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Semi-Degradable Poly(β -amino ester) Networks with Temporally-Controlled Enhancement of Mechanical Properties

David L. Safranski^{*,a,b}, Daiana Weiss^c, J. Brian Clark^c, W.R. Taylor^{c,d,e}, and Ken Gall^{b,f}

^aMedShape, Inc., 1575 Northside Drive NW Suite 440, Atlanta, GA 30318

^bSchool of Materials Science and Engineering, Georgia Institute of Technology, 771 Ferst Drive, Atlanta, GA 30332

^cDepartment of Medicine, Division of Cardiology, Emory University School of Medicine, 1639 Pierce Dr, Atlanta, GA 30322

^dAtlanta Veterans Affairs Medical Center, 1670 Clairmont Rd, Decatur, GA 30033

^eW.H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, 313 Ferst Drive, Atlanta, GA 30332

^fWoodruff School of Mechanical Engineering, Georgia Institute of Technology, 801 Ferst Drive, Atlanta, GA 30332

Abstract

Biodegradable polymers are clinically used in numerous biomedical applications, and classically show a loss in mechanical properties within weeks of implantation. This work demonstrates a new class of semi-degradable polymers that show an increase in mechanical properties through degradation via a controlled shift in a thermal transition. Semi-degradable polymer networks, poly(β -amino ester)-co-methyl methacrylate, were formed from a low glass transition temperature crosslinker, poly(β -amino ester), and high glass transition temperature monomer, methyl methacrylate, which degraded in a manner dependent upon the crosslinker chemical structure. *In vitro* and *in vivo* degradation revealed changes in mechanical behavior due to the degradation of the crosslinker from the polymer network. This novel polymer system demonstrates a strategy to temporally control the mechanical behavior of polymers and to enhance the initial performance of smart biomedical devices.

Keywords

Mechanical Properties; Glass Transition Temperature; Biodegradation; Acrylics; Biocompatibility

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*Tel: 1-404-249-9155, Fax: 1-404-249-9158, david.safranski@medshape.com.

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1. Introduction

Biodegradability is a highly desired property for biomedical polymers used clinically. It allows for tissue growth into the material as the material degrades, release of pharmaceutical agents, and negates the need for device removal [1–6]. Current clinically-used biodegradable polymers, such as poly(L-lactide) and poly(caprolactone), take years to degrade, and are associated with some late stage inflammatory reactions due to incomplete degradation [7–10]. Unfortunately, biodegradable polymers often experience a rapid loss of stiffness and strength upon implantation as the material begins to lose its structural integrity, calling into question their use as a structural support in load-bearing conditions [11–13]. Studies have focused on altering the chemistries of current clinical biodegradable polymers (e.g. by varying the ratio of poly(L-lactide) (PLA)/(poly(lactic-co-glycolic) acid (PLGA)) in an attempt to tailor their degradation rate and the subsequent decrease in mechanical properties [11, 14]. PLGA also has even been self-reinforced in another attempt to maintain mechanical properties, but the loss of mechanical properties occurs, albeit at a slower rate, because PLGA's chemical structure, the driving factor, has not been changed [13]. While the loss of stiffness and strength are inherently inevitable, it is important that the degradation rate be tuned to the rate of tissue infiltration, such that the decrease in mechanical properties occurs in parallel to the gradual shift of structural support provided by the surrounding tissue ingrowth [15–17].

