Genetics

Biology Dep.3rd Stage

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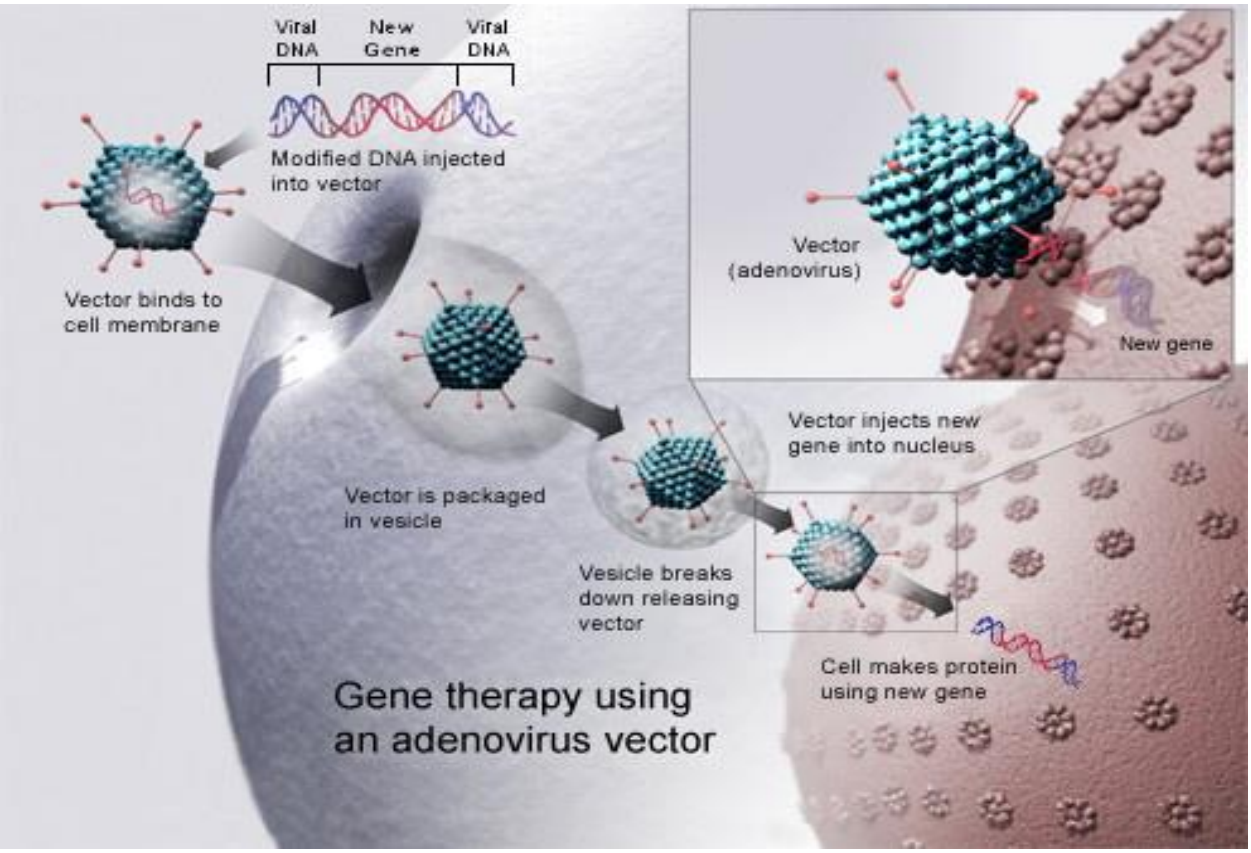
Lec-6

Gene Therapy - Correcting defective genes

- **Gene therapy** is the therapeutic delivery of nucleic acid polymers into a patient's cells as a drug to treat disease



Methods for treatment of human diseases

Current methods:

1 Medicine tablets by oral

2 Medicine by injection.

3.Medicine by nasal spray

4 Medicine by applying to skin.

Methods in the future:

5 Cell therapy

6 Gene therapy

The first attempt at modifying human DNA was performed in 1980 by Martin Cline, but the first successful nuclear gene transfer in humans, approved by the National Institutes of Health, was performed in May 1989 The first therapeutic use of gene transfer as well as the first direct insertion of human DNA into the nuclear genome was performed by French Anderson in a trial starting in September 1990

