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Chitosan-coated liposomes for enhanced skin permeation of resveratrol

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ABSTRACT

In this study, chitosan-coated liposomes were investigated for use in enhanced transdermal delivery of resveratrol. Particle size, entrapment efficiency, stability, and skin-permeation efficiency were evaluated. The particle size was seen to increase on coating with chitosan, with higher concentrations of coating solution forming larger particles. The zeta potential of the liposomes also followed the same trend, *i.e.*, it changed from a negative value for uncoated liposomes to increasingly positive values for the chitosan-coated ones. The chitosan coating was seen to increase the stability of the liposomes by preventing their aggregation. Transdermal delivery of uncoated and 0.1% chitosan-coated liposomes containing 0.1% resveratrol was investigated using Franz diffusion cells. The proportions of resveratrol that permeated the animal skin were 40.42% and 30.84% for the coated and uncoated liposomes, respectively. This increased skin-permeation efficiency with the coating could be explained by the tendency of positively charged liposomes to interact more strongly with the skin surface. These results indicate that chitosan-coated liposomes could be an effective transdermal delivery system for delaying skin aging using resveratrol.

1. Introduction

Resveratrol (3,5,4′-trihydroxy-trans-stilbene, Fig. 1) belongs to a class of polyphenolic compounds called stilbenes found in the skin of grapes [1]. It is beneficial for cardiovascular health as it prevents oxidative damage and platelet aggregation, promotes vasodilatation, and exhibits antimicrobial activity against dermatophytes and the herpes simplex virus [2–5]. Such beneficial effects of resveratrol have highlighted its potential applications throughout the world [6]. However, it has some limitations owing to its poor solubility in water, and its instability [7]. These shortcomings therefore need to be overcome if resveratrol is to fulfill its potential.

Human skin is a layered structure composed of three main layers: epidermis, dermis, and subcutaneous tissue. The outermost layer, the epidermis, which forms a barrier for protection of the underlying tissue from the environment, is known as the stratum corneum. This same structure, however, also provides a barrier to therapeutic agents that need to permeate the skin in order to achieve their objectives. Therefore, a major focus of transdermal drug delivery research is the development of methods to overcome this barrier and allow the active agents to permeate the skin.

Liposomes are spherical structures that consist of one or more phospholipid bilayers, and have been used as a transdermal drug delivery system owing to their interaction with the cell surface [8]. When the liposome reaches the cell surface, drug delivery is achieved *via* the fusion of the liposome to the cell membrane, protein-mediated transfer, or phagocytosis and pinocytosis [9]. However, the poor liposome stability can result in degradation, aggregation, and fusion, thereby leading to the leakage of the entrapped drug [10].

Polymer coating is a promising way to modify the surface characteristics of liposomes in order to improve their applicability. This can be achieved by simply mixing a liposome suspension with a polymer solution without chemically linking the two components [11]. On coating the liposomes with the polymers, long-range mutual repulsion between the adjacent bilayers arise, which improves their stability. In addition, coating with a positively charged polymer can improve the skin permeation because of the increased interaction between the liposomes and the skin [12,13].

Chitosan is a hydrophilic, biocompatible, biodegradable, and positively charged polysaccharide polymer. It has been previously used as a coating material for carrying hydrophilic drugs across the skin barrier, thereby enhancing the drug delivery [14,15]. Accordingly, coating the liposomes with chitosan can significantly improve skin permeation.

In this study, we investigated the particle size, zeta potential, entrapment efficiency, and skin permeation of chitosan-coated

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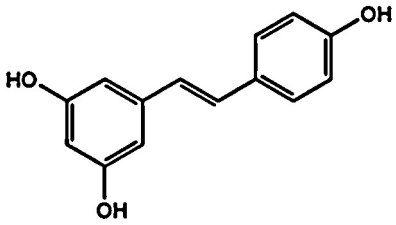

Fig. 1. Structure of resveratrol.

liposomes containing resveratrol in order to improve the application of this compound in cosmetics.

