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Loading efficiency and surface conductance of heparin-modified poly(lactide-*co*-glycolide) nanoparticles

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ABSTRACT

Poly(lactide-*co*-glycolide) (PLGA) nanoparticles (NPs) with surface modification of heparin were fabricated by microemulsion–diffusion method. These novel colloidal particles were stabilized by lecithin and Tween 80. The effects of lecithin on the loading of heparin onto PLGA NPs and on the surface conductance were analyzed. The electronic micrographs revealed that spherical colloids were prepared and the incorporation of heparin caused a slight coalescence of the particles. In addition, the average diameter of heparin-modified PLGA NPs was between 70 and 220 nm. An increase in the weight percentage of lecithin or in the concentration of heparin enlarged the average diameter. Based on constant amount of surfactants, the loading efficiency of heparin on the particle surfaces reached a maximum when the weight percentage of lecithin was 50%. Moreover, the surface conductance of heparin-modified PLGA NPs was improved by an increased weight percentage of lecithin. A high concentration of heparin in microemulsion also promoted the loading efficiency and surface conductance of heparin-modified PLGA NPs.

1. Introduction

Polymeric particles have been widely used in drug delivery system because of their efficacy in carrying pharmaceuticals [1]. Typical applications of the colloidal carriers include controlled release and drug targeting at tissue [2]. For satisfactory medical therapy, an understanding of physicochemical characteristics of the particles, such as size, loading efficiency, and surface charge, is often of critical concerns.

Various methods, including double emulsion [3], ion gelation [4], emulsion polymerization [5], nano-precipitation [6], and emulsification diffusion [7], have been developed for the fabrication of polymeric nanoparticles (NPs). Recently, homopolymeric polybutylcyanoacrylate (PBCA) NPs and co-polymeric methylmethacrylate–sulforopylmethacrylate (MMA–SPM) NPs were synthesized by emulsion polymerization and free radical polymerization, respectively [8]. The two polymeric NPs were employed to enhance the transport of antiviral agents across the blood-brain barrier [9–11]. In regard to biodegradable polymers, polylactide (PLA) was used in adsorbing two antigens, p24 and gp120 of human immunodeficiency virus-1, for the release of vaccine in vivo [12]. Also, the coating of Tween 80 on PLA NPs yielded improved brain-targeting delivery [13]. In tissue-engineering application, poly(lactide-*co*-glycolide) (PLGA) could be fabricated as porous three-dimensional scaffolds for cartilage regeneration [14,15]. It was worth to note that PLGA was highly biocompatible via physiological resorption and elimination.

Heparin is a primary anticoagulant for inhibiting thrombosis in clinical treatment and also an efficacious substrate for the functionalized surfaces of biomaterials [16]. From the viewpoint of biomolecular structure, heparin is one of the six-glycosaminoglycan chains in aggrecan and is composed of alternating D-glucosamine, L-iduronide, and D-glucuronic acid. It was observed that heparin-modified NPs caused slow release of vascular endothelial growth factor from particles [17]. Embedment of heparin-modified NPs in hydrogel could also regulate the release of adsorbed bone morphogenetic protein-2 [18].

Surface properties of synthetic NPs are very sensitive to the environmental condition. For example, electrokinetic behavior of PBCA and MMA–SPM NPs could be affected significantly by ionic species and ionic strength in the dispersion medium [19]. Using an ion condensation theory, the influences of pH value on the surface charge density and surface potential of biomimic solid lipid nanoparticles (SLNs) were evaluated [20]. In addition, cell models deriving from Happel's free surface and Kuwabara's zero vorticity were employed to analyze the effect of medium glutamate on the electrical characteristics of cationic SLNs containing amino groups [21]. An external electromagnetic field could reduce the Donnan potential and increase the softness of arginine-coated SLNs [22]. For negatively charged latex colloids with peripheral layers of temperature-sensitive hydrogel, an increase in

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Nomenclature

C_{hep}	concentration of heparin in microemulsion (mg/mL)
D	average diameter of heparin-modified PLGA NPs (nm)
K^{σ}	surface conduction of heparin-modified PLGA NPs (S)
r_{lec}	weight percentage of lecithin in surfactants (%)
θ	loading efficiency of heparin on PLGA NPs (%)
μ	electrophoretic mobility of heparin-modified PLGA NPs ($m^2\ s^{-1}\ V^{-1}$)
ζ	zeta potential of heparin-modified PLGA NPs (mV)

Abbreviations

NPs	nanoparticles
PLGA	poly(lactide-*co*-glycolide)

temperature enhanced the absolute value of electrophoretic mobility [23].

In this study, heparin was applied to the surface modification of PLGA NPs with the association of lecithin in the external layer. Effects of the composition of surfactants and concentration of heparin were considered. Since the loading property was crucial to the performance of heparin-modified PLGA NPs, the loading efficiency of heparin was studied. Also, surface conductance of biomimetic colloids played an important role in the behavior during pharmaceutical application. Hence, influences of lecithin and heparin on the surface conductance of heparin-modified PLGA NPs were investigated. Note that it is necessary to convert the electrokinetic results into the surface conductance because the conductance on particulate surface cannot be directly measured by commercially available instrument.

