

# **Treatment of Genetic Diseases**

- Stem cell therapy**

- Gene therapy**

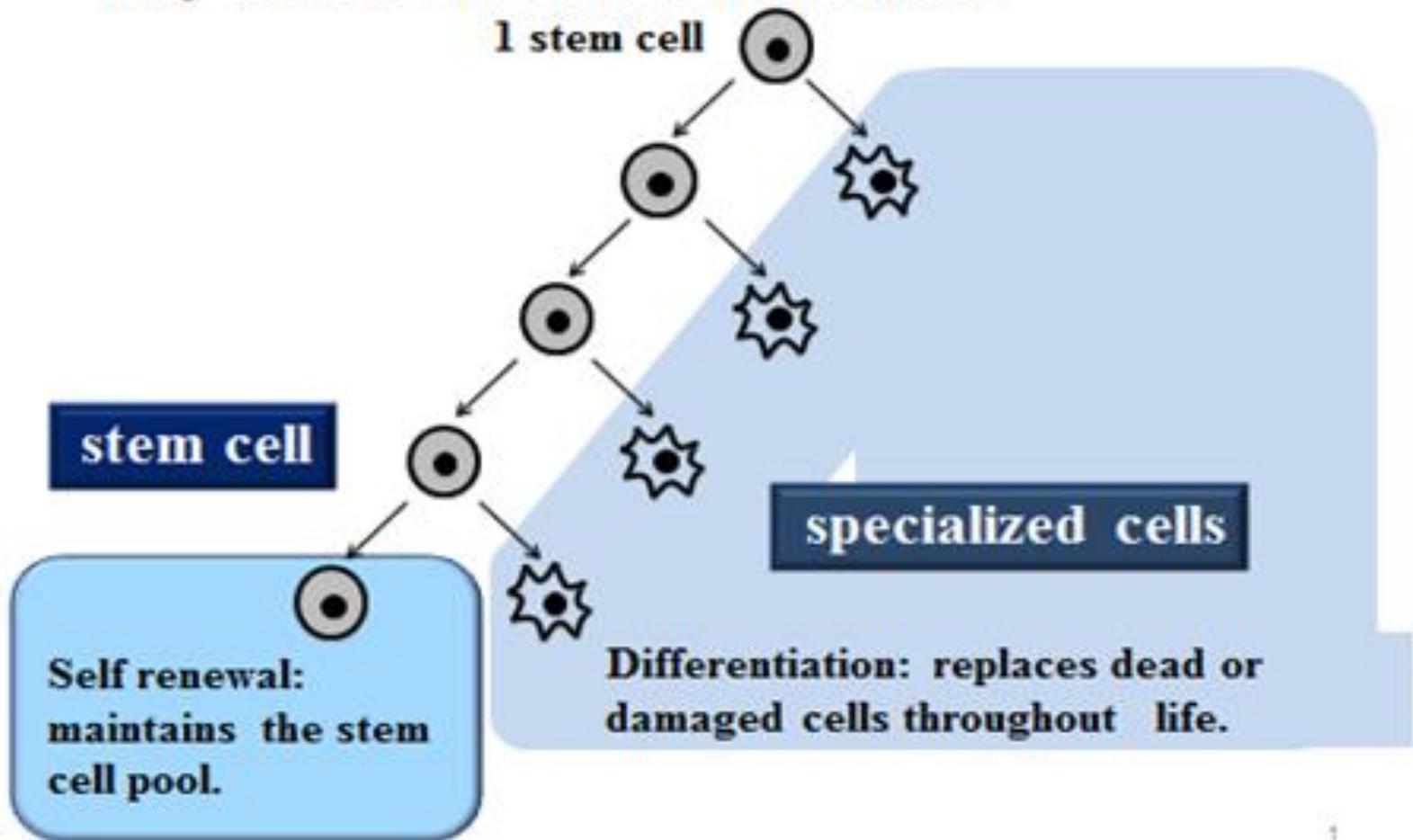
# I. Stem Cells

- They are **primal cells** which are the source, or “**stem,**” for all of the specialized cells that form organs and tissues.
- They retain the ability to **produce** through mitosis both:
  - a **self-renewing stem cell** and
  - a second cell with the **capacity to differentiate** into more specialized cells.

## Stem cell properties:

- 1- **Self-renewal** is the ability to go through numerous cycles of **cell division** while maintaining the **undifferentiated state**.
- 2- **Potency** is the capacity to **differentiate** into different cell types.

## Why self-renew and differentiate?



## Stem cell differentiation

- Development begins when a sperm fertilizes an egg and creates a single cell that forms an entire organism.
- In the first hours after fertilization, this cell divides into identical cells (morula).
- In humans, approximately four days after fertilization and after several cycles of cell division, these cells begin to specialize, forming a hollow sphere of cells, called a blastocyst.
- The blastocyst has
  - an outer layer of cells (trophoblast).
  - and inside this hollow sphere, there is a cluster of cells called the inner cell mass.
- The cells of the inner cell mass will go on to form virtual all of the tissues of the human body.

## Specialization of Stem Cells: Differentiation

- Differentiation: unspecialized stem cells give rise to specialized (differentiated) cells in response to external and internal signals

A- Internal signals: turn on specific genes causing differential gene expression, through which a stem can decide to self-renew or differentiate under regulation of a complex set of factors, including:

I. Transcription factors: as Oct4 (octamer-binding transcription factor 4), Sox2 (sex determining region Y box 2) which play a central role in the maintenance of “stemness”.

II. Chromatin modifications.

III. Signaling pathways.

IV. MicroRNAs which are small, non-coding RNAs regulating gene expression at the post-transcriptional level by:

- mRNA degradation or - translational repression.

B- External signals (stem cell niche):

I. Chemicals secreted by other cells such as growth factors, cytokines,...

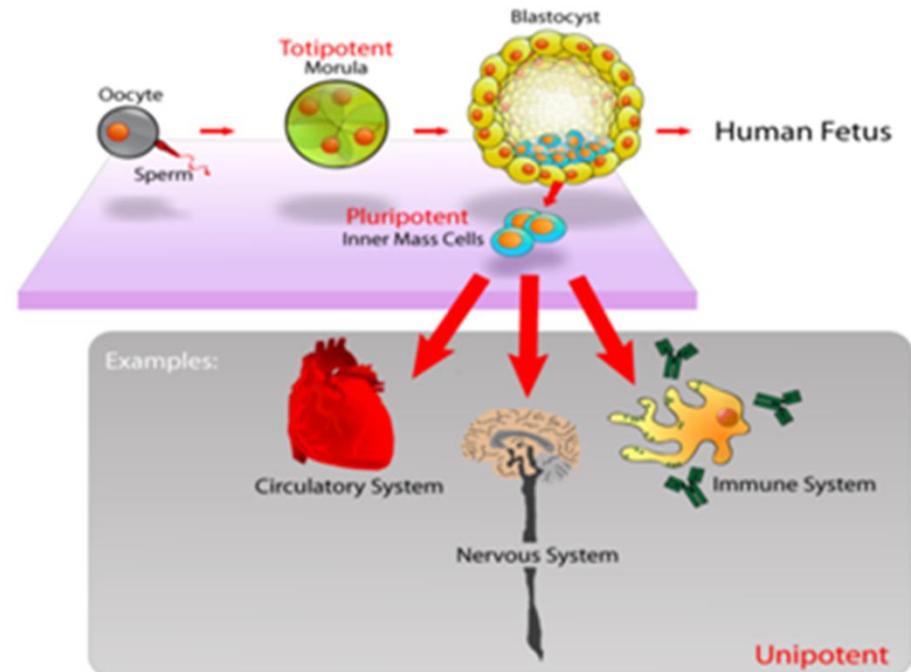
II. Physical contact with neighboring cells.

# Types: According to their potency (Differentiation capability), stem cells can be classified into:

## 1. Totipotent stem cells:

- Totipotent means entire because it has the potential to generate all the cells and tissues that make up an embryo.
- Such cells can construct a complete, viable, organism.
- These cells are produced from the fusion of an egg and sperm cell.
- Only the cells produced by the first few divisions of the fertilized egg (morula's cells) are totipotent.

### Totipotent, multipotent, unipotent stem cells



## 2. Pluripotent stem cells:

- Pluri” means **several** or many.
- They are the **descendants of totipotent cells**, derived from the inner cell mass of the blastocyst
- can **differentiate** into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm.
- These include each of the more than 220 cell types in the adult body.
- Although the cells of the inner cell mass can form virtually every type of tissue found in the human body, **they cannot form an organism.**
- Pluripotent stem cells undergo further specialization into multipotent progenitor cells.