A number of studies have detailed the influence of the glass transition temperature (T_g) on the mechanical properties of (meth)acrylate networks [18–22], especially under physiologically simulated conditions. The mechanical behavior of amorphous polymer networks is highly temperature dependent, where a polymer can exhibit glassy or rubbery behavior if cooled below or heated above its T_g , respectively. If heating or cooling is not possible in the desired environment, then immersion in water can be used to shift the T_g , effectively altering the modulus and mechanical behavior. Typically, immersion in water lowers the T_g relative to the testing temperature, which causes the modulus of the material to decrease. This softening of nondegradable plastics is broadly referred to as plasticization; however, this softening does not always lead to a reduction in the toughness, but may actually increase toughness [22]. These mechanical changes occur due to water-polymer interactions and the original thermal properties of the polymer. While these interactions do not degrade the network, they greatly influence the mechanical properties by reducing T_g . When exposed to aqueous conditions, the water-polymer interactions typically decrease T_g through the formation of hydrogen bonds via 'bound' water [23–25]. Immersion studies of nondegradable (meth)acrylate networks have shown that these polymers exhibit an initial decrease in T_g followed by an increase in T_g back to the original value over several months. This T_g shift corresponds to changes in the mechanical behavior, including a decrease in moduli from 500 to 15 MPa (i.e. "plasticization") [26]. In addition, the T_g can be predicted via a rule of mixtures, dependent upon each component's T_g and weight percentage, which allows for facile tailoring of the thermo-mechanical properties [27–28]. In order for the modulus of a copolymer to increase as it degrades, it can be hypothesized that the T_g should increase as the low- T_g component degrades.

A poly(β -amino ester) (PBAE) network containing degradable ester groups in its backbone and acrylate endgroups for photopolymerization can be produced from the step-growth polymerization of a diacrylate and a primary amine [29–31]. The molecular weight, degradation rate, and mechanical properties of these networks can be tailored by selecting from a large library of diacrylate and amine combinations. Previous studies have separately examined the combinatorial characterization of PBAE networks and the thermo-mechanical properties of (meth)acrylates [18, 29]. Previous work has also demonstrated that by mixing with varying amounts of methyl methacrylate (MMA), the thermo-mechanical properties of hydrophobic PBAE networks can be tuned to increase Tg, increase toughness, and maintain a rubbery state [32]. However, the relationships between hydrophilicity and thermo-mechanical behavior have yet to be assessed throughout the degradation process.

The objective of this study was to explore the effect of degradation on the temporal mechanical properties of semi-degradable PBAE-co-MMA networks with varying levels of initial hydrophilicity (Figure 1). The influence of environment was also examined, where samples were tested *in vitro* and *in vivo* to verify that this effect was not limited to an *in vitro* setting. Three biodegradable crosslinkers, poly(β -amino ester)s with acrylate endgroups, were synthesized with varying levels of hydrophilicity to alter mass loss rate [31, 33]. By combining them with a nondegradable, high Tg monomer, such as methyl methacrylate, the Tg of the network was increased to ambient conditions [32]. The biodegradable crosslinkers have shown previous biocompatibility *in vivo*, and PMMA is well known to be biocompatible [33–35]. In order for the PBAE-MMA network to have the same benefits of standard biodegradable polymers, it must elicit a favorable biological response, while also showing an increase in modulus during degradation. The results of this study present (1) how the chemical structure can be tailored to present an increase in mechanical properties with degradation time and (2) how this process provides a favorable *in vivo* response using a mouse model.

2. Materials and Methods

2.1 Materials and Polymer Synthesis

Poly(ethylene glycol) diacrylate $M_n \sim 700$ (PEGDA), hexanediol diacrylate (HDDA), 3-methoxypropylamine (3MOPA), methyl methacrylate, and 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959) were used as received from Sigma Aldrich. The formation of the semi-degradable network is shown in Figure 1. Briefly, PEGDA and HDDA were mixed in molar ratios of 0:100, 10:90, and 25:75 to create the diacrylate mixture. This diacrylate mixture was combined with 3MOPA at a molar ratio at 1.15:1 to ensure acrylate endgroups. The step-growth polymerization to form degradable PBAE macromers occurred for 24 hours at 200 rpm at 90°C on a JKEM reaction block (RBC-20 with BTS-1500 shaker) following [31,33]. The resulting macromers were verified by ^1H NMR on a Varian Mercury Vx 400 in deuterated chloroform and chemical structure can be found in [33], where the PEGDA units are incorporated between HDDA units of the macromer. The molecular weight of the PBAE macromers was determined by comparing the number of hydrogen specific to acrylate and amine groups as described in [31,33]. The average molecular weight for the macromers was 3343, 2602, and 2407 g/mol for the 25:75,

