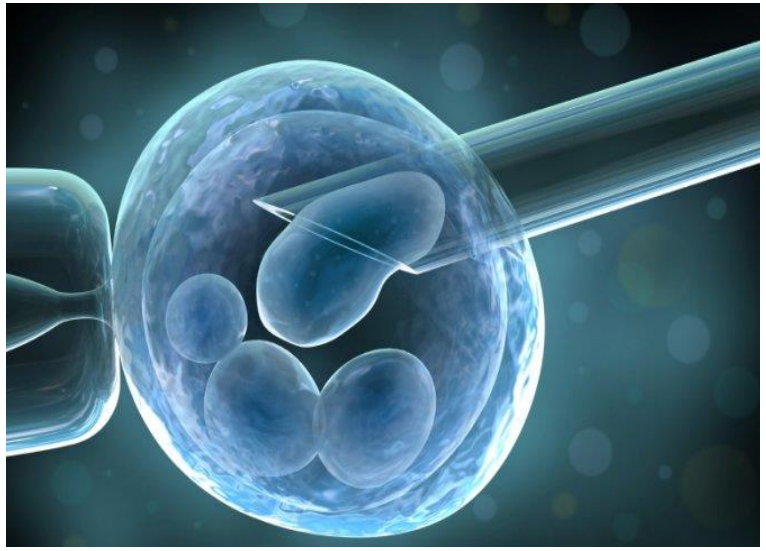
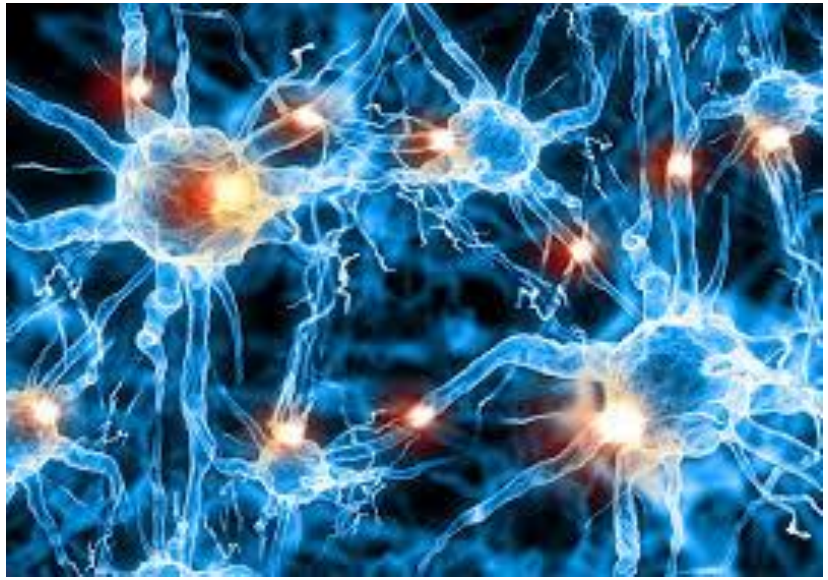


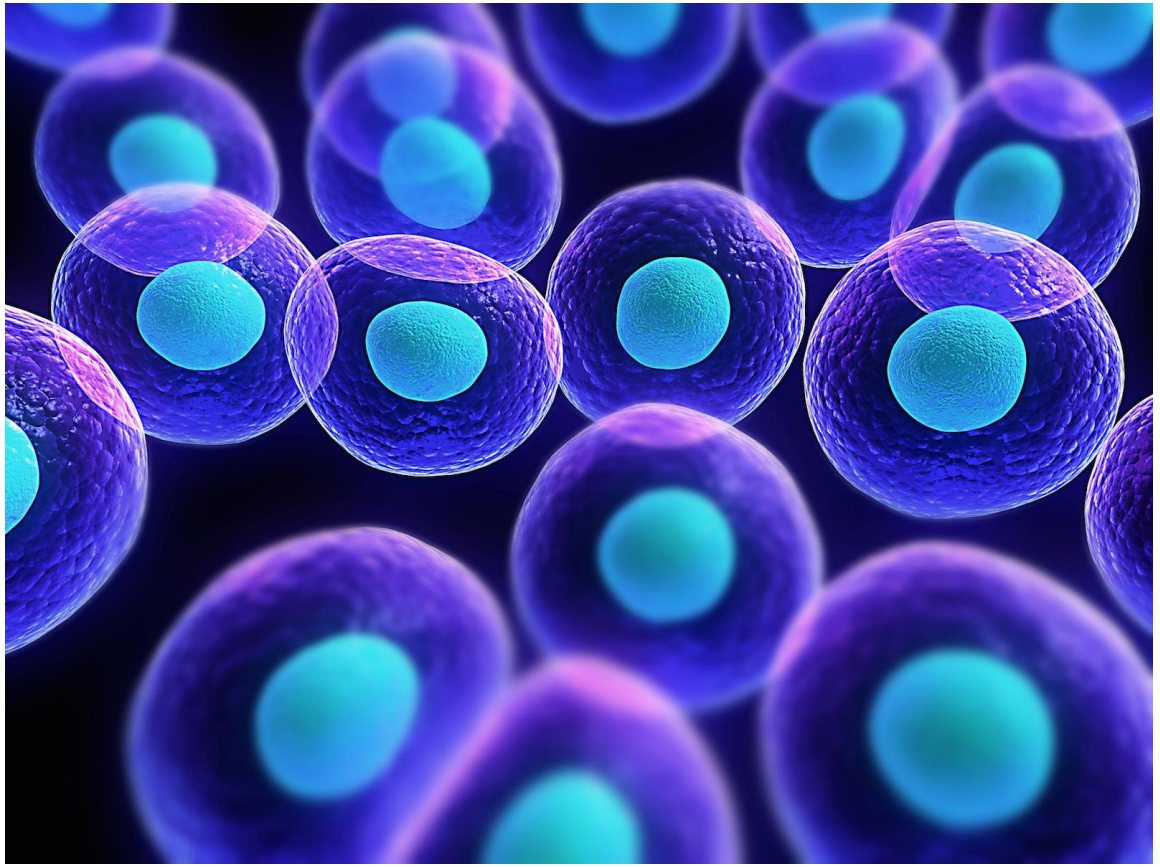
STEM CELLS

Assoc. Prof Dr. Murat Özmen
Assoc. Prof. Dr. Emrah Şefik Abamor

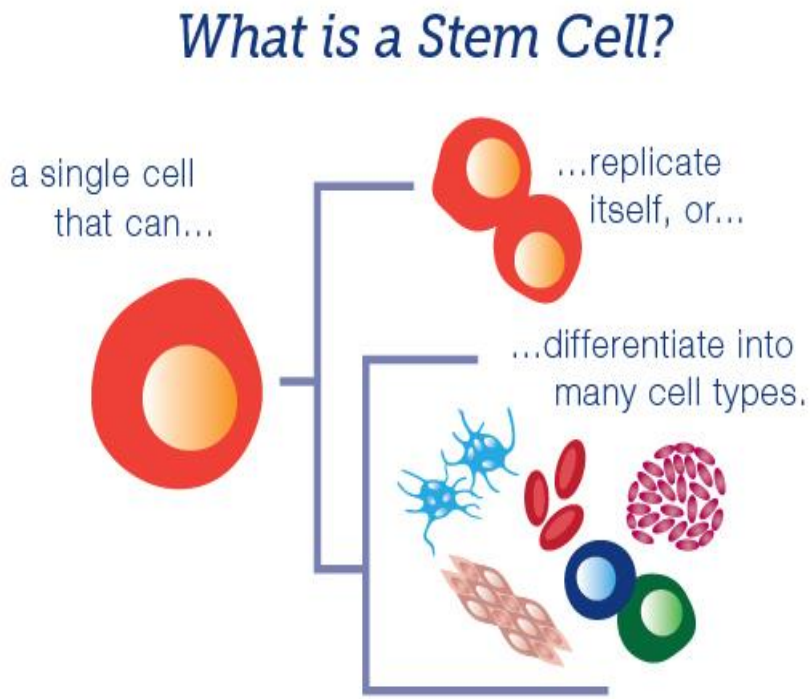


What is a Stem Cell?

- Stem cells are undifferentiated cells that have huge capacity to self-renew.
- All cells in the body come from stem cells



- They have not any function and are not yet specialized.
- They can differentiate into more than one cell type
- Stem cells have ability to divide for indefinite periods
- They should differentiate into appropriate cell types when transplanted to damaged recipients

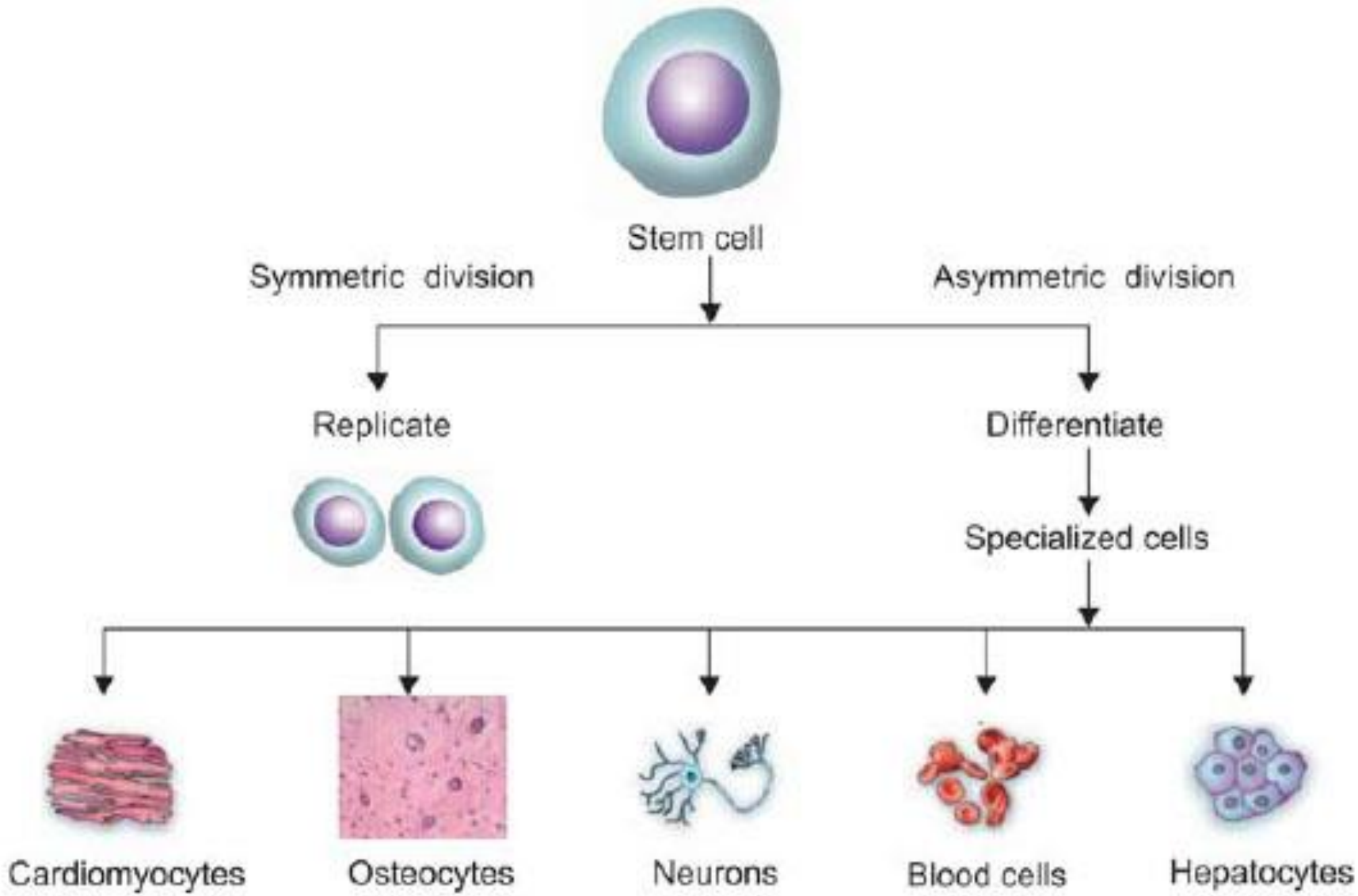


Unique characteristics of Stem Cells

- Stem cells can regenerate
  - Unlimited self renewal through cell division
  - This is unlike muscle, blood or nerve cells – which do not normally replicate themselves
- Stem cells can specialize
  - Under certain physiologic or experimental conditions
  - Stem cells then become cells with special functions such as:
    - Beating cells of the heart muscle
    - Insulin-producing cells of the pancreas

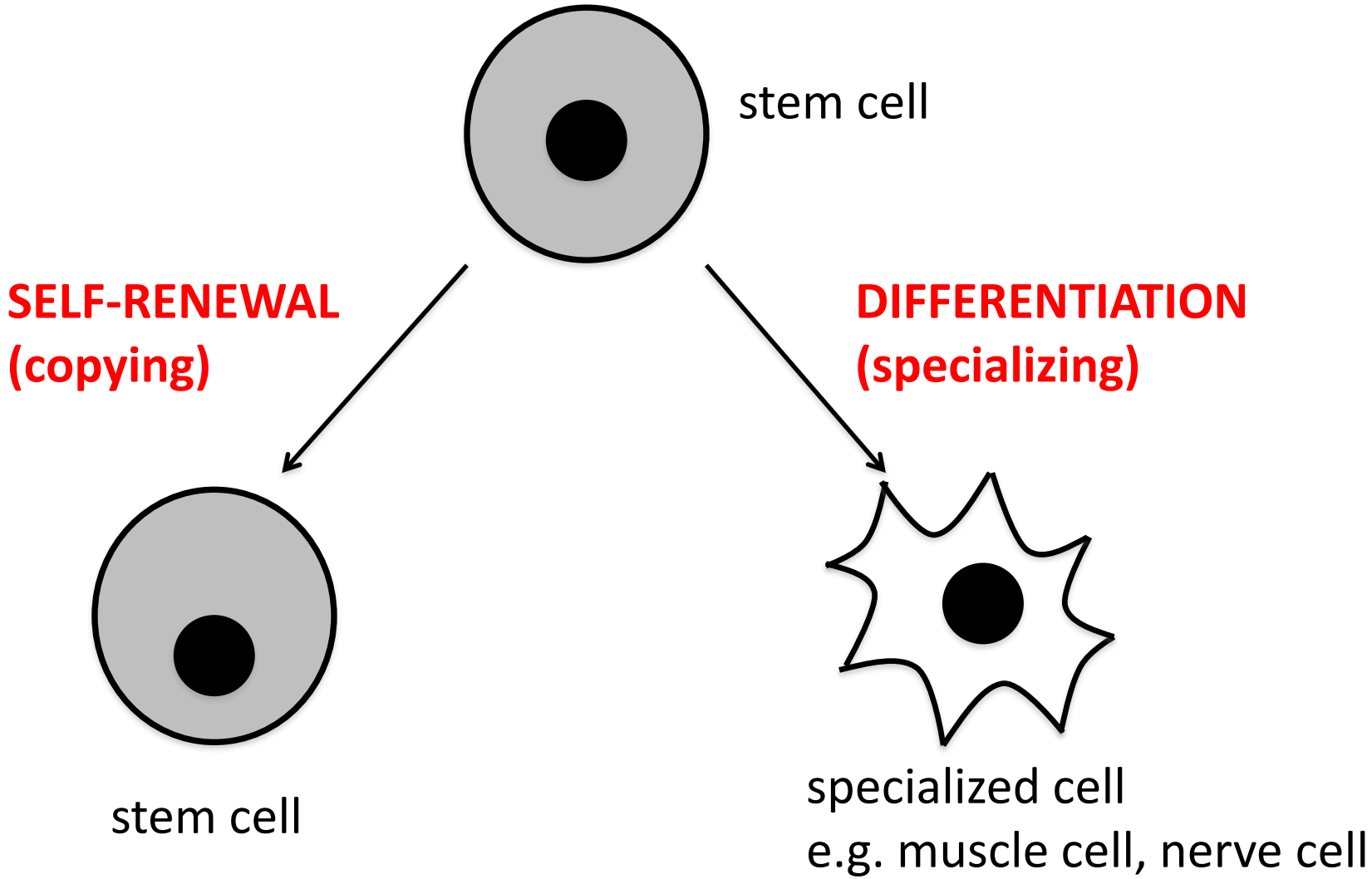
Specialization of Stem Cells: Differentiation

- Differentiation: unspecialized stem cells give rise to specialized (differentiated) cells in response to external and internal chemical signals
  - Internal signals: turn on specific genes causing differential gene expression
  - External signals include:
    - Chemicals secreted by other cells such as growth factors, cytokines, etc.
    - Physical contact with neighboring cells

