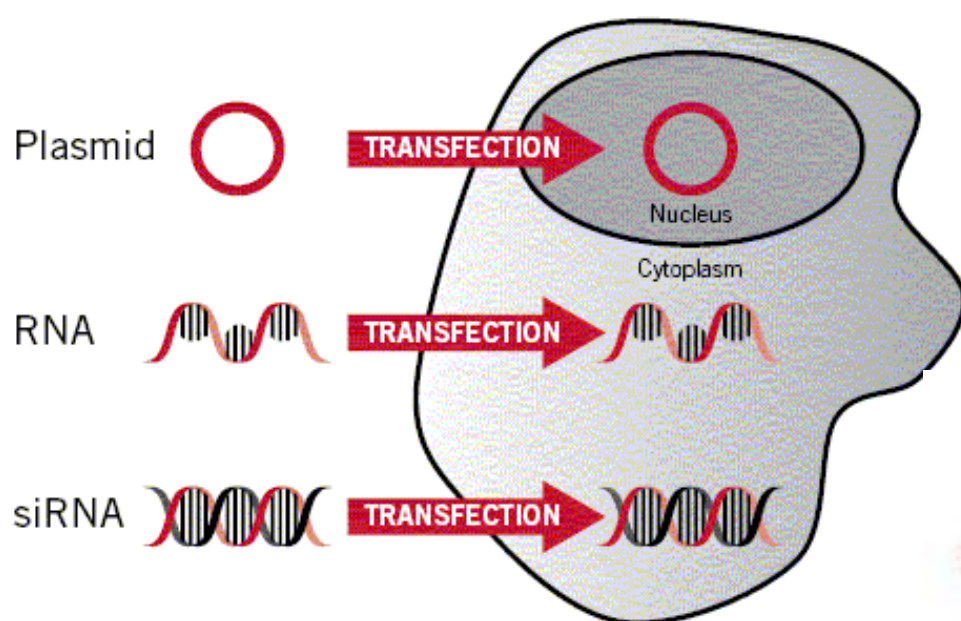


Transfection of Eukaryotic cells



Prof. Artatrana Pal
Department of Zoology
School of Life Sciences
Mahatma Gandhi Central
University, Bihar

Lipid-Mediated Transfection in Mammalian Cells

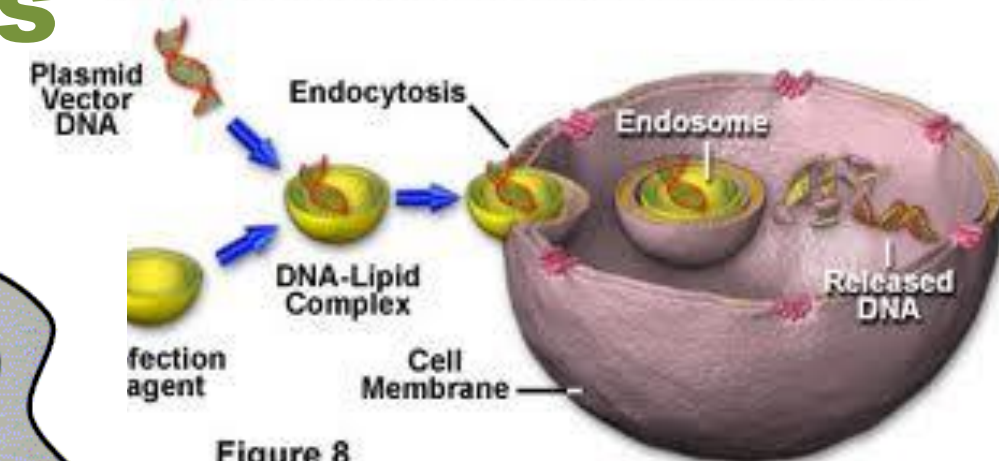
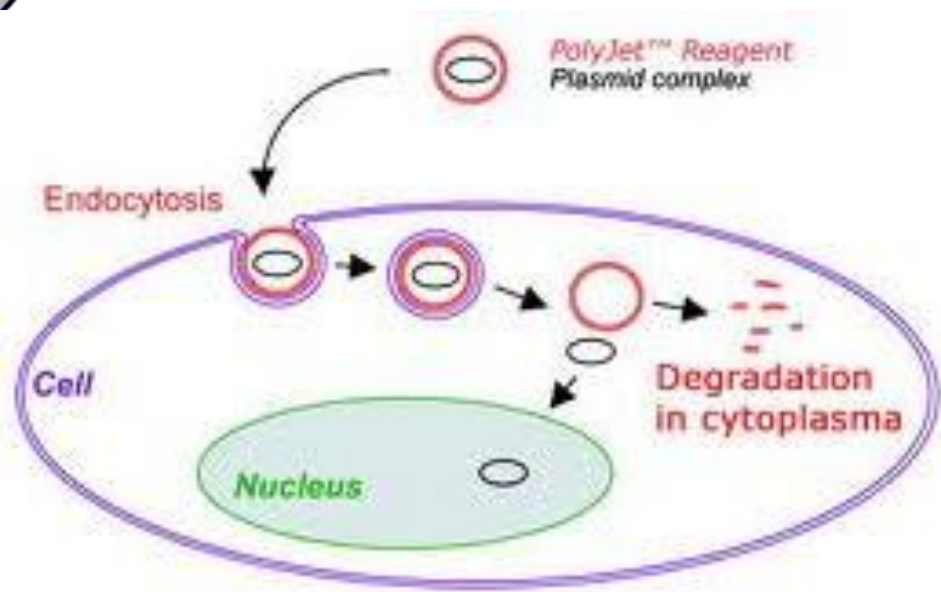


