Designing Responsive Materials with Chemically Amplified Sensitivity to NIR light

Nadezda Fomina, * Cathryn L. McFearin, * Marleen Sermsakdi, * Jose Morachis* Adah Almutairi.*,**

*Skaggs School Pharmacy and Pharmaceutical Sciences, **Department of NanoEngineering, Materials Science and Engineering and Biomedical Sciences Programs, University of California at San Diego, La Jolla, California 92093

ABSTRACT

We describe new UV- and Near-Infrared (NIR) light degradable polymers containing multiple light-sensitive triggering groups along the backbone and incorporating quinone-methide self-immolative moiety. NIR light can penetrate through tissues to an appreciable depth without scattering or damaging it. Thus, it may be used for targeted delivery of therapeutics and/or imaging agents in vivo. Chosing an appropriate triggering group alows disassembly of the materials in response to low power, biologically benighn NIR irradiation. It has been generally difficult to apply NIR light in vivo due to the limited number of photons that can reach a material to initiate a measurable response. We designed amplified response systems to overcome this limitation. Their design relies on a multiple cyclization and rearrangement reactions leading to unraveling of the entire material in response to NIR light.

Keywords: Near-Infrared, light, degradable, self-immolative

1 INTRODUCTION

Smart polymeric materials have become one of the main focuses in biomedical materials research . These materials respond to subtle changes in their environmental in a specific way, which renders them useful for tissue engineering[1], implants[2] and wound healing[3], drug delivery[4] and biosensors[4-5] applications. Both internal (pH[6], specific enzymes[7], temperature[8] and external (ultrasound[9-10], magnetic field[11-12] and light[13]) triggers are being explored. Optical stimulus provides high spatial and temporal precision of exposure and is, therefore, especially attractive. A large number of light-degradable materials (micelles, polymeric nanoparticles, and bulk hydrogels) have been reported recently[13]. However, most of the materials reported respond to NIR light by undergoing a hydrophobicity switch and the photodegradation products are high molecular weight linear or crosslinked polymer fragments that may be difficult to clear from the body. Additionally, most of the reported light-degradable materials respond efficiently to UV irradiation. Near infrared (NIR) light can penetrate up to 10 cm deep into tissue[14] with less damage and absorption or scattering and is more desirable for in vivo applications[15-17]. Despite these advantages, only a handful of organic materials reported to date can respond to high power NIR light due to the inefficient two photon absorption process. None are able to respond to low power NIR light which is important to biological applications because it causes less photodamage to tissues.[18] For in vivo applications, it would be more advantageous to have a material that degrades into small fragments upon light exposure, which can then be easily excreted, with less long term risks. Therefore, we designed a linear synthetic polymer with multiple pendant light-sensitive triggering groups in such a way that once these groups are cleaved, a cascade of cyclization and rearrangement reactions is triggered, leading to backbone degradation. One light degradable polymer utilizes a commercially available o-nitrobenzyl (ONB) triggering group. ONB groups are widely used in synthetic chemistry as protecting groups for alcohols and amines, which can be readily removed with UV light. They were also shown to photolyze upon NIR irradiation via two-photon excitation, although their two-photon uncaging cross-sections (a quantitative measure of the efficiency of a molecule to simultaneously absorb two photons of light and convert that energy into a chemical reaction) are low (0.1 GM at 750 nm) [19]. Another well-known photocleavable group is 4-bromo7-hydroxycoumarin (Bhc), which has an order of magnitude higher two-photon uncaging cross-section (1 GM at 740 nm) [20]. Introduction of this triggering group drastically increases the sensitivity of the material to both UV and NIR light.
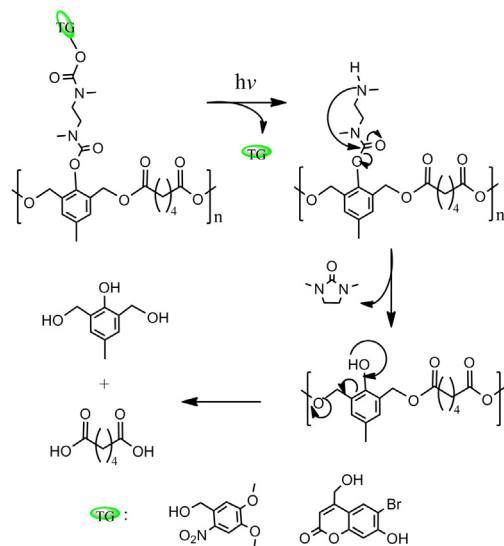
2 RESULTS AND DISCUSSIONS

