Published in final edited form as:

Acta Biomater. 2008 March ; 4(2): 207–217.

Controlling poly(β -amino ester) network properties through macromer branching

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Abstract

Photopolymerizable and degradable biomaterials are becoming important in the development of advanced materials in the fields of tissue engineering, drug delivery, and microdevices. We have recently developed a library of poly(β -amino ester)s (PBAEs) that form networks with a wide range of mechanical properties and degradation rates that are controlled by simple alterations in the macromer molecular weight or chemical structure. In this study, the influence of macromer branching on network properties was assessed by adding the trifunctional monomer pentaerythritol triacrylate (PETA) during synthesis. This led to a dose-dependent increase in the network compressive modulus, tensile modulus, and glass transition temperature, and a decrease in the network soluble fraction, yet led to only minor variations in degradation profiles and reaction behavior. For instance, the tensile modulus increased from 1.98 ± 0.09 MPa to 3.88 ± 0.20 MPa when the macromer went from a linear structure to a more branched structure with the addition of PETA. When osteoblast-like cells were grown on thin films, there was an increase in cell adhesion and spreading as the amount of PETA incorporated during synthesis increased. Towards tissue engineering applications, porous scaffolds were fabricated by photopolymerizing around a poragen and then subsequently leaching the poragen. Interconnected pores were observed in the scaffolds and observed trends translated to the porous scaffold (i.e., increasing mechanics with increasing branching). These findings demonstrate a simple variation during macromer synthesis that can be used to further tune the physical properties of scaffolds for given applications, particularly for candidates from the PBAE library.

Keywords

Macromer; Biodegradable; Tissue Engineering; Branching; Photopolymerization

1. Introduction

Radically polymerized materials are useful as biomaterials for numerous applications. For instance, bone cements and methacrylate monomers for filling dental caries have a history of use in medicine [1]. Light-initiated photopolymerizations are a type of radical polymerization that occurs at physiologically relevant temperatures (37°C) and with numerous wavelengths of light [2]. Photoinitiated polymerizations also offer spatial and temporal control over the reaction, which is useful in minimizing polymerization exotherms [3] and for fabricating complex structures [4,5].

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Many biomaterials have been developed that are photopolymerizable and degradable, including polyanhydrides [6], poly(propylene fumarate)s, [7], poly(ethylene glycol)s [8,9], and polysaccharides [10,11]. These materials have been investigated for a wide range of applications, including cartilage and bone regeneration [8,11,12], cardiovascular applications [13,14], drug delivery [15,16], and in microfluidic devices [17]. One limitation in engineering these materials toward a specific application is that tuning their properties iteratively or predicting their behavior can be tedious and difficult.

To accelerate the identification of potentially useful biodegradable and free-radically polymerized polymers, Anderson et al. developed a combinatorial approach for the synthesis of a library of PBAE macromers that are rapidly synthesized and form networks with a wide range of properties [18]. Specifically, network degradation times ranged from less than one day to over 4 months and the mechanics spanned two orders of magnitude (~2 MPa to ~200 MPa). The synthesis involves the reaction of commercially available diacrylates with amines and no purification of the synthesized macromers is necessary. Brey et al. [19] further expanded the library by investigating the influence of macromer molecular weight, through changes in the ratio of acrylate to amine, on network properties. This led to changes in network mechanical properties, degradation rates, and cell adhesion.

The primary objective of this work was to examine the importance of macromer branching on network properties, since branching can be easily altered during the synthesis of PBAEs by introduction of a reagent with higher functionality (e.g., triacrylate). This structural feature has only been minimally investigated and little is known about the influence of macromer branching on biodegradable polymers. To this end, one specific composition from the PBAE library was used and a triacrylate group was systematically introduced during synthesis, while maintaining the overall molar ratio of acrylates to amines. The influence of PBAE macromer branching on the overall bulk properties and initial cellular interactions of formed networks was investigated. Information obtained was also used to control scaffold mechanical properties. This will hopefully provide an additional tool to control scaffold properties for tissue regeneration.

