Highly elastomeric poly(glycerol sebacate)-co-poly(ethylene glycol) amphiphilic block copolymers

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A B S T R A C T

Poly(glycerol sebacate) (PGS), a tough elastomer, has been proposed for tissue engineering applications due to its desired mechanical properties, biocompatibility and controlled degradation. Despite interesting physical and chemical properties, PGS shows limited water uptake capacity (~2%), thus constraining its utility for soft tissue engineering. Therefore, a modification of PGS that would mimic the water uptake and water retention characteristics of natural extracellular matrix is beneficial for enhancing its utility for biomedical applications. Here, we report the synthesis and characterization of highly elastomeric poly(glycerol sebacate)-co-polyethylene glycol (PGS-co-PEG) block copolymers with controlled water uptake characteristics. By tailoring the water uptake property, it is possible to engineer scaffolds with customized degradation and mechanical properties. The addition of PEG results in almost 15-fold increase in water uptake capacity of PGS, and improves its mechanical stability under dynamic loading conditions. PGS-co-PEG polymers show elastomeric properties and can be subjected to serve deformation such as bending and stretching. The Young's modulus of PGS-co-PEG can be tuned from 13 kPa to 2.2 MPa by altering the amount of PEG within the copolymer network. Compared to PGS, more than six-fold increase in elongation was observed upon PEG incorporation. In addition, the rate of degradation increases with an increase in PEG concentration, indicating that degradation rate of PGS can be regulated. PGS-co-PEG polymers also support cell proliferation, and thus can be used for a range of tissue engineering applications.

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1. Introduction

Development of biodegradable materials has stimulated interest in a range of biotechnological and biomedical applications [1–5]. Amongst them, synthetic biodegradable polymers have been extensively investigated owning to their tunable physical and chemical properties, low batch-to-batch variation, ease of fabrication and modification, and low risk of disease transmission [6–10]. In the last few years, several biodegradable polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(e-caprolactone) (PCL), poly(hydroxybutyrate) (PHB) and their block copolymers have been explored for the development of emerging technologies in biomedical and biotechnological industries [10–12]. This is mainly attributed to their high mechanical strength, and in vivo biocompatibility. Despite the interesting physical and chemical properties, some of these polyster create an acidic local environment upon degradation that causes inflammation to the surrounding tissues [10,13]. Moreover, the conventional polyster follow the bulk degradation mechanism and display exponential decay in their mechanical properties with degradation [14]. Poly(glycerol sebacate) (PGS), a tough elastomer, has been proposed for tissue engineering applications due to controlled and

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linear degradation profiles [14–16]. The surface erodible nature of PGS makes it preferable and unique over the other polyesters for controlled drug delivery and scaffolding applications [16–20]. The elastic modulus of PGS can be easily tuned by controlling various parameters such as reaction time, reaction temperature and time of curing [16]. Additionally, both the reactants, glycerol and sebacic acid, used in the synthesis of PGS are inexpensive and approved by FDA for biomedical applications [21–23]. As a result, PGS has been explored for numerous tissue engineering applications such as myocardial tissue [17], vascular graft [24], cartilage tissue [25], nerve guide [26], retinal transplantation [27], and tissue [25], nerve guide [26], retinal transplantation [27], and surgical sealant [28].

To incorporate different functionalities and tailor physicochemical properties for specific tissue engineering applications, various PGS based copolymeric systems and blends were developed [15,28,29]. For example, lactic acid was incorporated within the PGS backbone and a range of copolymers were developed by varying the molar ratio of glycerol, sebacic acid and lactic acid [30]. The addition of lactic acid to PGS resulted in the increased mechanical properties and decreased degradation rates [30]. However, with an increase in lactic acid concentration, the surface degradation characteristic of PGS was compromised with the bulk erosion behavior [30,31]. In another study, poly(glycerol sebacate-co-glycolic acid) (PGS-GA) with different reactant ratios was developed [31]. The addition of glycolic acid to PGS decreases the elastic modulus and increases the degradation rate of the copolymer network [31]. Most of these studies aimed at tuning either the degradation behavior or the mechanical properties of PGS with a very limited focus on improving the hydration properties.

Hydration properties of biomaterials is an important parameter for tissue engineering applications as it directly determines the mechanical stability, degradation rate, and diffusion characteristic of the scaffolds under dynamic in vivo conditions [32–34]. For example, water uptake within a tissue engineered scaffold should be high enough to promote the mechanical deformation with minimum hysteresis under dynamic/cyclic stresses [32,33]. Moreover, the water uptake and diffusion characteristics of polymeric scaffolds also decide the degradation mechanism of scaffolds as well as cellular behavior [13,35]. For these reasons, there is a need to better control hydration properties of PGS to tailor the mechanical properties, and degradation characteristic.

Recently, citric acid was incorporated within PGS backbone to increase the hydrophilicity of the developed poly(sebacate-glycolate-citrate) (PGSC) copolymers [36]. The presence of additional carboxyl groups enhances the water uptake ability of the PGSC [36]. Another approach to tune the hydration property of polyesters is to design polyester-polyester amphiphilic block copolymers [9,37]. A range of polyesters such as PCL, PGA, PLAGA, PLA and PHB, have been copolymerized with polyethylene glycol (PEG), a polyether, to tune swelling degrees [38].

Here, we report synthesis of amphiphilic, biodegradable block copolymers from PGS and PEG. We hypothesized that the incorporation of PEG segments within PGS backbone will allow us to tune the hydrophilicity while maintaining the controlled degradation behavior of PGS. A range of PGS-co-PEG polymers from mechanically stiff to elastomeric soft was synthesized. The effect of addition of PEG to PGS on hydration properties was monitored by hydration kinetics and contact angle measurements. The elastomeric properties of the copolymers were studied by uniaxial tensile, unconfined compression, cyclic tensile and cyclic compression testing. In vitro behaviors of PGS-co-PEG polymers were evaluated by degradation rate, protein absorption/adsorption and cell adhesion properties. We aim to create PGS based amphiphilic block copolymers with tailored chemical and physical properties for a wide range of biomedical and biotechnological applications.

