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Self-healing supramolecular bioelastomers with shape memory property as a multifunctional platform for biomedical applications *via* modular assembly

Yaobin Wu [a, 1], Ling Wang [a, 1], Xin Zhao [a], Sen Hou [a], Baolin Guo [a, *], Peter X. Ma [b, c, d, e, **]

[a] Frontier Institute of Science and Technology, and State Key Laboratory for Mechanical Behavior of Materials, Xi'an Jiaotong University, Xi'an, 710049, China
[b] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA
[c] Department of Biologic and Materials Sciences, University of Michigan, Ann Arbor, MI 48109, USA
[d] Macromolecular Science and Engineering Center, University of Michigan, Ann Arbor, MI 48109, USA
[e] Department of Materials Science and Engineering, University of Michigan, Ann Arbor, MI 48109, USA

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ABSTRACT

Mimicking native functional dynamics for traditional biomaterials such as thermoset elastomers is limited due to their lack of responsiveness to biological stimuli and difficulties to incorporate biofunctionalities. Furthermore, the mechanical fracture of traditional thermoset elastomers caused by irreversible covalent bond rupture would lead to their permanent loss of properties. To overcome these challenges, degradable self-healed supramolecular bioelastomers are designed by an elastic poly(glycerol sebacate) (PGS) backbone and multiple hydrogen-bonding ureido-pyrimidinone (UPy) grafts. These supramolecular elastic polymers exhibit efficient self-healing, rapid shape-memory abilities and highly tunable mechanical properties due to the dynamic supramolecular interactions, and perform a good biocompatibility *in vitro* and a mild host response *in vivo*. By combining modular approaches, these supramolecular bioelastomers have been further assembled into a multifunctional platform to expand their applications in different biomedical fields. These include a complex 3D scaffold with shape-memory capacity and anisotropic mechanical properties, a controllable drug delivery model *via* a layer-by-layer technique, a surface antibacterial composite by physical modification, and a spatial oriented cell co-culture system *via* incorporating different cell-laden self-healing films, demonstrating their potential as building blocks in a wide range of biomedical applications where dynamic properties and biological functions are desired.

1. Introduction

In human body, natural materials such as double-helix DNA, functional proteins, and microfilaments exhibit the ordered structures assembled from biomolecules by supramolecular chemistry, and perform various biological functions *via* modular approaches in maintaining our life [1–4]. However, although traditional biomaterials show the potential for preparing similar mechanical properties or structures with native tissues, their ability to mimic native functional dynamics is still limited [5–7]. For instance, biodegradable thermoset elastomers such as poly(glycerol sebacate) (PGS) and its derivatives have been proposed as promising materials for various regenerative medicine applications [8–11], because of their good biocompatibility and the similar viscoelastic properties with several soft tissues [12–16]. However, the mechanical fracture and irreversible covalent bond rupture of these chemically crosslinked bioelastomers would lead to their permanent loss of properties, which limit their practical use within a complex *in vivo* environment. Furthermore, many thermoset bioelastomers lack of responsiveness to biological stimuli and are hard for the incorporation of biofunctionalities, which is an additional

* Corresponding author. Frontier Institute of Science and Technology, and State Key Laboratory for Mechanical Behavior of Materials, Xi'an Jiaotong University, Xi'an, 710049, China.
** Corresponding author. Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA.
E-mail addresses: baoling@mail.xjtu.edu.cn (B. Guo), mapx@umich.edu (P.X. Ma).
[1] These authors contributed equally to this work.

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limitation for their potential for a wide range of biomedical applications [13]. To overcome these limitations, developing biodegradable elastic materials with mechanical self-healing and the ability to mimic native functional dynamics are desperately needed, while remain an ongoing challenge.

As an effective reversible chemical strategy, supramolecular principles are a promising substitute to prepare non-covalent polymeric materials that not only show the mechanical properties similar with traditional covalent materials but also exhibit special dynamic properties [17–19]. Recently, various supramolecular materials with different functions such as self-healing [20–24], shape-memory [25–28], and stimuli-responsiveness [29–32] have been developed by combining different supramolecular recognition motifs and polymeric backbones [33–36]. Furthermore, some of these supramolecular materials have been used in a wide range of biomedical applications including drug delivery, biomimetic chemistry, and regenerative medicine [37–39]. In this regard, we hypothesize that incorporating elastic polyester backbone with supramolecular self-assembly motifs would be a creative approach to prepare a supramolecular elastic material with dynamic and reversible properties. Furthermore, by the elegant combination of supramolecular dynamic and reversible structures, incorporation of functional building blocks *via* supramolecular motifs has been regarded as an attractive and efficient modular approach to combine different functional properties into a biomaterial system [40–43]. As such, we suppose that developing supramolecular bioelastomers can not only overcome mechanical disadvantages of traditional thermoset bioelastomers, but also make the process of biological functions modification more easy and efficient *via* modular approaches, thereby showing potential as a promising multifunctional platform for widespread biomedical applications.

