New delivery carrier : Exosome–Liposome hybrid system

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Abstract

Exosomes are extracellular vesicles that contain a specific composition of proteins, lipids, RNA, and DNA. They are derived from endocytic membranes and can transfer signals to recipient cells, thus mediating a novel mechanism of cell-to-cell communication. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Exosomes and liposomes have many similarities as they are nanovesicles composed of one lipid bilayer. Exosomes has the advantage of being an endogenous nanocarrier, but it lacks deformation flexibility and has a low yield. On the other hand, Liposomes show exceptional flexibility to facilitate various engineering approaches, but lack endogenous functions. The purpose of this work was to develop a new type of liposomes with a unique mixture of phospholipids, similar to naturally occurring exosomes but overcoming their limitations of heterogeneity and low productivity, for therapeutic delivery of bioactive compounds. Exosome-liposome hybrid were designed by fusing the exosomes and liposomes extracted from Centella asiatica using evaporation and hydration methods. In addition, hybrid lipids were prepared by microfluidization to form a stable vesicle film without affecting the inherent properties of natural exosome and synthetic liposome nanoparticles. To further validate the exosome-liposome fusion, the DLS and Cryo-TEM were analyzed. TEM analysis showed that hybrids were morphologically similar to liposomes, and had an average diameter of less than 200 nm and zeta potential greater than

|30| mV. These results suggest that the engineered hybrid vesicles would be an exciting platform for applied to cosmetics.

Keywords: Exosome, Liposome, Nanoparticle, Hybrid material

Introduction.

Exosomes are cell-derived membrane vesicles with a diameter of 50-150 nm and can be easily found in blood, saliva, and other extracellular fluids, and cell culture media. They have excellent biocompatibility, non-immunity, organ circulation, and non-toxic properties [1]. These nanospores contain bioactive lipids and transport proteins, and their structures contain microRNAs. They act as messengers between cells that transmit biological signals to recipient cells to repair damaged or diseased cells. In other words, it plays an important role in mediating communication between cells and controlling immune responses. Recently, studies on various effects of exosomes derived from plants have been conducted, and studies on skin effects such as antioxidants and anti-inflammatory drugs have also been started. Plant-derived exosomes are natural nanoparticles that help move and absorb cells because they contain physiological activity and signal transmission substances secreted by plant cells, and plant-derived exosomes are known to be less toxic than mammalian-derived exosomes [2]. However, due to insufficient encapsulation of cargo, there are limitations in drug delivery applications such as low delivery rates and bio-synthesis of existing drug delivery systems.

Similar to exosomes, liposomes are also nanoscale lipid vesicles consisting of amphipathic phospholipid to form single or multiple bilayer membrane [3]. Liposomes have many advantageous features for drug delivery, including efficient drug loading, scalability, tunable size and surface charge, surface functionalization with easy control, and strong preclinical and clinical evidence for therapeutic relevance [4]. However, like most types of nanoparticles, liposomes are readily recognized by the immune system and rarely internalize into target cells. Although it is one of the most potential drug carriers, it has problems such as rapid drug pre-leakage and incomplete drug release, and further studies are needed to improve it.

Given these challenges, it will confer biological functions on liposomes and allow for broad application of drug delivery systems based on extracellular vesicles. The purpose of this exosome-liposome hybrid formulation is to take the advantages of both exosomes and

liposomes and overcome the disadvantages of each. Combining these two delivery systems can create an efficient hybrid skin delivery tool with exosome endogenous properties and liposome flexibility, which will ultimately help accelerate the development of cosmetically applicable skin functional materials.

Materials and Methods.

2.1. Cell culture

Centella asiatica (Cica) cells were kindly provided by the Xenohelix Research Institute (Incheon, Republic of Korea).

2.2. Preparation of exosomes

Prepare 100 µl of the preprocessed Cica sample and mix with 50 µl of Extracellular Vesicle lsolation (EVI) Pre-buffer. This centrifuge the mixture at 14,000 x g for 30 minutes at 4 °C. Transfer the supernatant to a new tube. here, Add 40 µl of XENO-EVI buffer and mix the sample by vortexing. Incubate the mixture at room temperature (15-25 °C) for 10 min, centrifuge the mixture for 10 minutes at 12,300 x g and discard the supernatant. next, centrifuge the tube for 15 seconds at 12,300 x g and discard the remaining supernatant. Resuspend the EV containing pellet thoroughly in the ≥ 50 µl of 1X PBS (for protein and other analysis) or EVARI buffer by vortexing (for EV RNA purification). Then exosome pellets were resuspended in phosphate buffer saline (PBS) or DMEM and stored at -20 °C.

