Virology (CC - 5)

Applications of Virology (Unit - 4)

Gene Therapy

- In gene therapy, human cells are engineered in order to treat a disease
- Gene therapy was used originally to describe methods that aim to cure an inherited disease by providing the patient with a correct copy of the defective gene
- But now the term has been extended to include attempts to cure any disease by introduction of a cloned gene into the patient
- There are two basic approaches to gene therapy: Germline therapy & Somatic therapy
- In **Germline therapy**, a fertilized egg is provided with a copy of the correct version of the relevant gene & reimplanted into the mother

- If successful, the gene is present & expressed in all cells of the resulting individual
- It is usually carried out by microinjection of a somatic cell followed by nuclear transfer into an oocyte & theoretically could be used to treat any inherited disease
- There are some **ethical issues** with germline therapy
- The techniques used for germline correction of inherited diseases are exactly the same techniques that could be used for germline manipulation of other inherited characteristics
- The development of this technique with animals has been prompted to improve farm animals by making genetic changes to lower fat content
- This type of manipulation, where the genetic constitution of an organism is changed in a directed, heritable fashion is clearly unacceptable with humans

- **Somatic cell therapy** involves manipulation of cells which either can be removed from the organism, transfected, and then placed back in the body or transfected *in situ* without removal
- This technique is widely used to treat inherited blood diseases (eg. Haemophilia & thalassaemia), with genes being introduced into stem cells from the bone marrow, which give rise to all the specialized cell types in the blood
- The strategy is to prepare a bone extract containing several billion cells, transfect these with a retrovirus-based vector, and then reimplant the cells

- Subsequent replication & differentiation of transfectants leads to the added gene being present in all the mature blood cells
- Somatic cell therapy also has the potential in the treatment of lung diseases such as cystic fibrosis, as DNA cloned in Adenovirus vectors or contained in liposomes is taken up by the epithelial cells in the lungs after introduction into the respiratory tract via an inhaler
- However, gene expressions occurs for only a few weeks & as yet, this has not been developed into an effective means of treating cystic fibrosis

TYPES OF GENE THERAPY

Somatic CELL gene therapy	Germ line gene therapy
– Therapeutic genes transferred into the **somatic cells.** – Eg. Introduction of genes into bone marrow cells, blood cells, skin cells etc. – **Will not be inherited** later generations. – At present all researches directed to correct genetic defects in somatic cells.	– Therapeutic genes transferred into the **germ cells.** – Eg. Genes introduced into eggs and sperms. – **It is heritable** and passed on to later generations. – For safety, ethical and technical reasons, it is not being attempted at present.

Gene Therapy successes

The first approved gene therapy experiment occurred on September 1990 in US to treat ADA-SCID

Some successfully treated diseases are -

- Melanoma (skin cancer)
- Severe Combined Immunodeficiencies
- Hereditary Blindness
- Sickle Cell Anemia
- Parkinson’s disease
- Alzheimer’s disease
- Cystic fibrosis

Steps Involved in Gene Therapy

To design and carry out a gene therapy treatment, a researcher must:

1. Identify the gene(s) responsible for the disorder.
2. Make copies of the normal gene.
3. Insert the copies into vectors.
4. “Infect” the affected cells with the vectors.
5. Activate the gene so that transcription and translation take place.

IN VIVO GENE THERAPY

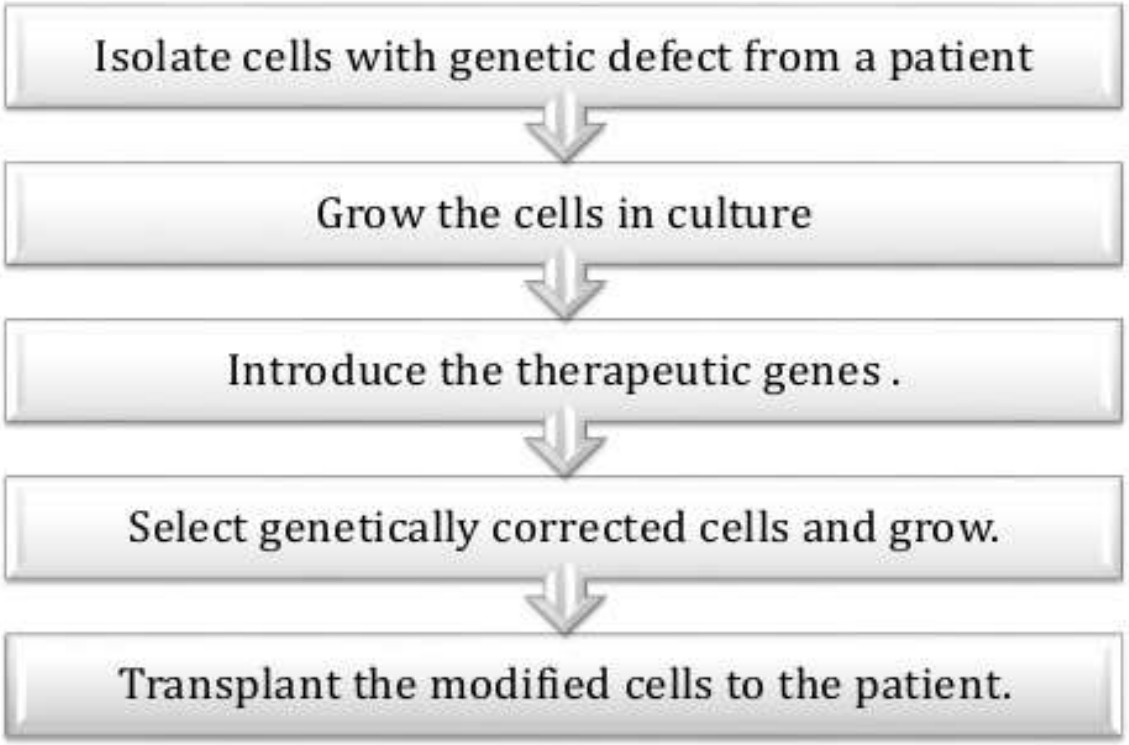
- **Direct delivery** of therapeutic gene into target cell into patients body.