Figure 8



Overview

- ☞ **What is Transfection?**
- ☞ **Transfection vs. Transformation**
- ☞ **Purpose of Transfection**
- ☞ **How it Works**
- ☞ **Experimental method/process**
- ☞ **Strengths and weaknesses of technique**

Transfection

☀ What is Transfection?

Transfection is a method of transporting DNA, RNA and/or various macromolecules into an eukaryotic cell by using chemical, lipid or physical based methods

☀ Methods: (few examples)

<u>Method</u>	<u>Application</u>
CaPO ₄ , DEAE	DNA Transfection
Liposome Based	DNA Transfection
Polyamine Based	DNA Transfection

Transfection methods

- **Calcium phosphate (CaPO₄) precipitation**
- **DEAE-dextran (dimethylaminoethyl-dextran)** Major variants: number of cells, concentration of DNA and concentration of DEAE-Dextran. Only method that cannot be used for stable transfections
- **Lipid mediated lipofection**
- **Electroporation**
- **Retroviral Infection**
- **Microinjection**

Method

CaPO₄, DEAE

Liposome Based

Polyamine Based

Application

DNA Transfection

DNA Transfectio

DNA Transfection

Transfection vs. Transformation

- ❖ **Transformation:** genetically altering cells by transporting in foreign genetic material
- ❖ **Transfection:** the process of transporting genetic material and/or macromolecules into eukaryotic cells through typically non-viral methods

