بسم الله الرحمن الرحيم

Stem cells

An Introduction

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Ph.D. study
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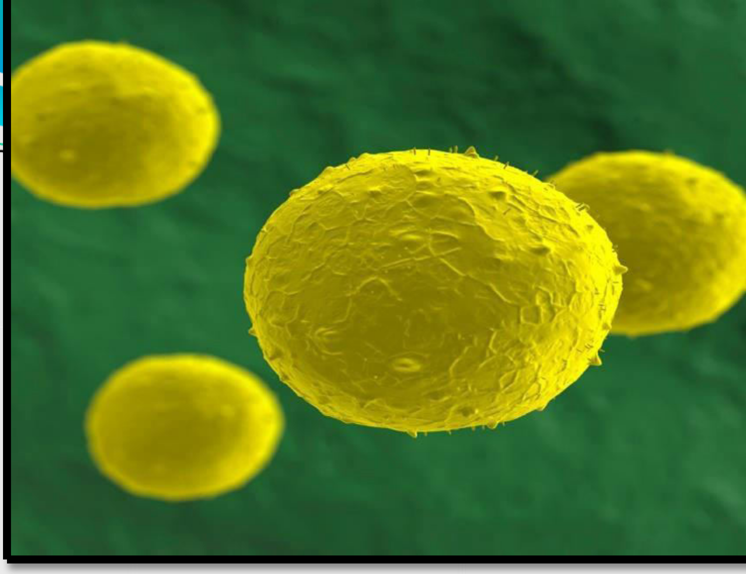
What are stem cells?



Electron micrograph of stem cells

- Stem cells are **un-differentiated/un-specialised/un-programmed** biological cells that are thought to be able to **reproduce themselves indefinitely** and, under the right conditions, to develop into a wide variety of **mature cells with specialized functions**.

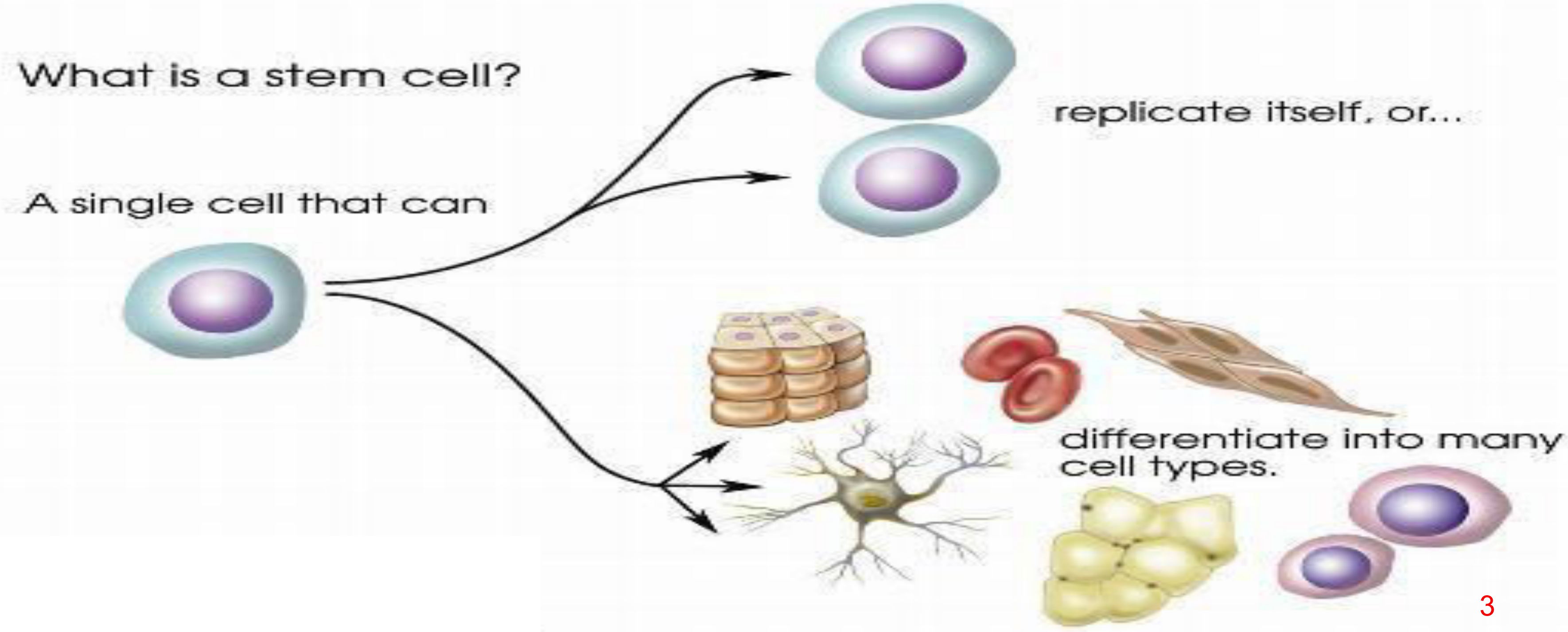
- They can **differentiate** into different type of tissue such as **skin**, **bone**, **cartilage, muscle, nerve** and other specialized type of cells.

- They can **divide** either asymmetrically or mitotically to produce more stem cells. They are found in **multicellular organisms.**

Why are stem cells special?

Stem cells can characterized by-

- **Stem cells are unspecialized which may differentiate into a specialized cell type**
- **Clonogenic/ proliferation i.e. Self-renew to make more stem cells**
- **Plasticity i.e. Stem cell from one tissue may be able to give rise to cell types of completely different tissue eg. Blood cells becoming neuron**
- **Leading to regeneration of tissues.**



How stem cell therapy works?

- When stem cells are **transplanted into the body** and **arrive into the injured part**,
- brain being targeted for tissue regeneration,
- the stem cells are coming in contact with growth chemical's (like EGF's , NGF's and HGF's)in the body.
- These chemicals **program the stem cells to differentiate into the tissue surrounding it**.

Epidermal growth factor (EGF) is **a single polypeptide of 53 amino acid residues which is involved in the regulation of cell proliferation**. Egf exerts its effects in the target cells by binding to the **plasma membrane located EGF receptor**. The EGF receptor is a **transmembrane protein tyrosine kinase**.