10:90, and 0:100 macromers, respectively. The PBAE macromers were mixed with MMA (45 wt% PBAE macromer: 55 wt% MMA) and 0.5 wt% Irgacure 2959. This solution was photopolymerized for 45 minutes under a UVP Blakray lamp ($\sim 10 \text{ mW/cm}^2$) [32]. A Bruker Vector 22 FTIR with Pike Technologies Miracle ATR attachment with ZnSe crystal was used to characterize the chemical structure of the networks after photopolymerization.

2.2 Mass Loss and Water Content

1 cm $\times$ 1 cm $\times$ 1 mm samples were massed, M_o , then soaked in phosphate buffered saline (PBS), pH 7.4, in an incubator at 37°C for up to 8 weeks. Samples were removed at time i and their wet mass taken, M_{wi} . The samples were dried for 24 hours at 80°C and the mass of the samples taken again, M_{di} , to determine mass loss from Equation 1 and water content from Equation 2.

$$\text{Mass Loss} = 1 - \frac{M_{di}}{M_o} \quad \text{Equation 1}$$

$$\text{Water Content} = \frac{M_{wi}}{M_{di}} - 1 \quad \text{Equation 2}$$

2.3 Thermal Properties

Dynamic mechanical analysis (DMA) (TA Instruments Q800) of rectangular samples from *in vitro* degradation conditions after drying 24 hours was run in tension under strain control, 0.1% strain at 1 Hz following: equilibrate at -100°C , isotherm for 2 minutes, ramp 3°C/min to 200°C . T_g was defined as the peak of the tan delta curve and the rubbery modulus was defined as the storage modulus at a temperature, $T_g + 75^\circ\text{C}$. Crosslinking density, ν , was calculated from the rubbery modulus, E_R , gas constant, R , and temperature in Kelvin, T , where $T = T_g + 75^\circ\text{C}$, using Equation 3.

$$\nu = \frac{E_R}{3RT} \quad \text{Equation 3}$$

Differential scanning calorimetry (DSC) (TA Instruments Q100) was used to determine the glass transition temperature of the samples under both *in vitro* and *in vivo* degradation conditions after drying 24 hours. The samples underwent the following: equilibrate at -90°C , isotherm for 2 minutes, then ramp at a rate of 3°C/min to 200°C . T_g was determined from the intersecting line method of the midpoint of the second order thermal transition.

2.4 Mechanical Properties

Strain-to-failure tensile tests were performed on a MTS Insight 2 with a 100 N load cell using ASTM D-638 Type IV half-sized dog bone samples at a strain rate of 10^{-3} s^{-1} in an environmental chamber filled with PBS and held at 37°C . For *in vitro* degradation conditions, dog bone samples were soaked in PBS in an incubator at 37°C for up to 8 weeks.

Toughness was defined as the energy required to break the materials and calculated as the area under the stress-strain curve.

2.5 In Vivo Assessment

All animal experiments were conducted in accordance with the IACUC guidelines of Emory University. Eight week old, male C57/BL6 mice were used in the present study. The mice were divided into three groups with networks of 0:100, 10:90, and 25:75 (PEGDA:HDDA molar ratio) networks as well as a control group with no implantation of the polymer network. Mice were anesthetized with Xylazine (13 mg/kg) and Ketamine (87 mg/kg). After creating a small incision in the back of the mouse, a small pocket was made to fit the implant. Animals were sutured closed and given Buprenex (0.01 mg) to alleviate pain. All the mice were on a standard chow diet (Purina, Certified Rodent Chow 5001). Animals were euthanized by CO₂ inhalation at 2 and 8 weeks post-surgery. The implants were removed and subjected to mechanical testing. The surrounding tissue was embedded in paraffin, and 5 μ m thick serial sections were used for histological analysis. Masson Trichrome (Sigma) and Hematoxylin and Eosin were used to visualize collagen/fibrosis and morphology, respectively. To image macrophages immunohistochemical antibodies against Mac3 were used. The primary antibodies were a monoclonal rat anti-mouse macrophage antibody (Mac3) diluted 1:50 (BD Bioscience Pharmingen). Goat anti-rat immunoglobulin diluted 1:200 were used as a secondary antibody. Immunoreactivity was visualized by using streptavidin conjugated QDots 605 (Invitrogen) diluted 1:100.

