9588 *Macromolecules* **2010**, *43*, 9588–9590
DOI: 10.1021/ma1020648

Macromolecules
COMMUNICATION TO THE EDITOR

Development of a PEG Derivative Containing Hydrolytically Degradable Hemiacetals

Branden Reid,* Stephany Tzeng, Andrew Warren, Kristen Kozielski, and Jennifer Elisseeff*

Johns Hopkins University, Smith Bldg. 5031, 400 N. Broadway, Baltimore, Maryland 21231, United States

Received September 6, 2010
Revised Manuscript Received October 26, 2010

Synthetic polymers are ubiquitous in the biomedical sciences, with applications in drug delivery, medical devices, and artificial matrices for tissue engineering.[1−4] Since the success of the first synthetic poly(glycolic acid)-based suture in the 1960s, vast effort has been devoted to designing synthetic biodegradable polymers.[5] Given the complexity of regenerative medicine, there is a need to tailor biomaterials for specific applications.[6] Among the hydrophilic synthetic polymers, poly(ethylene glycol) (PEG) is a widely used material due to its resistance to protein adsorption and its biocompatibility.[7] PEG and PEG-copolymers play critical roles ranging from PEGylation therapeutics to hydrogel scaffolds mimicking the natural extracellular matrix for cell culture and tissue regeneration. PEG or poly(ethylene oxide) (PEO) with molecular weight exceeding 20 kDa is a hydrolytically nondegradable polymer with excellent solubility in water and various organic solvents.[8] As a result of PEG's nondegradability, the entire polymer chain is excreted through the kidneys (< 30 kDa) or through the liver (> 30 kDa).[9] Therefore, only PEG of molecular weight less than 50 kDa is typically used in biomedical applications to ensure elimination from the body.[10] To overcome this limitation, efforts have been made to introduce biodegradability into PEG or to make copolymers of PEG and biodegradable polymer moieties such as esters.[11,12] Polyesters, as well as other similarly structured polymers, degrade via hydrolysis and give rise to products with carboxylic acid terminal groups.[13] As a result, their degradation may create an acidic environment that can induce tissue toxicity.[14] In this Communication, we report a synthetic scheme introducing hemiacetals randomly into the backbone of PEG which we will refer to as ROPEG, randomly oxidized PEG. This simple synthetic scheme of incorporating minimal random hemiacetals within the PEG backbone will retain beneficial characteristics of PEG while allowing hydrolytic degradation to nonacidic byproducts.[15,16]

Utilizing the Fenton reaction of hydrogen peroxide and ferric chloride at a neutral pH, we developed a simple method of introducing hemiacetals into the PEG backbone. Previous reports have shown the Fenton reaction to depolymerize PEG but particularly requiring highly acidic conditions to drive the reaction to completion.[17,18] Almkvist et al. oxidized PEG via hydroxyl radicals generated by Fenton's reagent ($Fe(II)/H_2O_2$) in aqueous solution and demonstrated PEG degradation products of alcohols, aldehydes, and formate esters using ^1H NMR.[17] This degradation pathway suggests that there are multiple steps of PEG oxidation and degradation into a variety of PEG oligomers, including PEG hemiacetals, an intermediate formed prior to complete depolymerization of the backbone.[17] We found that keeping the pH neutral during the Fenton reaction can oxidize the PEG backbone to ROPEG, a degradable polymer retaining the many advantages of PEG, while retaining higher molecular weight elements.

*Corresponding author. E-mail: jhe@jhu.edu.