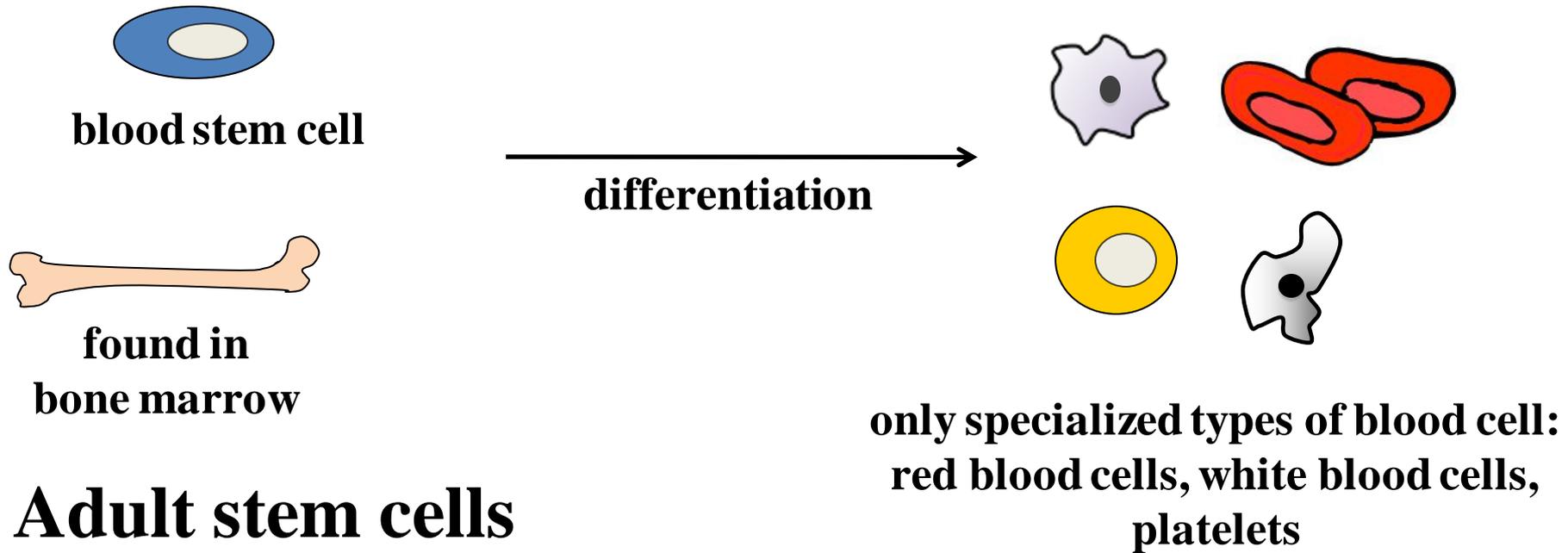
### 3. Multipotent stem cells:

- can produce only **cells of a closely related family** of cells.
- e.g.: **hematopoietic stem cells** differentiate into red blood cells, white blood cells, and platelets or **epithelial stem cells** that give rise to the various types of skin cells.

### 4. Unipotent cells:

- which means one.
- They can produce **only one cell type**, but have the property of **self-renewal** which distinguishes them from non-stem cells.
- eg.: -Muscle satellite cells that contribute to differentiated muscle tissue.

### 3. Multipotent Hematopoietic Stem cell



# Progenitor cells

- They are in the “**center**” between stem cells and fully differentiated cells.
- The most important difference between them is that **stem cells** can replicate indefinitely, whereas **progenitor cells** can divide only a limited number of times.
- Most progenitors are described as oligopotent.
- The characterization progenitor cells, is based on the **different cell markers rather than their morphological appearance**.

# Physiological functions of stem cells

- **In a developing embryo**, stem cells can differentiate into all of the specialized embryonic tissues.
- **In adult organisms**, stem cells act as a **repair system** for the body, replenishing specialized cells.

Stem cells are required wherever there is a need to replace differentiated cells that cannot divide themselves.

- In several tissues the terminal state of cell differentiation is incompatible with cell division.
- For example, degeneration of the nuclei of the cell of the outermost layer of the skin or extrusion of nuclei in the mammalian RBC.

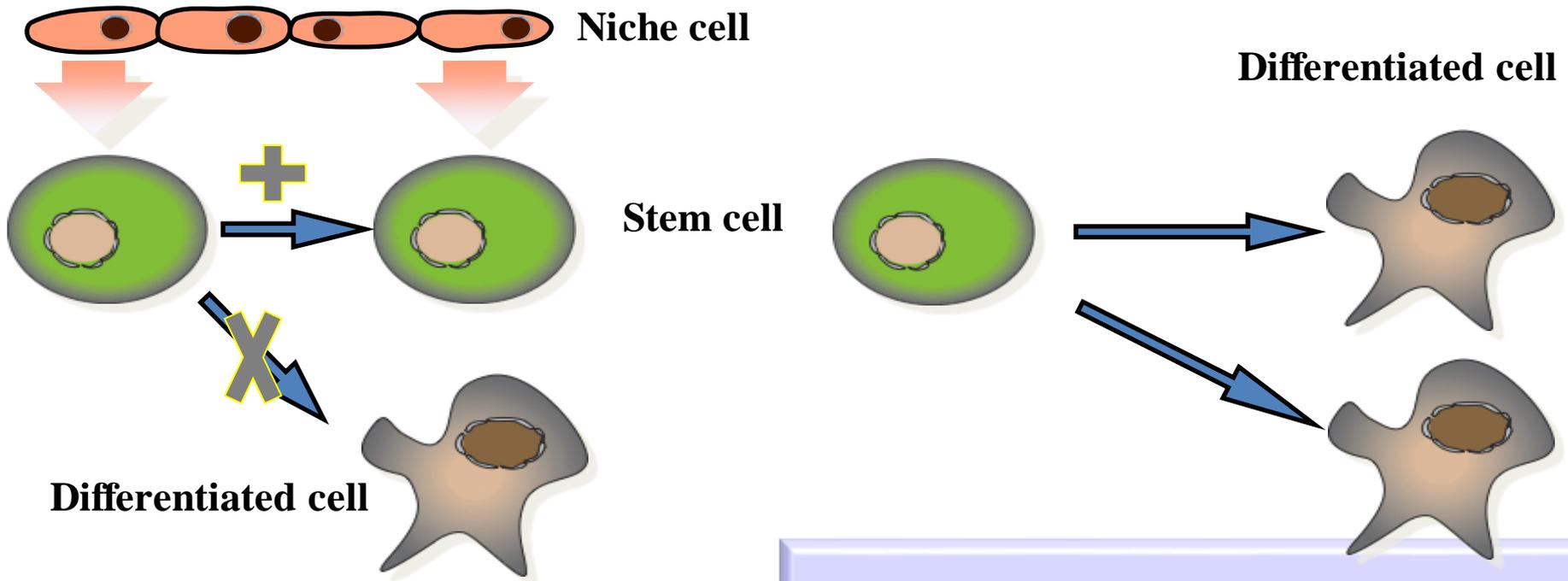
# Stem cell niche: definition, site

- Stem cells depend on local environmental factors to maintain their status as stem cells.
- **Stem cell niche**: it is the microenvironment that regulates the behavior of stem cells (regulating self-renewal and differentiation) and thus can teach us how to control stem cells in culture.
- **Stem cell niches occur in every organ** in the body that can regenerate this organ if damaged (**organ specific stem cells**).
- Niches are highly specialized for each type of stem cell, with a defined anatomical localization.
- **They are composed by:**
  - stem cells.
  - supportive stromal cells (which interact each other through cell surface receptors, gap junctions and soluble factors).
  - ECM in which they are located.

# Players in stem cell niche

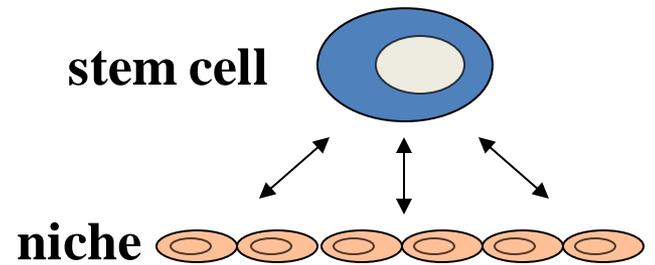
- Niche cells anchor stem cells with adherent junctions and provide cell surface and secreted proteins that regulate the cell cycle of the stem cell.
- Some of these factors **stimulate division**; others **inhibit differentiation**.
- **The niche can act on a stem cell by various mechanisms:**
  1. **Direct contact** between the stem cell and the niche cells.
  2. **Soluble factors** released by the niche that travel to the stem cell.  
**Biochemically, the ECM:**
    - can act directly by binding cell surface receptors or.
    - by growth factor presentation.
  3. **Intermediate cells** that ‘communicate’ between the niche and the stem cell.