Definition & history

- Normal gene inserted into the genome to replace non-functional gene
- Trials began in 1990
- Cystic fibrosis gene moderately successful
- **Gene therapy may be classified into two types:**
- **1-Somatic[**
- In somatic cell gene therapy (SCGT), the therapeutic genes are transferred into any cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell. Any such modifications affect the individual patient only, and are not inherited by offspring. Somatic gene therapy represents mainstream basic and clinical research, in which therapeutic DNA (either integrated in the genome or as an external episome or plasmid) is used to treat disease.
- Over 600 clinical trials utilizing SCGT are underway in the US. Most focus on severe genetic disorders, including immunodeficiencies, haemophilia, thalassaemia and cystic fibrosis. Such single gene disorders are good candidates for somatic cell therapy. The complete correction of a genetic

disorder or the replacement of multiple genes is not yet possible. Only a few of the trials are in the advanced stages.

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- **2-Germline[**
- In germline gene therapy (GGT), germ cells (sperm or eggs) are modified by the introduction of functional genes into their genomes. Modifying a germ cell causes all the organism's cells to contain the modified gene.

Gene therapy could be very different for different diseases

- **Gene transplantation** (to patient with gene deletion)
- **Gene correction** (To revert specific mutation in the gene of interest)
- **Gene augmentation** (to enhance expression of gene of interest)

