

# **Development of multifunctional envelope type artificial viral like gene delivery system**

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## **New concept for gene delivery**

For the efficient gene delivery into the nucleus of target cells, the non-viral vectors must overcome several barriers, such as the plasma membrane, the endosomal membrane and the nuclear membrane. Thus, to overcome the barriers, the non-viral gene delivery system must be equipped with various functional devices such as ligands for specific receptors, pH-sensitive fusogenic peptides for endosomal escape and a nuclear localization signal (NLS) for enhanced nuclear delivery.

## **Development of MEND**

The MEND is constructed by a novel assembly method, the “lipid film hydration method”. The lipid film hydration method is comprised of three steps: (i) DNA condensation with polycations, (ii) hydration of the lipid film for electrostatic binding of the condensed DNA, and (iii) sonication to package the condensed DNA with lipids. This packaging mechanism is based on electrostatic interactions between DNA, polycations and lipids. The transfection activity of the optimized R8-MEND was compared with Adenovirus, one of the strongest viral vectors, using human cervical cancer HeLa cells and a human lung epithelial carcinoma cell line A549. Compared with the highest transfection efficiency obtained by Adenovirus using  $1 \times 10^5$  particles/cell, the use of R8-MEND led to transfection activities as high as those for the Adenovirus. These high transfection activities result from the activation of macropinocytosis by high density or octaarginines on the surface of the MEND.

## **Quantitative analysis of intracellular trafficking: virus vs. non-virus**

Quantitative information as to how many exogenous DNA are taken up, released from endosomal/lysosome, and transferred to the nucleus is essential. This situation prompted us to establish a novel strategy to simply and reliably quantify the distribution of pDNA in the cytosol, endosomes/lysosomes and the nucleus simultaneously, with sequential Z-series images captured by confocal laser scanning microscopy, called Confocal Image-assisted 3-Dimensionally Integrated Quantification (CIDIQ). Based on this analysis, a systematic and quantitative comparison were performed on the cellular uptake and subsequent intracellular distribution (e.g. endosome/lysosome, cytosol and nucleus) of exogenous DNA transfected between viral and non-viral vectors in living cells. As a model, adenovirus (Ad) and LipofectAMINE PLUS (LFN) were used for comparison since they are highly potent and widely used viral and non-viral vectors, respectively. The findings indicate that the efficiency of cellular uptake for LFN is significantly higher than that for Ad. Once taken up by a cell, Ad exhibited comparable endosomal escape and slightly more higher nuclear transfer efficiency compared with LFN. In contrast, LFN requires three orders of magnitude more intra-nuclear gene copies to exhibit a trans-gene expression comparable to the Ad, suggesting that difference of transfection efficiency principally arises from differences in nuclear transcription efficiency, and not from a difference in intracellular trafficking between Ad and LFN. These results reveal the importance of understanding intranuclear disposition of exogenous pDNA.