## 2. Materials and methods

### 2.1. Synthesis of poly (glycerol sebacate) (PGS)

The PGS pre-polymer was synthesized by polycondensation of equimolar glycerol (Sigma–Aldrich, Milwaukee, WI) and sebacic acid (Sigma–Aldrich) according to previously published methods [16]. Briefly, an equimolar amount of glycerol and sebacic acid were mixed and stirred for 2 h in a 250 mL two necked round bottom reactor under Argon at 130 °C. The reaction pressure was slowly reduced to 50 mtorr over 5 h and the reaction was continued under vacuum for another 48 h at 130 °C. The pre-polymer samples were collected for spectroscopic analysis. The remaining prepolymer was poured into Teflon crucibles and thermally cured in the vacuum oven at 130 °C for 48 h.

### 2.2. Synthesis of poly (glycerol-sebacate-co-polyethylene glycol) (PGS-co-PEG polymer)

PGS-co-PEG pre-polymers were synthesized via two steps condensation polymerization. The first step involved the polycondensation of sebacic acid and polyethylene glycol (Alfa Aesar, Mw = 1000 g/mol) under stirring condition. PEG was dried in vacuum chamber at 90 °C before its use. The reaction was then carried out at 130 °C under the flow of Argon for 2 h and under vacuum of 50 mtorr for another 24 h. In the second step, a specific amount of glycerol was added into the reactor, mixed thoroughly under the flow of Argon and the reaction was further carried out at 130 °C under reduced pressure of 50 mtorr for 48 h. The samples of pre-polymers were collected for spectroscopic analysis. The viscous pre-polymer solutions were poured in Teflon crucibles and thermally cured in the vacuum oven at 130 °C for 48 h. The overall diol to dicarboxylic acid molar ratio was kept constant. Three molar ratios of PEG to glycerol (20/80, 40/60 and 60/40) were used to develop PGS-co-PEG polymers with different degrees of PEG segments within the resulting copolymer system.

### 2.3. Chemical characterization

The molecular weight of pre-polymer of PGS and PGS-co-PEG polymers was determined using gel permeation chromatography (GPC, Waters, Milford, MA). The samples were dissolved in tetrahydrofuran (THF) (0.5% w/v) and injected at the flow rate of 1 mL/min. Polyethylene standards were used for the calibration. Fourier Transform Infrared (FTIR) spectra of the samples were recorded using Alpha Bruker spectrometer. The average value of 48 scans at 4 cm⁻¹ resolutions were collected for each sample. The FTIR spectra were analyzed for PGS and PGS-co-PEG polymers before and after thermal curing. The pre-polymer samples of PGS and PGS-co-PEG polymers were also analyzed using Nuclear magnetic resonance (¹H NMR) spectroscopy (Varian Inova 500). The pre-polymer samples were dissolved in CDCl₃ and the spectra were recorded at 500 MHz. The resulting data were processed and analyzed using AC DLAB10/1D NMR software. The peak assignment in the NMR spectra for PGS and PGS-co-PEG pre-polymers are listed below. ¹H NMR (PGS) (500 MHz, CDCl₃) δ/ppm: 1.30 (37H, m, −CH₂), 1.62 (18H, d, −CH₂CH(OH)COO−), 2.35 (18H, m, −CH₂O(CH₂)₃CO−), 3.50-3.85 (6H, m, OCH²CH₂O), 3.94 (1H, m, −OCH₂CH(OH)OH), 4.05-4.35 (5H, m, −OCH₂CH(OH)O−), 5.26 (1H, m, −OCH₂CH(OH)O−)

¹H NMR (PGS-co-20PEG) (500 MHz, CDCl₃) δ/ppm: 1.30 (12H, m, −CH₂), 1.62 (6H, d, −CH₂CH(OH)COO−), 2.35 (6H, m, −CH₂O(CH₂)₃CO−), 3.64 (25H, m, −OCH₂), 3.94 (1H, m, −OCH₂CH(OH)OH), 4.05-4.35 (5H, m, −OCH₂CH(OH)O−), 5.09 (1H, s, OCH₂CH(OH)O−), 5.26 (1H, s, −OCH₂CH(OH)O−).

¹H NMR (PGS-co-40PEG) (500 MHz, CDCl₃) δ/ppm: 1.30 (19H, m, −CH₂), 1.62 (9H, d, −CH₂(OH)COO−), 2.35 (9H, m, −OCH₂CH(OH)COO−), 3.64 (76H, m, −OCH₂), 3.94 (1H, m, −OCH₂CH(OH)OH), 4.05-4.35 (9H, m, −OCH₂CH(OH)O−), 5.09 (1H, d, OCH₂CH(OH)O−), 5.26 (1H, s, −OCH₂CH(OH)O−).

¹H NMR (PGS-co-60PEG) (500 MHz, CDCl₃) δ/ppm: 1.30 (14H, m, −CH₂), 1.62 (7H, d, −CH₂(OH)COO−), 2.35 (7H, m, −CH₂O(CH₂)₃CO−), 3.64 (85H, m, −OCH₂), 3.94 (1H, m, −OCH₂CH(OH)OH), 4.05-4.35 (4H, m, −OCH₂CH(OH)O−), 5.09 (1H, s, OCH₂CH(OH)O−), 5.26 (1H, s, −OCH₂CH(OH)O−).