2. Materials and methods

2.1. Macromer synthesis and characterization

Acrylate terminated PBAEs were synthesized in parallel by the step-growth polymerization of a commercially available primary amine, 3-methoxypropylamine (1, TCI America), and different ratios of 1,6-hexanediol ethoxylate diacrylate (E, Sigma) and pentaerythritol triacrylate (PETA, Sigma). These liquid reagents were mixed at diacrylate:triacrylate ratios of 100:0, 95:5, and 90:10 while holding the molar ratio of acrylate end groups to amines constant (2.4:1) in glass scintillation vials at 90°C overnight while stirring (700 r.p.m., Telesystem HP15/RM, Variomag USA). The sample notation is consistent with our previous report on the development of the initial PBAE library [18]. Proton NMR spectra were recorded in deuterated chloroform on a Bruker Avance 300 MHz instrument and calibrated to the chloroform (7.27 p.p.m.) impurity as an internal standard.

2.2. Photopolymerization

The photoinitiator 2,2-dimethoxy-2-phenyl acetophenone (DMPA, Sigma) was added to the liquid macromers at a final concentration of 0.5% (w/w) by addition of a 10% (w/v) DMPA in methylene chloride. The solvent was removed in a vacuum desiccator overnight and all polymerizations were performed in bulk. The polymerization behavior was monitored using attenuated total internal reflectance – Fourier transform infrared (ATR-FTIR, Nicolet 6700, Thermo Electron) spectroscopy with a zinc selenide crystal collecting a spectrum every 17

seconds with a resolution of 3.86 cm^{-1} for 10 minutes. A drop of the macromer/initiator solution was placed directly on the horizontal crystal, covered with a glass cover slip, and exposed from above to ultraviolet light ($\sim 1.3 \text{ W cm}^{-2}$ at tip of light guide, distance of 24 cm, 365 nm, Omnicure Series 1000, Exfo). The change in area of the double-bond peak ($\sim 1630 \text{ cm}^{-1}$) was used to monitor double-bond conversion with light exposure. Values were normalized to the area of the carbonyl peak ($\sim 1730 \text{ cm}^{-1}$) and converted to double-bond conversion using the initial peak areas. Three samples for each macromer group were tested.

2.3. Network characterization

In order to test the bulk polymer properties, the macromer/initiator solution was placed between glass slides with a 1 mm spacer and polymerized with exposure to $\sim 10 \text{ mW cm}^{-2}$ ultraviolet light (365 nm, Blak-Ray B-100 AP, Ultraviolet Products) for 10 minutes. Polymer disks (5 mm diameter $\times$ 1 mm thick) were then punched from the resulting networks for testing. The soluble (sol) fraction of the various networks was determined by placing 3 disks of each polymer in methylene chloride. This allowed unreacted macromer to swell from the network. After drying, the sol fraction was calculated as the percent of initial mass lost during 48 hours of swelling. Negligible mass loss was observed with subsequent swelling and drying cycles, indicating that the sol fraction was removed during the first cycle.

For degradation analysis, 4 disks were punched from the slabs and weighed (initial mass). Samples were placed in histology cassettes and degraded in phosphate buffered saline (PBS) at 37°C (pH 7.4) on an orbital shaker with frequent changes of the PBS. Samples were removed after 2, 4, 7, and 10 weeks of degradation, dried in an oven, and weighed (final mass). The overall mass loss was calculated from the recorded initial and final mass values.

To investigate kinetic chain lengths, a previously reported isolation process was used [20]. Briefly, thin samples (0.56 mm thick $\times$ ~ 1.5 cm diameter) were rapidly degraded in 4 N NaOH over 3 days at 37°C . Thin samples were used to limit depth variations with light attenuation. The pH of the solution was then adjusted to 8–9 with the addition of 4 N HCL and filtered through a $0.45 \mu\text{m}$ syringe filter. Samples were then dialyzed (Spectra/Por® Biotech Cellulose Ester Membrane, molecular weight cut off: 500, Spectrum Laboratories) and lyophilized (Freezone 4.5, Labconco). Finally, samples were dissolved in DI water at $2 \mu\text{g ml}^{-1}$ and run in aqueous gel permeation chromatography (GPC, 1 ml min^{-1} , 515 HPLC Pump, Ultrahydrogel Linear and 250 columns, 2414 Refractive Index Detector, Waters) to determine the kinetic chain molecular weights compared to PEG standards (M_n , 35000, and 203000).

