

Biodegradable and Photopolymerizable Hydrogels for Tissue Engineering Based on Poly(ethylene glycol) and Sebacic acid

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Introduction

Poly (ethylene glycol, PEG) based macromers have been widely used in tissue engineering applications as hydrogel type biomaterials, mainly due to their well known hydrophilicity and biocompatibility [1,2]. In addition, PEG based biodegradable hydrogels have been reported [3] and have drawn more attraction for tissue engineering scaffolds and/or drug delivery systems [4,5]. However, the principle limitation to more extensive use of PEG hydrogels is their lack of mechanical strength [2]. We developed novel biodegradable and photopolymerizable hydrogels based on PEG and sebacic acid which have significantly greater mechanical strength and show low equilibrium swellings ratio. These novel biomaterials have different properties from existing PEG based hydrogels, and can be useful for drug delivery vehicles and/or tissue engineering scaffolds.

Materials and Methods

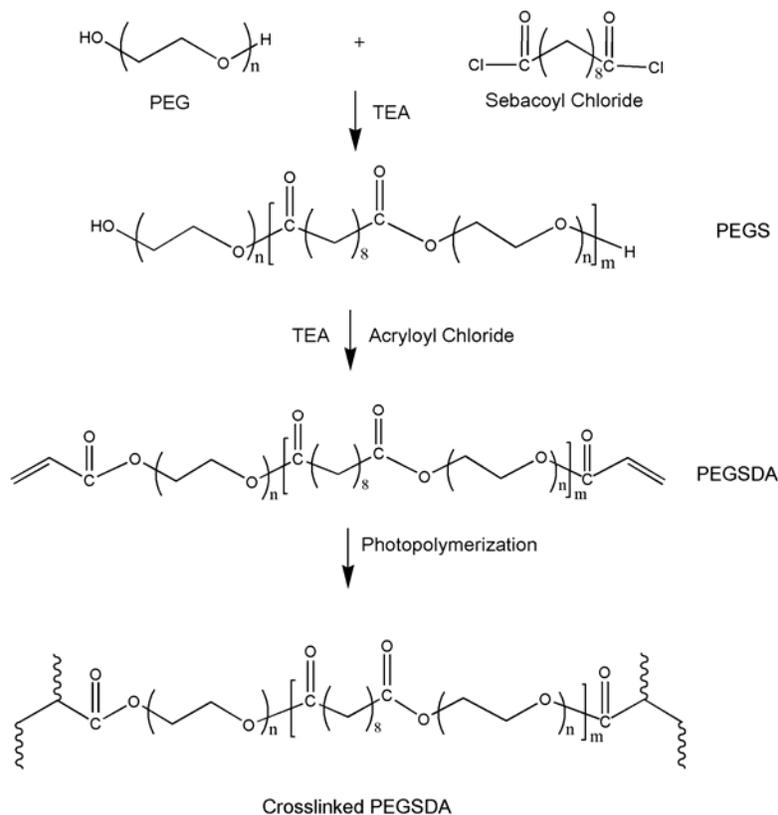
Photopolymerizable PEG based macromers were synthesized by the reaction represented in Scheme 1. Briefly, one hundred grams of PEG (MW: 1,000) was dissolved in anhydrous methylene chloride. Triethylamine (TEA) and sebacoyl chloride were subsequently added to the solution in an ice bath with vigorous stirring. The reaction mixture was stirred for at least 24 h at room temperature, filtered, precipitated in petroleum ether and dried under vacuum. A total of fifty grams of the above obtained macromer (PEG sebacate or PEGS) was dissolved in anhydrous methylene chloride and synthesized by adding triethylamine and acryloyl chloride subsequently. The reaction proceeded for another 24 h at room temperature and triethanolamine hydrochloride was removed by filtering the reaction mixture. The macromer (PEGSDA) was obtained by precipitation in petroleum ether and drying under vacuum. For further purification, the macromer was dissolved in methylene chloride and reprecipitated in petroleum ether. For comparison, PEG (MW: 8,000) diacrylate (PEGDA) was also synthesized by repeating the above procedure to make PEGSDA using PEGS and acryloyl chloride.

The final products were analyzed by gel permeation chromatography (GPC), FTIR-ATR and ¹H NMR. The swelling ratio was calculated by measuring initial dried weight and swollen weight after incubating hydrogels in PBS at 37 °C. Mechanical properties of the crosslinked polymers were analyzed with a dynamic mechanical rheometer using a parallel-plate with 8 mm diameter. Three-dimensional (3D) scaffold of PEGSDA hydrogel was fabricated as described previously [6].

Results and Discussion

Simple reaction between PEG and diacid monomers produces novel PEG based macromers, whose structures were deduced by analysis of their NMR and FT-IR spectra. The number-average molecular weight (M_n) and weight average molecular weight (M_w) of this

polymer (PEGSDA) measured by GPC was 11,000 and 20,200, respectively, with a polydispersity of 1.84. By comparison, M_n and M_w of conventional PEGDA are 11,200 and 12,800, with a polydispersity of 1.15. Similar number average molecular weights of those two polymers make it easy to compare physical properties with each other.



