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Urethane-based low-temperature curing, highly-customized and multifunctional poly(glycerol sebacate)-co-poly(ethylene glycol) copolymers

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ABSTRACT

Poly (glycerol sebacate) (PGS), a tough elastomer, has been widely explored in tissue engineering due to the desirable mechanical properties and biocompatibility. However, the complex curing procedure (high temperature and vacuum) and limited hydrophilicity (~90° of wetting angle) greatly impede its functionalities. To address these challenges, a urethane-based low-temperature setting, PEGylated PGS bioelastomer was developed with and without solvent. By simultaneously tailoring PEG and hexamethylene diisocyanate (HDI) contents, the elastomers X-P-mUs (X referred to the PEG content and m referred to HDI content) with a broad ranging mechanical properties and customized hydrophilicity were constructed. The X-P-mUs synthesized exhibited adjustable tensile Young's modulus, ultimate tensile strength and elongation at break in the range of 1.0 MPa–14.2 MPa, 0.3 MPa–7.6 MPa and 53.6%–272.8%, with the water contact angle varying from 28.6° to 71.5°, respectively. Accordingly, these elastomers showed favorable biocompatibility in vitro and mild host response *in vivo*. Furthermore, the potential applications of X-P-mU elastomers prepared with solvent-base and solvent-free techniques in biomedical fields were investigated. The results showed that these X-P-mU elastomers with high molding capacity at mild temperature could be easily fabricated into various shapes, used as reinforcement for fragile materials, and controllable delivery of drugs and proteins with excellent bioactivity, demonstrating that the X-P-mU elastomers could be tailored as potential building blocks for diverse applications in biomedical research.

Statement of Significance

Poly(glycerol sebacate) (PGS), a tough biodegradable elastomer, has received great attentions in biomedical field. But the complex curing procedure and limited hydrophilicity greatly hamper its functionality. Herein, a urethane-based low-temperature setting, PEGylated PGS (PEGS-U) bioelastomer with highly-customized mechanical properties, hydrophilicity and biodegradability was first explored. The synthesized PEGS-U showed favorable biocompatibility both in vitro and *in vivo*. Furthermore, the PEGS-U elastomer could be easily fabricated into various shapes, used as reinforcement for fragile materials, and controllable delivery of drugs and proteins with excellent bioactivity. This versatile, user-tunable bioelastomers should be a promising biomaterials for biomedical applications.

1. Introduction

Owing to the tunable physicochemical properties, easy fabrication and capacity to mimic viscoelasticity of different tissues, synthetic biodegradable elastomers have stimulated great interest in biotechnological and biomedical field [1–3]. Recently, several

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biodegradable elastomers, such as elastin-like polypeptides [4,5], polyurethanes [6–9] and poly (glycerol sebacate) [10–17] and their block copolymers [18,19] have been developed for soft tissue engineering. Among them, PGS, a crosslinked tough bioelastomer based on the polymerization of glycerol and sebacic acid, exhibited super elasticity and good biocompatibility, and has been applied in vascular [20], nerve regeneration [21] and myocardial tissue engineering [22] since 2002. Unfortunately, poor hydrophilicity and conventional high temperature thermo-curing (>130 °C, vacuum) process severely hampered the applications of PGS. Therefore, there is a great need to develop an alternative strategy for preparation of PGS-based elastomers that allows for low temperature crosslinking and desirable wettability, while preserving or improving its unique elasticity. As we know, hydrophilicity of biomaterials directly can determine their degradation rate, diffusion characteristic and biomedical properties in vitro and *in vivo* [2,23]. As one of promising and potential biodegradable elastomer, the formidable hydrophobicity of PGS in nature often led to undesirable cellular responses and thereby undermined its targeted properties for various biomedical applications. Therefore, the amelioration of hydrophilicity for PGS-based elastomer has attracted ever-increasing attention in recent years [23,24]. PEGylation, as an approach to introduce PEG segments [25,26], has stood out for its multifaceted regulation in hydration properties along with mechanical behaviors and degradation profiles. Alpesh Patel et al. [23] developed a series of PGS-co-PEG (PEGS) with gradient of PEG content, which exhibited controllable hydrophily, as well as desirable ductility and favorable cell attachment. Furthermore, our group [27] had found that compared with the PGS, PEGS could effectively improve the hydrophilicity and cell response of calcium phosphate scaffold. However, even these tempting performance, the exacting curing conditions (>150 °C, vacuum) and long reaction time (>24 h) required for the crosslinking of PEGylated PGS could trigger oxidized color and untoward molding, which greatly impaired its further applications in tissue engineering.