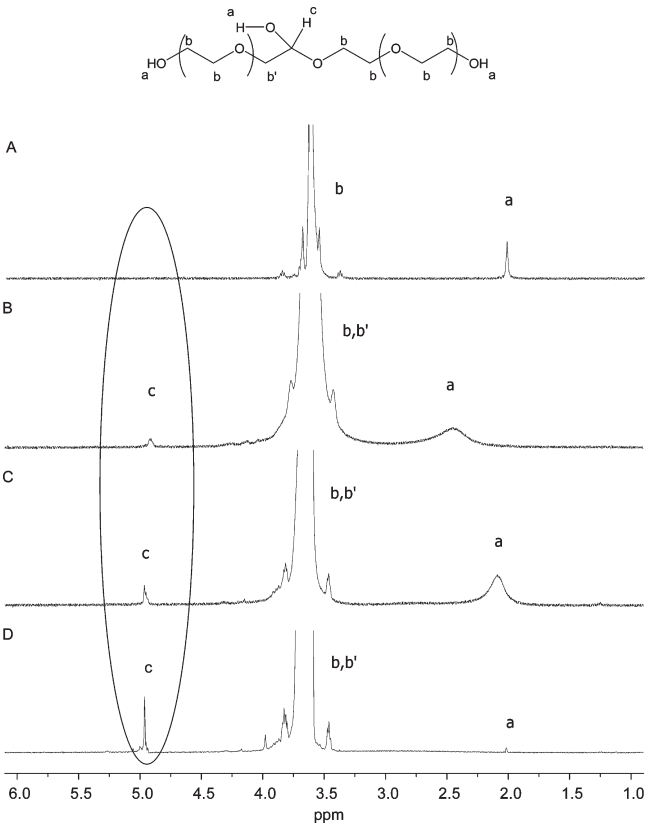
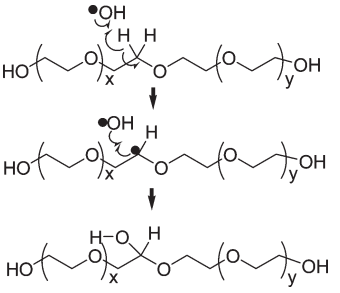


Figure 1. ^1H NMR spectra of PEG (A), ROPEG synthesis at 4 °C for 3 days (B), ROPEG synthesis at 4 °C for 5 days (C), and ROPEG synthesis at room temperature for 24 h (D).

Scheme 1. Depiction of Hypothesized Reaction Mechanism: Synthesis of Hemiacetals Randomly within the Backbone of PEG through Hydroxyl Radicals Produced from the Fenton Reaction



The Fenton reagent used in our studies consisted of 35% (v/v) hydrogen peroxide and 2.5 mM $FeCl_3$ buffered in 1X PBS, and the pH was adjusted to 7 with 5 M NaOH. PEG 3400 Da was added to the reagent at 10% (w/v), and oxidation was performed at either 4 °C or room temperature. Matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI-TOF MS) of PEG treated with the Fenton reaction at 4 °C and at neutral pH before and after dialysis with 1000 Da molecular weight cutoff membrane is provided in the Supporting Information. We demonstrate that some degradation of PEG does take place; however, ^1H NMR spectra show hemiacetal formation in the backbone of nondegraded PEG at 4 °C and room temperature (Figure 1). Resonance at 4.9 and 3.5−4 ppm corresponds to

 Published on Web 11/08/2010

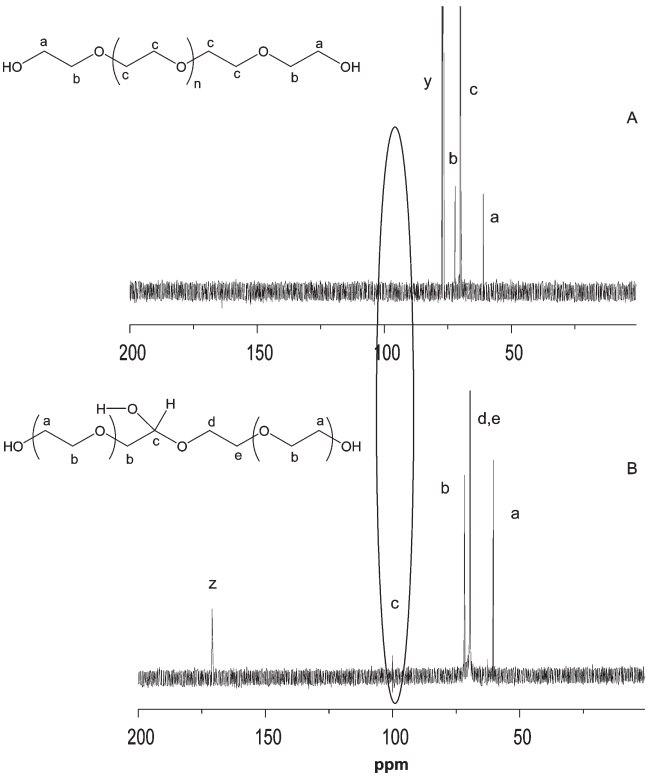


Figure 2. ^{13}C NMR spectra of PEG (A) and ROPEG synthesized at room temperature for 24 h depicting hemiacetal formation in ROPEG with resonance at 100 ppm (B). Z most likely represents resonance corresponding to degradation products of PEG-formate ester, and Y represents the solvent peak of $CDCl_3$.

