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Abstract

An amphiphilic poly((lactic acid)-b-hyaluronic acid) diblock copolymer, poly(LA-b-HA), was synthesized from short-chain hyaluronic acid and poly(lactic acid). The synthesis was conducted by coupling the N, N′ - dicyclohexylcarbodiimide activated poly(lactic acid) to a short-chain hyaluronic acid which was pre-aminated with 1, 2-ethylenediamine at the reducing end followed by $NaCNBH_3$ reduction. The poly (LA-b-HA) copolymers synthesized were verified by the spectral analyses of FTIR and ^1H NMR. The poly(LA-b-HA) molecules can self-assemble into micelles in aqueous solution. The average diameters of polymeric micelles were estimated to be 116 ± 17 and 98 ± 11 nm for the polymeric micelles derived from the poly(lactic acid)s of MW 3,200 and MW 16,900, respectively. The poly(LA-b-HA) copolymeric material is non-cytotoxic and can be used as micellar drug carriers. The drug encapsulation capabilities of these poly(LA-b-HA) micelles were demonstrated by using ellagic acid and lidocaine chloride as model compounds. These new biodegradable micelles have a great potential to be used as drug delivery carrier for biomedical applications.

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A novel biodegradable amphiphilic diblock copolymers based on poly(lactic acid) and hyaluronic acid as biomaterials for drug delivery

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Abstract An amphiphilic poly((lactic acid)-b-hyaluronic acid) diblock copolymer, poly(LA-b-HA), was synthesized from short-chain hyaluronic acid and poly(lactic acid). The synthesis was conducted by coupling the N, N′- dicyclohexylcarbodiimide activated poly(lactic acid) to a short-chain hyaluronic acid which was pre-aminated with 1, 2-ethylenediamine at the reducing end followed by $NaCNBH_3$ reduction. The poly (LA-b-HA) copolymers synthesized were verified by the spectral analyses of FTIR and ^1H NMR. The poly(LA-b-HA) molecules can self-assemble into micelles in aqueous solution. The average diameters of polymeric micelles were estimated to be 116±17 and 98±11 nm for the polymeric micelles derived from the poly (lactic acid)s of MW 3,200 and MW 16,900, respectively. The poly(LA-b-HA) copolymeric material is non-cytotoxic and can be used as micellar drug carriers. The drug encapsulation capabilities of these poly(LA-b-HA) micelles were demonstrated by using ellagic acid and lidocaine chloride as model compounds. These new biodegradable micelles have a great potential to be used as drug delivery carrier for biomedical applications.

Keywords Amphiphilic diblock copolymer · Poly(lactic acid) · Hyaluronic acid · Polymeric micelle · Skin penetration

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Introduction

One class of the drug and gene delivery systems that has received wide spread attention over the past few decades is the polymeric micelle delivery system. Polymeric micelles have recently emerged as a novel carrier for both hydrophobic and amphiphilic drugs [1]. AB and ABA type amphiphilic block-copolymers can spontaneously self-aggregate to form micelles in a selective solvent. Amphiphilic synthetic block copolymers have got tremendous impetus on the ongoing research in the area of drug delivery technology due to their capability to provide a delivery vehicle having a broad range of amphiphilic characteristics, as well as targeting the drugs to a specific site [2]. Amphiphilic block copolymer micelles are of special interest for a number of reasons. First of all, hydrophobic drugs can be physically entrapped in the core of micelle and transported at a concentration exceeding their intrinsic water solubility. Secondly, the hydrophilic blocks can form hydrogen bonds with the aqueous surrounding and form a tight shell around the micellar core. Lastly, the block copolymer micelle can also target their payload to specific tissues through either passive or active means [3]. The drug delivery from micelles can be divided into three different routes: (1) the micelles remain outside the cells where the drug is released [4, 5], (2) the micelles enter the cells and (3) the micelles enter the nucleus. The release rate of drug from micelles depends on the physical/chemical properties of both the drug and the block copolymer [6].

Poly(lactic acid) (PLA) has been widely studied for use in medical applications because of its bioabsorbable, biodegradable and biocompatible properties. Recently, biodegradable micelles prepared using the copolymers such as PLA or PLGA (poly(lactic-co-glycolic acid)) copoly-

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merized with PEG (poly(ethylene glycol)) have been employed extensively to deliver bioactive ingredients to the cells. The polymeric micelles thus formed have a PEG outer corona, which results in a prolonged plasma circulation times. Drugs using this type of approach, commonly known as "stealth" therapeutic strategy, can be delivered to the non-RES (reticuloendothelial system) sites with improved efficiency of immunospecific targeting [7–10]. Conjugation of proteins with PEG has been proved to result in prolonged protein circulation life and reduced immunogenicity and antigenicity [11–14]. In addition, PEG conjugated with an anti-tumor drug loaded microspheres were also of great interest since it could improve the water-solubility and stability of such microspheres, thus eliminating undesired side effects.

