MODULE 5- LECTURE 2

GENE TRANSFER TECHNIQUES: CHEMICAL METHODS

5-2.1. Introduction

Cell membrane is a sheet-like assembly of amphipathic molecules that separate cells from their environment. These physical structures allow only the controlled exchange of materials among the different parts of a cell and with its immediate surroundings. DNA is an anionic polymer, larger molecular weight, hydrophilic and sensitive to nuclease degradation in biological matrices. They cannot easily cross the physical barrier of membrane and enter the cells unless assisted.

Various charged chemical compounds can be used to facilitate DNA transfer directly to the cell. These synthetic compounds are introduced near the vicinity of recipient cells thereby disturbing the cell membranes, widening the pore size and allowing the passage of the DNA into the cell.

An ideal chemical used for DNA transfer should have the ability to-

- Protect DNA against nuclease degradation.
- Transport DNA to the target cells.
- Facilitate transport of DNA across the plasma membrane.
- Promote the import of DNA into the nucleus.

The commonly used methods of chemical transfection use the following,

1. Calcium phosphate
2. DEAE dextran
3. Cationic Lipid
4. Other polymers - poly-L-lysine (PLL), polyphosphoester, chitosan, dendrimers
5-2.1.1. Calcium phosphate mediated DNA transfer

5-2.1.1.1. Historical perspective

The ability of mammalian cells to take up exogenously supplied DNA from their culture medium was first reported by Szybalska and Szybalski (1962).

They used total uncloned genomic DNA to transfect human cells deficient for the enzyme hypoxanthine guanine phosphoribosyl transferase (HPRT). Rare HPRT-positive cells with fragments of DNA containing the functional gene were identified by selection on HAT medium. Till then, the actual mechanism of DNA uptake was not understood. It was later found that successful DNA transfer takes place by the formation of a fine DNA/calcium phosphate co-precipitate, which first settles onto the cells and is then internalized. This technique was first applied by Graham and Van Der Eb in 1973 for the analysis of the infectivity of adenoviral DNA.

5-2.1.1.2. Calcium phosphate transfection

This method is based on the precipitation of plasmid DNA and calcium ions by their interaction.

In this method, the precipitates of calcium phosphate and DNA being small and insoluble can be easily adsorbed on the surface of cell. This precipitate is engulfed by cells through endocytosis and the DNA gets integrated into the cell genome resulting in stable or permanent transfection.

Uses

- This method is mainly used in the production of recombinant viral vectors.
- It remains a choice for plasmid DNA transfer in many cell cultures and packaging cell lines. As the precipitate so formed must coat the cells, this method is suitable only for cells growing in monolayer and not for suspension cultures.
Figure 5-2.1.1.2. A schematic representation of transfection by Calcium Phosphate Precipitation.

**Advantages**

- Simple and inexpensive
- Applicability to generate stably transfected cell lines
- Highly efficient (cell type dependent) and can be applied to a wide range of cell types.
- Can be used for stable or transient transfection

**Disadvantages**

- Toxic especially to primary cells
- Slight change in pH, buffer salt concentration and temperature can compromise the efficacy
- Relatively poor transfection efficiency compared to other chemical transfection methods like lipofection.
- Limited by the composition and size of the precipitate.
• Random integration into host cell.

Optimal factors (amount of DNA in the precipitate, the length of time for precipitation reaction and exposure of cells to the precipitate) need to be determined for efficient transfection of the cells.

This technique is simple, expensive and has minimal cytotoxic effect but the low level of transgene expression provoked development of several other methods of transfection.

5-2.1.2. DEAE-Dextran (Diethylaminoethyl Dextran) mediated DNA transfer

• This method was initially reported by Vaheri and Pagano in 1965 for enhancing the viral infectivity of cell but later adapted as a method for plasmid DNA transfer.
• Diethylaminoethyl dextran (DEAE-dextran) is a soluble polycationic carbohydrate that promotes interactions between DNA and endocytotic machinery of the cell.
• In this method, the negatively charged DNA and positively charged DEAE – dextran form aggregates through electrostatic interaction and form apolypplex. A slight excess of DEAE – dextran in mixture results in net positive charge in the DEAE – dextran/ DNA complex formed. These complexes, when added to the cells, bind to the negatively charged plasma membrane and get internalized through endocytosis. Complexed DNA delivery with DEAE-dextran can be improved by osmotic shock using DMSO or glycerol.
• Several parameters such as number of cells, polymer concentration, transfected DNA concentration and duration of transfection should be optimized for a given cell line.
Advantages

- Simple and inexpensive
- More sensitive
- Can be applied to a wide range of cell types
- Can be used for transient transfection.

