Two-photon degradable supramolecular assemblies of linear-dendritic copolymers†

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Received (in Cambridge, UK) 5th February 2007, Accepted 28th February 2007
First published as an Advance Article on the web 15th March 2007
DOI: 10.1039/b701681f

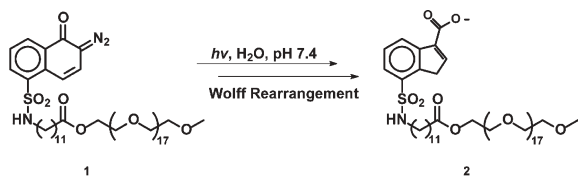
Micelles of dendritic-linear copolymers have been developed to release a payload after infrared stimulus.

Much attention has been focused on using synthetic polymers for biological applications,[1] including the delivery of anti-cancer drugs to tumors.[2] Encapsulation of drugs inside polymeric micelles can decrease the toxicity of the drug and help increase drug uptake to unhealthy cells if targeting can be achieved. However, in order to transport and then release the payload at a specific location, the micellar assembly must be capable of responding to a triggering event. In the past, pH-responsive polymeric micelles that encapsulate a payload and release it in a mildly acidic environment have been explored.[3] Another strategy is to use a spatially-directed external agent, such as light, which should allow for site-specific release in certain target areas. We have recently developed an infrared-sensitive micelle that utilizes IR irradiation as a stimulus for payload release.[4] This micelle was composed of amphiphiles that contained diazonaphthoquinone (DNQ) as the hydrophobic portion (**1**) (Scheme 1). Upon irradiation of the amphiphile with 795 nm light, the DNQ undergoes a Wolff rearrangement[5] to afford an indene carboxylic acid, thereby transforming the hydrophobic DNQ into a more hydrophilic moiety.[6] Unfortunately, this early system had a relatively high critical micelle concentration (CMC) and exhibited a rather high toxicity towards cells. Our target was to reduce both the CMC and the cytotoxicty of the amphiphile.

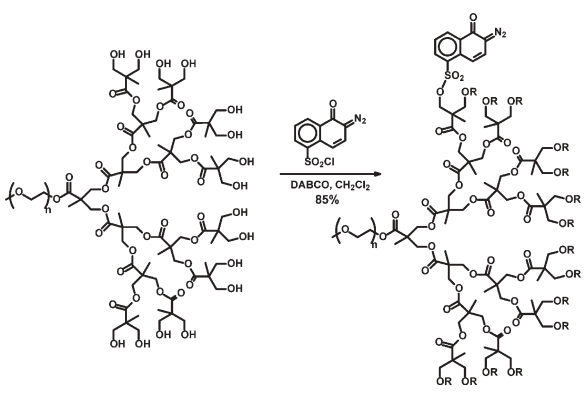
We have now developed poly(ethylene oxide) (PEO)-dendritic polyester copolymer hybrids that can self-assemble into supramolecular structures. These polymeric systems are attractive due to their facile synthesis and mulitvalency.[7] Changing the generation of the dendrimer or the length of the PEO chain leads to micelles of different sizes and stabilities. The size of the micelle can be important when trying to avoid rapid renal exclusion.[8] In addition, polymeric micelles generally have lower CMCs than small molecule micelles.[9] Finally, both PEO[10] and bis(hydroxymethyl)-propionic acid dendrimers[11] are known to be nontoxic. Here we report the synthesis and evaluation of the polymer amphiphile **4**, a PEO-10K-[G4]-polyester-OH (**3**) modified by reaction with sixteen DNQ-SO_2Cl groups at its periphery (Scheme 2). We show that **4** exhibits a better CMC and is less toxic than our previous micellar system **1** while maintaining adequate sensitivity to IR light.

As in previous work,[4] encapsulation studies with Nile Red proved convenient to determine micelle formation. In water, Nile Red exhibits a low fluorescence intensity at 660 nm due to excimer formation. When placed in a more hydrophobic environment, the fluorescence of the dye is blue shifted and the fluorescence intensity is markedly higher. When **4** is placed in an aqueous medium, the DNQ-polyester dendron acts as the hydrophobic portion of the amphiphile while the PEG acts as the hydrophilic portion. As expected, the amphiphiles self-assemble into micelles with the hydrophobic portion residing in the core. This produces a hydrophobic microenvironment inside the micelle into which Nile Red can enter. The CMC of the micelle from **4** can be determined by tracking the fluorescence intensity of Nile Red as a function of concentration of **4**. Fig. 1 shows that the relationship between the fluorescence intensity of Nile Red and the concentration of amphiphile **4** is non-linear and the observed inflection point corresponds to a CMC of 12 μg mL^{-1}. At concentrations lower then the CMC, a decrease in fluorescence intensity is observed due to Nile Red excimer formation.

After demonstrating that the polymeric micelle can successfully encapsulate a payload into a micellar assembly, we subjected these



Scheme 1 Original linear system (**1**) undergoing Wolff rearrangement upon IR laser irradiation at 795 nm.