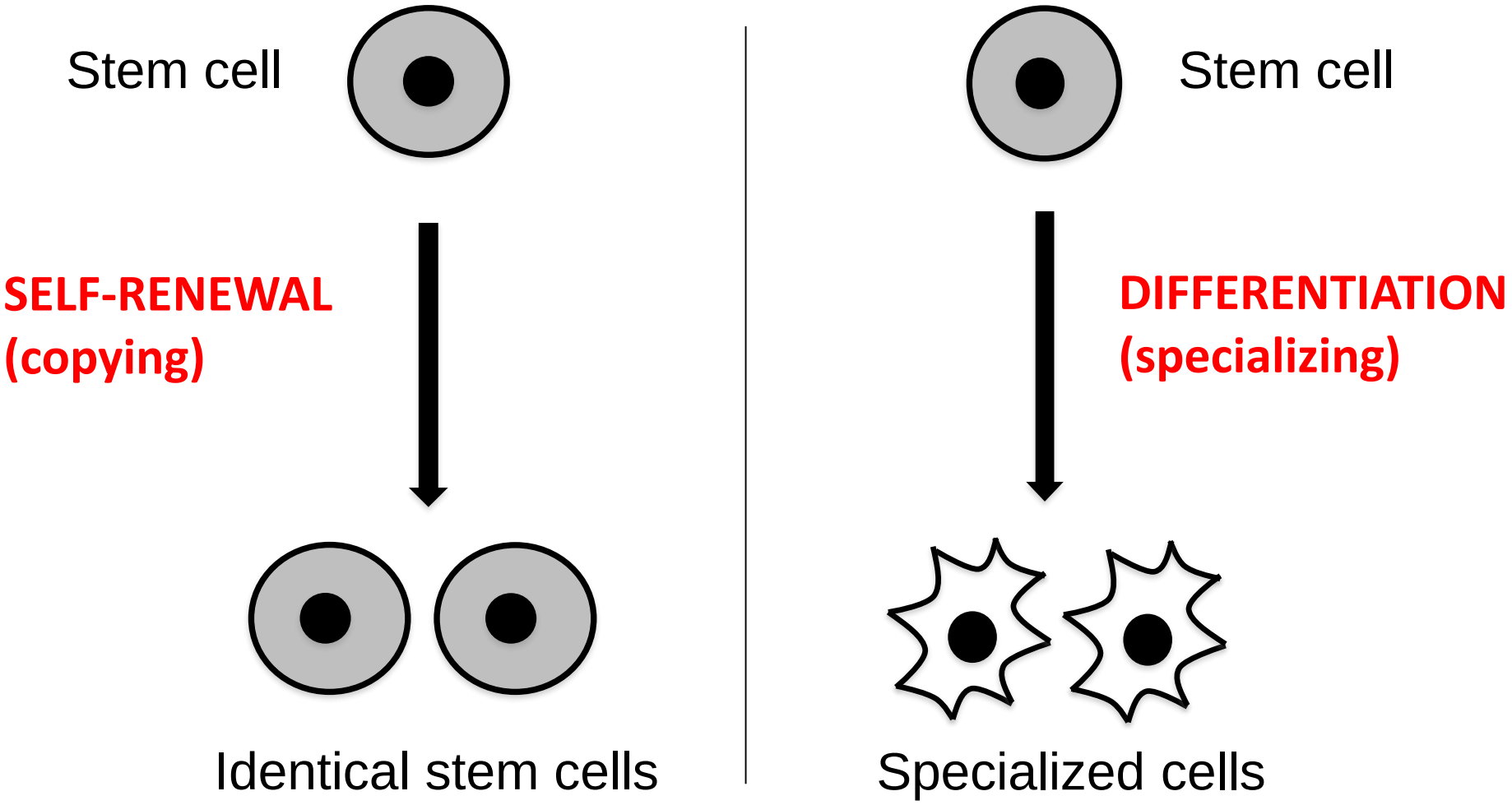

Stem cell
Symmetric division
Asymmetric division
Replicate
Differentiate
Specialized cells
Cardiomyocytes
Osteocytes
Neurons
Blood cells
Hepatocytes

What is a stem cell?

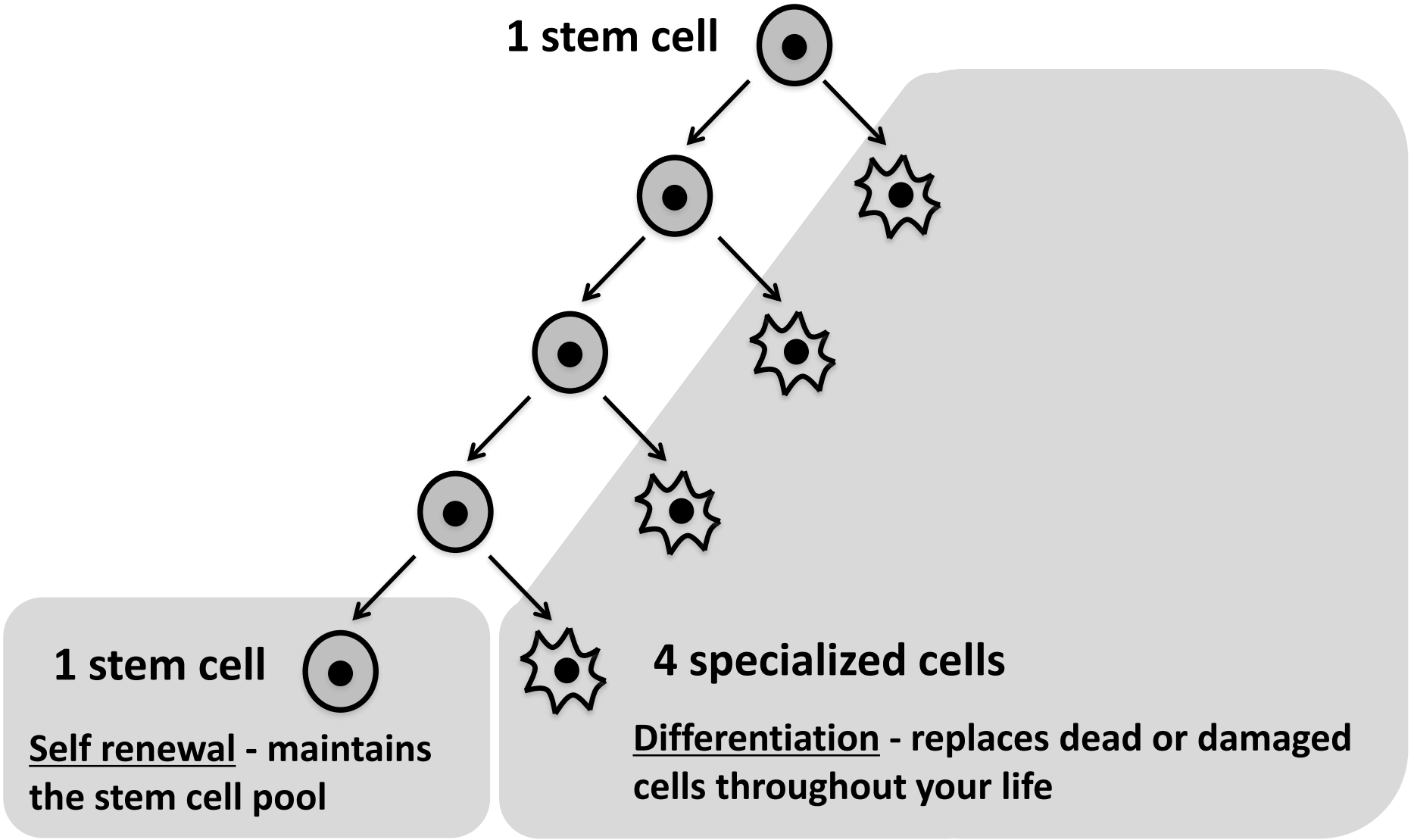
Asymetric Division



What is a stem cell?



Why self-renew AND differentiate?



Stem Cell Classification

Stem cells can be classified by two main criteria;

