molecular-weight species also appeared in the GPC chromatogram of the UV-exposed polymersomes (Figure S4). Peak deconvolution suggested that the shoulder corresponds to a species with approximately double the molecular weight of the starting polymer. Such dimerization could be due to side reactions that occur during the UV rearrangement,[9] though the exact mechanism is beyond the scope of this report.

As a demonstration of the potential utility of polymersomes that undergo UV-induced cleavage, release of encapsulated biocytin was monitored as a function of exposure time through a fluorescent capture assay (Figure 1b).[6] Release was observed over the entire 6 h exposure, while negligible release was observed for controls

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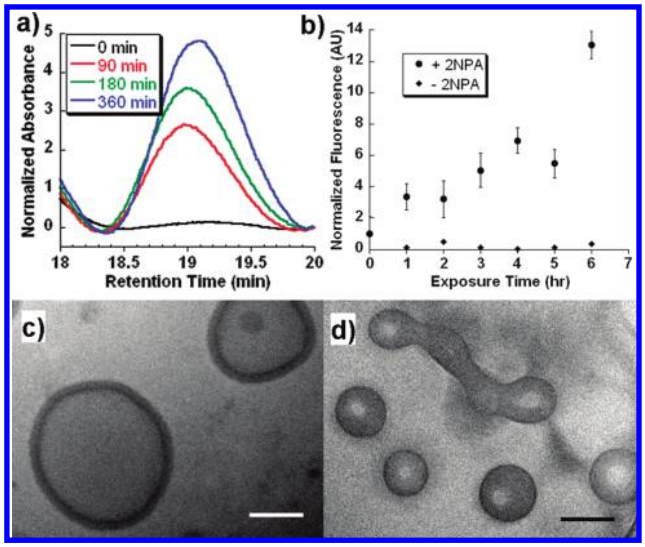

Figure 1. (a) GPC chromatograms of the eluted PEG peak shown with increasing UV exposure times. (b) Release of encapsulated biocytin. (c, d) Cryo-TEM images of 2NPA polymersomes (c) before and (d) after 6 h of UV exposure. Scale bars = 100 nm.

lacking the 2NPA group. To explore the possible mechanism of release, the change in the size of the polymersomes was monitored by dynamic light scattering (DLS). Intriguingly, only a slight decrease in the size of the species present was observed (Figure S5). To explore this phenomenon further, polymersomes were observed using cryogenic transmission electron microscopy (cryo-TEM) before and after UV exposure (Figure 1c,d and Figure S6). The images suggest that the decrease in size is caused by a thickening and gradual collapse of the membrane coupled with the expulsion of aqueous contents, which were not observed for control polymer lacking the 2NPA group (Figures S6 and S7). After sufficient PEG liberation, the remaining PCL aggregates (i.e., Figure 1d, top) and precipitates. The lower polymer density relative to solution could explain the lack of corroboration via DLS; photolyzed samples float out of the DLS light path. While polymersome systems that are responsive to UV light have been reported[6,10] and cleavable polymers have been applied to other self-assembled systems,[11] to the best of our knowledge, this is the first example of a polymersome for which UV-induced release is effected by chemical cleavage of the polymer.

As a second example of the utility of the modular synthesis of PCL−PEG diblock copolymers, we incorporated a fluorescent amino acid (fluorescein-conjugated lysine) between the blocks. Fluorescent polymersomes have been useful as imaging agents, but in all cases, the fluorophore has been dissolved in the polymersome rather than conjugated to it.[3,12] In the present study, polymersomes assembled from blends of fluorescein-labeled polymer and regular PCL−PEG exhibited membrane-localized fluorescence, confirming conjugation of the fluorophore (and PEG) to the PCL (Figure 2a inset). The absence of a PEG peak in the GPC of the polymer further ruled out the possibility that free fluorophore-conjugated PEG was partitioned into the membrane (Figure S8). To ensure a uniform distribution of the fluorescent polymer in the polymersomes, we performed flow cytometry on polymersomes containing various amounts of the fluorescent polymer (Figure 2a). Narrow distributions of fluorescence were observed, and the peak value increased with increasing amounts of fluorescent polymer. Finally, fluorescent polymersomes were fed to immature dendritic cells for 5 h and observed following washing to remove unassociated polymersomes (Figure 2b). While negative controls exhibited no fluorescence,

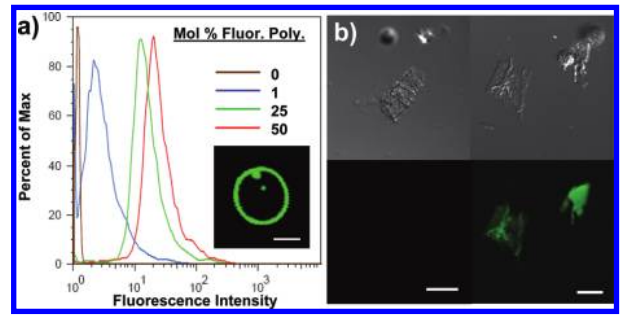

Figure 2. (a) Flow cytometry of PCL−PEG polymersomes blended with the noted amounts of fluorescent polymer. Inset: Confocal microscopy image of a 50 mol % fluorescent polymer polymersome. Scale bar = 5 μm. (b) Differential interference contrast (top) and fluorescence (bottom) microscopy images of immature dendritic cells following 5 h incubation with (left) PCL−PEG and (right) 50% fluorescent polymer polymersomes. Scale bars = 20 μm.

intense fluorescence was observed in cells incubated with fluorescent polymersomes. These results suggest that modularly synthesized fluorescent polymers could be useful for studies that require labeling or tracking of cells.

We have presented here a novel approach for synthesizing biodegradable block copolymers that allows for the incorporation of a variety of functional groups at the junctions of the two blocks. Photocleavable and fluorescent moieties are just two possible functionalities that could be incorporated. As our method relies on separate synthesis of the two blocks, this method could also be useful for applications in which systematic variation of only one block of the polymer is desired, such as in blended systems.

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Supporting Information Available: Detailed methodology and Figures S1−S8. This material is available free of charge via the Internet at http://pubs.acs.org.

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