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Determination of the *in vivo* degradation mechanism of PEGDA hydrogels[3]

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Abstract

Poly(ethylene glycol) (PEG) hydrogels are one of the most extensively utilized biomaterials systems due to their established biocompatibility and highly tunable properties. It is widely acknowledged that traditional acrylate-derivatized PEG (PEGDA) hydrogels are susceptible to slow degradation *in vivo* and are therefore unsuitable for long-term implantable applications. However, there is speculation whether the observed degradation is due to hydrolysis of endgroup acrylate esters or oxidation of the ether backbone both of which are possible in the foreign body response to implanted devices. PEG diacrylamide (PEGDAA) is a polyether-based hydrogel system with similar properties to PEGDA but with amide linkages in place of the acrylate esters. This provides a hydrolytically-stable control that can be used to isolate the relative contributions of hydrolysis and oxidation to the *in vivo* degradation of PEGDA. Here we show that PEGDAA hydrogels remained stable over 12 weeks of subcutaneous implantation in a rat model while PEGDA hydrogels underwent significant degradation as indicated by both increased swelling ratio and decreased modulus. As PEGDA and PEGDAA have similar susceptibility to oxidation, these results demonstrate for the first time that the primary *in vivo* degradation mechanism of PEGDA is hydrolysis of the endgroup acrylate ester. Additionally, the maintenance of PEGDAA hydrogel properties *in vivo* indicates their suitability for long-term implants. These studies serve to elucidate key information about a widely used biomaterial system to allow for better implantable device design and to provide a biostable replacement option for PEGDA in applications that require long-term stability.

Keywords

Poly(ethylene glycol); Hydrogel; In Vivo Degradation; Hydrolysis; Oxidation

1. Introduction

Poly(ethylene glycol) (PEG) hydrogels are one of the most adaptable biomaterials systems due to their exceptional tunability and biocompatibility. The soft tissue-like properties of PEG-based systems enables their use in a variety of tissue engineering and regenerative

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medicine applications. [1–5] Additionally, their intrinsic resistance to protein adsorption and cell adhesion provides a bioinert material that is ideal for use in drug delivery vehicles and blood-contacting devices. [6, 7] The range of mechanical and physical properties that can be achieved with simple alterations in PEG molecular weight, concentration, and functionality, as well as the ability to control bioactivity with the introduction of specific bioactive agents is well-characterized in current literature. [8–13] Similarly, PEG-based hydrogel devices with a breadth of *in vivo* lifetimes have been developed by modifying endgroup chemistries and/or by using co-polymerizations to adjust degradation profiles. [1, 10, 14–17]

Acrylate-derivatized PEG (PEGDA) is commonly utilized in the development of PEG hydrogels due to its ease of fabrication and use. As the poly(ether) backbone is hydrolytically stable, PEGDA hydrogels often serve as biostable controls in short-term, *in vitro* degradation studies. [1, 15, 18] However, it is widely recognized that PEGDA hydrogels are susceptible to slow degradation *in vivo* and are therefore unsuitable for long-term implants. [1, 10, 19, 20]In current literature, the *in vivo* degradation of PEGDA hydrogels is typically attributed to hydrolysis of the endgroup acrylate esters that are introduced upon acrylation of PEG diol. However, until recently, the hydrolytic degradation profile of PEGDA hydrogels was poorly characterized, as the majority of reports on PEGDA hydrolysis referred to a study with tri(ethylene glycol) diacrylate (TEGDA). [21] PEGDA hydrogel-based biomaterial systems are generally fabricated from pre-polymers of molecular weight between 2,000 and 10,000 Da. [16, 22] These higher molecular weight pre-polymers have a much lower crosslink density than low molecular weight TEGDA and higher ratios of hydrolysable esters relative to backbone groups. Thus, the degradation profile of TEGDA is not an ideal comparison. In an effort to develop a PEG-based gel with increased biostability and to identify PEGDA degradation mechanism, we synthesized PEG diacrylamide (PEGDAA) hydrogels with a hydrolytically stable amide group in place of the ester of PEGDA, Figure 1. [14] We previously reported on the properties of these gels and the *in vitro* hydrolytic stability relative to PEGDA hydrogels. These studies demonstrated that PEGDA gels undergo accelerated hydrolysis under alkaline conditions and that degradation rates can be increased by decreasing crosslink density (i.e. increasing molecular weight and/or decreasing concentration). In contrast, no measureable change of the PEGDAA hydrogels occurred under similar accelerated hydrolysis conditions.

Although PEG hydrogels are typically characterized as bioinert, PEG-based devices can promote a degree of non-specific protein adsorption and/or complement activation *in vivo*, which can result in macrophage recruitment, attachment and activation at the implantation site. [23–25] Macrophage adhesion and fusion to form foreign body giant cells (FBGCs) during frustrated phagocytosis of an implanted biomaterial generates a privileged microenvironment between the cell membrane and the material surface. Upon activation, macrophages and FBGCs secrete degradative agents including acids, reactive oxygen intermediates (ROIs), and enzymes into this microenvironment. [26, 27] The close cell-material interaction prevents buffering of these agents and results in a high concentration at the material surface to promote degradation. Acids present in the microenvironment can reduce the pH to as low as 4 to cause hydrolysis of the endgroup esters of PEG. [28] This is widely considered to be the source of *in vivo* degradation of PEGDA hydrogels; however, the ether backbone of PEG is also susceptible to oxidation that can be mediated by ROIs

released from adherent macrophages and FBGCs. [29] Thus, the observed degradation of PEGDA hydrogels could be a result of ester group cleavage via hydrolysis, ether cleavage via oxidation, or some combination of the two. [20, 24]

Reports in current literature lack a suitable control for determination of the relative contribution of hydrolysis and oxidation to the overall PEGDA degradation *in vivo*. PEGDAA hydrogels do not provide increased resistance to oxidative degradation compared to PEGDA hydrogels due to their ether backbone; however, they can serve as a hydrolytically-stable control for *in vivo* degradation studies. In this work, PEGDA and PEGDAA hydrogels were fabricated with similar initial compressive moduli and swelling ratios. First, *in vitro* degradation profiles under accelerated hydrolytic and oxidative conditions were characterized at 37°C by measuring changes in swelling ratio and modulus over time. Samples were then implanted subcutaneously using a standard rat cage model for up to 12 weeks. Swelling ratio and modulus were utilized as indicators of *in vivo* degradation over this time frame. These studies provide information about the relative extent of ester- and ether-based *in vivo* degradation of PEGDA for the first time and serve to enhance understanding of a widely utilized biomaterial system. Additionally, the evaluation of the *in vivo* degradation of PEGDAA serves to determine its suitability as a replacement for PEGDA for long-term implantable applications in which a biostable hydrogel system is desired.