The degree of crosslinked network was determined by sol and gel analysis. Here, samples (4 mm in diameter, 1.2–1.9 mm thickness and initial weight (Wₒ)) were allowed to swell in tetrahydrofuran (THF) for 24 h to elute out the sol contents. The remaining gel contents were weighed after drying (Wₕ) the sample overnight. The percentage of sol contents was calculated by Eq. (1).

\[
\text{Sol(%) = } \frac{W_o - W_h}{W_o} \times 100
\]

### 2.4. Mechanical properties

The mechanical properties of PGS and PGS-co-PEG polymers were evaluated using uniaxial tensile, unconfined compression, cyclic tensile and cyclic compression testing using Instron 5943 Materials Testing System Capacity (Norwood, MA, USA) equipped with 50 N load cell. For uniaxial tensile and cyclic tensile testing, thermally crosslinked samples were cut in a rectangular shape with 10 mm gauge length, 5 mm wide and approximately 1.2–1.9 mm thick. The...
mechanical properties were performed in both as-prepared and hydrated conditions (soaked in PBS at 37 °C for 24 h). For uniaxial tensile test, samples were stretched until failure at the crosshead speed of 10 mm/min. Force-displacement curves that is obtained from the machine were converted to stress-strain curves. The stress (σtens, MPa) was obtained by dividing the applied force (N) with cross-section area (mm²) and strain was obtained from the displacement using (1−L_/Lo)*100/(L_/Lo), where L_/Lo was initial gauge length and L_ was instantaneous gauge length. Young's Modulus was calculated from the linear stress-strain region by fitting a straight line between 5% and 15% strain and toughness of the copolymer network was determined by total area under the stress-strain curve. The ultimate tensile stress, fracture stress and failure strain were also calculated. The uniaxial compression testing was performed with a crosshead speed of 1 mm/min on circular samples with 4 mm in diameter and 1.2–1.9 mm thickness. The 5–15% strain region was used to measure the compressive modulus of the samples and instantaneous drop in more than 20% stress was considered as a fracture point.

For cyclic testing, 5 loading and unloading cycles were performed between 0 and 20% strain. To emphasize the elastic behavior of the copolymer network, the cyclic stress-strain curves (tensile and compression) were represented in Mooney-Rivlin plot using Eq (2) and Eq (3) [39]. For tensile test, the Mooney's stress (σMooney) is plotted as a function of 1/εtens = 1/Lsh, where L is the instantaneous gauge length and L_/Lo is the initial gauge length, and the Mooney stress (σMooney) was calculated as follows [39–41]:

$$\sigma_{\text{Mooney}} = \frac{\sigma_{\text{tens}}}{\varepsilon_{\text{tens}}} - 1/12$$

(2)

For compression test, compressive stress ($\sigma_{\text{Comp}}$, MPa) is obtained by dividing applied force (N) by cross-section area (mm²). Mooney's stress is plotted as a function of 1/εcomp = 1/Lsh, where ε is defined as 1/εcomp and εcomp = h/ho (ho is the initial height and h is the current height of the sample), Mooney stress was calculated as follow [39–41]:

$$\sigma_{\text{Mooney}} = \frac{\sigma_{\text{Comp}}}{\varepsilon_{\text{Comp}}} = \frac{\sigma_{\text{Comp}}}{L/Lo} - 1/12$$

(3)

2.5. Hydration properties and physiological stability

The hydration properties of copolymeric network were determined by contact angle measurements, and hydration kinetics. The contact angle of water on cross-section area (mm²) of Mooney’s plot using Eq (2) and Eq (3) [39]. For tensile test, the Mooney’s stress (σMooney) is plotted as a function of 1/εtens = 1/Lsh, where ε is defined as 1/εtens and εtens = h/ho (ho is the initial height and h is the current height of the sample). Mooney stress was calculated as follow [39–41]:

$$\sigma_{\text{Mooney}} = \frac{\sigma_{\text{tens}}}{\varepsilon_{\text{tens}}} - 1/12$$

2.6. Protein adsorption/absorption

Sample disks (n = 3) of PGS and PGS-co-PEG polymers (20%, 40% and 60% PEG) having 4 mm diameter were soaked at 37 °C in PBS for 24 h. The PBS was aspirated and disks were soaked in 500 μl of protein solution for 24 h at 37 °C. For protein adsorption from fetal bovine serum (FBS, Gibco, USA), 10% (v/v) FBS in 1 × PBS was used, whereas for fibronectin adsorption study, 50 μg/mL of fibronectin in 1 × PBS was used. The samples were then washed 3 times in PBS to extract any non-specific adsorbed proteins. The samples were then treated with 2% SDS solution for 6 h in a shaker maintained at 50 rpm to extract the adsorbed proteins. The supernatant was collected separately by centrifuging the samples and the eluted proteins were analyzed using micro Bicinchoninic acid (BCA) protein assay reagent (Pierce BCA, Thermo Scientific) and quantified using a UV/VIs spectrophotometer (Epoch Biotech Instruments) at 562 nm.

2.7. In vitro studies

The cell adhesion properties of the polymers were assessed by seeding NIH 3T3 fibroblast cells on different compositions of PGS-co-PEG polymers. Briefly, the cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM, Gibco, USA), supplemented with 10% FBS, and 1% antibiotic (penicillin/streptomycin, Gibco, USA) in a humidified atmosphere with 5% of CO₂ at 37 °C. When the culture reached 80% confluence, the cells were trypsinized (0.05% Trypsin/EDTA, Gibco, USA) from the culture tissue flask, subsequently re-suspended in culture medium and seeded on PGS-co-PEG polymers at a density of 5 × 10⁴ cells per sample in a low cell-adhesive 24-well plate. Cells were allowed to adhere for 1 h and then 500 μl of medium was added. The proliferation rate of the adhered cells on day 1, 4 and 10 was assessed using an Alamar Blue assay (Invitrogen) following standard protocol. Tissue culture polyethylene (TCP) surface was used as a positive control.

