Polymer 86 (2016) 32–41

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Reduction- and pH-Sensitive lipoic acid-modified Poly(L-lysine) and polypeptide/silica hybrid hydrogels/nanogels

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ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form
7 January 2016
Accepted 12 January 2016
Available online 14 January 2016

Keywords:
Polypeptide-based hydrogel/nanogel
Stimuli-sensitive
Hybrid hydrogel/nanogel

ABSTRACT

We report the preparation of biocompatible, dual stimuli-sensitive lipoic acid-modified poly(L-lysine) (PLL-g-Lipo) hydrogels/nanogels by chemical cross-link and polypeptide/silica hybrid hydrogels/nanogels by simply depositing silica in the gel matrix. Hydrogels were prepared by ethylene oxide sterilization through the formation of inter-/intramolecular disulfide cross-link. Their gelation and mechanical properties were dictated by the degree of inter-/intramolecular disulfide cross-link and amphiphilic nature, which was influenced by the chain conformation. These hydrogels exhibited reduction- and pH-sensitive properties as demonstrated by the accelerated drug release upon changing the solution pH from neutral to acidic condition and/or cleaving disulfide bonds. Swelling studies demonstrated that the genipin and disulfide cross-linked nanogels prepared by miniemulsion exhibited redox- and pH-sensitive properties. Silica deposition resulted in hybrid nanogels exhibiting excellent colloid stability. Silica deposition can not only stabilize the gel network but also tune their mechanical properties as well as their payload release and colloidal properties.

1. Introduction

Hydrogels, which are three-dimensional cross-linked hydrophilic polymeric networks stabilized through physical or chemical cross-links, have been studied extensively for biomedical applications including drug delivery, tissue engineering, and intelligent devices [1–9]. A variety of biocompatible natural and synthetic polymers have been utilized to prepare hydrogels/nanogels. Comparing to natural polymer-based hydrogels/nanogels, synthetic polymer-based hydrogels/nanogels exhibited the versatility in tailoring macromolecular chemistry to tune the materials property and functionality. It provides the opportunity to develop stimuli-responsive hydrogels/nanogels by properly choosing polymer building block and cross-linking agent to meet the criteria for specific biomedical applications. These hydrogels/nanogels can stimulate structural and/or morphological responses such as sol–gel transition, swelling/shrinking, or degradation in response to the changes in the environmental conditions, which could be physical changes such as temperature [10–12] and light [13,14], or (bio)chemical stimuli such as pH [15–17] and chemical agents [17–19].

Among these stimuli, pH and temperature are the most common ones for the development of hydrogels/nanogels for biomedical applications. Particularly, hydrogels/nanogels exhibiting swelling-shrinking responses or sol–gel transition to temperature and/or pH triggers have been extensively studied as controlled drug delivery systems [1,4,8,9]. However, most of these chemically cross-linked hydrogels/nanogels can only be utilized for the delivery of cargoes through a degradation- or diffusion-controlled release, which led to a long-term and incomplete drug release due to their slow degradation and cannot satisfy the on-demand delivery for certain applications. Incorporation of various decomposable cross-linkers including peptide [20] and some labile bonds [7] can overcome this problem. Disulfide bond, which can be cleaved by redox agents including glutathione (GSH), dithiothreitol (DTT), and reactive oxygen species (ROS), can serve not only as a degradable cross-linker but also as a reduction trigger for drug delivery. It has been demonstrated that it is possible to achieve controlled drug release of payloads by incorporation of disulfide bonds in the gel network [18,21–23].

It would be desirable to prepare stimuli-responsive hydrogels/nanogels from polymers that contain natural building blocks such

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http://dx.doi.org/10.1016/j.polymer.2016.01.030
0032-3861/

as saccharides and amino acids because it could generate polymers with inherent biocompatibility, biodegradability, and rich functionalities for facile modification. Polypeptides can adopt ordered conformations such as α-helices and β-sheets, and their molecular weight and composition can be precisely controlled via the living ring opening polymerization (ROP) of *N*-carboxyanhydrides (NCAs) [24–26]. Polypeptide-based block copolymers can form hydrogels through reversible physical interactions such as hydrophobic interactions and hydrogen bonding between polymer chains and their gel properties can be tuned by varying their chain conformation and amphiphilicity [27–31]. Chemically cross-linked polypeptide hydrogels can also be prepared by forming covalently bonded networks and their gel properties can be tuned by varying molecular weight, chain composition, and cross-linking degree [10,32–34].

Herein we report a simple approach to prepare reduction/pH dual sensitive lipoic acid-modified poly(L-lysine) (PLL-*g*-Lipo) hydrogels through ethylene oxide (EO) sterilization and nanogels via miniemulsion. In addition, PLL-*g*-Lipo/silica hybrid hydrogels/nanogels were also prepared by depositing silica in the gel matrix. For the preparation of hydrogels, this apporach is advantageous since EO sterilization is a common procedure to sterilize biomaterials and devices. A series of PLL-*g*-Lipo graft copolypeptides was synthesized by varying PLL molecular weight and lipoic acid grafting ratios. The genipin and disulfide cross-linked nanogels were prepared by miniemulsion The resultant hydrogels/nanogels are expected to exhibit redox- and pH-sensitive property due to the presence of disulfide linkages and pH-sensitive PLL. Lipoic acid, which is a naturally occurring compound generated by human body, is currently applied for the treatment of varying diseases including Alzheimer's disease and diabetes [35,36]. While polypeptide-based hydrogels/nanogels have gained increasing attention recently, there are only a few studies on dual stimuli-responsive hydrogel/nanogel system based on polypeptide-based biomaterials [10,17]. The gel morphology and mechanical properties of the as-prepared polypeptide and polypeptide/silica hybrid hydrogels were studied by varying the polypeptide chain length, lipoic acid grafting ratio, and silica weight percentage. A preliminary investigation on the cytotoxicity of these hydrogels/nanogels was carried out in order to evaluate the biocompatibility of these materials. The reduction- and/or pH-sensitive properties of polypeptide and polypeptide/silica hybrid hydrogels/nanogels were studied in order to evaluate the feasibility of these materials for drug delivery application. It is expected that silica deposition in the gel network can be a means to further stabilize the gel network and simultaneously tune their mechanical properties as demonstrated in our previous study [37], as well as the drug release behaviors and colloidal properties.