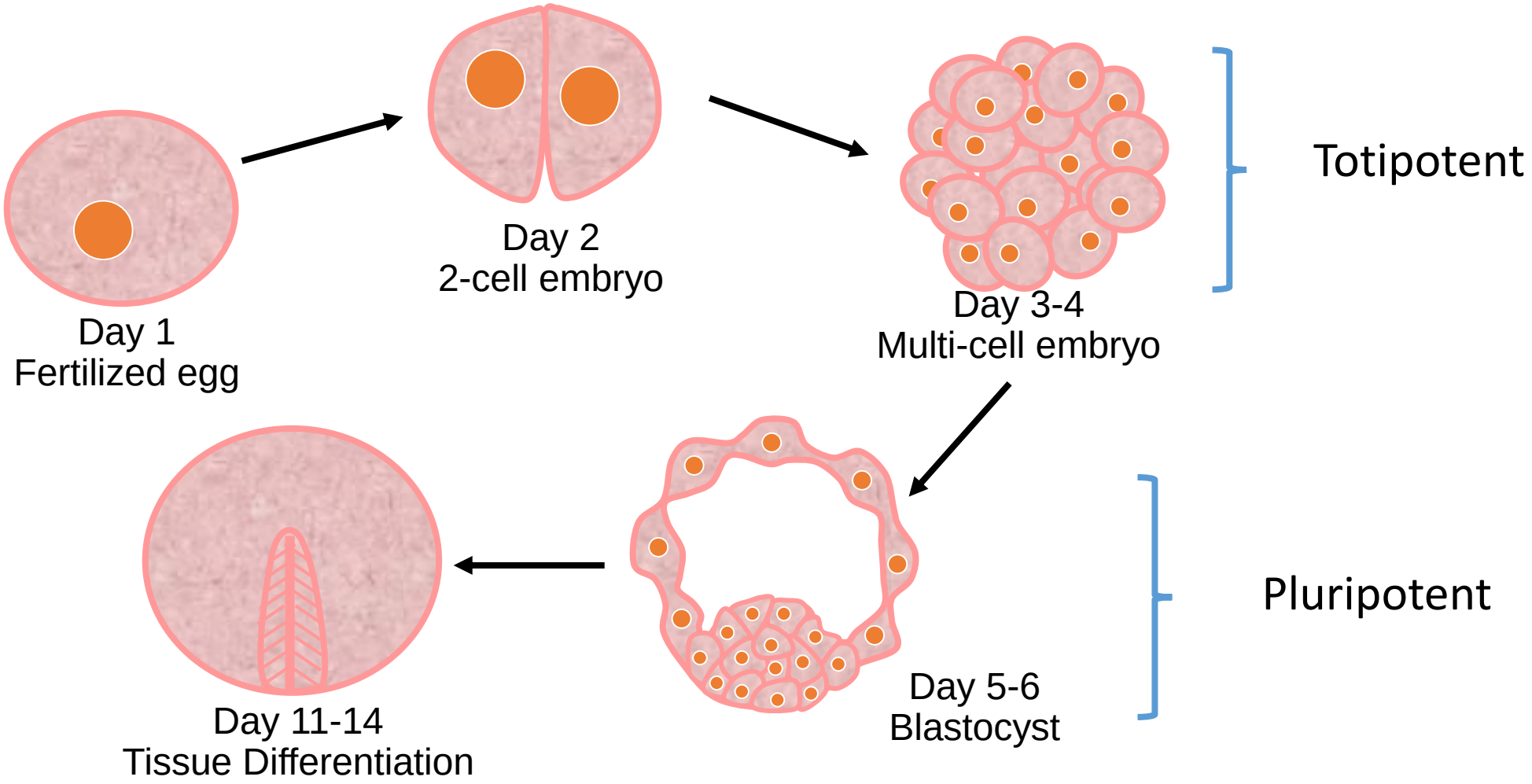
1. Differentiation potential

- Totipotent
- Pluripotent
- Multipotent

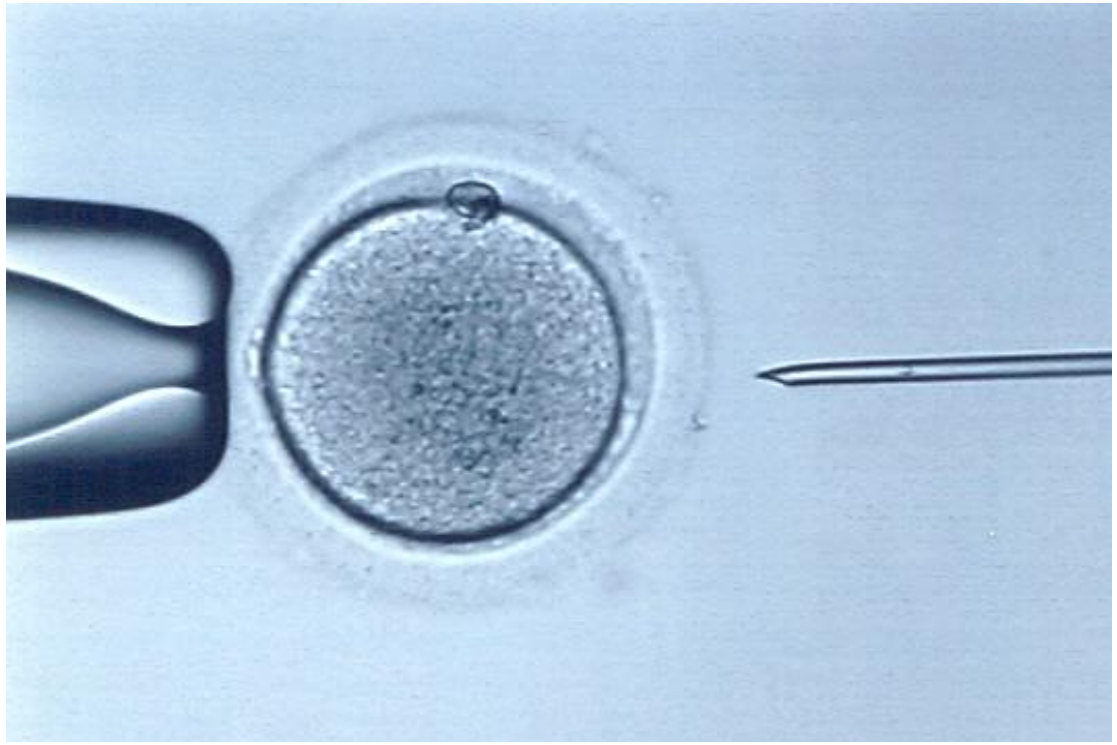
2. Source

- Embryonic stem cells
- Adult stem cells
- Induced Pluripotent stem cells

Stages of Embryogenesis



Intra-Cytoplasmic Sperm Injection

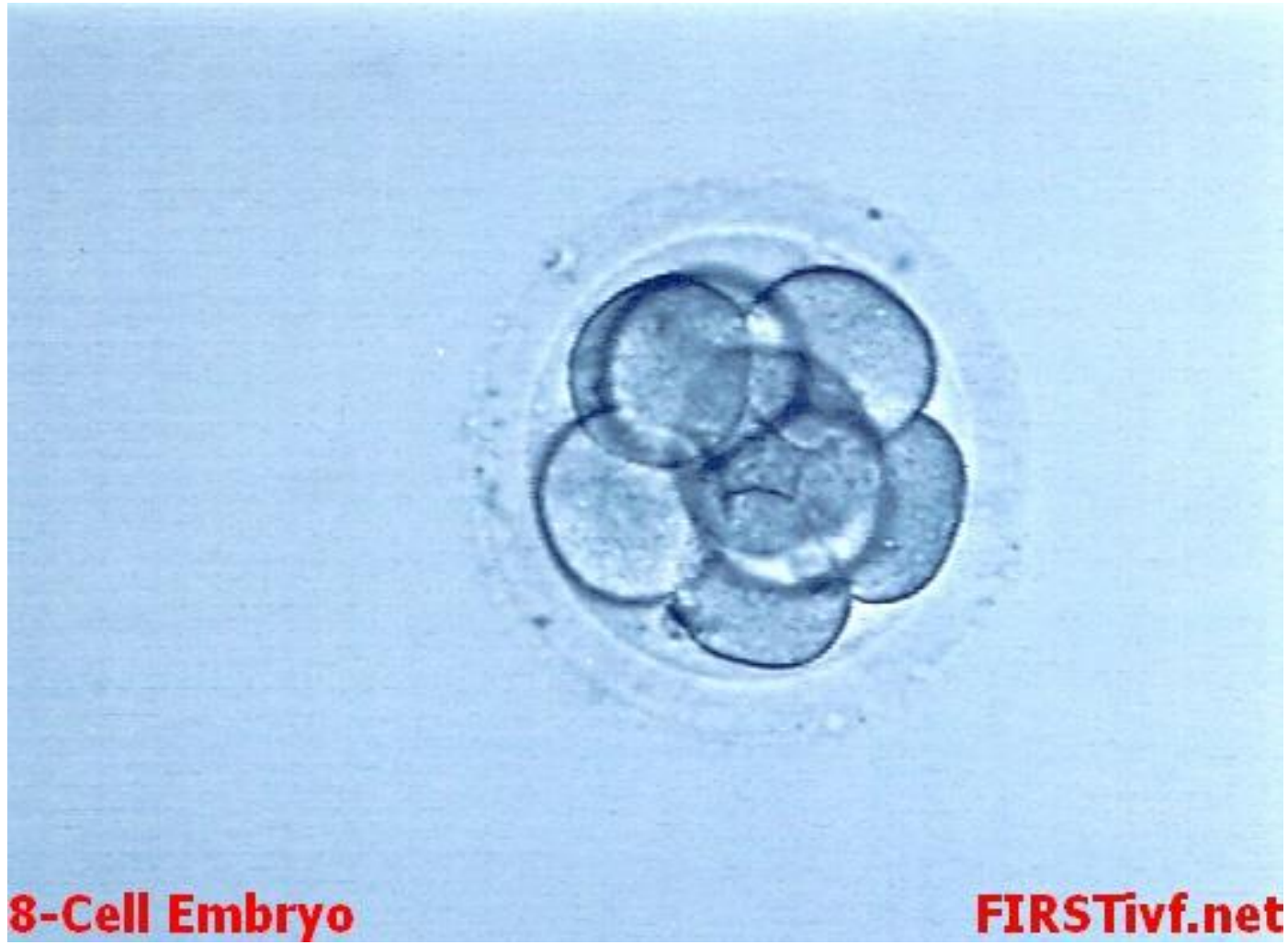


Intra-Cytoplasmic Sperm Injection

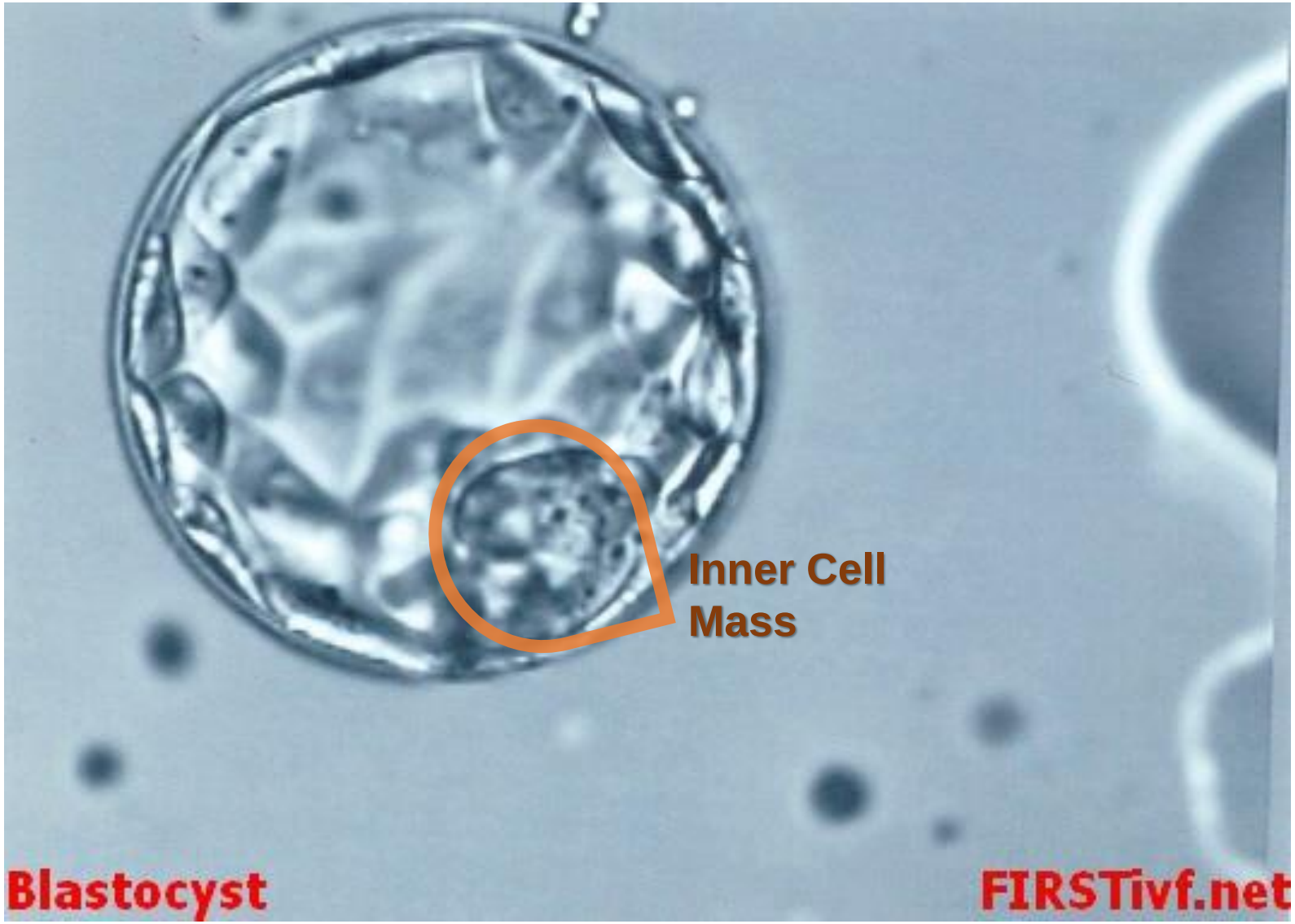

Fertilized Egg
FIRSTivf.net


2-Cell Embryo
FIRSTivf.net


4-Cell Embryo
FIRSTivf.net


8-Cell Embryo
FIRSTivf.net


Morula
FIRSTivf.net


Inner Cell
Mass
Blastocyst
FIRSTivf.net

Classification of stem cells:

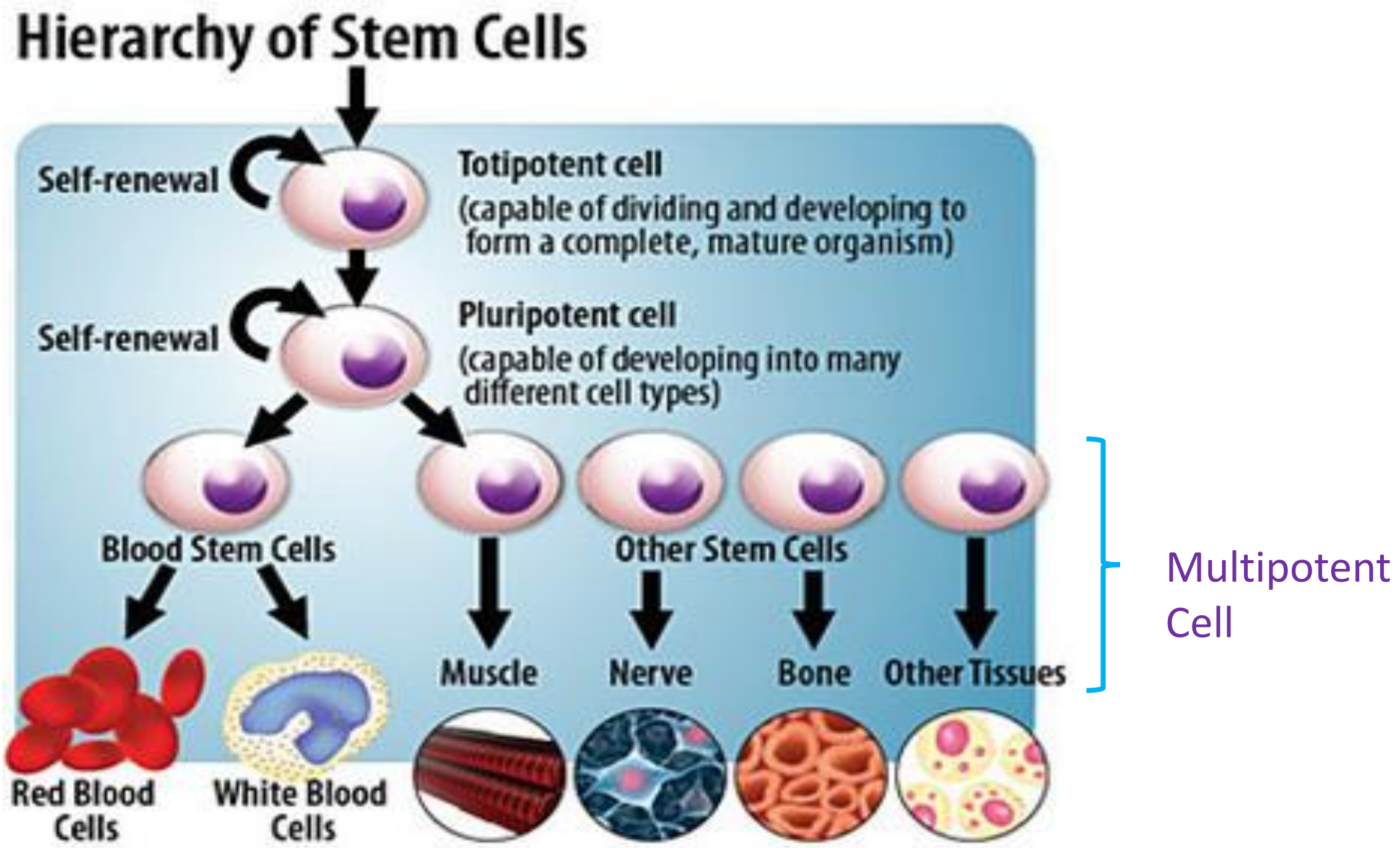
MIDDLE EAST FERTILITY SOCIETY

- According to their potency:

Totipotent	Pluripotent	Multipotent	Progenitor cells
• Embryonic blastomeres. • From fertilization up to 8 cells stage • Give complete organism including extra embryonic tissue.	• From embryonic tissue layers; (ectoderm, endoderm and mesoderm). • Two types: ✓Embryonic stem (ES) ✓Embryonic Germ (EG)	• Differentiate into more than 1 type of specialized cells. • Example: ✓ Hematopoietic stem cells	• Produce terminally differentiated or specialized cells.

Kinds of Stem Cells

Stem cell type	Description	Examples
Totipotent	Each cell can develop into a new individual	Cells from early (1-3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5 to 14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and adult stem cells


Hierarchy of Stem Cells
Self-renewal
Totipotent cell
(capable of dividing and developing to form a complete, mature organism)
Self-renewal
Pluripotent cell
(capable of developing into many different cell types)
Blood Stem Cells
Other Stem Cells
Muscle
Nerve
Bone
Other Tissues
Red Blood Cells
White Blood Cells
Multipotent Cell

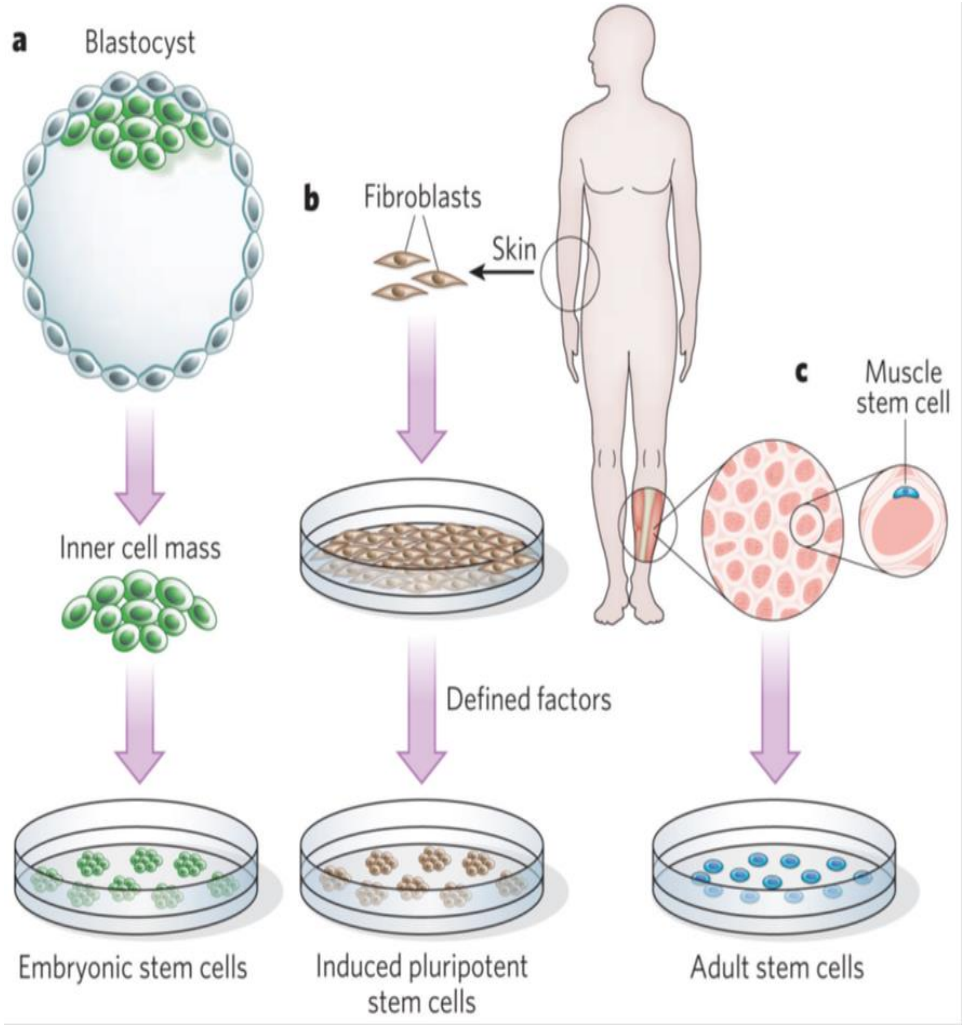
Stem Cell Classification

According to Source

Embryonic Stem Cells

Induced Pluripotent Stem Cells(iPS)

Adult Stem Cells



Embryonic type stem cells

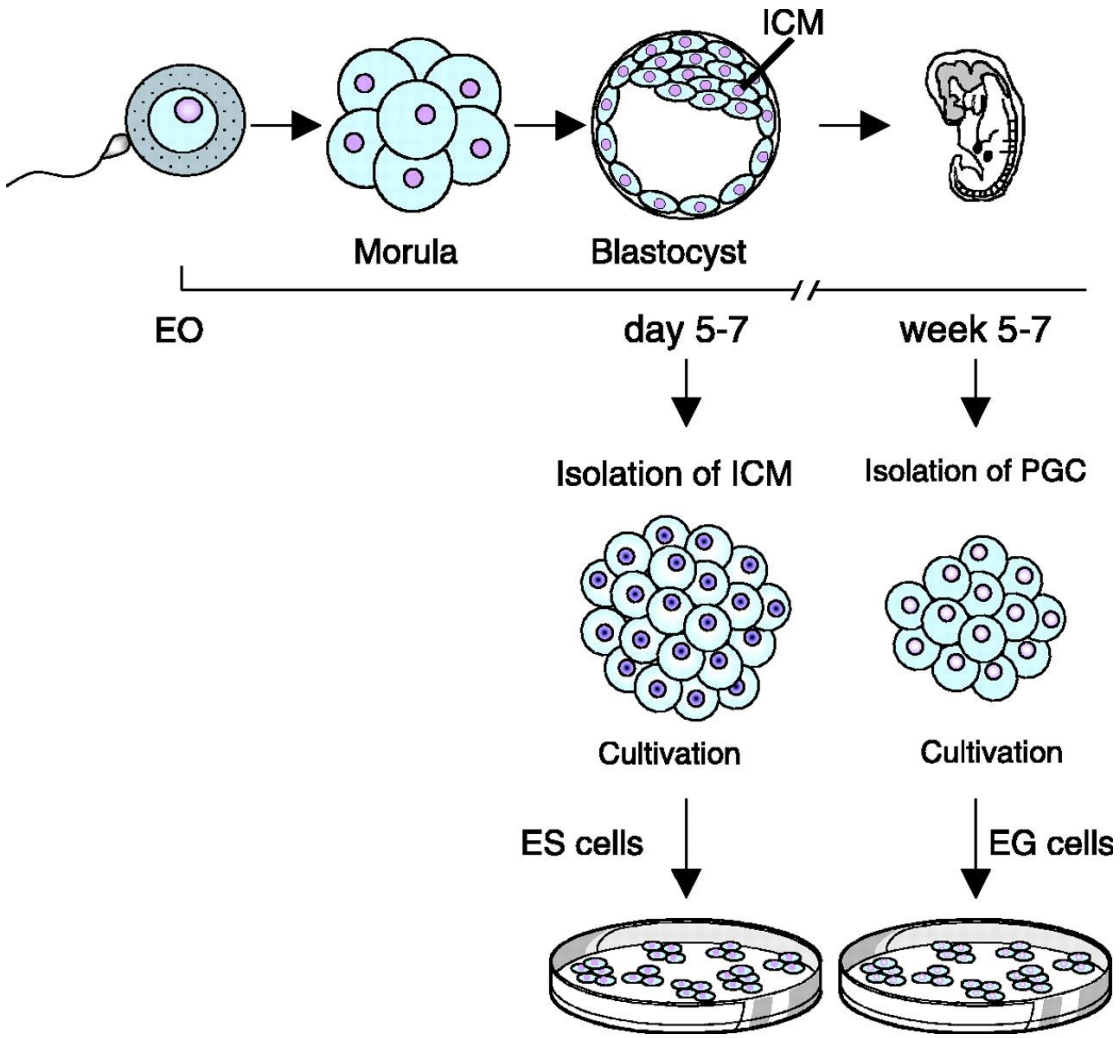
Embryonic stem cells come from a five to six-day-old embryo. They have the ability to form virtually any type of cell found in the human body.

