

**Yeungnam Univ**

## Key Technology Highlights

\* Non-confidential information

### Enhanced virus or gene delivery using proteoliposome for therapeutics

The delivery of genes or viruses using liposomes is a common approach used to enhance delivery efficiency. In the current study, to enhance delivery efficiency, proteoliposomes (PLs) containing adenovirus (Ad) were synthesized using dimyristoylphosphatidylcholine (DMPC), cholesterol, and apolipoprotein (apo) A-I. Wildtype-apoA-I (WT) or V156K-apoA-I (V156K) was then used as an apolipoprotein to compare the structural and functional differences of the PL. The particle diameter of V156K-PL-Ad was slightly larger than that of WT-PL-Ad based on native gel electrophoresis. V156K showed more rapid phospholipid bilayer formation than the WT based on DMPC-clearance. In addition, V156K exhibited a maximum fluorescence that was more blue than the WT in the PL state. Moreover, isothermal denaturation in response to the addition of guanidine hydrochloride (Gnd-HCl) revealed that V156K was more resistant, with no denaturation until 3 M Gnd-HCl was added. Additionally, electron microscopy revealed that the viral particle was well-associated with PL particles, which had a discoidal structure and a rouleaux shape. In addition, treatment of Ad in the PL state showed enhanced GFP expression when compared to treatment with Ad alone or with DMPC-Ad in hepatoma and brain glioma cells. Cells treated with WT-PL-Ad and V156K-PL-Ad showed approximately 50% more GFP expression than cells treated with Ad alone or with DMPC-Ad after 24 hr of incubation at 37°C, indicating that viral stability was highly increased in the PL state. Furthermore, V156K-PL-Ad showed the highest expression of GFP in adult zebrafish (9 weeks old) at 5 days post-injection (10.5- and 3.8-fold more GFP expressed than Ad only and DMPC-Ad, respectively). In conclusion, the efficiency of viral delivery and the stability of the virus were significantly enhanced when the PL containing apoA-I was used in cellular and zebrafish models.

## Technology Overview

### Technology platform

Recombinant adenoviruses (Ad) are highly efficient at transferring genes into a wide range of cells and species (McConnell *et al.*, 2004). However, even though Ad has a high level of gene delivery and expression, use has been limited because Ad can induce inflammation (Yang *et al.*,

1994) and Ad is not stable when stored at room temperature. Conversely, the use of non-viral vectors, such as cationic liposome containing a fragment of DNA (Felgner, 1996), to mediate gene delivery has become common because non-viral vectors are less immunogenic, although they have a lower delivery efficiency and specificity.

To determine if the advantages of viral and non-viral vectors could be combined, the use of an Ad and liposome was combined in the current study by creating a proteoliposome (PL) containing Ad as a new gene therapy. PL, which is also known as reconstituted high density lipoprotein (rHDL), has been developed for the delivery of small molecules, including hydrophobic biomolecules, such as those used to produce anti-cancer (Lacko *et al*, 2002; Lou *et al*, 2005) and antifungal drugs (Oda *et al*, 2006). The results of studies evaluating the use of rHDLs have indicated that they may be efficient drug delivery vehicles for therapeutic agents due to the ability of peripheral cells to acquire HDL core components.

In the current study, we created a new PL that comprised of a phospholipid, cholesterol, apoA-I, and Ad in an attempt to maximize viral stability, infection ability, and gene delivery efficiency, while minimizing unwanted side-effects. [The efficiency of delivery and expression was tested in human cells \(hepatoma and brain glioma cells\) and in a vertebrate animal model using zebrafish \(\*Danio rerio\*\).](#)

## Specific Patent Information

PCT Application	Korea patent 10-0863068.

## Specific Publication Information

No.	Journal	Title
1	<a href="#">Human Gene Therapy, 2010 in press</a>	Enhanced delivery of an adenovirus using proteoliposomes containing WT or V156K apolipoproteinA-I and dimyristoylphosphatidylcholine.

## Enhanced Delivery of Adenovirus, Using Proteoliposomes Containing Wild-Type or V156K Apolipoprotein A-I and Dimyristoylphosphatidylcholine

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### Abstract

The delivery of genes or viruses via liposomes is a common approach used to enhance delivery efficiency. In the current study, to enhance delivery efficiency, proteoliposomes (PLs) containing adenovirus (Ad) were synthesized with dimyristoylphosphatidylcholine (DMPC), cholesterol, and apolipoprotein A-I (apoA-I). Wild-type apoA-I (WT) or V156K-apoA-I (V156K) was then used as an apolipoprotein to compare the structural and functional differences of the PLs. The particle diameter of V156K-PL-Ad was slightly larger than that of WT-PL-Ad, based on native gel electrophoresis. V156K showed more rapid phospholipid bilayer formation than did the WT, based on DMPC clearance. In addition, V156K exhibited maximal fluorescence that was more blue than that of WT in the PL state. Moreover, isothermal denaturation in response to the addition of guanidine hydrochloride (Gnd-HCl) revealed that V156K was more resistant, with no denaturation until 3 M Gnd-HCl was added. In addition, electron microscopy revealed that the viral particles were well associated with PL particles, which had a discoidal structure and were shaped like rouleaux. In addition, treatment of Ad in the PL state showed enhanced green fluorescent protein (GFP) expression when compared with treatment with Ad alone or with DMPC-Ad in hepatoma and brain glioma cells. Cells treated with WT-PL-Ad and V156K-PL-Ad showed approximately 50% more GFP expression than cells treated with Ad alone or with DMPC-Ad after 24 hr of incubation at 37°C, indicating that viral stability was highly increased in the PL state. Furthermore, V156K-PL-Ad showed the highest expression of GFP in adult zebrafish (9 weeks old) at 5 days postinjection (10.5- and 3.8-fold more GFP expressed than by Ad only and DMPC-Ad, respectively). In conclusion, the efficiency of viral delivery and the stability of the virus were significantly enhanced when PLs containing apoA-I were used in cellular and zebrafish models.

### Introduction

RECOMBINANT ADENOVIRUS (Ad) is highly efficient at transferring genes into a wide range of cells and species (McConnell and Imperiale, 2004). However, even though Ad has a high level of gene delivery and expression, use has been limited because Ad can induce inflammation (Yang *et al.*, 1994) and Ad is not stable when stored at room temperature. Conversely, the use of nonviral vectors, such as cationic liposomes containing a fragment of DNA (Felgner, 1996), to mediate gene delivery has become common because nonviral vectors are less immunogenic, although they have lower delivery efficiency and specificity.

To determine whether the advantages of viral and non-viral vectors could be combined, Ad and liposome were combined in the current study by creating a proteoliposome (PL) containing Ad as a new gene therapy. PL, which is also known as reconstituted high-density lipoprotein (rHDL), has been developed for the delivery of small molecules, including hydrophobic biomolecules, such as those used to produce anticancer drugs (Lacko *et al.*, 2002; Lou *et al.*, 2005) and antifungal drugs (Oda *et al.*, 2006). The results of studies evaluating the use of rHDLs have indicated that they may be efficient drug delivery vehicles for therapeutic agents because of the ability of peripheral cells to acquire HDL core components.

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