2. Materials and Methods

2.1 Materials

All chemicals were purchased from Sigma Aldrich (Milwaukee, WI) and used as received unless otherwise noted.

2.2 Polymer Synthesis

PEGDA was synthesized according to a previously described method. [30] Briefly, acryloyl chloride was added drop-wise to a solution of PEG (10 kDa) diol and triethylamine (TEA) in anhydrous dichloromethane (DCM) under nitrogen. The molar ratios of PEG, TEA, and acryloyl chloride were 1: 2: 4, respectively. After 24 hours of stirring, the reaction solution was washed with 8 molar equivalents of 2M potassium bicarbonate and dried with anhydrous sodium sulfate. The product was precipitated in cold diethyl ether, filtered, and dried under vacuum overnight.

PEGDAA was prepared using a protocol adapted from a previously described method. [14, 31] Briefly, acryloyl chloride was added to a solution of PEG (10 kDa) diamine and TEA in anhydrous DCM under nitrogen. The molar equivalent of PEG diamine, TEA, and acryloyl chloride was kept at 1: 2: 4, similarly to the PEGDA reaction. After reacting for 24 hours, the solution was washed with 8 molar equivalents of 2M potassium bicarbonate. Then, it was dried with anhydrous sodium sulfate, and the polymer product was precipitated in cold diethyl ether, filtered, and dried under vacuum.

Successful formation of PEGDA and PEGDAA was confirmed using Fourier transform infrared (FTIR) spectroscopy and proton nuclear magnetic resonance (^{1}H-NMR)

spectroscopy. Control and functionalized polymers were solution cast directly onto KBr pellets for acquisition of transmission FTIR spectra using a Bruker TENSOR 27 spectrometer. An ester peak at 1730 cm^{-1} in the PEGDA spectra indicated successful acrylation of PEG diol, and amide peaks at 1640 cm^{-1} and 1675 cm^{-1} signified successful acrylamidation of PEG diamine, Figure 2. Proton NMR spectra of control and functionalized polymers were recorded on Mercury 300 MHz spectrometer using a TMS/solvent signal as an internal reference. PEGDA: ^{1}H-NMR ($CDCl_3$): 3.6 ppm (m, -OC*H*$_2$C*H*$_2$-), 4.3 ppm (t, -C*H*$_2$OCO-) 6.1 ppm (dd, -C*H*=CH_2), 5.8 and 6.4 ppm (dd, -CH=C*H*$_2$). PEGDAA: ^{1}H-NMR ($CDCl_3$): 3.6 ppm (m, -OC*H*$_2$C*H*$_2$-); 6.7 ppm (s, -CH_2-N*H*-); 6.4 ppm (m, -C*H*=CH_2); 5.6 and 6.1 ppm (m, -CH=C*H*$_2$). All candidate polymers had greater than 90% endgroup functionalization.

2.3 Hydrogel Characterization

2.3.1 Hydrogel Preparation—PEGDA and PEGDAA were further purified via dialysis against reverse osmosis (RO) water for 24 hours (2 kDa molecular weight cut off) to ensure full removal of reaction by-products prior to *in vivo* use. The dialyzed solutions were sterile-filtered (0.2 μm cellulose acetate syringe filters) and lyophilized to obtain the final purified product. Hydrogels were prepared by dissolving PEGDA or PEGDAA (10 kDa; 10 wt%) in sterile water. A photoinitiator solution (1 mg Irgacure 2959 per 0.1 ml 70% ethanol) was added at 1 vol% of precursor solution. Solutions were sterile-filtered (0.2 μm cellulose acetate syringe filters) and crosslinked between 1.5 mm spaced plates under sterile conditions via 6 minute exposure to long-wave UV light (Intelli Ray Shuttered UV Flood Light, Integrated Dispensing Solutions, Inc., 365 nm, 4 mW/cm^2).

2.3.2 Hydrogel Modulus—For modulus measurements, 10 specimens (2 mm × 2 mm rectangular prisms) were cut from crosslinked sheets and swelled in RO water for 24 hours. Specimens were subjected to mechanical testing using a dynamic mechanical analyzer (RSAIII, TA Instruments) equipped with a parallel-plate compression clamp. Testing was performed under unconstrained compression at room temperature. The linear viscoelastic range for each hydrogel formulation was determined using dynamic strain sweeps. Then, a strain within the upper end of the linear viscoelastic range was used in a constant strain frequency sweep. Tests were conducted between 0.79 and 79 Hz, and the compressive storage modulus was taken at 1.25 Hz.

2.3.3 Hydrogel Swelling Ratios—To measure swelling ratio, 10 hydrogel specimens (2 mm by 10 mm rectangular prisms) were cut from each sheet directly after polymerization. Specimens were swelled in RO water for 24 hours and weighed to determine the equilibrium swelling mass (W_s). Then, specimens were dried under vacuum for 24 hours and weighed to assess dry (polymer) mass (W_d). The equilibrium volumetric swelling ratio, Q, was calculated from the equilibrium mass swelling ratio:

2.4 Hydrogel *In Vitro* Degradation

Specimens (2 mm by 12 mm rectangular prisms) were cut from each hydrogel formulation and incubated at 37°C with shaking for up to 12 weeks or until complete dissolution occurred. For accelerated hydrolytic degradation, PEGDA gels were incubated in a 5 mM sodium hydroxide (NaOH) solution with daily solution changes. Swelling ratio was monitored daily until complete dissolution occurred. PEGDAA gels were incubated in a 5 mM NaOH solution for 12 weeks with twice-weekly solution changes. Swelling ratio and modulus were measured every two weeks. Oxidative degradation was accelerated using a solution of 3% hydrogen peroxide with 1.25 mM cobalt chloride. Specimen swelling ratios were monitored daily until complete dissolution occurred. Solutions were changed daily to maintain ROI concentrations. For the degradation studies that were carried out to 12 weeks, swelling ratio was measured as described above with sample dry (polymer) mass obtained at each time point. For faster-degrading samples (PEGDA in hydrolytic solution, PEGDA and PEGDAA in oxidative solution), swelling ratio was calculated at each time point relative to initial dry (polymer) mass. All swelling ratio measurements were normalized to the initial swelling ratio at time 0.

