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Development of Biodegradable and Injectable Macromers Based on Poly(Ethylene Glycol) and Diacid Monomers

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Abstract

Novel biodegradable injectable poly(ethylene glycol) (PEG) based macromers were synthesized by reacting low molecular weight PEG (MW: 200) and dicarboxylic acids such as sebacic acid or terephthalic acid. Chemical structures of the resulting polymers were confirmed by Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy characterizations. Differential scanning calorimetry (DSC) showed that these polymers were completely amorphous above room temperature. After photopolymerization, dynamic elastic shear modulus of the crosslinked polymers was up to 1.5 MPa and compressive modulus was up to 2.2 MPa depending on the polymer composition. The in vitro degradation study showed that mass losses of these polymers were gradually decreased over 23 weeks of period in simulated body fluid. By incorporating up to 30 wt% of 2-hydroxyethyl methacrylate (HEMA) into the crosslinking network, the dynamic elastic modulus and compressive modulus was significantly increased up to 7.2 MPa and 3.2 MPa, respectively. HEMA incorporation also accelerated degradation as indicated by significantly higher mass loss of up to 27% after 20 weeks of incubation. Cytocompatibility studies using osteoblasts and neural cells revealed that cell metabolic activity on these polymers with or without HEMA was close to the control tissue culture polystyrene. The PEG based macromers developed in this study may be useful as scaffolds or cell carriers for tissue engineering applications.

Keywords

Polyethylene glycol; dicarboxylic acid; HEMA; tissue engineering; biodegradation

1. Introduction

During the past few decades, numerous biomaterials have been developed for tissue engineering applications and drug delivery systems ranging from naturally derived to synthetic biopolymers^{1–5}. Natural biomaterials such as collagen have been used in tissue engineering mainly due to their excellent bioactivity and biocompatibility^{6,7}. However, those materials have difficulties in handling and offer limited control over their chemical and physical properties which are crucial to intended applications⁸.

To this end, synthetic biopolymers with improved critical properties have been developed^{4,9}. Biocompatibility is a critical design parameter for candidate materials for tissue engineering and drug delivery since it has been one of the major hurdles for synthetic

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biomaterials to be approved by the regulatory agencies ¹. Ideally, biocompatible materials should not promote inflammatory reaction such as platelet activation/fragmentation or leukocyte adhesion/activation but most synthetic materials eventually do promote these processes, perhaps due to adsorbed proteins on the material ^{10,11}. For that purpose, hydrophilic materials have drawn great attention as reasonable candidates for producing biocompatible materials ^{12,13}, since hydrophilic materials have been shown to reduce protein adsorption and cellular adhesion, thus improving biocompatibility ¹⁴.

Biodegradability should also be considered as a critical design parameter since most available traditional biomaterials such as polyurethanes, woven polyethylene terephthalate, and silicone rubber are non-biodegradable over a reasonable time scale ^{2,15}. Mechanical properties of tissue engineering scaffolds are important considerations not only for hard tissue such as bone regeneration, but also soft tissue such as nerve regeneration ¹⁶. In addition, successful tissue engineering materials with appropriate mechanical strength should be easily fabricated to 3D structures for supporting cell infiltration and tissue regeneration.

Among synthetic biomaterials, poly(ethylene glycol) (PEG) based macromers have been widely used in tissue engineering and drug delivery applications as hydrogel type biomaterials, mainly due to their well known low toxicity and good biocompatibility ^{12,17,18}. In the early 1990s, Sawhney et al. have developed PEG based hydrogels coupled with poly(α -hydroxy acid) to make them biodegradable ¹⁹. Since then, PEG based biodegradable hydrogels have been more attractive candidates for these applications. However, the main challenge to more extensive use of PEG based macromers for tissue engineering scaffolds is their lack of proper mechanical strength ^{12,20}. Up to date however, few non-hydrogel type biomaterials based on PEG have been investigated.