Scheme 1. Synthesis reaction for PEG-co-sebacic acid diacrylates and photopolymerization

After eight weeks of incubation, total weight losses of 25% and 50% (w/v) PEGSDA were 41.4 % and 35.2 %, respectively, without significant difference between the two. Such degradation profiles confirmed that these polymers are biodegradable in physiological conditions, compared with other PEG-based hydrogels which are considered non-biodegradable over a reasonable time scale [7]. This novel hydrogel can degrade even faster *in vivo* as other investigators reported with various biodegradable polymers due to enzymatic and/or cellular effects [8]. The equilibrium swelling ratio of 25% and 50% PEGSDA were 1.84 and 2.15, respectively after 24 h without significant difference (Fig 1A). This data confirmed that PEGSDA absorbed much less water than PEGDA (whose equilibrium swelling ratio was 12.6) mainly due to the presence of hydrophobic segment (sebacic acid) in the polymer backbone.

Mechanical properties were evaluated with dynamic mechanical analysis using a torsional rheometer. Dynamic shear modulus (G') of PEGSDA (Fig. 1B) was almost one order of magnitude greater than that of PEGDA throughout the tested frequency, indicating that PEGSDA was a much stronger hydrogel compared with the conventional PEGDA hydrogel.

The average moduli under the tested frequencies of PEGSDA and PEGDA were 90 and 10 kPa, respectively. As the amount of PEGSDA macromer increased from 25% to 50%, the G' of the hydrogel increased from 90 kPa to 230 kPa, which is comparable to the complex modulus G^* of the cartilage tissue under minimal testing torsional frequency (200 kPa at 0.1 rad/s [7]). It is also possible to further enhance the mechanical properties by incorporating co-monomers such as N- vinyl pyrrolidone (NVP) which has shown no adverse effect of the biocompatibility of synthetic macromers [9].

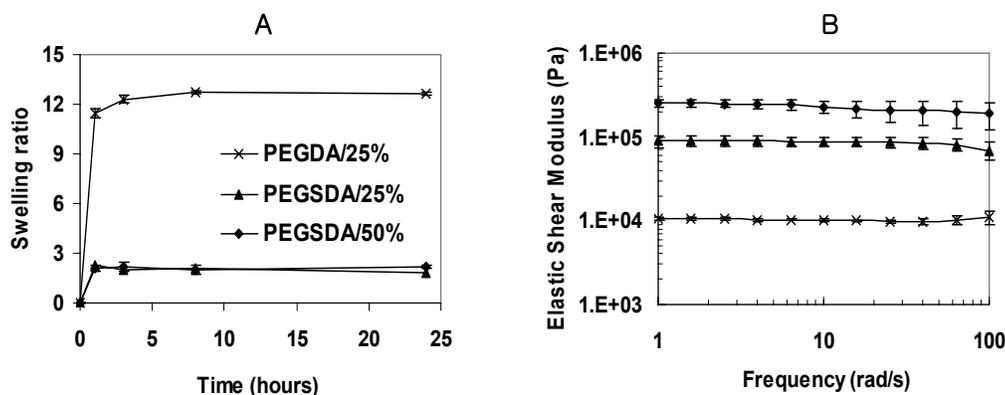


Figure 1. (A) Swelling ratio of crosslinked PEG based macromers. Swelling ratio was determined by the equation; swelling ratio = $(W_s - W_d)/W_d$, where W_s : swollen weight of polymers, W_d : dry weight. (B) Dynamic shear modulus of PEGSDA hydrogels. Dynamic shear modulus was determined by frequency sweeping using a torsional rheometer at 0.1% of the strain at room temperature.

It is known that hydrogels are hydrophilic and relatively resistant to protein adsorption or cell adhesion, so that hydrogels have often been modified with cell adhesion peptides to induce cell adhesion [10]. For possible application of PEGSDA hydrogel to bone tissue engineering, cell affinity and proliferation were evaluated with bone marrow stromal cells on PEGSDA hydrogel with cell adhesion peptides such as RGDS peptide. The cells on unmodified PEGSDA hydrogel after 24 h were well attached, but did not spread and maintained a spherical shape (Fig. 2A), whereas the cells on RGD peptide modified hydrogel spread extensively similar to the control (TCPS) (Fig. 2B & C). Interestingly, there was no significant difference in proliferation rate between the cells on unmodified and on RGDS peptide modified hydrogels (Fig. 2D). These results indicate that unlike other hydrogels which do not support cell attachment, PEGSDA hydrogel itself induces cell attachment and proliferation, but do not induce extensive spreading. Overall, PEGSDA hydrogel is cytocompatible and modulates cell adhesion and spreading with the immobilization of cell adhesion peptides similar to other hydrogels.