2.5 Hydrogel *In Vivo* Degradation

All procedures were approved by the Texas A&M University Institutional Animal Care and Use Committee. Sterile hydrogel samples were obtained from the same slabs used in the *in vitro* degradation studies to ensure consistency between studies. The chosen formulation was determined via scouting studies with gels fabricated from a range of PEG molecular weights (3.4, 6, and 10 kDa; 10 wt%) to ensure that measureable degradation would occur within the timeframe of the study. Specimens (2 mm by 12 mm) were cut and enclosed in cylindrical stainless steel mesh cages (1.5 mm length, 3 mm diameter). [32] Three month old female Sprague Dawley rats were anesthetized before and during the procedure with 2% isoflurane. Their sides were shaved, and incision sites were scrubbed with chlorhexidine. Incisions (approximate 1 cm long) were made on the side of the rats, and blunt dissection was used to prepare implant pockets (2 per side). Specimens were introduced through the incision and positioned within the pocket away from the incision site. The incisions were then closed with stainless steel surgical wound clips. Clips were removed 7 days post-implantation. After 4, 8, or 12 weeks, rats were euthanized via carbon dioxide inhalation. A subsequent bilateral thoracotomy was performed to ensure death. The stainless steel cages were explanted, and hydrogel specimens were carefully removed from the stainless steel cages. Swelling ratio and modulus of explants were measured as described above to measure the extent of degradation.

3. Results

3.1 Initial Hydrogel Properties

Initial swelling ratio and modulus of 10% PEG (10k) DA and DAA hydrogels were measured to ensure that the two systems were comparable, Figure 3. Both swelling ratio and modulus were statistically similar ($p<0.05$) between the two gel systems prior to degradation. It should be noted that the PEGDAA gel had a slightly higher initial modulus (94 ± 11 vs. 77 ± 19) than the PEGDA gel. However, the lack of statistical difference in

these measurements indicates that the two systems had similar initial crosslink densities and can be directly compared in subsequent degradation studies.

3.2 Hydrogel *In Vitro* Degradation

3.2.1 Accelerated Hydrolytic In Vitro Degradation—Hydrogel degradation occurs via cleavage of networked groups and results in a reduced crosslink density over time. These changes in crosslink density can be monitored via measurements of swelling ratio and/or modulus. [19] To this end, swelling ratio and compressive modulus measurement were utilized to assess the extent of sample degradation in the varied media at the selected time points. Only swelling ratio was measured for samples that exhibited rapid declines in structural integrity that prevented accurate measurement of compressive modulus, including PEGDA under accelerated hydrolytic conditions and both PEGDA and PEGDAA under accelerated oxidative conditions. Thus, to analyze PEGDA accelerated hydrolytic degradation, swelling ratio of samples in 5 mM NaOH was measured every 24 hours, Figure 4. PEGDA hydrogels experienced significant increases in swelling each day, indicating that rapid degradation was occurring under these alkaline conditions. A loss of mechanical integrity was observed at 5 days, and complete dissolution occurred within 10 days. Singificant increases in swelling occurred every day except for day 5; the insignificant change in swelling ratio between days 4 and 5 may indicate that some samples were beginning to physically break apart, which may have prevented measurement of full swollen weight due to loss of smaller pieces. Both swelling ratio and modulus of PEGDAA hydrogels were measured every 2 weeks over 12 weeks of accelerated hydrolytic degradation in alkaline 5 mM NaOH at 37°C, Figure 5. PEGDAA hydrogels remained stable for the full 12 weeks in the alkaline solution, with no significant increases in swelling or decreases in modulus. The stability of PEGDAA gels after 12 weeks in the accelerated solution is especially significant when looking at the 55% increase in swelling that occurred in PEGDA gels after only 1 day. These results demonstrate that PEGDAA has significantly increased hydrolytic stability relative to PEGDA, which correlates with our previous hydrolytic degradation studies comparing the two gel systems. [14]

3.2.3 Accelerated Oxidative In Vitro Degradation—Oxidation of the polyether background of both PEGDA and PEGDAA was accelerated using an aqueous solution with 3% hydrogen peroxide with 1.25 mM cobalt chloride. The cobalt ions are expected to rapidly decompose the hydrogen peroxide molecules via the Haber-Weiss reaction. [33] The increased concentration of ROIs within the degradation medium was expected to help simulate a longer degradation period *in vivo* and identify potential oxidative rate differences between the two hydrogels. PEGDA and PEGDAA gels experienced similar oxidative degradation profiles, demonstrated by comparable daily swelling increases, Figure 6. This was expected, as the common ether backbone of both systems has similar susceptibility to oxidative cleavage. PEGDA hydrogels experienced a loss of mechanical integrity at 3 days and underwent complete dissolution in 4 days whereas PEGDAA hydrogels completely dissolved within 5 days. The increased speed of PEGDA dissolution in the oxidative solution was likely due to the aqueous environment that allowed hydrolysis to occur as well. The transition into a loss in mechanical integrity occurred more rapidly in the oxidative study compared with the hydrolytic study. In the hydrolytic study, the PEGDA sample

swelling ratio increased by over 100% prior to a loss in integrity whereas in the oxidative study, the swelling ratio only increased by 20%. This is possibly due to differences in degradation of the polymer backbone versus the endgroups, as there are many more oxidatively-labile groups relative to hydrolytically-labile groups. The combined results of the accelerated *in vitro* studies demonstrate that PEGDAA hydrogels have the expected increased hydrolytic stability compared to PEGDA and similar susceptibility to oxidation. Thus, they serve as a suitable control for determination of the *in vivo* degradation mechanism of PEGDA hydrogels by isolating potential degradation to oxidation independent of hydrolysis.

