European Polymer Journal 67 (2015) 304–313

Contents lists available at ScienceDirect

European Polymer Journal

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

Macromolecular Nanotechnology

Hydrolyzed gelatin-based polymersomes as delivery devices of anticancer drugs

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ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form 16 March 2015
Accepted 23 March 2015
Available online 1 April 2015

Keywords:
Polymersomes
Protein conjugate
Methotrexate
Drug delivery
Lung cancer

ABSTRACT

Monodispersed polymersomes based on a hydrophobically-modified protein with dimensional range between 154 and 254 nm were prepared employing a water addition/solvent evaporation method. Gelatin hydrolyzate and PEG_{40}-stearate, acting as hydrophilic and hydrophobic blocks, respectively, underwent radical coupling and three materials with different hydrophilic/lipophilic ratio were obtained by varying the amount of PEG_{40}-stearate in the reaction feed. Critical aggregation concentrations of each amphiphilic polymer were found ranged from 3.9 to 10.2 μg mL^{-1}, with lower values recorded for conjugate with lower hydrophilic/lipophilic ratio. Anticancer drug methotrexate was loaded into the vesicular systems with efficiency strictly dependent on the gelatin hydrolyzate content and the *in vitro* releasing profile assessed and analyzed by suitable mathematical models. To prove the applicability of the proposed delivery vehicle, the biocompatibility properties were assessed in normal human lung fibroblasts cells MRC-5, while the anticancer efficiency was tested on H1299 lung cancer cells, proving that the drug encapsulation into the polymersomes dramatically reduced the cytotoxicity on healthy cells while preserving its efficiency in killing cancer cells.

1. Introduction

Performing drug delivery systems (DDS) possess the potential of enhance the therapeutic efficacy of a loaded drug through the improvement of its therapeutic index, protection from degradation processes, and reduction of toxicity in the body [1]. Polymeric materials, by virtue of their highly tunable physico-chemical properties, offer great advantages in the design and development of advanced DDS, obtaining long circulation times in blood, precise targeting or specific recognition of bioactive compounds. Numerous types of polymers have been developed for these purposes, including micro- and nano-particles [2] polymer conjugates [3], micelles [4] and polymeric vesicles, also named polymersomes [5].

Polymersomes are bilayer systems composed of an aqueous core with mean size ranging from 50 nm to 50 μm and a wall thickness of ~10–50 nm. They derive from the self-assembly of macromolecular amphiphiles of various architectures including diblock [6,7], triblock [8], graft [9], and dendritic copolymers [10]. In the last decades, they have broaden the range of the potential biomedical applications due to the versatility of amphiphiles in terms of type of polymers, molecular weight, polydispersity, hydrophobic/hydrophilic ratio, and chemical feature by incorporation of responsive or functional species [11,12]. When applied in drug delivery and

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http://dx.doi.org/10.1016/j.eurpolymj.2015.03.058
0014-3057/

diagnostic, they offer great advantages because of high stability [13], tunable membrane properties, versatility and capacity of transporting hydrophilic as well as hydrophobic species, including anticancer drugs, genes, proteins and diagnostic probes [14,15–17].

Amphiphilic copolymers have been prepared from synthetic biodegradable and non-biodegradable hydrophilic and hydrophobic blocks [18,19]. Nevertheless, with the advances in the areas of formulation and drug delivery technology, several recent research works focused on the development of polymeric vesicles based on hydrophobically modified natural polymers, such as polypeptides [20] and polysaccharides [21].

Gelatin is a protein obtained by hydrolytic degradation of naturally occurring collagen. It is a readily and economically available material used for decades in various forms in food and pharmaceutical industries due to its non-toxic, nonirritant and biodegradability properties, and good living body compatibility [22]. Gelatin was widely used as starting material for the preparation of micro and nanoparticles in drug delivery field [23] but, to the best of our knowledge, no examples of gelatin-based polymersomes have been reported. In this work, hydrolyzed gelatin (HGel) was hydrophobically modified by radical grafting with polyoxyethylene$_{40}$ stearate ($PEG_{40}ST$), a FDA-approved polyoxyl ester widely used in cosmetic and pharmaceutical formulations [24,25]. The scope is the obtainment of a surfactant derived from two of the most biocompatible compounds used in the pharmaceutical industry, which can act as starting materials for bilayered vesicles. The grafted proteins were characterized by FT-IR, ^{1}H NMR studies and critical aggregation concentration. Then, polymersomes were prepared by a water addition/solvent evaporation method and characterized by dynamic light scattering (DLS) and Transmission Electron Microscopy (TEM). The efficiency of the obtained nanovesicles as drug delivery devices was verified by Methotrexate (MTX) and evaluating the releasing profile, which was analyzed by suitable mathematical models. Finally, cytotoxicity was investigated in an *in vitro* model of lung cancer using H1299 cells, with MRC-5 acting as a model for healthy cells.