2.3. Liposome preparation

Thin-film hydration technique is commonly used to prepare liposomes. In some articles also reported as “Hand Shaking Method” [5]. To prepare liposomes by this method, an organic solvent is taken in a round bottom flask and to this, Lipoid S 75-3 (Lipoid GmbH, Ludwigshafen, Germany) and phospholipids (DSPC) are added together. Afterward, the organic solvent is evaporated in a rotary vacuum evaporator. On evaporation, a thin layer forms on the inner surface of the round bottom flask. The residual trace solvent was completely removed in vacuo to yield a thin film on the wall of a glass flask.

2.4. Synthesis of Exosome-Liposome hybrid

Previously isolated Cica exosomes were used to hydrate the dry lipid layer. On hydration, the layer swells and formation of multilamellar vesicles, containing drug takes place [6]. Phospholipid and exosome added at the same content were added to the lipid film in a final volume of 1 mL as shown in Table 1. It was then vortexed and sonicated (30% amplitude, 30 sec pulse on/off, for 30 min) for proper mixing. Thus formed multilamellar hybrid solution was passed through twice using a homogenizing device, the Microfluidizer, to reduce the particle size.

Phospholipid	Exosome	Thin film
+	+	1
+	+	2
+	+	3
+	+	5
+	+	7
+	+	9

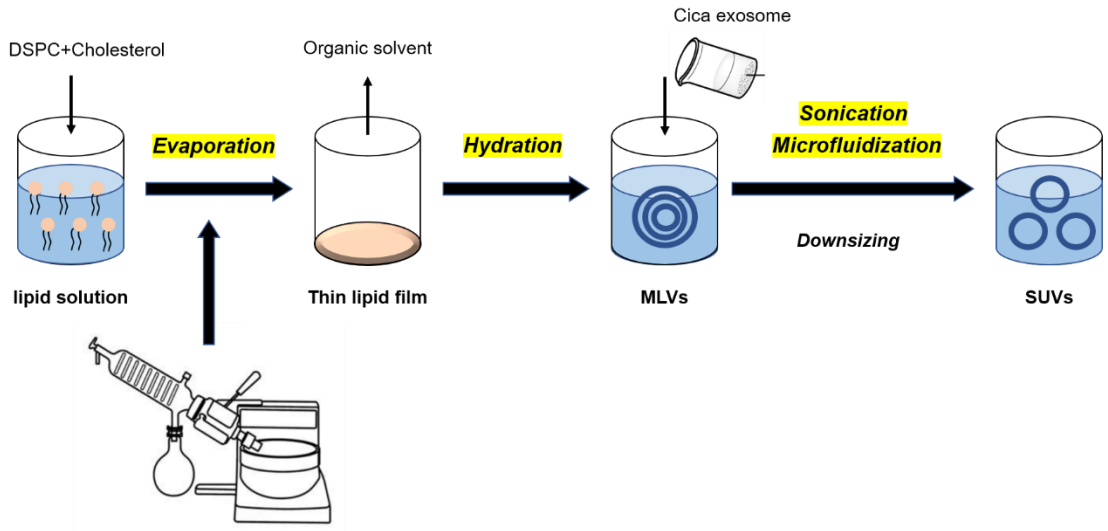
Table 1. Ratio of thin films used for exosome-liposome hybrid synthesis.

Results.

The ratio of the liposome to Cica exosome was optimized to 3:1. Exosome was quantified based upon protein content whereas liposome was quantified based upon lipid weights. 1000 mg protein equivalent of exosome dispersed in 1 mL Phosphate buffer saline (PBS) was added to 300 mg of lipid film. This initiates the hydration of lipid film to lipid cake which after microfluidization results in the formation of exosome-liposome hybrid as shown in Scheme 1.