3.3 Hydrogel *In Vivo* Degradation

PEGDA and PEGDAA *in vivo* degradation was measured using a subcutaneous cage implant system in a rat, wherein swelling ratio and modulus were assessed at 4 week intervals over 12 weeks, Figure 7. PEGDA hydrogels experienced significantly increased swelling and decreased modulus at each time point, indicating progressive degradation. Both swelling ratio and modulus followed linear trends (R^2 = 0.95 and 0.91, respectively). Based on the linear interpolation, swelling ratio increased at an average rate of 8% per week while modulus decreased at an average rate of 6% per week. Thus, both measurements provided relatively comparable trends. PEGDAA hydrogels remained stable throughout the course of the study with no significant increases in swelling or decreases in modulus at any time point. This indicates that PEGDAA hydrogels are suitable for use as a biostable replacement for PEGDA gels in long-term applications. Additionally, the lack of PEGDAA degradation indicates that neither of the PEG-based systems underwent measurable oxidation over the course of this study and that the observed *in vivo* degradation of PEGDA was primarily due to ester hydrolysis.

4. Discussion

The results from these studies demonstrate that PEGDAA is a suitable replacement for PEGDA in hydrogel-based devices for long-term implantable applications. Additionally, the use of the hydrolytically stable PEGDAA as a control in PEGDA *in vivo* degradation demonstrates for the first time that PEGDA degrades primarily due to hydrolysis of the endgroup ester linkages rather than via oxidation of the ether backbone. It should be noted that it has been previously shown that synthetic biomaterials can be susceptible to enzymatic degradation via cholesterol esterase (CE), an additional product of activated macrophages that catalyzes the hydrolysis of esters. [34] Although this has not been tested in hydrogel systems, the CE hexamer has a hydrodynamic radius as approximately 6 nm. [35, 36] We have previously measured the mesh size of a 10% PEG (10k) DA gel to be approximately 90 nm using a diffusion-based system. [9] Thus, it is possible that CE could have diffused into the gels used in these studies to promote ester cleavage and that some of the previously observed *in vivo* degradation of low crosslink density PEGDA gels could have been partially caused by CE. However, the work presented here still demonstrates that the primary cause of PEGDA hydrogel *in vivo* degradation is not oxidation. This information allows for improved design of biostable and biodegradable PEG-based systems.

Additionally, it allows for better estimation of the *in vivo* lifetime of a PEGDA implant, as one only needs to account for the effects of hydrolytic degradation.

Some discrepancies were found between the results in this study and those of a previous *in vivo* degradation study with PEGDA hydrogels. [24] In this study, Lynn et al. saw complete degradation of 80% of PEGDA specimens (20%, 3 kDa) after 4 weeks of subcutaneous implantation in a mouse model. Due to the increased concentration and decreased molecular weight of the PEGDA used in the Lynn study, this hydrogel formulation was expected to have a higher crosslink density than the gel used in our studies (10%, 10 kDa). It follows that the gel with the higher crosslink density should degrade at a slower rate; however, we observed a slower rate of degradation of our gels despite the presumed lower crosslink density. [14] Given that similar gel dimensions were used for both studies, potential differences in expected crosslink density were examined. Hydrogel moduli has a strong correlation to crosslink data but hydrogel modulus was not reported by Lynn et al. and so no comparison could be made with the present study. Alternatively, accelerated *in vitro* oxidative degradation rates have also been correlated with crosslink density. Lynn et al. carried out an accelerated oxidative study in 20% H_2O_2/0.1M $CoCl_2$, which resulted in complete gel degradation within 4 days. In previous scouting studies, we observed complete dissolution of a similar PEGDA gel composition (20%, 3.4kDa) in 13 days in 20% H_2O_2/0.1M $CoCl_2$ solution. As the degradation profiles differ greatly between gels of similar formulations under the same conditions, it can be assumed that the PEGDA gels fabricated in the Lynn study had a lower crosslinking efficiency than our PEGDA gels. This could be due to reduced reaction ratios between the macromer and acryloyl chloride (1: 4 vs. 1: 2.6) and/or reduced reaction temperatures (room temperature vs. on ice) to result in lower acrylation of the PEG diol; however, similar acrylation efficiencies are reported based on NMR spectra (87% vs. 90%). Additionally, Lynn et al. crosslinked their gels with a slightly higher amount of photoinitiator (0.0125 wt% vs. 0.01 wt%) for longer times (10 min. vs. 6 min.) than we did in our degradation studies. Both of these conditions would be expected in increase crosslink density. Thus, the reason for the incongruities in the relative crosslinking efficiencies here are unclear. We performed a scouting study of accelerated oxidative degradation of our 10% PEG (10k) DA gels in the same solution that was used by Lynn, et al (20% H_2O_2/0.1M $CoCl_2$). Our gel formulation completely degraded within 2 days in this solution, which is approximately half the rate of the gels fabricated by Lynn, et al. that degraded within 4 days. This indicates that although the crosslinking efficiency of the previously presented system was most likely lower than that of our system, the relative crosslinking density of the chosen formulation was likely higher as is expected based on the concentrations and molecular weights utilized.

Alternate reasons for the faster degradation rate seen by Lynn et al. could be differences in sterilization methods, the use of cages in our studies, or the use of mice vs. rats. Lynn et al. utilized an ethanol soak to sterilize their gels prior to implantation. Here, we dialyzed our final PEG product to remove any additional small reactant debris and sterile-filtered our gel solutions prior to crosslinking. The use of dialysis could account for some of the difference seen in crosslinking efficiency, as the lyophilized polymer used was likely more pure and therefore more accurate in making the solutions. Furthermore, more stringent sterilization methods (i.e. filtration vs. ethanol soak) could help to remove more foreign debris which

would in turn reduce the overall inflammatory response and result in lower levels of degradative agents around the samples. The authors did, in fact, see a thick layer of inflammatory cells surrounding the PEGDA implant surfaces in histology. To obtain an initial idea of the potential effect of the cage, we implanted one sample from each of our formulations (10% PEG (10 kDa) DA and DAA) without a cage and compared it to caged samples at 4 weeks. The PEGDA sample implanted without a cage was broken up into smaller pieces, especially at the ends, and more difficult to retrieve relative to the caged samples. Additionally, it had a higher swelling ratio (~67% increase from caged samples), indicating that the degradation rate was in fact increased without the cage. The swelling ratio of the PEGDAA sample that was implanted without a cage was unchanged relative to the initial gel properties and to the caged samples (19 vs. 21 ± 2 (initial) and 19 ± 1 (caged)). Thus, it is hypothesized that the presence of the cage in these studies did not affect the overall trends seen or the hypothesized degradation mechanism. Although there was evidence of increased degradation of our PEGDA samples at 4 week without cages, the magnitude of this effect still did not correlate with those seen by Lynn et al., in which they were unable to retrieve 80% of their samples at 4 weeks due to complete degradation. Finally, the differences between the murine and rat inflammatory responses may have contributed to some of the noted inconsistences between the two studies. It is most likely that the combination of reduced crosslinking efficiency, lack of cage, and change in animal model contributed to the increased degradation observed by Lynn et al.

