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New delivery carrier : Exosome-Liposome hybrid system

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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.

The purpose of this exosome-liposome hybrid formulation is to take the advantages of both exosomes and liposomes and overcome the disadvantages of each.

Materials & 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 Isolation (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.

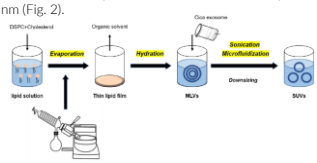
Phospholipid	Exosome	Thin film
+[1]	+[1]	1[1]
+[2]	+[2]	2[2]
+[3]	+[3]	3[3]
+[4]	+[4]	5[4]
+[5]	+[5]	7[5]
+[6]	+[6]	9[6]

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

Results & Discussion:

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. 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).



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.

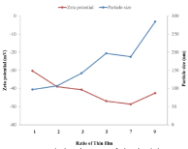


Fig. 2. Particle size and zeta potential plots of hybrids made by proportion of lipid films.

The morphological changes of hybrid after downsizing were also analyzed by cryo-transmission electron microscopy (Cryo-TEM). TEM image (Fig. 3) showed a general distribution of nanoparticles with vesicular structure. Additionally, a stabilized transparent liquid was formed by reducing the size using microfluidization (Fig. 4).

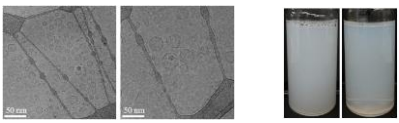


Fig. 3. Cryo-TEM images of Exosome-Liposome hybrid obtained by membrane fusion and high-pressure microfluidization techniques.

Fig. 4. Influence of microfluidization process on hybrid lipid appearance.

Conclusions:

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.

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