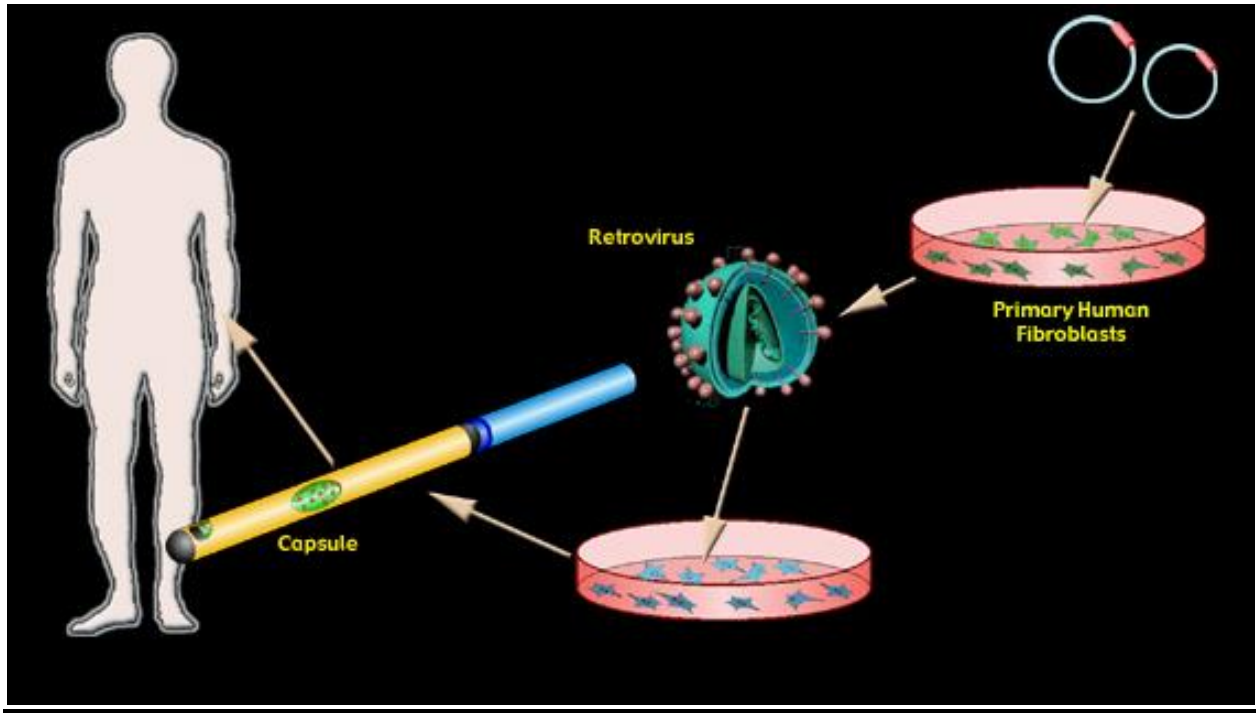
Diseases for applying gene therapy

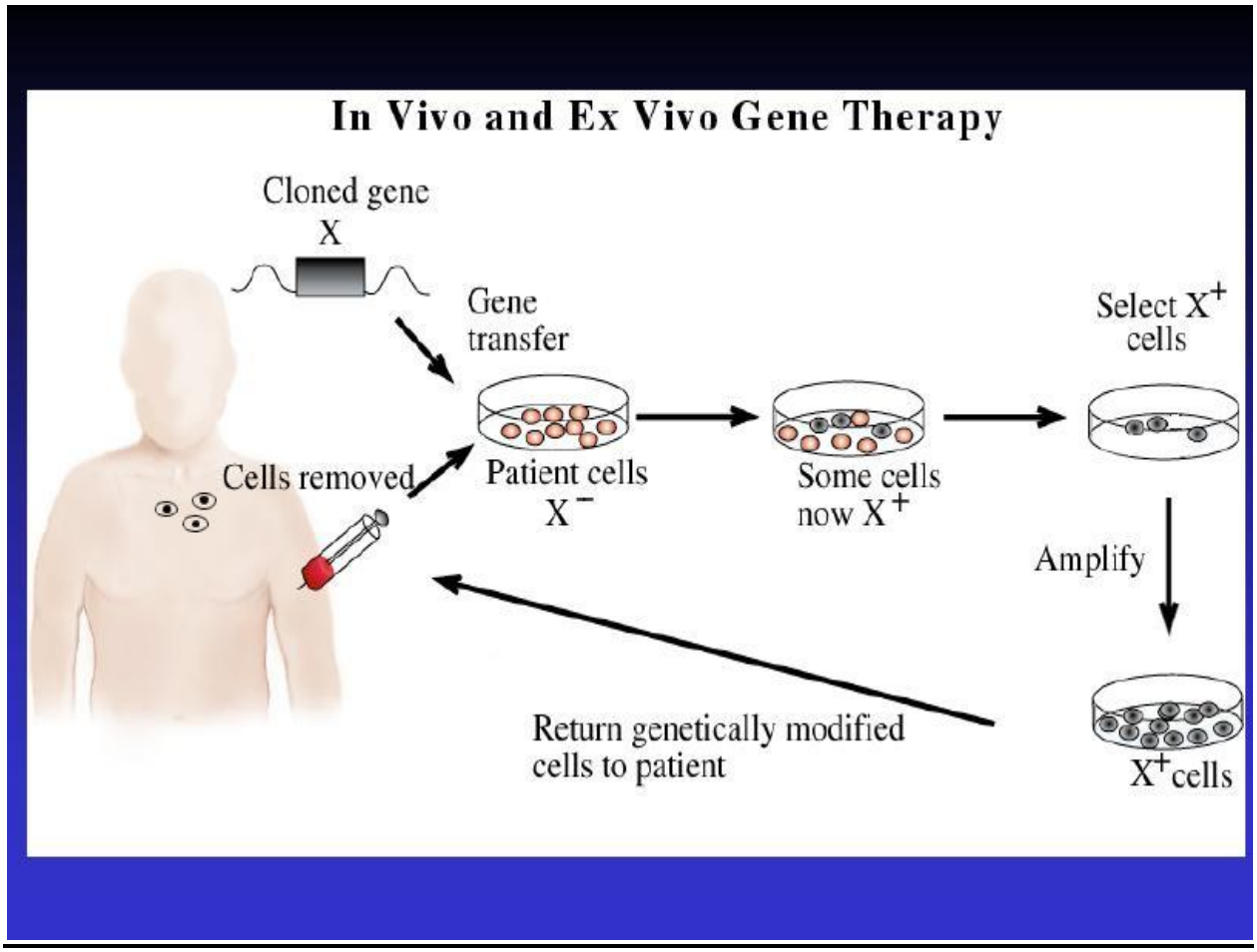
T-lymphocytes		**immunodeficiency**
Hemophilia	**Factor VIII, Factor IX deficiency**	**Liver, muscle,**
Airspaces in the lung	**Loss of CFTR gene**	**Cystic fibrosis**
Bone-marrow cells	**α or β globulin gene**	**Hemoglobulinpathies**
Lung or liver cells	**α1-antitrypsin**	**α1-antitrypsin deficiency**
Many cell types	**Many causes**	**Cancer**
Direct injection into the brain	**Parkinson's, Alzheimers**	**Neurological diseases**
Vascular endothelium	**Restenosis, arteriosclerosis**	**Cardiovascular**
T cells, macrophages,	**AIDS, hepatitis B**	**Infectious diseases**