**Self-renewal** is proliferation coupled to **blocking differentiation**, controlled by signals.

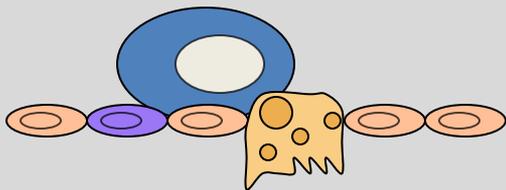


**In the absence of niche signals,** adult stem cells will **differentiate**, by default

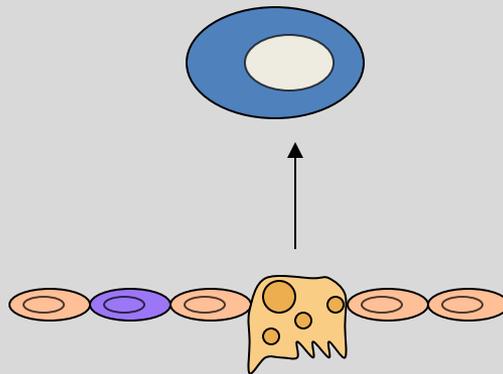
# Stem cells & niches



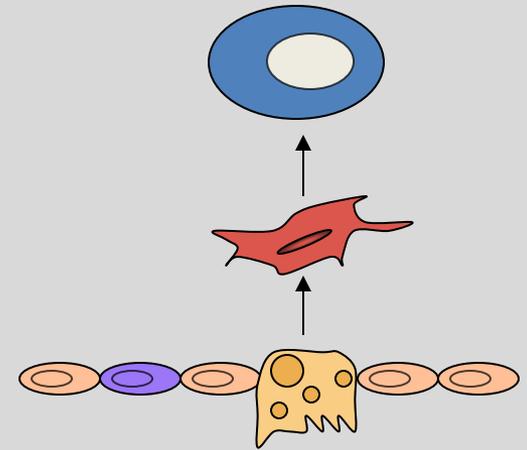
**Direct contact**



**Soluble factors**



**Intermediate cell**

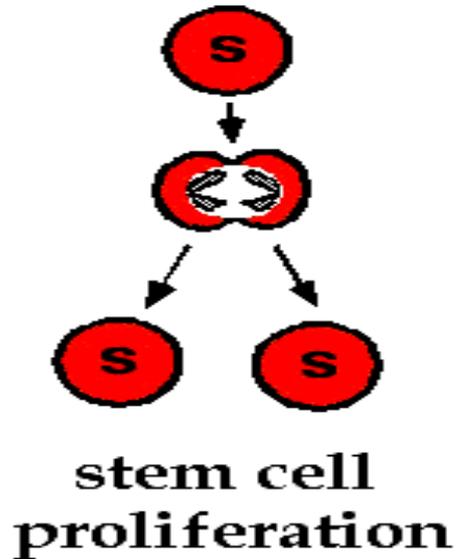


# Stem cell choice??

- The choice of a stem cell to undergo **self-renewal** is carried out by two cell division mechanisms, which fulfill two different **requests by the tissue**:
- **i) Asymmetric self-renewal**, in which each stem cell divides into one stem and one differentiated cell, allows maintaining a constant number of stem cells, which is generally sufficient **under physiological conditions**.
- **ii) Symmetric self-renewal**, in which each stem cell originates two daughter stem cells, leads to an expansion of the stem cell pool, a condition required **after tissue injury**.
- In these niches, the regulation of the balance between symmetric and asymmetric divisions is critical for maintaining proper stem cell number.

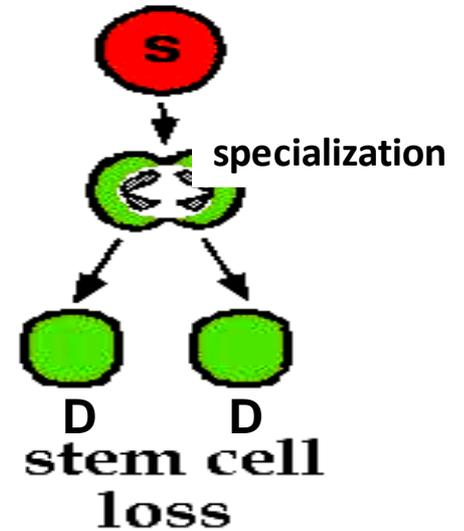
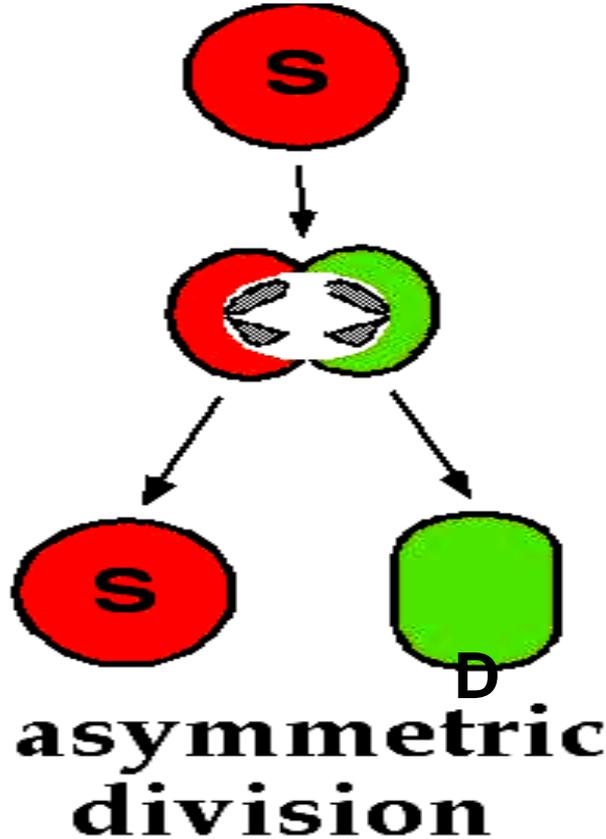
# Alternate Stem Cell Fates

**Embryonic  
Stem Cells**



**After tissue injury**

**Adult  
Stem Cells**

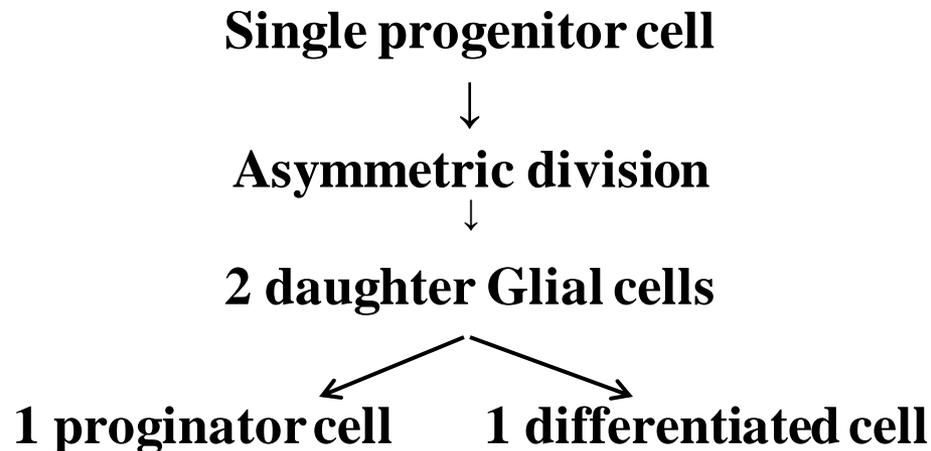


**S: stem cell**  
**D: differentiated cell**

**Under physiological conditions**

# Asymmetric division in stem cells and progenitors

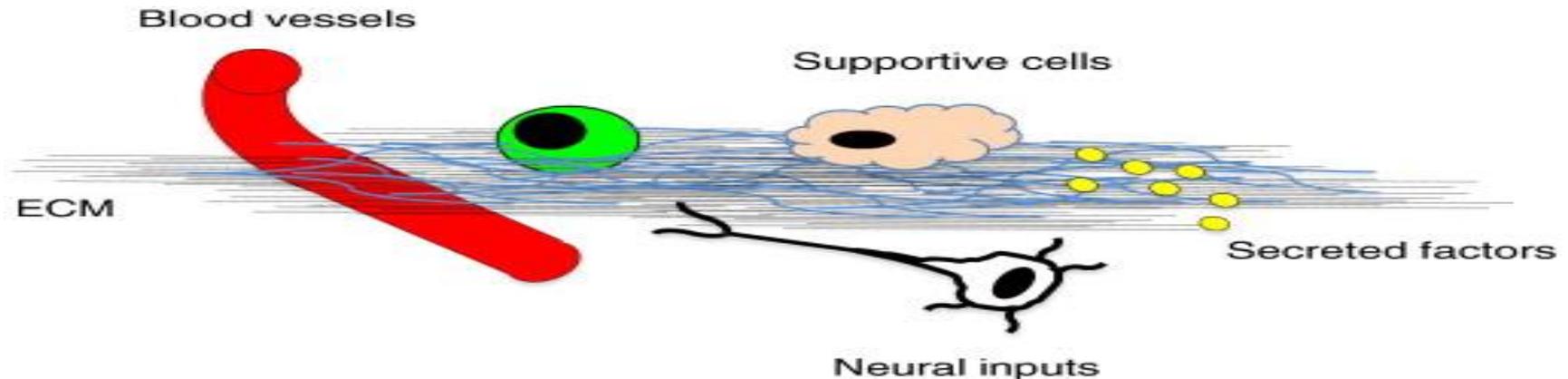
- **Intrinsic factors:** involve differing amounts of cell-fate determinants being distributed into each daughter cell.
- **Extrinsic factors**