- Carried out by viral or non viral vector systems.

- It can be the only possible option in patients where individual cells cannot be cultured in vitro in sufficient numbers (e.g. brain cells).

- In vivo gene transfer is necessary when cultured cells cannot be re-implanted in patients effectively.

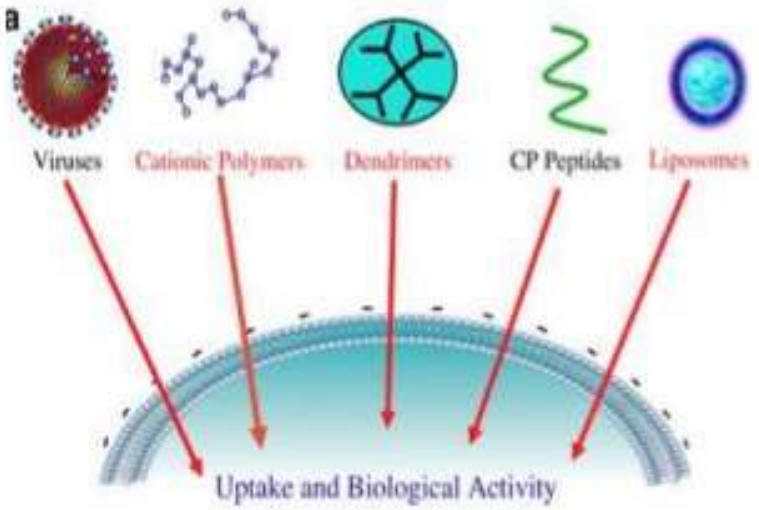
EX VIVO GENE THERAPY



VECTORS IN GENE THERAPY

- To transfer the desired gene into a target cell, a carrier is required. Such vehicles of gene delivery are known as **vectors**.

- 2 main classes
  - **Viral** vectors
  - **Non viral** vectors



METHODS OF GENE DELIVERY
PHYSICAL METHOD

Gene Gun

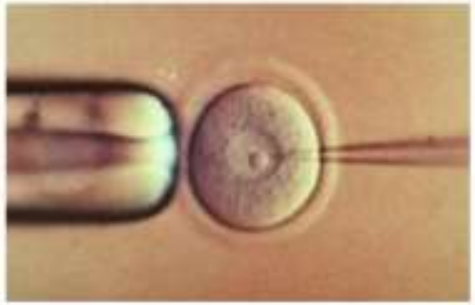
- Employs a high-pressure delivery system to shoot tissue with gold or tungsten particles that are coated with DNA

Gene Gun

Microinjection

- Process of using a glass **micropipette** to insert microscopic substances into a single living cell.