The purpose of this study was to synthesize and evaluate non-hydrogel type injectable macromers with improved mechanical properties, which are based on low molecular weight PEG and dicarboxylic acid monomers. Sebacic acid or terephthalic acid was chosen as diacid monomers since they are shown to be biocompatible and readily available ^{21,22}. It is expected that these PEG based macromers can take advantage of the excellent biocompatibility of PEG and the rigidity (which provides mechanical strength) of hydrophobic segments of the polymers. In addition, we also investigated the effects of 2-hydroxy ethylmethacrylate (HEMA) incorporation into the polymer network on the physical and mechanical properties of such PEG based biomaterials. HEMA is known to be biocompatible ¹² and since it has vinyl group at one end, it allows easy incorporation into acrylic polymers by polymerization reaction. It also has hydroxyl group at the other end, which renders it hydrophilic and may lead to increased degradation rate.

2. Materials and methods

2.1. Synthesis of PEG based macromers

All chemicals were purchased from Sigma-Aldrich (Milwaukee, WI) unless otherwise noted. PEG based macromers were synthesized by the reaction represented in Scheme 1. Briefly, PEG with nominal molecular weight of 200 Da was dried by azeotropic distillation in toluene and remaining toluene and traces of water were further removed under reduced pressure. Sebacoyl chloride was purified by distillation at boiling point (168 °C) under vacuum (6mm Hg). A total of 100 g of dried PEG was dissolved in 500 ml of anhydrous methylene chloride. Then 50% molar excess of triethylamine was added dropwise into the solution with vigorous stirring followed by dropwise addition of sebacic chloride (0.9 mole/mole of PEG) solution in an ice bath. The reaction mixture was stirred for 24 h at room temperature. The solvent was removed by rotovaporation and the product was dissolved in

warm ethyl acetate. Triethylamine hydrochloride salt was removed by filtration until the solution was clear and the final product (namely PEGS) was precipitated in petroleum ether and dried under vacuum.

To prepare PEG sebacate diacrylate (PEGSDA), 50 g of PEGS was dissolved in methylene chloride. Again 50% molar excess triethylamine was added dropwise into the solution, followed by dropwise addition of dried acryloyl chloride (50% molar excess of PEGS). The reaction was run for another 24 h at room temperature and the product was dissolved in ethyl acetate, filtered, and precipitated in petroleum ether. To obtain the final product, the macromer was dissolved in methylene chloride, reprecipitated in dry petroleum ether twice, and dried under vacuum overnight.

PEG terephthalate (PEGT) was similarly synthesized by using PEG (MW: 200) and terephthaloyl chloride and PEG terephthalate diacrylate (PEGTDA) was obtained by end capping PEGT with acrylate group as described above.

2.2. Macromer characterization

Molecular weight of the synthesized polymers were measured by gel permeation chromatography (GPC, Waters, Milford, MA) equipped with a HPLC pump and a refractive index detector by injecting 20 μ l of the sample and eluting at 1 ml/min of flow rate in tetrahydrofuran (THF) (Fisher, Pittsburgh, PA). Monodisperse polystyrene standards (Polyscience, Warrington, PA) with number average molecular weight (M_n) of 0.474, 6.69, 18.6, and 38 kDa and polydispersity index (PDI) of less than 1.1 were used to construct the calibration curve. M_n and weight average molecular weight (M_w) were calculated from the standard curve.

The characterization of hydroxyl terminated polymers (PEGS and PEGT) and acrylate terminated polymers (PEGSDA and PEGTDA) were performed by using Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR, Nicolet 8700, Madison, WI) and proton nuclear magnetic resonance (^1H -NMR, Bruker AC 500, Billerica, MA) spectroscopy. For FTIR measurement of crosslinked PEG based networks, all samples were soaked in methylene chloride for 24 h at room temperature to completely remove all possible contaminants or unreacted monomers and then the spectra were obtained.

Thermal properties of the macromers were characterized using a differential scanning calorimeter (DSC, TA instruments, New Castle, DE). To keep the same thermal history, the samples were first preheated from room temperature to 100 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}/\text{min}$ in a nitrogen atmosphere and then cooled at a rate of 5 $^{\circ}\text{C}/\text{min}$ to -90 $^{\circ}\text{C}$ and the scan was then recorded from -90 $^{\circ}\text{C}$ to 100 $^{\circ}\text{C}$.