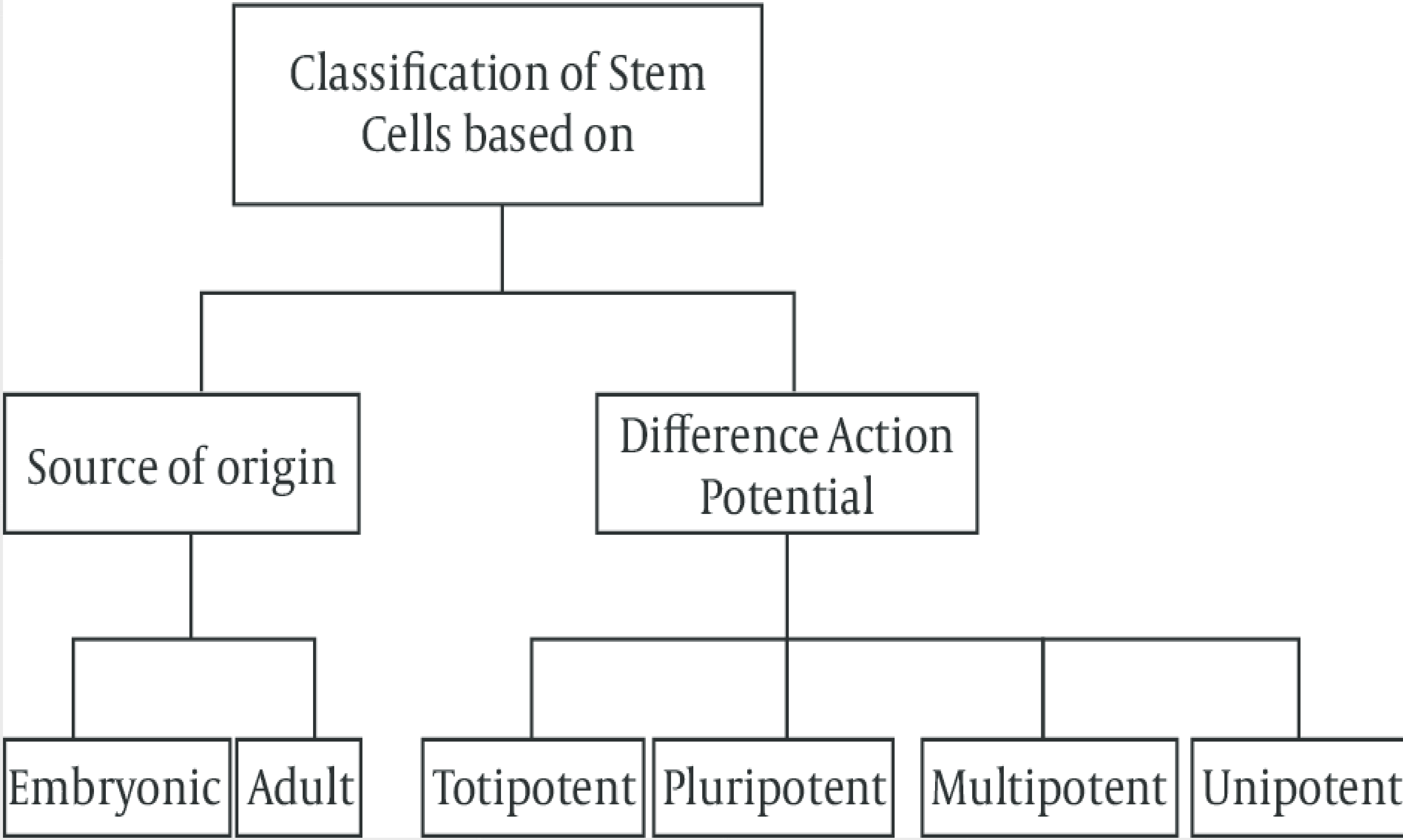
Nerve growth factor (NGF) is a **neurotrophic factor** and **neuropeptide** primarily involved in the regulation of growth, maintenance, proliferation, and survival of certain target neurons.

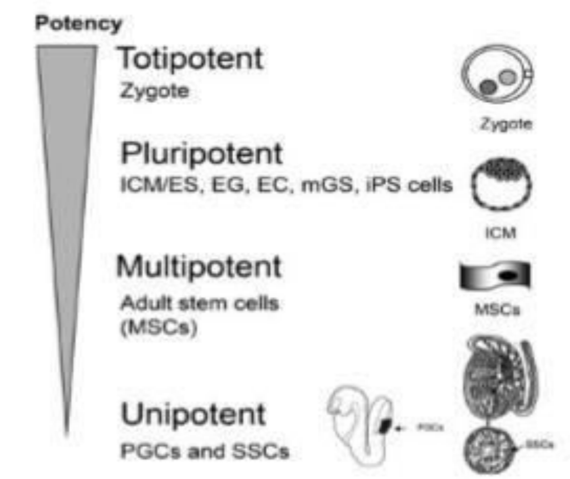
Hepatocyte growth factor (HGF) is **a mesenchyme-derived pleiotropic factor which regulates cell growth, cell motility, and morphogenesis of various types of cells**, and is thus considered a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development ...

Historical Perspectives

- 1908: The term **"stem cell"** was coined by **Alexander Maksimov**
- 1963: **McCulloch and Till** illustrate the presence of **self-renewing cells** in mouse bone marrow.
- **1968**: **Bone marrow transplant (BMT)** between two siblings successfully treats SCID.
- 1978: **Haematopoietic stem cells** are discovered in human cord blood.
- **1990**: The Nobel Prize **was awarded jointly to Thomas ED, Lochte HL, Lu WC, Ferrebee JW** for "Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy first successful" (**Haematopoietic stem cell transplantation (HSCT) in treatment of acute leukemias**)
- 2000: Several reports of **adult stem cell plasticity** are published.
- **2007**: The Nobel Prize **was awarded jointly to Mario R. Capecchi, Sir Martin J. Evans and Oliver Smithies** "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells".
- **2008**: Development of human cloned blastocysts following somatic cell nuclear transfer **(SCNT) with adult fibroblasts**
- **2012**: The Nobel Prize **was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka** "for the discovery that mature cells can be **reprogrammed** to become **pluripotent**"