(**Extrinsic factors** causes physical displacement of one of the daughter cells out of the original stem cell niche, exposing it to signalling molecules such as chondroitin sulfate which stimulate it to differentiate while the other daughter cell remains in the original niche in a quiescent state)

# Players in stem cell niche

- **The stem cell niche** is a dynamic microenvironment in which a number of inputs regulate stem cell behavior:
  - **Supportive stromal cells** (which interact each other through cell surface receptors, gap junctions and soluble factors).
  - **ECM** in which stem cells are located.
  - **Blood vessels** carry systemic signals to stem cells.
  - **Neural inputs** transmit distant physiological impulses to the stem cell microenvironment.

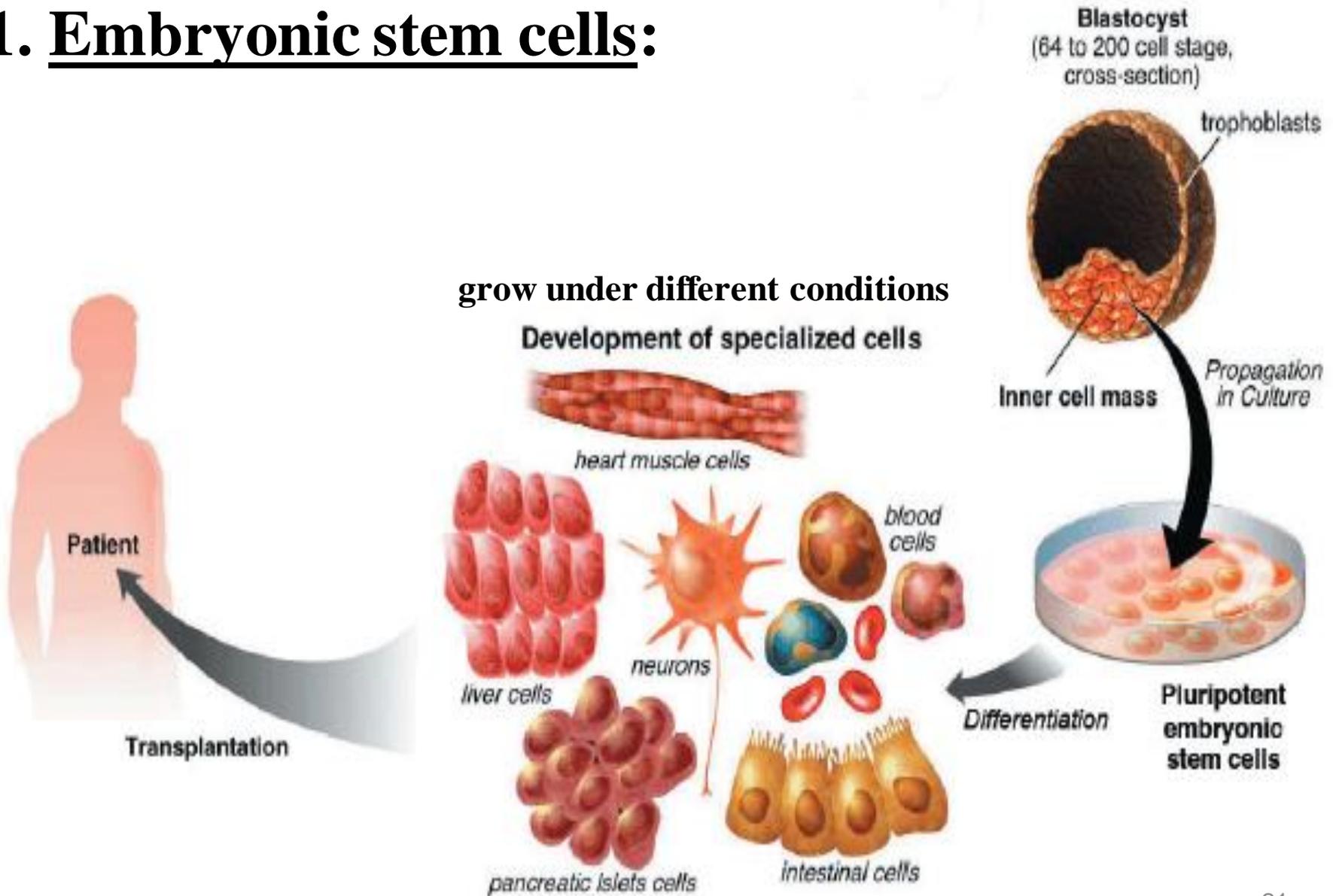


# SOURCES OF STEM CELLS FOR CLINICAL APPLICATION

## 1. Embryonic stem cells (ES cells):

- These stem cells come from embryos that are 4 to 5 days old.
- At this stage, an embryo is called a **blastocyst**.
- These are **pluripotent** stem cells, meaning they can divide into more stem cells or they can specialize and become any type of body cell (e.g. blood cells, heart cells, brain cells, etc).
- Embryonic stem cells have the highest potential for use to regenerate or repair diseased tissue and organs in people.
- Although ES cells represent an **ideal source for tissue regeneration** as they are immunologically inactive, yet they are not commonly used in routine stem cell therapy.

