Materials Science and Engineering C 42 (2014) 111–115

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Synthesis of methylprednisolone loaded ibuprofen modified inulin based nanoparticles and their application for drug delivery

Luzhong Zhang [a,1], Yue Li [b,1], Caiping Wang [a,1], Guicai Li [a], Yahong Zhao [a], Yumin Yang [a,*]

[a] Jiangsu Key Laboratory of Neuroregeneration, Nantong University, Nantong 226001, PR China
[b] School of Education Science, Nantong University, Nantong 226001, PR China

ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 8 April 2014
Accepted 6 May 2014
Available online 22 May 2014

Keywords:
Methylprednisolone
Nanoparticle
Inulin
Ibuprofen
Drug delivery

ABSTRACT

Ibuprofen modified inulin was synthesized through a direct esterification linkage in which the in situ activation of the carboxylic acid with *N,N′*-carbonyldiimidazole was carried out. The critical aggregation concentration of the ibuprofen modified inulin was determined by using pyrene as the fluorescence probe. Methylprednisolone loaded nanoparticles were prepared by the self-assembly of the ibuprofen modified inulin copolymer and methylprednisolone. In vitro release of the methylprednisolone and the cytotoxicity of the methylprednisolone loaded nanoparticles against RSC-96 cells were evaluated. Since the ibuprofen and methylprednisolone could stimulate a significant neurite growth and diminish the human neurological deficits after the spinal cord injury, the methylprednisolone loaded nanoparticles based on the ibuprofen modified inulin copolymer may have a great potential in the synergetic effect treatment for spinal cord injury.

1. Introduction

Spinal cord injury has a devastating impact on the life quality of patients, such as the loss of neurons, axonal degeneration, serious neurological deficits, life-long motor and sensory disability [1,2]. Although much effort has been expended, there is still no effective treatment for the spinal cord injury [3]. Up to now, methylprednisolone is the only common used agent for treatment of acute spinal cord injury, and high-dose systemic administration could diminish the human neurological deficits after the spinal cord injury [4,5]. Although the underlying therapeutic mechanism was unclear, people believed that the main therapeutic benefits after the spinal cord injury might be related to the inhibition of lipid peroxidation and inflammatory response of methylprednisolone [6,7]. Unfortunately, a high-dose systemic methylprednisolone used in the acute spinal cord injury could result in serious side effects, such as gastric bleeding, sepsis, pneumonia, acute corticosteroid myopathy and wound infection, which accompany only with modest improvements in the neurological recovery [8–10]. The side effects of methylprednisolone therapy as just mentioned may be related to the high systemic dosage and its toxicity. Therefore, the targeted delivery of methylprednisolone to the injury site is likely the major obstacle to the effectively and widespread clinical use of methylprednisolone.

Recently, increasing interest and intensive effort have been devoted to biomedical used drug delivery carriers in nanoscale [11–13]. Nanoparticle based drug delivery system could easily penetrate deeply into tissues and fine capillaries because of their sub-cellular and submicron size [14,15]. In particular, natural polysaccharide based bionanoparticles are widely used for drug delivery systems, such as anti-inflammatory drugs, antibiotics, proteins, gene, peptides and hormones, all due to their remarkable superiority in biodegradability and biocompatibility [16–20]. On the other hand, suitable modification of polysaccharides allows for an improvement in the properties of natural polymers [21]. For example, these modified polysaccharides could self-assemble into nanoparticles with the hydrophobic segments forming the core and hydrophilic segments forming the shell in the aqueous solution. These polysaccharide based nano-drug delivery systems have significant advantages, such as stabilizing the therapeutic agents, improving the solubility of hydrophobic drugs, prolonging the circulation life-time and reducing the side effects of the drugs [20,22].

In present work, we report a synthetic method of methylprednisolone loaded nanoparticles, specifically, the non-steroidal anti-inflammatory drug ibuprofen decorated inulin nanoparticles. The ibuprofen modified inulin was prepared by a direct esterification linkage between the hydroxyl groups of inulin and the carboxylic acid groups of ibuprofen with the help of *N,N*-carbonyldiimidazole which in-situ activates the carboxylic acid group. The critical aggregation concentration of ibuprofen modified inulin copolymer was investigated using pyrene as a fluorescence probe. The methylprednisolone loaded nanoparticles were prepared by a process of nanoprecipitation. The size and morphology of obtained nanoparticles were characterized with dynamic light scattering and transmission electron microscopy. In vitro release of the methylprednisolone was evaluated in the phosphate buffer solution.

* Corresponding author. Tel./fax: +86 513 85511585.
E-mail address: yangym@ntu.edu.cn (Y. Yang).
[1] These authors contribute to this work equally.

http://dx.doi.org/10.1016/j.msec.2014.05.025
0928-4931/

The obtained ibuprofen nanoparticles have great application potentials in the treatment for spinal cord injury. Furthermore, ibuprofen was chosen as the biologically active carboxylic acids, which could surmount axon growth restrictions from myelin and proteoglycans and stimulate a significant neurite growth in the cultured dorsal root ganglion neurons [23]. It has a great potential in the synergetic effect treatment for spinal cord injury.

2. Materials and method

2.1. Materials and characterization

N,N′-carbonyldiimidazole was purchased from Sigma-Aldrich. Ibuprofen [2-(4-isobutylphenyl) propanoic acid] was obtained from Acros Organics Company. Inulin was bought from Shanghai Alladin Reagent Company (China). Methylprednisolone was purchased from Yueyang Huanyu Pharmaceutical Co., Ltd. (China).