2.4. Mechanical testing

For mechanical testing, slabs were prepared as described above with either a 1 mm (tensile, dynamic, degradation samples) or 2 mm (compression samples) spacer. The polymers were then cut into $25 \text{ mm} \times 5 \text{ mm}$ samples for tensile and dynamic testing and 5 mm disks were punched for compression testing. Mechanical properties with degradation were determined through tensile and compressive analysis of samples prepared as described above after 2, 4, and 8 weeks of degradation, using the previously described degradation procedure.

Tensile testing was performed on a materials testing machine (Microtester 5848, Instron) with a constant strain rate of 0.1% per second until macroscopic failure. The elastic modulus was then calculated as the slope of the linear portion of the stress versus strain plot. Unconfined compression testing was completed on a custom-made mechanical testing device designed as described in Soltz et al. [21]. Samples were creep-tested under a 2 g load until equilibrium and then stressed to 10% strain at $1 \mu\text{m s}^{-1}$ and allowed to relax. The compressive moduli were calculated as the slope of the stress versus strain plots between 5 and 10% strain.

The viscoelastic behavior of the samples was determined using a Dynamic Mechanical Analyzer (Q800 TA Instruments). Rectangular strips (25 mm × 5 mm × 1 mm) of polymer were cut from polymer slabs and tested in a controlled strain mode at 1 Hz, an amplitude of 10 μm, and a heating rate of 3°C min⁻¹ from -100°C to 25°C. The glass transition temperature, T_g , is reported as the inflection point of the tan δ (the storage modulus over the loss modulus) curve. The cross-linking density, ν_c , was calculated using the theory of rubber elasticity [22]:

$$\nu_c = E' / 3RT$$

where E' is the storage modulus in the rubbery plateau region at a given temperature T , R is the gas constant (8.314 J K⁻¹ mol⁻¹), and assumes that Poisson's ratio for a rubber is 0.5.

2.5. Cell interaction studies

To prepare films for cell interaction studies, the macromer/initiator solutions were dissolved in ethanol at a 1:2 (w/v) ratio and pipetted (35 μl) into 24-well plates. The ethanol was allowed to evaporate off overnight to leave a thin film of the macromer and initiator. The plates were placed in a transparent chamber being purged with nitrogen and polymerized for 10 minutes with ultraviolet light (Blak Ray). To sterilize the films, the plates were placed under a germicidal lamp in a laminar flow hood for 30 min. Wells were incubated with PBS overnight to allow unreacted monomer to be removed. After washing with PBS, the wells were incubated with growth media for 30 minutes prior to cell seeding.

Human sarcoma osteoblast-like cells (SaOS-2, ATCC) were grown in media comprised of Modified McCoy's Medium (ATCC) with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin/streptomycin (Invitrogen). SaOS-2 cells were seeded on the polymer films at 50 000 cells per well (24-well plate). The cells were cultured for 2 and 7 days, fixed in 2.5% glutaraldehyde (Polysciences) for 15 minutes, and cell nuclei were stained with DAPI (1:2500). Phase contrast and fluorescent photomicrographs were taken using an inverted microscope (Axiovert, Zeiss) and a digital camera (Axiovision, Zeiss). The total cell number was determined by counting adhered cell nuclei in at least five random fields on three individual films for each composition at each time point. Cell area was measured using NIH ImageJ software (3 samples per polymer/ratio, 5 pictures per well, 20 cells per picture, at least 300 cells per composition).

2.6. Scaffold fabrication and characterization

Porous scaffolds were fabricated using previously developed techniques of salt leaching [23] and sintered poly(methyl methacrylate) (PMMA) microbeads [24]. Briefly, a solution of 2:1 (w:v) macromer:ethanol containing DMPA and NaCl crystals (sieved to 100–300 μm) were mixed together (80:20 mass ratio NaCl:macromer) and packed into teflon molds. The ethanol was evaporated overnight in a vacuum desiccator, and the resulting salt-macromer mixture was cross-linked with ultraviolet light exposure (as described above) between glass slides. Samples were then cut, the salt was leached with several washes in DI water, frozen in liquid nitrogen, and lyophilized for scanning electron microscopy (SEM) and mechanical testing in compression as describe above.

For spherical porosity, PMMA microbeads (250 μm, Polysciences) teflon molds, clamped between glass slides, and placed in an oven at 120°C for 22 hours [24]. The glassy top layer was then scraped away, and the voids between the sintered spheres were filled with macromer/DMPA/ethanol solutions. The ethanol was evaporated overnight in a vacuum desiccator, the constructs were cross-linked with ultraviolet light exposure (as described above) between glass slides, the PMMA was removed with several washes in methylene chloride, and the methylene

chloride was removed with several washes in water. Samples were then frozen and lyophilized before SEM analysis and compressive mechanical testing as described above.

