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Brief communication

High performance and reversible ionic polypeptide hydrogel based on charge-driven assembly for biomedical applications

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ABSTRACT

In the pursuit of new strategies for the design and synthesis of high performance, physically associated hydrogels, dynamic materials formed through electrostatic interactions can serve as a powerful model. Here, we introduce a convenient strategy to obtain biodegradable hydrogels from ABA triblock ionic polypeptides formed by mixing poly(L-glutamic acid)–block-poly(ethylene glycol)–block-poly(L-glutamic acid) (PGA–PEG–PGA) with poly(L-lysine)–block-poly(ethylene glycol)–block-poly(L-lysine) (PLL–PEG–PLL). The hydrogels showed tunable physical properties, high strength and reversible response. The reactive function groups in the ionic blocks can conjugate with oppositely charged drugs or proteins and allow for further modification. These ionic ABA triblock polyelectrolytes can also encapsulate intact cells without significantly compromising cell viability, suggesting that the hydrogels have excellent cytocompatibility. In vivo evaluation performed in rats with subcutaneous injection indicated that the gels were formed and degraded, and hematoxylin and eosin staining suggested good biocompatibility in vivo. In addition, these advantages, combined with the synthetic accessibility of the copolymer, make this cross-linking system a flexible and powerful new tool for the development of injectable hydrogels for biomedical applications.

1. Introduction

Hydrogels are three-dimensional (3-D) networks with high water content which maintain their structural integrity. Due to their good biocompatibility, flexibility, variable composition and desirable physical characteristics which mimic physical properties of tissues, potential responsiveness to certain stimulus and effective loads for chemicals and organisms, the hydrogels are perfect candidate soft materials for many biomedical applications, including as cell scaffolds for tissue regeneration, as carriers for cell encapsulation or drug/gene delivery and so on [1–5].

In particular, in situ formed hydrogels have recently been widely studied for their superiority in moldability and operation [1,6]. They can be easily applied through mild gelation conditions and in a minimally invasive manner. Following injection in vivo, the hydrogels form constructs in situ, providing local biological and mechanical cues that may enhance tissue regeneration or responding to local surroundings that may control drug release [7,8]. In situ gelation can be obtained via chemical cross-linking or via self-assembly by reversible interaction. Temperature, pH, hydrogen bonding, π–π stacking and electrostatic or hydrophobic interaction are examples of the most widely studied external stimuli for self-assembly [1,9–11]. Typically, drawbacks of such self-assembled hydrogels are poor mechanical properties and stability due to their generally weak intermolecular interactions.

Unlike the direct self-assembly of ligand molecules such as metal ions and small molecules as cross-linkers [12] or recombinant peptides and proteins as cross-linkers [13], the hydrogels formed by mixing the synthetic block copolyelectrolytes with oppositely charged polymer blocks showed tunable physical properties, reversible response, high stability, strength and resilience [14–16]. However, in the previous studies, these synthetic copolyelectrolyte hydrogels lacked biodegradability, limiting their biomedical applications.

Herein, we present a convenient strategy for the formation of biodegradable hydrogels from water-soluble ABA triblock ionic polypeptides, which formed by mixing poly(L-glutamic acid)–block-poly(ethylene glycol)–block-poly(L-glutamic acid) (PGA–PEG–PGA) with poly(L-lysine)–block-poly(ethylene glycol)–block-poly(L-lysine) (PLL–PEG–PLL) (Scheme 1). The key to this strategy was the phase separation of ion-rich regions from the aqueous environment, which occurred on mixing two ABA triblock copolymers with oppositely charged anionic and cationic blocks

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[15,16–18]. The hydrogel assembled and disassembled reversibly through a pH-trigger operation at both high and low pH controlling the level of electrostatic interactions. In contrast to mixing directly the pure poly(glutamic acid) and polylysine, only the precipitate or coacervate was obtained [19,20].

Synthetic ionic polypeptide hydrogels based on electrostatic interaction have several advantages: (1) good water solubility and a low concentration of the unmixed copolyelectrolyte, which facilitate the encapsulation and injection; (2) rapid gelation and high modulus; (3) well-controlled ring-opening polymerization of the amino acid NCA, producing symmetric triblock copolymers with a well-defined mass and composition, providing the resulting materials with a high and tunable density of ionic groups; (4) the ability of the charged ionic block to conjugate with oppositely charged drugs or proteins to achieve high-efficiency encapsulation and controllable release; (5) the presence of the reactive function units for the post-polymerization modification through EDC condensation chemistry. Not limited to the pairs of poly(glutamic acid) and polylysine blocks, this strategy is rather flexible and can lead to a library of well-defined, ABA triblock ionic copolyelectrolytes of diverse block lengths and nature of ionic groups, such as polyarginine, poly(aspartic acid) and their copolymers. Therefore, through the use of charge-driven assembly, a new design strategy for robust hydrogels is obtained that could be widely used in a variety of materials and potentially useful in biomedical applications, such as cell scaffolds and drug carriers.