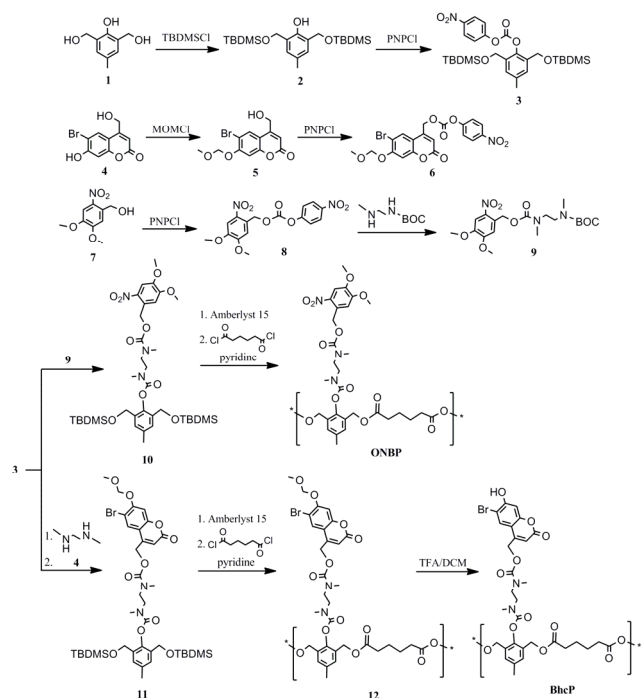### 2.1 Synthesis of Light-sensitive Polymers

The synthesis of ONBP and BhcP is described in Scheme 1. We started with commercially available 2,6-bis-(hydroxymethyl)-p-cresol, 1, and selectively protected the benzylic alcohols with TBDMSCl in 87.5% yield. Activated carbonate 3 was obtained in 85% yield by reacting compound 2 with PNPCl in the presence of DMAP and Et$_3$N in DCM. To instal ONB triggering group, commertially available alcohol 7 was activated by reacting

it with PNPCl (83% yield) and coupled with mono-BOC protected diamine linker to afford compound 9 in 95% yeild. Reacting 3 with 9 afforded compound 10 (56% yield), which was deprotected with Amberlyst-15 (87% yield) and polymerized with adipoyl chloride in the presence of pyridine, affording polymer ONBP. In order to install the 7-hydroxy-4-bromocoumarin triggering group, N,N,-dimethylethylene diamine was reacted with compound 3 at a stoichiometric ratio of 3 to 1 to achieve conversion of only one amino group of the diamine into a carbamate. Excess N,N,-dimethylethylene diamine was removed and the coumarin derivative 6 was added into the reaction mixture to obtain compound 11 in 51% yield. The TBDMS protecting groups were removed with Amberlyst-15 (74% yield) and the monomer was copolymerized with adipoyl chloride to afford polymer 12. Finally, the MOM protective groups were removed in DCM/TFA solution to afford the final polymer, BhcP. Low molecular weight oligomers were removed from polymers ONBP and BhcP by precipitation into ice-cold MeOH. The molecular weights (Mw) of ONBP and BhcP were determined by GPC to be 65,000 (PDI = 1.54) and 31,500 Da (PDI = 1.09), respectively, relative to PS standards.

Scheme 1. Synthesis of ONBP and BhcP.

2.2 UV and NIR Light Induced Degradation of Polymers

Scheme 2 shows the mechanism of degradation of light-sensitive polymers containing a quinone-methide self-immolative moiety [21]. The degradation starts when a triggering group is cleaved upon irradiation with either UV or NIR light, releasing an amino group. N,N-Dimethylethylene diamine linker cyclizes, unmasking an unstable phenol. The quinone-methide rearrangement of the phenol results in the cleavage of the polymer backbone.