When comparing the *in vivo* data to the accelerated hydrolytic data in our studies, 1 day in 5 mM NaOH at 37°C was approximately equivalent to 3 weeks *in vivo* in the cage implant system. Thus, it is likely that if the study had included an additional time point at 16 weeks, a loss in mechanical integrity would have been observed at that time, and complete dissolution of implants would have occurred at approximately 28–30 weeks. While these comparisons are useful for this system, one should keep in mind that differences in degradation rates exist between implant locations and that device geometry can also impact degradation. [20] In particular, Reid et al. recently demonstrated that PEG hydrogels elicit an enhanced immune response when implanted in contact with adipose tissue than they do when implanted subcutaneously. This enhanced immune response would result in increased concentrations of degradative agents and is hypothesized to increase *in vivo* degradation rates. Thus, the correlations found in these studies cannot be directly utilized for all types of PEGDA implants. However, this is the first *in vivo* degradation study with PEGDA hydrogels that was carried out to a point of significant degradation to confirm that they are not useful for long-term implantable applications, and it provides a general time frame over which PEGDA gels can retain their initial properties *in vivo*.

An additional consideration is the impact of these findings on the more recently developed hydrolytically degradable PEG hydrogels. These can be formed via Michael-type addition of multifunctional thiol or amine moieties with PEGDA or in a two step reaction. In the latter, an initial Michael-type addition between PEGDA and a dithiol or amine is used to form acrylate-terminated PEG chains which is then followed by photocrosslinking step to form the hydrogel network. The resulting gels degrade on a much faster time scale than photocrosslinked PEGDA hydrogels. [37, 38] Degradation rates can be tuned from weeks to months with the two step reaction introducing more esters and undergoing faster

degradation. Differences in the resulting network and the hydrophobicity of the netpoints can potentially influence degradation rate; however, Schoenmakers et al. reported decreasing hydrolysis half life with decreasing number of methylene units between the ester bond and the sulfide which was also correlated with increased atomic charge on the carbon atom of the ester bond. [39, 40] Therefore, it is likely that the presence of the thiols and amines acting as intramolecular catalysts contributes more to the rapid hydrolysis of the adjacent acrylate ester bonds in these gels rather than the differences in network formation. [37, 38, 41, 42] Given that our findings indicate that hydrolysis is likely the primary mechanism of degradation of PEGDA-based hydrogels, we would expect that gels containing sulfide and amine moieties adjacent to acrylate esters would degrade hydrolytically at a rate more rapid than PEGDA counterparts *in vivo*.

5. Conclusions

These studies utilize a hydrolytically stable PEG-based hydrogel system with similar mechanical, swelling and oxidative degradation properties to PEGDA to determine the dominant degradation mechanism of PEGDA *in vivo*. The significant degradation of PEGDA samples that occurred over 12 weeks *in vivo* combined with the no measurable degradation of PEGDAA over the same time frame suggest that the endgroup esters of PEGDA are undergoing hydrolysis while the backbone ethers are resistant to oxidation within this time frame. This allows for more rational design of both degradable and biostable PEG-based devices, as one only needs to account for the effects of hydrolytic degradation when tuning the *in vivo* lifetime of a PEGDA hydrogel. Additionally, PEGDAA can serve as a suitable replacement for PEGDA in long-term biostable applications without the need for major design changes or the sacrifice of any of the valuable properties of the PEGDA system. These results provide a fundamental advance in the understanding of one of the most widely utilized synthetic biomaterial systems and further extend its utility.