2. Experimental

2.1. Materials

l-α-Phosphatidylcholine (egg PC, ~60%) and chitosan (low molecular weight, viscosity 20–300 cP) were purchased from Sigma–Aldrich (USA). Resveratrol was purchased from TCI (Japan). All other chemicals were of analytical grade.

2.2. Preparation of liposomes

Egg PC (2%, w/v) and resveratrol (0.1%, w/v) were dissolved in a mixture of chloroform (10 mL) and methanol (5 mL). The solution was then poured into a round-bottom flask, and the solvent was removed by rotary evaporation (Buchi, Switzerland), resulting in the formation of a thin layer of lipid film on the walls of the flask. Distilled water (10 mL) was then added to the flask and the solution was sonicated using a probe-type sonicator (Branson, USA) to obtain uniformly sized submicron particles.

2.3. Preparation of chitosan-coated liposomes

Chitosan was dissolved in 1% v/v solution of acetic acid in water in various concentrations (0.1%, 0. 3%, and 0.5%, w/v). The liposome suspension was then added dropwise to an equal volume of the chitosan solution with stirring. The mixture was incubated for 1 h with continuous stirring and then left overnight at 4 °C. The resulting chitosan-coated liposomes were harvested from the suspension by centrifugation at 15,000 rpm for 30 min at 4 °C (Labogene 2236HR, Korea), which was then resuspended in distilled water.

2.4. Particle size and determination of zeta potential

The particle size and zeta potential of the chitosan-coated liposomes were measured using an ELSZ analyzer (Otsuka Eletronics, Japan).

2.5. Entrapment efficiency of resveratrol in liposomes

Free resveratrol was removed from the liposome or chitosan-coated liposome suspension by filtering through 1.2 μm cellulose acetate filters (Minisart, CA, 26 mm). The liposome vesicles were resuspended in ethanol (15 mL) and the solvent was then removed by rotary evaporation. The remaining resveratrol was redissolved in ethanol and its content was measured using HPLC (Shimazu, Japan). The following equation was used to calculate the entrapment efficiency:

$$\text{Entrapment efficiency}\,(\%) = \frac{P}{T} \times 100$$

T is the initially added resveratrol content;P is the content of resveratrol passed through the 1.2 μm filter.

2.6. In vitro *skin-permeation study*

The *in vitro* skin-permeation study was carried out using Franz diffusion cells (Permegear, USA). Full-thickness skin was removed from the dorsal side of a freshly excised ICR albino mouse (8 weeks, female). Subcutaneous fat and bristles were carefully removed from the skin, which was then stored at -70 °C. A specimen of the skin was sandwiched between the two halves of the Franz cell, with stratum corneum facing the donor compartment. The receptor compartment had a volume of 5 mL and was filled with a receptor phase (HCO-60:ethanol:phosphate buffered saline (PBS) = 2:20:78, w/w/w). The compartment was kept at 37 °C by circulating water through it. An aliquot (0.2 mL) of the sample was introduced into the donor compartment, which was then covered with parafilm to prevent the evaporation of the solvent. The receptor phase was continuously stirred by a spinning bar magnet. Receptor phase samples (0.5 mL) were withdrawn through the sampling port of the receptor compartment at intervals of 4 h throughout the 24 h experimental period, and the receptor compartment was refilled with the receptor phase to maintain a constant volume. The test samples were concentrated, dissolved in 100% ethanol, and then analyzed for resveratrol content (Fig. 7, Transdermal) using HPLC equipped with a UV detector and computer integrating apparatus. The column was a Shim-pack VP-ODS C18 (250 mm × 4.6 mm). The skin surface was then washed on each side with 3 mL PBS solution to remove the residual donor sample. The stratum corneum was subsequently removed by the stripping method using three strips of 3M scotch tape (3M, Korea). The tape strips were dissolved in 100% ethanol at 70 °C, and analyzed for resveratrol content using HPLC. The remaining skin was cut into small pieces and dissolved in 100% ethanol at 70 °C and analyzed for resveratrol content using HPLC.