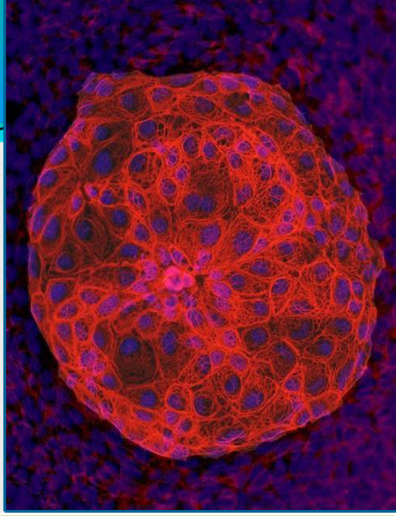
Classification of Stem cells



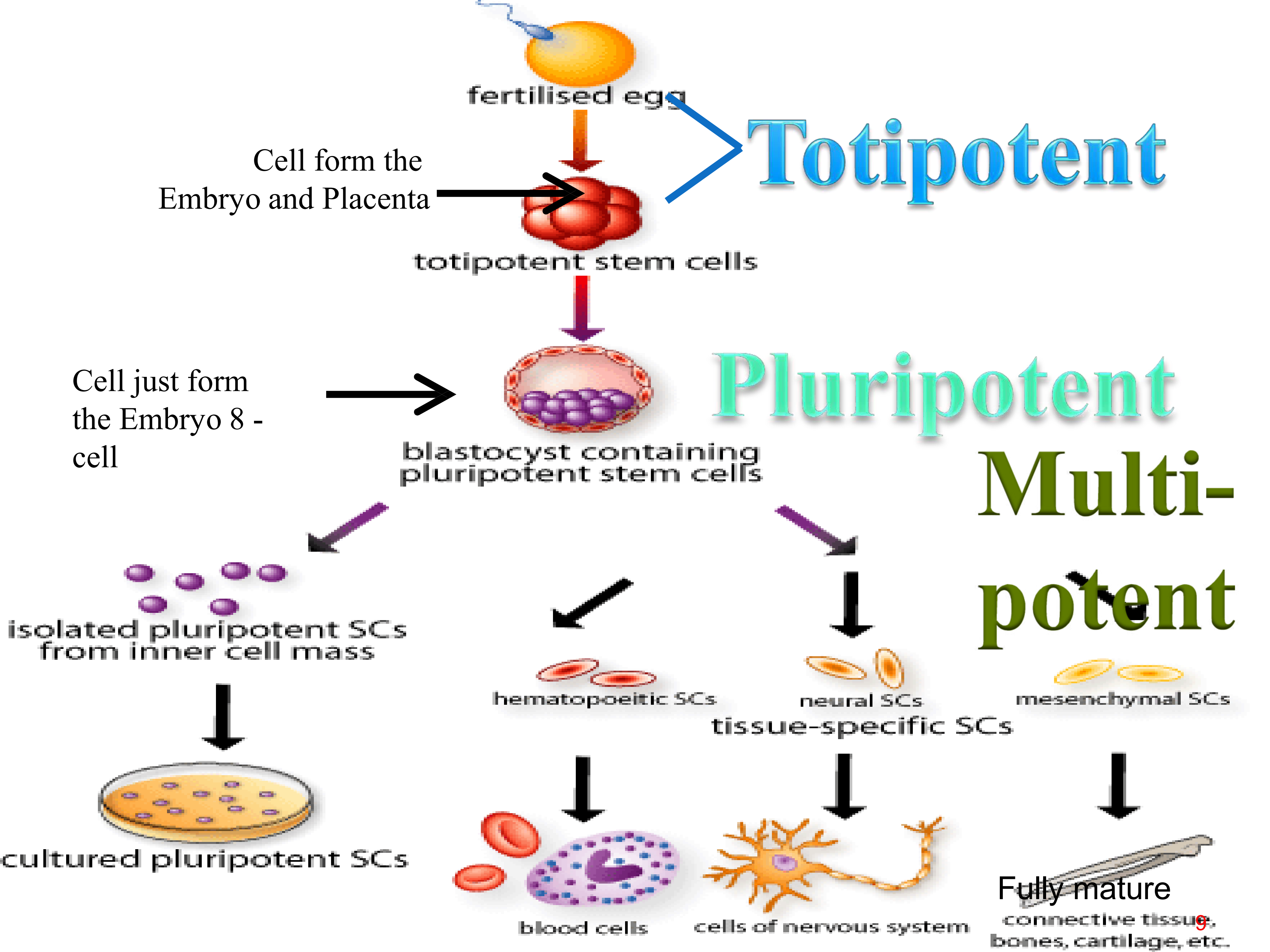


Type of stem cells-Potency

Pluripotent stem cells



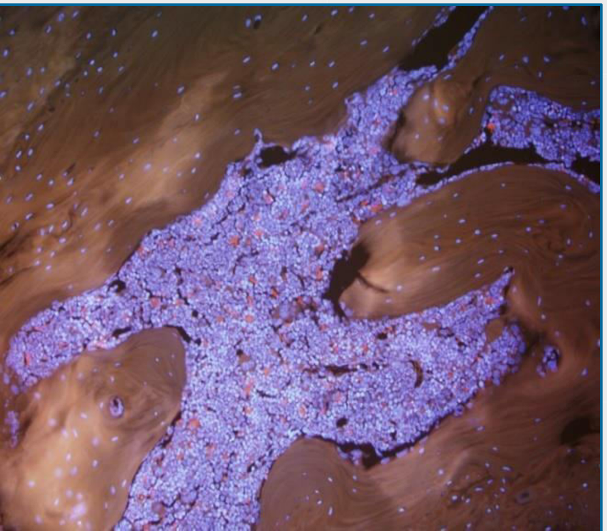
Totipotent	Pluripotent	Multipotent	Unipotent
➢ Cells from early embryo	➢ Cells from blastocyst	➢ Fetal tissue, cord blood & adult stem cell	➢ Adult cells
➢ Able to become whole individual **including a extra-embryonic structures i.e. placenta**	➢ Can become any type of tissue in the body **excluding a placenta**	➢ Produce only cells of a **closely related family of cells**	➢ Unipotent stem cells can produce only **one cell** type
➢ E.g. **8-16 cell embryo**	➢ E.g. inner cell mass (**ICM**), **iPSC**	➢ E.g. **MSC, hematopoietic stem cells**	➢ E.g. muscle stem cells, **SSC**


fertilised egg
Totipotent
Cell form the Embryo and Placenta
totipotent stem cells
Cell just form the Embryo 8 - cell
Pluripotent
blastocyst containing pluripotent stem cells
Multi-potent
isolated pluripotent SCs from inner cell mass
hematopoeitic SCs
neural SCs
mesenchymal SCs
tissue-specific SCs
cultured pluripotent SCs
blood cells
cells of nervous system
Fully mature
connective tissue, bones, cartilage, etc.

