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.

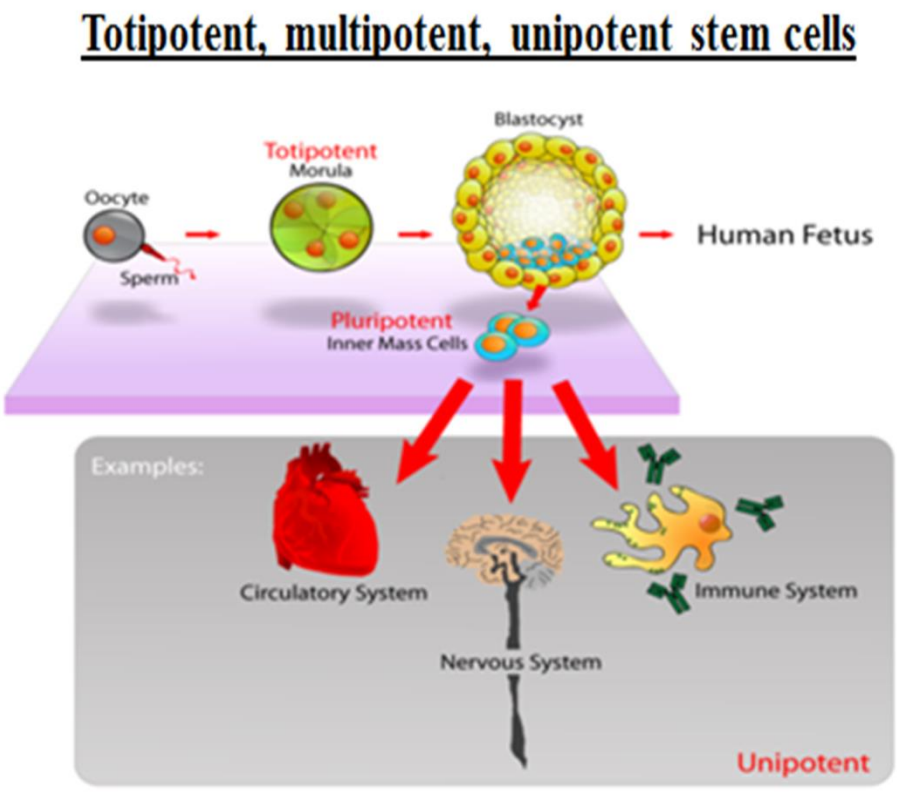
Stem cell differentiation

- 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.

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.



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.

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:**

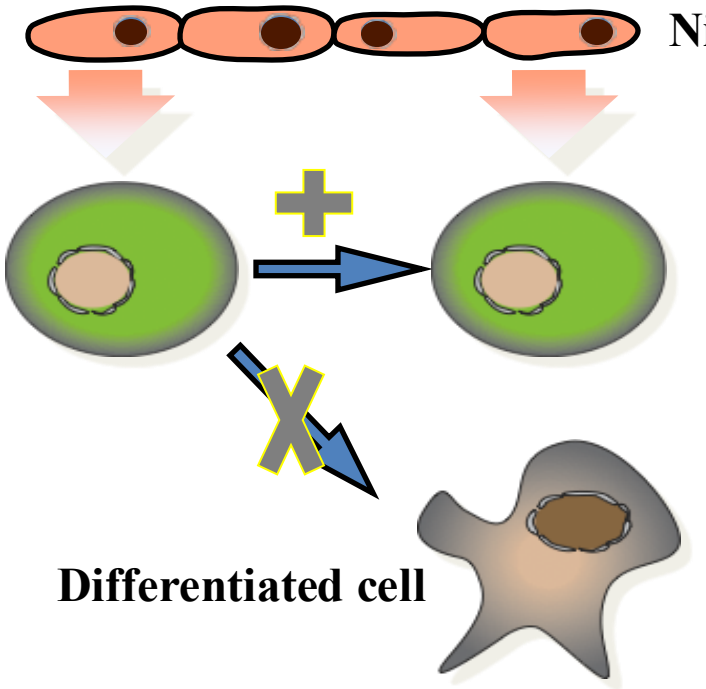
1. **Direct contact** between the stem cell and the niche cells.
2. **Soluble factors** released by the niche that travel to the stem cell. **Biochemically,** the ECM:

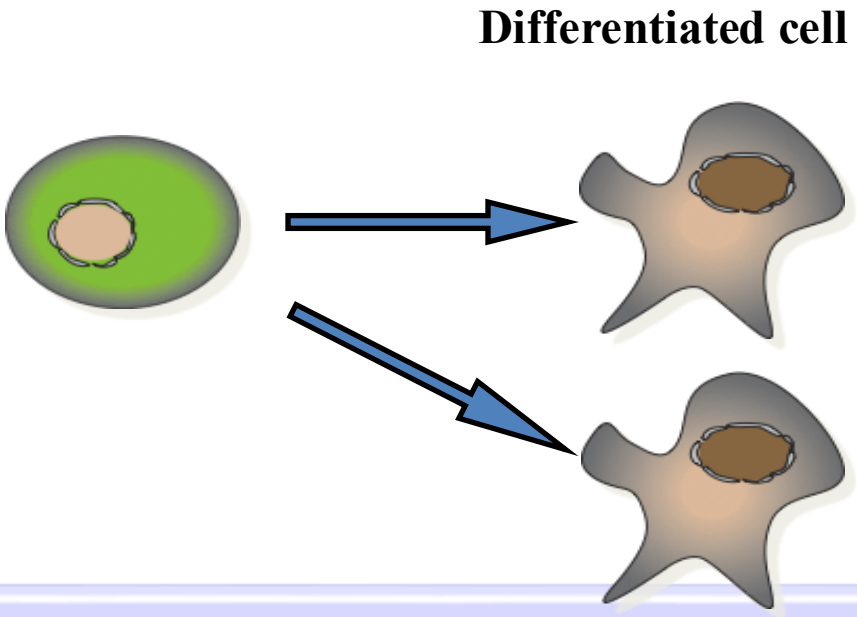
➢ can act directly by binding cell surface receptors or.

➢ by growth factor presentation.

3. **Intermediate cells** that 'communicate' between the niche and the stem cell.

<u>Self-renewal</u> is proliferation coupled to **blocking differentiation**, controlled by signals.





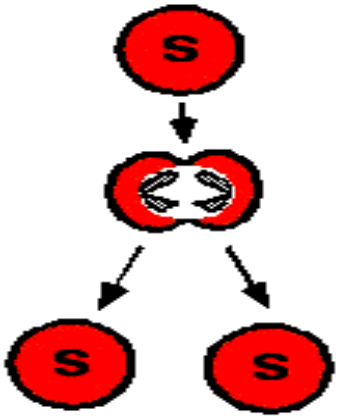
<u>In the absence of niche signals</u>, adult stem cells will **differentiate**, by default

Stem cell choice??

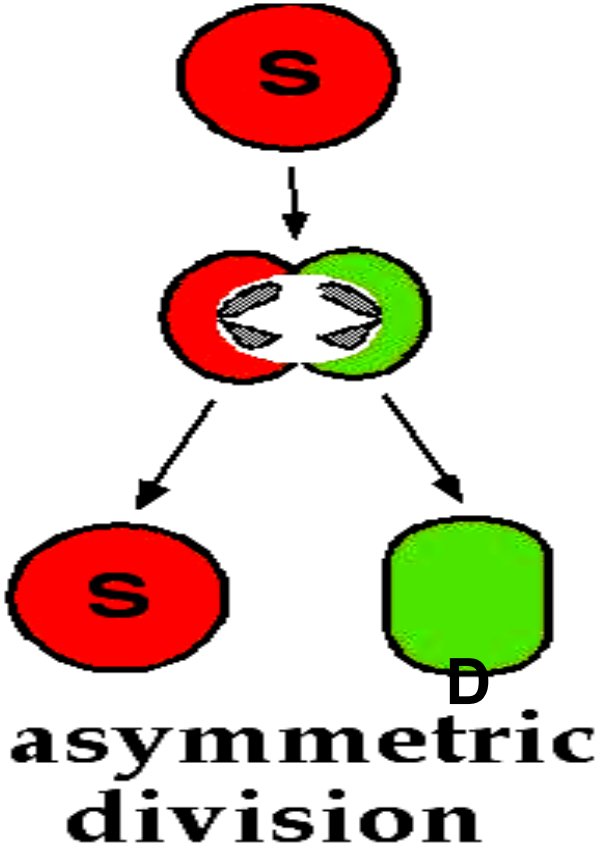
- The choice of a stem cell to undergo **self-renewal** is carried out by two cell division mechanisms, which fulfill two different **requests by the tissue**:
- **i) Asymmetric self-renewal**, in which each stem cell divides into one stem and one differentiated cell, allows maintaining a constant number 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.