Hepatocyte growth factor **Fibrogenesis** **Liver cirrhosis**

MHC, β2-microglobulin **Lupus, diabetes** **Autoimmune disease**

In vivo gene therapy





In vivo gene therapy

1. The genetic material is transferred directly into the body of the patient

2. More or less random process;

small ability to control; less manipulations

3. Only available option for tissues that can not be grown in vitro; or if grown cells can not be transferred back

Ex vivo gene therapy

1. The genetic material is first transferred into the cells grown in vitro

2. Controlled process; transfected cells are selected and expanded; more manipulations

3. Cells are usually autologous; they are then returned back to the patient

Methods of gene delivery (therapeutic constructs)

1- Injection of naked DNA into tumor by simple needle and syringe

DNA coated on the surface of gold pellets which are air-propelled into the epidermis (gene-gun), mainly non applicable to cancer

2 DNA transfer by liposomes (delivered by the intravascular, intratracheal, intraperitoneal or intracolonic routes)

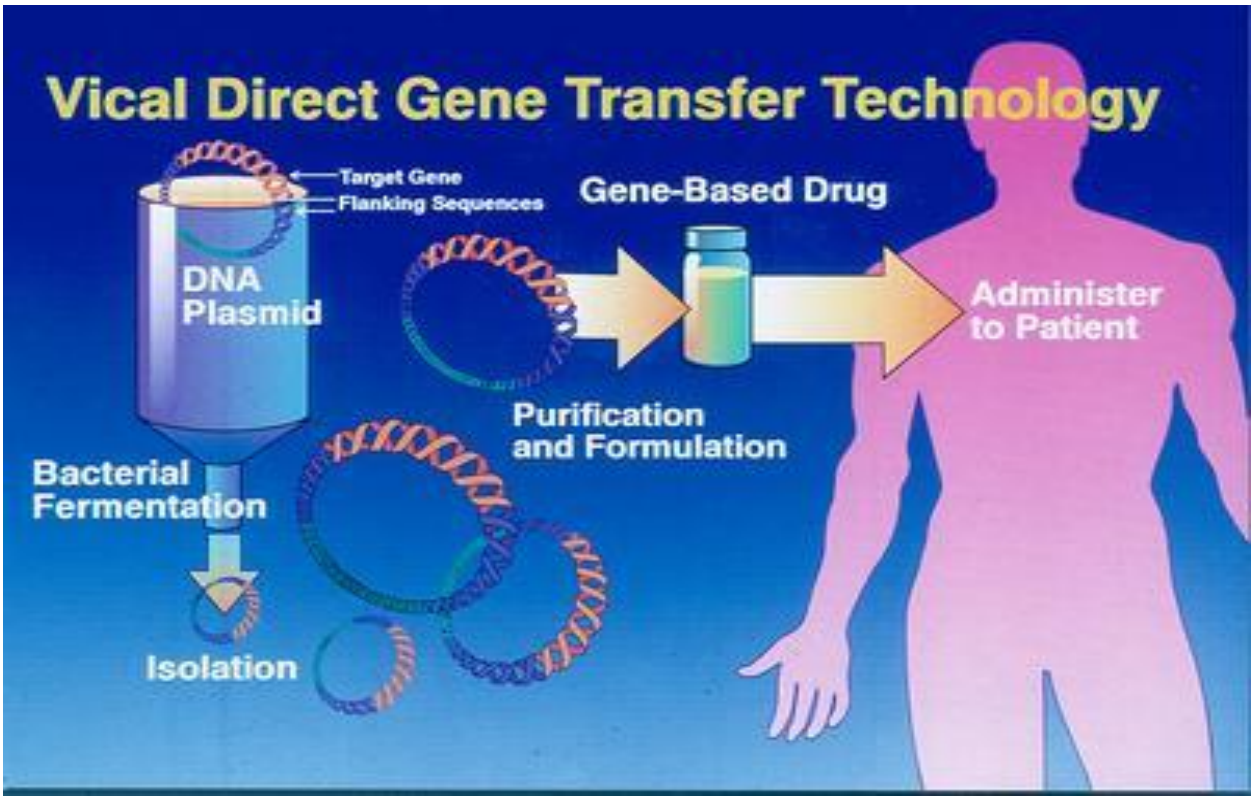
3 Biological vehicles (vectors) such as viruses and bacteria.