the hemiacetal protons in the ROPEG and all methylene protons, respectively. The peak ratio of hemiacetal protons to methylene protons for synthesis at 4 °C for 3 days, 4 °C for 5 days, and room temperature for 24 h was 1:557, 1:372, and 1:135, respectively; this corresponds to approximately 0.7%, 1%, and 3% oxidation of methylene subunits to hemiacetals. This suggests the possibility of tuning the number of hemiacetals in the PEG backbone by modifying reaction time and temperature and, as a result, tuning the rate of hydrolytic degradation. Difficulties arise, however, with elevated oxidation temperatures leading to increased degradation rate. Consequently, higher oxidation temperatures produce degradation products that are difficult to separate with dialysis or column purification. ^{13}C NMR spectra of the ROPEG (not purified) synthesized at room temperature for 24 h corroborate this, demonstrating hemiacetal formation in ROPEG as indicated with resonance at 100 ppm, but also the presence of what is most likely PEG-formate ester degradation products, demonstrated by resonance at 170 ppm (Figure 2).

As previously mentioned, hemiacetals can be hydrolyzed to aldehydes and alcohols, a process that can be catalyzed via acidic environments.[19] Thus, we sought to provide further evidence of hemiacetal formation within the backbone of ROPEG by treating dialyzed ROPEG synthesized at 4 °C and a control group of unmodified PEG with 0.5 M hydrochloric acid for 24 h (Figure 3). Analysis by MALDI-TOF MS shows a bimodal molecular weight distribution due to hydrolysis of the hemiacetals in ROPEG synthesized at 4 °C, while the molecular weight distribution of the unmodified PEG demonstrates that no degradation occurred.

Further analysis of hemiacetal formation with the Fenton reaction at a neutral pH was evaluated with a smaller molecule, tri(ethylene glycol) dimethyl ether (TEGDME). Similarly to 3400 Da PEG, hemiacetals were formed in the backbone of TEGDME

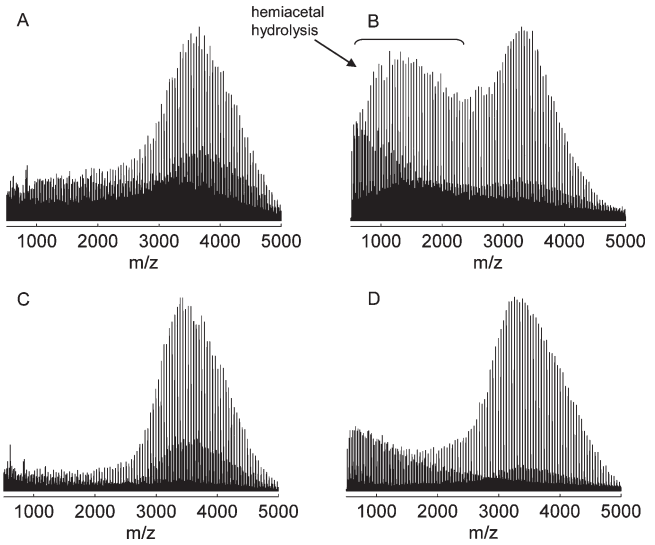


Figure 3. MALDI-TOF MS of 3 day oxidized ROPEG dialyzed with 1000 Da cutoff in order to remove most of the degraded PEG products (A), 3 day oxidized ROPEG treated with 0.5 M hydrochloric acid to verify hemiacetals in the backbone (B), PEG 3400 Da (C), and PEG 3400 Da treated with 0.5 M hydrochloric acid depicting no degradation as expected (D).

(178 Da) at room temperature as depicted in ^1H NMR spectra of Figure S2.