2.8. Statistics

Experimental data were presented as mean ± standard deviation. Statistical differences between the groups were analyzed using one-way ANOVA using Tukey post-hoc analysis and two-way ANOVA. Statistical significance was represented as *p < 0.05, **p < 0.01, ***p < 0.001.

3. Results and discussion

3.1. Synthesis of PGS-co-PEG polymers

The synthesis of PGS-co-PEG polymers was performed in three steps (Fig. 1a). In the first step, polycondensation reaction of PEG and sebacic acid was carried out in order to get a linear prepolymer and to avoid any crosslinking. In the second step, glycerol was added to obtain a block copolymer of PGS-co-PEG (pre-polymer) with different ratio of PEG segments. In the third step, the pre-polymer was thermally crosslinked. The crosslinking density of the pre-polymers can also be altered by varying the time and temperature of curing [15], however the curing conditions were kept constant in this study to investigate the effect of PEG on PGS network.

A series of PGS-co-PEG polymers was designed by altering the glycerol/PEG molar ratios. The nomenclature of synthesized PGS-co-PEG polymers was based on the glycerol/PEG molar ratios and was represented as PGS-co-xPEG, where “x” represents the molar concentration of PEG within the PGS. For example, the PGS-co-20PEG represents the copolymer with 20% PEG and 80% PGS. The hydrophilicity of the final block copolymer was tuned by adding PEG in three different ratios (20%, 40% and 60%). The presence of PEG chains within materials increases hydrophilic nature (Fig. 1b). Furthermore, the hydrophilic nature of copolymer network will facilitate the hydrolysis of ester bond. Thus, it is expected that the chemical, mechanical and degradation properties can be tailored by altering the amount of PEG within PGS backbone.

3.2. Chemical characterization of PGS-co-PEG polymers

The structure of pre-polymer (after the second step of poly-condensation) was investigated using 1H NMR spectroscopy (Fig. 2a and Fig. S1). In the 1H NMR of PGS, the methylene peaks related to sebacic acid were identified at 1.30, 1.62 and 2.35 ppm, whereas peaks between 4.05–4.35 ppm and 5.05–5.30 ppm were observed for glycerol. The presence of an additional methylene peak from PEG segment was observed in the 1H NMR spectra of PGS-co-PEG polymers, indicating the presence of PEG segment within pre-polymer solution. The ratio of methylene hydrogen within PEG and sebacic acid were calculated from NMR data (Fig. 2b). The experimental ratio from NMR correlates well with the theoretical estimation indicating close control over the polymer synthesis process. Furthermore, the presence of ester bonds in the pre-polymer was investigated by using FTIR. A strong peak around 1730 cm⁻¹
corresponding to the ester group was observed which confirms that all the pre-polymer contain PGS (Fig. S2).

The molecular weight of pre-polymer of PGS and PGS-co-PEG polymer was determined by using gel permeation chromatography (GPC). The results indicate that the molecular weight \( M_w \) of PGS was 5012 Da (polydispersity index \( PDI = 2.64 \)). The addition of 20, 40 and 60% PEG resulted in copolymer with \( M_w \) of 4998 Da \( (PDI = 1.48) \), 4037 Da \( (PDI = 1.42) \) and 3789 Da \( (PDI = 1.4) \), respectively, indicating close control over the condensation reaction.

A fully crosslinked copolymer was obtained by subjecting the pre-polymer solution to thermal curing process at \( 130 \, ^\circ C \) for 48 h. The additional hydroxyl groups \( (-OH) \) present on PGS backbone react with unreacted sebacic acid to form crosslinked networks (Fig. 3a). The effect of thermal crosslinking on the polymer chains was investigated by monitoring FTIR spectra before and after the curing process (Fig. 3b). The results indicated that the peaks at 1350 cm\(^{-1}\) (-COOH) and 1100 cm\(^{-1}\) (-OH) decreased and the peak at 1150 cm\(^{-1}\) (-COO) increased after the thermal crosslinking.

Fig. 1. Synthesis of PGS and PGS-co-PEG polymers. (a) PGS was synthesized by polycondensation of equimolar glycerol and sebacic acid. The synthesis of PGS-co-PEG polymers involves polycondensation of PEG and sebacic acid to obtain a linear polymer chain, followed by addition of glycerol to obtain a block copolymer of PGS-co-PEG. The ratio of glycerol to PEG was altered to obtain copolymers with different degree of amphiphilicity. (b) The addition of PEG reduces available hydroxyl group on copolymer network and increases hydrophilicity of PGS-co-PEG copolymers. The increase in PEG concentration renders dissolution of copolymer in water.
The FTIR spectra of the cured PGS-co-PEG polymers were shown in Fig. 3c. The reduction in the hydroxyl peak at 3500 cm⁻¹ was observed with an increase in the PEG segment. In PGS, the ratio of methylene (-CH₂) to hydroxyl (-OH) peak was 3.57. The addition of PEG results in an increase in CH₂/OH ratio to 5.94, 7.39 and 7.5 for PGS-co-20PEG, PGS-co-40PEG and PGS-co-60PEG, respectively. At higher PEG concentrations (40 and 60% PEG), the CH₂/OH ratio was similar. This indicates that the number of additional hydroxyl groups were quite limited in copolymer containing above 60% PEG and result in lower crosslinking density.