2.7. Statistical analysis

Statistical analysis was performed using ANOVA with Tukey's post-hoc among the groups with significance defined as a confidence level of 0.05. All values are reported as the mean and the standard deviation of the mean.

3. Results and discussion

3.1. Synthesis of macromers

Recently, a large library of PBAEs, synthesized via a step growth polymerization of liquid amines and diacrylates, was developed for biomaterial applications [18]. The biggest advantage to this system is that the reaction scheme is simple since there is no purification necessary and that a large number of networks formed from the PBAEs can be rapidly synthesized and screened for applications in tissue regeneration. Although the diversity in properties through alterations in the macromer chemistry and molecular weight [19] was explored, no investigations have been completed on the influence of structural features, namely branching, on network properties.

Thus, we synthesized one macromer system (Fig. 1), with a variety of branching structures, to illustrate the diverse properties possible through this structural variation. The macromer made from the diacrylate E and the amine 1 (E1) was selected because it did not have optimal cell interactions, and thus could illustrate the potential improvement in properties with the addition of branching. The system involved the synthesis of a diacrylate (E) and a primary amine (1) to form a linear diacrylated macromer, or alternatively, a system of branched multiacrylated and linear diacrylated macromers with the addition of small quantities of triacrylate (PETA). This was performed in ratios of diacrylate to triacrylate (E:PETA) of 100:0, 95:5, or 90:0, while maintaining the overall molar ratio of acrylate end groups to amines constant. Higher E:PETA ratios (e.g., 85:15 or 80:20) led to cross-linking during synthesis (insoluble in organic solvents) and could not be examined, as opposed to the tested macromers, which were viscous liquids and soluble in organic solvents. The number of repeat units in the linear E1 was determined by NMR using end group analysis to be 5.66 ($\bar{M}_n$), assuming that all end groups are acrylates. This technique was not used for the branched system since assumptions on the functionality of the macromers would be necessary. Incorporation of PETA into the macromer was confirmed by NMR spectroscopy through the disappearance of the acrylate peak specific to the PETA monomer at 5.9 p.p.m. (Fig. 2). Moreover, previous efforts to obtain gel permeation chromatography (GPC) data for these macromers were unsuccessful due to their highly cationic nature [19].

Polymer branch content can be determined using various techniques such as GPC, NMR, rheology, and small-angle scattering [25]. Based on the structures of the starting reagents, it is anticipated that the macromers will contain short-chain branches as opposed to long-chain branches (molecular weight indistinguishable from the main chain). GPC, small-angle scattering, and rheology are techniques that are useful for determining long-chain branching as opposed to short-chain branching [25] and could not be used for macromer characterization in this work. Although high-frequency (188.6 MHz) ^{13}C NMR can be used to quantify short-chain branching, it is limited to high levels of short-chain branching because it is very difficult to assign chemical shift values to side chains greater than six or more carbon atoms in length [25]. Therefore, based on the chemical structures of the starting reagents, it is very difficult to quantify the branch content of this system via traditional techniques.

3.1. Photopolymerization and degradation behavior

The photopolymerization behavior of the macromers was monitored in real time using ATR-FTIR and the results are shown in Fig. 3. The rate of double-bond conversion (Fig. 3a) was slightly faster for the most branched system (90:10 E:PETA) initially ($p < 0.05$ at 2 min, $50.7 \pm 2.0\%$ for 90:10 vs $31.2 \pm 6.8\%$ for 95:5 and $32.3 \pm 2.5\%$ for 100:0) and conversion plateaued for all samples at approximately 5 min. However, there was no statistical difference between all macromer groups in ultimate double bond conversion after 10 min (Fig. 3b). Although the ultimate conversions are similar, the amount of unreacted macromer molecules may be higher for the less branched system, since the probability is higher that a branched macromer with a greater number of functional groups is incorporated into the network. This is apparent in our sol fraction calculations shown in Table 1. The linear macromer (100:0 E:PETA) had almost twice the sol fraction that our most branched system (90:10 E:PETA) had. The release of unreacted macromer is very important in the application of these polymers to biological systems, where released molecules may be toxic to the surrounding cells and tissue. Thus, macromer branching may be useful in limiting this effect.