Types of Stem cell-Origin

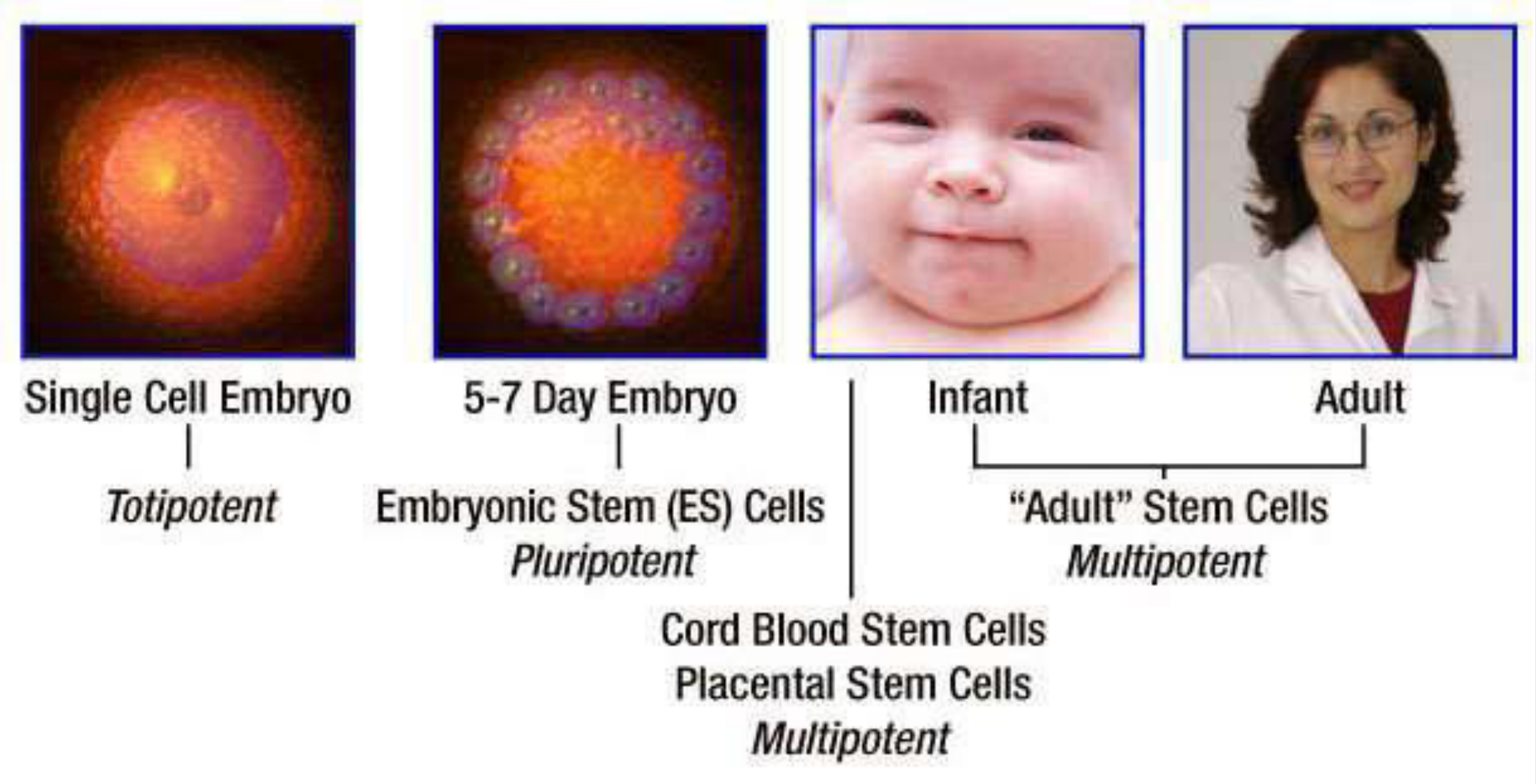
Stem Cells

- Embryonic stem Cells (ESCs)
- Adult / Tissue/ Somatic stem Cells
  - Hematopoietic stem cells (HSC)
  - Mesenchymal stem cells (MSC)
  - Spermatogonial stem cells (SSC)



Tissue stem cells/
Adult Stem Cell

Stem Cell Timeline



ES CELLS	**ADULT STEM CELLS**
Blastocyst (Inner cell mass)	Adult tissues (Bone marrow, liver, brain, etc)
Pluripotent	Multipotent

Advantages

- ES cells:
  - Easily grown in cultures
- Adult stem cells:
  - Potential transplant source
  - **Low risk of rejection**

Disadvantages

- ES cells:
  - Availability
  - **Ethics**
- Adult stem cells:
  - **Limited plasticity**
  - Rare and hard to isolate & purify
  - Difficult to culture and maintain

The proliferation time of somatic stem cells is longer than that of ESCs

Embryonic stem cells (ESCs)

Around 3–5 days after a sperm fertilizes an egg, the embryo takes the form of a blastocyst or ball of cells.

The blastocyst contains stem cells and will later implant in the womb.

Embryonic stem cells come from a blastocyst that is 4–5 days old.

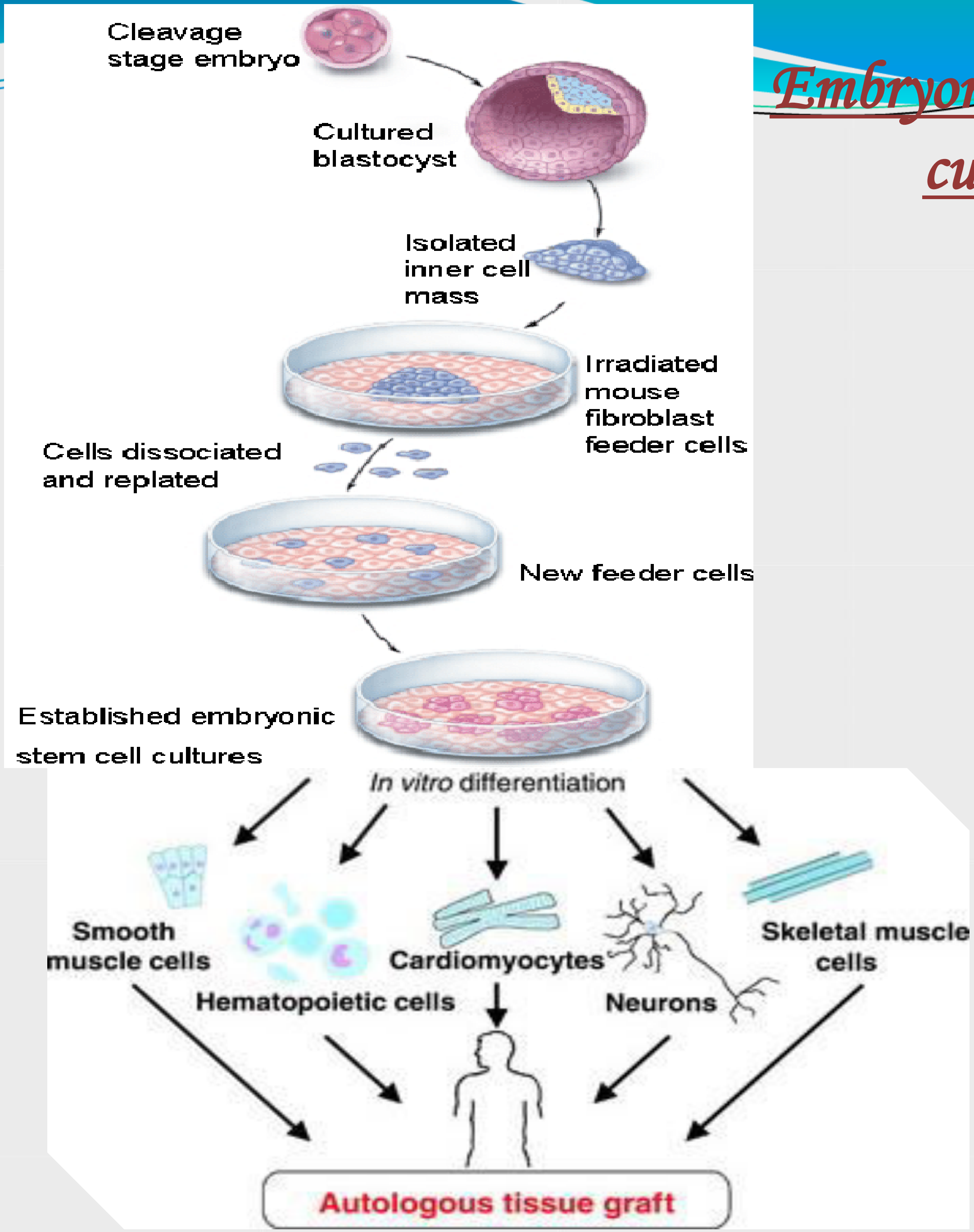
The embryo (blastocyst), contains an outer cell mass that become part of placenta and an inner cell mass that is capable of generating all the specialized tissues that develop into the human body.

ESCs are derived from the inner cell mass of an embryo that has been fertilized *in vitro* . It is not derived from eggs fertilized in a woman's body.

This is known as pluripotent stem cells have the potential to become any cell type and are only found during the first stages of development.