The decrease in the crosslinking density due to the addition of PEG was also investigated by determining the sol content (unreacted pre-polymer) within the covalently crosslinked PGS-co-PEG polymer network. The presence of sol content within the crosslinked network was evaluated by swelling the network in THF. The high swelling degree of copolymers within THF allows the sol to diffuse-out. The gel content (crosslinked network) can be determined by obtaining the dry weight of remaining copolymer network (Fig. S3). The results indicate that the thermally crosslinked PGS comprises of 9.9 ± 4.3% sol content. The presence of low amount of sol content indicates high crosslinking density. This was mainly attributed to the presence of free hydroxyl groups on the polymer (PGS) backbone that can be used to form covalently crosslinked network. The addition of PEG decreased the amount of free hydroxyl groups and thus decreased the crosslinking density. This was shown by an increase in the sol contents of PGS-co-PEG copolymers compared to PGS.

### 3.3. Highly elastomeric and tough PGS-co-PEG polymers

Evaluation of new polymeric biomaterials for tissue engineering application requires extensive mechanical characterization under various in vivo conditions. Earlier studies reported that the mechanical properties of PGS could be tuned by changing the curing temperature and time [15–17]. For example, by increasing the curing temperature of PGS from 110 °C to 130 °C, Young’s modulus, ultimate stress and elongation can be altered from 1.2 kPa to 56 kPa, 230 kPa—470 kPa, and 448% to 41%, respectively. In our study, we used curing temperature of 130 °C and curing time of 48 h, and the mechanical properties of PGS reported here are similar to the previously published results [17].

PGS is an elastomeric polymer [16], and the addition of PEG further enhances its elastomeric properties (Fig. 4). For example, PGS-co-PEG polymers can be subjected to severe deformation such as bending and stretching without fracturing the structure (Fig. 4a). We investigated the mechanical properties of PGS and PGS-co-PEG polymers using uniaxial tensile test in dry (as-prepared) and hydrated conditions. From hydration kinetics data, we observed that the addition of PEG to PGS significantly increases water uptake capacity of the copolymeric network. In order to evaluate the effect of water uptake on the tensile properties of the copolymeric network, we allowed the samples to hydrate in PBS for 24 h and then subjected it to uniaxial tensile test. Fig. 4b represents the stress-strain curve of PGS and PGS-co-PEG polymers in dry and hydrated conditions. The mechanical properties such as Young’s modulus, ultimate stress, fracture stress and ultimate elongation were calculated from the stress-strain curves.

More than a two-fold increase in elongation was observed due to the addition of 60% PEG (107.9 ± 9.8%) when compared to pure PGS (42.2 ± 5%) in dry conditions (Fig. 4d). Whereas, in fully hydrated conditions, almost a six-fold increase in elongation was observed (PGS = 313.3 ± 3.2% and PGS-co-60PEG = 192.3 ± 20%). This is mainly attributed to high water uptake capacity of PGS-co-PEG polymers that result in higher chain flexibility (Fig. 4c).

The effect of adding PEG to PGS on the mechanical strength and the toughness of the copolymer network was also investigated (Fig. 4e). In dry conditions, PGS has Young’s modulus, tensile...
Fig. 3. Thermal crosslinking of PGS-co-PEG polymer network. (a) Schematic showing that the crosslinking of pure PGS results in the formation of a highly crosslinked network due to presence of hydroxyl group on PGS backbone. The addition of PEG to PGS significantly reduces the crosslinking density due to a decrease in the number of hydroxyl groups. (b) The FTIR spectra of PGS and PGS-co-PEG polymers (20, 40 and 60% PEG) were obtained before and after the thermal crosslinking process. The decrease in carboxyl (COOH), and hydroxyl (OH) group at 1350 and 1100 (and at 3400) cm\(^{-1}\) respectively and increase in ester (COO\(^{-}\)) at 1150 cm\(^{-1}\) indicates thermal crosslinking of PGS. (c) FTIR spectra of PGS and PGS-co-PEG polymers (20%, 40% and 60% PEG) after curing indicate that the CH\(_2\)/OH peak ratio increases.
strength and toughness of 2.39 ± 0.29 MPa, 690 ± 160 kPa and 190 ± 68 kJ/m³, respectively. The mechanical properties of PGS reported here are similar to the previously published results [16]. The addition of PEG to PGS, significantly decreases the modulus, tensile strength and toughness. For example, the addition of 60% of PEG to PGS decreases the Young’s modulus, tensile strength and toughness to 40 ± 10 kPa, 26 ± 4 kPa and 17 ± 3 kJ/m³, respectively. This was mainly attributed to the decrease in crosslinking density due to a lower number of hydroxyl groups in the PGS backbone.

It was observed that in PGS, both tensile strength (500 ± 76 kPa) and toughness (84 ± 26 kJ/m³) decrease significantly without affecting the Young’s modulus (2.26 ± 0.20 MPa) and elongation (31.3 ± 3.2%) when subjected to physiological conditions. Whereas, more than a three-fold decrease in modulus (13 ± 2 kPa) and a two-fold (12 ± 2 kPa) decrease in ultimate strength was observed in PGS-co-PEG compared to the dry conditions. In PGS, almost a two-fold decrease in toughness was observed after hydration, whereas PGS-co-60PEG had a similar toughness in both dry and hydrated conditions.

The previous study on mechanical properties on polyesters such as PLA, PLGA, PCL and copolymers blended with PGS showed high mechanical properties in dry conditions, which drastically decreases after soaking in saline buffer or media [29,30]. The change in the mechanical properties of the conventional polyesters in dry and wet conditions, may induce an inflammatory response that enhances fibrous capsule formation [15]. Moreover, high mechanical strength of these polymers/copolymers limit their application for soft tissue engineering (such as cartilage, cardiac and vocal fold), where good stiffness along with highly elastomeric properties are required [15]. The PGS-co-PEG polymer networks reported here...
have high stiffness along with elastomeric properties, making them suitable to engineer scaffolds for a range of soft tissues. For example, the tensile modulus of PGS-co-PEG polymers is similar to human cardiac muscles (0.02–0.15 MPa) [42,43]. Hence, PGS-co-PEG polymers can potentially be used to engineer cardiac patches.