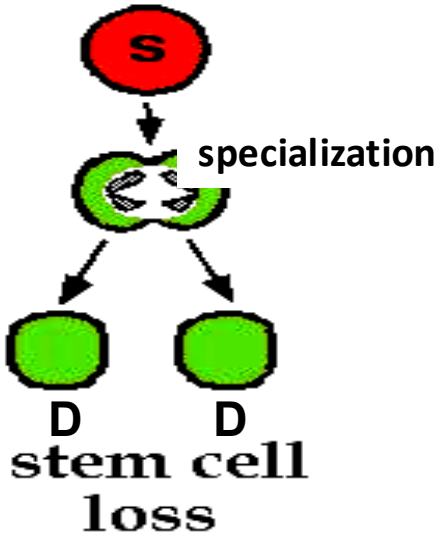
Alternate Stem Cell Fates

Embryonic Stem Cells



Adult Stem Cells





After tissue injury

S: stem cell
D: differentiated cell

Under physiological conditions

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 (ESC): sources:

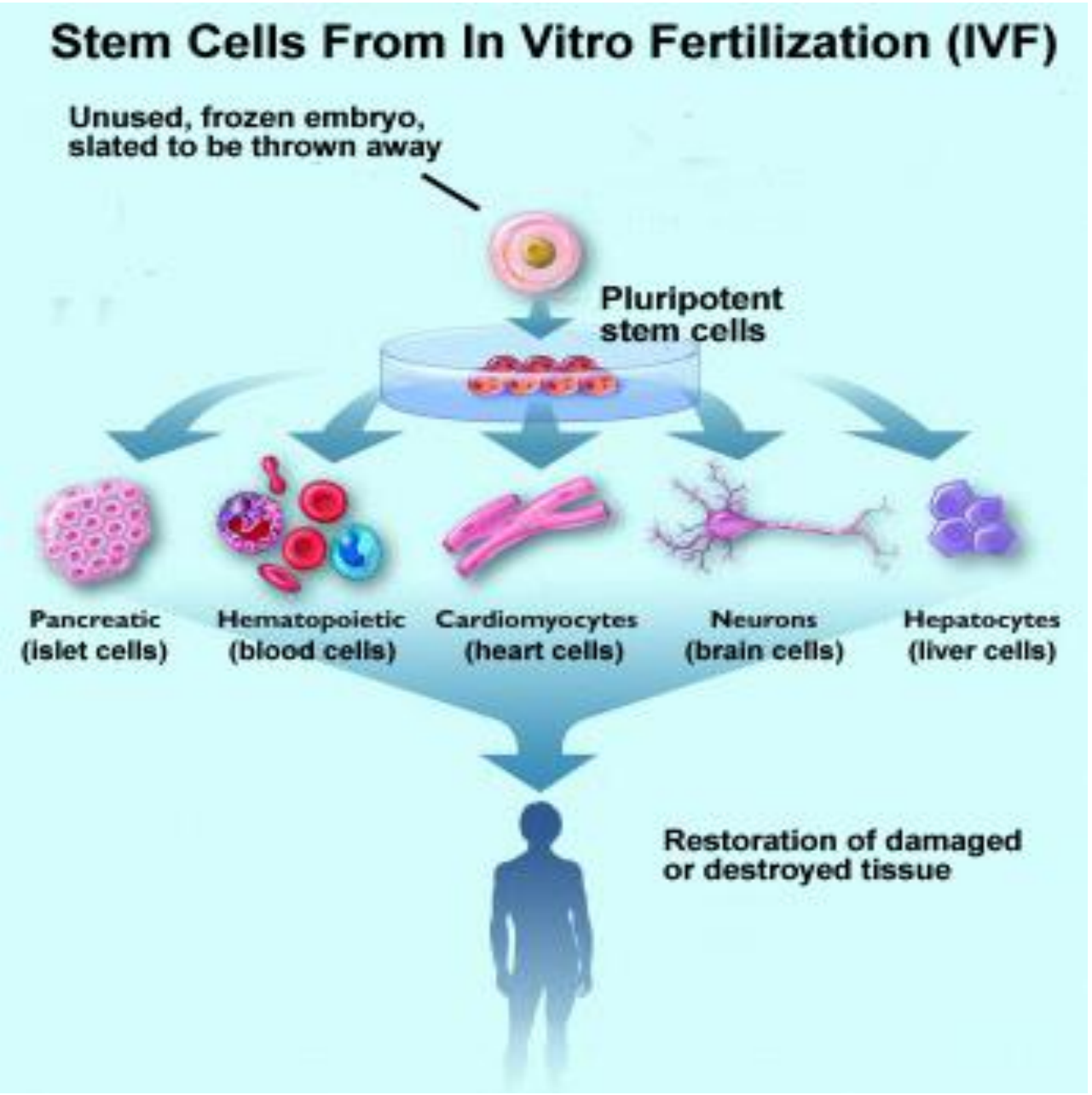
A. In Vitro Fertilization

- The source of **blastocysts** for stem cell research is from in vitro fertilization (IVF) clinics.