These stem cells grown in vitro such as nerve, skin, intestine, liver, etc for transplantation.

Embryonic stem cell culture



Adult stem cells (ASCs)

A body contains stem cells throughout their life. The body can use these stem cells whenever it needs them.

ASCs are **undifferentiated**, **multipotent cells** **found in living differentiated tissues in our bodies** that can renew themselves or generate new cells that can replace dead or damaged tissue.

It is also **called tissue specific or somatic stem cells**, adult stem cells exist throughout the body from the time an embryo develops.

ASCs are present in different tissue such as the **brain**, **bone marrow**, **blood** and **blood vessels**, **umbilical cord**, **placenta**, **skeletal muscles**, **skin**, the **liver**, **fat tissue** etc.

ASCs generate new cells to replace those that are lost through normal repair, disease, or injury.

Types of Adult Stem Cells (multipotent cells)

Hematopoietic Stem Cells (Blood Stem Cells)
Mesenchymal Stem Cells.
Neural Stem Cells.
Epithelial Stem Cells.
Skin Stem Cells.

Hematopoietic stem cells culture

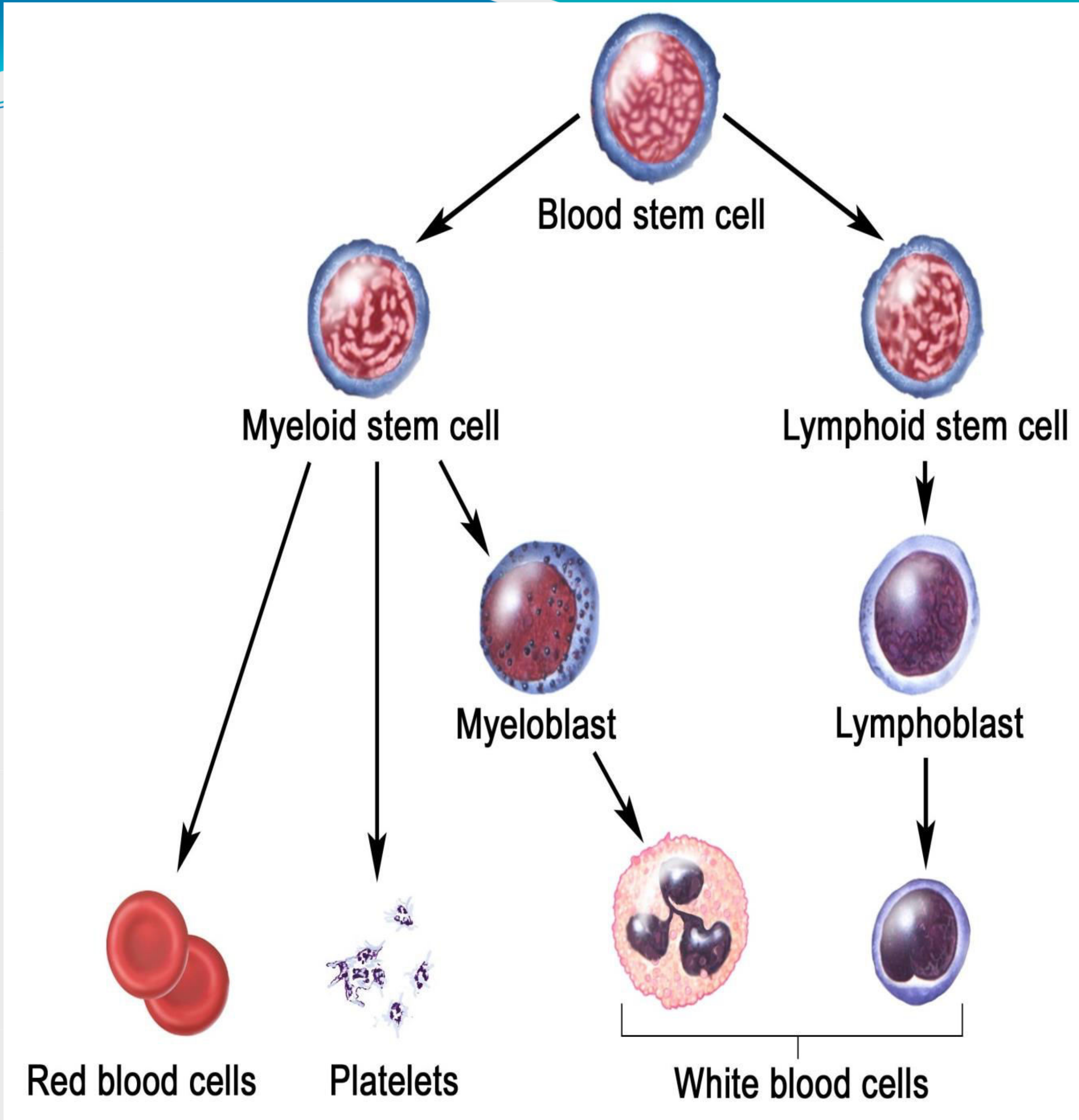
Hematopoietic stem cells (HSCs) are multipotent, have self renewal capacity and the ability to regenerate all the different blood forming cells.

hPSC **culture requires growth factors**, **cell to cell interactions** and **cell to matrix adhesions**. Media enriched with growth factors found in fetal bovine serum (FBS) or defined serum replacements.

hPSCs are grown in aggregates, or colonies, which helps create this **niche**.

hPSC culture systems utilize support cells such as an inactivated mouse embryonic fibroblast (MEF) feeder layer to support growth and prevent differentiation.

These cells provide necessary intercellular interactions, extracellular scaffolding and factors creating a stable hPSC culture environment.


Blood stem cell
Myeloid stem cell
Lymphoid stem cell
Myeloblast
Lymphoblast
Red blood cells
Platelets
White blood cells

Mesenchymal stem cells (MSCs)

MSCs exhibit the potential for differentiation into a variety of different cells/tissue lineages.