2. Experimental

2.1. Preparation of heparin-modified PLGA NPs

Heparin-modified PLGA NPs were fabricated by the following microemulsion–diffusion method. Briefly, the organic phase was prepared by 0.6% (w/w) PLGA (Sigma, St. Louis, MO) in 38.8% (w/w) acetone (Mallinckrodt, Hazelwood, MO). The aqueous phase was prepared by 2.7% (w/w) surfactants, 3.5% (w/w) 1-butanol (Riedel-de Haen, Seelze, Germany), and heparin (164 USP unit/mg, Sigma) in 54.4% (w/w) ultrapure water (Barnstead, Dubuque, IA). The surfactants included lecithin (L-α-phospatidylcholine type II-S, Sigma) and Tween 80 (FisherScientific, Fair Lawn, NJ). For the diffusion between the two phases, the organic phase was added slowly into the aqueous phase under magnetic stirring at 600 rpm and 25 °C for 10 min. The emulsified fluid was continuously stirred for 3 h for the evaporation of acetone and then filtrated through a filter paper with pore diameter of 1 μm. The filtrate was centrifuged by a super-speed refrigerated centrifuge (Hitachi Koki, Tokyo, Japan) at 50200 × *g* for 30 min. The bottom pellet was resuspended in ultrapure water with 2% (w/v) D-mannitol (Sigma), placed in an ultra-low temperature freezer (Sanyo, Osaka, Japan) at −80 °C for 4 h, and lyophilized by a freeze dryer (Eyela, Tokyo, Japan) at 2–4 Torr and −80 °C. The powder products were stored in a refrigerator at 4 °C.

2.2. Loading efficiency of heparin on PLGA NPs

The loading efficiency was determined by the supernatant after centrifugation. Briefly, heparin in the supernatant was separated by a high performance liquid chromatograph (HPLC, Jasco, Tokyo, Japan) at 25 °C with a reverse-phase BDS Hypersil C-18 column (Thermo Hypersil-Keystone, Bellefonte, PA). The mobile phase, containing 90% (v/v) acetonitrile (BDH, Poole, England) and 10% (v/v) ultrapure water, was driven by two high-pressure pumps (PU-2080 Plus, Jasco, Tokyo, Japan) at a flow rate of 0.1 mL/min. The elution time of heparin was about 18 min. The concentration of heparin in the supernatant was obtained by a UV–visible spectrophotometer (UV-2075 Plus, Jasco, Tokyo, Japan) at absorbance wavelength of 190 nm. The loading efficiency of heparin on PLGA NPs, θ, was defined by the following formula: (total weight of heparin − weight of heparin in the supernatant)/(weight of heparin-modified PLGA NPs).

2.3. Electronic microcopy

Heparin-modified PLGA NPs were mainly composed of carbon, hydrogen, oxygen, and nitrogen. These highly electron-transmissible elements required negative staining of phosphotungstic acid (PTA, Sigma) for a strong contrast of the images by a transmission electron microscope (TEM, JEM-2010, Jeol, Tokyo, Japan). 0.2% (w/v) heparin-modified PLGA NPs in 0.1 M Tris hydroxymethyl aminomethane (Tris, Riedel-de Haen) buffer was deposited on a carbon-coated 200-mesh copper grid. Then, 2% (w/v) PTA solution was dripped down on the sample in complete darkness for 24 h. Images of heparin-modified PLGA NPs could also be visualized by a scanning electron microscope (SEM, Jeol, Tokyo, Japan). The samples were spread on an adhesive carbon tape, vacuum-dried, and coated with platinum at 8 kV for 200 s in a sputtering device.

2.4. Average diameter

The cumulant Z-average diameter of heparin-modified PLGA NPs, D, was obtained by a Zetasizer 3000 HS_A with a photo correlation spectroscope (version 1.41, Malvern, Worcestershire, UK) at 25 °C. 4 mL of the suspension with the concentration of 0.2% (w/v) heparin-modified PLGA NPs in Tris buffer was added little by little into a quartz tube to avoid the formation of bubbles.

2.5. Electrophoretic mobility

Electrophoresis of heparin-modified PLGA NPs was performed by a P/ACE-2100 at 25 °C with Gold data acquisition software (Beckman Coulter, Palo Alto, CA) followed by a UV detector (Beckman Coulter) at 214 nm. Before filling up with Tris buffer, the internal surface of a capillary was activated by 0.1 N HCl (Hanawa, Osaka, Japan) for 3 min, washed by ultrapure water for 3 min, rinsed by 3% (w/v) poly(ethylene oxide) (M_n = 600,000, Aldrich, Milwaukee, WI) for 2 min, and washed by ultrapure water for 1 min. The samples containing 0.2% (w/v) heparin-modified PLGA NPs were driven into the capillary with high-pressure nitrogen. Electrophoretic mobility of the heparin-modified PLGA NPs, μ, could be obtained by $\mu = (lL/tV)$. In this formula, l, L, t, and V were the effective migration length, the total capillary length, the elution time, and the applied electrical potential, respectively. In this study, l = 29.9 cm, L = 36.6 cm, V = 10 kV, and the inner and outer diameter of the capillary were, respectively, 50 μm and 375 μm.

2.6. Zeta potential and surface conductance

Zeta potential of heparin-modified PLGA NPs, ζ, was converted from the electrophoretic mobility by the Henry formula [24]:

$$\zeta = \frac{3\eta\mu}{2\varepsilon_0\varepsilon_r f(\kappa D)}, \quad (1)$$