# 1. Embryonic stem cells:



# 1. Embryonic Stem Cells (ESC):

## sources:

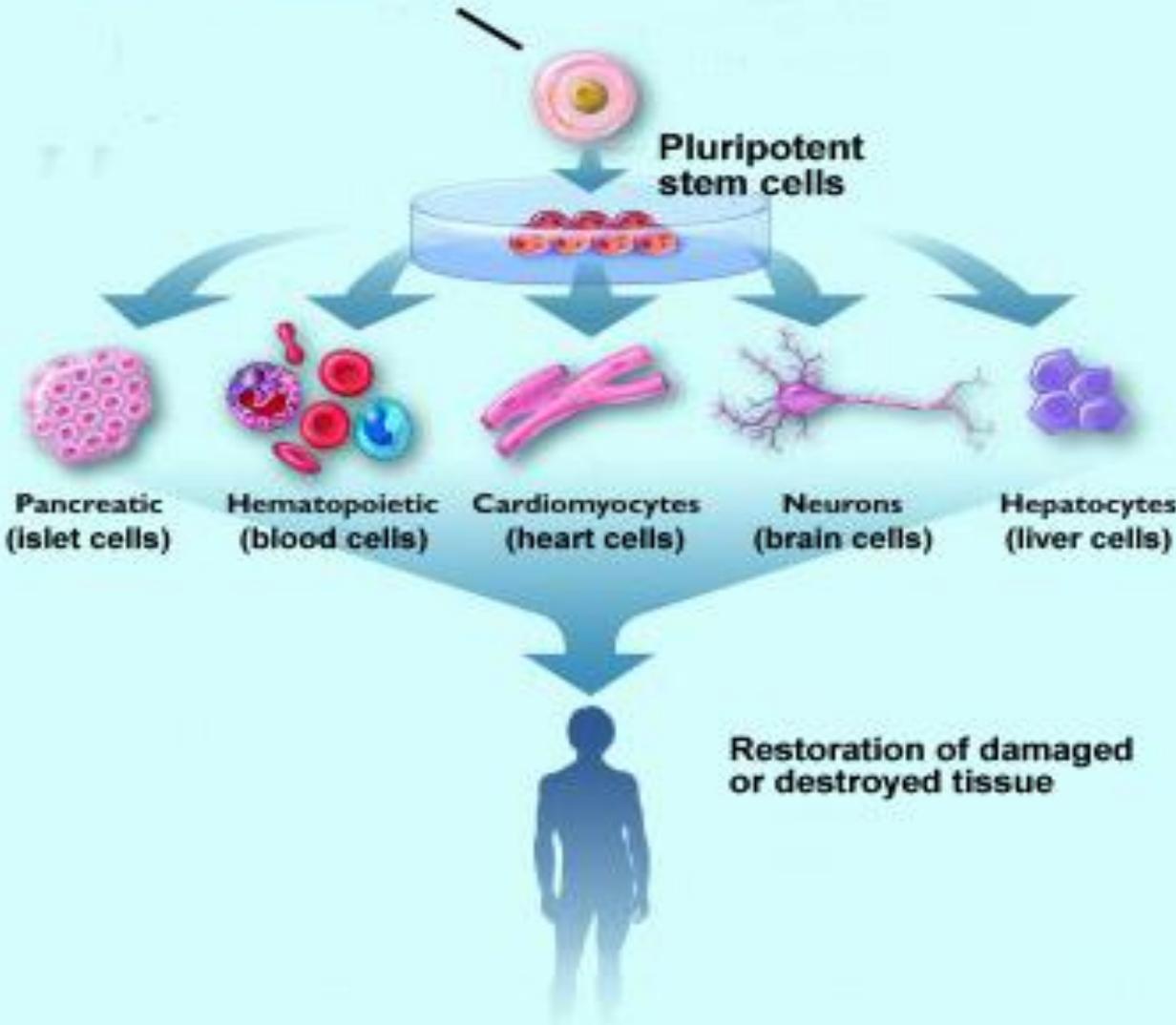
### A. In Vitro Fertilization

- The source of blastocysts for stem cell research is from in vitro fertilization (IVF) clinics.
- When IVF is used for reproductive purposes, doctors typically fertilize all of the donated eggs in order to maximize their chance of producing a viable blastocyst that can be implanted in the mother.
- Because not all the fertilized eggs are implanted, this has resulted in a **large bank of "excess" blastocysts** that are currently stored **in freezers**.

A.

## Stem Cells From In Vitro Fertilization (IVF)

Unused, frozen embryo,  
slated to be thrown away



● frozen embryos are routinely destroyed when couples finish their treatment.

● These embryos can be used to produce stem cells.

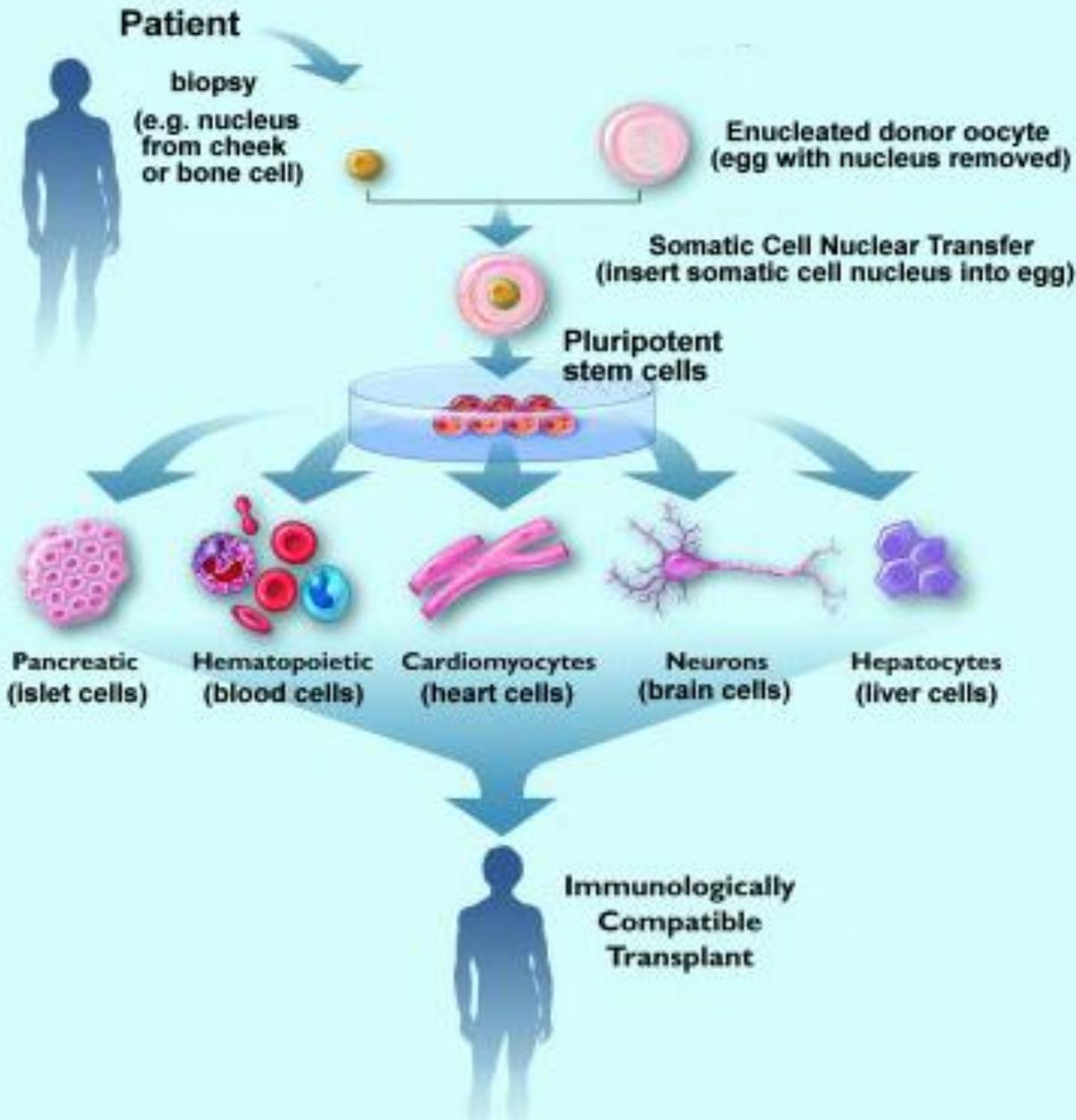
● Regenerative medical research aims to develop these cells into new, healthy tissue to heal severe illnesses.

# 1. Embryonic Stem Cells (ESC): sources:

## B. Nuclear Transfer

- In animals, nuclear transfer has been accomplished by inserting the nucleus of an already differentiated adult cell-for example, a skin cell-into a donated egg that has had its nucleus removed.
- This egg, which now contains the genetic material of the skin cell, is then stimulated to form a blastocyst.
- The stem cells that are created in this way are therefore copies or "clones" of the original adult cell because their nuclear DNA matches that of the adult cell.
- In the future, scientists may be able to create "*personalized*" stem cells that contain only the DNA of a specific patient.
- The ESC created by nuclear transfer would be genetically matched to a person needing a transplant, so that the patient's body would not reject the new cells.
- Ethical considerations include egg donation, which requires informed consent.

## B. Human Therapeutic Cloning (SCNT)



## B. Somatic Cell Nuclear Transfer

❖ The nucleus of a donated egg is removed and replaced with the nucleus of a **mature, "somatic cell"** (a skin cell, for example).

❖ **No sperm** is involved in this process, and **no embryo** is created to be implanted in a woman's womb.

❖ The resulting stem cells can potentially develop into specialized cells that are useful for treating severe illnesses.

# Difficulties & Challenges:

## The limited use of ES cells is due to:

- Potential for **tumor formation**
- Questions regarding **functional differentiation**
- **Immune rejection.**
- **Ethical debate:**
  - **In favor of ESC research:**
    - Embryonic stem cells are used to **alleviate human suffering.**
    - **excess IVF embryos will be discarded anyway**, isn't it better that they be used in valuable research?
  - **Against ESC research:**
    - stem cells are taken from a human **blastocyst**, which is then destroyed. This means **“murder.”**
    - There is a risk of **commercial use** of the human participants in ESC research.

# SOURCES OF STEM CELLS FOR clinical application

## 2. Adult stem cells

- An **adult stem cell** is an **undifferentiated** cell, found **among differentiated cells** in a tissue or organ. The adult stem cell can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ.
- Scientists use the term **somatic stem cell** instead of adult stem cell, where somatic refers to cells of the body (not the germ cells).

Examples:

- a. **Pluripotent adult stem cells** are rare but can be found in a number of tissues including **umbilical cord blood**. At delivery, cord blood is collected, stored and frozen. (It contains RBC, WBC, lymphocytes, platelets).
  - b. **Most adult stem cells are multipotent** and can only produce a limited number of cell types. These stem cells are found in children, in some adult tissues, such as **bone marrow**.
- **Fetal stem cells** collected from the organs of fetuses at a later stage of development.

# Tissue stem cells:

- Tissue stem cells can **mostly** make the kinds of cell found in the **tissue** they belong to.
- So,
  - **Blood stem cells** can ~~only~~ make the different kinds of cell found in the blood.
  - **Brain stem cells** can ~~only~~ make different types of brain cell.