Embryonic germ cells are derived from the part of a human embryo or fetus that will ultimately produce eggs or sperm (gametes).

Embryonic Stem Cells

- Embryonic Stem Cells are derived from embryos that develop from eggs that have been fertilized *in vitro*.
- Embryonic Stem Cells are never derived from eggs fertilized inside of a woman's body.
- The embryos from which Human Embryonic Stem Cells are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst

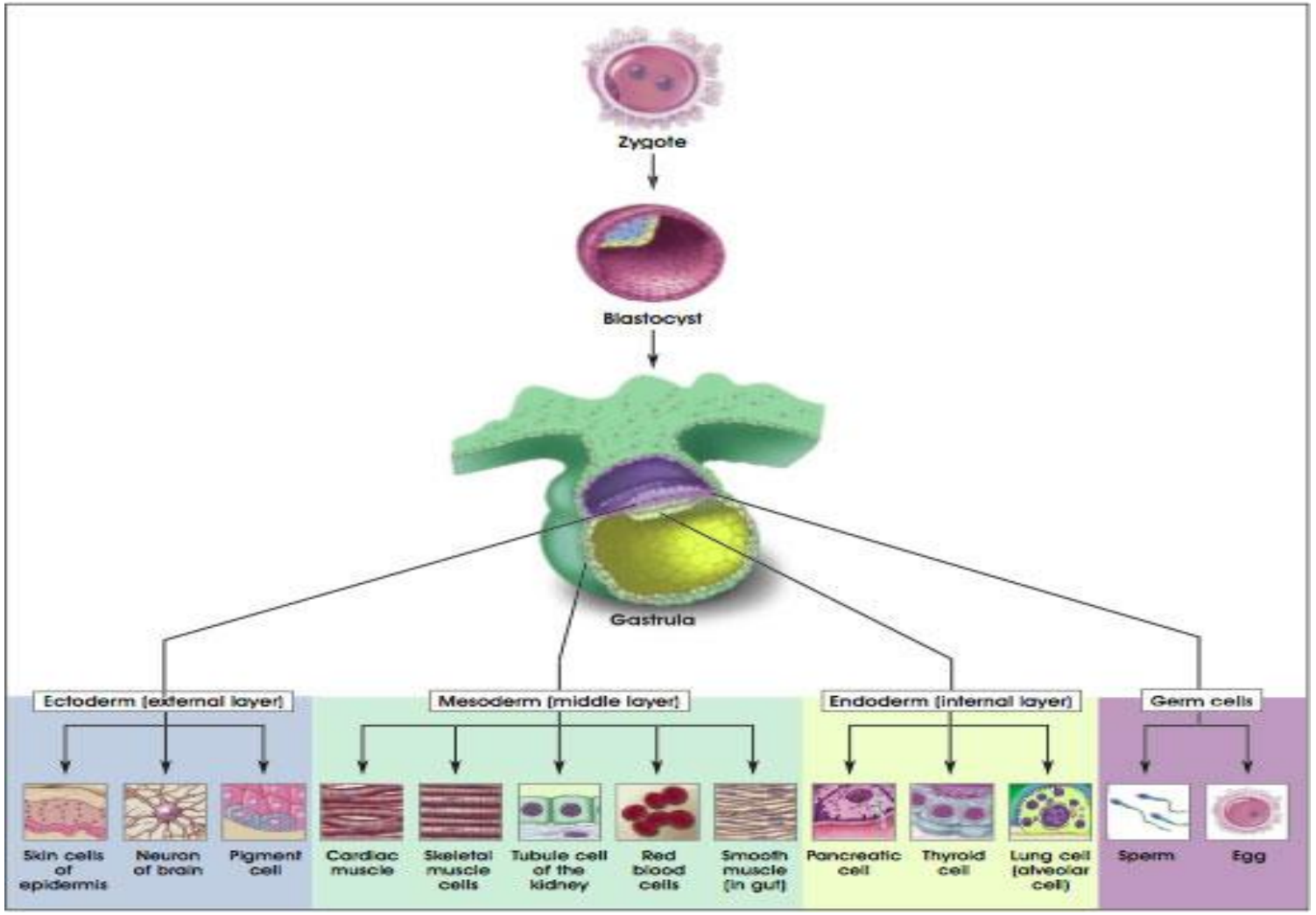
Embryonic Stem Cell Collection



Wobus, A.M. et al. (2005)

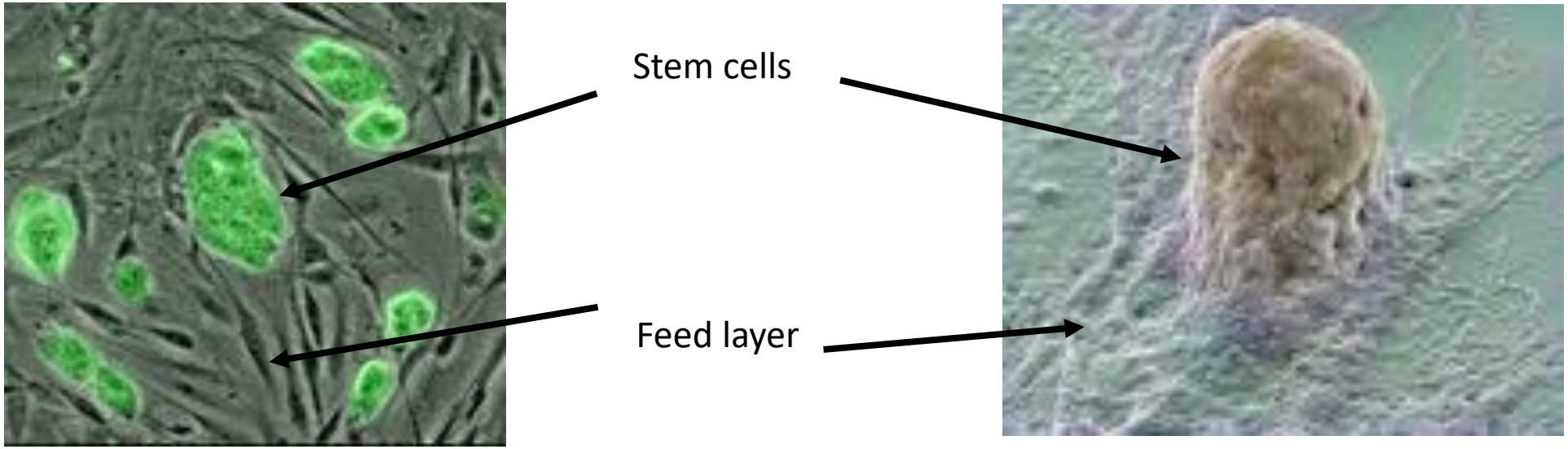
Why are Embryonic Stem Cells So Special?

- Pluripotent stem cells
- Can differentiate into three main tissue: ectoderm, mesoderm and endoderm
- Can form more than 200 types of cells



Embryonic Stem Cells

- In order to control in vitro differentiation of embryonic stem cells, feed layer cells and some cytokines must be used
- Human embryonic stem cells can only preserve their special features by culturing together with mouse embriyonic fibroblast cells and leukemia inhibitory factor (LIF)

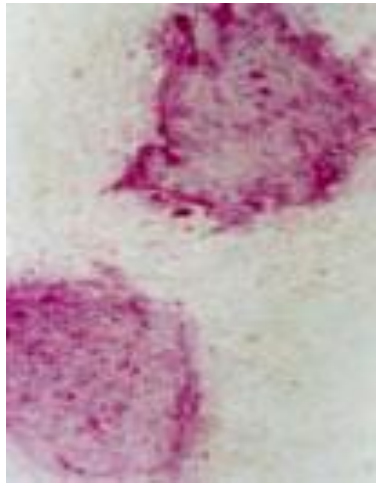


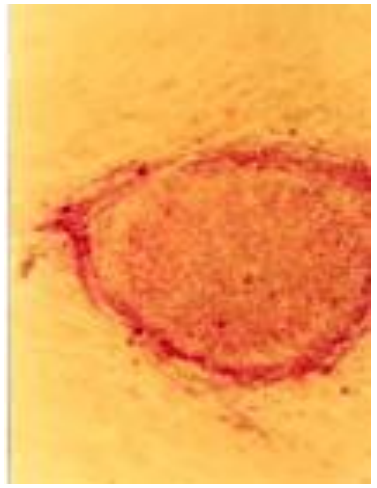
Superior features of ESCs

- Embryonic stem cells are easier to identify, isolate and harvest.
- There are more of them.
- They grow more quickly and easily in the lab than adult stem cells.
- They can be more easily manipulated (they are more plastic)

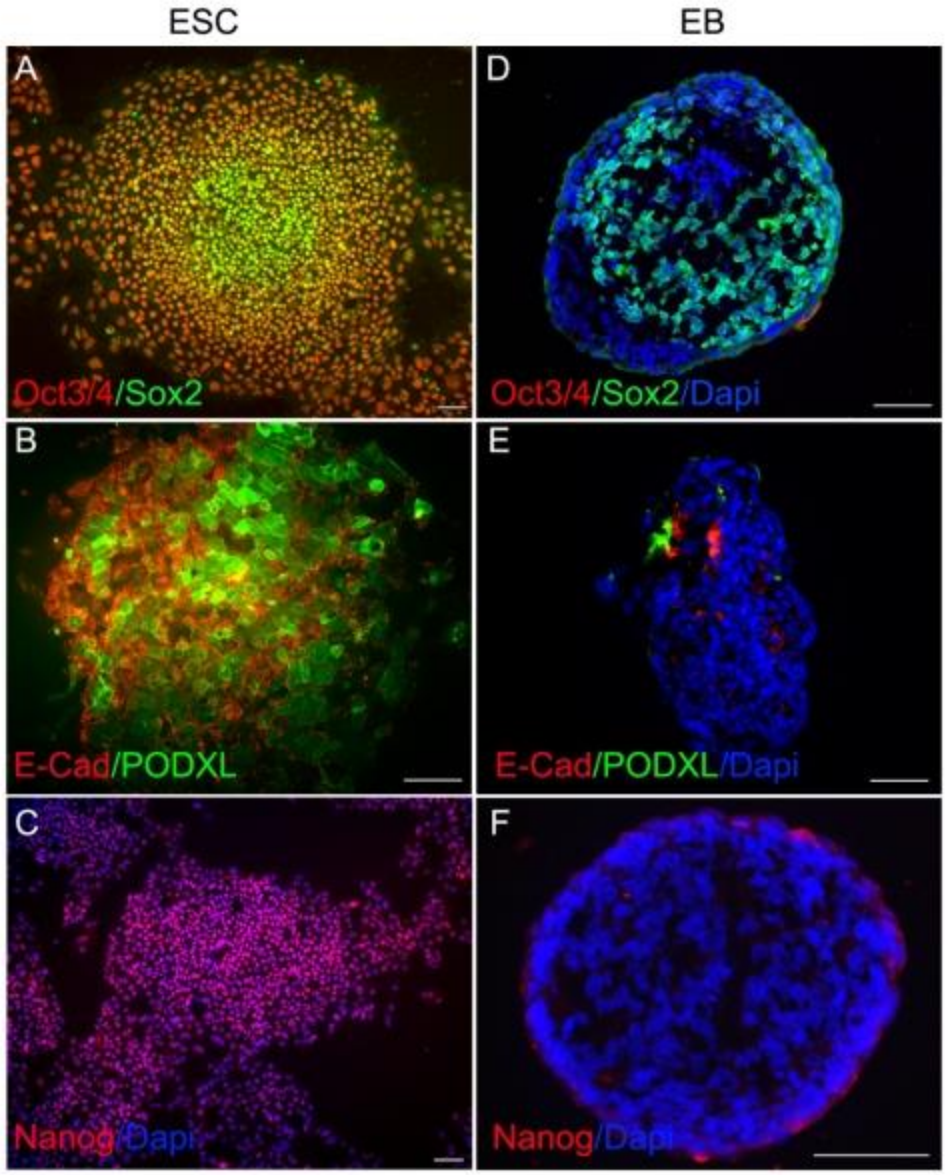
Characterization of Embryonic Stem Cells

- Embryonic stem cells possess higher nucleus/cytoplasm volume ratio in contrast to somatic cells within the body
- They include an apparent pronucleus structure
- These cells compose three dimensional (3D) colonies during in vitro cultivation with feed-layer cells
- For characterization of embryonic stem cells, researchers benefit from structures special to undifferentiated cells.
- Cell surface markers: SSEA-3, SSEA-4
- Proteoglycans: TRA-1-60, TRA-1-81
- Alkaline phosphatase reaction
- Oct-4, E-Cad, Nanog, SOX2, PODXL expression
- High telomerase activity





Alkaline phosphatase staining



A, B and C stem cells

D, E and F differentiated embryoid bodies

Comparison of embryonic and adult stem cells

- **Advantages of Embryonic Stem Cell**

  1. Flexible – appear to have the potential to make any cell.

  2. Immortal – one embryonic stem cell line can potentially provide an endless supply of cells with defined characteristics.

  3. Availability – embryos from *in vitro* fertilization clinics.

Disadvantages of Embryonic Stem Cell

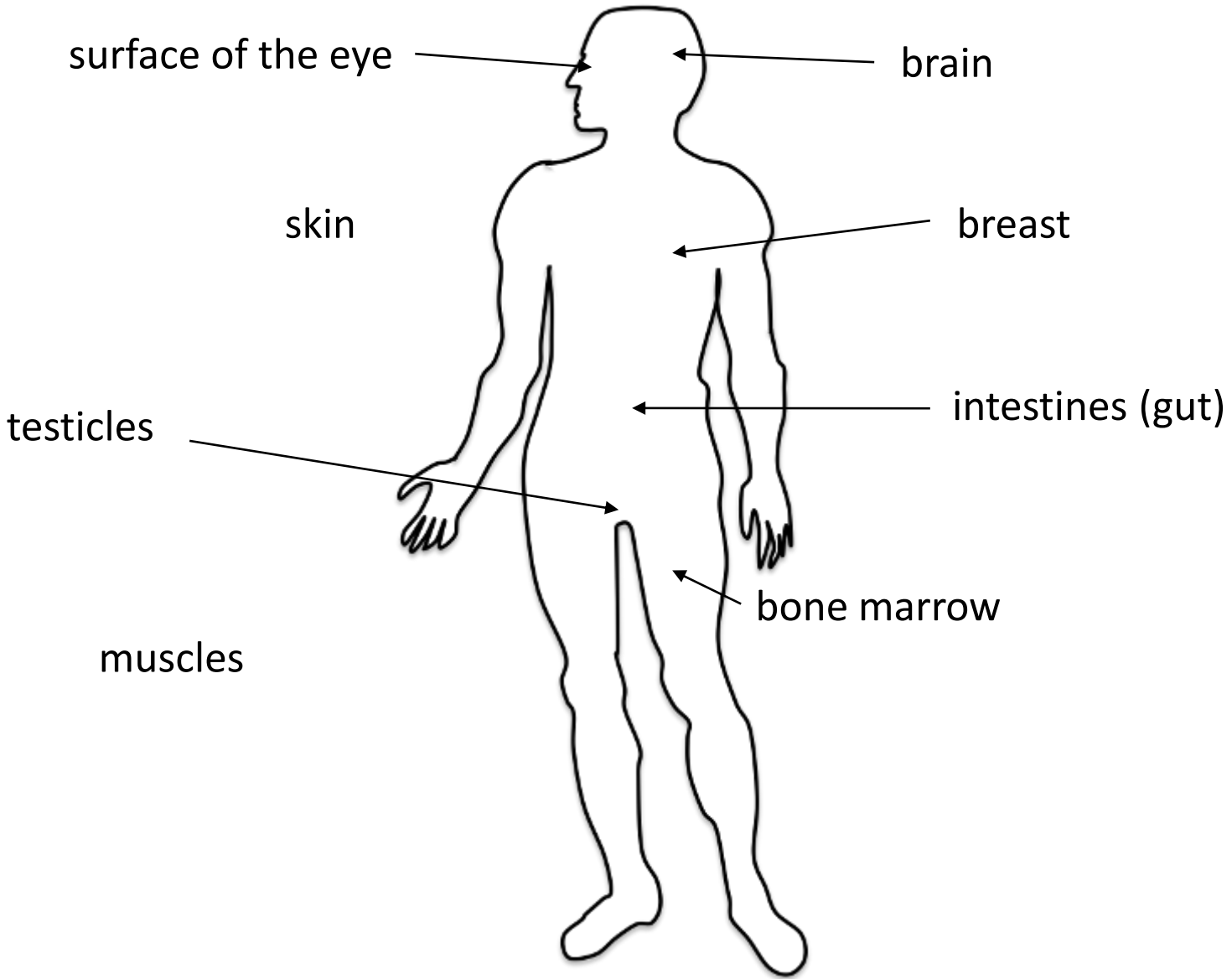
1. Difficult to differentiate uniformly and homogeneously into a target tissue.
2. Immunogenic - embryonic stem cells from a random embryo donor are likely to be rejected after transplantation
3. Tumorigenic - capable of forming tumors or promoting tumor formation.
4. Destruction of developing human life.