Scheme 2 Synthesis of the PEO-dendritic DNQ (**4**).

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† Electronic supplementary information (ESI) available: General procedures and materials, syntheses, critical micelle concentration studies, one- and two-photon kinetic studies, dynamic light scattering and cytotoxicity testing. See DOI: 10.1039/b701681f

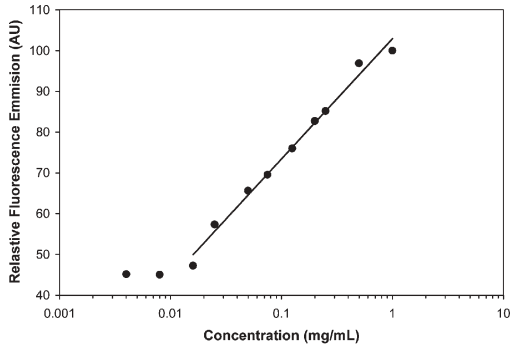

Fig. 1 Fluorescence intensity of Nile Red (λ_{ex} = 550 nm) at 642 nm *vs.* log conc. (mg mL^{-1}) of **4**. Line indicates linear fit of data at concentrations greater than 0.01 mg mL^{-1}.

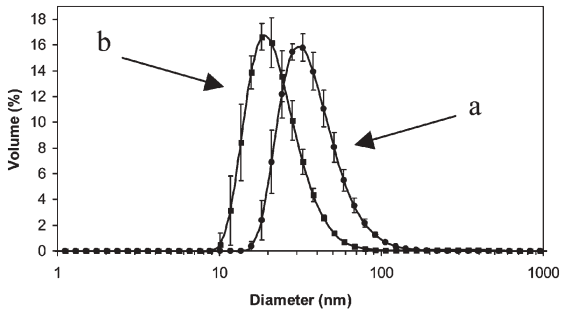

Fig. 2 DLS results before (a) and after (b) 3 min irradiation of **4** using 355 nm light.

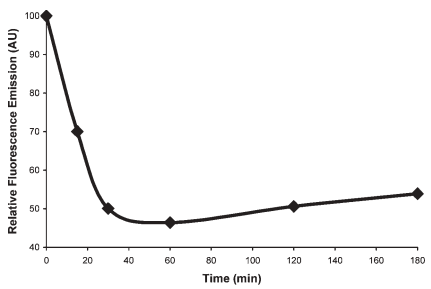

Fig. 3 Fluorescence intensity of Nile Red in **4** after irradiation with infrared laser light at 795 nm.

assemblies to irradiation with 355 nm UV light. As expected, the DNQ moieties undergo the Wolff rearrangement under UV irradiation and the Nile Red fluorescence decreases as irradiation time increases since the micellar assemblies break down (see ESI†). A similar trend can be observed by monitoring the reduction using dynamic light scattering (DLS). Before UV irradiation the average size of the micelles is *ca.* 40 nm (Fig. 2). After 3 min of UV irradiation this value falls to *ca.* 20 nm. A similar size range was found for the unimer in chloroform (see ESI†).

Next, a 0.5 mg mL^{-1} sample of **4** with encapsulated Nile Red was irradiated with a Ti-sapphire pulsed laser at 795 nm (Fig. 3). After 30 min, the fluorescence emission decreased to less than half of its original intensity. Control experiments revealed that no photobleaching of Nile Red occurs with up to 3 h of exposure. We

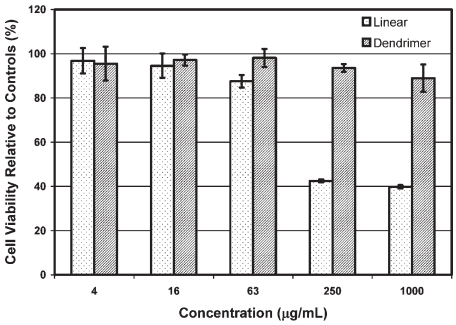

Fig. 4 Cell toxicity results of linear system **1** (light) and dendritic system **4** (darker).

also tested a sample of **4** below the CMC, which resulted in a constant low fluorescence intensity.

In vitro cell viability studies were performed in order to compare the cytotoxicity of **4** to our original linear system **1**. The amphiphiles were incubated at varying concentrations with 293T HEK cells for a period of 48 h, and cell viabilities were evaluated using a MTS assay. As expected, the linear system showed significant toxicity at higher concentrations while the dendritic construct showed little cytotoxicity even at 1 mg mL^{-1} (Fig. 4).

An IR-sensitive PEO-dendritic polyester copolymer was synthesized and evaluated for its potential as a polymeric micellar system for the IR triggered delivery of a hydrophobic payload. This system exhibited a CMC of 12 μg mL^{-1} and showed little cytotoxicity at concentrations as high as 1 mg mL^{-1}. Investigations are underway on the potential use of these and analogous polymeric moieties for the two-photon IR triggered delivery of therapeutic agents and other externally triggered processes.

We thank the NIH (HL080729) and the US Department of Energy-BES (DE-AC03-76SF00098) for support of this research.

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