The mass loss with degradation in PBS at 37°C showed similar profiles between the macromers (Fig. 4a). The networks degrade via hydrolysis of ester groups in the cross-links into low-molecular-weight degradation products and kinetic chains (Fig. 1). In all groups, there was an initial period of more rapid mass loss and then a slowing of the rate of mass loss with time. In the initial 2 weeks there was greater mass loss in the network formed from the linear macromer (E:PETA 100:0), possibly due to the greater amount of sol fraction that is present in that system. After 10 weeks, networks formed from the E:PETA 90:10 macromers had the greatest mass loss ($p < 0.05$), but the difference was still only ~5% between the different networks.

During polymerization, nondegradable linear kinetic chains (Fig. 1) form from the free-radical polymerization of the acrylate groups. These kinetic chains are a degradation product and the molecular weight of these chains can give some insight into the network formation and structure [20]. When isolated and analyzed (Fig. 4b), a trend of an increase in molecular weight (both M_n and $\bar{M}_w$) with an increase in branching was observed; however, there was no statistically significant differences between groups. In this process, all low-molecular-weight degradation products are removed and only the kinetic chains remain. It should also be noted that kinetic-chain lengths change with sample depth [20] and thin films were used in this study to eliminate this factor.

3.2. Mechanical properties

The mechanical properties of the networks were investigated in both tension and unconfined compression and the results initially as well as with degradation are found in Fig. 5. A statistically significant difference in moduli was found initially between all of the different macromers used and the 90:10 E:PETA macromer formed networks with a modulus almost double that formed from the 100:0 E:PETA macromer. Similar trends were observed for the networks in both tension and compression. The more complete cross-linking (lower sol fraction) and the greater chance of a macromer forming cross-links between kinetic chains due to higher functionality likely contribute to this increase.

With degradation, the mechanical properties decreased with time. This is expected from bulk eroding networks and was observed regardless of the macromer branching. However, the moduli of the higher branched networks actually decreased more quickly with degradation. For instance, the moduli of networks formed from the 90:10 E:PETA macromer was actually the weakest at 8 weeks, potentially due to trends towards greater mass loss for this composition (statistically greater at 10 weeks).

The T_g was also measured using dynamic mechanical testing. A representative plot of the temperature-dependent mechanics is shown in Fig. 6 and the data is listed in Table 1. There was a small but statistically significant change in T_g between networks formed from the 100:0 and 95:5 E:PETA macromers, and networks formed from the 90:10 E:PETA macromer had a more pronounced and higher T_g ($p < 0.05$) than the other groups. The cross-linking density (Table 1) is also significantly ($p < 0.05$) larger as that amount of triacrylate is increased during synthesis. This potentially reduces the amount of free volume as the chains are cross-linked together, preventing the microstructure from transitioning freely from the glassy to rubbery state and could explain variations in the T_g between groups.

Others have reported the branching effects on the physical properties of polymers [26–28], yet not in the manner that is reported here. For example, Tian et al. [28] melt-grafted PETA and peroxide to linear polypropylene (PP) to form long-chain-branching PP. They found that increased branching led to increased zero-shear viscosity and shear thinning at lower temperatures. Additionally, the shear modulus (G') and loss angle ($\tan \delta$) were increased and decreased, respectively, at lower temperatures with increased branching. Previous work by Nivasu et al. [27] incorporated PETA as a monomer during network formation, but not into macromer synthesis as is shown here. They found a reduction in polymerization efficiency, but did not investigate the influence of branching on mechanics and degradation. A review by McKee et al. [29], discussed the effect of short-chain branching on T_g , where short-chain branching was found to lower T_g . These are somewhat different than our case because the end groups are not cross-linked, so they are able to move. From free volume theory, non-cross-linked branches have increased free volume due to these branches, which decreases T_g . In the case of cross-linked polymers, the free volume is reduced since the chains are actually bound together and not allowed to move independent of one another.