2.7. Statistical analysis

All reported data are presented as mean ± S.E.M. Statistical significance was determined by Student's *t*-test.

3. Results and discussion

3.1. Particle size of chitosan-coated liposomes containing resveratrol

The effects of chitosan addition on the physical properties of liposomes containing 0.1% resveratrol were investigated. Liposomes containing 0.1% resveratrol were coated with 0.1%, 0.3%, and 0.5% chitosan solution (w/v). The particle size of uncoated (typical) liposomes was found to be 212.83 nm. After coating with chitosan solution, this increased to 279.85, 432.58, and 558.35 nm, for 0.1%, 0.3%, and 0.5% chitosan solutions, respectively (Fig. 2). These significant increases in the size of liposomes were attributed to the adhesion of the chitosan to the liposome surface *via* strong interactions, thereby forming a layer on the surface [16,17]. In addition, the particle size was found to be highly dependent on the concentration of chitosan. These results suggest that large aggregated structures self-assembled on the liposome surface, with higher chitosan concentrations forming a thicker layer [18].

3.2. Zeta potential of chitosan-coated liposomes containing resveratrol

Zeta potential measurements are commonly used to investigate certain physical properties of colloidal drug delivery systems. They give an indication of the surface electrical charge of the particles,

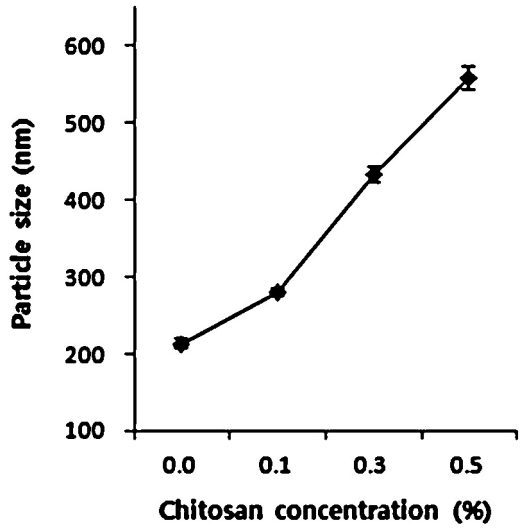

Fig. 2. Variations in the size of chitosan-coated liposomes with varying chitosan concentration.

which is a particularly important parameter that affects liposome behavior [19]. Fig. 3 shows the measured zeta potentials of the chitosan-coated and uncoated liposomes containing 0.1% resveratrol. The uncoated liposomes had a negative charge of −9.4 mV; whereas, 0.1%, 0.3%, and 0.5% chitosan-coated liposomes had positive charges of 26.5, 34.5, and 39.2 mV, respectively. These results indicate that the major interaction between the chitosan and the liposomes was through electrostatic attraction. The zeta potential of the coated liposomes increased with rising concentration of the chitosan, which was attributed to the adsorption of the cationic polymer increasing the density of the positive charge around the liposomal surface. In addition, the zeta potential of uncoated liposomes was almost neutral, and it has been previously shown that neutral liposomes undergo hydrogen bonding between their phospholipid head group and the chitosan [13].

3.3. Entrapment efficiency of resveratrol

The entrapment efficiency of uncoated and chitosan-coated liposomes containing resveratrol was investigated (Fig. 4). The

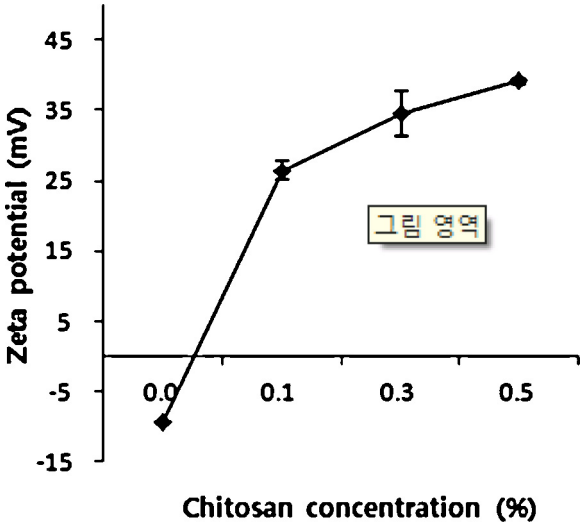

Fig. 3. Variations in zeta potentials of chitosan-coated liposomes with varying chitosan concentration.

