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Gene Delivery System: Non-Viral Mediated Chemical Approaches

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Abstract

Employing cationic lipids and polymers, inorganic nanoparticles, etc. as DNA carriers for gene delivery systems are gaining interest with the passage of time such chemical vectors form condensed complexes or aggregates with negatively charged DNA to not only protect the DNA from nucleases but also facilitate its intracellular uptake and site specific delivery.

Keywords: Cationic polymers; Chitosan; Cationic lipids; Calcium chloride; Transfection

Introduction

Chemical based non-viral methods employs chemical carriers for *in vivo* gene delivery to accomplish high-level of transgene expression [1] (Figure 1).

Chemical carriers can be characterized into numerous types:

- Carriers forming conjugate complexes with DNA to protect from endonucleases and other blood components;
- Target specific carriers for precise gene delivery;
- Customized carriers for DNA delivery to cytosol or nucleus;
- Carriers designed to dissociate from DNA in the cytosol or release DNA in the tissue to attain a continuous or precise expression [2,3].

The most widely used chemicals carries used for gene delivery is Cationic lipids/polymers and Inorganic nanoparticles.

Cationic Lipids for Gene Delivery

Cationic lipid-based gene delivery system (lipofection/liposome) was introduced by Felgner et al. in 1987. Presently used lipids possess a positively charged hydrophilic head linked by means of ether, ester, carbamate or amide linker structure with a hydrophobic tail.

Hydrophilic head

Hydrophilic head consists of positively charged groups such as quaternary ammonium salts, primary, secondary, tertiary amines, pyridinium, guanidine, phosphorus, imidazole, arsenic, etc. essential for binding with negatively charged phosphate groups in nucleic acids.

Hydrophobic tail

Composed of aliphatic chains, cholesterols and steroid rings [4].

Cationic lipids mediated gene delivery transfection efficiency depends on lipid structural configuration (geometric shape, presence of charged groups per molecules, etc.), type of lipid linker structure and charge ratio for the formation of co-lipids (DNA-lipid aggregates) [5].

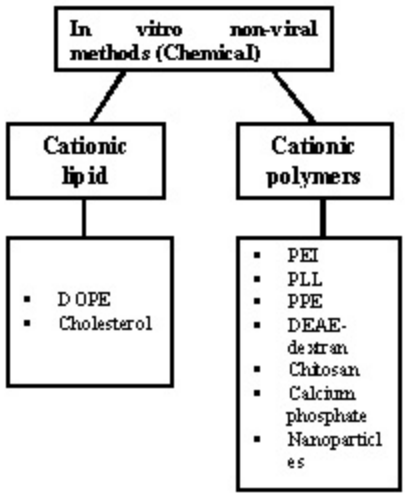

Figure 1: Non-viral chemical methods of gene delivery.

Delivery Mechanism

Positively charged lipids form compacted lipoplexes (DNA-lipid complex) when binds with negatively charged DNA. Lipoplexes structure protects the DNA from the degrading activity of endonucleases and also interacts with the negatively charged glycoproteins and proteoglycans, present in the plasma membrane of the target cell to allow its intracellular uptake [6,7]. The most frequently used colipids are Dioleoylphosphatidylethanolamine (DOPE) and Cholesterol.

DOPE (Dioleoylphosphatidylethanolamine)

It enables lipoplexes endosomal escape by destabilizing the membrane through flip-flop mechanism by disposition of anionic

lipids which forms neutral lipids by interacting with cationic lipids that successively assist in dissociation of DNA-lipid complex to release the gene into the cytoplasm as a lipoplex. Since DOPE is affected by the serum components which disrupt the lipoplex so its function is rendered effectively in the absence of serum [8].

Cholesterol

Stabilization of cationic lipid membrane structures against serum components requires cholesterol and provides improved activity for *in vivo* transfection in the presence of serum [9].

Drawbacks

- Short half-life of cationic lipids in blood due to excessive surface charge.
- Fast elimination of cationic lipids from circulation upon large aggregates formation confines their efficacy in delivering genes to cells located beyond vascular endothelial cells.
- Treatment related serious toxicity and short-lived expression of gene [10,11].

Applications

- Enables DNA delivery to interstitially located cell populations.
- Efficaciously deliver DNA and siRNA to tumor cells
- Possible therapeutic intervention for cancer gene therapy by delivering anticancer therapeutic genes.
- Widely used therapeutic approach for neuronal and brain tumor
- Treatment of cystic fibrosis, acute lung injuries and cornea tissues for degenerative ocular diseases [12-14].