Disadvantages

- Toxic to cells at high concentrations
- Transfection efficiency varies with cell type
- Can only be used for transient transfection but not for stable transfection
- Typically produces less than 10% delivery in primary cells.

Another polycationic chemical, the detergent Polybrene, has been used for the transfection of Chinese hamster ovary (CHO) cells, which are not amenable to calcium phosphate transfection.

5-2.1.3. Lipofection

- Lipofection is a method of transformation first described in 1965 as a model of cellular membranes using liposomes.
- Liposomes are artificial phospholipid vesicles used for the delivery of a variety of molecules into the cells. They may be multi-lamellar or unilamellar vesicles with a size range of 0.1 to 10 micrometer or 20-25 nanometers respectively.
- They can be preloaded with DNA by two common methods- membrane-membrane fusion and endocytosis thus forming DNA- liposome complex. This complex fuses with the protoplasts to release the contents into the cell. Animal cells, plant cells, bacteria, yeast protoplasts are susceptible to lipofection method.
- Liposomes can be classified as either cationic liposome or pH-sensitive.
5-2.1.3.1. Cationic liposomes

- Cationic liposomes are positively charged liposomes which associate with the negatively charged DNA molecules by electrostatic interactions forming a stable complex.

Neutral liposomes are generally used as DNA carriers and helpers of cationic liposomes due to their non-toxic nature and high stability in serum. A positively charged lipid is often mixed with a neutral co-lipid, also called helper lipid to enhance the efficiency of gene transfer by stabilizing the liposome complex (lipoplex). Dioleoylphosphatidyl ethanolamine (DOPE) or dioleoylphosphatidyl choline (DOPC) are some commonly used neutral co-lipids.

- The negatively charged DNA molecule interacts with the positively charged groups of the DOPE or DOPC. DOPE is more efficient and useful than DOPC due to the ability of its inverted hexagonal phase to disrupt the membrane integrity.
- The overall net positive charge allows the close association of the lipoplex with the negatively charged cell membrane followed by uptake into the cell and then into nucleus.
- The lipid: DNA ratio and overall lipid concentration used in the formation of these complexes is particularly required for efficient gene transfer which varies with application.

5-2.1.3.2. Negatively charged liposomes

- Generally pH-sensitive or negatively-charged liposomes are not efficient for gene transfer. They do not form a complex with it due to repulsive electrostatic interactions between the phosphate backbone of DNA and negatively charged groups of the lipids. Some of the DNA molecules get entrapped within the aqueous interior of these liposomes.
- However, formation of lipoplex, a complex between DNA and anionic lipids can occur by using divalent cations (e.g. Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, and Ba$^{2+}$) which
can neutralize the mutual electrostatic repulsion. These anionic lipoplexes comprise anionic lipids, divalent cations, and plasmid DNA which are physiologically safe components.

- They are termed as **pH sensitive** due to destabilization at low pH.

The efficiency of both *in vivo* and *in vitro* gene delivery using cationic liposomes is higher than that of pH sensitive liposomes. But the cationic liposomes get inactivated and unstable in the presence of serum and exhibit cytotoxicity. Due to reduced toxicity and interference from serum proteins, pH-sensitive liposomes are considered as potential gene delivery vehicles than the cationic liposomes.

5-2.1.3.3. Liposome Action

![Figure 5-2.1.3](source: Pleyer U, Dannowski H. 2002. Delivery of genes via liposomes to corneal endothelial cells. Drug News Perspect, 15(5): 283)
In addition, liposomes can be directed to cells using monoclonal antibodies which recognize and bind to the specific surface antigens of cells along with the liposomes. Liposomes can be prevented from destruction by the cell’s lysosomes by pre-treating the cells with chemicals such as chloroquine, cytochalasin B, colchicine etc. Liposome mediated transfer into the nucleus is still not completely understood.