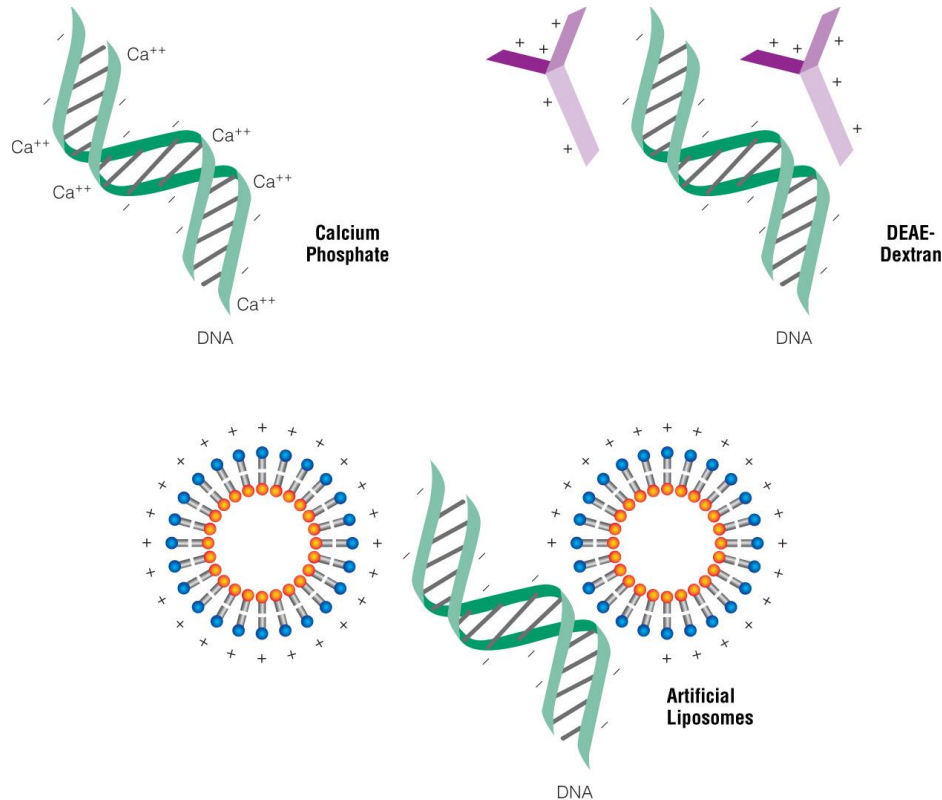
Purpose/uses of Transfection

- **Study gene function**
- **Study protein expression**
- **Transfer DNA into embryonic stem cells**

How it works ???

Utilize Chemical, lipid or physical methods (direct microinjection, electroporation, biolistic particle delivery) for transportation of genetic materials or macromolecules

How it works – Chemical and lipid methods



➔ **Neutralize negative charges on DNA**

➔ **Chemical method: CaPO_4 ; creates precipitates that settle on cells and are taken in**