Hyaluronic acid (HA), a linear polysaccharide composed of repeating units of N-acetyl-glucosamine and D-glucuronic acid, is a major component of ECM. It has been widely used in biomedical applications such as scaffolding for wound healing and tissue engineering, ophthalmic surgery, arthritis treatment and as a component in implant materials [15–17]. Copolymer of HA and PLA is expected to be of great use for medical applications since both HA and PLA can be degraded into the metabolites. Recently, a graft copolymer consisted of HA as a hydrophilic backbone and PLA as hydrophobic polyester branches has been synthesized by Fabio et al. [18]. However, the capability of micelle formation of this branched copolymer has never been demonstrated.

The goal of our research was to synthesize the novel poly(LA-b-HA) block copolymers with unique physico-chemical property. PLA is a hydrophobic, biodegradable polymer which will become amphiphilic when covalently bonded with HA. We believed that these amphiphilic diblock copolymers could self assemble into micelles of hydrophilic shell and hydrophobic core and thus useful as potential drug carriers. Two poly(LA-b-HA) copolymers were synthesized and designated as poly(sLA-b-HA) and poly(lLA-b-HA) derived from two different molecular weights of PLA (sPLA, MW 3,200 and lPLA, MW 16,900). The synthesized poly(LA-b-HA) linear diblock copolymers were characterized and used to encapsulate ellagic acid and lidocaine chloride to demonstrate their potential biomedical applications.

Materials and methods

Materials

Stannous octoate, N, N′-dicyclohexylcarbodiimide ,1,2-ethylenediamine, sodium cyanoborohydride, and MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide thiazolyl blue) were all purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Hyaluronic acid (HA) of low molecular weight (~MW 6,500) was obtained from Lifecore Biomedical Inc. Dialysis membranes (Spectra Por_6 regenerated cellulose, MWCO 1,000 and 6,000~8,000) were purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). Poly(lactic acid) with different molecular weights were synthesized by the direct condensation of lactic acid using stannous octoate as a catalyst [19, 20] and the molecular weight distributions of the polymers were determined in tetrahydrofuran by size exclusion chromatography using polystyrene as the standard (sPLA; MW~3,200 and lPLA; MW~16,900).

Experimental procedures

Poly(LA-b-HA) linear diblock copolymers were synthesized by two steps (Scheme 1), a reductive amination of hyaluronic acid by 1,2-ethylenediamine followed by the coupling of aminated HA with PLA through the amide bond coupling reaction. For the coupling reaction of diamine to hyaluronic acid, there is only one coupling site at the reducing end of HA. The occurrence of the HA dimerization by 1,2-ethyldiamine could be eliminated by raising the molar ratio of diamine to HA in the reaction mixture [21]. In this study, an excess amount of 1,2-ethyldiamine was reacted with hyaluronic acid.

Poly(lactic acid) activated by DCC/NHS

Poly(lactic acid)(PLA) and DCC (N,N′-dicyclohexylcarbodiimide,1.1 equiv.) were loaded into a flask, dissolved by DMSO (dimethyl sulfoxide) and then mixed with a magnetic stirrer. After 4 h mixing, an aliquot of NHS (N-hydroxy-succinimide, 1.1 equiv.) was added to activate the PLA and then the reaction was conducted overnight at ambient temperature.

Reducing-end amination of hyaluronic acid

An aliquot of 1, 2-ethylenediamine (~10 equiv of the HA) was first dissolved in PBS buffer (pH~9.5) and the HA (MW~6,500) solution (10 mg/ml) was then added dropwisely into the pre-mixed solution and stirred for more than 4 h [22–25]. Sodium cyanoborohydride was added into the mixture incubated in an ice-bath and then the reaction proceeded at room temperature for overnight. The excess diamine and water were evaporated by rotary evaporator and then dialyzed (Spectra Por_6 regenerated cellulose, MWCO 1000) in PBS buffer solution for two days. After dialysis, the solution was dried by lyophilization to obtain the modified HA oligomers (hyaluronic acid-amine).

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