In conclusion, PEG was randomly functionalized with hemiacetals (ROPEG) via the Fenton reaction. We have demonstrated a simple method of synthesizing a degradable PEG polymer capable of hydrolyzing into nonacidic byproducts. This new technique of ROPEG synthesis can be utilized in a variety of areas such as PEGylation therapeutics and tissue engineering scaffolds. PEGylation has emerged as an effective strategy to alter the pharmacokinetic profiles of a variety of drugs.[20] Unfortunately, this technology has been limited to lower molecular weights of PEG which can be excreted by the kidneys, given that high molecular weights will accumulate in the liver.[9] We have demonstrated synthesis of degradable hemiacetal moieties in the backbone of PEG within a small molecule of 3 repeat units (TEGDME) as well as a larger molecule (PEG 3400 Da) of ~77 repeat units. Therefore, ROPEG synthesis can be employed to improve PEGylation therapeutics. In tissue engineering, biomaterials are designed to be degradable to allow growing tissue to replace the scaffold. This normally requires using degradable polymers such as PLA and PGA, which have been previously observed to cause inflammatory responses.[21] ROPEG will provide a simple method of designing a degradable scaffold that will have the biocompatible properties of PEG while also allowing degradation. Further investigation of tuning hemiacetal formation for injectable hydrogel scaffolds will be studied and reported in forthcoming papers.

Acknowledgment. The authors acknowledge the financial support from NIH/NIDCR (3R01DE016887-03S1). We also acknowledge Professor Robert Moriarty (Department of Chemistry, Univeristy of Illinois at Chicago) and Professor Krzysztof Matyjaszewski (Department of Chemistry at the Mellon College of Science, Carnegie Mellon University) for their critical review and valuable discussions.

Supporting Information Available: Details of all procedures along with MALDI-TOF MS and ^1H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hubbell, J. A. *Curr. Opin. Solid State Mater. Sci.* **1998**, *3* (3), 246–251.
(2) Hubbell, J. A. *Nature Biotechnol.* **1995**, *13* (6), 565–576.
(3) Elbert, D. L.; Hubbell, J. A. *Annu. Rev. Mater. Sci.* **1996**, *26* (1), 365–294.
(4) Langer, R.; Vacanti, J. P. *Science* **1993**, *260* (5110), 920–926.
(5) Nair, L. S.; Laurencin, C. T. *Prog. Polym. Sci.* **2007**, *32* (8−9), 762–798.
(6) Chan, G.; Mooney, D. J. *Trends Biotechnol.* **2008**, *26* (7), 382–392.
(7) Lin, C.-C.; Anseth, K. *Pharm. Res.* **2009**, *26* (3), 631–643.
(8) Tessmar, J. K.; Göpferich, A. M. *Macromol. Biosci.* **2007**, *7* (1), 23–39.
(9) Veronese, F. M.; Pasut, G. *Drug Discovery Today* **2005**, *10* (21), 1451–1458.
(10) Yamaoka, T.; Tabata, Y.; Ikada, Y. *J. Pharm. Sci.* **1994**, *83* (4), 601–606.
(11) Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. *Macromolecules* **1993**, *26* (4), 581–587.
(12) Kretlow, J. D.; Klouda, L.; Mikos, A. G. *Adv. Drug Delivery Rev.* **2007**, *59* (4−5), 263–273.
(13) Kaihara, S.; Matsumura, S.; Fisher, J. P. *Macromolecules* **2007**, *40* (21), 7625–7632.
(14) Falco, E.; Patel, M.; Fisher, J. *Pharm. Res.* **2008**, *25* (10), 2348–2356.
(15) Moreau, J. L.; Kesselman, D.; Fisher, J. P. *J. Biomed. Mater. Res., Part A* **2007**, *81A* (3), 594–602.
(16) Kaihara, S.; Matsumura, S.; Fisher, J. P. *J. Biomed. Mater. Res., Part A* **2009**, *90A* (3), 863–873.
(17) Almkvist, G.; Persson, I. *Holzforschung* **2008**, *62* (6), 704–708.
(18) Lynn, A. D.; Kyriakides, T. R.; Bryant, S. J. *J. Biomed. Mater. Res., Part A* **2009**, *93A* (3), 941–953.
(19) Barkakaty, B.; Matsumoto, K.; Endo, T. *Macromolecules* **2009**, *42* (24), 9481–9485.
(20) Fishburn, C. S. *J. Pharm. Sci.* **2008**, *97* (10), 4167–4183.
(21) Athanasiou, K. A.; Niederauer, G. G.; Agrawal, C. M. *Biomaterials* **1996**, *17* (2), 93–102.