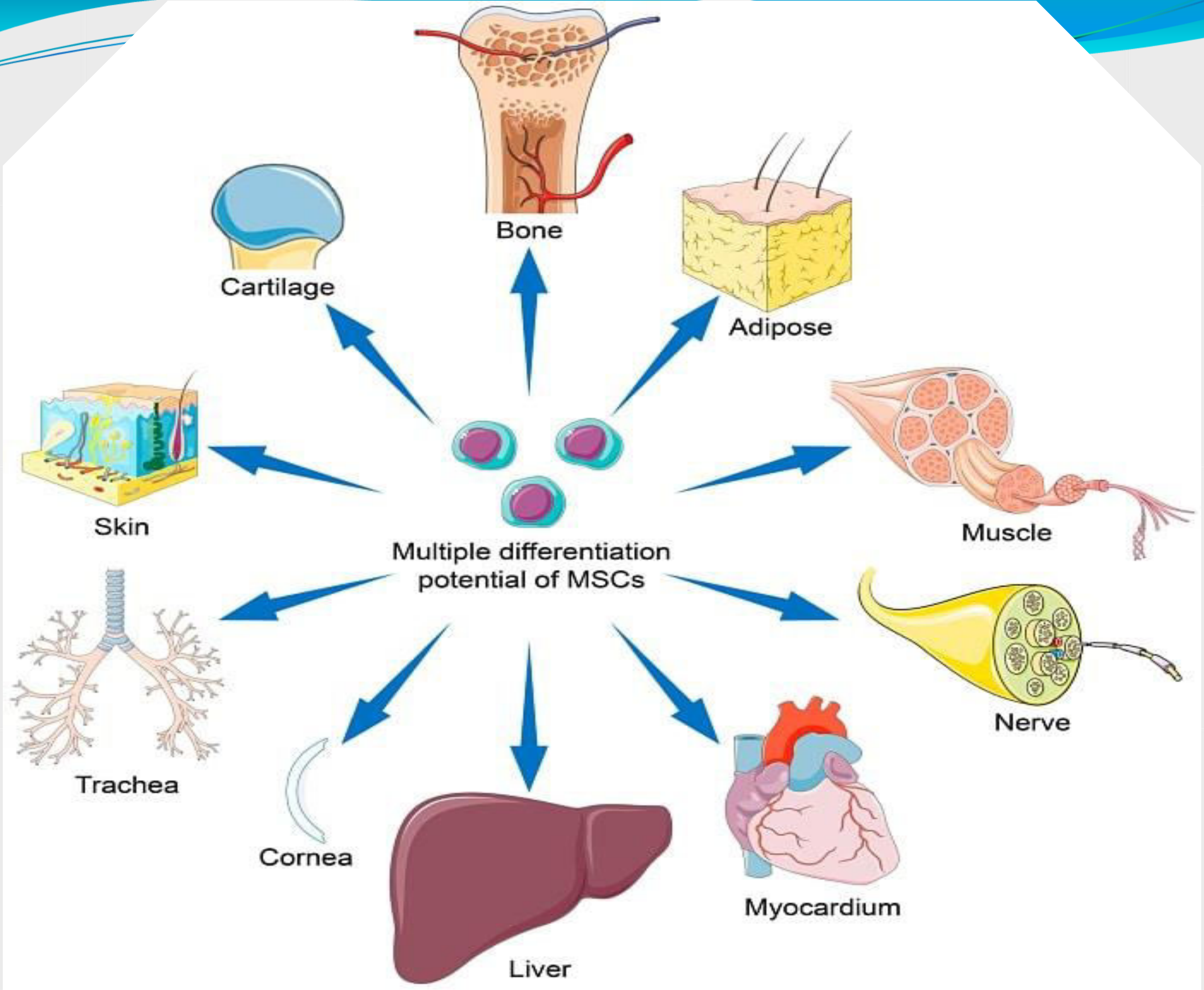
(MSCs) are multipotent stem cells **found in bone marrow** that are important for **making and repairing skeletal tissues**, such as **cartilage**, **bone** and **the fat** found in bone marrow.

These are not to be confused with haematopoietic (blood) stem cells that are also found in bone marrow and make our blood.

MSCs come from the **connective tissue** or **stroma** that **surrounds the body's organs and other tissues**.

MSCs has to be used to create new body tissues, such as bone, cartilage, and fat cells.

They play a role in solving a wide range of health problems.


Bone
Cartilage
Adipose
Skin
Muscle
Multiple differentiation potential of MSCs
Trachea
Nerve
Cornea
Myocardium
Liver

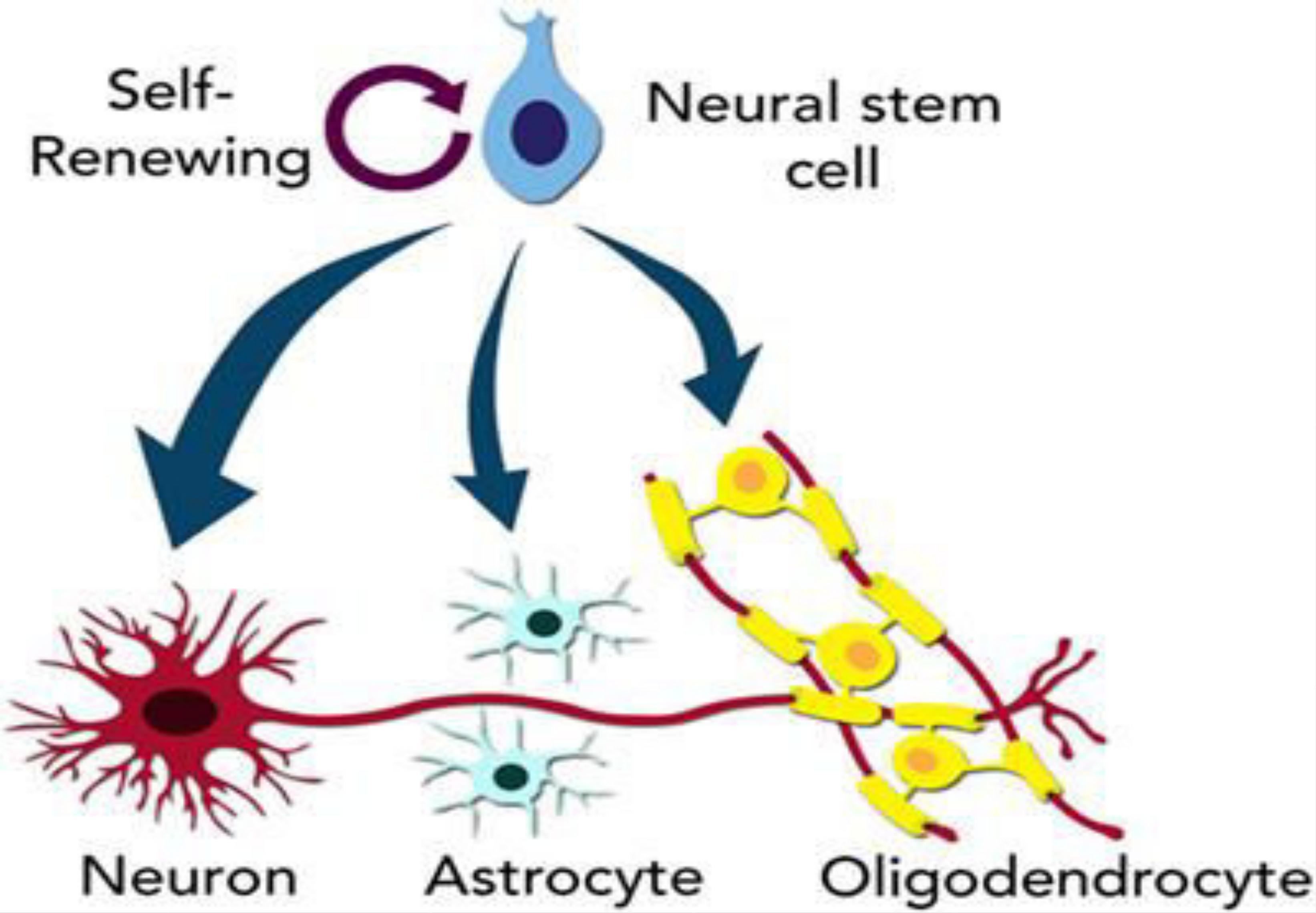
Neural stem cell (NSCs)

Neural stem cell, largely undifferentiated, self-renewing, multipotent cells **originating in the central nervous system**.