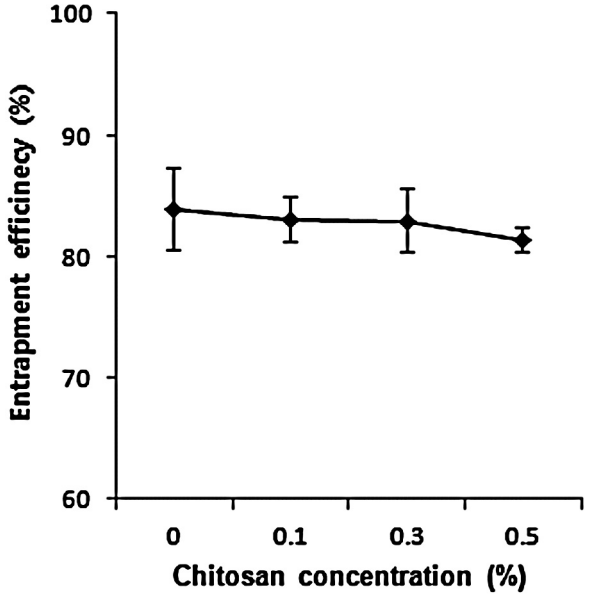

Fig. 4. Variations in the entrapment efficiency of resveratrol with varying chitosan concentration.

entrapment efficiency was calculated to be 83.91% for the uncoated liposomes, with values for 0.1%, 0.3%, and 0.5% chitosan-coated liposomes being 82.95%, 82.9%, and 81.36%, respectively. These results demonstrate that the addition of chitosan decreased the entrapment efficiency, though not to a significant level.

3.4. Stability of chitosan-coated liposomes containing resveratrol

In order to investigate the stability of the uncoated and chitosan-coated liposomes containing resveratrol, the change in the particle size was measured at room temperature over the course of 1 week (Fig. 5). As shown in Fig. 2, coating with chitosan immediately increased the particle size of the liposomes, with the higher concentrations producing larger particles. A week after

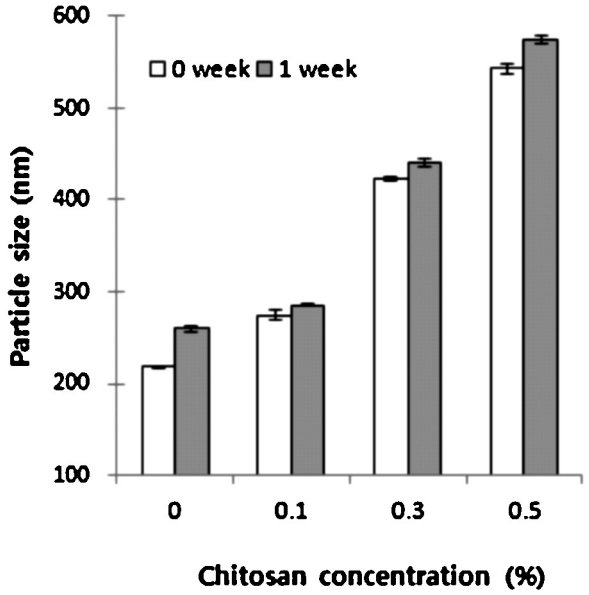

Fig. 5. The influence of chitosan concentration on the size of chitosan-coated liposomes after 1 week.