Three dimensional tissue engineering scaffold was easily fabricated from PEGSDA hydrogel using SFF techniques such as 3D printing. After 24 h of incubation in PBS at 37 °C, the structural integrity of the swollen 3D structure of PEGSDA was well maintained. Highly porous interconnected and well-controlled internal microarchitecture can be easily seen after drying (Fig 3). Using 3D printing technique, we have obtained pore sizes of ~600 μm at

swollen state and $\sim 300 \mu\text{m}$ at dry state. This range of pore sizes have shown most satisfactory outcome especially for bone tissue engineering due to the promotion of the ingrowth and distribution of osteoprogenitor cells throughout the

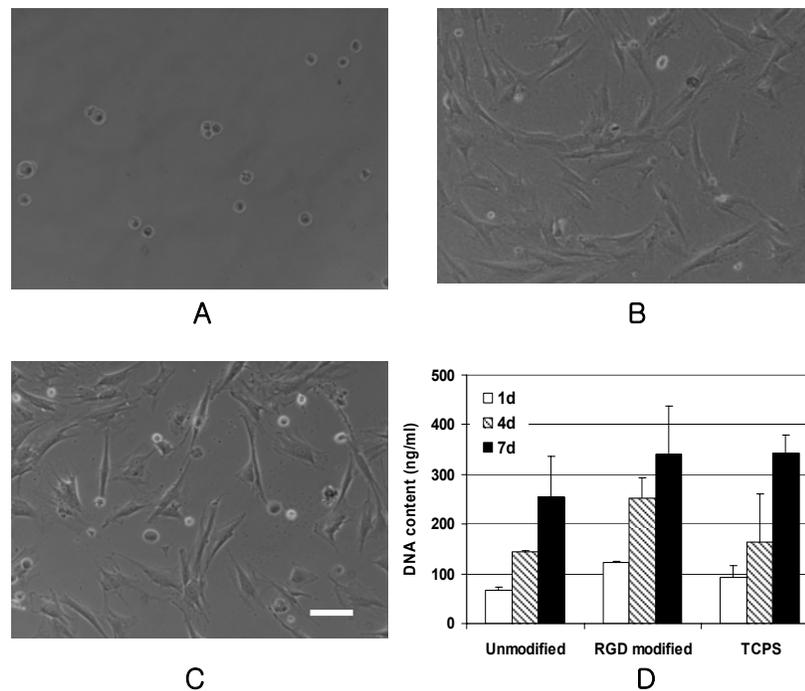


Figure 2. Morphology of rat-derived bone marrow stromal cells (BMSC) on various substrates: (A) Unmodified PEGSDA hydrogel, (B) Modified PEGSDA hydrogel with RGDS peptide, and (C) control, tissue culture polystyrene (TCPS) and (D) Cell proliferation quantified by DNA analysis. Scale bar = $100 \mu\text{m}$

matrix as well as the migration of endothelial cells into the matrix, which are crucial to new bone formation [11].

Conclusions

We developed novel biodegradable and photopolymerizable hydrogels based on PEG and sebacic diacid. Their synthesis and post synthesis processing are straightforward. Compared with conventional PEG based hydrogels (e.g. PEG diacrylate), PEGSDA is a relatively strong biodegradable hydrogel, thus greatly reduces handling difficulties. Physical properties including biodegradation rate are easily tailored by using different dicarboxylic acids or incorporating co-monomers into the polymer network. Bioactive molecules such as cell adhesion peptides were also readily coupled to the hydroxyl groups of the polymer in order to control the cell adhesion and proliferation on the hydrogel. Furthermore, complex 3D structures which are indispensable to especially tissue engineering application, was well fabricated and maintained its structural integrity due to enhanced mechanical properties.

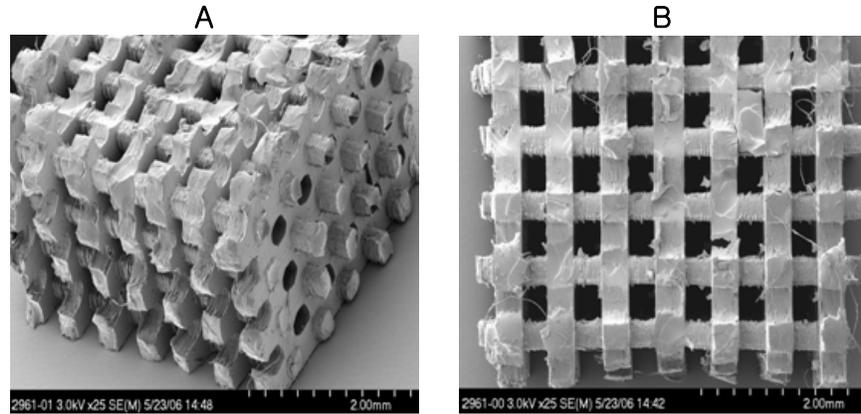


Figure 3. SEM micrographs of a dried, multi-layered PEGSDA hydrogel scaffold fabricated by 3D printing technique (A) side view and (B) top view.

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