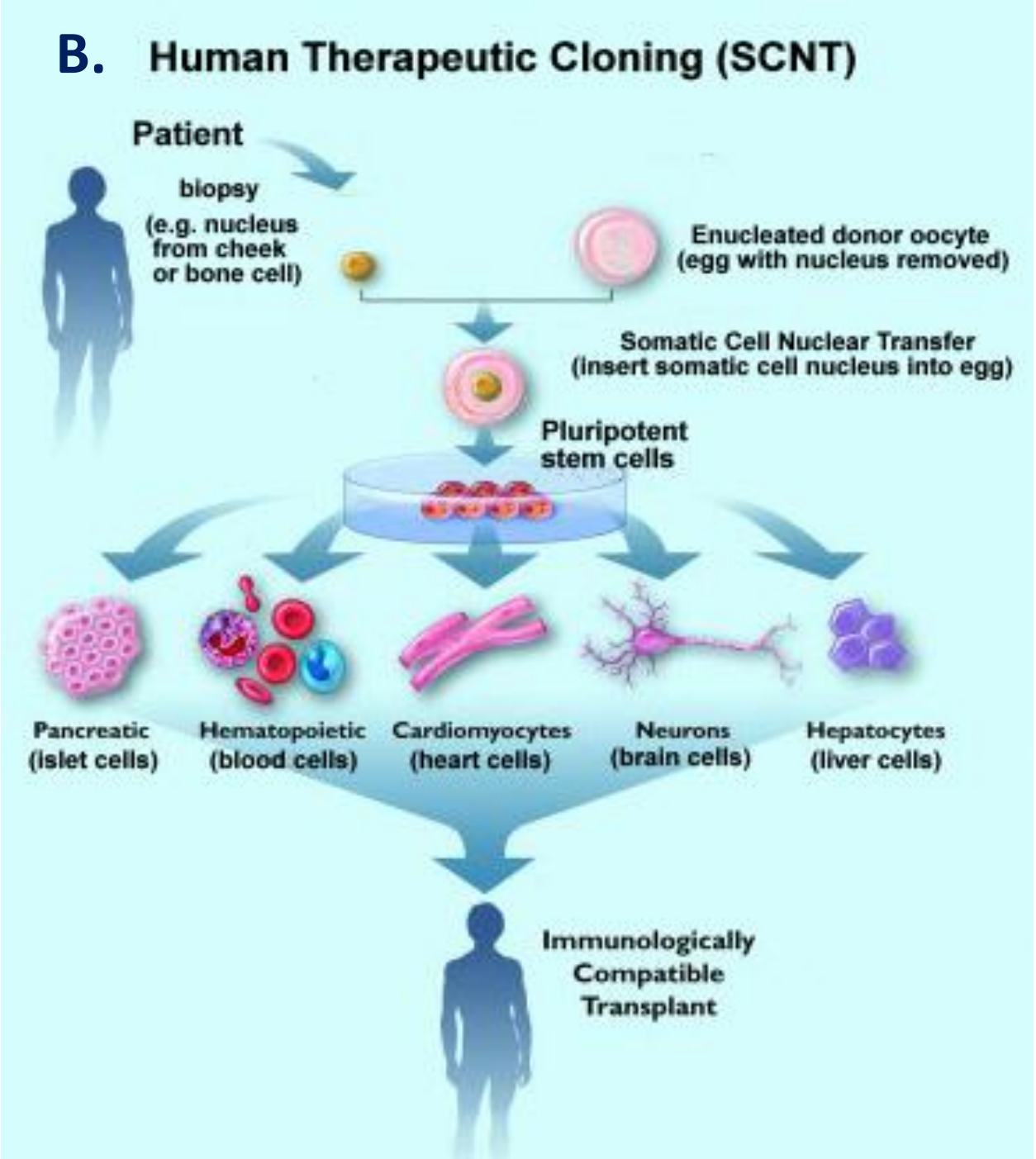
- When IVF is used for reproductive purposes, doctors typically fertilize all of the donated eggs in order to maximize their chance of producing a viable blastocyst that can be implanted in the mother.

- 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.



- 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.



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.

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

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**.