2.3. Photopolymerization

The photoinitiator, Irgacure 2959 (Ciba Specialty Chemicals, Tarrytown, NY), was added to the polymer at a final concentration of 0.05% (w/v). The mixture was then incubated in the convection oven at 60 $^{\circ}\text{C}$ for 10 min to remove air bubbles. The solution was pipetted into glass plates with a 0.8 mm spacer and exposed to UV light ($\lambda = 310 - 400$ nm, Blak-Ray®, Upland, CA) for 10 min. The crosslinked polymers were then removed from the glass plate and cut into disks (0.8 cm $\times$ 0.8 mm) with a cork borer.

To incorporate HEMA into the polymer network, up to 30 % (w/w) of HEMA was added into the melted polymer (PEGSDA or PEGTDA) then the mixture was photopolymerized as described above. To calculate the conversion of crosslinked network, initial solution was weighed as W_{bc} and the final weight of the crosslinked network was weighed as W_{ac} and the percent conversion was then calculated as $W_{ac}/W_{bc} \times 100$. The sol fraction was also

determined to measure the fraction of unreacted macromer. The crosslinked discs were weighed as W_{is} and the dry discs were soaked in methylene chloride for 24 h at room temperature to completely dissolve the unreacted macromers and weighed as W_{fs} . The percent sol fraction was then calculated as $(W_{is} - W_{fs})/W_{is} \times 100$.

2.4. Swelling and degradation

After photopolymerization, the samples were incubated in pH 7.4 phosphate buffered saline (PBS) at 37 °C with gentle shaking. Swollen samples were weighted as W_s at different time points and dry weight (W_d) was measured after lyophilization under vacuum for 24 h. The swelling ratio was then calculated as $(W_s - W_d)/W_d$. In separate experiments, the swollen samples were lyophilized and weighed (W_i) and the dried samples were incubated at 37 °C in PBS or in 1.0 N NaOH solution for accelerated degradation on an orbital shaker. At certain time points, specimens were rinsed with deionized water, lyophilized and weighed (W_{d2}). The percent weight loss after lyophilization was calculated as $(W_i - W_{d2})/W_i \times 100$.

2.5. Mechanical properties

Mechanical properties of the crosslinked polymers were analyzed with a dynamic mechanical rheometer (TA Instruments, New Castle, DE) using the parallel-plate setup and a dynamic mechanical analyzer (TA instruments). All samples were tested at room temperature after 24 h of equilibration in deionized water. Dynamic shear strain sweep experiment was first performed at a frequency of 10 rad/s to determine the linear stress-strain range of the different samples. The results indicated that 0.1% of shear strain was in the linear stress-strain range of tested samples. The dynamic shear elastic modulus of the samples was then obtained by the frequency sweep test with a frequency range from 1 to 100 rad/s at a strain of 0.1%.

The crosslinking density of these macromers was then calculated by the following equation (1) ^{16,23}:

$$n = \frac{G}{RT} = \frac{\rho}{M_c} \quad (1)$$

Where n represents the crosslink density which is moles of active network chains per unit volume, M_c is the molecular weight between crosslinks, R is the universal gas constant (8.3144 J/mol·K), T is the absolute temperature (K), ρ is the macromer density (g/m³) and G is the shear modulus.

Static elastic compressive modulus was measured for cylindrical samples by a dynamic mechanical analyzer (TA instruments). Before testing, initial cross-section area and dimension of each sample were measured. All samples were submerged in de-ionized water and compressed at a loading rate of 2 N/min up to 15 N. Compressive modulus was determined by dividing applied forces and dimensional changes into initial cross-section area and dimension, and then calculating the slope of linear region in the stress-strain curve.