2.6 In vivo Degradation

ASTM D-638 type IV quarter sized dog bone polymer samples were massed prior to subcutaneous implantation for 2 or 8 weeks. The samples were sterilized under a UV light at 254 nm for 60 minutes. After extraction, tensile tests were performed, followed by drying for 24 hours at 80°C, massing to determine mass loss via Equation 1, followed by FTIR-ATR to determine chemical changes, and thermal characterization by DSC.

2.7 Statistical Analysis

One-way ANOVA with Tukey's HSD package in Igor Pro was used to determine if any statistically significant difference was present. $p < 0.05$. The mean $\pm$ standard deviation is given, where $n=3$, unless otherwise specified.

3. Results

3.1 Mass Loss and Water Content

Two diacrylates, PEGDA and HDDA were mixed in varying molar ratios to control the hydrophilicity of the PBAE crosslinker and resulting PBAE-MMA networks. The mass loss profiles are detailed by showing mass loss over time from *in vitro* samples, which were immersed in PBS at 37°C (Figure 2a) or *in vivo* samples, where were implanted subcutaneously (Supplemental Figure 1). For *in vitro* samples at 1 week, the average mass loss (g/g) of the 25:75 network was 0.11, which is greater than the average mass loss of the 10:90 and 0:100 networks, 0.016 and 0.011, respectively. At 8 weeks, the average mass loss of the 25:75 network was 0.30, while the average mass loss of the 10:90 and 0:100 networks

was 0.124 and 0.029, respectively. At 4 weeks, the average mass loss of the 10:90 network, 0.055, had increased above that of the 0:100 network, 0.023. Similar to the *in vitro* samples, the mass loss of the *in vivo* samples increased over the 8 weeks. The 25:75 network's mass loss was higher than the 10:90 and 0:100 networks at 2 weeks and at 8 weeks. Also, the 10:90 network's mass loss was higher than the 0:100 at 8 weeks. For the first 2 weeks, the overall water content of the networks increased with time and the water content increased for each network as the PEGDA:HDDA ratio increased. At 2 weeks, water content was 0.436, 0.333, and 0.094 for 25:75, 10:90, and 0:100 networks, respectively. Beyond 2 weeks, each network's water content increased with time, but the 10:90 network had a higher water content than the 25:75 network (Figure 2b). At 8 weeks, water content was 0.6, 0.661, and 0.14 for 25:75, 10:90, and 0:100 networks, respectively. Hydrolysis of the polymer was also observed by monitoring the carbonyl group and carboxyl group concentration via ATR-FTIR. Over 8 weeks, the carbonyl group concentration decreased and the carboxyl group concentration increased (Supplemental Figure 2).