Here, we present a highly tunable supramolecular bioelastomer with self-healing and shape-memory properties by combining an elastic polyester backbone and hydrogen-bonded supramolecular motifs, and further assemble this bioelastomer as a multifunctional platform *via* modular approach for a range of biomedical applications. PGS is chosen as the elastic backbone due to its good elasticity and biocompatibility [9,44], meanwhile ureido-pyrimidinone (UPy) is used as supramolecular self-assembly motif grafted on PGS polymer chain due to its strong non-covalent interactions formed by the well-defined quadruple H-bonding structure [45–49]. Given the complementary advantages of PGS elastic chains and UPy strong H-bonding motifs, the novel supramolecular bioelastomers poly(glycerol sebacate)-graft-UPy (PGS-U) not only exhibited highly tunable mechanical properties but also showed self-healing and shape-memory properties. Furthermore, the *in vitro* biocompatibility and the *in vivo* biological response of these PGS-U polymers have been investigated. Taking advantages of dynamic supramolecular properties, we demonstrated these supramolecular bioelastomers as the building blocks to develop a multifunctional platform *via* modular approaches for expanding their biomedical applications, which included fabricating a complex 3D biomaterial scaffold with anisotropic properties, preparing a controllable drug delivery model, constructing a surface antibacterial composite, and establishing a cell co-culture system for multiple cell types with spatial orientation.

2. Materials and methods

2.1. Materials

Glycerol, sebacic acid, N, *N*-Dimethylformamide (DMF) (anhydrous, ≥ 99.9%), anhydrous dimethyl sulfoxide (DMSO) (anhydrous, ≥ 99.9%), epsilon-poly-L-lysine (EPL), 2-Amino-4-hydroxy-6-methylpyrimidine, hexamethylene diisocyanate (HDI), stannous octoate ($Sn(Oct)_2$), lipase enzyme from *Thermomyces lanuginosus* (≥100,000 U/g), and 5-aminosalicylic acid (5-ASA) were all obtained from Sigma-Aldrich and used without further purification. Diethyl ether, pentane, and tetrahydrofuran (THF) were used as received. Minimum Essential Medium alpha, Dulbecco's Modified Eagle Medium, phosphate buffered saline (PBS), fetal bovine serum and horse serum were all purchased from Gibco, life technologies. CellTracker Green CMFDA and CellTracker Red CMTPX reagents were obtained from Molecular Probes, life technologies.

2.2. Synthesis of PGS-U polymers and preparation of PGS-U polymer films

The synthesis of PGS prepolymer and UPy-HDI synthon were carried out by following the previous reports [10,50,51], and the details are available in SI Materials and Methods. PGS-U polymer was synthesized using a one-pot reaction. The PGS prepolymer was dissolved in anhydrous DMF (10 wt%), and the UPy-HDI synthon was then added into the reaction solution with a strict molar ratio (Table S1). After addition of catalyst $Sn(Oct)_2$ (0.3% w/v), the solution was stirred at 85 °C under nitrogen atmosphere for 12 h. The products were precipitated from DMF in diethyl ether, and then dried in a vacuum oven for 3 days, resulting in pale-yellow materials. PGS-U films were prepared by dissolution of PGS-U polymers in DMF (15 wt%) while stirring the solution for 2 h at 80 °C. The polymer solution was dropped into Teflon mold or on glass cover slips (14 mm diameter), and the films were obtained by solvent evaporation in an oven at 50 °C for 24 h and in a vacuum oven at 40 °C for another 3 days. The chemically crosslinked PGS elastomer films were prepared according to the previous reports [8,10]. The details are available in SI Materials and Methods.

2.3. Characterization

The gel permeation chromatography (GPC), nuclear magnetic resonance (^{1}H NMR), fourier transform infrared spectroscopy (FT-IR), UV–vis spectroscopy, differential scanning calorimetry (DSC), rheological studies, and viscosity measurements were used to investigate the chemical and physical properties of polymer samples. The details are available in SI Materials and Methods.

2.4. Mechanical properties

Tensile measurements were performed by using a MTS Criterion tester at an extension rate of 50 mm/min until specimen failure ($n > 6$ per sample), and Young's modulus calculated as the slope at 5% strain. For the cyclic tensile test, both loading and unloading cycles were performed at constant velocity of 50 mm/min with the maximum strain of 50%. The PGS-U polymer film specimens were prepared with 30 mm × 6 mm × 0.8 mm, and the tensile tests were performed at room temperature.

2.5. Self-healing properties

The self-healing properties of PGS-U polymer were performed as following methods. For mechanical healing analysis, the samples (30 mm × 6 mm × 0.8 mm) were cut with a razor blade from the middle, and then the cutting faces were pressed together for 1 min and left for set times (0.5 h, 1 h, 2 h, and 12 h) at a certain temperature (37 °C, 55 °C, or 75 °C) before tensile testing. In order to visualize the self-healing process, Nile red and crystal violet were mixed respectively in PGS-U polymer solution before film preparation to label individual polymer film. The films with Nile red and films with crystal violet were cut from the middle, and then the