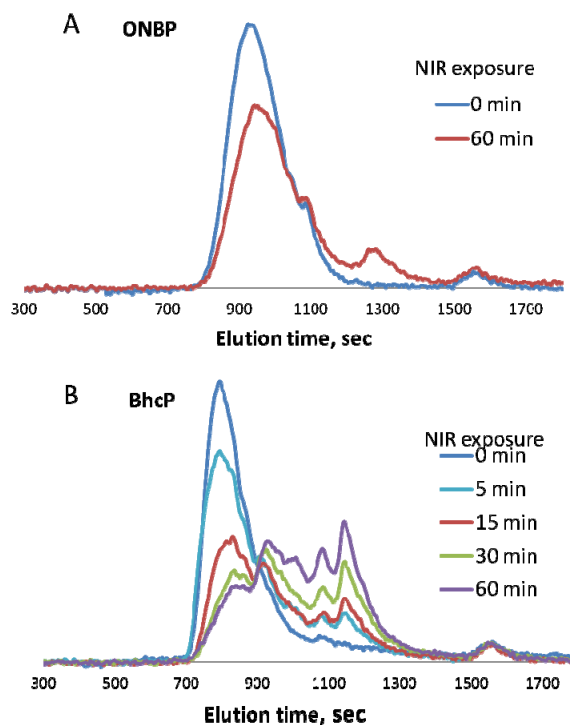Scheme 2. Light-triggered polyme degradation.

Figure 1. UV light triggered degradation of ONBP (A) and BhcP (B).

Degradation of the polymers containing ONB and Bhc triggering groups was studied in acetonitrile: PBS pH 7.6 (9:1 and 7:3, respectively). These combinations of solvents were found suitable to fully dissolve the polymers. The

solutions were irradiated with UV light for 0, 10, 20, 60 and 300 sec, incubated at 37°C for 96 hrs and analyzed by GPC. The chromatograms of ONBP and BhcP after UV exposure are shown in Figure 1. For both polymers, the GPC traces shift to longer elution times after irradiation and smaller fragments are formed. However, much shorter irradiation times are required to produce a significant reduction in the molecular weight of BhcP compared to ONBP. Thus, M_w of BhcP decreases by 50% after 25 sec of UV irradiation compared to 300 sec in the case of ONBP.

The NIR light induced degradation was studied in the same way. The polymers were irradiated for 5, 15, 30 and 60 min and incubated for 96 h at 37°C. The GPC chromatograms after 96h of incubation are shown in Figure 2. Similar to UV irradiation, much shorter irradiation times were required to produce significant fragmentation of BhcP, compared to ONBP. Figure 2B shows 50% molecular weight loss was achieved after 21 min of NIR irradiation of BhcP, while for ONBP one hour of continuous irradiation only resulted in 20% weight loss.
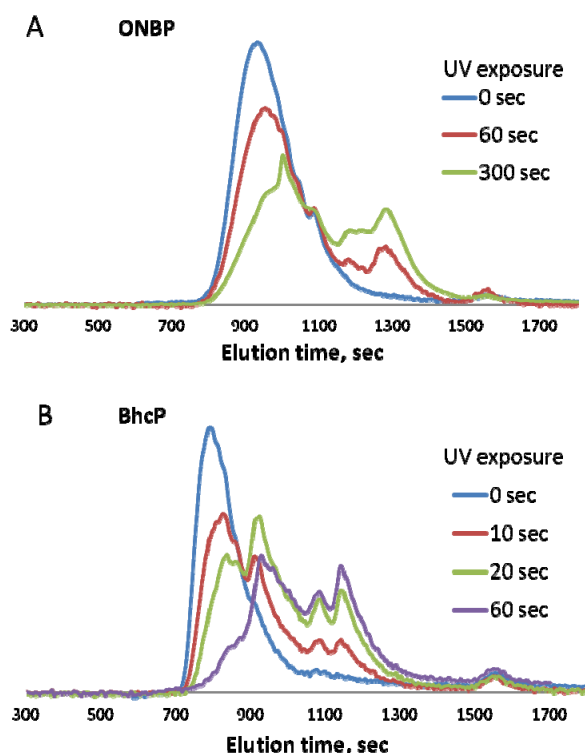

Figure 2. NIR light induced degradation of ONBP (A) and BhcP (B).

Even though NIR irradiation is considered more benign than UV wavelengths, there is a certain energy threshold above which photodamage will occur. Watanabe et al demonstrated that laser energies between 2 nJ/pulse and 4 nJ/pulse did not produce any damage to living cells[18]. Therefore, we attempted NIR light degradation of BhcP within this range (200 mW, corresponding to 2.5 nJ/pulse) to further demonstrate the practicality of using this material

for in vivo applications. Exposure of the BhcP solution to low power NIR irradiation for 60 min resulted in the 29% drop in the molecular weight, confirming that the polymer degradation is caused by the two-photon absorption process and not simply by possible heat generated by the laser, since at 200 mW heat generation is negligible.