3.2 Thermo-mechanical Properties

The Tg of each PBAE-MMA network was tracked by DMA for samples degraded under *in vitro* conditions and dried for 24 hours (Figure 3). DSC was used to track Tg for samples degraded under both *in vitro* and *in vivo* conditions and dried for 24 hours (Supplemental Figure 3). DMA and DSC were run after drying in order to eliminate any effects of water on the thermo-mechanical properties. Two methods of examining Tg were used in order to confirm the increase in Tg, where the DMA values are higher than the DSC values due to differences in how the Tg is defined. The Tg is defined as peak of tan delta from DMA, which can be up to 25°C higher than the DSC values [36–37]. Over 8 weeks, the Tg of each network increased for samples degraded both *in vitro* and *in vivo*. As the PEGDA:HDDA ratio increased, the upward shift in Tg increased for each network (e.g. the 25:75 network showed a greater increase in Tg than the 10:90 network). The Tg of the 25:75 network shifted from 48.4°C to 95.0°C, the Tg of the 10:90 network shifted from 50.1°C to 72.8°C, and the Tg of the 0:100 network shifted from 56.5°C to 64.2°C over 8 weeks. By using Equation 3 and the rubbery modulus of the DMA curves (Figure 4a), the crosslinking density was calculated. The crosslinking density decreases over 8 weeks (Figure 4b). As the PEGDA:HDDA ratio increased, the crosslinking density decreased at each time point. Over 8 weeks, the 0:100 network decreased from 309 mol/m³ to 290 mol/m³, the 10:90 network decreased from 223 mol/m³ to 131 mol/m³, and the 25:75 network decreased from 209 mol/m³ to 81 mol/m³.

3.3 Mechanical Properties

The mechanical behavior of the three PBAE-MMA networks was followed over 8 weeks under both *in vitro* (Figure 5) and *in vivo* (Supplemental Figure 4) degradation conditions. The 0:100 network maintained rubbery behavior over 8 weeks under both degradation conditions. The 10:90 network initially exhibited rubbery behavior, but by 8 weeks the mechanical behavior had a defined elastic region and plastic region under both *in vitro* and *in vivo* conditions. The 25:75 network displayed rubbery behavior at early time points, but by 2 weeks the network displayed defined elastic and plastic regions that lasted through week 6; however, week 8 shows a more brittle response *in vitro*. The *in vivo* mechanical

behavior of the 25:75 network shows a similar response over the 8 weeks with initial rubbery behavior, followed by defined elastic and plastic regions, then ultimately brittle behavior.

The elastic modulus profile for *in vitro* degradation is presented in Figure 6 and for *in vivo* degradation in Supplemental Figure 5. The 0:100 network's modulus remained below 5 MPa over the course of 8 weeks with no significant changes. The 10:90 network maintained its modulus over the first 6 weeks *in vitro*, but showed a significant increase from 6.1 MPa to 18.2 MPa at 8 weeks compared to 6 weeks. Also, the 10:90 network degraded *in vivo* maintained a rubbery modulus for the first two weeks and showed an increase in modulus at 8 weeks. The 25:75 network displayed the largest increase in modulus by increasing by two orders of magnitude within the first two weeks *in vitro* and further increased over the remainder of the 8 week study. The 25:75 network showed a significant increase in modulus at 2 weeks, 115 MPa, compared to the prior time point at 1 week, 13.3 MPa, and a significant increase in modulus at 8 weeks, 234 MPa, compared to the prior time point at 6 weeks, 101.6 MPa. The 25:75 networks degraded *in vivo* also showed a large increase in modulus during the first two weeks and maintained this higher modulus at 8 weeks.

Toughness profiles over 8 weeks for *in vitro* degradation conditions are shown in Supplemental Figure 6. All networks showed an increase in toughness during the first week followed by a decrease at two weeks, and then changes in toughness that were composition dependent. The toughness of the 0:100 network increased from 2.5 to 4.7 MJ/m³ during the first week, then decreased to 2.1 MJ/m³ at two weeks and maintained toughness to 2.3 MJ/m³ at eight weeks. The toughness of the 10:90 network increased from 0.8 to 5.5 MJ/m³ during the first week, then decreased to 1.2 MJ/m³ at two weeks, followed by an increase to 3.4 MJ/m³ at six weeks. The toughness of the 25:75 network increased from 1.0 to 7.6 MJ/m³ during the first week, then decreased to 4.0 MJ/m³ at two weeks, followed by an increase to 10.1 MJ/m³ at four weeks, and then ultimately dropping to 0.6 MJ/m³ by eight weeks.

