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Improved stability and skin permeability of sodium hyaluronate-chitosan multilayered liposomes by Layer-by-Layer electrostatic deposition for quercetin delivery

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ABSTRACT

Layer-by-Layer (LbL) technology, based on the electrostatic interaction of polyelectrolytes, is used to improve the stability of drug delivery systems. In the present study, we developed multilayered liposomes with up to 10 alternating layers based on LbL deposition of hyaluronate-chitosan for transdermal delivery. Dihexadecyl phosphate was used to provide liposomes with a negative charge; the liposomes were subsequently coated with cationic chitosan (CH) followed by anionic sodium hyaluronate (HA). The resulting particles had a cumulative size of 528.28 ± 29.22 nm and an alternative change in zeta potential. Differential scanning calorimetry (DSC) and transmission electron microscopy (TEM) revealed that the multilayered liposomes formed a spherical polyelectrolyte complex (PEC) after deposition. Observations in size distribution after 1 week found that the particles coated with even layers of polyelectrolytes, hyaluronate and chitosan (HA-CH), were more stable than the odd layers. Membrane stability in the presence of the surfactant Triton X-100 increased with an increase in bilayers as compared to uncoated liposomes. An increase in the number of bilayers deposited on the liposomal surface resulted in a sustained release of quercetin, with release kinetics that fit the Korsmeyer–Peppas model. In an *in vitro* skin permeation study, negatively charged (HA-CH)-L and positively charged CH-L were observed to have similar skin permeability, which were superior to uncoated liposomes. These results indicate that multilayered liposomes properly coated with polyelectrolytes of HA and CH by electrostatic interaction improve stability and can also function as potential drug delivery system for the transdermal delivery of the hydrophobic antioxidant quercetin.

1. Introduction

Transdermal drug delivery systems (TDDS) are an alternative route through which an effective amount of drugs can be delivered across the skin. One of the main advantages of these systems is the non-invasive administration, in comparison with parenteral routes. Some of the challenges include low permeability of drugs, due to skin barrier such as the stratum corneum, the outermost layer of epidermis. Thus, the development of a robust TDDS is required to enhance the delivery of active compounds [1–4]. Liposomes, which are spherical vesicles composed of a bilayer of phospholipids, are similar to cell membranes in the body, and are most commonly used in drug delivery systems. Both hydrophilic and hydrophobic drugs can be accommodated in the internal core and lipid membranes [5,6]. However, the poor stability of liposomes can lead to rapid leakage of incorporated drugs [7]. Several researchers have proposed modifying the surface of the liposome in order to overcome this limitation, including using a polymer coating [8,9] or cell-penetrating peptide [10], or conjugating the surface with poly(ethylene glycol) [11].

Layer-by-Layer (LbL) technology is a simple and versatile method used to develop multilayer films by alternating the deposition of oppositely charged polyelectrolytes *via* electrostatic attraction. It improves stability by forming a polyelectrolyte complex (PEC), and has general biomedical or biotechnology applications [12–14]. In particular, drug delivery systems fabricated by LbL deposition of polyelectrolytes could significantly contribute to the delivery of the therapeutic protein by increasing tolerance to extended shelf storage and drug loading [15], as well as to oral administration by protecting drugs against external environments such as low gastric pH due to the functionality of the surface

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[16–18]. They could also enhance *in vivo* biological performance by improving characteristics such as stability and pharmacokinetic profiles [19]. Xavier et al. [20] observed the slow release rate of aloin when incorporated into liposomes immobilized in LbL films, and suggested a possible use in patches for transdermal delivery. However, the functionality of a multilayer of polyelectrolytes deposited on the liposome in transdermal delivery is unknown; further analysis of skin permeability is also needed.

Chitosan is a cationic polysaccharide present in nature that is biocompatible and has low toxicity; hence, it is commonly used as a biomaterial [21], particularly in drug delivery systems [22]. Hyaluronate is a negatively charged polysaccharide, and the main component of the extracellular matrix (ECM). It has versatile properties, including viscoelasticity, biocompatibility, water absorption and water retention, proving that it can be used in transdermal delivery in spite of it being an anionic macromolecule [23–26]. A chitosan-hyaluronate complex assembled using electrostatic attractions based on LbL liposomes has not been investigated yet in transdermal delivery.

In this study, we improved the stability of the liposome by fabricating multilayered liposomes using LbL deposition of polyelectrolytes. We also investigated the potential and roles of alternate deposition of chitosan and hyaluronate on the liposome as carriers for the transdermal delivery of drugs. Quercetin, widely known as a hydrophobic antioxidant, was loaded onto the lipid membranes of liposomes. The physical characteristics of multilayered liposomes were observed using dynamic light scattering (DLS), zeta potentials, differential scanning calorimetry (DSC), and transmission electron microscopy (TEM) analysis. Additionally, the storage stability and membrane stability against an external stimuli factor, Triton X-100, were evaluated. The release kinetics of quercetin from multilayered liposomes was analyzed using several mathematical models. Finally, we performed *in vitro* skin permeation to investigate interactions between the skin and multilayered liposomes.

