NOVEL POLYMERIC MATERIALS FOR LIGHT –TRIGGERED RELEASE

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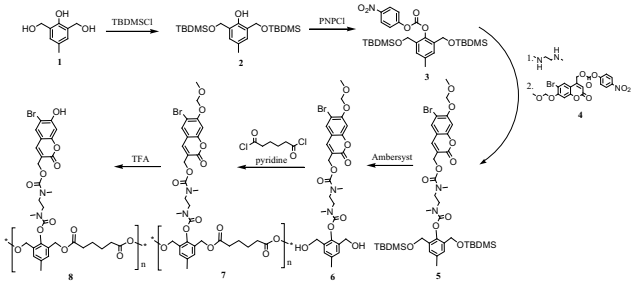
Introduction

Polymeric materials that can be remotely disassembled in a controlled fashion upon an external stimulus, but otherwise are stable under physiological conditions are of great interest for the medical and pharmaceutical fields.[1] Various internal and external stimuli, such as pH[2], specific enzymes[3], temperature[4-6], ultrasound[7,8] and light[9] are being explored. Optical stimulus is especially attractive as it can be remotely applied for a short period of time with high spatial and temporal precision. Near-infrared (NIR) light can penetrate deeper into tissue and has many *in vivo* applications.[10] Despite these advantages, there is a dearth of biomaterials that can efficiently respond to light. For practical applications, the sensitivity of light-triggered systems needs to be improved.[11,12] To increase the efficiency of response we designed a polymer with self-immolative monomers.[13-16] We have previously reported a polymer system based on such self-immolative quinone-methide units containing 4,5-dimethoxy-2-nitrobenzyl triggering group.[17] However, nitrobenzyl group has a very low two-photon uncaging cross-section (0.01 GM at 740 nm).[18] Furthermore, the byproduct of 4,5-dimethoxy-2-nitrobenzyl group cleavage, 4,5-dimethoxy-2-nitrozobensaldehyde, is toxic.[19] Herein we describe a new degradable polymer with NIR photolabile 6-bromo-7-hydrohycoumarin as the triggering group. 6-Bromo-7-hydrohycoumarin is widely used in biological systems as it has a two orders of magnitude higher two-photon uncaging cross-section (0.9 GM at 740 nm) and no toxic byproducts are generated upon its cleavage.[11]

Results and Discussion

Polymer synthesis. The monomer synthesis was modified from our previously published procedure (**Scheme 1**).[17] Commercially available compound **1** was protected with TBDMSCl to yield compound **2**, which was activated with PNPCl to form reactive carbonate **3**. Compound **3** was reacted with excess of N,N-dimethylethylene diamine in DCM. Upon completion of the reaction the solvent and excess diamine were removed, the intermediate was redissolved in DMF and reacted with 1 equivalent of **4** to yield compound **5**. Compound **4** was synthesized according to the reported procedure.[11] TBDMS groups were removed with Amberlyst-15 to afford monomer **6**. Copolymerization of **6** with adipoyl chloride yielded polymer **7**, which was deprotected with TFA to produce the desired polymer **8**. The low molecular weight oligomers were removed by repeated precipitation of the polymer with cold ethanol. The molecular weight of **3** was determined to be 60kDa relative to polystyrene standards.



Scheme 1. Synthesis of the light-sensitive self-immolative polymer.

Polymer degradation. Degradation of polymer **8** was monitored by GPC in a mixture of acetonitrile and water (1:1). The solution was irradiated with 350 nm light for 15 min and the degradation of polymer **8** was evident from a shift in the polymer GPC trace towards longer elution time (**Figure 1**).

Figure 2 shows the ^{1}H NMR spectrum of polymer **8** in DMSO-d_6/D_2O (6/1) before and after 15 min irradiation with 350nm light and incubation at 37˚C for 2 days. The spectrum shows cleavage of the triggering group and polymer backbone degradation. Thus, the signal at 5.2 ppm corresponding to the 7-protons of the coumarin attached to the polymer backbone (labeled as **d** on **Figure 2A**) is significantly decreased after irradiation. The new peaks corresponding to the degradation products, namely, 2,6-bis(hydroxymethyl)-*p*-cresol and 1,3-dimethyl-2-imidazolidinone, appear at 1.52, 2.01, 2.61 and 6.26 ppm (**Figure 2B**).