Viruses are genetically engineered

so as not to replicate once inside the host.

They are currently the most efficient means of gene transfer.

Injections of naked DNA



Naked DNA gene therapy

Intramuscular delivery

Intravascular delivery → liver and muscle

Covalently closed circular form is more stable that open plasmid

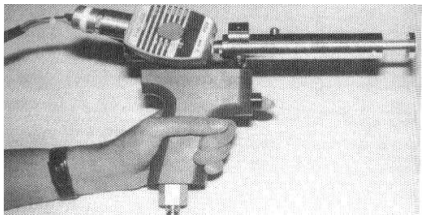
-- Results in a prolonged low level expression in vivo

-- Very cheap

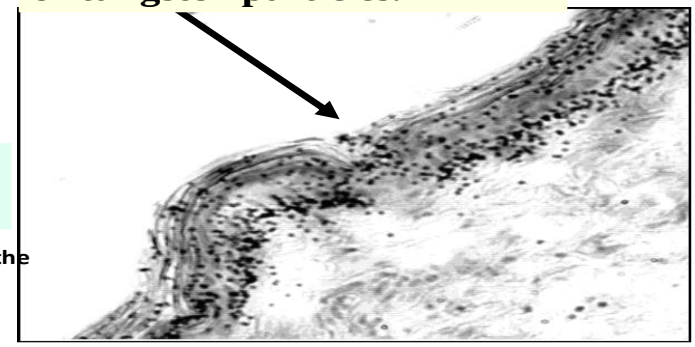
Ballistic DNA Injection,
particle bombardment,
microprojectile gene transfer (gene guns)

Invented for DNA transfer to plant cells

Fully applicable to mamalian cells



plasmid DNA is precipitated onto 1-3 micron sized gold or tungsten particles.

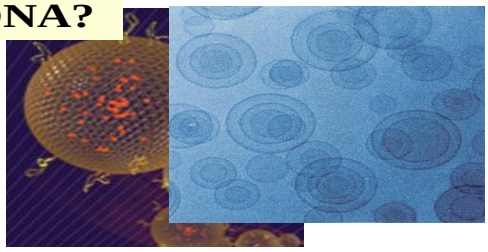


Discharge: helium pressure, or high-voltage electronic

Light micrograph of DNA-coated gold beads in the skin after gene-gun vaccination

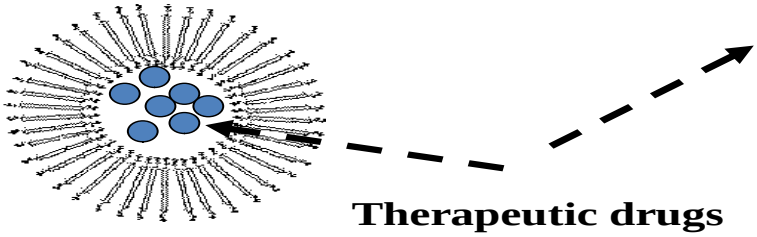
Liposomes

Next level idea – why naked DNA?



Lets' wrap it in something safe to increase transfection rate

Lipids – is an obvious idea !

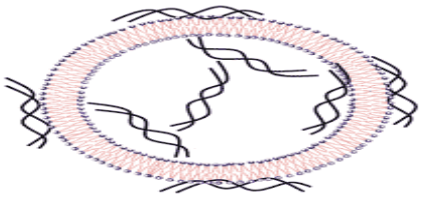


delivery of genes by liposomes

Cheaper than viruses

No immune response

Especially good
for in-lung delivery (cystic fibrosis)



100-1000 times more plasmid DNA needed
for the same transfer efficiency as for viral vector

Vectors

- **Viruses eg retro viruses, adenoviruses (commonly used)**
- **Direct introduction ("golden bullets")**
- **Liposomes**
- **Endocytosis of DNA bound to cell surface receptors (low efficiency)**
- **Artificial chromosome (under development))**

Applications

- **Curing genetic diseases**
- **Correcting cancer genes**
- **Inducing cancerous cells to make toxins so they kill themselves**
- **Blocking viral genes (e.g. HIV)**
- **Creating stem cells from somatic cells**

Ethical problems

- **Gene therapy for serious genetic diseases OK but for other health problems?**
- **Somatic cell treatment stays with the individual, germ cell treatment passes down the germ line (becomes immortal)-Very costly. Who pays? Who is eligible?**