3.4 In Vivo Assessment

Along with mechanical testing, the inflammatory reaction was examined for each network. The sections show the four layers of the skin: subcutaneous muscle, subcutaneous fat (minimal in the mouse), dermal and epidermal regions. Figure 7 (two week time point) and Supplemental Figure 7 (eight week time point) suggest a minimal inflammatory response with all three networks compared to the control tissue at the two- and eight weeks time points with no difference observed amongst the various compositions. No gross morphological changes were seen in the tissue over the time course, and collagen deposition was minimal, indicating that the material would not be highly encapsulated. At both time points, collagen content and fibrosis were comparable to the control tissue. At two weeks, some macrophage infiltration was observed, suggesting an initial inflammatory response to the presence of the network. However, macrophage infiltration fell to negligible levels eight weeks after implantation.

4. Discussion

The objective of this study was to evaluate the temporal change in mechanical properties of a semi-degradable polymer network under aqueous conditions as a function of the networks' initial hydrophilicity (i.e. PEGDA concentration). A biodegradable polymer's standard course of action in the body is to degrade in order to provide drug release or tissue integration; however, a decrease in mechanical properties typically occurs alongside this process. The PBAE-MMA networks presented in this current study represent a novel 'semi-degradable system' with mechanical properties that can be controlled during degradation via control of a thermal shift. These networks are defined as 'semi-degradable' as only the PBAE crosslinks are expected to undergo hydrolysis and subsequently leave the network, while the nondegradable MMA portion of the network remains intact (Figure 1). Here we emphasized the control of mass loss by tailoring the network hydrophilicity (via altering the PEGDA:HDDA ratio). Similar to a previous study, networks of PBAE crosslinker with higher PEGDA concentrations had higher rates of mass loss and increased water absorption due to the more hydrophilic nature of PEGDA compared with HDDA [33].

The degradation process of the PBAE crosslinkers from the PBAE-MMA networks can be broken down into three steps: (1) water uptake, (2) hydrolytic cleavage of the PBAE crosslinks, (3) mass loss of the PBAE degradation products from the network. The rate and amount of water uptake is dependent not only on PEGDA concentration, but also on changes in the crosslinking density. During the first two weeks, the water content of the 25:75 network is greater than the 10:90 network because of the higher concentration of PEGDA. During those first two weeks, the PEGDA segments undergo hydrolysis, the crosslinking density decreases, and PEGDA leaves the network. At four weeks, the 25:75 network has a lower water content than the 10:90 network because more PEGDA has left the 25:75 network than the 10:90 network. Interestingly, the 25:75 network's water content does not decrease, but continues to increase because the decrease in crosslinking density provides more volume in the network for further water uptake and hydrophilic acrylic acid groups remain in the non-degraded chains with MMA.

Crosslinked networks made solely of PBAE crosslinkers degrade by hydrolysis into bis(β -amino acids), diols, and poly(acrylic acid) kinetic chains [29]. Since this is a network composed of PBAE crosslinkers and MMA, there would not be pure poly(acrylic acid) kinetic chains. After hydrolysis, the acrylic acid groups would be expected to remain with the MMA chains. The hydrolysis of the PBAE-MMA networks were verified through FTIR by an increase in intensity of carboxyl groups and decrease of carbonyl groups (Supplemental Figure 2) as well as by a decrease in the crosslinking density determined from DMA (Figure 4b). The decrease in the carbonyl group is representative of the hydrolytic cleavage of the PBAE crosslinker's ester groups, which results in the production of the small molecular weight degradation products. The increase in carboxyl group from the FTIR shows that the acrylic acid groups are being produced and remain in the network. Since PEGDA is considered more hydrophilic than HDDA, the crosslinks containing more PEGDA are expected to undergo hydrolysis before the crosslinks containing only HDDA. The crosslinking density decreases rapidly within the first two weeks for the networks with PEGDA, but then decreases at a slower rate for the remaining time due to the remaining

crosslinks with HDDA. The 0:100 network, which contains only crosslinks based upon HDDA, still undergoes slow, gradual hydrolysis as seen in the decrease in its crosslinking density over the eight weeks due to its hydrolytically cleavable ester bond.