Adult Stem Cells

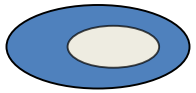
- An adult stem cell is an **undifferentiated** (or partially-differentiated) cell found in tissues and organs
- They can **self-renew** and **differentiate** to become most or all of the **specialized** cell types within their specific tissue lineage.
- Adult stem cells
  - Maintain cell populations
  - Help you heal
  - Play a role in aging

Tissue stem cells:

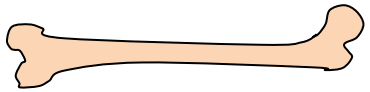
Where we find them



Tissue stem cells:
What they can do



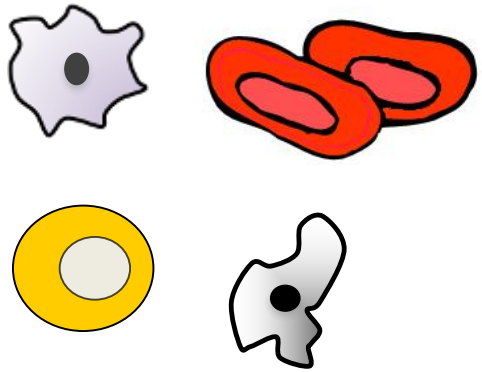
blood stem cell



found in
bone marrow

MULTIPOTENT

differentiation



only specialized types of blood cell:
red blood cells, white blood cells,
platelets

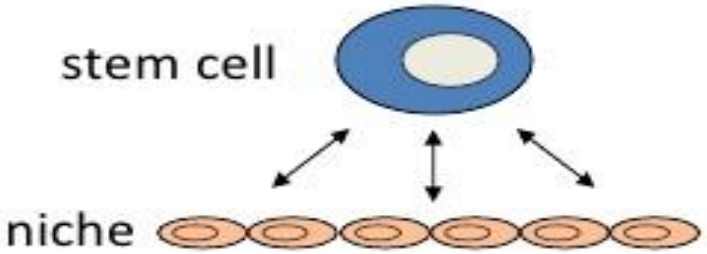
Location of Adult Stem Cells

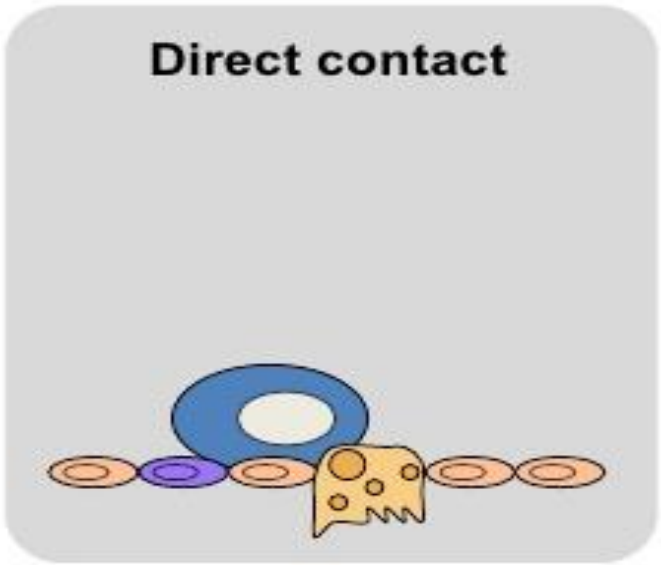
- Adult stem cells and progenitor cells reside through out your body
- These stem cells reside in a specific area of each tissue called the "stem cell niche"
- This niche is a particular microenvironment that fosters the growth of resident stem cells
- Mutations in cells, signals they receive, and changes in the microenvironment can activate a stem cell

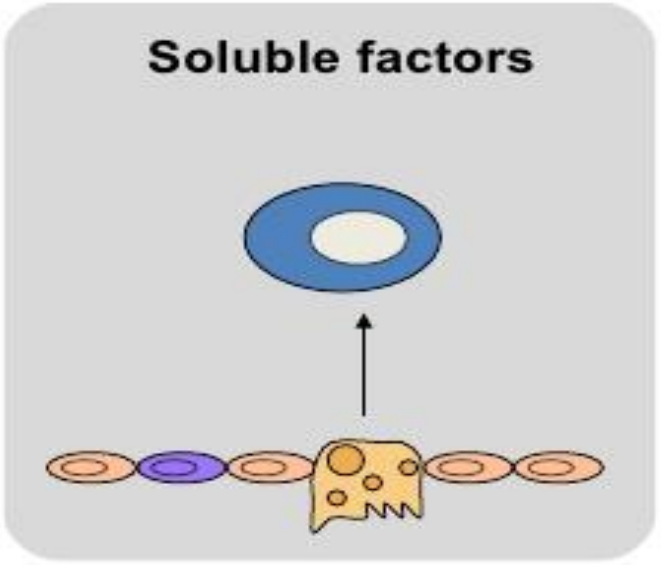
Stem cell niches

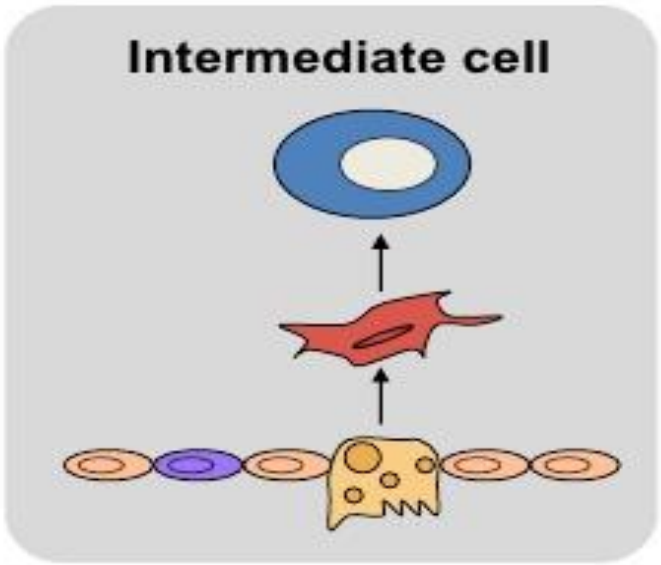
Niche

Microenvironment around stem cells that provides support and signals regulating self-renewal and differentiation









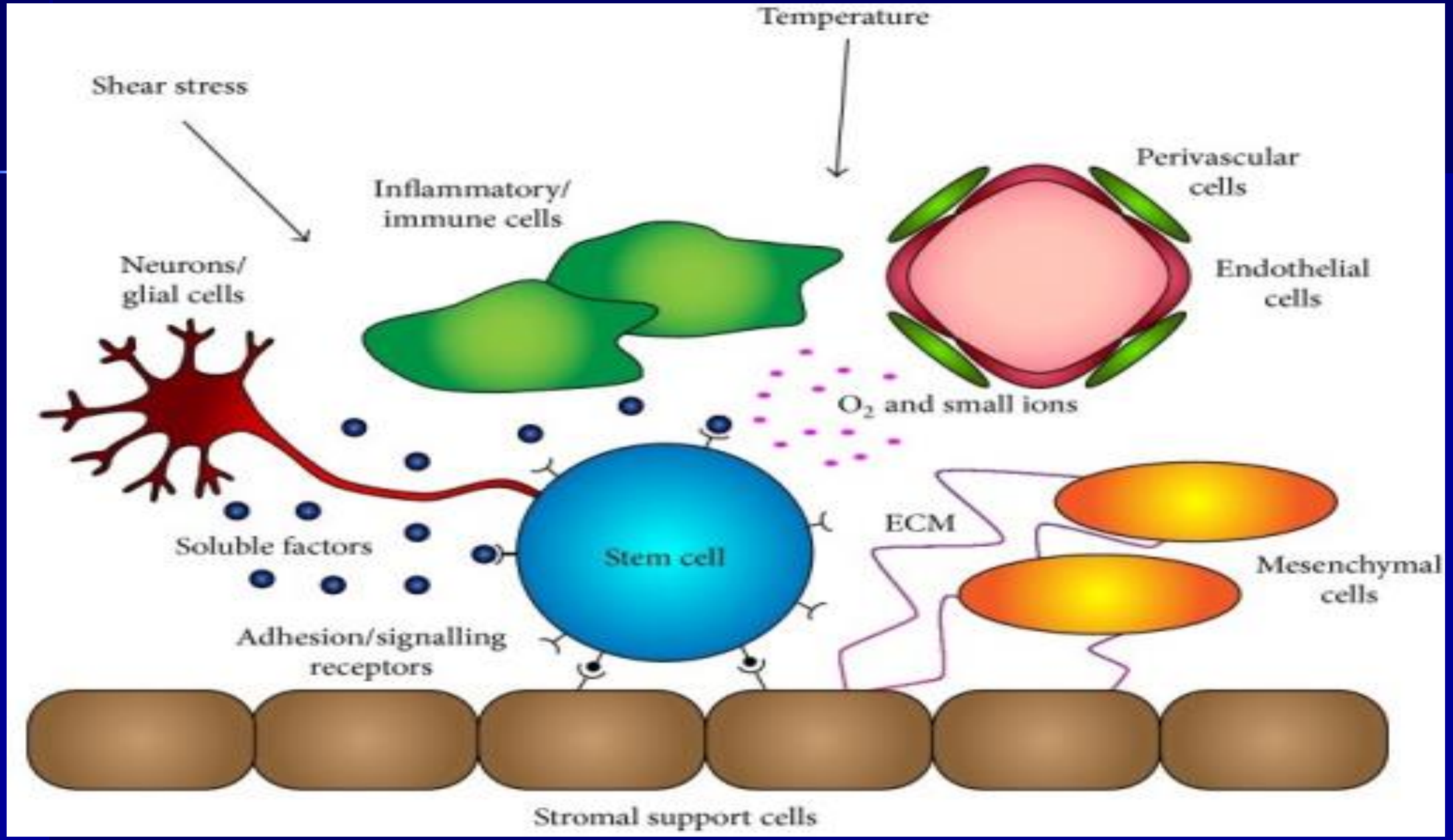
Centre for Regenerative Medicine

MRC

THE UNIVERSITY of EDINBURGH

EuroStemCell

SEVENTH FRAMEWORK PROGRAMME


Temperature
Shear stress
Perivascular cells
Inflammatory/ immune cells
Neurons/ glial cells
Endothelial cells
O_2 and small ions
ECM
Soluble factors
Stem cell
Mesenchymal cells
Adhesion/signalling receptors
Stromal support cells

Types of Adult Stem Cells

1. Hematopoietic stem cells: blood and immune system
2. Mesenchymal stem cells: bone, cartilage, fat, muscle, tendon/ligament
3. Neural stem cells: neurons, glial cells
4. Epithelial stem cells: skin, linings

Hematopoietic stem cells

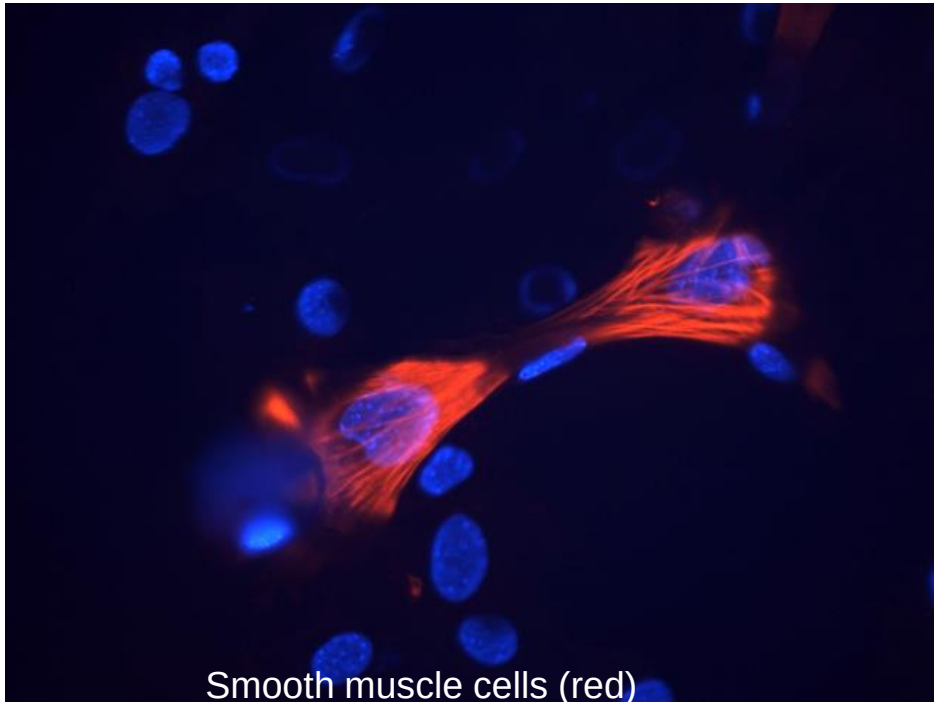
Give rise to all the blood cell types:

- **Myeloid** (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells)
- **Lymphoid** (T-cells, B-cells, NK-cells)

Found in the bone marrow from very early on in development, as well as in umbilical cord blood, peripheral blood and placental tissue

Mesenchymal stem cells

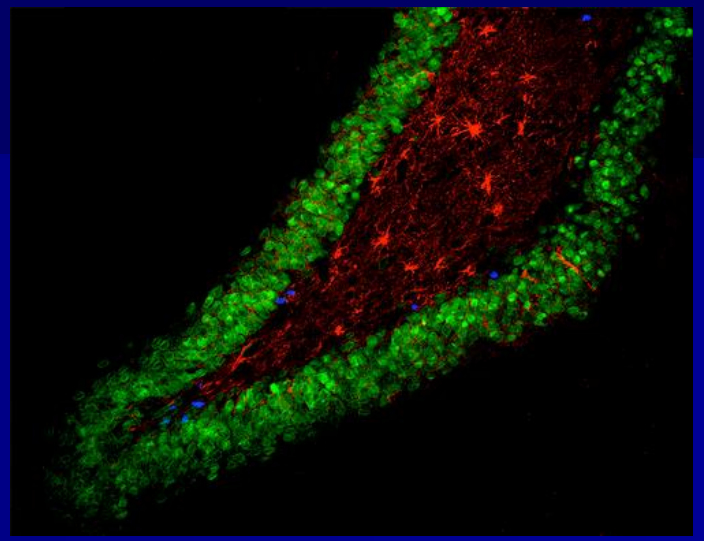
- These stem cells will differentiate into:
  - cartilage cells (chondrocytes)
  - muscle cells (myocytes)
  - fat cells (adipocytes)
  - tendons, ligaments, and connective tissue (epithelial cells including osteoblasts)
- These cells are located throughout the body
  - Bone marrow, fat, and cord blood are easiest to isolate