Neural stem cells (NSCs) have the potential to give rise to offspring cells that grow and differentiate into **neurons** and **glial cells**.

When transplanted, NSCs are able to improve the phenotype in different transgenic models of motor neuron disease.

Neural stem cells can help to treat things such as **stroke**, **spinal cord injury**, and **Parkinson's disease** (a disease in which cells that contribute to control body movements progressively stop working and die).


Self-
Renewing
Neural stem
cell
Neuron
Astrocyte
Oligodendrocyte

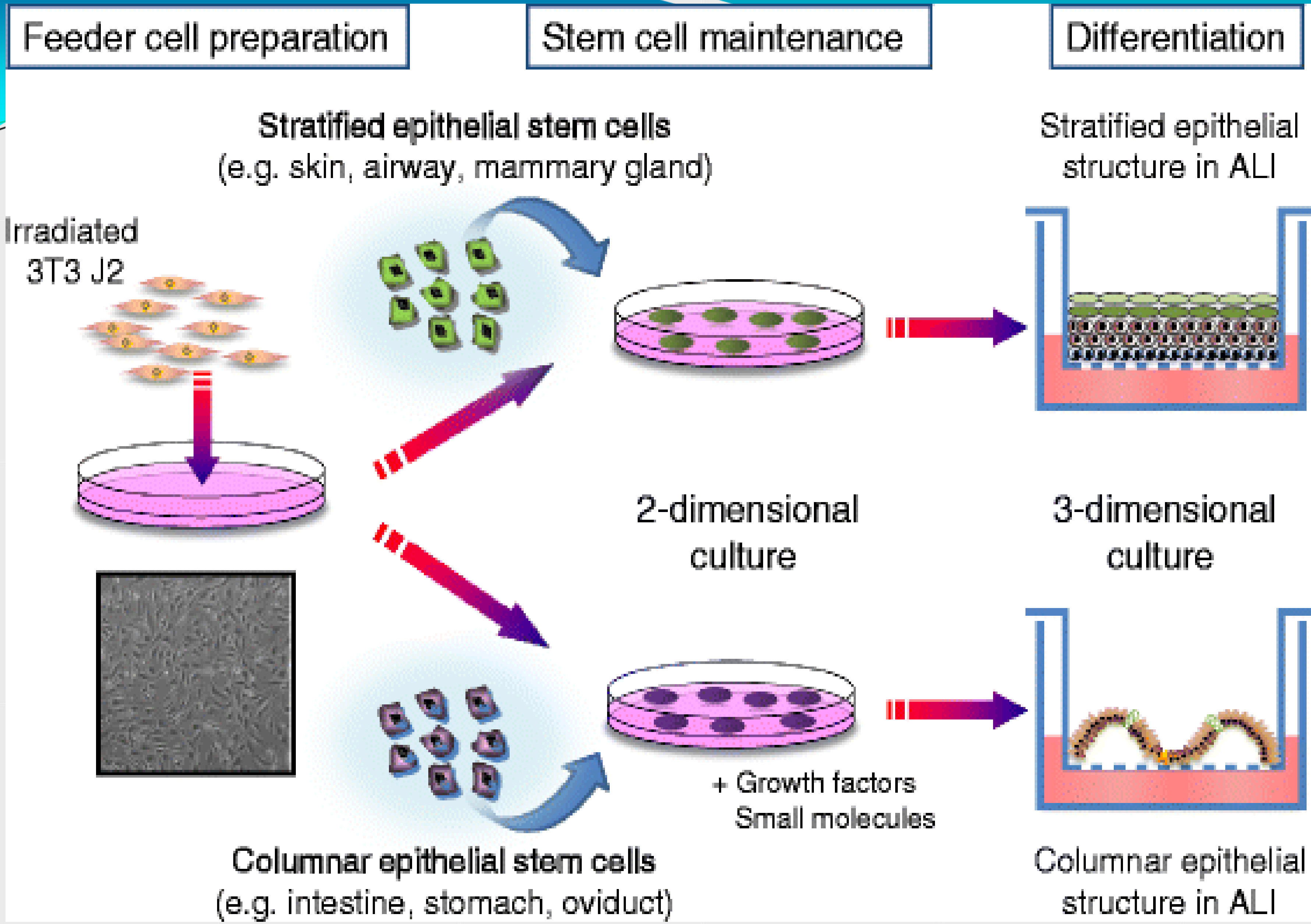
Epithelial stem cells

Epithelial stem cells (EPSCs) are a multipotent cell and self renewal.

Epithelial stem cells are **responsible for everyday regeneration of the different layers of the epidermis**.

These stem cells are **found in the basal layer of the epidermis**.

Many epithelial tissues are capable of regeneration, that is, they are capable of rapidly replacing damaged and dead cells.



Epithelial stem cell culture

Skin stem cells

Skin stem cells are multipotent adult stem cells present in the adult skin.

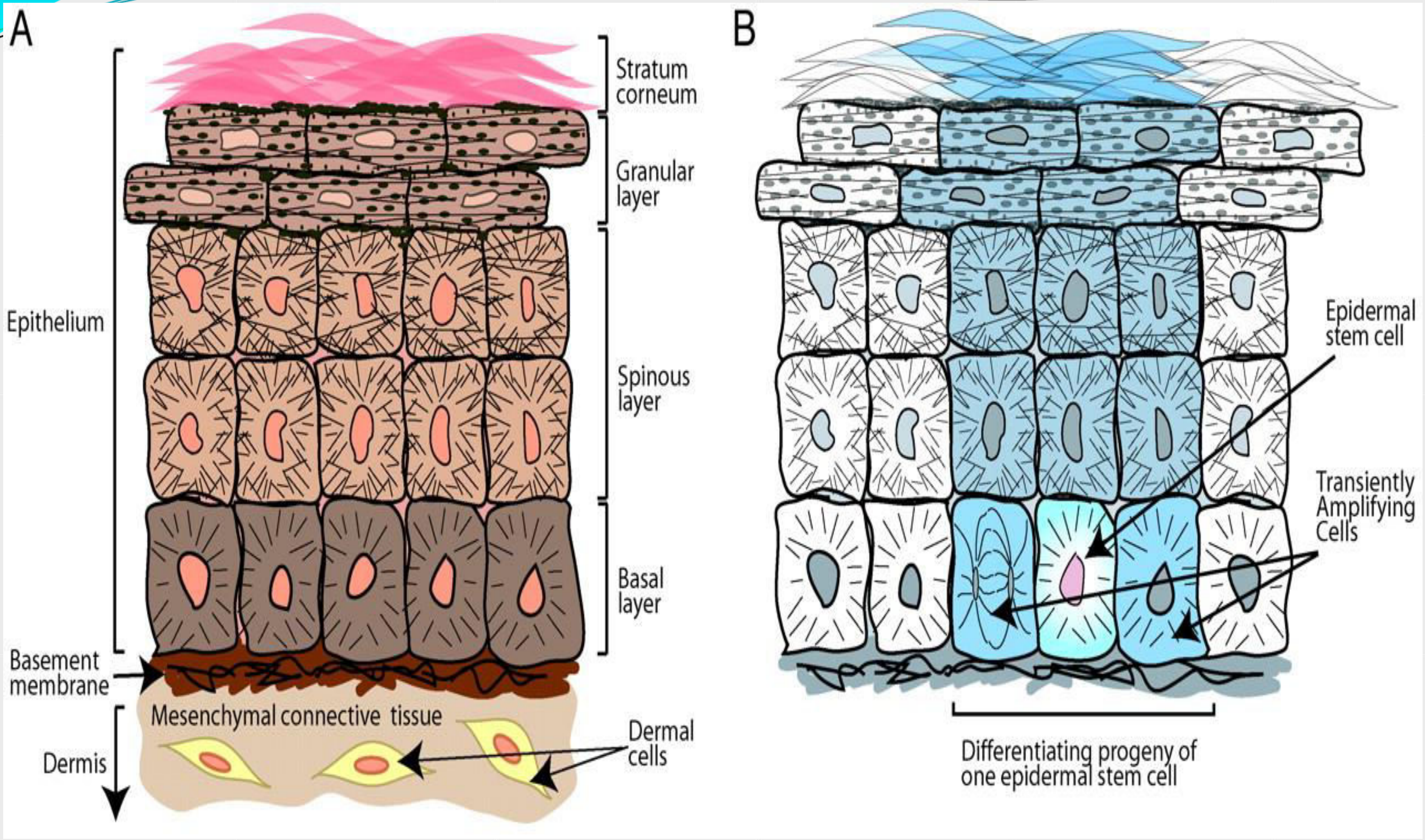
It can self-renew and differentiate into different cell lineages of the skin.