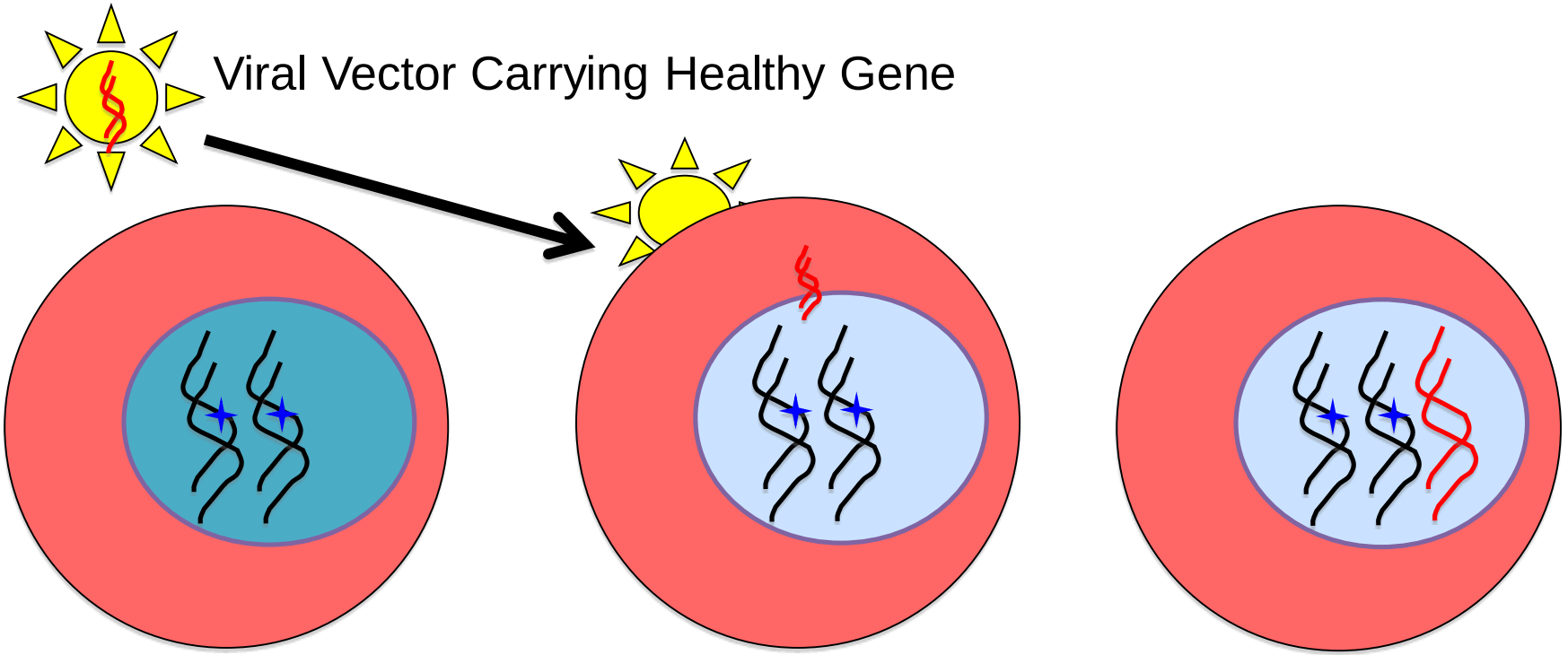
- Normally performed under a specialized optical microscope setup called a **micromanipulator**.



CHEMICAL METHODS

- **USING DETERGENT MIXTURES**
  - Certain charged chemical compounds like Calcium phosphates are mixed with functional cDNA of desired function.
  - The mixture is introduced near the vicinity of recipient cells.
  - The chemicals disturbs the cell membrane, widens the pore size and allows cDNA to pass through the cell.
- **LIPOFECTION**
  - It is a technique used to inject genetic materials into a cell by means of liposomes.
  - Liposomes are **artificial phospholipid vesicles** used to deliver a variety of molecules including **DNA** into the cells.

Use of Viral Vector



Cell with mutated gene(s)