2.6. Cytocompatibility

Rat bone marrow stromal cells (MSCs) were obtained as previously described ²⁴. Briefly, MSCs were isolated from femurs of five month old male Sprigui-Dawley rats (Harlan, Indianapolis, IN). The femora were harvested, soft tissue was removed, and the femora were placed in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (50 µg/ml), streptomycin (50 µg/ml), neomycin (100 µg/ml), and fungizone (25 µg/ml). The femora were rinsed with sterile PBS, and the epiphyses were

cut off. The bone marrow was flushed out using a syringe with an 18 gauge needle inserted into the diaphysis. The marrow was flushed with medium into a sterile 50 ml centrifuge tube, titrated with the syringe to form a single cell suspension, and plated in two T-75 flasks. The cells were then cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C in a 5% CO₂ atmosphere. Cells were used between passages three to six. Additionally, PC-12 cells were routinely cultured in DMEM supplemented with 10% FBS and 10% horse serum at 37 °C in a 5% CO₂ atmosphere.

The cells (MSCs or PC-12 cells) were seeded at a density of 20,000 cells/cm² on the bottom of the 24-well transwell tissue culture plates (Costar, membrane pore size: 0.3 μm, Pittsburgh, PA) and incubated for 24 h. PEGSDA and PEGSTA disks (5 mm diameter, 0.8 mm thick) were then placed into the inserts of the transwells and the cells were further cultured for up to 7 days in the presence of the polymers. The polymer disks were sterilized by incubation with 70% ethanol for 1 h followed by extensive washes with sterile PBS. Transwell inserts without the polymer disks were also transferred to the culture plates containing the cells and used as the positive control.

On days 4 and day 7, cell viability was evaluated using a colorimetric cell metabolic assay (CellTiter 96 Aqueous One Solution, Promega, Madison, WI), based on the MTS tetrazolium compound, 3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium. The reagent solution was added to each well and incubated for 1 h at 37 °C. The supernatant from each well was transferred to a 96 well plates. The UV absorbance at 490 nm was measured using a plate reader (SpectraMax Plus, Molecular Devices, Sunnyvale, CA), which is correlated to the viable cell number, and inversely correlated to the toxicity of the crosslinked polymer disks.

2.7. Statistical analysis

All data are represented as means ± standard deviations for n = 3. Statistical analysis was performed using single factor analysis of variance (ANOVA) with Scheffe's posthoc test at a significance level of 0.05.

3. Results and Discussion

The main objective of this study is to develop non-hydrogel type injectable and biodegradable PEG based macromers with less swelling thus improved mechanical strength compared to existing PEG based hydrogels such as PEG diacrylate (PEGDA). We chose sebacic acid and terephthalic acid as dicarboxylic acid monomers for the following reasons: (1) sebacic acid and terephthalic acid have been shown to be biocompatible both in vitro and in vivo ^{21,22}, (2) they have appropriate molecular weights to render the synthesized polyester polymers biodegradable within a reasonable time scale ^{23,25}, and (3) they are readily available and relatively cheap for large scale-up applications.

3.1. Macromer synthesis and characterization

Simple reaction between PEG and diacid monomers as shown in scheme 1 produced novel non-hydrogel type injectable and biodegradable PEG based macromers. Before crosslinking, these macromers were viscous liquids at room temperature which can be used as injectable polymers. They were insoluble in water but soluble in most common solvents such as acetone or methylene chloride. Mn, Mw, and polydispersity (PDI) of these polymers were characterized by GPC analysis (Table 1). The molar ratio (r) between diacid monomers and PEG was 0.9. The end group of the first resulting products, PEG sebacate (PEGS) or PEG terephthalate (PEGT) has to have a hydroxyl group at the end of the macromer, which can be easily modified with an acrylic group by adding an acryloyl chloride ¹⁹. In order to

analyze functional groups of the macromers, we used FTIR spectroscopy equipped with zinc selenide ATR crystal and $^1\text{H-NMR}$. The FTIR spectrum of PEGS clearly showed an absorption band at 3510 cm^{-1} , indicating the presence of terminal hydroxyl group (Fig. 1A). However, this band was substantially reduced when the hydroxyl group was modified with acrylic group and additional band for the presence of double bond was also seen at 1650 cm^{-1} in the spectrum of PEGSDA. Another strong characteristic band at 1725 cm^{-1} which represents the ester group, was seen in the FTIR spectra of PEGS as well as PEGSDA suggesting that these macromers were polyester polymers. $^1\text{H-NMR}$ spectrum of PEGS (Fig. 2A) shows the presence of PEG and sebacic acid and the spectrum of PEGSDA (Fig. 2B) shows characteristic proton peaks at 5.9, 6.2 and 6.4 ppm which represent acrylate group in the polymer in addition to PEG and sebacic acid related proton peaks. Triplet at 4.3 ppm and singlet at 2.1 ppm were likely from the solvent or unreacted macromers.