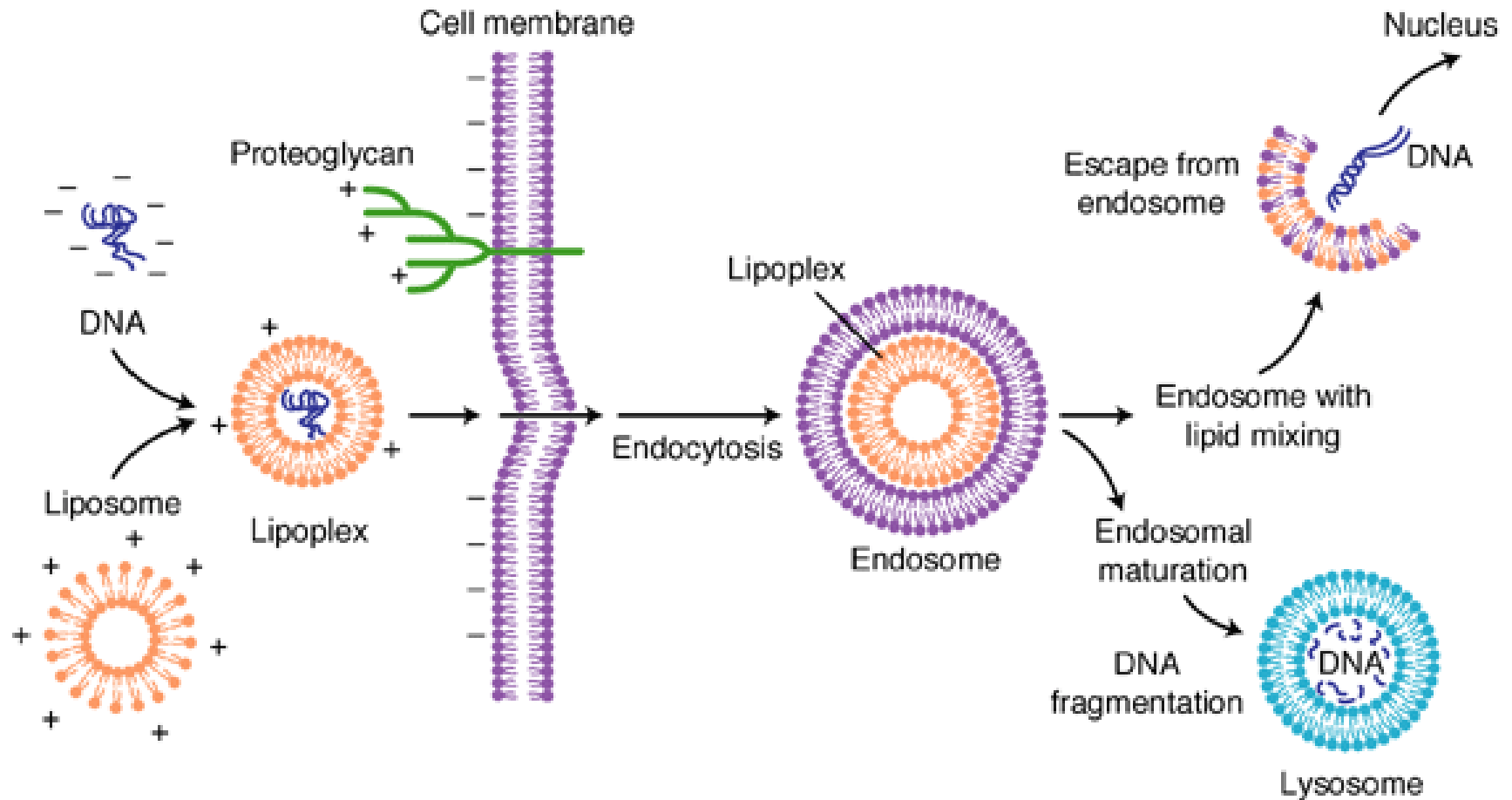
➔ **Lipid or Polymer methods:**

interact with DNA, promotes binding to cells and uptake via endocytosis

Transfection method : liposome-mediated

- ⊗ **Use of cationic lipids**
 - chemical and physical similarities to biological lipids
 - spontaneous formation of complexes with DNA, called lipoplexes
- ⊗ High efficiency for *in vitro* transfections
- ⊗ Can carry larger DNA than viruses
- ⊗ Safer than viruses
- ⊗ Low *in vivo* efficiency

Lipid or Polymer methods



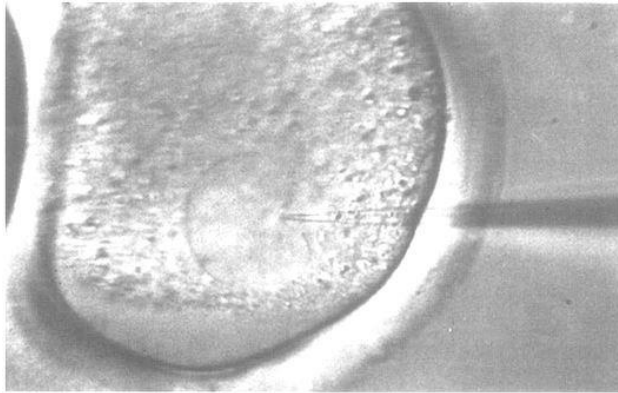
Lipoplex-mediated transfection and endocytosis

Expert Reviews in Molecular Medicine © 2003 Cambridge University Press

How it works – Physical methods



- **Electroporation:** use of high voltage to deliver nucleic acids; pores are formed on cell membrane



- **Direct Microinjection:** Use of a fine needle and used for transfer of DNA into embryonic stem cells



- **Biolistic Particle delivery:** Uses high-velocity for delivery of nucleic acids and penetration of cell wall

Transfection method : electroporation

- Use of high-voltage **electric shocks** to introduce DNA into cells
- Cell membranes: **electrical capacitors** unable to pass current
- Voltage results in temporary breakdown and **formation of pores**

Harvest cells and resuspend in electroporation buffer



Add DNA to cell suspension...for stable transfection
DNA should be linearized, for transient the DNA may be supercoiled