3.4. Compressive properties of PGS-co-PEG polymers

The compressive properties of PGS and PGS-co-PEG polymers were investigated using unconfin ed compression testing in dry (as prepared) and hydrated conditions (Fig. 5a). The stress-strain curve of PGS indicates similar compressive modulus in dry (6.70 ± 0.84 MPa) and hydrated (6.51 ± 0.47 MPa) conditions. Similarly, no significant difference in fracture strain, fracture stress and toughness was observed between dry and hydrated PGS samples (Fig. 5b). As expected, with an increase in PEG content, the compressive modulus of PGS-co-PEG polymers decreases. However, no significant difference was observed for PEG-co-20PEG in dry and hydrated conditions. At higher PEG concentrations (PGS-co-40PEG and PGS-co-60PEG), significant decrease in modulus was observed in dry and hydrated conditions. This can be mainly attributed to an increase in the water uptake ability of the copolymer at higher PEG content.

The fracture stress and fracture strain were only observed for PGS, PGS-co-20PEG and PGS-co-40PEG samples, as the addition of more than 40% PEG to PGS results in the formation of a highly elastomeric network (more than 80% strain). PGS-co-60PEG displays a unique stress-strain behavior that is typically observed in highly elastomeric soft tissues (such as cartilage [44]). In particular, it displays a plateau at low strain (0–60% strain) and almost vertical increase in stress was observed at higher strain (75–85% strain). Moreover, compressive modulus of PGS-co-PEG polymers can be tuned between 3.2 MPa and 9 kPa by varying the PEG concentration, which is in the range of cartilage (0.4–0.8 MPa) [44]. Hence, PGS-co-PEG polymers can potentially be used to engineer cartilage or osteochondral tissues.

3.5. Cyclic tensile properties of PGS-co-PEG polymers

The applicability of PGS-co-PEG polymers to engineer elastomeric tissues that are subjected to repeating or pulsating in vivo mechanical forces, was investigated by evaluating the mechanical properties of the copolymeric network under cyclic tensile conditions. From the uniaxial tensile test, it was observed that, both PGS and PGS-co-PEG polymers display linear stress-strain curve until 20% strain. We subjected the fully hydrated samples to 20% cyclic tensile strain and monitored the loading and unloading stress-strain curve for five consecutive cycles. Pure PGS and PGS-co-PEG polymers showed elastomeric characteristics (Fig. 6 and Fig. S4).

The area between the loading and unloading curve was used to determine the amount of energy absorbed by the crosslinked network and percentage recovery during the deformation cycle [45,46]. Fig. 6a shows that the addition of PEG significantly reduces the amount of energy absorbed (hysteresis). For example, PGS absorbs 6.17 ± 1.23 kJ/m^3 during the first cycle and 2.33 ± 1.53 kJ/m^3 during the fifth cycle, whereas PGS-co-60PEG absorbs 0.019 ± 0.006 kJ/m^3 during the first cycle and 0.011 ± 0.004 kJ/m^3 during the fifth cycle.
during the fifth cycle. Similar trend was observed for the recovery of the crosslinked network. After the first cycle, PGS and PGS-co-PEG polymers show more than 90% of recovery. These results indicate that the energy adsorbed (and recovery of network) in the first cycle was not equivalent to the subsequent cycles. This is mainly due to the plastic deformation of the crosslinked network during the first cycle. Whereas, the energy absorbed (and recovery of network) between second to fifth cycles was almost constant and can be attributed the elastic deformation of the network. This also indicates that after the first cycle, the crosslinked network showed little energy dissipation at the molecular level and the copolymer network had high recoverability.

The normal tensile stress–strain curves of PGS and PGS-co-PEG polymers were transformed into Mooney’s representation that is classically used for rubbers [39,40,47]. Moreover, Mooney’s representation from second tensile cycle will allow us to visualize hysteresis between loading and unloading cycle and help us to correlate the cyclic tensile data with the cyclic compression data. As expected the peak Mooney’s stress decreases with an increase in PEG concentration indicating the softening of the polymeric network. The Mooney’s curve (Fig. 6a) indicates that PGS and PGS-co-PEG polymers show very limited hysteresis and all the samples return to their original shape after secession of tensile stress. The results also show that Mooney’s stress for both PGS and PGS-co-PEG polymers reach a plateau phase within $1/\lambda_{tens} = 0.95$. This indicates that strain hardening of the polymer network occurs rapidly and the entire sample was under constant stress. This behavior highlights the elastomeric property of the copolymer network and highlights its versatility in engineering scaffolds for a range of elastomeric tissues.

**Fig. 6.** Cyclic tensile and compressive properties of PGS-co-PEG polymers. The elastomeric properties of the copolymeric network were investigated under (a) cyclic tensile and (b) cyclic compression conditions. The fully hydrated samples were subjected to cyclic strain and the loading and unloading curves were monitored for five consecutive cycles. Both, PGS and PGS-co-PEG polymers showed highly elastomeric characteristic in tensile and compression tests. All the copolymeric network display a plateau in energy absorbed and recovery of the network after the first cycle indicating elastomeric properties. Mooney’s representation from second cycle was used to visualize hysteresis between loading and unloading cycle. The Mooney’s stress decreases with an increase in PEG concentration indicating the softening of the polymeric network. The Mooney’s curve indicates that PGS and PGS-co-PEG polymers show very limited hysteresis and all the samples return to their original shape after secession of tensile stress. The data represented as mean ± standard deviation ($n = 5$).
3.6. Cyclic compressive properties of PGS-co-PEG polymers

The mechanical behavior polymeric network under unconfined cyclic compression can be used to evaluate the applicability of the copolymeric networks for soft tissue engineering [45,46]. PGS and PGS-co-PEG polymers display elastomeric properties under compression, which is similar to our observations in tensile test (Fig. 6b). The amount of energy absorbed by the PGS network was constant between first and fifth compressive cycles. The addition of 20% of PEG significantly increased the amount of energy that was absorbed. This might be due to the deformation of swollen surfaces of the samples that do not return instantaneously to the original shape after cessation of compressive stress. Whereas, at higher PEG concentrations (PGS-co-40PEG and PGS-co-60PEG), negligible hysteresis was observed and the network displayed high recoverability (>95%). This is mainly attributed to the formation of softer structures that are able to absorb energy during the loading cycle and release it almost completely during the unloading cycle.