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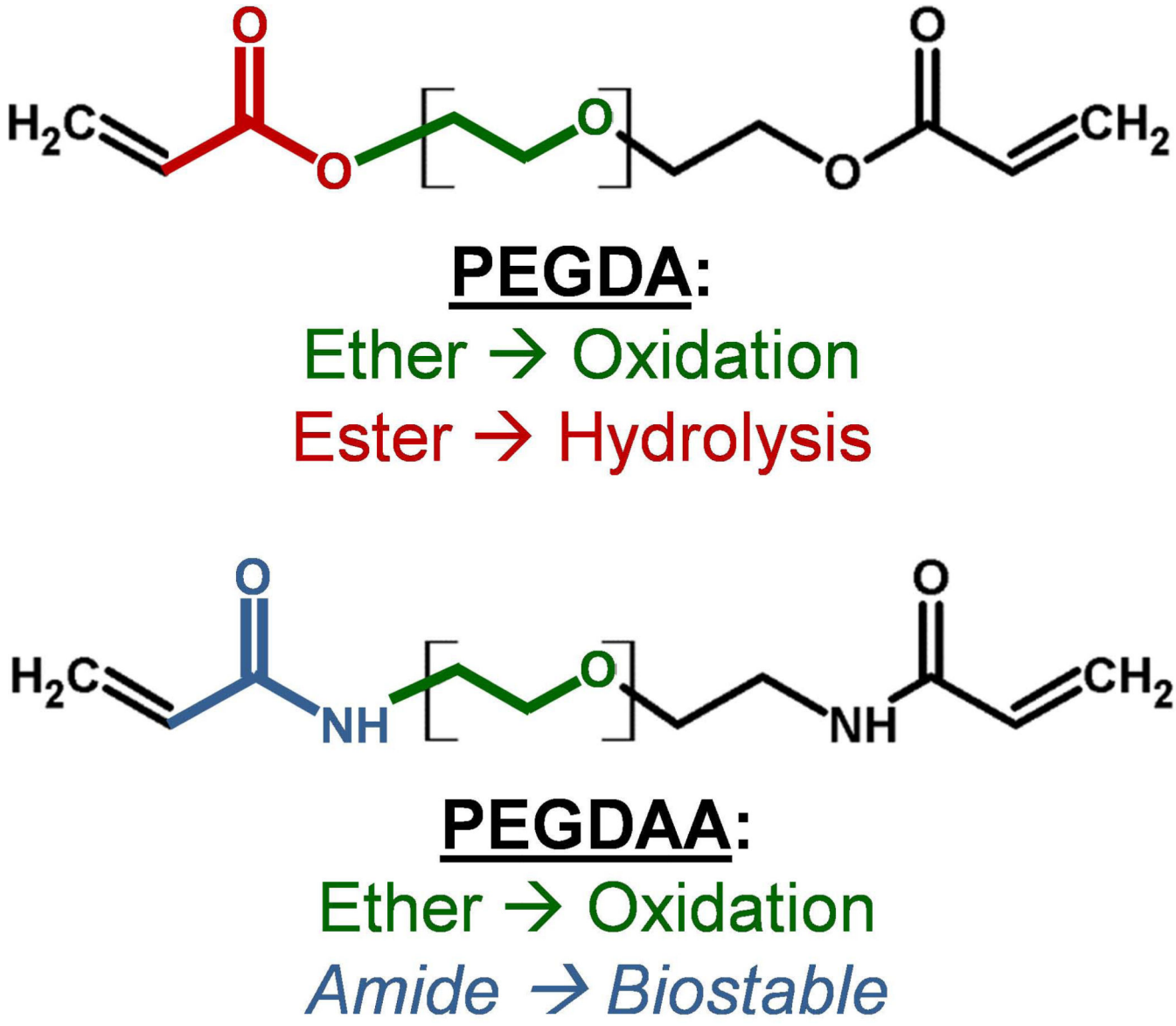

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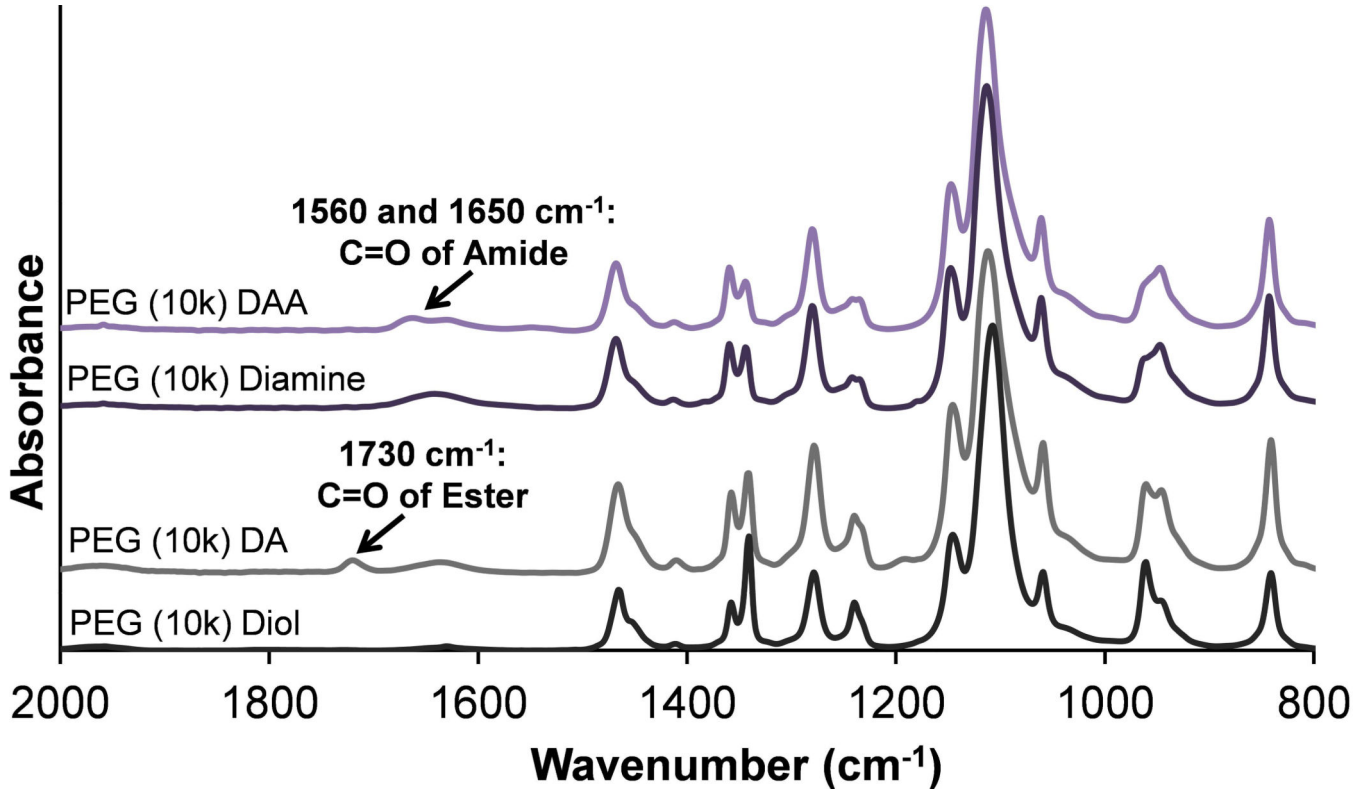