Embryonic vs Adult Stem Cells

- 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

- 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

II. Gene therapy

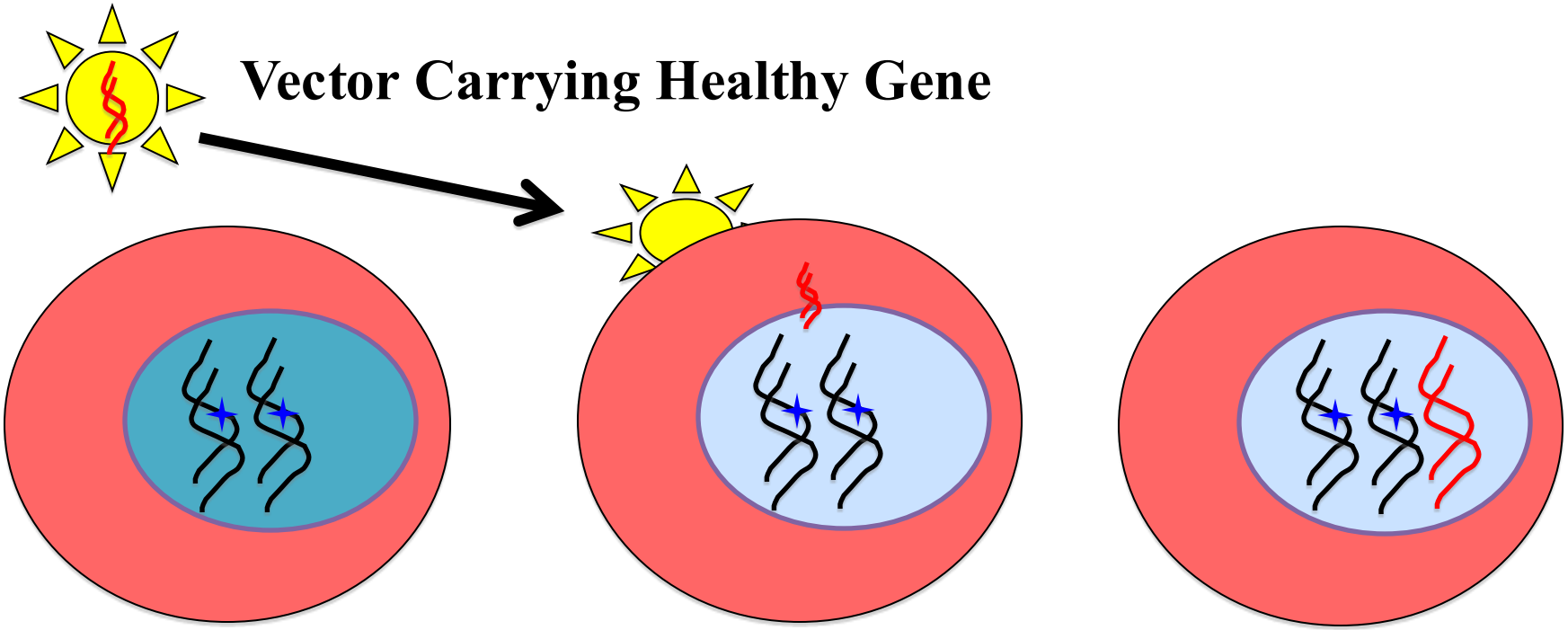
What is Gene Therapy?

- 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?



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.

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**

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.

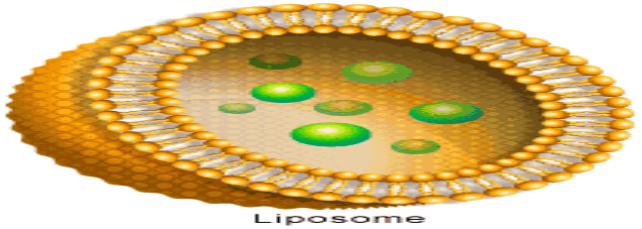
Routes of delivery of genes into humans:

1. Non viral options

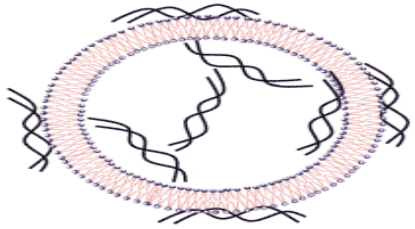
a. Direct introduction of therapeutic DNA into target cells. Can be used only **with certain tissues** and requires **large amounts** of DNA. improving the efficiency DNA uptake: by **"gene gun",** which shoots DNA coated gold particles into the cell using high pressure gas.

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

- **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.**