To provide an easy comparison between uniaxial compression and uniaxial stretching we used biax as our deformation, since uniaxial compression is equivalent to biaxial stretching in terms of deformation [41]. The Mooney’s representation indicate that crosslinked network behave similarly in cyclic tensile and cyclic compression conditions. The rapid increase in Mooney’s stress at smaller strain, indicates strain hardening of the polymeric network. The extent of strain hardening directly depends on the amount of PEG within the copolymer networks. Overall, PGS-co-PEG polymers exhibit unparalleled elastomeric properties that can be used to engineer a range of scaffolds for soft tissues that are subjected to pulsating/cyclic mechanical forces such as blood vessels, cardiac, cartilage and muscle tissues.

3.7. Hydration property of PGS-co-PEG polymers

The hydration property of biomaterials is an important factor in determining their targeted applications for various biomedical and biological applications [48–50]. The addition of PEG within the hydrophobic backbone induces hydrophilic characteristic to the block copolymers [38,51]. In the current approach, we expect that the addition of PEG would facilitate uptake of water by the cross-linked network (Fig. 7a). We first investigated surface characteristics of PGS and PGS-co-PEG polymers using optical tensiometry (goniometry) (Fig. 7b). In this technique, a sessile water drop on the polymeric surface was observed and the contact angle was

![Fig. 7. Effect of PEG on hydrophilicity of PGS-co-PEG polymers. (a) Schematic showing that the addition of PEG to PGS results in a decrease in crosslinking density and increased water absorption in PGS-co-PEG polymers. (b) The contact angle measurements indicate an increase in surface hydrophilicity due to the addition of PEG as determined by the decrease in contact angle of water on PGS-co-PEG polymers. (c) The hydration kinetic of PGS-co-PEG copolymers strongly depends on the amount of PEG within the network. All the copolymer reaches saturated hydration degree within 72 h. (d) The equilibrium water content significantly increases with an increase in PEG concentration. The data represented as mean ± standard deviation (n = 5) (**p < 0.01, ***p < 0.001, ANOVA with Tukey’s multiple comparison test).](image-url)
determined by measuring the angle between the polymer surface and a tangent to the water drop surface. Pure PGS shows a contact angle of 77.5 ± 1.7° with water. As expected, an increase in the PEG segment increases the hydrophilic nature. Addition of 60% PEG to PGS reduces the contact angle to 66.2 ± 0.1°.

The hydration properties of PGS and PGS-co-PEG polymers were further investigated by evaluating bulk hydration characteristic (Fig. 7c). The crosslinked polymer samples were subjected to physiological conditions (37 °C and PBS) and uptake of water was monitored. The swelling study reveals the maximum water uptake within 48 h for all PGS-co-PEG polymers, while PGS reaches the equilibrium water content (EWC) within 24 h (Fig. 7d). The EWC for PGS was 2.11 ± 0.88% whereas for PGS-co-60PEG was 32.98 ± 3.17%. Addition of 60% PEG results in almost 15-fold increase in water uptake capacity. Moreover, as the PEG content in the block copolymer increases, the network becomes translucent in the swollen conditions, which is the characteristic of an amphiphilic copolymer network.

The hydration kinetics of PGS-co-PEG polymers was analyzed by fitting the initial hydration data ($W_t/W_{eq} < 0.6$) to Eq. (6) [50].

$$f = W_t/W_{eq} = Kt^n$$

Where, 'K' is the characteristic swelling constant, 'n' is the hydration exponent that describes the mode of solvent transport, 'W_t' is hydrated weight at time 't' and 'W_{eq}' is saturated hydrated weight. For PGS, the characteristic swelling constant was not obtained due to a very low degree of swelling. While, the copolymer containing 20, 40 and 60% PEG had characteristic swelling constants of 0.38, 0.41 and 0.5 respectively, indicating Fickian diffusion. Thus, the water transport through the polymeric network was diffusion limited and the relaxation of the copolymeric network had no significant interference with the solvent diffusion. This property is an asset for fabricating scaffolds with a controlled drug release properties.

**Fig. 8.** The physiological stability of copolymeric network determined by in vitro degradation of PGS-co-PEG polymers under physiological conditions (PBS, 37 °C). (a) The optical images of copolymer network at day 0 and day 21 demonstrate that PGS and PGS-co-PEG polymers degrade via surface erosion mechanism. (b) The weight loss of polymeric network was monitored over the period of 21 days. The increase in hydrophilicity due to addition of PEG results in higher water uptake that accelerates the hydrolysis of the copolymer. The degradation rate directly depends on the amount of PEG within the PGS-co-PEG polymers network. The data represented as mean ± standard deviation ($n = 5$). Two-way ANOVA indicates significant ($p < 0.0001$) effect of composition and time on the mass loss of polymeric network.
3.8. In vitro degradation of PGS-co-PEG polymers

Chemical structures of polymeric materials play an important role in determining the degradation characteristic of the biomaterials [13,52]. The degradation rate and byproducts of degradation will determine the suitability of the polymeric materials for biomedical applications [51,53]. PGS is a biodegradable polymer and is composed of glycerol and sebacic acid [14]. Glycerol is the basic building block of lipids and sebacic acid is a natural metabolic intermediate in ω-oxidation of various fatty acids [21–23]. Whereas, PEG is an inert polymer that is extensively used in designing various biomedical products and devices. Hence, it is expected that copolymers made from PEG and PGS can potentially be used for biomedical and biotechnological applications.