2. Experimental section

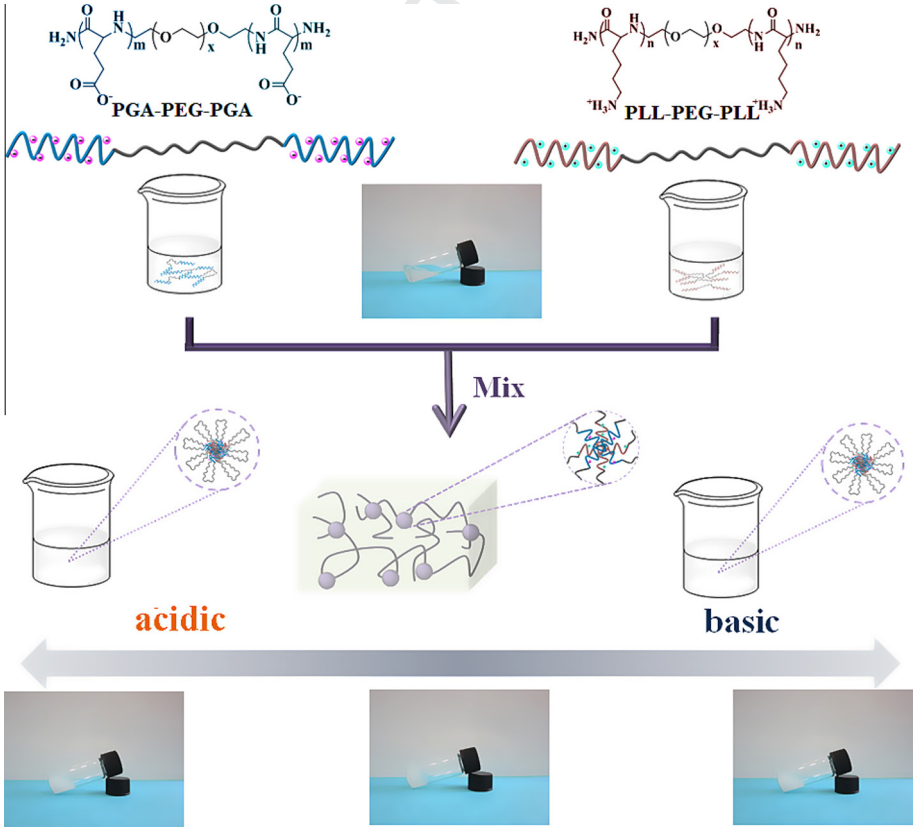
2.1. Materials

PEG (Mn = 1500 and 4000 Da) was purchased from Sigma–Aldrich without further purification. γ-Benzyl-L-glutamate (BLG) and ε-benzyloxycarbonyl-L-lysine (ZLL) were purchased from GL Biochem Co. Ltd. Amino-terminated poly(ethylene glycol) (NH_2–PEG–NH_2), γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) and ε-benzyloxycarbonyl-L-lysine-*N*-carboxyanhydride (ZLL-NCA) were synthesized as described in previous work with slight modification [21–23]. *N*,*N*-dimetylformamide (DMF) was dried over calcium hydride (CaH_2) before vacuum distillation. All the other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd and used as received. All chemicals were of analytical grade or higher.

2.2. Synthesis of PGA–PEG–PGA and PLL–PEG–PLL copolymers

As shown in Scheme S.1, PGA–PEG–PGA (G) and PLL–PEG–PLL (L) were synthesized through ring-opening polymerization (ROP) of BLG-NCA and ZLL-NCA in DMF using NH_2–PEG–NH_2 as initiator and followed by deprotection reaction, respectively.

A typical procedure for the preparation of PGA_{44}–PEG_{91}–PGA_{44} was as follows: NH_2–PEG_{91}–NH_2 (4.0 g, 1.0 mmol) was dissolved in toluene (50 ml) and the residual water was removed by azeotropic distillation. Then a certain amount of BLG–NCA (21.0 g, 40.0 mmol) and anhydrous DMF (100 ml) were added to the dried NH_2–PEG–NH_2. The reaction mixture was stirred at 25 °C for 3 days under a dry nitrogen atmosphere. The different reactant feeding molar ratios are listed in Table 1. The solution was precipitated into an excess amount of diethyl ether to give the $PBLG_{44}$–PEG_{91}–$PBLG_{44}$ triblock copolymers. Subsequently, the copolymer was dissolved in dichloroacetic acid (mass/vol. 1/10) and HBr/acetic acid (33 wt.%, mass/vol. 1/3) was added. The deprotection reaction was conducted at 30 °C for 2 h and then the mixture was precipitated into excessive diethyl ether. After dried under vacuum, the precipitate was dissolved in 0.1 M NaOH, dialyzed with distilled water and freeze-dried to give a PGA–PEG–PGA product



Scheme 1. Schematic representation of the general macromolecular design of oppositely charged ionic ABA triblock copolyelectrolytes and the formation of reversible hydrogels based on charge-driven assembly.

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