Liposomes, Exosome, and Hybrids were characterized for size, surface property, and protein content. Fig. 1 shows a comparative study on the hydrodynamic size distribution and zeta potential along with the stability of theses nanovesicles. Hydrodynamic size of liposome and exosome was found to be 447 ± 20 nm and 139 ± 20 nm with zeta potential of -42 ± 1 and -12 ± 1 mV, respectively. Whereas that of hybrid was found to be 142 ± 40 nm and -40 ± 6 mV. To reduce the size of lipid vesicles, high pressure microfluidization gave a large change in the hydrodynamic size of the hybrids and the size of the hybrids was found to increase to 142 nm (Fig. 2). The increase in hybrid size is probably due to the insertion of

Centella asiatica exosomes into the bilayer of synthetic liposomes, increasing the interaction points of water molecules, thereby increasing the hydration layer. The size of the hybrid is smaller than that of liposomes and larger than that of Centella asiatica exosomes, but the most important factor is the homogeneity of the size distribution.



Scheme 1. Schematic representation of the fabrication of Exosome-Liposome hybrid.

	Size (nm)	Zeta Potential (mV)
Exosome-Liposome hybrid	142 ± 40	-40 ± 6
Exosome	139 ± 20	-12 ± 1
Liposome	447 ± 20	-42 ± 1

Fig. 1. Comparison between nanovesicles in terms of size and surface charge.

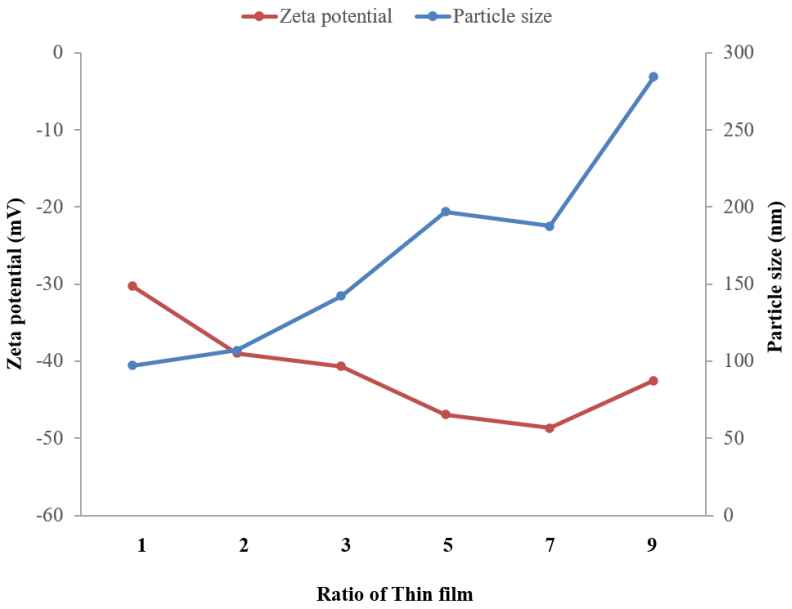


Fig. 2. Particle size and zeta potential plots of hybrids made by proportion of lipid films.

The optical microscope study could not show the average morphology, but there was a clear morphological difference before and after by microfluidization process (Fig. 3). Also, the morphological changes of hybrid after downsizing were also analyzed by cryo-transmission electron microscopy (Cryo-TEM). TEM image (Fig. 4) showed a general distribution of nanoparticles with vesicular structure. Additionally, a stabilized transparent liquid was formed by reducing the size using microfluidization (Fig. 5).

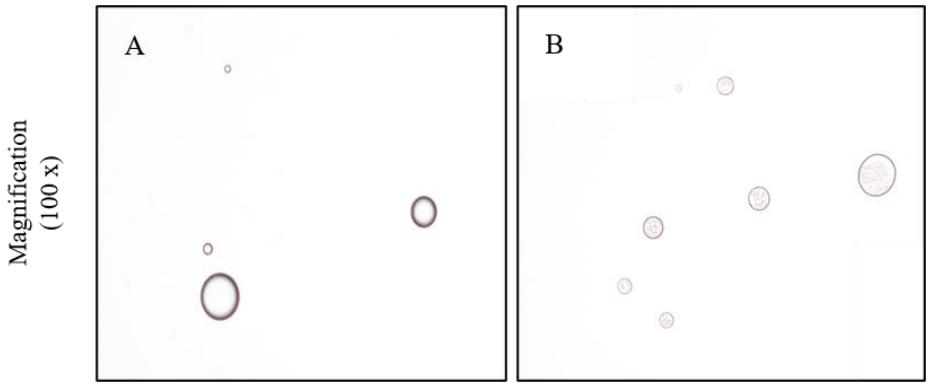


Fig. 3. Optical microscope image of nanovesicles. (A) Before microfluidization process of Exosome-Liposome hybrid, (B) After microfluidization process of Exosome-Liposome hybrid.

