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Surface biofunctionalization of PLA nanoparticles through amphiphilic polysaccharide coating and ligand coupling: Evaluation of biofunctionalization and drug releasing behavior

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Abstract

The purpose of present study was to conceive small-sized nanoparticles which can be easily functionalized with ligands and meanwhile minimized drug leakage. For this purpose, cholesterol-modified dextran dialdehyde was synthesized and used to prepare indomethacin loaded PLA nanoparticles containing aldehyde groups on surface. Transferrin (TF) was coupled to their surface by taking advantage of the Schiff's base reaction and the effect of ligand coupling on drug leakage was evaluated. The results show that the coupling process reached equilibrium within 5 min and less than 20% drug was leaked after $NaBH_4$ reduction. TF coupled nanoparticles was fluorescence labeled with FITC, and cell uptake experiment was performed in vitro. The result demonstrated the bioactivity of TF after binding on nanoparticles and the ability of the nanoparticles targeting to tumor cell mediated by ligand–receptor interaction.

Keywords: Amphiphilic polysaccharide; Nanoparticles; Transferrin; Biofunctionalization; Drug leakage

1. Introduction

Poly(lactic acid) (PLA) and its copolymer nanoparticles have shown great potential as carrier systems for an increasing number of active molecules, largely due to the excellent biocompatibility and the controlled biodegradability properties. However, the main drawback of these carriers is their non-specific interaction with cells and proteins, leading to drug accumulation in non-target tissues (Lemarchand, Gref, & Couvreur, 2004; Bazile et al., 1995; Mora & Baraldi, 2002). To overcome this problem, many studies have been devoted to surface functionalization of PLA nanoparticles, namely, active target delivery system (ATDS).

The active target delivery of drug need the carriers (i) minimize interaction with plasma and (ii) target cell surface receptors (Nobs, Buchegger, Gurny, & Allemann, 2004). Poly(ethylene glycol) (PEG) coating could delayed the phagocytosis of nanoparticles, avoiding the mononuclear phagocyte system (MPS) sequestration. But PEG-coated nanoparticles cannot provide specific targeting, and their final destination was always the MPS (Mora & Baraldi, 2002). In order to achieve an active targeting, specific ligands must be attached to nanoparticles surface to enable molecular recognition. However, chemical coupling of such ligands is usually very difficult because of the absence of reactive groups at the surface of pegylated carriers (Stella et al., 2000).

Polysaccharides constitute an important class of physiological materials. They display well-documented biocompatibilities and biodegradabilities, which are the basic

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characteristics for polymers used as biomaterials (Dumitriu, 2001). The surface modification by polysaccharide increased the *in vivo* half-life of nanoparticles in the same way as PEG did (Woodle & Lasic, 1992; Allen, 1994; Rouzes, Gref, Leonard, & Dellacherie, 2000; Choi, Kim, & Kim, 2003), and the enrichment in reactive groups make them are more easily to couple ligands.

Although some polysaccharide decorated PLA nanoparticles have been prepared successfully, the ligand conjugation is still a challenge work, because inappropriate conjugation method will lead to, on the one hand, the loss of bioactivity of ligand (Nobs et al., 2004), and on the other hand, the leakage of the pre-entrapped drug (Lundberg, Griffiths, & Hansen, 2000), resulting in the fail in the clinic application. To our knowledge, however, only a few papers have addressed this issue so far. The present paper focus on the biofunctionalization of polysaccharide decorated PLA nanoparticles and its effect on the drug-loading and release behavior. For these purposes, cholesterol hydrophobic modified dextran dialdehyde (Chol-Dex-CHO) was synthesized. Indomethacin (IMC) loaded PLA nanoparticles coated with Chol-Dex-CHO were prepared using diafiltration method, and transferrin (TF), chosen as model ligands, was coupled to nanoparticles *via* Schiff's base reaction. The optimum conditions for the surface functionalization and drug-loading were investigated, and the tumor cell targeting was evaluated by glioma cell uptake experiment in vitro.