Ultimately, the loss of these PBAE crosslinks from the network is shown in the mass loss profiles. There is limited mass loss for the 0:100 network because it is more hydrophobic, has a lower water content, and undergoes less hydrolysis than the other networks. Networks created with only HDDA-based PBAE crosslinkers and no MMA undergo very limited mass loss, 20% by 8 weeks, thus with the added 55 wt% MMA, the mass loss is further reduced. The 10:90 and 25:75 networks have higher concentrations of PEGDA, thus have higher water contents, and undergo more hydrolysis with larger decreases in crosslinking density, which leads to greater mass loss. Mass loss continues throughout the entire eight weeks, even though most of the hydrolysis occurs in the first two weeks most likely due to the hydrolyzed PEGDA segments are in the process of leaving the network with the remaining HDDA segments still undergoing hydrolysis.

The increase in T_g observed over the eight week degradation period can be attributed to the rate at which the PBAE crosslinker left the network. As a binary polymer system, a copolymer network's T_g can be determined using a rule of mixtures. The increase in T_g can be explained by the PBAE crosslinker component, with a T_g far lower (−40°C) than MMA (120°C), leaving from the network, which leaves the “high T_g” MMA component in the network and thus effectively raises the overall network's T_g. As the PEGDA:HDDA ratio increased, the magnitude of the T_g shift increased because networks with PEGDA lost mass at a faster rate than those without PEGDA (partially related to the water content of the networks). Drying the samples prior to analysis by DMA or DSC may be a considered a limitation of the study; however, the samples were dried in order to eliminate any shifts in T_g due to water and to observe how the T_g had changed due to mass loss. If wet samples had been run in DMA or DSC, the T_g may have shown a decrease in T_g instead of an increase in T_g at early timepoints.

This increase in T_g with degradation time resulted in the thermomechanical behavior of the networks shift from a rubbery to viscoelastic to glassy state at a rate dependent upon network composition. For example, the 25:75 network demonstrated a 46.4°C increase in T_g over 8 weeks corresponding to a rapid shift from rubbery to glassy state over the same time course. In stark contrast, the 0:100 network demonstrated a 7.7°C increase in T_g over the same time course corresponding to minimal changes in stress-strain behavior between 2 and 8 weeks of immersion. Unlike traditional degradable polymers, continued plasticization (decrease in modulus, strength, and failure strain) was not observed despite the water content increasing over time, but rather all networks increased in modulus, further suggesting that the T_g-temperature relationship is still driving the mechanical behavior of the networks, even under aqueous conditions.

Toughness is an important property of biomaterials that serve a mechanical function *in vivo*. Toughness broadly reflects the ultimate stress and strain a material can withstand and can be considered an indicator for material durability [26, 38]. While most degradable polymer systems lose their toughness as they degrade, the toughness of the PBAE-MMA networks

actually exhibit an increase in toughness with degradation time. Previous studies have demonstrated that toughness is maximized when the T_g of a network aligns closely with environmental temperature corresponding to when the network is in its viscoelastic state [22]. Similarly, the 25:75 PBAE-MMA networks exhibited their maximum toughness at a later time point that corresponded to the stress-strain behavior demonstrating large failure strain with a high yield stress. As T_g continues to increase (i.e. the network continues to lose mass), the network's toughness begins to decrease as more of the PBAE departs from the system and the network transitions to a more glassy, brittle state. Comparatively, these PBAE-MMA networks have toughness similar to the initial toughness of nondegradable crosslinked acrylic polymers, 0.14 to 50 MJ/m³, crosslinked polyurethanes, 50.2 MJ/m³, swollen chitosan-PVA hydrogels, 1.76 MJ/m³, and PLA, 2.13 MJ/m³ [22, 39–43]. To the authors' knowledge, there are few comparable studies of degradable polymers that examine the toughness of degradable polymers over the degradation time course. One study found that poly(anhydride ester) fibers only retained 4.8% of their initial toughness, 20 MJ/m³, after 24 hours of degradation [44].

