

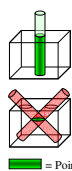
Abstract

A critical aspect of designing biomaterial carriers for cells and drug delivery is tuning and controlling the material's degradation behavior. In the last decade, there has been considerable interest in using photochemistry to produce biomaterials because of the ability to form scaffolds in situ under physiological conditions¹; integrating photochemistry as a degradation mechanism should be equally biocompatible and give spatial and temporal control. In this project, we are synthesizing a model compound that degrades via both single and two-photon photolysis. In single-photon photolysis, any volume exposed to light is susceptible to degradation. Two-photon degradation only occurs where two photons are simultaneously absorbed (the laser focal point), allowing for 3D control over degradation. The model compound contains a nitrobenzyl ether (NBE) moiety linked to a coumarin fluorophore, which imparts two-photon photolysis sensitivity. The model compound also contains two polymerizable groups (acrylates) for network formation. We have synthesized both the NBE linker and the coumarin fluorophore, which will be coupled to form the model compound, 2-(acryloyloxy)ethyl 7-(1-(4-(4-(2-(acryloyloxy)ethoxy)-4-oxobutoxy)-5-methoxy-2-nitrophenyl)ethoxy)-6-chlorocoumarin-3-carboxylate. We will characterize the single-photon degradation of this model compound using long-wave UV light at 365 nm. The degradation will be followed using ¹H NMR spectroscopy. The model compound will be polymerized into a 3D network, which we will spatially pattern in 2D and 3D, using UV light or a two-photon microscope. Photodegradable biomaterials should support on-demand degradation, resulting in a sophisticated platform that allows encapsulation of cells and provides unprecedented external control of the cellular (micro)environment (chemical and physical) in both time and space.

Background

- Nitrobenzyl ether (NBE) caged coumarins give uncaging cross sections, a measure of the efficiency of photolysis, at 365 nm two orders of magnitude better than previously studied caged fluorophores²
- Also efficiently photodegrades using two-photon photolysis at 740 nm²
- From these studies, we expect the linkage of a coumarin fluorophore to a 2-nitrobenzyl ether (NBE) group, a single-photon degrading compound by itself, will form a compound susceptible to two-photon photolysis as well
- Single-photon photolysis is degradation that occurs upon the absorption of a single photon while two-photon photolysis is degradation upon the absorption of two photons
- Two-photon photolysis
 - Occurs at the focal point of a pulsed infrared laser where there is a high concentration of photons
 - In a 3D material containing groups susceptible to two-photon photolysis, the degradation occurs only at the focal point
 - Allows for the possibility of etching a pathway in the material.
 - Single-photon photolysis degrades the entire volume of a 3D material exposed to light
 - There are many applications for 3D control over degradation in tissue engineering and drug delivery.
- Use of infrared radiation minimizes photo toxicity to cells²

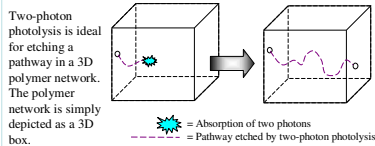
3D Degradation: Single- & two-photon photolysis



Single-Photon Photolysis
 •Only the volume exposed to light is degraded

Two-Photon Photolysis
 •Simply depicted as degradation only where two infrared laser beams intersect to represent the absorption of two photons

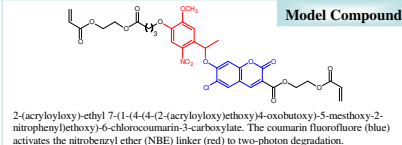
■ = Point of degradation



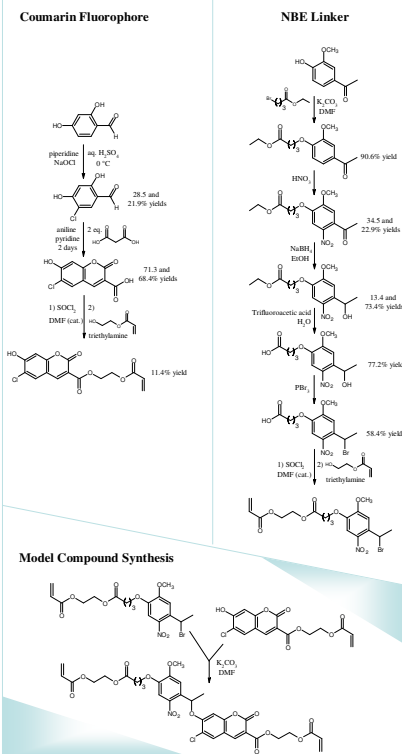
Hypothesis and Approach

- Goal:** Synthesize a model compound susceptible to single and multi-photon photolysis
- Previously studied photodegradable groups have included a nitrobenzyl ether (NBE) moiety that degrades using single-photon photolysis
 - Linking the NBE moiety to a coumarin fluorophore will allow for two-photon photolysis in addition to single-photon
 - Because of the coumarin, degradation can be easily visualized—the fluorescence intensity of the coumarin increases by three orders of magnitude as the compound photodegrades
- Experiments**
- Perform organic synthesis to form model compound
 - React model compound to form a polymer network
 - Perform degradation experiments
 - Monitor degradation using proton nuclear magnetic resonance imaging (¹H NMR)
 - Perform single photon degradation experiments using UV light at 365 nm on model compound
 - Spatially pattern polymer network with UV light or a two-photon microscope