The in vitro degradation of PGS and PGS-co-PEG polymers was investigated under physiological conditions (PBS, 37 °C) over the period of 21 days. The microscopic images indicate that all samples showed surface degradation characteristics (Fig. 8a). All samples follow a linear mass loss and the rate of degradation increases with an increase in PEG concentration (Fig. 8b). After 21 days, PGS showed 8.69 ± 1.64% mass loss, whereas PGS-co-20PEG, PGS-co-40PEG and PGS-co-60PEG indicate mass loss of 15.58 ± 0.81 and 35.91 ± 5.06°C and 61.2 ± 4.39°C respectively. The increase in degradation rate can be attributed to an increase in hydrophilicity of copolymer network with an increase in PEG concentration. The increase in hydrophilicity results in higher water uptake that accelerates the hydrolysis of PGS. This indicates that the slow degradation rate of PGS can be improved and tuned by incorporating PEG segments. The surface degradation characteristic combined with the diffusion controlled hydration kinetics of PGS-co-PEG polymers also suggests possible applications of PGS-co-PEG polymers elastomers for controlled drug release application.

3.9. Protein absorption and cells—matrix interactions

The adsorption of protein on a biomaterial surface plays a significant role in controlling cell—matrix interactions [32–34]. The cell adhesion and spreading on a biomaterial surface is mediated by the presence of an adsorbed layer of protein which is strongly influenced by substrate chemistry. Under physiological conditions, the surrounding media contains a wide-range of proteins that are adsorbed on the biomaterial surface in competitive or sequential manner. Thus, it is important to investigate adsorption of protein on PGS-co-PEG surfaces. The effect of PEG concentration on protein adsorption was evaluated by immersing PGS and PGS-co-PEG in 10% FBS at 37 °C for 24 h. It is recognized that the protein adsorption is favored by a hydrophobic surface compared to a hydrophilic surface [54] and more than 60–70% proteins in FBS are hydrophilic albumin [55]. The addition of PEG to PGS increases hydrophilicity of the polymeric network. The results indicate that the addition of PEG to PGS results in a significant increase in the amount of protein adsorption/absorption (Fig. 9a). This might be attributed to the absorption of proteins within the hydrated copolymer structure. These results support earlier finding that some of the hydrated and porous polymer structures facilitate protein absorption instead of protein adsorption [55]. To further investigate surface properties of the polymeric network, adsorption of cell-adhesive proteins such as plasma fibronectin was analyzed. Plasma fibronectin is vital for initial cell attachment on the biomaterials surface. The increase in PEG content within the copolymer network (PGS-co-20PEG, PGS-co-40PEG), reduced fibronectin adsorption compared to PGS (Fig. 9a). In case of PGS-co-60PEG, an increase in fibronectin was observed. This was mainly attributed to high water uptake capacity of PGS-co-60PEG that results in fibronectin absorption.

To determine the feasibility of copolymer network for biomedical applications, preliminary study to evaluate cell—matrix interactions was performed. NIH 3T3 Fibroblasts were seeded on PGS and PGS-co-PEG surfaces. Due to the autofluorescence of the PGS and PGS-co-PEG polymers, immunostaining images of the adhered cells were difficult to obtain. Therefore, we monitored the metabolic activity of adhered cells using Alamar Blue assay in low cell adhesion plates for 10 days (Fig. 9b). Tissue culture polystyrene (TCPs) surface was used as a positive control. Both PGS and PGS-co-PEG polymers support cell proliferation. The data represented as mean ± standard deviation (n = 3) (*p < 0.05, **p < 0.01, ***p < 0.001, ANOVA with Tukey’s multiple comparison test).

4. Conclusion

We successfully synthesized a range of PGS-co-PEG polymers by altering the molar ratios of glycerol to PEG. The incorporation of hydrophilic PEG chains result in increased water uptake by the copolymer network. The addition of PEG reduced the number of hydroxyl groups within the copolymeric network and decreased crosslinking density. PGS-co-PEG polymers show elastomeric properties and can be subjected to severe deformation such as...
bending and stretching without fracturing the structure. Under tension, PGS-co-PEG polymers displayed a stress-strain behavior that is typically observed in highly elastomeric soft tissues. The Young's modulus of PGS-co-PEG can be tuned from 13 kPa to 2.2 MPa by altering the amount of PEG within the network. Compared to PGS, more than a six-fold increase in elongation was observed in PGS-co-60PEG. Similarly, under compression, the addition of PEG results in a soft and elastomeric network. Both surface and bulk characterization indicates that the addition of PEG increases the hydrophilicity and the rate of degradation of the copolymer network. Moreover, PGS-co-60PEG supports protein adsorption/absorption and cell proliferation, and thus can be used for a range of tissue engineering applications.

Authors contributions

AP, AKG and GI contributed equally. AP, AKG and AK conceived the idea and designed the experiments. AP, AKG and GI synthesized PGS and PGS-co-PEG polymers and performed the chemical (NMR, FTIR, GPC), mechanical (tensile, compression and cyclic testing), and structural characterizations (sol–gel, hydration kinetics, contact angle, degradation studies). SMM performed cells proliferation studies, HZ and SM performed protein adsorption studies. AP, AKG, GI and AK analyzed experimental data and wrote the manuscript. All authors discussed the results and commented on the manuscript.

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Supporting Information

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.01.045.

References