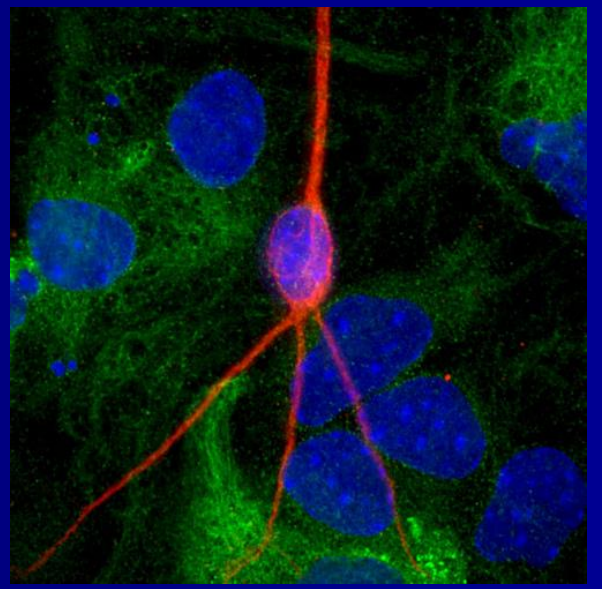


Smooth muscle cells (red)

Neural stem cells

- They are located in:
  - *Subventricular zone* lining the lateral ventricles, where they give rise to newly-born neurons that migrate to the olfactory bulb via the rostral migratory stream
  - *Subgranular zone,* part of the dentate gyrus of the hippocampus
- Neural stem cells (also called Neural precursor cells) give rise to neurons, oligodendrocytes, and astrocytes



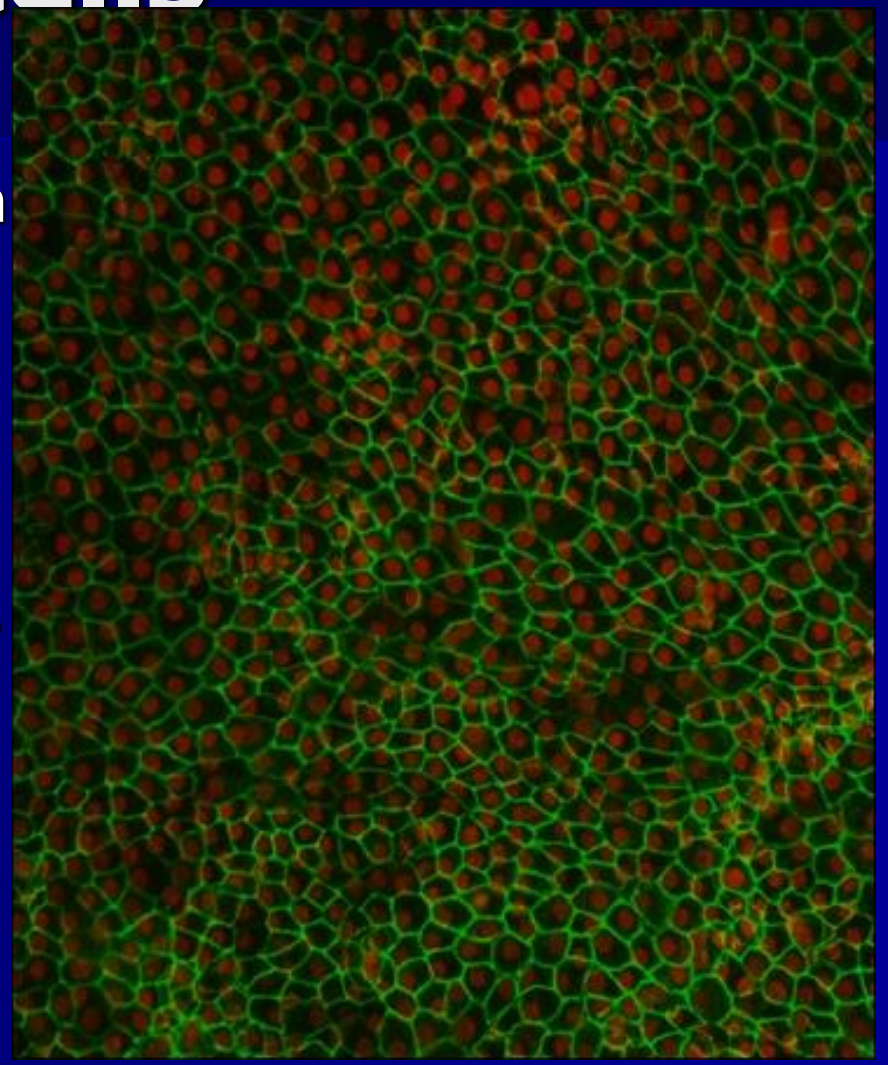


Top: Section of the hippocampus, blue dots are neural stem cells

Left: Mature neuron (red)

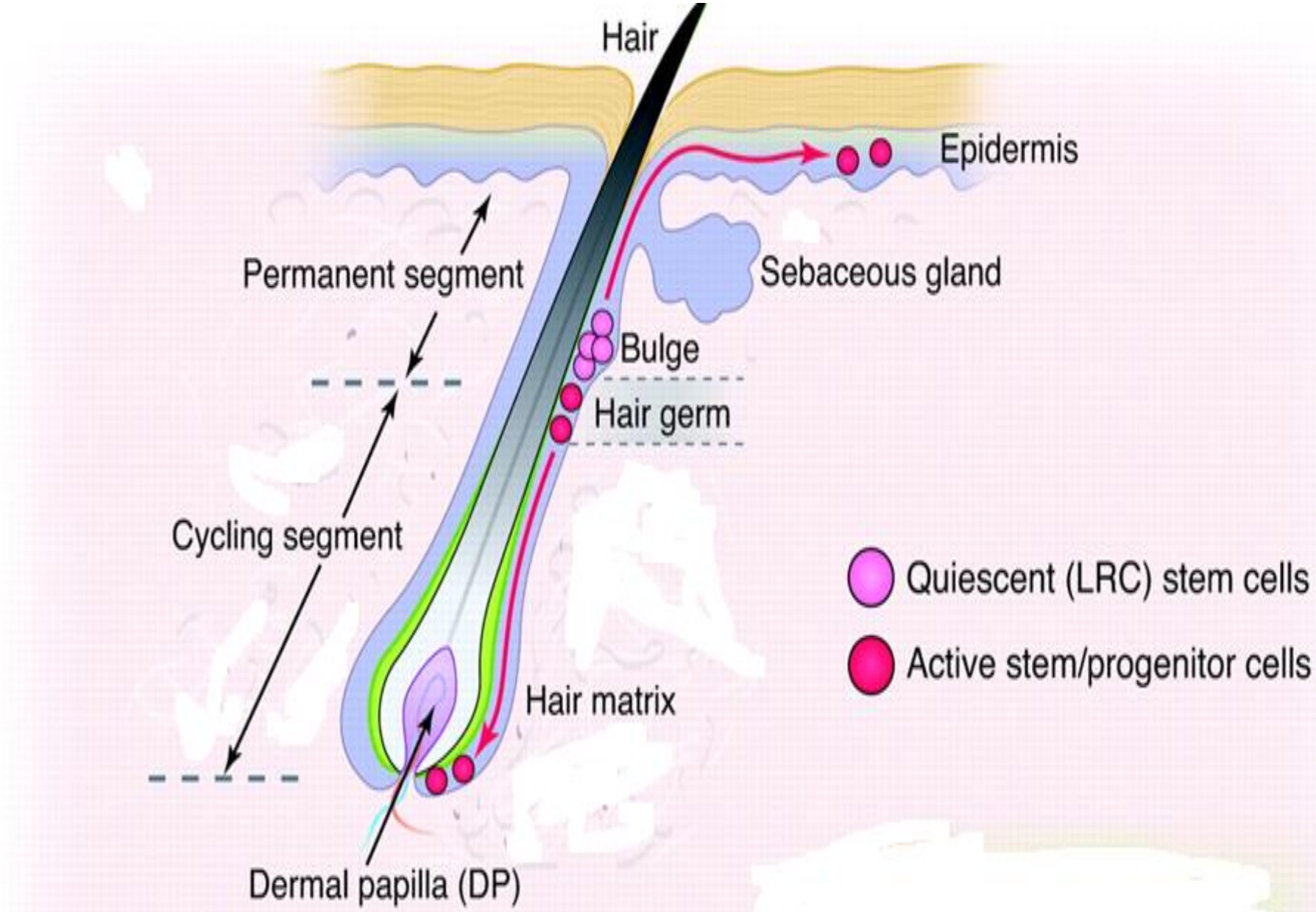
Epithelial stem cells

- Give rise to **epithelial cells** which constitute 60 percent of the differentiated cells in the body.
- Responsible for covering the internal (i.e. intestinal lining) and external surfaces (i.e. skin) of the body, including the lining of vessels, glands, and other cavities.
- Epithelial stem cells are also found in the bulge region of the hair follicle



Retinal pigment epithelial cells

Stem cell locations in hair follicle



Advantages of Adult Stem Cell

1. Adult stem cells from bone marrow and umbilical cords appear to be as **flexible as the embryonic type**
2. **Somewhat specialized -** inducement may be simpler.
3. **Not immunogenic -** recipients who receive the products of their own stem cells will not experience immune rejection.
4. **Relative ease of procurement** - some adult stem cells are easy to harvest (skin, muscle, marrow, fat)
5. **Non-tumorigenic**-tend not to form tumors.
6. **No harm done** to the donor.

Disadvantages of Adult stem cells

1. **Limited quantity** - can sometimes be difficult to obtain in large numbers.
2. **Finite** - may not live as long as embryonic stem cells in culture.
3. **Less flexible** - may be more difficult to reprogram to form other tissue types

Why are adult stem cells preferable to embryonic stem cells?

- Adult stem cells are naturally exist in our bodies, and they provide a natural repair mechanism for many tissues.
- They belong in the microenvironment of an adult body, while embryonic stem cells belong in the microenvironment of the early embryo, where they tend to cause tumors and immune system reactions.

Induced Pluripotent Stem Cells (IPSCs)

- Induced pluripotent stem cells (iPSCs) are pluripotent stem cells generated from adult cells by reprogramming.
- iPSCs have the same properties as embryonic stem cells, and therefore self-renew and can differentiate into all cell types of the body except for cells in extra-embryonic tissues such as the placenta.

Induced Pluripotent Stem Cells (IPSCs)

- It was reported that only four transcription factors (Oct4, Sox2, Klf4, and c-Myc) were required to reprogram mouse fibroblasts (cells found in the skin and other connective tissue) to an embryonic stem cell–like state by forcing them to express genes important for maintaining the defining properties of ESCs

Induced Pluripotent Stem Cells (IPSCs)

- The primary advantages of iPSCs compared to other stem cells are:

a) iPSCs can be created from the tissue of the same patient that will receive the transplantation, thus avoiding immune rejection,

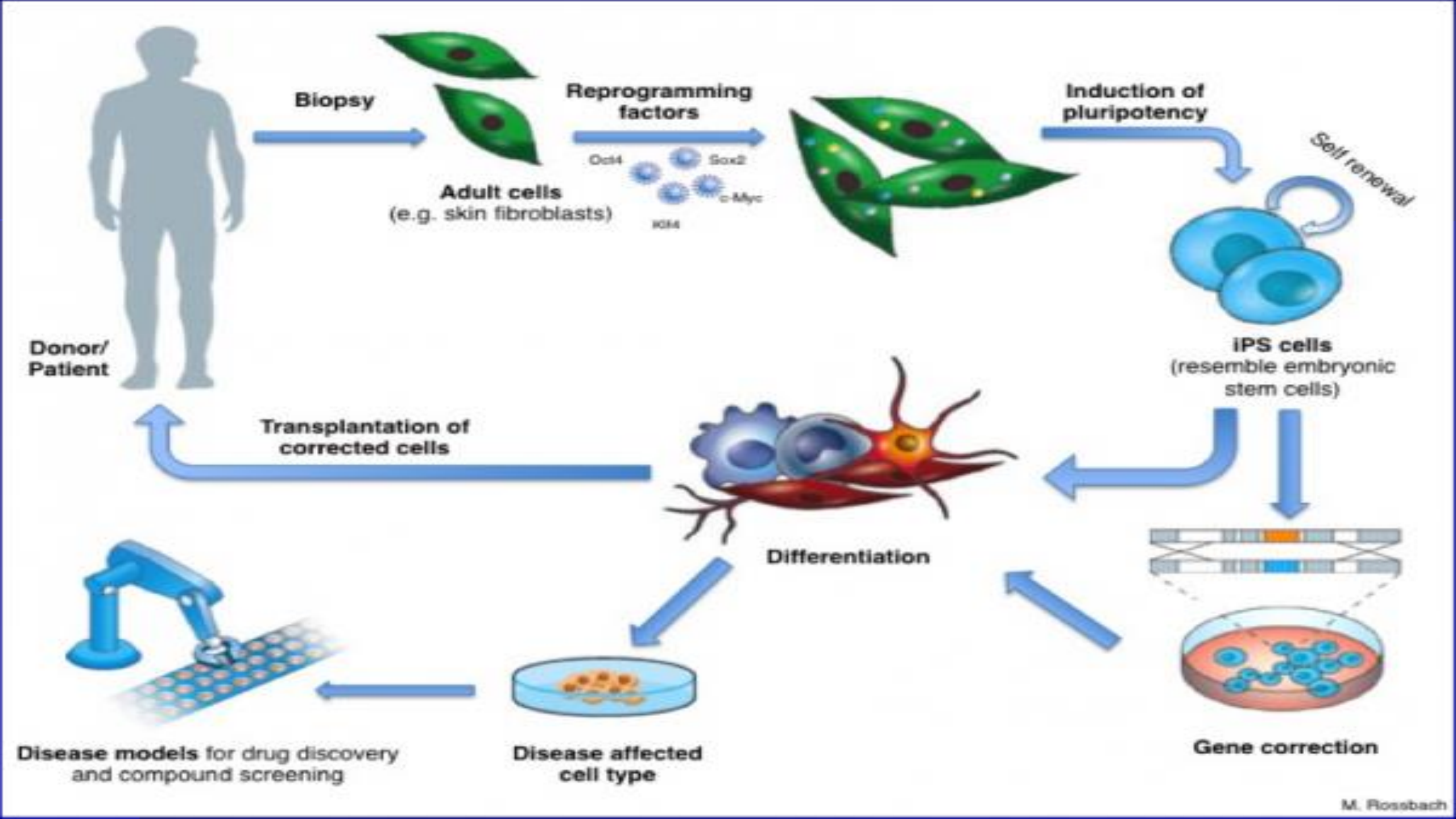
b) the lack of ethical implications because cells are harvested from a willing adult without harming them.

Induced Pluripotent Stem Cells (IPSCs)

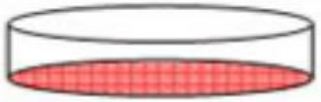
- Four key genes are transfered into fibroblasts by retroviruses
- Retroviruses can carry target DNA that is inserted into a host cell's genome upon injection, making them ideal for incorporating the four genes into target cells.

Summary of the Process – Yamanaka Approach

- Human fibroblast isolation from patient's skin
- Retroviral/Lentiviral production of the reprogramming factors (Oct4, Sox2, Klf4 & c-Myc) – production, collection & titration
- Viral transduction of human fibroblasts (overnight)
- Replace media with fresh fibroblast media. Fibroblast media is replaced every 2 days up to 1 week post-transduction
- Passage transduced fibroblasts onto mouse embryonic feeders (MEFs) and culture in human ES cell media for 3-4 weeks until hiPS cell colonies form.