In the control experiment, molecular weights of the non-irradiated polymers remained unchanged, demonstrating that backbone fragmentation is controlled exclusively by the removal of the triggering groups and no dark hydrolysis takes place during this time.

2.3 Formulation and Release from Nanoparticles

To evaluate the properties of the new ONBP polymer for controlled light-triggered release, nanoparticles were formulated by the single emulsion method (Figure 3), encapsulating the small hydrophobic molecule dye Nile Red. The Z-average diameter of the nanoparticles was 170 nm as determined by dynamic light scattering (DLS). The 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 irradiating ONBP nanoparticles with 350 nm light for 1 min, the fluorescence intensity dropped by 67%, indicating burst release of the dye from the nanoparticles into a more polar medium (Figure 3 and 4A). On the other hand, a suspension of nanoparticles that was not irradiated exhibited unchanged fluorescence intensity over several days. Interestingly, prolonged irradiation of nanoparticles did not result in a further drop of fluorescence signal. We also explored the possibility of triggering the release of Nile Red by NIR light through two-photon absorption. The suspension of nanoparticles in PBS pH 7.4 was irradiated at 750 nm for 20 min intervals followed by 10 min of incubation at 37ºC. A gradual decrease in the fluorescence intensity of Nile Red was observed during the 4 hours of irradiation (Figure 4B).
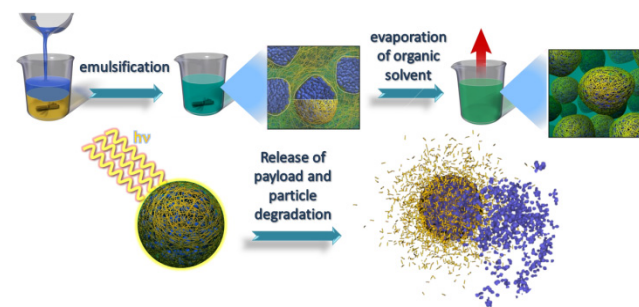

Figure 3. An illustration of the formulation of nanoparticles, their degradation and light-triggered release of their encapsulated Nile Red payload.

The rapid and efficient unmasking of a large number of the secondary amino groups may make the particles rapidly

more permeable to water. This may explain the rapid release of Nile Red upon UV irradiation. However, the two-photon unmasking process is much less efficient which could explain the slower Nile Red release in the NIR two-photon regime. Notably, the final degradation of the nanoparticles is an important property for *in vivo* biological applications that require materials to degrade into easily excretable fragments.

While ONB triggering group can be removed with light in organic solvents or in bulk, Bhc group is only photoactive in aqueous environment. Therefore, we didn't expect that BhcP would maintain its high light sensitivity in bulk, since in this case the polymer backbone would create a local hydrophobic environment. Therefore, we envision further applications of this material in hydrogel systems where unrestricted access of water will allow for high photolysis quantum yields.
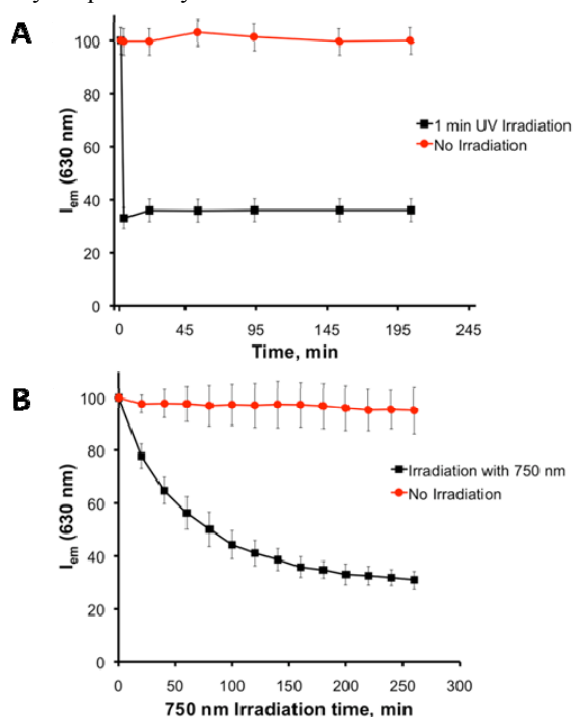

Figure 4. Release of Nile Red from ONBP nanoparticles upon UV (A) and NIR (B) exposure.