electroporate



Selection process for
transfectant

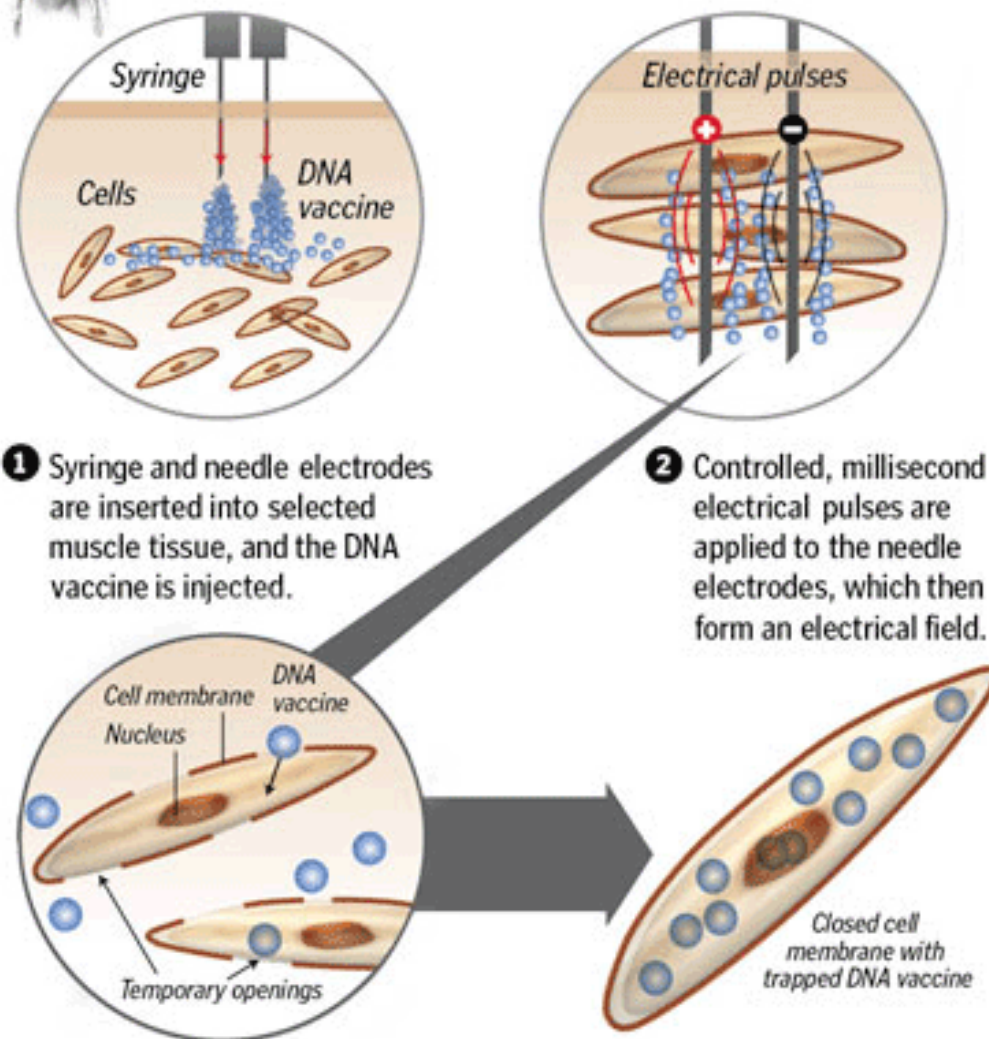
Electroporation

- ❖ **Variants: amplitude and length of electric pulse**
- ❖ **Less affected by DNA concentration- linear correlation between amount of DNA present and amount taken up**
- ❖ **Can be used for stable transfections**

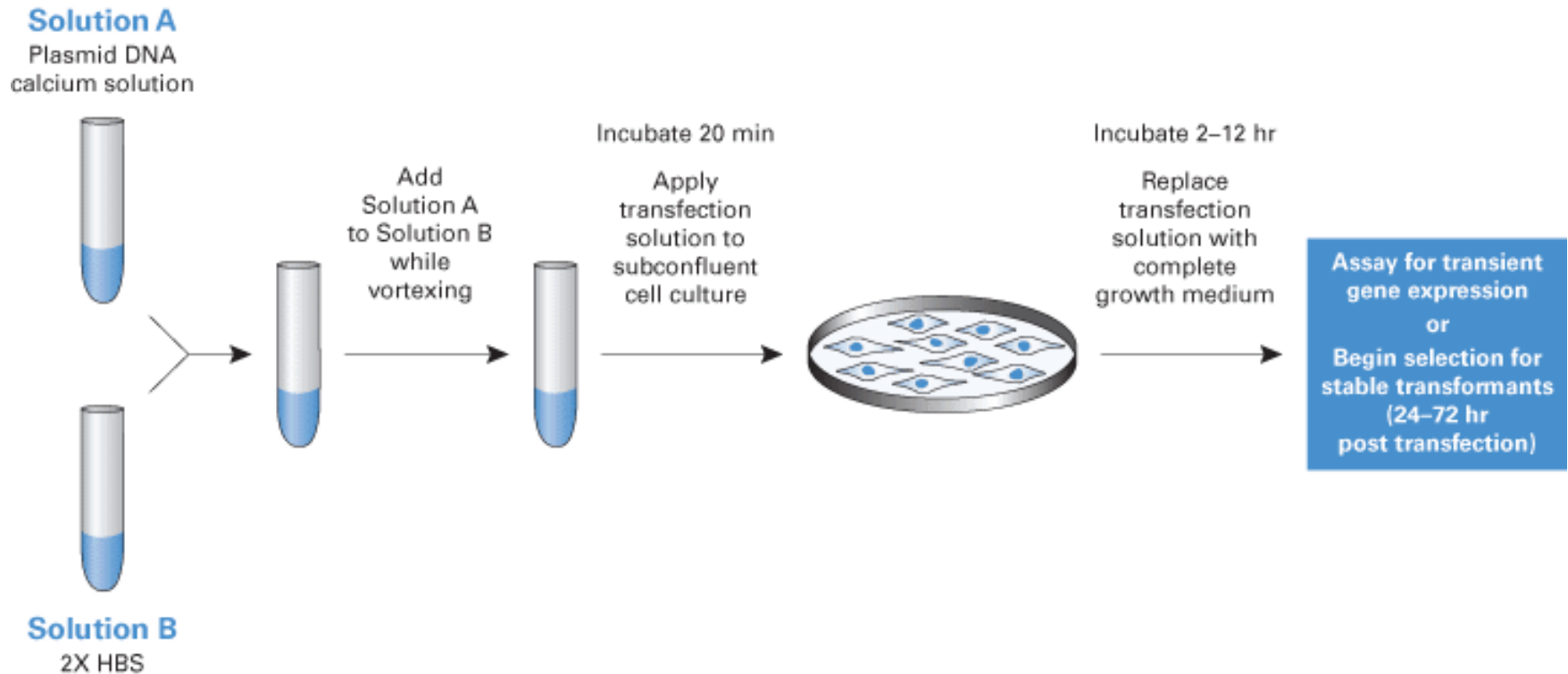
[Unlike transient transfection, in which introduced DNA persists in cells for several days, stable transfection introduces DNA into cells long-term. Stably transfected cells pass the introduced DNA to their progeny, typically because the transfected DNA has been incorporated into the genome, but sometimes via stable inheritance of nongenomic DNA]