2. Experimental section

2.1. Synthesis of PLL-g-Lipo graft copolypeptides

Poly(Z-L-lysine) (PZLL) was synthesized using the zero valent nickel initiator 2,2′-bipyridyl-Ni(1,5-cyclooctadiene) (BpyNiCOD) to polymerize Z-L-lysine NCA by following the literature reported procedures [38,39]. The PZLL polypeptides with different molecular weights were synthesized and characterized by GPC (Table. S1). The Z group was deprotected by using HBr to obtain PLL, which were PLL_{50}, PLL_{90} and PLL_{180}. PLL-*g*-Lipo copolypeptides were synthesized by reacting lipoic acid (98+%, Alfa Aesar) with the amino group on PLL using 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 98+%, Alfa Aesar) and *N*-hydroxysuccinimide (NHS, 98%, Aldrich). In a glove box, PLL and lipoic acid were weighted out based on the designated grafting ratio of lipoic acid and dissolved in anhydrous methanol. EDC and NHS with two folds of the mole of lipoic acid were added to the solution. The reaction mixture was then taken out of the glove box and left to stir at room temperature for two days. Then the solution was placed in a cellulose membrane dialysis tube (MWCO 12000–14,000 g mL^{-1}, Membrane Filtration Product), dialyzed against deionized (DI) water for two days, and then freezed-dried to yield the product as a white spongy material.

2.2. Preparation of PLL-g-Lipo and PLL-g-Lipo/silica hybrid hydrogels

For the preparation of polymer hydrogels, PLL-*g*-Lipo graft copolypeptides were sterilized by ethylene oxide vapor in an autocleave (pressure: −2.7 kg/cm^2, sterilization time: 8.5 h, aeration time: 7.5 h). Hydrogels were prepared by dissolving PLL-*g*-Lipo samples in DI water and a vortex mixer was used to enhance the mixing and dissolution process. For the preparation of polypeptide/silica hybrid hydrogels, the calculated amount of tetramethyl orthosilicate (TMOS, 99%, Fluka) was added to the as-prepared hydrogel samples. The resultant samples were mixed by using a vortex mixer and then aged at 40 °C for at least two days. The drug loading capacity (LC) was calculated by the following equation:

$$LC(\%) = W_{Dox}/(W_{S_p} + W_{Dox}) \times 100$$

where W_{Dox} is the weight of the Dox loaded in hydrogels and W_{SP} is the weight of the freezed-dried gel specimen.

2.3. Preparation of PLL-g-Lipo and PLL-g-Lipo/silica hybrid nanogels

Nanogels were synthesized in a water-in-oil miniemulsion. The oil phase was prepared by using Span 80 (75 mg) and Tween 80 (25 mg) dissolving in 4 mL of n-hexane. The aqueous phase was prepared by dissolving PLL-*g*-Lipo (5 wt%) in 0.5 mL of DI water. The aqueous phase was then mixed immediately with the oil phase, stirred for 10 min, and pulsed through a probe sonication (SonicsÒ VCX750, Vibra-Cell Ultra Sonics Processor) with energy levels of 700 kW for 5 min with ice bath. Genipin (4.2 or 8.4 mg) was added to the emulsified solutions and pulsed again through a probe sonication for 5 min with ice bath. The reaction mixture was stirred at 350 rpm for 12–24 h. The nanogels were purified by centrifuging the emulsified solutions at 7000 rpm (Hitachi, ct15e) for 1 min before removing the supernatant. The aqueous phase was extracted by adding 1 mL of n-hexane and the above process was repeated until the oil phase is colorless. After removing the oil phase, 1 mL of DI water was added to the solution and centrifuged at 7000 rpm (Hitachi, ct15e) for 1 min before removing the supernatant. 10 mol% of GSH was added to the solution and stirred for 2 h. The aqueous phase was then dialyzed extensively using a cellulose membrane dialysis tube (MWCO 100 000 g mL^{-1}) for two days. The polypeptide/silica hybrid nanogels were prepared by adding 0.015 mL of TMOS to 1 mL of nanogel solution. The resultant solutions were then mixed by using a vortex mixer, placed still for 2 h, and dialyzed extensively using a cellulose membrane dialysis tube (MWCO 100 000 g mL^{-1}) for at least one day.

2.4. In vitro cytotoxicity tests

The fibroblast 3T3 cells were cultured onto a 48-well plate (1×10^4 cells/mL) using DMEM (Dulbecco's modified eagle medium, Gibco) with 10% FBS (fetal bovine serum, Gibco) under a humidified atmosphere of 5% CO_2 at 37 °C for 24 h. For hydrogel samples, the medium was replaced with extract fluids obtained by