2. Materials and methods

2.1. Synthesis of gelatin hydrolyzate

Hydrolyzated gelatin (HGel) was prepared according to the literature with some modifications [26]. A reaction mixture containing 40 g of gelatin (Ph Eur, Bloom 160, Sigma–Aldrich, St. Louis, MO, USA) were taken up in 60 g of water and, after the addition of 1.6 g of NaOH (Carlo Erba Reagent, Milan, Italy), the solution was heated for 16 h to 130 °C. After the reaction time, the mixture was cooled to room temperature and adjusted with HCl (Carlo Erba Reagent, Milan, Italy) to a pH 7.0. The obtained functional hydrolyzate was precipitated in an excess volume of acetone (Carlo Erba Reagent, Milan, Italy) at room temperature under stirring. The suspensions were filtered by sintered glass filter funnel (Pyrex, Ø30 mm; porosity 3) and washed with diethyl ether (Carlo Erba Reagent, Milan, Italy) and the recovered HGel was dried in a vacuum oven at 40 °C. The number average molar mass (M_n) of the gelatin hydrolyzate was determined by potentiometric titration [27,28] in accordance with the Eq. (1).

$$\frac{W_{(g)}}{M_n} = N \times \frac{V}{1000} \quad (1)$$

W is the weight of gelatin hydrolyzate, M_n is the number average molar mass, N and V the concentration and the volume of the HCl used during titration, respectively.

HGel: FT-IR (KBr disk, a Jasco FT-IR 4200), ν (cm^{-1}): 3600–3100 (s, O—H; s, N—H), 3018 (s, CH_2), 1700–1640 (s, C=O), 1554 (b, N—H), 1405 (s, C—N). ^{1}H NMR (D_2O, 500 MHz): 1.0 (CH_3), 1.4–1.6 (CH_2), 5.0–5.6 (CONH).

2.2. Synthesis of $PEG_{40}STMA$

$PEG_{40}STMA$ was prepared as follows: $PEG_{40}ST$ (2.04 g, 1 mmol, Sigma–Aldrich, St. Louis, MO, USA) was dissolved in dry 1,4-dioxane (Carlo Erba Reagent, Milan, Italy). Then, an excess of MA (2 mmol, Sigma–Aldrich, St. Louis, MO, USA) and 2 mmol 4-dimethylaminopyridine (DMAP, Sigma–Aldrich, St. Louis, MO, USA) were added to this solution, stirring at room temperature for 24 h. The solution was precipitated in diethyl ether/*n*-hexane (1/1, vol/vol, Carlo Erba Reagent, Milan, Italy) and the white product (1.82 g) dried under vacuum for 24 h.

$PEG_{40}ST$: FT-IR (KBr disk), ν (cm^{-1}): 2975 (s, C—H); 1744 (s, C=O ester); 1120 (s, C—O—C PEG); 970 and 840 (s, C—C). ^{1}H NMR (D_2O, 500 MHz): 0.8 (CH_3 stearic); 1.3 (CH_2 stearic); 3.5–3.8 (CH_2 PEG).

$PEG_{40}STMA$: FT-IR (KBr disk), ν (cm^{-1}): 2975 (s, C—H); 1732 (s, C=O stearate ester); 1686 (s, C=O methacrylate ester; 1626 (coupling of s, C=C methacrylic moieties); 1120 (s, C—O—C PEG); 970 and 840 (s, C—C). ^{1}H NMR (D_2O, 500 MHz): 0.8 (CH_3 stearic); 1.3 (CH_2 stearic); 3.5–3.8 (CH_2 PEG); 5.6 (CH_3 methacrylic) and 6.0 (CH_2 methacrylic).

2.3. Synthesis of HG samples

HGel-*g*-$PEG_{40}ST$ graft polymers, coded HG samples, were synthesized according to the following procedure. HGel (0.2 g) was dissolved in 4 mL of distilled water containing 0.05 g of potassium persulfate (Sigma–Aldrich, St. Louis, MO, USA) and the mixture was maintained under stirring at 40 °C under nitrogen. After 1 h, in separate experiments, different amounts of $PEG_{40}STMA$ (Table 1) were added and the mixture allowed to react for 24 h. Then the solution of copolymer was introduced into dialysis tubes with a cut-off molecular weight of 3.5 kDa and dipped into a glass vessel containing distilled water at 20 °C for 48 h with eight changes of water. The resulting solution was frozen and dried with a freeze drier to afford a vaporous solid.

HG3: FT-IR (KBr disk), ν (cm^{-1}): 3350–3500 (s, O—H; s, N—H); 2940 cm^{-1} (s, C—H); 1744 (s, C=O ester); 1554 (b, N—H); 1130 (s, C—O—C PEG); 970 and 840 (s, C—C). ^{1}H NMR (D_2O, 500 MHz): 0.8 (CH_3 stearic); 1.3 (CH_2 stearic); 3.5–3.8 (CH_2 PEG); 5–5.6 (CONH).