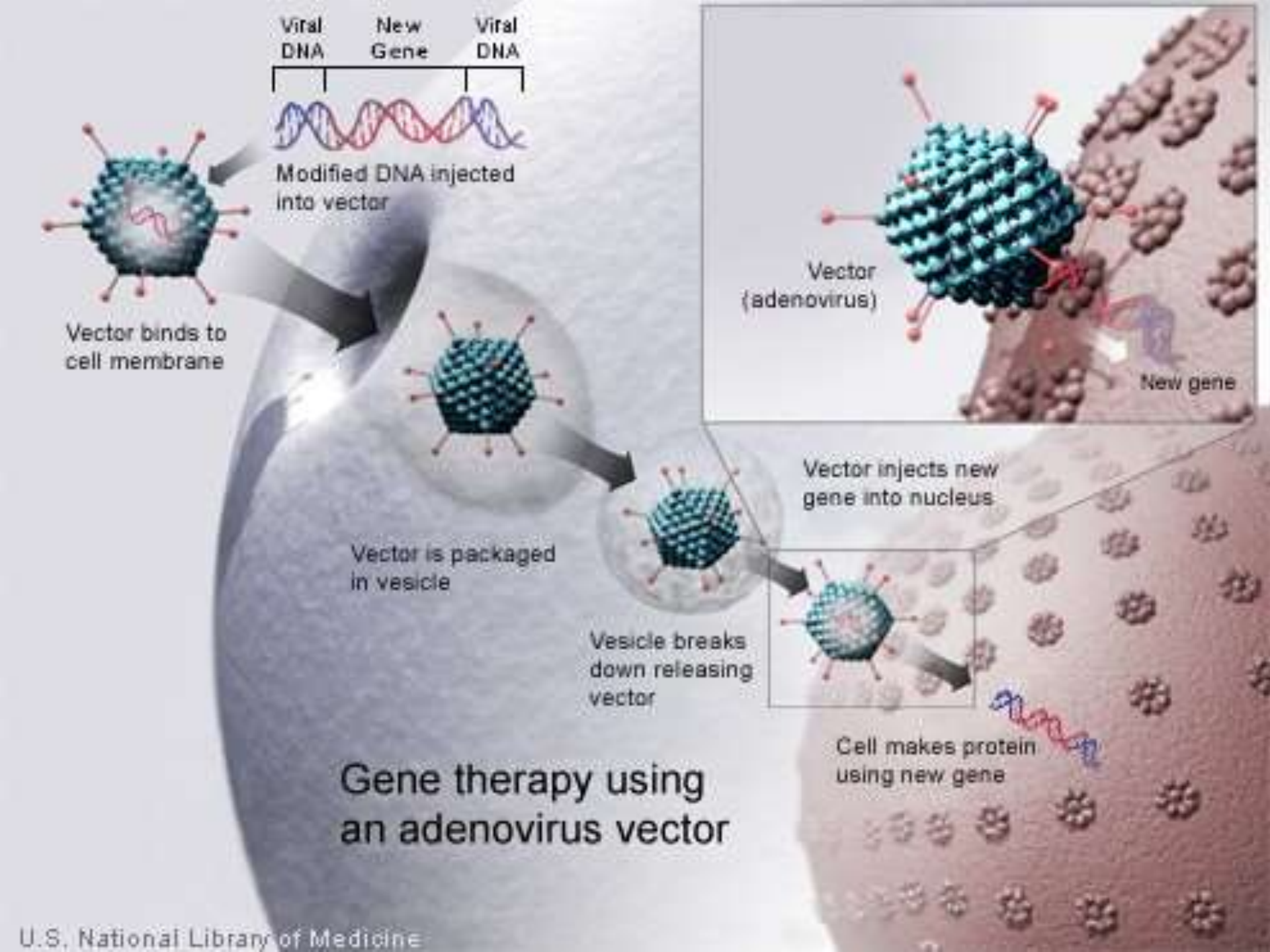
Vector inserts healthy gene into cell

New gene in the cell along with original genes

Functional proteins are created from the therapeutic gene causing the cell to return to a **normal** state.

Viruses as Vectors

- Replicate by inserting their DNA into a host cell
- Gene therapy can use this to insert genes that encode for a desired protein to create the desired trait
- Four different types
  - Adenovirus
  - Adeno-Associated Virus (AAV)
  - Retrovirus
  - Herpes Simplex Virus (HSV)



Vector Advantages and Disadvantages

- Adenovirus
  - \+ Infects many cell types
  - − Does not integrate into host genome and can be lost.
- Retrovirus
  - \+ High transfection frequency
  - \+ Integrates into host genome and cannot be lost
  - − Integrates into host genome and can cause cancer
- Adeno-Associated Virus (AAV)
  - \+ Integrates into host genome and cannot be lost
  - − Difficult to work with.
- Herpes Simplex Virus (HSV)
  - \+ DNA stays in nucleus without integrating into host genome.
  - − Only infects cells of the nervous system.

ADVANTAGES

- Gene therapy has the potential to eliminate and prevent hereditary diseases such as cystic fibrosis, ADA- SCID etc.
- It is a possible cure for heart disease, AIDS and cancer.
- It gives someone born with a genetic disease a chance to life.
- It can be used to eradicate diseases from the future generations.

Current Research

- The most intensive area of current research regarding the application of gene therapy is to use it in cancer treatment
- Most cancers result from activation of an oncogene that leads to a tumour formation, or inactivation of a gene that normally suppresses formation of a tumour
- Possible means of cancer treatment using gene therapy include introduction of a gene –

1. Encoding an antisense RNA copy of an oncogene to reduce or prevent its expression & reverse its tumourigenic activity
2. Capable of selective killing of cancer cells
3. To improve natural killing of cancer cells by the patient's immune system

CONCLUSION

- Theoretically, gene therapy is the permanent solution for genetic diseases.
- But it has several complexities. At its current stage, it is not accessible to most people due to its huge cost.
- A breakthrough may come anytime and a day may come when almost every disease will have a gene therapy
- Gene therapy have the potential to revolutionize the practice of medicine.