2. Materials and methods

2.1. Materials

L-α-Phosphatidylcholine (from egg yolk, ≥60%, egg PC), cholesterol (≥99%, Chol), dihexadecyl phosphate (DHP), triton X 100 (BioXtra), quercetin, and chitosan (low molecular weight, MW 50,000 ~ 190,000, CH) were purchased from Sigma–Aldrich (USA). Sodium hyaluronate (MW 490,000, HA) was obtained from Bioland Co., Ltd. (Korea). Solvents such as 1,3-butylene glycol, methanol, ethanol, chloroform, acetic acid, and sodium hydroxide were of analytical grade.

2.2. Preparation of liposomes

Liposomes were prepared using the thin-film hydration method. Egg PC, Chol, DHP (10:2.5:1, molar ratio) and 500 μM quercetin were dissolved in a chloroform-methanol mixture (2:1). The solvent was removed by a rotary evaporator (Buchi, Switzerland), resulting in the formation of a lipid film over the inner surface of a round-bottom flask. The film was hydrated with distilled water (DW), and then the solution was homogenized using a probe sonicator (Branson, USA). It was filtered by passing through 1.20 μm filter (Minisart, CA, 26 mm).

2.3. Preparation of multilayered liposomes

2.3.1. Preparation of polysaccharide solutions

CH solutions (1 mg/ml) were prepared by dissolving chitosan in 1% acetic acid aqueous solution. The final pH was adjusted with 0.05 M NaOH to 4.0. Sodium hyaluronate solutions (1 mg/ml) were prepared in DW. Both the solutions were stirred overnight [27].

2.3.2. Deposition of polysaccharides onto liposomes

Multilayered liposomes were coated with a series of polysaccharides using LbL assembly, mediated by ionic interactions. The first layer of CH solution (1:1, v/v) was deposited onto the negatively charged liposomes and gently mixed for 10 min with stirring (HSD 120-03P, Misung Scientific Co., Ltd., Korea) at 500 rpm. Excess polyelectrolytes were then removed by ultracentrifugation (2236R, Gyrozen Co., Ltd., Korea) at 36,000 × *g* for 90 min followed by a single washing with DW. The resulting CH-coated liposomes were resuspended in DW. The subsequent layer was fabricated by adding HA solutions to the CH-coated liposomes (1:1, v/v) using the same procedure. The resulting HA-CH-coated liposomes were referred to 1 bilayered liposomes (HA-CH-L). To successfully build up each layer, the liposomes were coated with alternating layers of positively charged CH and negatively charged HA until the 10 layers ((HA-CH)5-L) were achieved (Scheme 1). All the formulations were obtained at pH 4.5 ± 0.3 [28].

2.4. Characterization of multilayered liposomes

2.4.1. Particle size and zeta potential measurement

The particle size and distribution of liposomes were determined by DLS (Otsuka ELS-Z2, Otsuka Electronics, Chiba, Japan) at 25 °C, with a scattering angle of 165°, with an Argon laser. The average particle size is indicated by cumulative analysis, and distribution is resolved using the Contin method. The zeta potentials of the liposomes were measured based on electrophoretic mobility under an electric field (Zetasizer, Malvern Instruments, UK).

2.4.2. Differential scanning calorimetry (DSC)

The thermal analysis of the CH solution, HA solution, uncoated liposomes, and (HA-CH)-L were studied by DSC using a JADE DSC (PerkinElmer, USA). A sample was placed in aluminum pan and heated from 10 °C to 150 °C at a heating rate of 1 °C/min with continuous purging of nitrogen at 20 ml/min.

2.4.3. Shape and surface morphology

The morphology of the multilayered liposomes was observed using TEM (JEOL-JEM1010 instrument, JEOL Ltd., Japan). Samples were dropped into a carbon-coated copper grid and dried for 1 min. Excess sample was removed using filter paper. Then, the samples were instantly stained with 0.4% (w/v, %) phosphotungstic acid, allowed to stand for 1 min, and drained. The analysis was performed at an accelerating voltage of 80 kV.

2.5. Membrane stability of multilayered liposomes against Triton X-100

The nonionic surfactant Triton X-100 is generally used as a good solubilization agent of the lipid membrane [29]. So, liposome membranes were destabilized under different Triton X-100 concentrations. To evaluate the stability of multilayered liposomes, we measured optical density (OD) of the liposome suspension at 500 nm using ultraviolet spectrophotometry (Cary 50, Varian, Australia) with a 1 cm path-length cell at room temperature. The % loss in OD after the addition of Triton X-100 can reflect the % solubilization of lipid membrane [30]. Different concentrations of Triton X-100 (10 μL) were added to 1 ml of the liposome suspension. This measure of turbidity was normalized against the control absorbance values (before the addition of Triton X-100) to quantify the effect of Triton X-100 on the liposomes [31].