3.3. Cellular interaction studies

As an initial step towards investigation of branching influences on tissue engineering scaffolds, osteoblast-like cells (SaOS-2) were plated on thin films of networks formed from the various branched macromers and cultured for up to 1 week. Representative photomicrographs of the cells after 2 and 7 days are shown in Fig. 7. While cells do adhere to the films in all cases, the attachment was much greater and the cells were more spread as the branching increased. After one week, a clear difference can be seen in cellular morphology, where almost all of the cells on films formed from the 100:0 E:PETA are rounded, as compared to many viable and spread cells that are found on films formed from the 90:10 E:PETA macromers. These observations are also inherent in the quantification of cell adhesion and spreading reported in Fig. 8. There is a statistical increase in adhesion and proliferation (2 and 7 days) on networks formed from the branched macromers over the linear macromer at both time points, but none of the values reaches that of control TCPS. Again, the attachment and spreading are most similar to TCPS for that of the most branched macromer (90:10 E:PETA). For spreading results, the percentage of cells that are less than $300 \mu\text{m}^2$ drops from 88% to virtually zero on films formed from the 100:0 to 90:10 E:PETA macromers. There are still not as many large cells ($>1500 \mu\text{m}^2$) as there are on the controls, but the cell morphology is much more similar.

These changes in cellular interactions with macromer branching may be attributed to a number of factors such as the presence of unreacted end groups (as demonstrated by FTIR conversion $< 100\%$) and the mechanics of the polymer. As shown here, the linear macromers led to networks with a greater amount of potentially unreacted molecules, which may be toxic to cells. Additionally, others have reported the relationship between substrate mechanical properties and cellular attachment and function [30–32], which may play a limited role. Ultimately, these results illustrate the potential in using macromer branching to alter network properties (e.g., mechanics, sol fraction), which can influence cellular interactions with

biodegradable polymers. Mechanical properties can control cellular interactions, an important factor in the success of scaffolding in many tissue engineering approaches. It should be noted that these networks were not chosen based on their superior interactions with cells, but because they are useful in illustrating these branching effects. This approach will be useful for many polymers, and especially for candidates from the PBAE library.

3.4. Tissue engineering scaffolds

As a final step towards illustration of the impact of macromer branching on the development of scaffolds for tissue regeneration, two different methods were investigated to develop interconnected, porous scaffolds. Two methods were used to illustrate the diversity in using branching to influence scaffold properties and that changes in these properties do not depend on the scaffold fabrication process. These include both poragen leaching [23,33] and sintered-PMMA microbead [24] techniques that have been used successfully for scaffold fabrication from other polymers and are particularly appropriate for the formation of scaffolds from photoreactive macromers. The polymerization around poragens allowed for simple control over the size of pores through the size of the salt crystals or microbeads and the interconnectivity was created by the packing of the salt-macromer mixture or through the sintering of the beads. The porosity, pore size, and pore interconnectivity are important for the transport of nutrients and waste by diffusion, as well as the infiltration and growth of cells and vasculature [34–36].

The scaffolds created with the sintered microspheres are shown in Fig. 9a. These scaffolds contain very regular, interconnected pores, created by the sintering of PMMA beads together, photo-cross-linking of the macromer in the void space, and dissolution of the PMMA using a solvent. The use of spheres may lead to a more regular structure than alternative poragens, and consequently, greater connectivity and mechanical properties [37]. When the scaffolds were tested in compression (Fig. 9c), similar mechanical property trends were noted that were found with the slabs, with the 90:10 samples significantly ($p < 0.05$) greater than the 95:5 and 100:0. Specifically, an increase in macromer branching led to scaffolds with greater moduli (though not significantly between 100:0 and 95:5), illustrating that the effects of macromer structure translate to scaffold properties.

As an alternative technique, a salt-leaching process, was also used to obtain porous scaffolds. Fig. 9b shows an example of the porous structure obtained with this technique, with most of the pores following the shape of the salt crystals and an interconnectivity in the structure. Again, the compressive moduli (Fig. 9d) follows the same trend seen in the bulk polymer with an increase in moduli with increased branching. The overall values are greatly reduced from the bulk polymer, but this is expected as there is only ~20% of the polymer remaining. The samples made with salt crystals had considerably higher moduli than their PMMA microbead counterparts. Observations of the SEM images indicate that the scaffolds formed from salt crystals are less interconnected and have greater wall thicknesses, both of which can influence scaffold properties through polymer density and distribution. Additionally, others have enhanced the mechanics of spherical porosity scaffolds through the uniformity of the microspheres prior to cross-linking [37].