Detail **How electroporation delivers DNA vaccines**



Experimental method/process (chemical methods)



HBS = HEPES-Buffered Saline

Advantages/Disadvantages

Advantages

- **Provides the ability to transfer in negatively charged molecules into cells with a negatively charged membrane**
- **Liposome-mediated transport of DNA has high efficiency. Good for long-term studies requiring incorporation of genetic material into the chromosome**

Disadvantages

- **Chemical Reagents: not useful for long-term studies**
- **Transfection efficiency is dependent on cell health, DNA quality, DNA quantity, confluency (40-80%)**
- **Direct Microinjection and Biolistic Particle delivery is an expensive and at times a difficult method**

Another interesting use

- “...dermal patches consisting of gene-transfected cultured skin, which secrete endogenous antimicrobial peptides such as B-defensins instead of exogenous antibiotics, can be a new DDS for the treatment of severe burns without decrease in cell viability.”

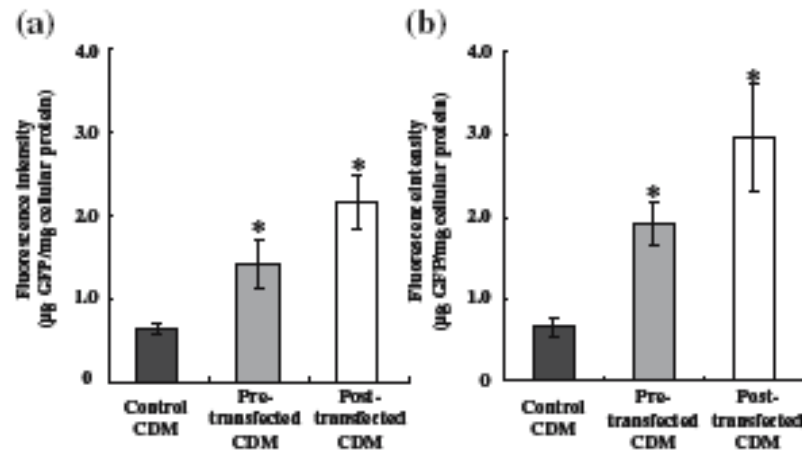


Fig. 6. Measurement of GFP expression in the control, pre-transfected, and post-transfected rat (a) and human (b) CDMs by fluorescence spectrophotometry. Data are shown as the mean \pm SE ($n=4-6$). * $p < 0.05$ vs control CDM.

Nobuko Hada, Hiroaki Todo, Fusao Komada, & Kenji Sugubayashi. (2007). Preparation and Evaluation of Gene-Transfected Cultured skin as a Novel Drug Delivery System for Severely Burned Skin. *Pharmaceutical Research*, Vol. 24, No. 8, p.1473-1479.