Thermal properties were characterized by DSC and summarized in Table 1. The apparent glass transition temperatures (T_g) for PEGSDA and PEGTDA were $-62.1\text{ }^\circ\text{C}$ and $-40.8\text{ }^\circ\text{C}$, respectively. The crystallization (T_c) and melting temperature (T_m) of PEGSDA were $-38.1\text{ }^\circ\text{C}$ and $-34.1\text{ }^\circ\text{C}$, respectively, whereas no T_c and T_m were found for PEGTDA samples in the temperature studied. Both macromers are completely amorphous at the operation temperature ($37\text{ }^\circ\text{C}$) and appeared to be elastomer-like material at the temperature similar to a vulcanized rubber^{23,26}.

3.2. Properties of crosslinked networks

After crosslinking of the macromers in the presence of photoinitiator (Irgacure 2959), the equilibrium swelling ratios of PEG based networks were obtained after 24 hrs of incubation in PBS at $37\text{ }^\circ\text{C}$ (Fig. 3). Compared to existing biodegradable polymers such as poly(lactide-glycolide) (PLGA) and poly(caprolactide) (PCL) whose swelling ratios are often well over 1.0²⁷, these polymers are less swellable mainly due to the presence of strong hydrophobic segments as well as low molecular weight of PEG in the crosslinked networks. Regarding the effect of HEMA incorporation on swelling of the macromers, the equilibrium swelling ratios of HEMA incorporating networks (0.35 and 0.17 for PEGSDA/30H and PEGTDA/30H, respectively) were significantly greater than those of non-HEMA networks (0.25 and 0.12 for PEGSDA and PEGTDA, respectively) after 24 h of equilibration mainly due to the increased hydrophilicity of the networks (Fig. 3)

Mechanical properties were evaluated by measuring dynamic shear modulus using a torsional rheometer²². The ranges of elastic shear modulus of PEGSDA and PEGTDA were $0.58 - 0.61\text{ MPa}$ and $1.5 - 1.8\text{ MPa}$, respectively (Fig. 4A) throughout the tested frequencies ($1 - 100\text{ rad/s}$), which are comparable to the complex modulus of the cartilage tissue ($0.2 - 2.5\text{ MPa}$ under the frequencies from 0.1 to 100 rad/s ²⁸). By comparison, the range of viscous shear modulus of PEGSDA and PEGTDA were $11 - 89\text{ kPa}$ and $40 - 330\text{ kPa}$, respectively, indicating that the elastic modulus dominates over the viscous modulus, which is an additional evidence for the elastomeric behavior of the polymers. As expected, the modulus of PEGTDA was significantly higher throughout the tested shearing frequency mainly because the aromatic unit (terephthalic acid) of the PEGTDA building block increases hydrophobicity of polymer, thus decreases water absorption and leads to the greater mechanical strength as compared to PEGSDA²⁹. Elastic compressive moduli of these polymers were also measured (Fig. 4B) and showed the similar trend compared to dynamic shear modulus data.

In order to compare the temperature dependence of the polymers on mechanical properties, dynamic moduli of PEGSDA and PEGTDA were measured at room temperature ($25\text{ }^\circ\text{C}$) and at physiological temperature ($37\text{ }^\circ\text{C}$). The moduli were almost identical at both temperatures, indicating that the mechanical properties were not affected by the testing

temperature (data not shown). The elastic modulus of these polymers was almost constant throughout the shearing frequencies, indicating that they are highly crosslinked without any slippage³⁰.