## **Transdifferentiation:**

- **In culture**, certain **adult stem cell types** can **differentiate** into cell types seen in organs or tissues other than those expected from the cells' predicted lineage (i.e., **brain stem cells** that **differentiate** into **blood cells**)

# SOURCES OF STEM CELLS FOR CLINICAL APPLICATION

## Amniotic fluid stem cells:

- A type of cell that floats freely in the amniotic fluid of pregnant women has been found to have many of the same traits as embryonic stem cells.
- The cells, **shed by the developing fetus** and are easier to maintain in laboratory dishes than embryonic stem cells.
- because the cells are a **genetic match to the developing fetus**, tissues grown from them in the laboratory will **not be rejected** if they are used to treat birth defects in that newborn.
- the cells **could be frozen**, providing a **personalized tissue bank** for use later in life.

## SOURCES OF STEM CELLS FOR clinical application

### Induced pluripotent stem cell (iPS cells)

- **Adult somatic cells** are altered (a process known as dedifferentiation) to have properties of embryonic stem cells.
- By **altering the genes** in the adult cells, researchers were able to reprogram the cells to **act similarly to embryonic stem cells.**
- **Principle:**
  1. Take cells from the body (like skin cells from a patient).
  2. Make iPS cells.
  3. Use those iPS cells to grow the specialized cells the patient needs to recover from the disease.
- These cells would be made from the patient's own skin cells so the body **would not reject them.**
- This new technique **avoids the controversies that come with embryonic stem cell.**

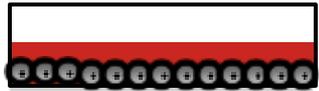
# Induced pluripotent stem cells (iPS cells)



cell from the body

**'genetic reprogramming'**  
= add certain genes to the cell

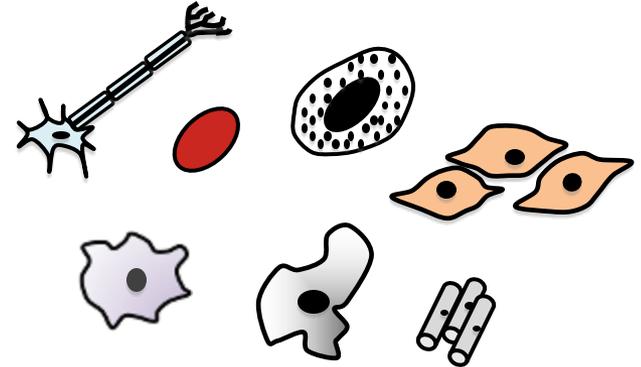
induced pluripotent stem (iPS) cell  
behaves like an embryonic stem  
cell



culture iPS cells in the lab

differentiation

Grow in special media  
to differentiate to  
a specific tissue



all possible types of  
specialized cells

**Advantage: no need for embryos**

# Embryonic VS

- Totipotent or pluripotent
  - Differentiation into ANY cell type; can become >200 cell types.
- Culture: easy to culture, reproduce for long periods.
- Source:
  - a. frozen embryo (IVF).
- Large numbers.
- May cause immune rejection

# Adult Stem Cells

- Multi or pluripotent
  - Differentiation into some cell types, limited outcomes
- Culture: difficult to find, hard to culture. Some can be reprogrammed.
- Source:
  - a. umbilical cord & placenta.
  - b. adult/child tissue (bone, teeth, skin, hair, brain, liver, ..).
- Limited numbers
- Less likely to cause immune rejection, since the patient's own cells can be used

# Stem cells and their clinical applications

## 1. In transplant therapy: Regenerative medicine

- e.g. to **replace or restore tissue** that has been damaged by disease or injury.
- examples of potential treatments include:
  - regenerating **bone** using cells derived from bone marrow stroma.
  - developing **insulin-producing cells** for type 1 diabetes.
  - Genetic based disease as **Cystic fibrosis**.

## 2. Develop model systems to study **normal growth** and **identify the causes of birth defects**.

## 3. Advance knowledge about how an organism develops from a single cell and **how healthy cells replace damaged cells in adult organisms**.

# II. Gene therapy

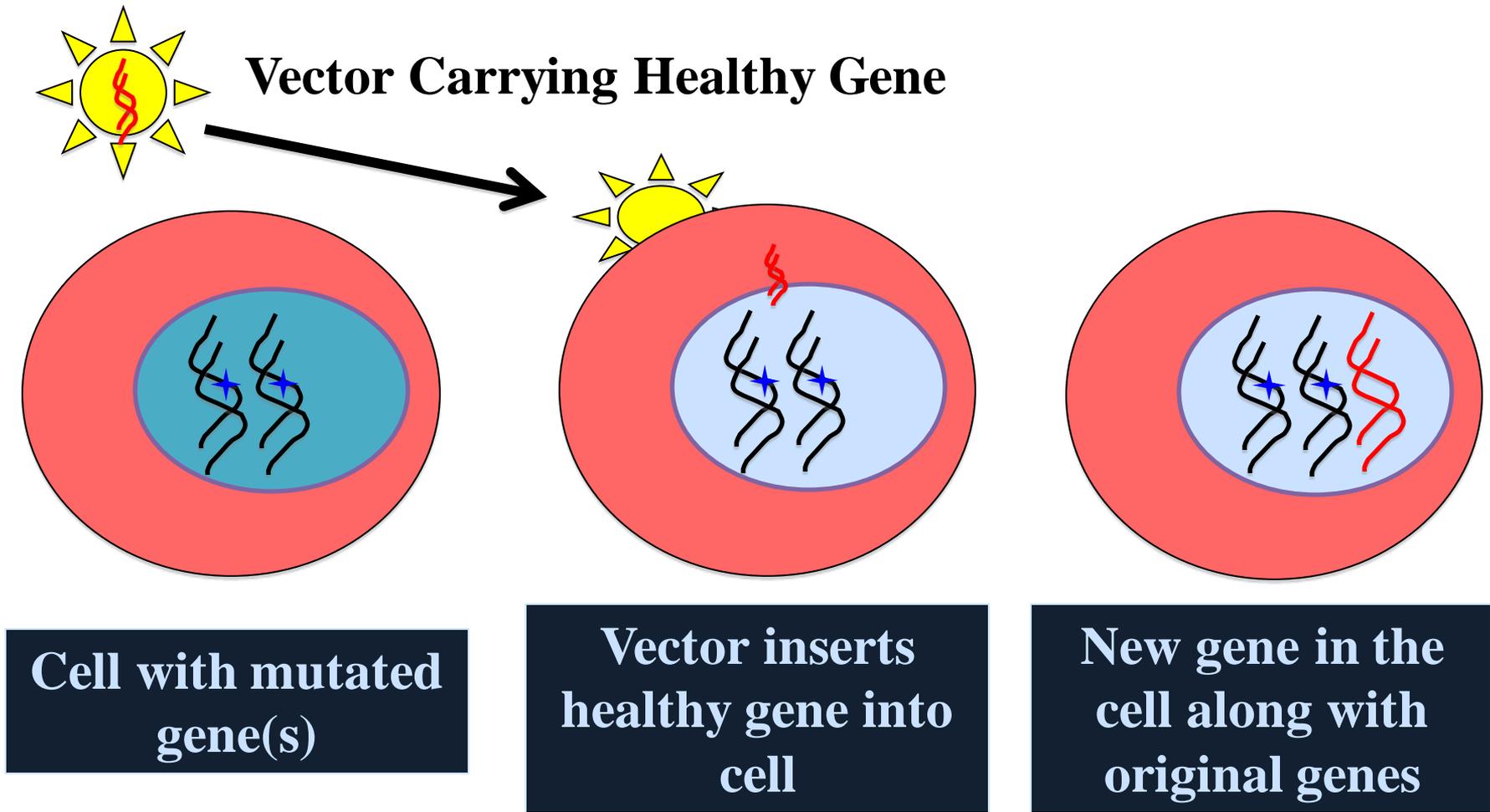
## What is Gene Therapy?

- Gene therapy is a **treatment** or **cure** for disorders caused by mutated genes.
- It involves adding a normally functioning copy of the gene(s) to enough affected cells to restore normal function.