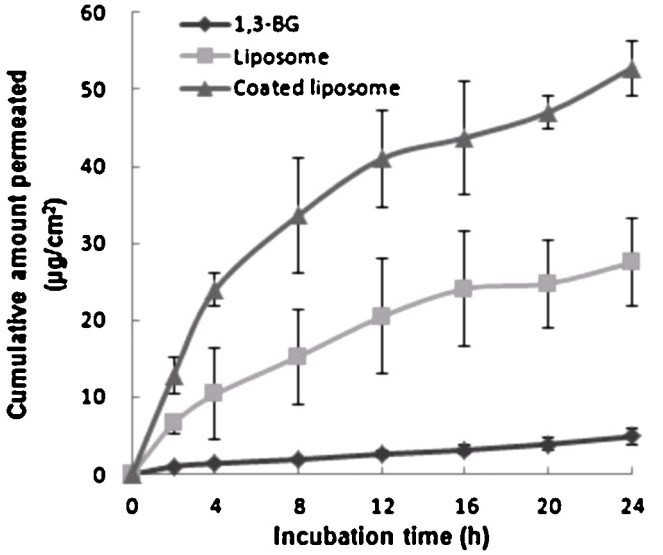

Fig. 6. *In vitro* skin-permeation profiles of 1,3-butylene glycol solution (1,3-BG), uncoated liposomes (liposome), and chitosan-coated liposomes (coated liposome) containing resveratrol through ICR albino mouse skin. Mean ± SD (n = 3).

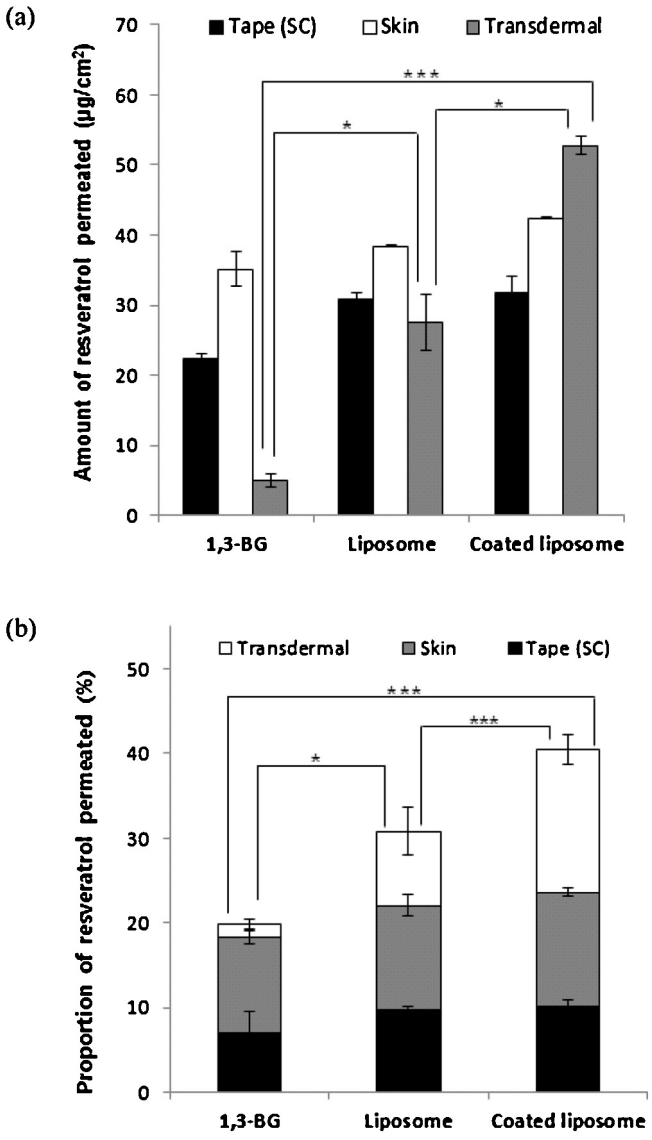

Fig. 7. Proportions of permeated amount of 1,3-butylene glycol solution (1,3-BG), uncoated liposomes (liposome), and chitosan-coated liposomes (coated liposome) containing resveratrol through ICR albino mouse skin after 24 h of incubation (Tape, stratum corneum; Skin, epidermis + dermis without stratum corneum; Transdermal, receptor chamber). $^{*}p < 0.05$; $^{***}p < 0.005$.