Model Compound

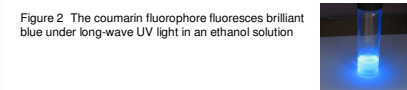
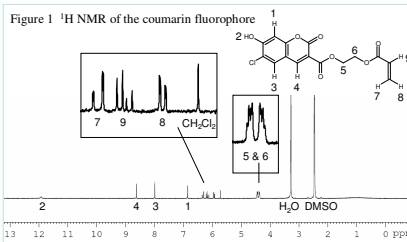


Organic Synthesis



Results

- The coumarin fluorophore (Figure 1, 2) was successfully synthesized according to Scheme 1, collecting 40 mg



- NBE linker synthesis; conjugation of 2-hydroxyethyl acrylate to the NBE linker did not go to completion, but all the other synthetic steps worked
- Bromination was successful (Figure 3)
- Etherification with 2-hydroxyethyl acrylate only had about 75% reaction and was not completely purified (Figure 4)
- Tried washing with aqueous sodium bicarbonate to remove starting material, but NMR continued to show 75% product

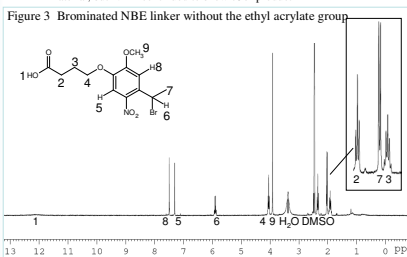
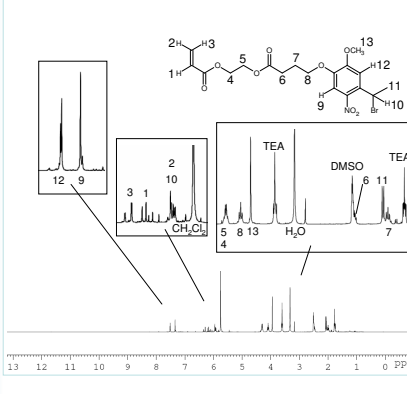
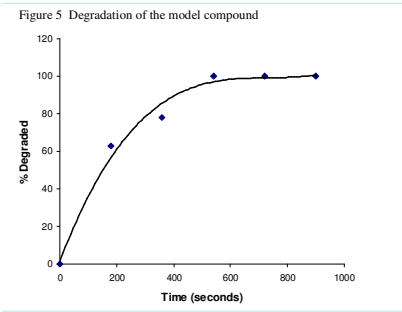


Figure 4 Brominated NBE linker with the ethyl acrylate group (75% reacted) (TEA = triethylamine).



Degradation Results

- A small sample of the NBE linker was purified on a column, and the coupling reaction was performed with the coumarin fluorophore (0.074 mmol scale). The resulting product was not purified because of time constraints, but the desired product was shown in the NMR and degradation experiments were performed.



Conclusions

- The coupling reaction between the coumarin fluorophore and the NBE linker was performed
- Degradation is successful in two dimensions
- The procedure and work-up of the NBE linker synthesis needs to be adjusted to ensure all of the starting material reacts
 - Avoid water and humidity
 - Try larger amounts of starting material as larger scaled reactions tend to have higher yields (only 0.25 and 0.3 g were reacted in each of the two setups performed)
- Synthesize the model compound on a larger scale to allow further study
 - Perform patterning experiments using UV and IR radiation
 - Determine whether or not 3D etching is successful with the model compound

Future Directions: Biomedical applications

- Drug delivery and tissue engineering**
- Evaluate biomaterial-cell interactions
 - Directing tissue growth along a specified 3-D pathway, which would be etched using two-photon photolysis
 - Drug delivery—using both single and two photon degradation
 - Spatially and temporally controlled
 - Integration of different photodegradable groups into the 3D network would allow more tailoring of the degradation behavior
 - Different compounds degrade at different rates; therefore, one could degrade only certain parts of the biomaterial at a time

References

¹Bryant, S. J.; Nuttelman, C. R.; Anseth, K. S. Cytocompatibility of UV and visible light photoinitiating systems on cultured NIH/3T3 fibroblasts in vitro. *Journal of Biomaterials Science-Polymer Edition* 2000, 11, (5), 439-457.

²Zhao, Y. R.; Zheng, Q.; Dakin, K.; Xu, K.; Martinez, M. L.; Li, W. H. New caged coumarin fluorophores with extraordinary uncaging cross sections suitable for biological imaging applications. *Journal of the American Chemical Society* 2004, 126, (14), 4653-4663.

Acknowledgments

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