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Remarkably enhanced stability and function of core/shell nanoparticles composed of a lecithin core and a pluronic shell layer by photo-crosslinking the shell layer: In vitro and in vivo study

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ABSTRACT

A core/shell nanoparticle system with a lecithin core and a pluronic shell has been previously reported, and it was shown to act as an effective sustained release system for positively charged proteins. Here, to provide improved stability of the core/shell nanoparticle system in a physiological environment, we prepared the core/shell nanoparticle system with a photo-crosslinked shell layer by using a lecithin liposome as the core and pluronic F 127 diacrylate (DA-PF 127) as the shell layer. The DA-PF 127 was then photo-polymerized. Compared with a purely physical system, chemical crosslinking of the shell layer resulted not only in significantly increased structural stability of the core/shell nanoparticles in both an organic co-solvent and in serum but also several remarkably enhanced functioning as a protein delivery system. First, the chemically crosslinked systems were resuspended in aqueous solution after lyophilization without using a cryo-protectant. Second, target proteins were efficiently loaded into the nanoparticles by simple co-incubation in aqueous solution at a low temperature (4 °C) and the dried powder form of the protein-loaded nanoparticles was obtained. The loading capacity of the system was increased by more than 10 times compared with that of a purely physical system. Most importantly, the chemically crosslinked system showed more sustained release of the loaded proteins, and the release rate was not noticeably affected by the presence of serum proteins, whereas sustained release of loaded vascular endothelial growth factor (VEGF) in a purely physical system was greatly reduced by serum proteins. In an in vivo corneal angiogenesis assay the chemically crosslinked system loaded with VEGF resulted in more efficient new blood vessel formation than the physical system.

1. Introduction

A variety of colloidal and nanoparticulate carriers, such as liposomes [1–3], micelles [4,5], polymeric nanoparticles [6–8] and micro-emulsion droplets [9], have been investigated for sustained release of drugs [10]. Although such delivery systems are still being actively studied as efficient delivery vehicles for proteins or small molecular weight organic drugs, various problems associated with their mechanical/biological instability and with toxicity from remaining organic solvent are often encountered, thereby limiting their in vivo application [11,12].

Polymeric core/shell nanoparticles have recently been suggested for many biomedical applications, such as gene delivery [13], targeting the brain [14,15], targeting tumors [16,17], and oral vaccine formulations [9,18]. Also, these core/shell nanoparticles may effectively protect the enclosed bioactive compounds against hydrolytic degradation and aggregation, and hydrophobic drugs can be encapsulated inside the core/shell nanoparticles. Previously, a core/shell nanoparticle system with a lecithin core and a pluronic shell was reported by Oh et al. [19], which could act as an effective sustained release system for positively charged proteins based on the negative charge of the lecithin core. Despite its advantages, including an intrinsically excellent biocompatibility and a simple preparation method, this system may have potential, inherent limitations, such as in vivo instability, the use of a cryo-protectant during lyophilization and complications in drug loading, due to its purely physical association state.

As an efficient way to overcome these drawbacks we report that a core/shell nanoparticle system with a photo-crosslinked shell layer can be prepared by using lecithin as the core and pluronic F 127 diacrylate (DA-PF 127) as the shell material, followed by photo-polymerization of the shell layer in a simple way. Because of the photo-polymerized shell layer, the core/shell nanoparticle

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system shows remarkably improved functioning and better stability in serum compared with a purely physical system. The characteristic features of the improvements include: (1) resuspension after lyophilization without use of a cryo-protectant; (2) significantly enhanced structural stability in the presence of serum proteins and organic solvents; (3) over a 10 times increase in the loading capacity of protein drugs; (4) more sustained release of loaded proteins, as compared with a purely physical, unmodified core/shell nanoparticle system, especially in serum-containing medium. These enhanced properties of the chemically crosslinked core/shell nanoparticles also resulted in statistically more significant corneal neovascularization than the physical system in vivo.

2. Materials and methods

2.1. Materials

Pluronic F 127 (E100P65E100, molecular weight 12,600) (PF 127) was a donation from BASF Corp. (Seoul, Korea). L-α-Phosphatidylcholine (lecithin) from soy beans, lysozyme from chicken egg whites, potassium phosphate monobasic, sodium phosphate dibasic, phosphotungstic acid and sodium azide were obtained from Sigma (St. Louis, MO). 4-(2-Hydroxyethoxy) phenyl-(2-hydroxy-2-propyl) ketone (Irgacure 2959, photoinitiator) was purchased from Ciba Specialty Chemicals Inc. (Basel, Switzerland). Recombinant human vascular endothelial growth factor (rhVEGF) and a rhVEGF ELISA kit were obtained from PeproTech (Rocky Hill, NJ). Sodium chloride and potassium chloride were purchased from Merk (Darmstadt, Germany). Nanosep® centrifugal devices (molecular weight cut-off 300,000) were obtained from Pall Life Sciences (Ann Arbor, MI) and the dialysis bag (cellulose ester, molecular weight cut-off 300,000) was a product of Spectrum (Houston, TX). The 0.2 μm cellulose sterilization syringe filters were purchased from Whatman (Florham Park, NJ). All reagents were used as received without further purification.