Cationic Polymers for Gene Delivery

Cationic polymers form stable polyplexes (nanosize polymer-DNA complexes) with the desired DNA. Cationic polymer mediated gene delivery involves DNA complexation, complex mediated transversion of cell membrane to the cytoplasm, release of DNA, transfer of DNA into the nucleus [15,16].

Commonly used cationic polymers are as follows:

PEI (Polyethylenimine)

Introduced in 1995, either branched or linear structure.it consists of high number of nonprotonated amine groups which exerts proton sponge effect to efficiently cease endosomal pH acidification by deactivating the ATPase pumped protons. Eventually creates chloride counter ions influx within the compartment that increases the osmotic pressure resulting in swelling and rupture of the endosomal membrane [17].

Properties of PEI

- PEI with branched configuration and high molecular weight (>25,000 Da) is toxic with low transfection efficiency as compared to linear PEI with molecular weigh between 5,000-25,000 Da are less toxic.
- Non-specific interactions of PEI is minimized by conjugation to neutral polymers such as pluronic triblock polymers (P127), dextran, PEG (5,000 Da), etc. [18,19].

DEAE-dextran (Dimethylaminoethyl-dextran)

Cationic water soluble polymer which enables DNA uptake by endocytosis [20].

PLL (Poly-L-lysine)

High DNA condensation and protection from nucleases makes PLL to be widely used for delivering genes. But its low transfection and high cytotoxicity is still a problem. Furthermore, PLL-DNA complexes aggregate under physiological conditions. To increase PLL solubility hydrophilic groups such as dextran and PEG is used, e.g. PEG-g-PLL and PEG-b-PLL. Both offers low cytotoxicity and improved transfection efficiency than PLL [21].

Chitosan

Biodegradable polysaccharide (consists of D-glucosamine repeating units) with effective DNA binding and nuclease protection with good compatibility and low toxicity makes it a suitable vector for gene delivery in numerous types of target cells. However it offers low transfection efficiency [22].

PPE (Polyphosphoesters)

Its good biocompatibility, biodegradability, DNA binding capability and pendent chain functionality make them suitable carriers. Transfection effectiveness could be improved by incorporating chloroquine. PPE exhibits lower cytotoxicity than PEI or PLL *in vivo* and *in vitro* [23].

Calcium phosphate

DNA is mixed in a phosphate buffer. Later addition of aqueous calcium chloride which forms the insoluble calcium phosphate aggregates which co-precipitates with the DNA. Calcium phosphate-DNA precipitate is absorbed by the cell through phagocytosis. Transfection efficiency of about 20% is achievable with this transfection method [24,25].

Advantages

- Simple and cost-effective approach to produce stably transformed cell lines.
- Applicable for transfection of numerous cell types.

Disadvantages

- Non-specific integration into the host cell with low transfection efficiency as compared to other chemical methods for gene delivery.
- Slight alteration in buffer salt concentration, pH and temperature can reduce the efficiency rate.
- Restricted by the composition and size of the precipitate [26].

Inorganic Nanoparticles

Inorganic nanoparticles of metallic elements, inorganic salts, or ceramics produce complexes of size 10-100 nm. Nanoparticles mediated gene transfer enables effective DNA binding and precise DNA delivery [27].

Advantages

- Capable of bypassing physiological/cellular barriers.
- High transfection rate.
- Direct delivery to the nucleus via specific membrane receptor or nucleolin skipping the endosomal-lysosomal degradation.
- Proficiently transfect postmitotic cells *in vivo* and *in vitro*.
- Exhibit no or low toxicity and are inert to immune responses.
- Super paramagnetic nanoparticles provide magnetic field guided targeted delivery [28,29].

Advantages of Non-Viral Chemical Gene Delivery Methods

- No viral components.
- Low or no immunogenicity.
- No limit to DNA inserts size.
- Cell specificity possible with targeted ligands.
- Relatively simple preparation procedures.
- Standardized homogenous, stable reagents.
- Scale up possible [30].

Disadvantages of Non-Viral Chemical Gene Delivery Methods

- Low transfection efficiency.
- Transient gene expression-episomal expression.
- Intracellular barrier- may require additional agents.
- Cellular toxicity with some vectors (PEI,liposomes).
- Inflammation due to unmethylated CpG DNA sequences [31].

Conclusion

Thus the use of non-viral cationic lipids/polymers or inorganic nanoparticles as gene carriers shows effective transfection of target cells with low cytotoxicity making them applicable to a wide range of different types of cells. However further improvements still required to develop a more efficient gene delivery system.

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