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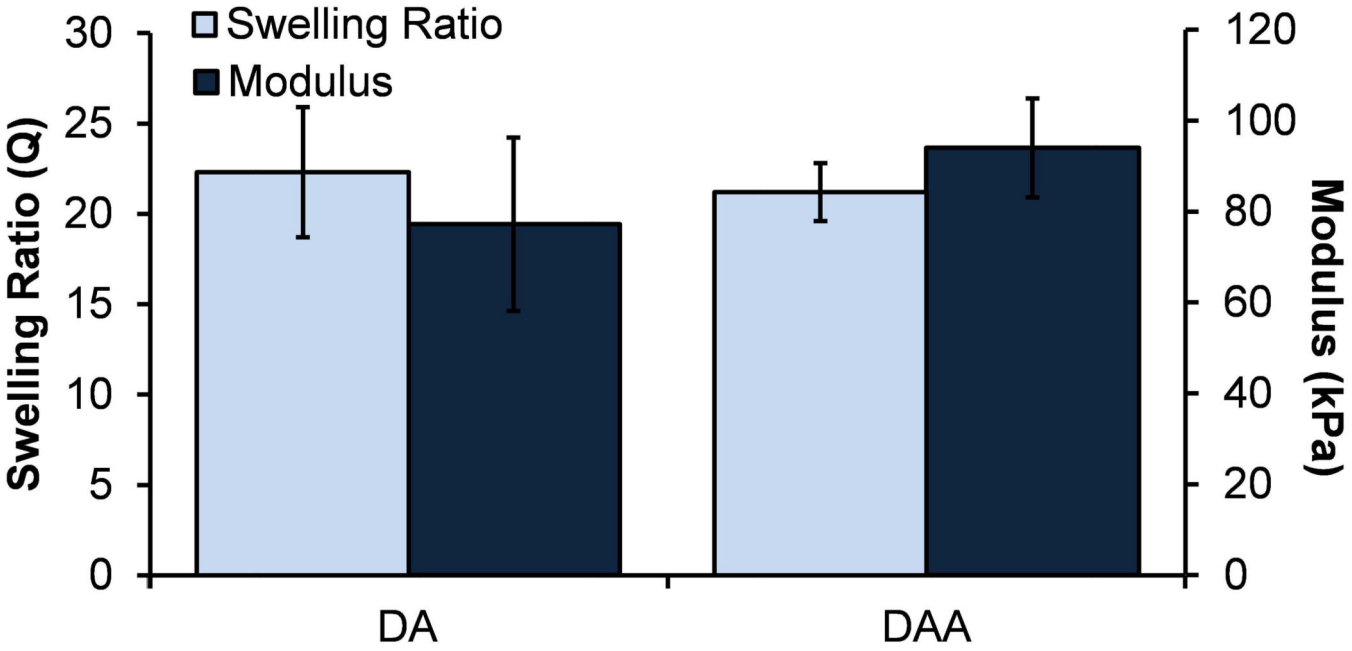

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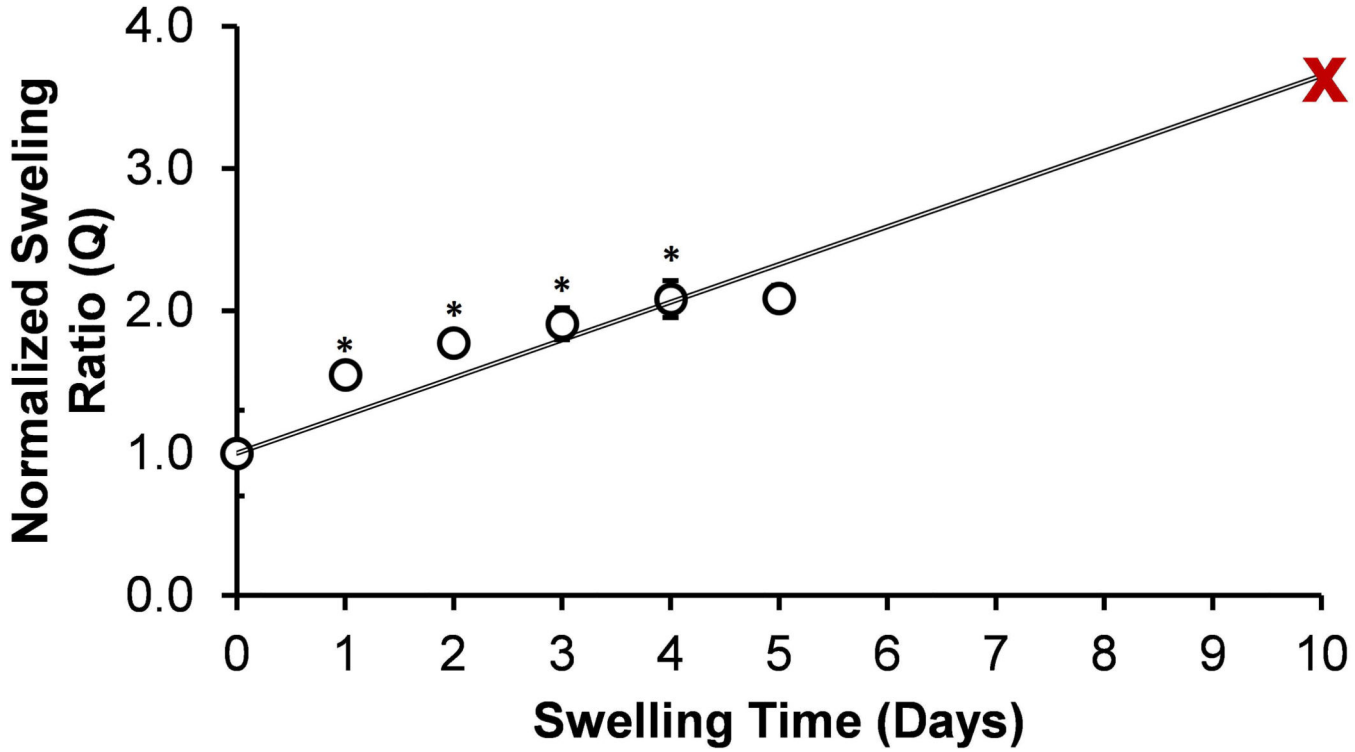