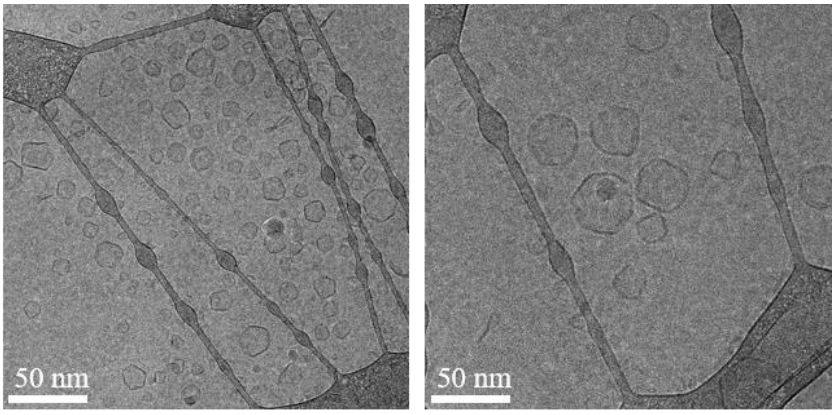


Fig. 4. Cryo-TEM images of Exosome-Liposome hybrid obtained by membrane fusion and high-pressure microfluidization techniques.

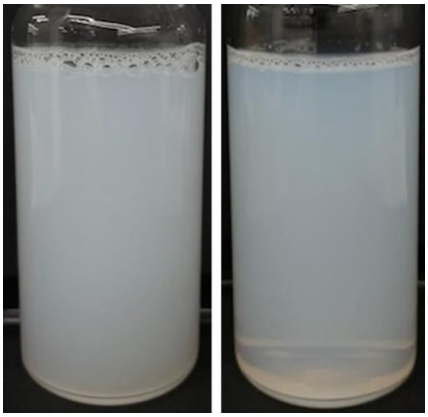


Fig. 5. Influence of microfluidization process on hybrid lipid appearance.

Discussion.

More recently, biohybrid nano-DDSs, obtained through the fusion of conventional DDSs with cellular components (e.g. Extracellular vesicles), have emerged as promising next generation DDSs in view of their potential ability to overcome the shortcomings of each individual system [7].

Characterization of hybrid particles may be influenced by additional liposomes and exosomes and the purification would be rather difficult as liposomes, exosomes and the hybrid are very similar in multiple aspects. However, one strength of the hybrid approach is that the hybrid possesses natural components from exosomes, despite dilution, those hybrid nanocarriers may have improved delivery efficiency than liposomes and higher stability than

exosomes, leading to high applicability [8]. To this end, it is necessary to standardize and establish the extensive characterization of exosome-like nanoparticles as carriers with active ingredients and the delivery of novel regulations, which remain very challenging tasks. Nevertheless, we believe that in the future, the continuous expansion of knowledge about the biological properties of exosomes and the rapid development of nanotechnology will provide a new class of safe and efficient exosome-like nanoplatforms.

Centella asiatica has long been used as a medicinal plant. As an active ingredient, pentacyclic triterpenes (PTs) including asiaticoside, madecasosside, asiatic acid, and madecassic acid are known. These ingredients are reported to be excellent in collagen formation and antioxidant activity in skin cells, anti-aging effect, anti-inflammatory effect, skin photoaging improvement, and wound healing, and are used as raw materials for cosmetics and wound healing ointments [9]. Further studies are required in the future for the detailed mechanism and efficacy as an active ingredient for the centella asiatica-derived hybrid nanoparticles mentioned in this study. Membrane fusion technology utilizing these characteristics of plants is expected to be developed as a raw material suitable for the green bio era as it has the characteristics of being eco-friendly, sustainable, and capable of enhancing efficacy.

Conclusion.

Here, the aim of hybrid engineering was to merge the advantage of exosome and liposomal Transdermal delivery system. Plant-derived exosomes are natural products, and since the exosomes themselves have a double lipid membrane structure, they can be absorbed into the skin as well as other cells, which is a great advantage as a cosmetic preparation. In this study, exosome-liposome hybrid with uniform size distribution were formulated by fusioning exosomes derived from Centella asiatica with synthetic liposomes.

Acknowledgments.

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