To overcome the drawbacks of traditional high-temperature crosslinking on thermoset bioelastomer, several strategies have been proposed, such as photocurable methacrylated PEGS (PEGS-M) [28], diacrylated PEG (PEGDA) [29,30] and CinA-based hydrogels [31]. As for these photo-induced crosslinking researches, though, these materials could be synthesized under mild conditions, it is difficult to protect sensitive molecules from UV light (e.g. growth factors delivery) and the obtained elastomers were typically weaker. In contrast, diisocyanate and its derivates have been demonstrated as highly effective chemical crosslinkers to improve the mechanical properties and multifunction of elastomer under mild conditions [8,32–34]. For example, urethane crosslinked intrinsically multifunctional silica-poly (citrate) (CMSPC)-based hybrid elastomers [33] and poly(glycerol sebacate urethane) (PGS-U) [34] exhibited highly tunable mechanical properties and could be synthesized facilely. Furthermore, previous studies [32,34,35] have utilized diisocynate in drug-delivery applications as well. In this regard, diisocyanate might be a favorable crosslinker to cure PEGS under low temperature and endow it with the ability to deliver bioactive molecules.

Based on the above rationale, in this study, we aimed to develop a urethane-based, low-temperature setting PEGylated PGS (PEGS-U) bioelastomer in the presence or absence of solvent. In order to optimize the structure and properties of PEGS-U, a series of elastomers with different HDI ratios and PEG contents were developed and the effects of dual variables on mechanical and degradation properties, as well as cell attachment, proliferation, in vitro and *in vivo* cytotoxicity were assessed. Given the synergy effect of two factors (PEG and HDI content), the elastomer PEGS-U exhibited highly tunable mechanical behavior, hydrophilicity and degradation. In addition, the mild reaction conditions of isocyanate and free hydroxyl group allowed the elastomer to be applied in encapsulating bioactive macromolecules and controlling release. Finally, according to the features of PEGS-U, several potential applications were also investigated, including multiformity matrix for tissue engineering, reinforcement of fragile materials and drug or protein delivery.

2. Materials and methods

2.1. Synthesis of PGS and PEGS pre-polymer

PGS and PEGS pre-polymer were synthesized according to our previous study [27]. Briefly, PGS in this study was synthesized by polycondensation of 0.06 mol sebacic acid (Aladdin) and 0.04 mol glycerol (Aladdin) at 130 °C under argon for 24 h. The reaction was kept at 60 mTorr and 160 °C for 4 h. For PEGS, mole percentage ratio of PEG in PEGS pre-polymers were set as 20% and 40% in this work, thus the pre-polymer samples were respectively coded as PEGS20 and PEGS40 in the following discussion. The synthetic process of PEGS consisted of two steps. At first, PEG (Aladdin, Mw = 1000 g/mol) was dried to be molten and then mixed with sebacic acid at 130 °C under the flow of Argon for 2 h before reacting under vacuum for another 24 h. In the second step, a specific amount of glycerol and sebacic acid was added into the flask with the flow of Argon until the mixture became clarified, then the reaction was further carried out at specific reaction temperature (130 °C for PEGS20 and 140 °C for PEGS40) and under vacuum for 48 h. The overall carboxyl to hydroxyl molar ratio was kept as 1:1. The final pre-polymer was obtained after dialyzing by 3500 D dialysis bag.

2.2. Preparation of X-P-mUs

The pre-polymer was dissolved in anhydrous dimethyl formamide (DMF, 10% w/v) (Aladdin) within the catalyst Tin (II) 2-ethyl-hexanoate (0.05% w/v) (Sigma) in a flask. Hexamethylene diisocyanate (HDI) (Sigma) was then added drop-wise after heating the reaction mixture to 55 °C. With the flow of Argon, the flask was sealed and reacted with stirring for 5 h. The solution was transferred to a Teflon mold for 2 days at room temperature and then evaporated in a vacuum oven at 30 °C for another 2 days [34]. The products we synthesized were coded as X-P-mU for different PEG content and ratio of HDI to glycerol, where 'X' referred to the mole percentage of PEG in PEGS pre-polymer and 'm' referred to the molar ratio of HDI to glycerol moiety. The PEG content 'X' would be 0%, 20%, and 40%, while the ratio 'm' of HDI to glycerol would be 0.5 and 1.0. Additionally, the process of the elastomers prepared by solvent-free approach was shown in supporting and these samples were coded as X-P-mU-F.

2.3. Chemical characterization

The molecular weight of PEGS and PGS pre-polymers were determined by gel permeation chromatography (GPC, Shimadzu Prominence, Kyoto, Japan). The samples were dissolved in tetrahydrofuran (THF) (0.5% w/v) (Aladdin) and injected at the flow rate of 1 mL/min. Polyethylene glycol standards were used for the calibration.

Fourier transform infrared (FT-IR) spectra (Nicolet 5700, Thermo) of pre-polymers and X-P-mU elastomers were recorded in the range of 800–4000 cm^{-1}. The components of pre-polymers and X-P-mUs were also identified by nuclear magnetic resonance (NMR) (Brukeravance II 600, Bruker Corporation, Switzerland). The resulting data were processed and analyzed using MestReNova NMR software. Differential scanning calorimeter (DSC, modulated

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