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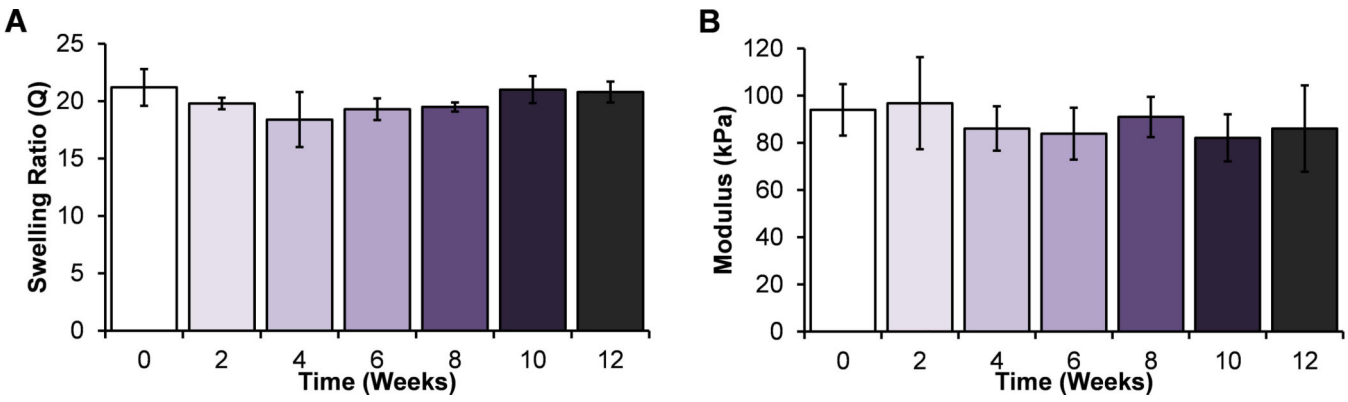

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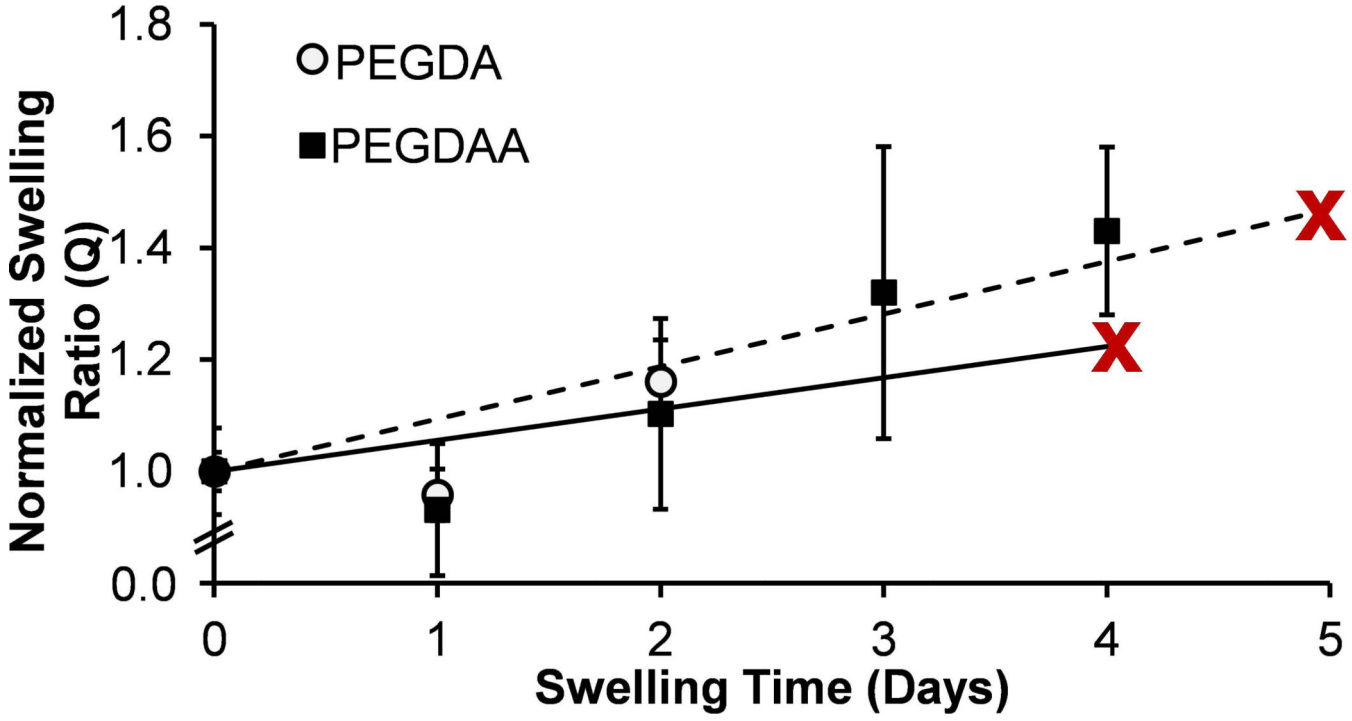

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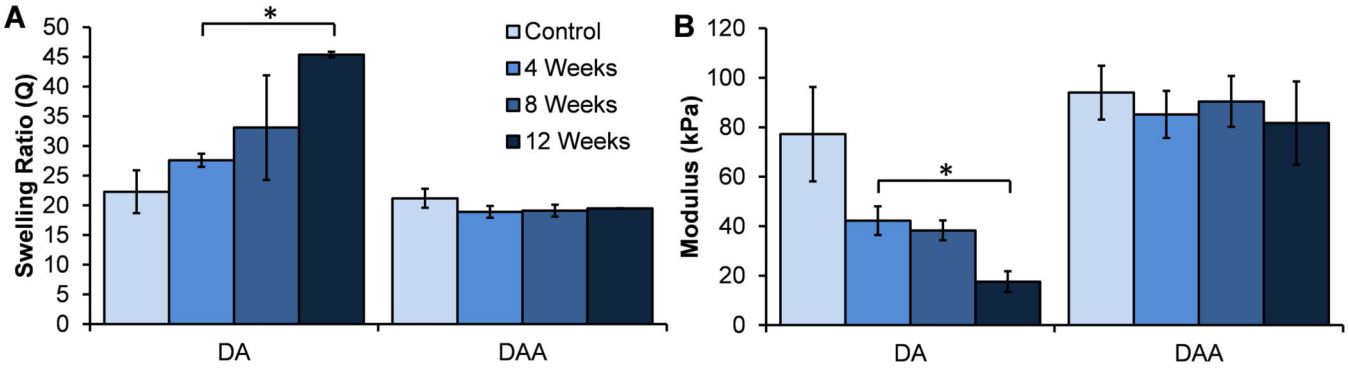

Figure 7.