These PBAE-MMA networks have the potential to be used in a number of translational research areas. The low macrophage infiltration and minimal collagen deposition noted in the histology indicate that the networks elicit a low long-term inflammatory response, regardless of the composition. The similar changes in mechanical behavior after implantation suggest the same relationship between hydrophilicity, degradation, and mechanical properties is upheld under *in vivo* conditions. Furthermore, these results suggest that the network's ability to shift in T_g and change in modulus does not produce an unfavorable inflammatory response. These PBAE-MMA networks offer the opportunity to explore the effect of increasing stiffness over time on cellular behavior. These networks could be incorporated into orthopedic scaffolds to help tissue regeneration by stiffening once implanted. It has been shown that stiff scaffolds enhance tissue regeneration when compared to soft degradable scaffolds with a modulus less than the native tissue [45]. Due to the acrylate endgroups, *in situ* photopolymerization would be possible, which would allow for the delivery of drugs into complex spaces *in vivo* [5]. These polymer networks can also display shape-memory characteristics if their thermo-mechanical properties are tuned properly, allowing for their use as an implant material in minimally invasive procedures [46].

5. Conclusions

PBAE-MMA networks allow for a novel approach to temporally control the mechanical properties during degradation. The thermo-mechanical properties are controlled by the change in T_g that shifts as water uptake and subsequent degradation occurs. This work establishes a platform of polymer networks that can be synthesized from a large number of possible combinations of PBAE and (meth)acrylate monomers. Temporal control and the ability to increase the modulus and change the toughness of the polymer network will serve to enhance the design of novel materials for medical implants and tissue engineering applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. (top) Synthesis schematic for PBAE crosslinkers. (bottom) Network formation with PBAE (blue) and MMA (red). As degradation occurs, PBAE leave the networks, which increases overall MMA content, increases Tg, and changes mechanical behavior.

Figure 2. (A) Mass loss profile of PEGDA:HDDA 1.15 +55%MMA networks of varying PEGDA:HDDA ratio over eight weeks under *in vitro* degradation conditions. (B) Water Content for *in vitro* degraded samples. Each point is the mean $\pm$ standard deviation, n=3.

Figure 3. Glass transition temperature profile for PEGDA:HDDA 1.15 + 55%MMA networks of varying PEGDA:HDDA ratio over 8 weeks under *in vitro* degradation conditions determined by DMA. Each point is the mean $\pm$ standard deviation, n=3.

Figure 4. (A) Exemplary DMA curves for the 25:75 PEGDA:HDDA 1.15 + 55%MMA network over 8 weeks of *in vitro* degradation. (B) Crosslinking density profile for PEGDA:HDDA 1.15 + 55%MMA networks of varying PEGDA:HDDA ratio over eight weeks of *in vitro* degradation. Each point is the mean $\pm$ standard deviation, n=3.

Figure 5. Exemplary stress-strain curves of PEGDA:HDDA 1.15 +55%MMA networks of varying PEGDA:HDDA ratio over eight weeks under *in vitro* degradation conditions. Ratio 0:100 (A), 10:90 (B) 25:75 (C).

Figure 6.

Elastic modulus profile for PEGDA:HDDA 1.15 +55%MMA networks of varying PEGDA:HDDA ratio over eight weeks under *in vitro* degradation conditions. Each point is the mean $\pm$ standard deviation, n=3. *p value <0.05 vs. previous timepoint of same network. ^p value <0.05 for 25:75 network vs. 10:90 network and for 25:75 network vs. 0:100 network at given timepoint.

Figure 7. Photomicrographs of skin from the back of mice after two weeks of implantation. Sections were obtained and stained for H&E, trichrome, and with a murine macrophage-specific antibody (Mac3).