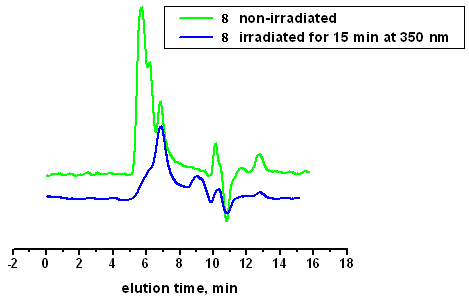


Figure 1. GPC traces of polymer **8** before and after UV irradiation

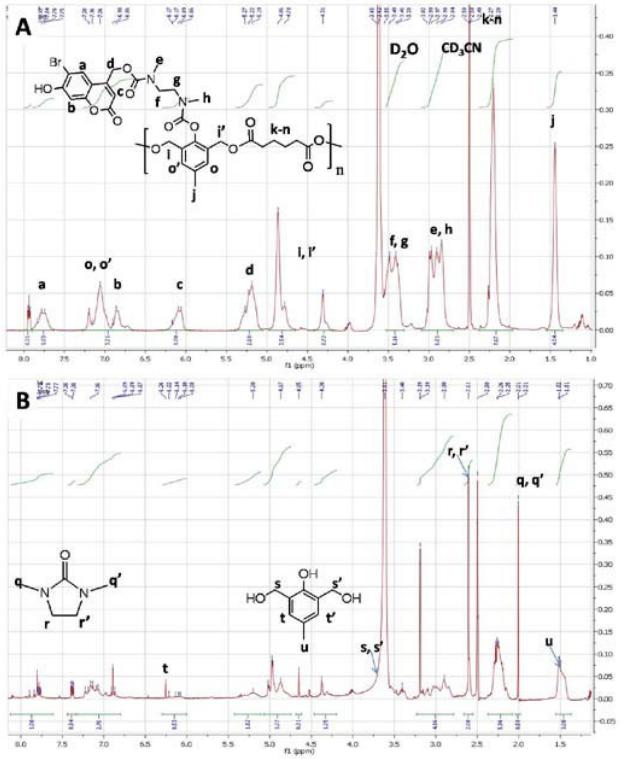


Figure 2. ^{1}H NMR spectrum of polymer **8** in DMSO-d_6/D_2O (6/1) before irradiation (A) and after irradiation with 350nm light and incubation at 37˚C for 2 days (B).

Light-triggered release. In order to demonstrate the potential of the new polymeric material for controlled light-triggered release, nanoparticles encapsulating the small hydrophobic molecule Nile Red were formulated by

the single emulsion method. The Z-average diameter of the nanoparticles was 136 nm and PDI = 0.157, as determined by dynamic light scattering (DLS).

Release of the Nile Red payload upon irradiation was observed by fluorescence spectroscopy. Nanoparticles were redispersed in PBS pH 7.4 and the fluorescence intensity of the suspension was recorded. After irradiation with 350 nm light for 2 min, the fluorescence intensity dropped by 50%, indicating release of the dye from the nanoparticles into a more polar medium (Figure 4).[20,21] Next round of irradiation (2min) produced a further decrease in fluorescence intensity of Nile Red to 20%. Prolonged irradiation did not produce any significant decrease in fluorescence signal. On the other hand, a suspension of nanoparticles that was not irradiated exhibited stable fluorescence intensity.

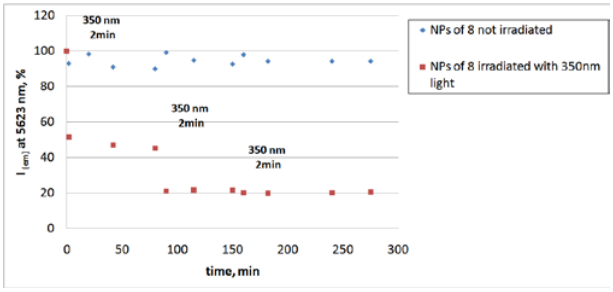


Figure 2. Release of Nile Red from nanoparticles of **8** monitored by fluorescence intensity at 623 nm.

Conclusions

In conclusion, we have developed a new light-sensitive polymer incorporating the 6-bromo-7-hydrohycoumarin triggering group. Nanoparticles formulated from this polymer are capable of controlled triggered degradation and release of small hydrophobic molecules upon exposure to UV light. Current efforts are exploring a possibility of triggered response upon irradiation with NIR light. Cell viability studies are underway.

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