While the sintered microspheres lead to the desired scaffold microstructure and the material is able to support cell growth in two-dimensional culture, this is not possible as an injectable system. The PMMA spheres must be formed into a rigid sintered structure prior to implantation and removed using toxic solvents. The use of salt-leaching may not be optimal, but alternative poragens, such as cytocompatible sugar crystals, may be explored for use as injectable formulations. Thus, the composite could be used to fill a tissue defect, polymerized in situ, and then the porogen would dissolve to leave an interconnected porous structure behind.

4. Conclusions

The enhanced branching of network precursors, through the introduction of reagents with higher functionality during macromer synthesis, allows for further diversity of properties from a library of PBAs. Increased macromer branching led to an increase in the initial polymerization rate, but little change in the ultimate double-bond conversion and degradation behavior. However, macromer branching significantly ($p < 0.05$) decreased the network sol fraction and increased the mechanical properties of networks and adherence and spreading of osteoblast-like cells on polymer films. Finally, the macromers were used to form interconnected porous scaffolds using poragen leaching techniques, and scaffold properties followed the same trends in mechanical properties that were found in the bulk polymers. Ultimately, this work indicates that the introduction of branching during macromer synthesis can lead to variations in scaffold properties towards tissue engineering applications.

Acknowledgements

The authors acknowledge assistance from Sarah Grieshaber and Xinqiao Jia for GPC measurements, Isaac Erickson for FTIR measurements, and funding from the NIH (K22 DE-015761) and a University of Pennsylvania University Research Foundation Award to JAB.

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Figure 1.

Schematic of macromer synthesis, network formation, and degradation. The poly(acrylic acid) kinetic chains are shown as dashed lines and are formed during the radical polymerization of acrylate end groups.

Figure 2.

(a) ^1H -NMR spectra of E and PETA acrylate groups prior to synthesis. (b) ^1H -NMR spectra of mixtures of E, PETA, and 1 before and after synthesis, demonstrating the incorporation of PETA into the macromer structure by the disappearance of the peak at 5.9 p.p.m. (indicated by arrow).

Figure 3. Macromer reaction behavior. Real-time double-bond conversion with ultraviolet light exposure for: (a) E:PETA ratios of 100:0, 95:5, and 90:10, and (b) maximum double-bond conversion after 10 minutes. There was no statistical difference in the maximum conversion with changes in macromer branching.

Figure 4. Degradation of networks (in PBS at 37°C) formed with various macromers at ratios of (a) E:PETA of 100:0, 95:5, and 90:10, and (B) molecular weights (M_n and M_w) of kinetic chains isolated from degraded networks formed from the same macromers.

Figure 5.

(a) tensile and (b) compressive moduli initially and with degradation for networks formed with various macromers at ratios of E:PETA of 100:0, 95:5, and 90:10. * denotes significance from other groups (0 weeks).

Figure 6. Example plot of storage modulus (solid line) and $\tan \delta$ (dashed line) for networks formed with E:PETA of 90:10. T_g is defined as the temperature at the peak of the $\tan \delta$ curve.

Figure 7. Osteoblast-like cells on control TCPS (a,b) and on films formed from macromers with E:PETA ratios of 100:0 (c,d), 95:5 (e,f), and 90:10 (g,h) after 2 days (a,c,e,g) and 1 week (b,d,f,h). The scale bar represents 100 μm.

Figure 8.

(a) Thousands of cells per cm² after 1 week, † denotes significance from other groups, †† denotes significance from all other groups, * denotes significance to all other groups, and ** denotes significance from 100:0 at 7 days. (b) Histogram of cell areas (µm²) after 24 hours.

Figure 9. Representative scaffolds fabricated with E:PETA of 95:5 for the sintering technique (a) and salt-leaching techniques (c) and compressive moduli of scaffolds from the sintering (b) and salt-leaching techniques (d) from various E:PETA ratios. * denotes significance from the all other groups.

Table 1

Properties of networks formed of E, 1, and PETA with varying ratios of diacrylate and triacrylate.

E:PETA	Sol fraction (%)	T _g (°C)	Storage modulus (MPa)	Cross-link Density (mol m ⁻³)
100:0	19.5 ± 1.1	-46.5 ± 1.0	2.30 ± 0.20	337.6 ± 28.7
95:5	9.4 ± 0.4	-45.1 ± 0.3	2.99 ± 0.05	438.5 ± 7.1
90:10	10.0 ± 1.2	-40.5 ± 0.4	3.92 ± 0.12	575.0 ± 18.0