## **Gene therapy could be very different for different diseases:**

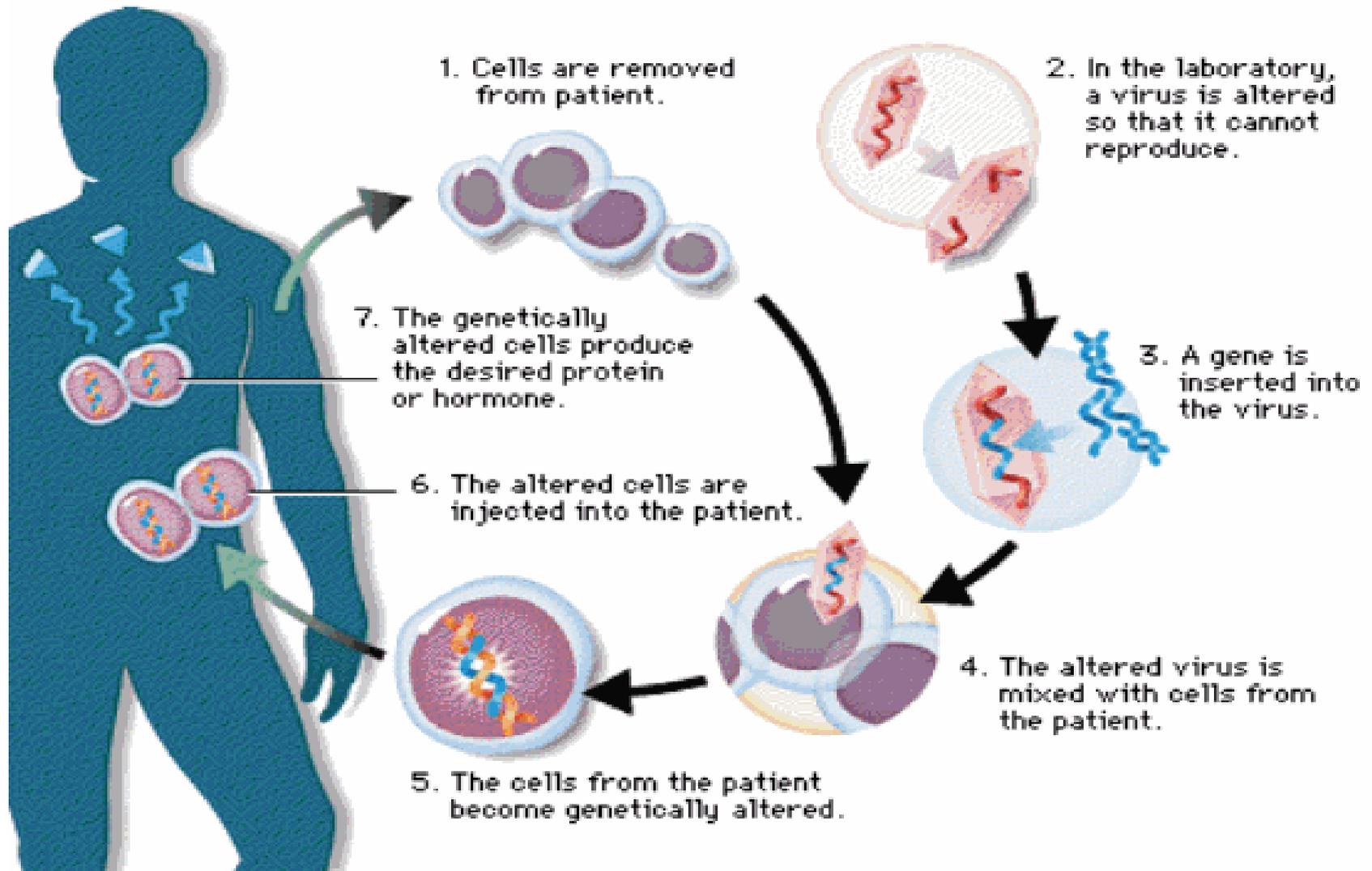
- **Gene transplantation** : (to patient with gene deletion)
- **Gene correction:** (To revert specific mutation in the gene of interest)
- **Gene augmentation:** (to enhance expression of gene of interest)

# How is gene therapy done?

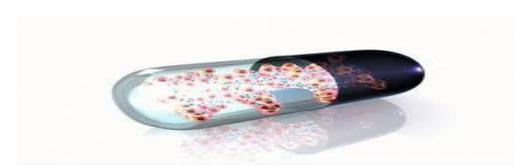


**Functional proteins are created from the therapeutic gene causing the cell to return to a normal state.**

# Gene therapy



# Gene Therapy: principle



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

**Routes of delivery of genes into humans by Vectors.**

- **Vectors are carrier molecules.** They deliver the therapeutic gene into a patient’s target cell.
- Both viral and non-viral vectors have been used, though non-viral have a decreased transfer efficiency.

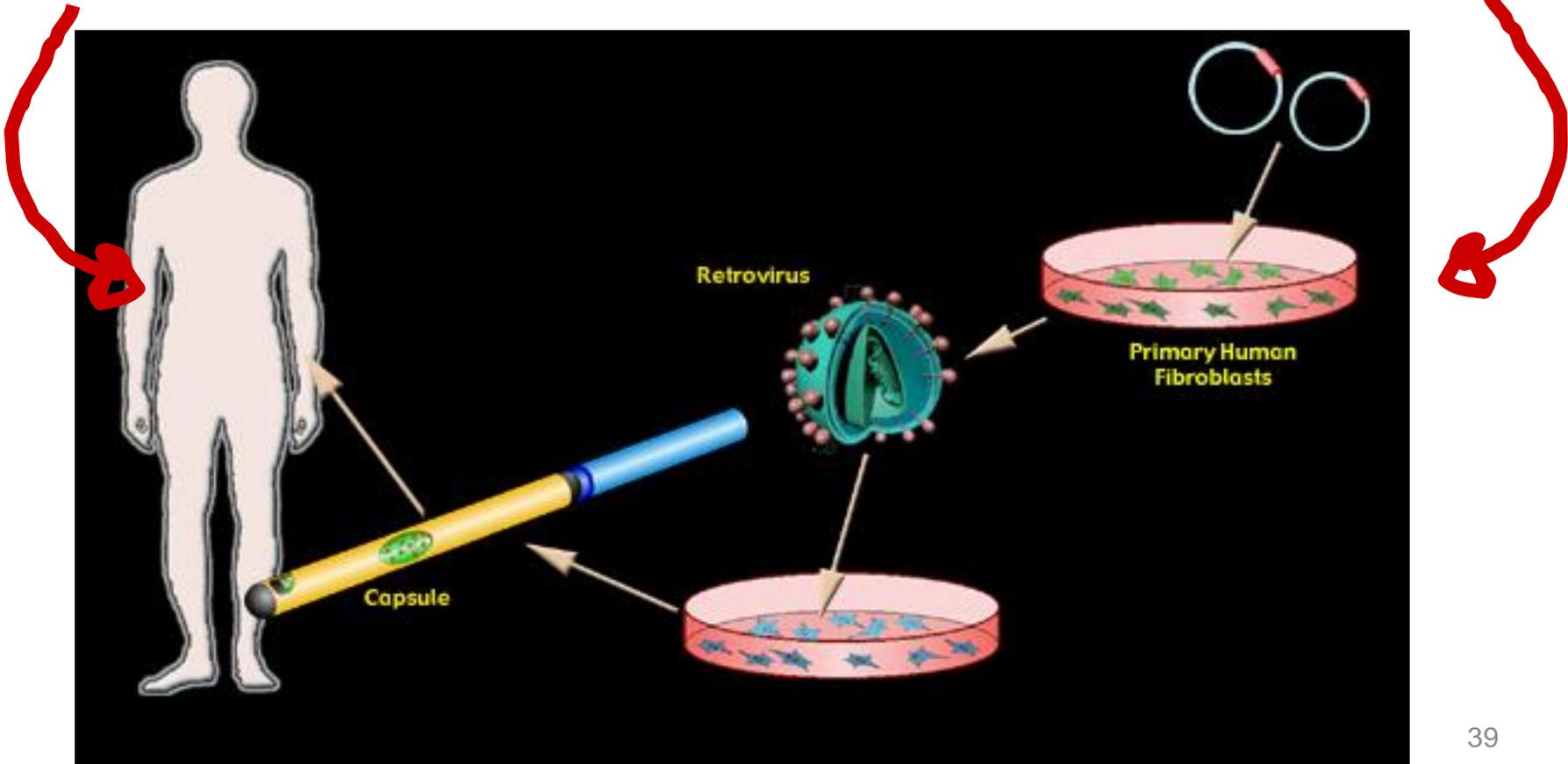
# Types: Somatic & Germ

- **Germline gene therapy** would be the permanent transfer of a gene into **sperm or egg cells**.
  - Future generations would be “cured”.
- **Somatic cell (body cell) gene therapy** is ideally only the transfer of genes to the affected cells. (Somatic cells are cells that form the body and cannot produce offspring).
- **Only somatic gene therapy is permissible in humans**

# Gene therapy: techniques

**In vivo**

**Ex vivo**



# 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.
3. Only available option **for tissues that can not be grown in vitro**.

# **Ex vivo gene therapy**

1. The genetic material is first transferred **into the cells grown in vitro**.
2. **Controlled process**; Genetically altered cells are selected and expanded .
3. **Cells are** then returned back to the patient.