The ^{1}H NMR spectra were recorded with a Bruker DPX-300 at a frequency of 300 MHz. The size of nanoparticles was measured using a Brookheaven BI9000AT system (Brookheaven Instruments Corporation, U.S.A.). The fluorescence spectra were taken with a RF-5301PC spectrofluorometer (Shimadzu, Japan). High performance liquid chromatography (HPLC) analysis was performed on a Shimadzu LC-15A (Shimadzu, Japan) HPLC system equipped with a Shimadzu UV detector and a C-18 Wondasil-HPLC analysis column. The mobile phase consisted of a mixture of acetonitrile:water (34:66, *v/v*, pH = 3.4), and delivered at a flow of 1.0 mL/min at 25 °C. Detection was performed at a wavelength of 243 nm [24]. Transmission electron microscope (TEM) analyses were performed on a JEOL JEM-1010 after the sample stained with a phosphotungstic acid solution (2%, w/v).

2.2. Synthesis of inulin–ibuprofen conjugates

First, 0.43 g (2.66 mmol) of *N,N′*-carbonyldiimidazole was added to a 5 mL DMSO solution consisting of 0.5 g (2.42 mmol) ibuprofen, and the reaction mixture was stirred at room temperature for 1 h. Subsequently, 0.5 g (3.1 mmol, the fructose unit) of inulin was added to the mixture and the reaction was allowed to react for 24 h at 80 °C under stirring. The resultant solution was precipitated into 100 mL cold water. The precipitates were filtered out, washed several times with water and dried under vacuum to obtain inulin–ibuprofen conjugates.

2.3. Determination of the critical aggregation concentration

The critical aggregation concentration of the inulin–ibuprofen conjugates was determined by using pyrene as a fluorescence probe. A pyrene solution (1.8×10^{-4} mol/L) in acetone was added to 3.0 mL of samples with different concentrations ranging from 1×10^{-5} to 1 mg/mL in 20 mM of phosphate buffer solution (pH = 7.4). The final concentration of pyrene was 6×10^{-7} M. Then, the mixtures were incubated for 15 h at room temperature in dark. The fluorescence spectra were recorded by using a fluorescence spectrometer at the excitation wavelength of 330 nm with the emission and excitation slit widths of 5 nm. The emission fluorescence of 372 and 383 nm were monitored and the intensity ratio of pyrene at 372 and 383 nm was plotted against the polymer concentration to determine the critical aggregation concentration.

2.4. Preparation of drug-loaded nanoparticles

Methylprednisolone-loaded nanoparticles were prepared by a modified nanoprecipitation method. 5 mg of inulin–ibuprofen conjugates with 1 mg of methylprednisolone was dissolved in acetone (1 mL), and then, dropped into 10 mL of hot water (50 °C) under a moderate stirring. Later on, the acetone was removed under a reduced pressure at room temperature. Subsequently, this suspension was filtered by a cellulose acetate filter with the average pore size of 0.22 μm to remove the unloaded methylprednisolone (72%, the yield obtained after centrifugation).

2.5. Drug loading content and encapsulation efficiency

The lyophilized nanoparticles were accurately weighted before dispersing in methanol. The concentration of methylprednisolone in the resulting methanol solution was determined by HPLC and using a predetermined calibration curve. The drug loading content and encapsulation efficiency was calculated by the following formulas, respectively:

$$\text{Drug loading content}\% = \frac{\text{Weight of the drug in the nanoparticles}}{\text{Weight of the nanoparticles}} \times 100\%$$

$$\text{Encapsulation efficiency}\% = \frac{\text{Weight of drug in the nanoparticles}}{\text{Weight of the feeding drug}} \times 100\%.$$

2.6. In vitro release of methylprednisolone-loaded nanoparticles

The lyophilized methylprednisolone-loaded nanoparticles (1.1 mg) containing 150 μg of methylprednisolone was dialyzed with 10 mL of phosphate buffer solution (PBS) at 37 °C in a 15 kDa MWCO membrane. After certain periods, 1 mL of aliquot was withdrawn and then the same volume (1 mL) of medium was replenished to the suspension. The released concentrations of methylprednisolone in the sample medium were measured by HPLC with a pre-established calibration curve. The measurements were repeated three times and the shown results were the average of three measurements.

2.7. In vitro cytotoxicity

The cytotoxicity of samples was tested by using a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Rat Schwann cell line (RSC-96) cells were grown in a Dulbecco's modified Eagle's medium (DMEM) with 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin with 10% fetal bovine serum in a 5% CO_2 atmosphere at 37 °C. The cells were seeded into a 96-welled plate at a density of 5000 cells per well, and incubated with 100 μL of culture medium containing a series of doses of samples at 37 °C for 48 h. After the incubation, the culture media in each well were removed and the cells were washed three times with PBS. Then, 20 μL of MTT solution (5 mg/mL) was added to each well and cultured for another 4 h. The supernatant was discarded and 100 μL of DMSO was added then to each well. The OD values of plates were observed on a microplate reader at 570 nm (Safire, Tecan). The results were expressed as the percentage of cells relative to the control cells after various treatments without any modifications.

3. Results and discussion

3.1. Synthesis and characterization of the inulin esters

Inulin is a naturally occurring dietary fiber composing of polysaccharides, mostly existed in plants of *Compasitae* family including dahlia, Jerusalem artichoke and chicory [25]. Generally, the polymer consists of linear chains of fructose unit chains linked by glycosidic bonds and terminated at a single glucopyranoside ring. Thanks to the biocompatibility and biodegradability of inulin, it is a good candidate for biomedical applications including tissue engineering and biodegradable drug carriers [26,27]. It would be a significant progress to prepare the copolymers of polysaccharides and hydrophobic drug, and the copolymers