It is well known that the increase of hydrophobicity of the polymer can decrease water absorption thus results in increasing its mechanical properties³¹. Early studies from hydrogel based copolymers demonstrated that either altering polymer composition with more hydrophobic comonomer or increasing the hydrophobic segment fraction in the polymer can significantly increase mechanical properties^{32,33}. Extensive studies have also shown that the incorporation of hydrophobic blocks (e.g., poly α -hydroxy acid) into PEG based hydrogels can substantially improve the mechanical properties of resulting polymers^{19,34} and also provide biodegradability by hydrolysis of ester linkage between PEG and poly(α -hydroxy acid). Additionally, biological properties (e.g. cell interaction with the material) are known to be closely related to the mechanical properties of the material³⁵. In this work, the incorporation of hydrophobic segment (e.g. dicarboxylic acids) into the macromers is expected to allow maintenance of their structural integrity and mechanical strength during the regeneration process of various soft tissues such as vascular grafts or nerves and prevent excessive swelling of the structures which can cause failure of the degrading biomaterials³⁶.

3.3. Effects of co-monomer

The properties of the macromers can be further modulated by incorporating co-monomers³¹. For this purpose, we incorporated HEMA, a well known monomer in commercial contact lenses¹⁸, into the PEG-based macromers. The rationale for choosing HEMA are: (1) it is a readily available monomer and potentially biocompatible when incorporated into a crosslinked network¹², (2) it has vinyl group at one end, allowing easy incorporation into acrylic polymers such as PEGSDA by crosslinking reaction, and (3) it is hydrophilic due to hydroxyl group at the other end, which is expected to increase the hydrophilicity of the copolymer and thus increase its degradation rate. HEMA was incorporated into the polymer network at up to 30 wt% by adding it into the solution containing PEGSDA or PEGTDA before photopolymerization.

FTIR spectra of the crosslinked copolymers showed broad absorbance peaks in the range of 3100 – 3600 cm⁻¹, which corresponds to the O-H stretching vibration (Fig. 1B), indicating that HEMA was well conjugated into the PEG based polymers. Figure 4A shows the mechanical strength of PEG based macromers containing up to 30% HEMA by measuring the dynamic shear modulus. Compared to the PEG based macromers in the same figure, the moduli of the macromers with HEMA were significantly increased throughout the frequency range (1 – 100 rad/s). The moduli of PEGTDA incorporated with 30% HEMA were monotonically increased 3.8 – 7.2 MPa under the given frequencies which are comparable to those of porcine aortic heart valve (6.4 ± 0.9 MPa³⁷). There appeared to be some upward drift of dynamic shear modulus data, especially for the modulus of HEMA containing PEG based network, which may be an artifact of drying during the measurement. Elastic compressive moduli of these polymers were also measured under hydrated condition (Fig. 4B) and confirmed that the incorporation of HEMA into PEG based macromer network substantially increased the modulus of copolymers.

To assess the effect of HEMA incorporation on crosslinking reactivity of PEG based macromers, the conversion and sol fraction were measured during crosslinking reaction (Fig. 5). The conversions of PEG based macromers into crosslinked networks were not significantly different between the networks with and without 30% HEMA, and all macromers showed highly crosslinked networks (conversion > 95%). However, the sol fractions (e.g., unreacted macromers) of the final networks without HEMA were

significantly greater than those of the networks incorporating HEMA. It is likely that HEMA increased the mobility of the chains during the crosslinking reaction leading to low sol fraction, thus more macromers were covalently incorporated into the crosslinked network, whereas in the case of the network without HEMA, substantial amount of macromers are inside the crosslinked network yielding similar conversion, but not covalently incorporated into the network and thus dissolved in the solvent. These results revealed that by increasing the amount of HEMA in the crosslinked network, the initial mechanical properties could be increased due to the resulting highly crosslinked structure (Table 2).