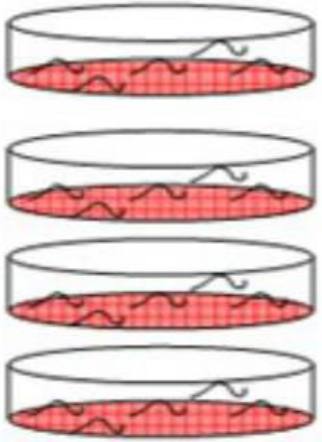

Biopsy
Adult cells
(e.g. skin fibroblasts)
Reprogramming factors
Oct4
Sox2
c-Myc
Induction of pluripotency
Self renewal
iPS cells
(resemble embryonic stem cells)
Donor/ Patient
Transplantation of corrected cells
Differentiation
Gene correction
Disease affected cell type
Disease models for drug discovery and compound screening
M. Rossbach

Creating induced pluripotent stem (iPS) cells

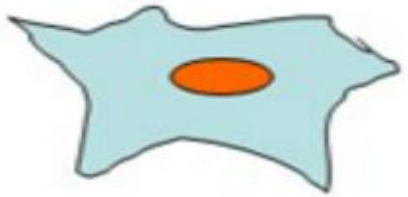


'Packaging' cells produce the virus

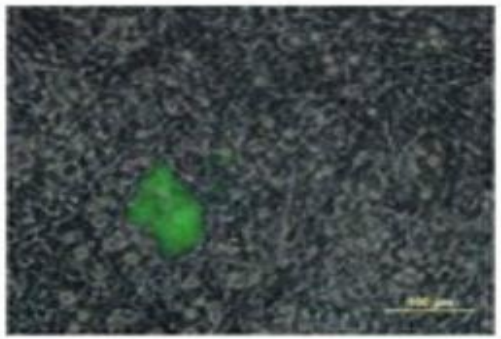
The virus is modified with four different genes



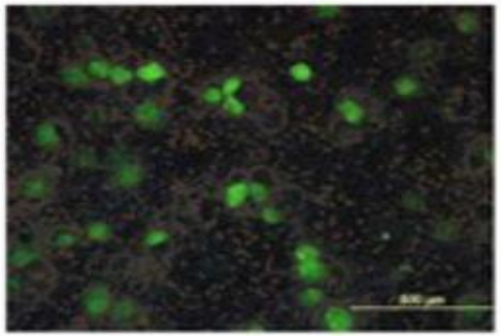
The genes and virus are mixed together



Cells, such as human skin cells, are then 'infected' with the virus



8-9 days post-infection, colonies of cells with similar properties to embryonic stem cells can be identified



Colonies further magnified

Advantages & Disadvantages

Advantages

- Generation of iPS cells does not require embryonic tissue
- Can generate an autogenic (patient-specific) pluripotent stem cells
- Reduces the potential for rejection when implanted into patients
- Creation of disease-specific lines allows for generation of primary cell lines for drug discovery & basic research

Disadvantages

- Safety concerns due to use of virus to reprogram the cells
- Safety concerns due to the use of transgenes to reprogram cells
- High costs associated with generation and characterization of each iPS cell line

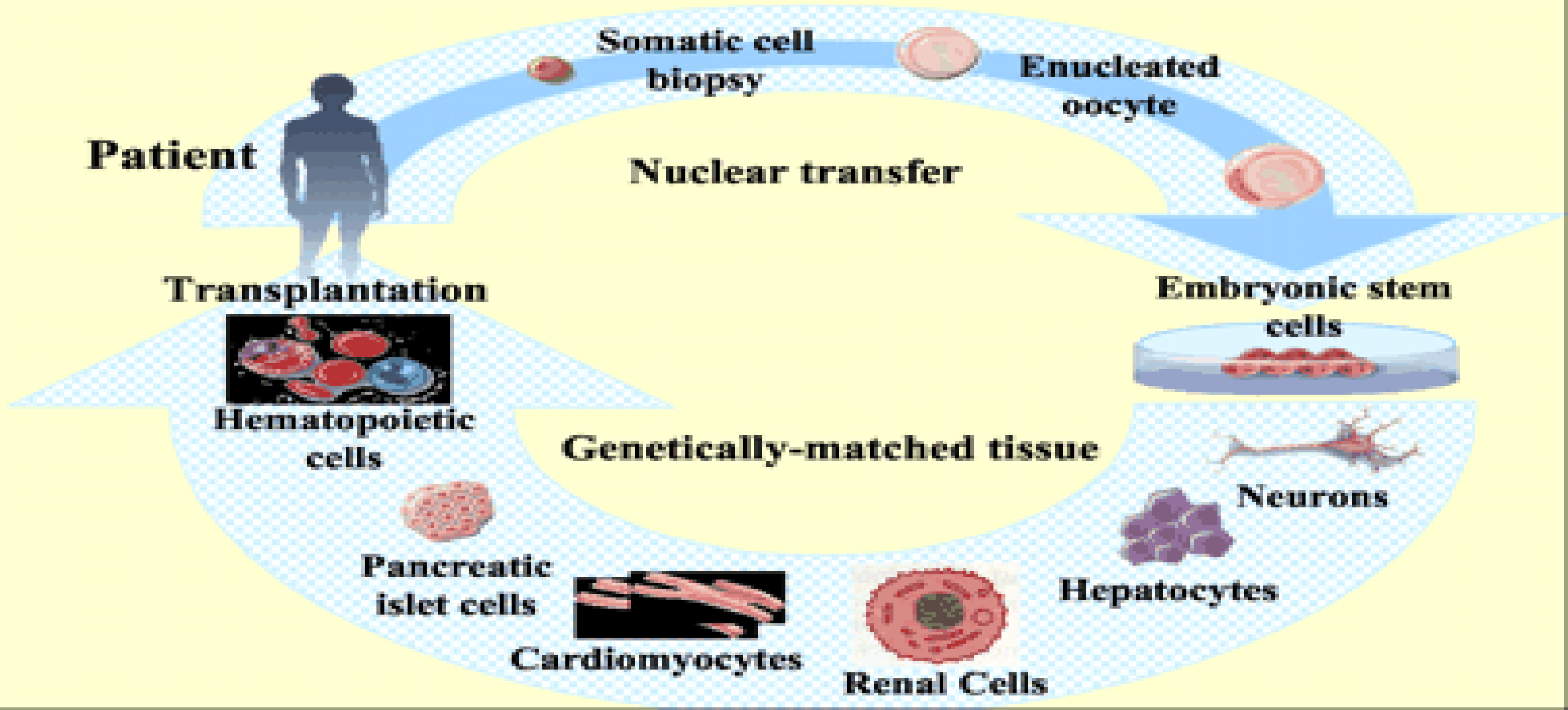
Therapeutic cloning/ Somatic Cell Nuclear Transfer

- Scientists first remove the nucleus from a normal egg cell of a woman. They then extract a nucleus from a somatic cell - that is, any body cell other than an egg or sperm—from a patient who needs an infusion of stem cells to treat a disease or injury, and insert the nucleus into the egg.
- The egg, which now contains the patient's genetic material, is allowed to divide and soon forms a hollow sphere of cells called a blastocyst.
- Cells from the inner cell mass are isolated and used to develop new embryonic stem cell (ESC) lines.

Therapeutic Cloning

- DNA is extracted from a human's cell
- inserted into a woman's ovum
- develop and produce stem cells.
- stem cells are removed from the pre-embryo
- grown into specific organ
- transplanted into the patient.

Therapeutic Cloning Strategies



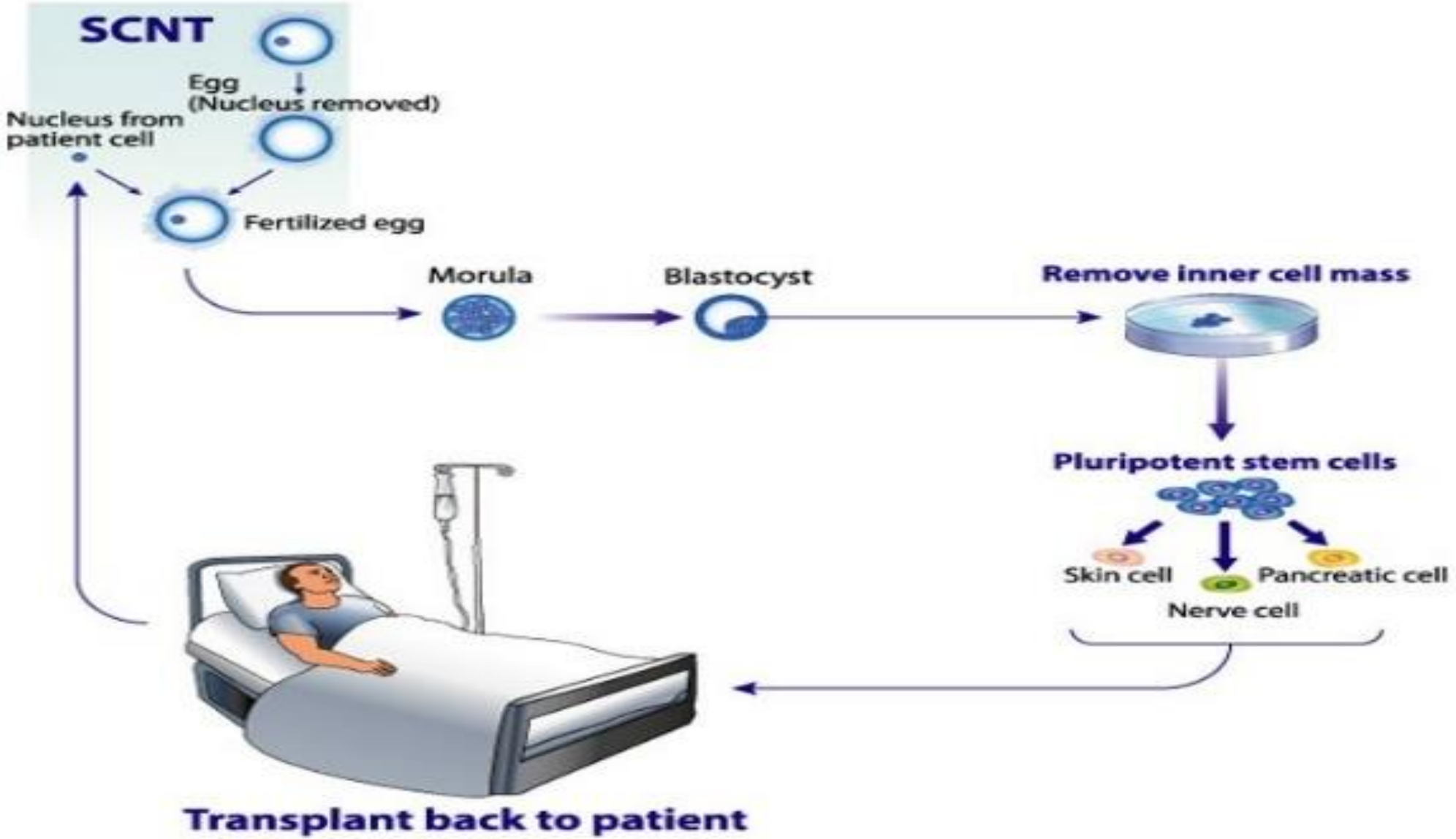
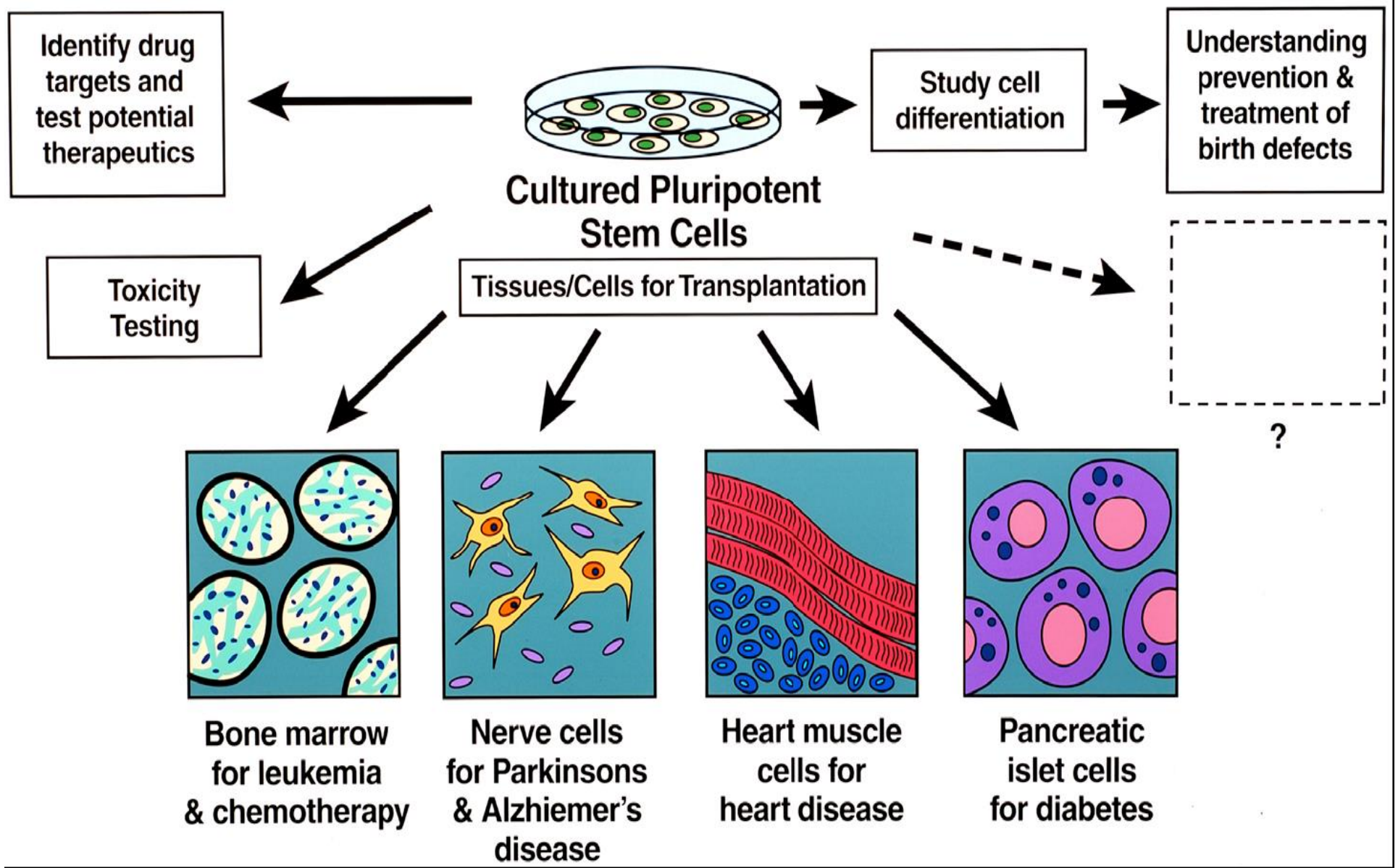


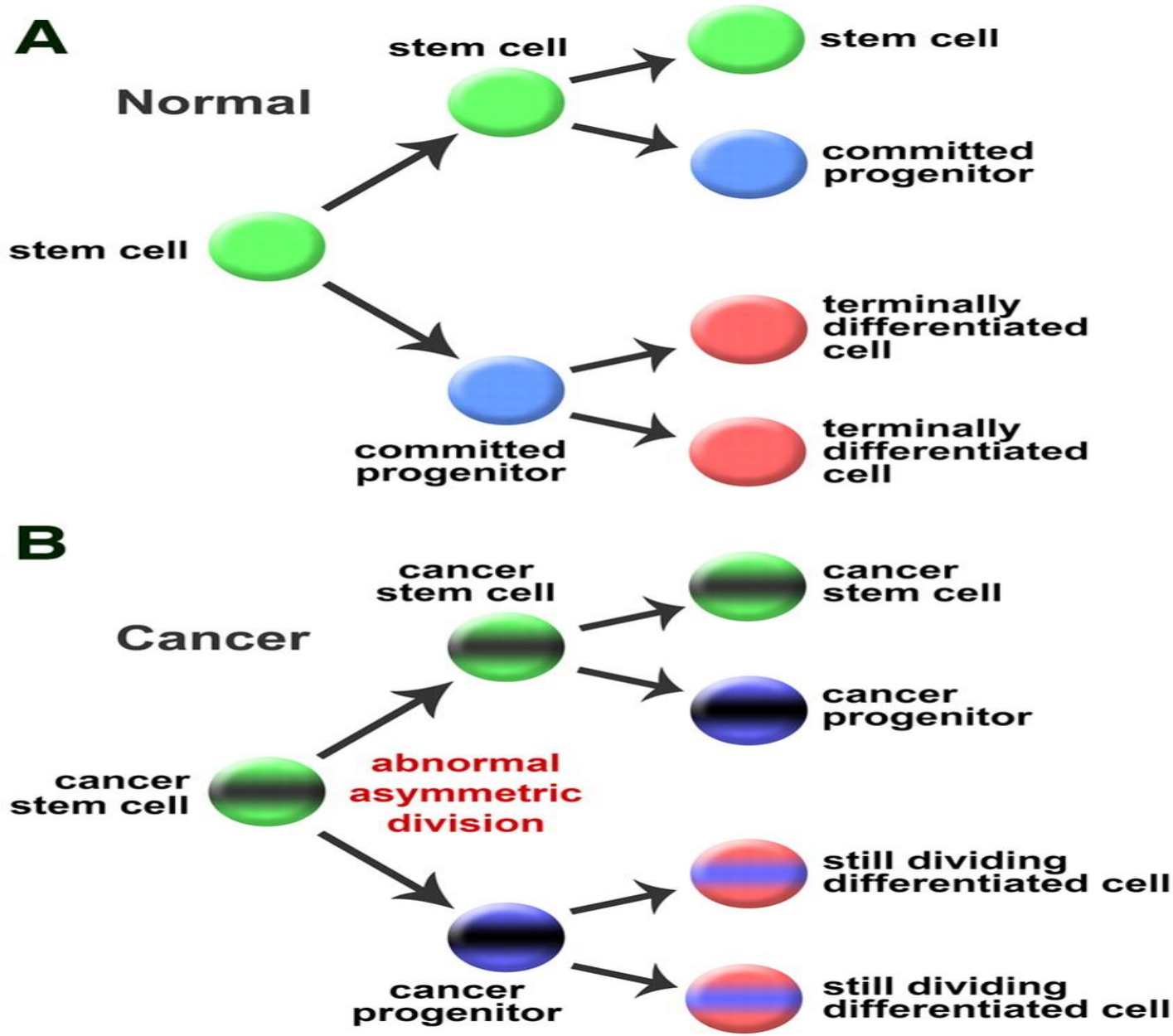
Illustration by Cell Imaging Core of the Center for Reproductive Sciences.