2.2. Preparation of core/shell nanoparticles with a photo-crosslinked shell layer

Core/shell nanoparticles with a photo-crosslinked shell layer were prepared by using a lecithin liposome as the core and pluronic F 127 diacrylate (DA-PF 127) as the shell layer, followed by photo-polymerization, as depicted in Fig. 1. To prepare the lecithin liposome a 20.0 wt.% aqueous solution of lecithin was sonicated for 5 min using a probe-type ultrasonication system (Sonics VCX 750, Newtown, CT, power 750 W, frequency 20 kHz). DA-PF 127 (>98% degree of acrylation) was synthesized as previously reported [20], and dissolved in deionized water at 1.0 wt.%. Then, to form a physically associated core/shell state lecithin liposome solution (20.0 wt.%) and DA-PF 127 solution (1.0 wt.%) were mixed at several ratios after filtering through a 0.2 μm sterilization syringe filter. Unbound DA-PF 127 that might be present were removed by spin filtration (12,000 rpm, 5 min) using Nanosep® centrifugal devices (molecular weight cut-off 300,000 K). Then, a photoinitiator solution dissolved in 70 vol.% ethanol at 0.05 wt.% was added to the physically associated core/shell nanoparticle solution and the solution was UV-irradiated for 5 min at 1.3 mW cm^{-2} intensity using an unfiltered UV lamp (VL-4.LC, 8 W, Vilber Lourmat, France). Finally, the core/shell nanoparticles were further purified by spin filtration, as mentioned above. As a control group, purely physical core/shell nanoparticles were prepared using lecithin liposome as the core and pluronic F 127 (PF 127), instead of DA-PF 127, at the same concentrations as the shell. The purely physical core/shell nanoparticles were also purified by spin filtration.

2.3. Characterization of core/shell nanoparticles with a photo-crosslinked shell layer

The hydrodynamic diameters and surface charges (zeta potential) of the core/shell nanoparticles were measured using an electrophoretic light scattering spectrophotometer equipped with a 10 mW He–Ne laser (632.8 nm, ELS-8000, Otsuka Electronics Co., Japan) at a 90° scattering angle. The temperature was controlled from 4 to 37 °C. The measurements were carried out in triplicate.

The sizes and the morphologies of the core/shell nanoparticles were characterized by transmission electron microscopy (TEM) (JEM-2100, JEOL, Japan). The TEM sample was prepared by drop-drying 20 μl of the core/shell nanoparticle suspension onto a 200 mesh carbon-coated copper grid at room temperature. The average nanoparticle size was determined by counting more than 50 nanoparticles in the TEM images. To identify the core/shell structure of the nanoparticles the nanoparticles were also measured after negative staining with 2% (w/v) phosphotungstic acid solution.

2.4. Stability analysis of core/shell nanoparticles

The stabilities of both types of core/shell nanoparticles (those with a crosslinked shell layer and those with physical association only) were analyzed using the particle size distribution either in an organic co-solvent or in 100% fetal bovine serum (FBS). For stability evaluation of the core/shell nanoparticles in an organic co-solvent the nanoparticles were mixed with an aqueous solution containing 4% methylene chloride and the particle size distribution of the core/shell nanoparticles was subsequently measured using a dynamic light scattering (DLS) instrument.

The core/shell nanoparticles were also suspended in 100% FBS to evaluate serum stability. The nanoparticles were placed in FBS with 0.1% NaN_3 and were kept in a shaking rocker at 100 rpm and 37 °C. The size distributions of the core/shell nanoparticles

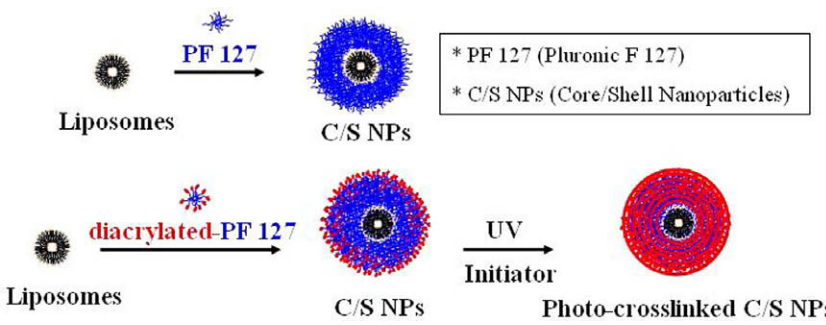

Fig. 1. Schematic representations of the preparations of the purely physical core/shell nanoparticles and of the core/shell nanoparticles with a chemically crosslinked shell formed by photo-polymerization.