The effect of HEMA on the degradation rate was also evaluated by measuring the weight loss of the polymers over time by incubating polymers in PBS (pH 7.4) or 1.0 N NaOH to rapidly obtain relative degradation rates. At physiological condition, PEGSDA and PEGTDA degraded slowly with weight losses of $8.8 \pm 2.9\%$ and $12.6 \pm 6.6\%$, respectively, after 23 weeks of incubation (Fig. 6). Degradation rate of the polymers can be modulated by copolymerization with other biodegradable vinyl monomers such as HEMA. There was a general trend of increasing the amount of HEMA with increasing degradation rate of both PEG based macromers. This trend was more obvious under the accelerated degradation condition in 1 N NaOH (Fig. 7A). For the initial 24 h of incubation, there was no significant difference between the macromers with and without HEMA, however, mass losses of HEMA containing PEG based macromers (89.8% and 100% for PEGSDA and PEGTDA after 48 h of the incubation, respectively) were significantly greater than those of the macromers without HEMA afterwards (57.6% and 61.8% for PEGSDA/30H and PEGTDA/30H, respectively). These results suggest that the introduction of HEMA into the network may increase the degradation rate of the crosslinked polymer, likely due to increased hydrophilicity which attracts more water into the network to accelerate hydrolysis¹⁹. This result is consistent with the increased swelling ratios of HEMA-containing macromers in Fig. 3.

To assess the mechanical properties of the crosslinked networks during degradation, compressive modulus of the PEG based networks was also measured under the accelerated degradation condition (Fig. 7B). Mechanical properties of HEMA containing networks during degradation were not significantly different from the networks without HEMA, except at 24 h of incubation, indicating that faster degradation compromised mechanical properties of crosslinked PEG based networks. Overall, HEMA incorporation into PEG based networks may increase the initial mechanical properties of the networks, however they exhibited similar mechanical strength during degradation due to faster degradation rates as compared to PEG based macromers without HEMA incorporation. Regarding the effects of diacid monomer on physical properties, there was no significant difference in mass loss throughout the incubation time between PEGSDA and PEGTDA, except at 36 h of incubation. Mechanical strength of PEGTDA networks was significantly greater than that of PEGSDA throughout the degradation periods likely due to increased rigidity of the polymer.

3.4. Cytocompatibility test

The major building blocks of current PEG based macromers are PEG, dicarboxylic acids, and acrylate group at the end of the polymers. PEG and dicarboxylic acids have proven to be biocompatible as mentioned earlier, it is however, critical to evaluate the cytocompatibility of the resulting crosslinked polymers for biomedical applications². Cell viability was examined in the presence of any unreacted monomers and/or the photoinitiator which can leach out from the polymers placed in the transwell with a membrane pore size of 3.0 μm . Both cell types, bone marrow stromal cells (MSCs) and neuron-like cell line cells (PC-12 cells) were used for the evaluation since these macromers could be candidate materials for orthopedic or neural tissue engineering. Fig. 8 shows percent metabolic activity of the cells in the presence of the crosslinked polymers normalized to that of cells cultured on control

tissue culture polystyrene (TCPS) without the polymers. The metabolic activity of MSC cells with PEGSDA was similar to the control at both 4 and 7 days. Although a statistical difference was detected for PEGTDA and HEMA incorporated macromers as compared to TCPS, the metabolic activity of cells with these materials was over 85 % at any given incubation time. For PC-12 cell cultures, cell metabolic activity for in the presence of all PEG-based macromers with or without HEMA was either significantly higher than or similar to the control TCPS. These results indicate that the PEG-based macromers have relatively good cytocompatibility to the control. Finally, these macromers can be further modulated by incorporating various crosslinking molecules. For example, bioactive molecules such as cell adhesion peptides can be easily immobilized on these novel biomaterials due to their functional end groups^{38,39}. Overall, the macromers described in this work have different properties from existing PEG based hydrogels such as PEGDA, and may be useful as tissue engineering scaffolds or drug delivery vehicles.

4. Conclusions

We have successfully synthesized novel injectable and biodegradable macromers based on PEG and diacid monomers. The swelling, mechanical, and degradation properties of these macromers could be varied by changing the type of diacid. Incorporation of HEMA into the crosslinking network further improves initial mechanical strength and degradation rate. The macromers are also cytocompatible with bone marrow stromal cells and neuron like cells, thus may be useful for tissue engineering applications and/or drug delivery systems.

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Figure 1.

(A) FTIR spectra of PEG based macromers after dissolving the polymers in acetone at room temperature. (B) FTIR spectra of crosslinked PEG based networks after swelling the samples in methylene chloride for 24 h and by contacting the sample to the micro-ATR crystal (zinc selenide) at room temperature. Arrows indicate O-H stretching vibration due to the presence of HEMA.