2. Experimental

2.1. Materials

PLA ($M_w = 30$ kDa) was purchased from Sigma. Dextran ($M_n = 40$ kDa) was from Amersham Biosciences (Uppsala, Sweden). Cholesterol was obtained from Tianjin Chemical Reagent Co., China, and recrystallized in ethanol before use. Indomethacin was purchased from Tianjin Pharmaceutical Group Corporation, China. Transferrin and fluorescein isothiocyanate (FITC) was obtained from Sigma. The water used in these studies was double distilled and all other reagents and organic solvents used were reagent grade or better. Fuchsin solution was prepared as follows.

0.5 g powdered basic fuchsin was dissolved in about 100 mL hot water in a 200 mL flask, and 20 mL sodium sulphite aqueous solution (0.15 g/mL) was added. After 10 min 6 mL hydrochloric acid was added and the solution turned colorless, the flask was capped tightly and stored at room temperature in the dark for 72 h, shaking occasionally to dissolve pink precipitate.

2.2. Synthesis and characterization of cholesterol hydrophobically modified dialdehyde dextran derivatives

Cholesterol-modified dextran derivates containing aldehyde groups (Chol-Dex-CHO) was synthesized by esterifying of hydroxyl groups of dextran dialdehyde with cholesterol 3-hemisuccinyl chloride. The detail processes were described as follows.

2.2.1. Synthesis of cholesterol 3-hemisuccinyl chloride

One gram of succinic anhydride was added per gram of cholesterol and dissolved in 30 mL of pyridine. The mixture was stirred for 3 h at 70 °C. The crude product was dissolved in a minimum amount of H_2O/ethanol (1:10,v/v), and cholesterol 3-hemisuccinate was crystallized from H_2O/methanol (1:10,v/v), then recrystallized from ethanol (Kuhn, Schrader, Smith, & O'Malley, 1975).

One gram of cholesterol 3-hemisuccinate was dissolved in 30 mL anhydrous chloroform, and an excess of $SOCl_2$ (10-fold) in 10 mL anhydrous chloroform was added dropwise under nitrogen protecting. The reaction mixture was stirred vigorously at 60 °C for 4 h, then the solution was evaporated under vacuum to remove solvent and the remained $SOCl_2$, the residue was kept in anhydrous chloroform airproofly.

2.2.2. Synthesis of Chol-Dex-CHO

Oxidation of dextran was performed in 30 mL distilled water (Schacht, Bogdanov, Van Der Bulcke, & De Rooze, 1997; Svetlana, Ludmila, Alexander, & Elena, 1996), which contained dextran (2 g) and $NaIO_4$ (IO_4^-/glucopyranosidic units = 1/20). The mixture was stirred vigorously at room temperature for 8 h followed by dialyzed against deionized water for 72 h. Then the dextran dialdehyde (Dex-CHO) was obtained by freeze-drying. The content of aldehyde groups was estimated to be 0.235 mmol/g Dex-CHO by Schales' method with the calibration curve of glutaraldehyde (Imoto & Yanagishita, 1971).

For the synthesis of Chol-Dex-CHO, 500 mg Dex-CHO was dissolved in 40 mL anhydrous DMSO, and 0.3 mL triethylamine was added. After the mixture was heated up to 80 °C, 0.3 mmol Chol-succ-COCl in 4.4 mL $CHCl_3$ was added dropwise. The reaction was performed for 8 h under nitrogen protecting. Then Chol-Dex-CHO was obtained by dialysis of product against deionized water and freeze-drying.

The structure of Chol-Dex-CHO was confirmed by FT-IR (Bio-Rad FTS 135, Perkin-Elmer Co., US) and ^1H NMR (DMSO-d_6, Varian UMTY plus400 NMR spectrometer).

2.2.3. Surface tension measurement

The surface tensions were measured with a Dataphysics DCTA-21 dynamic contact-angle analyzer (Bad Vilbel, Germany) using the Wilhelmy plate method. The instrument was calibrated against distilled water, and before each measurement the platinum plate was cleaned by heating to a red/orange color with a alcohol burner. Measurements were taken at 25 ± 0.1 °C. The 20-mL Chol-Dex-CHO solutions were prepared at concentrations ranging from 1×10^{-5} to 1 mg/mL using distilled water. All sample solutions were aged before use.