The Promise of Stem Cell Research



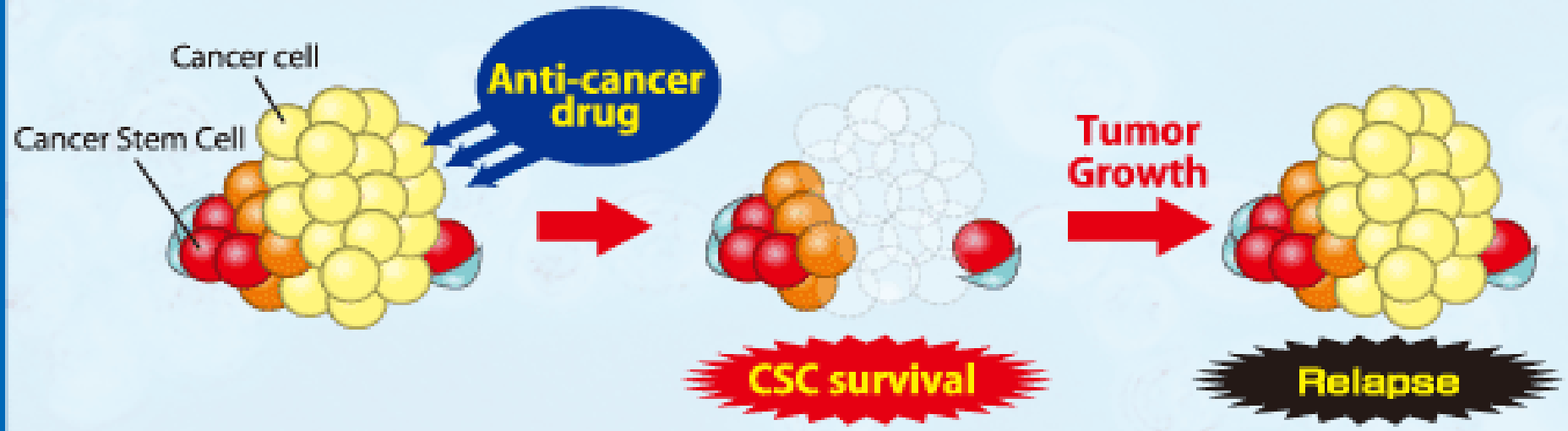
CANCER STEM CELL

- Cancer stem cells are defined as those cells within a tumour that can self-renew and drive tumorigenesis.
- cancer stem cells have been isolated from a number of human tumours, including haematopoietic, brain, colon and breast cancers
- The cancer stem-cell concept has important implications for cancer therapy


A
Normal
stem cell
stem cell
stem cell
committed progenitor
terminally differentiated cell
committed progenitor
terminally differentiated cell
B
Cancer
cancer stem cell
cancer stem cell
cancer progenitor
cancer stem cell
abnormal asymmetric division
still dividing differentiated cell
cancer progenitor
still dividing differentiated cell

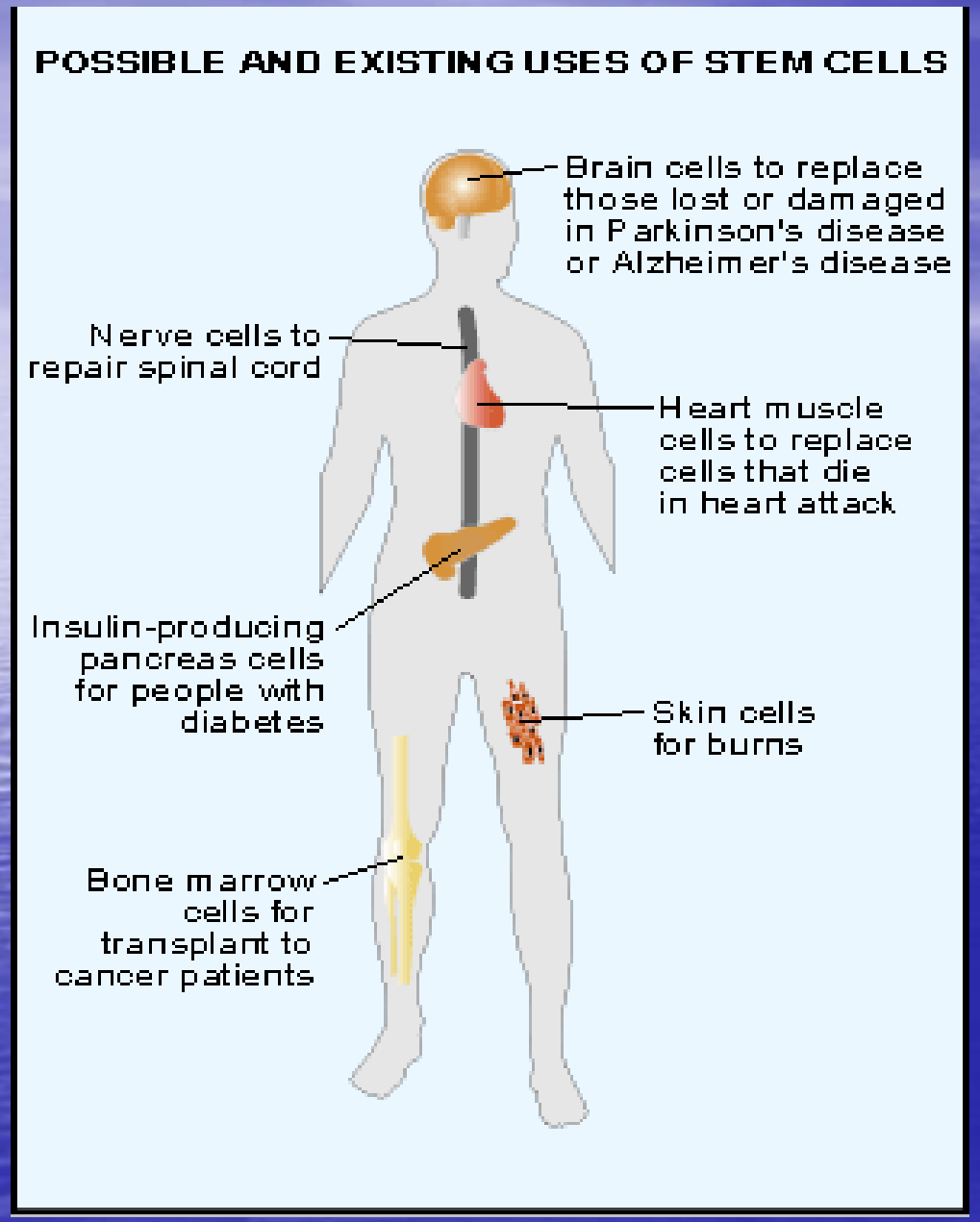
Cancer Stem Cell [CSC] Characteristics

- Minor population in tumor : 0.1 - a few percent
- Self-renewing; infinite proliferative potential.
- Enhanced resistance to drugs, radiation, cell stress.
- Tumorigenic; give rise to other cell types in tumor.
- Associated with **metastasis** and **relapse.**



Metastasis and relapse are involved in more than 90% of all cancer deaths

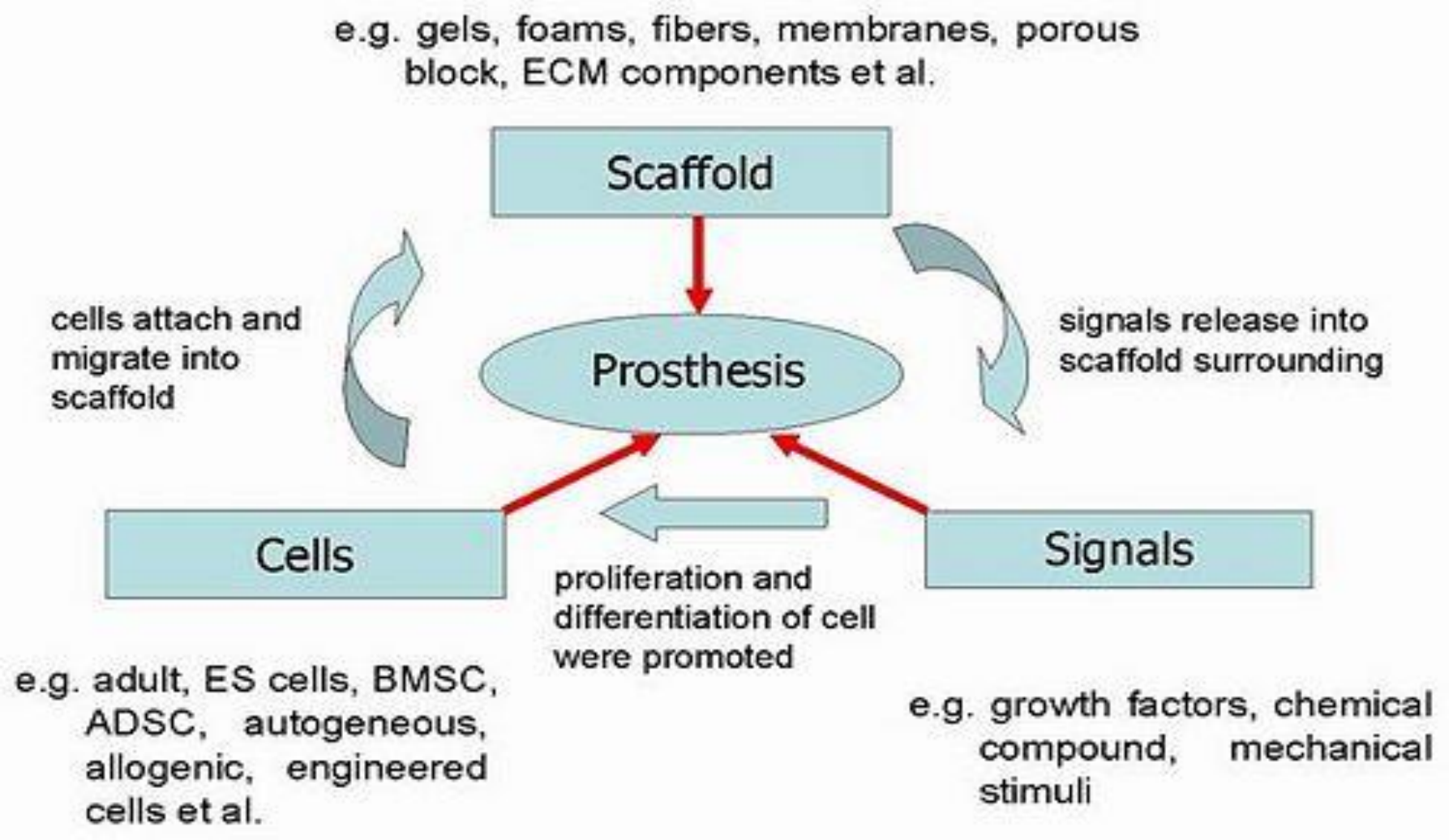
Strategies to eradicate CSCs are an urgent topic in cancer research



Today stem cell based therapy has mainly focused on;

- Leukemia and other cancers,
- Diabetes,
- Parkinson,
- Alzheimer,
- Spinal Cord Injury,
- Heart Diseases

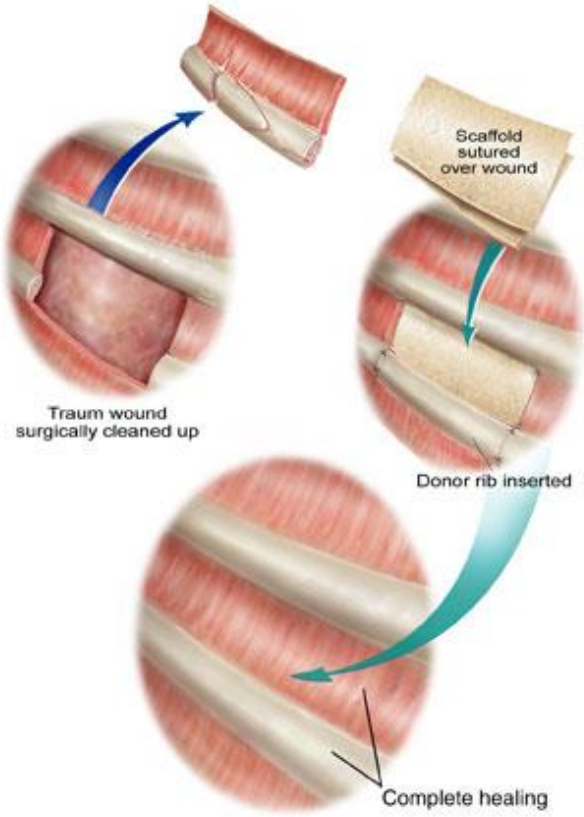
Tissue Engineering



Nanotechnology and Tissue Engineering: The Scaffold, CRC Press; 1 edition (June 16, 2008)

Scaffold

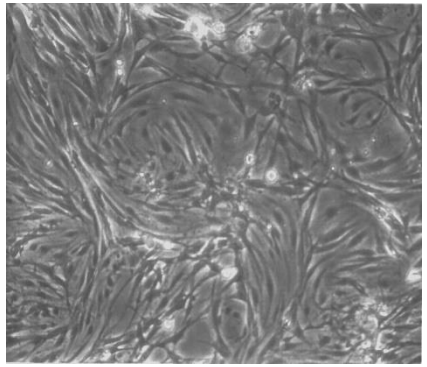
- Allow cell attachment and migration
- Deliver and retain cells and biochemical factors
- Enable diffusion of vital cell nutrients
- Exert certain mechanical and biological influences to modify cell behaviour



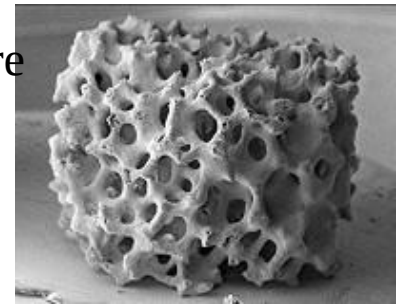
Stem Cell, Niche and Tissue Engineering Strategies

biopsy

in vitro cell expansion



Scaffold seeding



Static culture

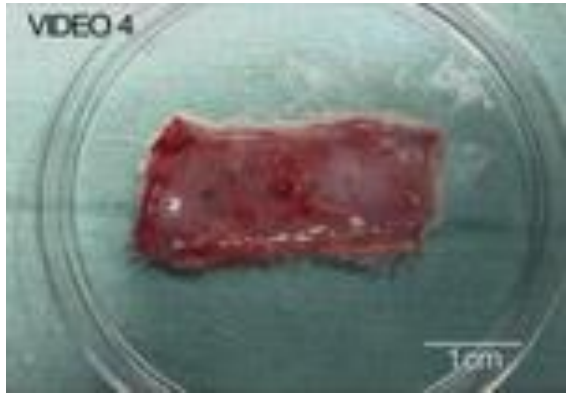
Implantation

Dynamic culture

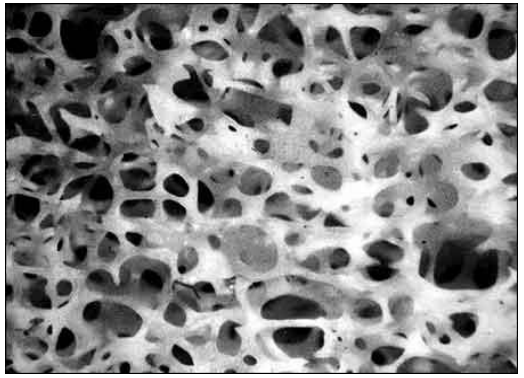
Use of Stem Cells in Tissue Engineering

Skin

Bladder

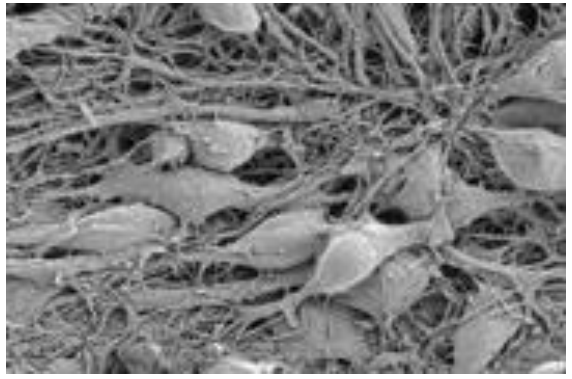


Muscle



Bone

Cartilage



Nerve