Figure 2.

^1H -NMR spectra of PEG based macromers (A) PEGS and (B) PEGSDA. Proton labels on the schematic formula of PEGSDA are assigned for ^1H -NMR analysis. NMR measurements were carried out by dissolving the polymers in CDCl_3 containing TMS as an internal standard at room temperature.

Figure 3. Equilibrium swelling ratio of crosslinked PEG based macromers. Swelling ratio was determined as $(W_s - W_d)/W_d$, where W_s : swollen weight of polymers, W_d : dry weight.

Figure 4. (A) Dynamic shear modulus and (B) Elastic compressive modulus of different crosslinked PEG based networks. PEGSDA/30H represents PEGSDA with 30% HEMA. Dynamic shear modulus was determined by frequency sweeping using a torsional rheometer at 0.1% of the strain at room temperature after 24 h equilibration in water. Elastic compressive modulus was measured under hydrated condition. All samples were hydrated for 24 h at room temperature before testing. Error bars represent means $\pm$ standard deviation for $n=3$.

Figure 5. (A) Percent conversion and (B) sol fraction of crosslinked PEG based networks. Sol fraction was determined by soaking the crosslinked network into methylene chloride for 24 h. Error bars represent means $\pm$ standard deviation for $n=3$. * indicates significant difference ($p < 0.05$) from each other.

Figure 6.

Degradation profiles of PEG based macromers at various time points in PBS at 37 °C. The percentage of remaining mass was determined by $(W_i - W_{d2})/W_{d2} \times 100$, where W_i : initial dry weight of polymers, W_{d2} : dry weight of polymers at various time points.

Figure 7. Accelerated degradation evaluation of crosslinked PEG based macromers. (A) Degradation profiles and (B) elastic compressive moduli of the PEG based networks. Elastic compressive modulus was measured under hydrated condition. Error bars represent means $\pm$ standard deviation for $n = 3$.

Figure 8.

Mitochondrial metabolic activities of (A) rat derived bone marrow stromal cells (MSCs) and (B) PC-12 cells, cultured in the presence of crosslinked PEG based macromers (PEGSDA and PEGTDA) with or without 30% (w/w) HEMA after 4 and 7 days of culture. The metabolic activity was determined by a tetrazolium (MTS) colorimetric assay. UV absorbance at 490 nm of each sample was normalized against the control TCPS. Error bars represent means $\pm$ standard deviation for $n = 3$. * indicates a significant difference ($p < 0.05$) from the control TCPS.

Scheme 1.

Synthesis and photopolymerization of PEG based macromers incorporated with dicarboxylic acids and terminated with acrylate group.

Characterization of PEG based macromers. Number average molecular weight (Mn), weight average molecular weight (Mw), and polydispersity index (PDI = Mw/Mn) of synthesized PEGSDA and PEGTDA macromers were measured by GPC and thermal properties were measured by DSC analysis.

Table 1

	Mn (g/mol)	Mw (g/mol)	PDI	Tg (°C)	Tc (°C)	Tm (°C)
PEGSDA	6,857 ± 88	18,768 ± 390	2.74	-62.1 ± 1.1	-38.1 ± 0.4	-34.1 ± 0.4
PEGTDA	6,226 ± 43	17,784 ± 102	2.85	-40.7 ± 0.6	-	-

Table 2

Mechanical properties of PEG based macromers.

Samples	G'(MPa) ^a	density(g/cm ³)	n (mol/m ²)	Mc (g/mol)
PEGSDA	0.59 ± 0.11	1.23 ± 0.06	237 ± 43	4,765 ± 868
PEGSDA/30H ^b	3.67 ± 0.64	1.32 ± 0.05	1,122 ± 258	730 ± 157
PEGTDA	1.58 ± 0.03	1.10 ± 0.03	636 ± 12	1933 ± 36
PEGTDA/30H	5.13 ± 0.50	1.32 ± 0.07	2,070 ± 201	641 ± 62

^a dynamic shear modulus at a frequency of 10 rad/s^b PEGSDA/30H represents PEGSDA containing 30 wt% of HEMA