3   CONCLUSIONS

In conclusion, new polymeric materials capable of triggered disassembly upon UV and NIR irradiation were developed. The material bearing Bhc triggering group disassembles in aqueous solution with unprecedented sensitivity to NIR light. A 29% decrease in Mw of BhcP was observed after irradiation with 200 mW NIR light. To the best of our knowledge, this is the first example of a polymeric material capable of disassembly into small molecules in response to harmless levels of irradiation. While less sensitive to NIR light, ONBP retains its activity in bulk and, when formulated into nanoparticles, allows release of payload on demand.

4   REFERENCES

[1] Prabhakaran, M. P.; Venugopal, J.; Kai, D.; Ramakrishna, S. *Mater. Sci. Eng.C* **2011**, *31*, 503.
[2] Zhang, Q. W.; Mochalin, V. N.; Neitzel, I.; Knoke, I. Y.; Han, J. J.; Klug, C. A.; Zhou, J. G.; Lelkes, P. I.; Gogotsi, Y. *Biomaterials* **2011**, *32*, 87.
[3] Sabolinski, M. L.; Alvarez, O.; Auletta, M.; Mulder, G.; Parenteau, N. L. *Biomaterials* **1996**, *17*, 311.
[4] Duceppe, N.; Tabrizian, M. *Exp. Opin. Drug Del.* **2010**, *7*, 1191.
[5] Lin, G.; Chang, S.; Hao, H.; Tathireddy, P.; Orthner, M.; Magda, J.; Solzbacher, F. *Sensors and Actuators B-Chemical* **2010**, *144*, 332.
[6] Murthy, N.; Xu, M. C.; Schuck, S.; Kunisawa, J.; Shastri, N.; Frechet, J. M. J. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 4995.
[7] Saez, J. A.; Escuder, B.; Miravet, J. F. *Tetrahedron* **2010**, *66*, 2614.
[8] Liechty, W. B.; Kryscio, D. R.; Slaughter, B. V.; Peppas, N. A. *Ann. Rev. Chem. Biomol. Eng.* **2010**, *1*, 149.
[9] Yu, X. D.; Liu, Q. A.; Wu, J. C.; Zhang, M. M.; Cao, X. H.; Zhang, S.; Wang, Q.; Chen, L. M.; Yi, T. *Chem. Eur. J.* **2010**, *16*, 9099.
[10] Huang, J. W.; Xu, J. S.; Xu, R. X. *Biomaterials* **2010**, *31*, 1278.
[11] Hu, J. M.; Liu, S. Y. *Macromolecules* **2010**, *43*, 8315.
[12] Ruiz-Hernandez, E.; Baeza, A.; Vallet-Regi, M. *ACS Nano* **2011**, *5*, 1259.
[13] Fomina, N.; Sankaranarayanan, J.; Almutairi, A. *Adv. Drug Delivery Rev.*, *doi: 10.1016/j.addr.2012.02.006*.
[14] Weissleder, R. *Nat Biotechnol* **2001**, *19*, 316.
[15] Chen, C. C.; Lin, Y. P.; Wang, C. W.; Tzeng, H. C.; Wu, C. H.; Chen, Y. C.; Chen, C. P.; Chen, L. C.; Wu, Y. C. *J. Am. Chem. Soc.* **2006**, *128*, 3709.
[16] Braun, G. B.; Pallaoro, A.; Wu, G. H.; Missirlis, D.; Zasadzinski, J. A.; Tirrell, M.; Reich, N. O. *ACS Nano* **2009**, *3*, 2007.
[17] *Near-Infrared Applications in Biotechnology*; Raghavachari, R., Ed. New York, 2001; Vol. 25.
[18] Watanabe, W.; Arakawa, N.; Matsunaga, S.; Higashi, T.; Fukui, K.; Isobe, K.; Itoh, K. *Opt. Express* **2004**, *12*, 4203.
[19] Aujard, I.; Benbrahim, C.; Gouget, M.; Ruel, O.; Baudin, J. B.; Neveu, P.; Jullien, L. *Chem. Eur. J.* **2006**, *12*, 6865.
[20] Furuta, T.; Wang, S. S. H.; Dantzker, J. L.; Dore, T. M.; Bybee, W. J.; Callaway, E. M.; Denk, W.; Tsien, R. Y. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 1193.
[21] Amir, R. J.; Pessah, N.; Shamis, M.; Shabat, D. *Angew. Chem. Int. Ed.* **2003**, *42*, 4494.