**Advantages**

- Economic
- Efficient delivery of nucleic acids to cells in a culture dish.
- Delivery of the nucleic acids with minimal toxicity.
- Protection of nucleic acids from degradation.
- Measurable changes due to transfected nucleic acids in sequential processes.
- Easy to use, requirement of minimal steps and adaptable to high-throughput systems.

**Disadvantages**

- It is not applicable to all cell types.
- It fails for the transfection of some cell lines with lipids.

**5-2.1.4. Other Methods**

Other methods of chemical transfection involve the use of chemicals such as polyethylenimine, chitosan, polyphosphoester, dendrimers.

**5-2.1.4.1. Polyethylenimine**

- Polyethylenimine (PEI) is a non-degradable, high molecular weight polymer which may accumulate in the body.
- PEI, due to its polycationic nature, condenses with the DNA molecule resulting in the formation of PEI-DNA complex which enters the cell by endocytosis, thus mediating gene transfer.
- PEI exhibit cytotoxicity due to its ability to permeabilize and disrupt cell membranes leading to necrotic cell death.
• The cytotoxicity may be reduced using various methods e.g. PEGylation and conjugation of low molecular weight polyethylenimine with cleavable cross-links such as disulfide bonds in the reducing environment of the cytoplasm.

5-2.1.4.2. Chitosan

• Chitosan, a biodegradable polysaccharide is composed of D-glucosamine repeating units and can be used as a non-viral gene carrier.
• It can efficiently bind and protect DNA from nuclease degradation.
• The biocompatibility and low toxicity profile makes it a safe biomedical material for clinical applications.
• Chitosan DNA nanoparticles can transfect several different cell types with relatively low transfection efficiency.
• Modified chitosans such as trimethylated chitosan and chitosan conjugated with deoxycholic acid have been developed to increase the solubility of chitosan at neutral pH which can efficiently transfect COS-1 cells.
• Chitosans with different molecular weights exhibit different DNA binding affinities. The efficiency of transfection is determined by the particle stability which is one of the rate-limiting steps in the overall transfection process.

5-2.1.4.3. Polyphosphoester

• Polyphosphoesters (PPE) are biocompatible and biodegradable, particularly those having a backbone analogous to nucleic acids and teichoic acids and used in several biomedical applications. They may result in extracellular persistent release of the DNA molecules thus enhancing the expression of transgene in the muscle as compared to naked DNA intake.
• Several polyphosphoesters with positive charges both in the backbone and in the side chain can be used as non-viral gene carriers.
• They can efficiently bind and protect DNA from nuclease degradation.
• They exhibit a significantly lower cytotoxicity than Poly-L-Lysine or polyethylenimine both in vitro and in vivo.
• It is a cell type dependent transfection method the efficiency of which can be enhanced using chloroquine.
• The transfection using polyphosphoestersis found to be effective in many cell lines, with some of them comparable to Liposome-mediated transfection.

5-2.1.4.4. Dendrimers

• Dendrimers are a new class of polymeric materials that are highly branched and monodisperse macromolecules. Due to their unique behaviour, they are suitable for a wide range of biomedical applications.
• They have positively charged amino groups (termini) on their surface which interact with the negatively charged phosphate groups of the DNA molecule to form a DNA-dendrimer complex.
• This DNA-dendrimer complex has an overall net positive charge and interacts with negatively charged surface molecules of the cell membrane thus allowing the entry of complex into the cell through non-specific endocytosis.
• Once inside the cell, these complexes are then transported to the endosomes where these are protected from nuclease degradation by being highly condensed within the DNA-dendrimer complex.
• The unprotonated amino groups on the dendrimers at neutral pH can become protonated in the acidic environment of the endosome leading to buffering of the endosome and thus inhibiting pH-dependent endosomal nucleases.

Figure 5-2.1.4.4. Structure of a dendrimer.
Bibliography


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