# Routes of delivery of genes into humans:

## 1. Non viral options

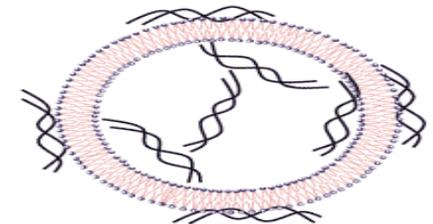
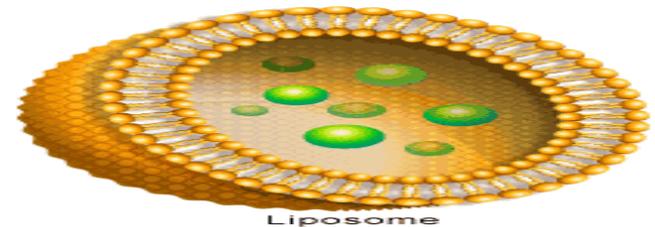
a. **Direct introduction** of therapeutic DNA into target cells. Can be used only **with certain tissues** and requires **large amounts** of DNA. improving the efficiency DNA uptake: by "**gene gun**", which shoots DNA coated gold particles into the cell using high pressure gas.

### b. **An artificial lipid sphere (liposome)**

which carries the therapeutic DNA and is capable of passing the DNA through the target cell's membrane.

- **DNA delivery of genes by liposomes:**

- Cheaper than viruses.
- No immune response.
- Especially good for in-lung delivery (cystic fibrosis).
- Less transfer efficiency than viral vector.



# Routes of delivery of genes into humans:

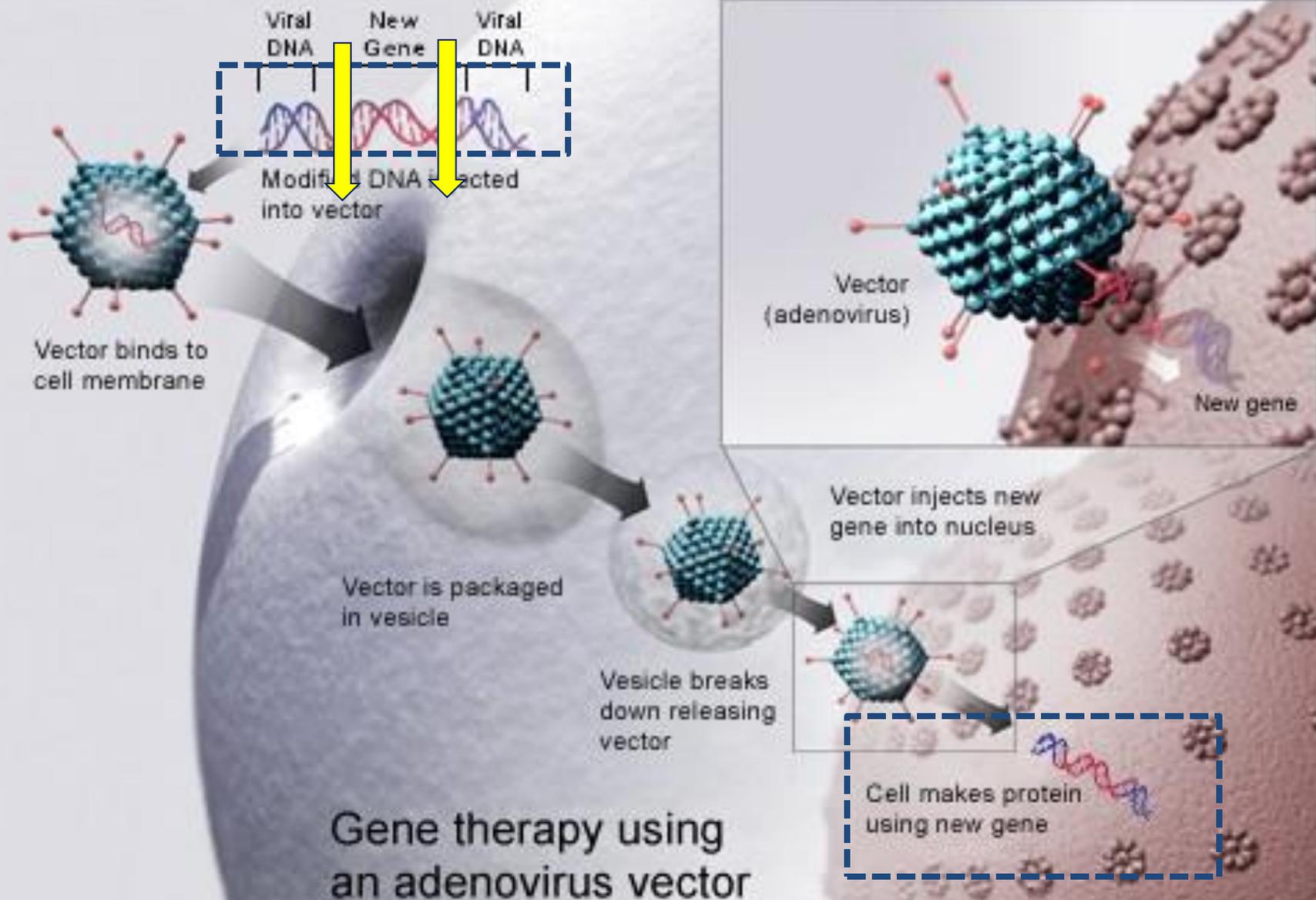
## 2. Viruses as Vectors

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- **different types:** Adenovirus, Retrovirus, Herpes Simplex Virus (HSV).

### a. Adenovirus:

- Are **double stranded DNA** genome.
- The inserted DNA is **not incorporate** into genome.
- **Not replicated.** So, Has to be **reinserted** when more cells divide.
- **How adenoviruses work?**
  - Adenovirus vector binds to target cell membrane.
  - Vector is packaged in vesicles.
  - Vesicle breaks down releasing vector.
  - Vector injects new gene into nucleus.
  - Target cell forms normal protein from new gene. (normal functioning gene).



## **b. Retroviruses**

- They contain **RNA** genome.
- They form double stranded DNA copies from **RNA genome** through **reverse transcription** using **reverse transcriptase enzyme**.
- the double stranded viral genome **integrates** into the human genome using **integrase**. Integrase inserts the gene anywhere . So, may cause insertional mutagenesis.
- Vectors used are derived from the human immunodeficiency virus (**HIV**) and are being evaluated for safety.

**Viral RNA genome**

↓ (RT)

**dsDNA**

↓ (integrase)

**Inserts DNA into human genome**

# Vector: Advantages (+) and Disadvantages (-)

- Adenovirus

- + Infects **many** cell types. + efficient.
- **Does not integrate** into host genome and can be lost.
- **Have immunological response.**

- Retrovirus

- + **Integrates** into host genome and cannot be lost (permanent expression).
- Integrates into host genome and can cause **cancer**

- Herpes Simplex Virus (HSV)

- + DNA stays in nucleus without integrating into host genome.
- **Only infects** cells of the **nervous system.**

# Applications - trials

- Although no gene therapies have been approved by the FDA for sale, some diseases have been **experimentally successful**:
  - Melanoma (skin cancer).
  - Severe Combined Immunodeficiencies (SCID).
  - Sickle Cell Anemia.
  - **The First Case**: The first gene therapy was performed on **1990**
    - Ashanti DeSilva was treated for SCID
    - Doctors removed her white blood cells, inserted the missing gene (**Adenosine deaminase**) into the WBC, and then put them back into her blood stream.
    - This strengthened her immune system
    - Only worked for a **few months**.

## Gene therapy: success

- **Problem:** restenosis of coronary arteries after they have been opened by coronary bypass surgery.
- **Treatment:** 13 patients with restenosis were injected in the heart with **DNA encoding vascular endothelial growth factor**, which stimulate angiogenesis (vessel formation).  
**Result:** all patients had improved heart function.

## Gene therapy: failure

- Four children have developed **cancer** due to a retrovirus gene therapy vector.

## Bone grafts

- Bone cancers, as osteosarcoma, are treated by removing the diseased bone and replacing it with a donated section of bone from a dead body.
- **In mice.** They found that the transplanted “dead” bone did not express two key genes normally expressed in bone, **RANKL** and **VEGF**.
- To do this, **they inserted the RANKL and VEGF genes into a modified, harmless virus.** This virus was then **applied** in a paste **directly onto the mouse bone graft during surgery.** The viral vector was absorbed into the tissue surrounding the dead bone graft, activating the RANKL and VEGF genes. **As a result the mouse’s body began to treat the bone graft as if it was its own tissue.** New blood vessels began to grow around the graft and the dead bone was converted to new, healthy bone.

# Problems with Gene Therapy

- **Short Lived**
  - Hard to rapidly integrate therapeutic DNA into genome and rapidly dividing nature of cells prevent gene therapy from long time
  - Would have to have multiple rounds of therapy
- **Immune Response**
  - new things introduced leads to immune response
- **Viral Vectors**
  - patient could have toxic, immune, inflammatory response
  - also may cause disease once inside
- **Multigene Disorders**
  - Heart disease, high blood pressure, and diabetes are hard to treat because you need to introduce more than one gene
- **May induce a tumor** if integrated in a tumor suppressor gene because insertional mutagenesis.