preparation, the particle size of all liposomes was observed to have increased. However, this increase was lower for the chitosan-coated liposomes than the uncoated ones, with the 0.1% chitosan coating in particular displaying very little change in size. The zeta potential values gave an indication of the potential stability of the colloidal system, and therefore, the use of chitosan to increase the zeta potential should improve the stability of the liposomes. Repulsion between the particles would increase with increasing zeta potential. If all particles in a suspension have a large negative or positive zeta potential, they will tend to repel each other, thereby discouraging aggregation and providing a more stable colloidal dispersion [19].

3.5. In vitro *skin-permeation efficiency of resveratrol*

The chitosan-coated liposome system was applied to skin-permeation studies by using Franz diffusion cells. The 0.1% chitosan-coated liposome system was used as it consisted of the smallest (279.85 nm) and most stable particles. The control groups consisted of uncoated liposome and a 1,3-butylene glycol (1,3-BG) solution containing 0.1% resveratrol. 1,3-BG is widely used as an ingredient for dissolving active components in cosmetics, and also has skin moisturizing properties. The skin permeation of resveratrol 24 h after application was shown to be the highest for the chitosan-coated liposomes (52.75 μg/cm^2), followed by the uncoated liposome system (27.52 μg/cm^2), and finally, the 1,3-BG solution (4.94 μg/cm^2) (Fig. 6). Fig. 7(a) shows the amount of resveratrol deposited in the stratum corneum (Tape), the skin layer without stratum corneum (Skin), and the receptor phase after permeation (Transdermal) after 24 h. The amount of resveratrol deposited in the stratum corneum (Tape) was determined to be the highest for the chitosan-coated liposomes, followed by the uncoated, and then the control 1,3-BG solution. The same trend was also evident for the amount of resveratrol deposited in the skin layers without the stratum corneum (Skin) and for that which permeated into the receptor phase (Transdermal). Fig. 7(b) shows the amount of resveratrol in each of the three positions as a proportion of the initial amount used, which was 314.37 μg/cm^2 in every system. Overall, the permeation of resveratrol, in terms of the total mass per area and the percentage of the initial amount, was determined to be as follows: chitosan-coated liposomes (126.93 μg/cm^2, 40.42%) > uncoated liposomes (96.85 μg/cm^2, 30.85%) > 1,3-BG solution (62.37 μg/cm^2, 19.86%). Thus, the transdermal skin-permeation studies indicate that the chitosan-coated liposome system was superior for the transdermal delivery of resveratrol. The negatively charged lipid present in the lipid layer of the stratum corneum could interact with the positively charged chitosan coating, resulting in increased permeation of the drug through the skin [20–22].

4. Conclusion

Although resveratrol has many benefits, its applications are limited because of its poor solubility in water and its instability. Therefore, in this work, we investigated the physical characteristics and skin permeation of chitosan-coated liposomes containing resveratrol as a potential transdermal drug delivery system.

The particle size of the resveratrol-containing liposomes increased on being coated with chitosan, with higher concentrations of polysaccharide solution forming thicker coating layers. The zeta potential of the liposomes also demonstrated a similar trend, changing from a negative value for the uncoated, to increasingly positive values for the chitosan-coated liposomes. By assessing changes in particle size over the course of a week-long incubation, it was observed that the liposomes coated with 0.1% chitosan were extremely stable and did not undergo significant aggregation. These liposomes were then investigated for their ability to permeate animal skin by using Franz diffusion cell studies. The chitosan-coated liposomes were seen to have superior permeation as compared to the uncoated sample.

These results demonstrate that coating liposomes containing resveratrol with chitosan could increase their stability and improve the skin permeation of the compound. Therefore, we suggest that the chitosan-coated liposome can be used as an effective transdermal delivery system for an antioxidant defense system to delay skin aging.

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