RAMA UNIVERSITY

www.ramauniversity.ac.in

FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

DNA DELIVERY METHODS

Gene delivery is the process of introducing foreign DNA into host cells.
A **transgene** is a gene or genetic material that has been transferred naturally, or by any of a number of genetic engineering techniques from one organism to another.

There are two methods of gene delivery.

1. **Physical Methods**
2. **Chemical Methods**

1. Physical Methods:

a. **Microinjection:** This technique applied to cultured cells that are recalcitrant to other transfection, but its principle use is to introduce DNA and other molecules into large cells, such as oocytes, eggs, and the cells of early embryo.
Microinjection delivers nucleic acids into the cytoplasm or the nucleus one cell at a time by means of a fine needle; therefore, this method is limited to *ex vivo* applications such as the transfer of genes into oocytes to engineer transgenic animals or the delivery of artificial chromosomes (Cappechi, 1980; Cappechi, 1989; Telenius et al., 1999). Although direct microinjection is nearly 100% efficient, it demands considerable technical skill, is extremely labor-intensive, and often causes cell death. As such, this method is not appropriate for studies that require the transfection of large number of cells.

a. **Particle bombardment:** This involves coating small metal particles with DNA and then accelerating them into target tissues using a powerful force, such as a blast of high-pressure gas or an electric discharge through a water droplet.
Particle bombardment, involves projecting microscopic heavy-metal particles (often gold or tungsten) coated with nucleic acids into recipient cells at high velocity using a ballistic device (i.e., “gene gun”). Biolistic particle delivery can be used to transiently transfect dividing and non-dividing cells in culture as well as cells in vivo, and it is often used for genetic vaccination and agriculture applications (Klein et al., 1992; Ye et al., 1990; Burkholder et al., 1993). While this technique is reliable and fast, it requires costly equipment, causes physical damage to the samples, and necessitates high cell numbers due to high mortality

DNA DELIVERY METHODS

c. **Electroporation:** Electroporation involves the generation of transient, nano-meter-sized pores in the cell membrane, by exposing cells to a brief pulse of electricity. DNA enters the cell through these pores, and is transported to the nucleus.
The main advantage of electroporation is its applicability for transient and stable transfection of all cell types. Furthermore, because electroporation is easy and rapid, it is able to transfect a large number of cells in a short time once optimum electroporation conditions are determined. The major drawback of electroporation is substantial cell death caused by high voltage pulses and only partially successful membrane repair, requiring the use of greater quantities of cells compared to chemical transfection methods.

d. **Ultrasound transfection:** Ultrasound transfection involves the exposure of cells to a rapidly oscillating probe, such as the tip of a sonicator.

2. Chemical Methods

a. **Calcium phosphate method**: The calcium phosphate method involves the formation of a co-precipitate which is taken up by endocytosis. It is thought thatsmall granules of calcium phosphate associated withDNA are taken up by endocytosis.

Diethylaminoethyl (DEAE)-dextran is a polycationic derivative of the carbohydrate polymer dextran, and it is one of the first chemical reagents used to transfer nucleic acids into cultured mammalian cells (Vaheri and Pagano, 1965).

The cationic DEAE-dextran molecule tightly associates with the negatively charged backbone of the nucleic acid, and the net positive charge of the resulting nucleic acid-DEAE-dextran complex allows it to adhere to the cell membrane and enter into the cytoplasm via endocytosis or osmotic shock induced by DMSO or glycerol.
The advantages of DEAE-dextran method are its relative simplicity, reproducibility, and low cost, while its disadvantages include cytotoxicity and low transfection efficiency for a range of cell types (typically less than 10% in primary cells), as well as the requirement for reduced serum media during the transfection procedure. In addition, this method is limited to transient transfections, and is not suitable for generating stable cell lines.

DNA DELIVERY METHODS

Liposomal Transfection:

An alternative chemical transfection procedure is to package DNA inside a fusogenic phosopholipid vesicle, which interacts with the target cell membrane and facilitates DNA uptake. Lysozyme was used to remove the cell walls, and the resulting protoplasts were gently centrifuged onto a monolayer of mammalian cells and induced to fuse with them using polyethylene glycol.
More widespread use has been made of artificial phospholipid vesicles, which are called liposomes.

Biological Methods:

Biological methods that rely on genetically engineered viruses to transfer non-viral genes into cells (also known as transduction) and include: Viral delivery