Skin stem cells are active during skin renewal, which occurs throughout life, and in skin repair after injury.

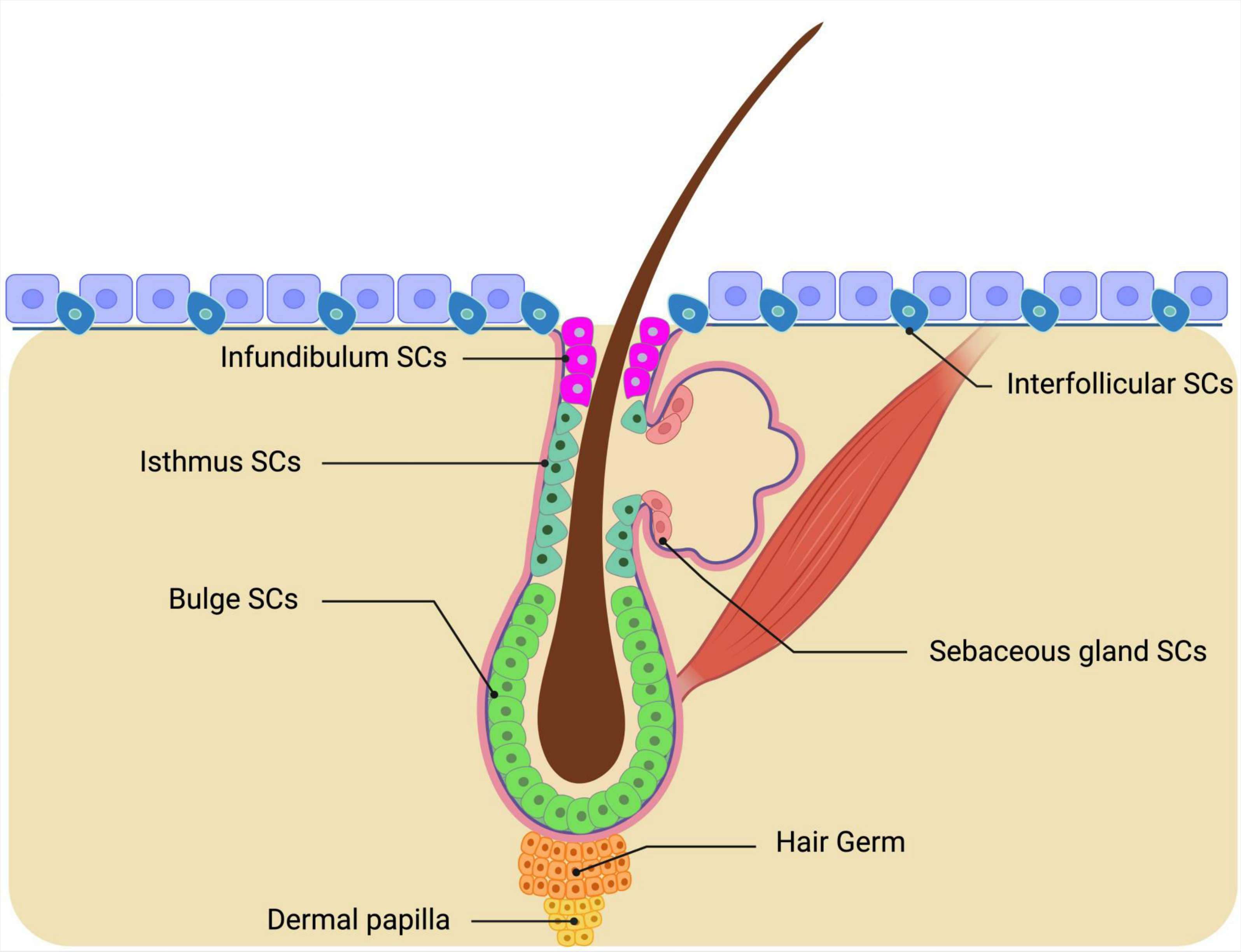
The epidermis of the skin contains **layers of cells called keratinocytes**. Only the **basal layer**, next to the dermis, contains cells that divide.

Hair follicle stem cells ensure constant renewal of the hair follicles.

The stem cells at the base of the skin stop proliferating and start differentiating into the cells that form the skin itself.


A
Stratum corneum
Granular layer
Epithelium
Spinous layer
Basal layer
Basement membrane
Mesenchymal connective tissue
Dermis
Dermal cells
B
Epidermal stem cell
Transiently Amplifying Cells
Differentiating progeny of one epidermal stem cell

Hair-follicle stem cells



Pluripotent stem cells (PSCs)

- **Pluripotent stem cells (PSCs) form cells of all germ layers but *not* extraembryonic structures, like placenta, e.g.,**
  - **Embryonic stem cells (ESCs) – derived from the inner cell mass of preimplantation embryos, and**
  - **Induced pluripotent stem cells (iPSCs)** – are a type of pluripotent stem cell derived from adult somatic cells that have been genetically reprogrammed to an embryonic stem (ES) cell-like state..
  - Their culturing and utilization are very promising for present and future **regenerative medicine**.

Induced pluripotent stem cells (iPS cells)

What are iPS cells?

In 2006, scientists discovered that it is possible to make a new kind of stem cell in the laboratory. They found that they could transform **skin cells from a mouse into cells that behave just like embryonic stem cells**. In 2007, researchers did this with human cells too. The new stem cells that are made in the lab are called **induced pluripotent stem cells**. Just like embryonic stem cells, they can make all the different types of cell in the body – so we say they are **pluripotent**.

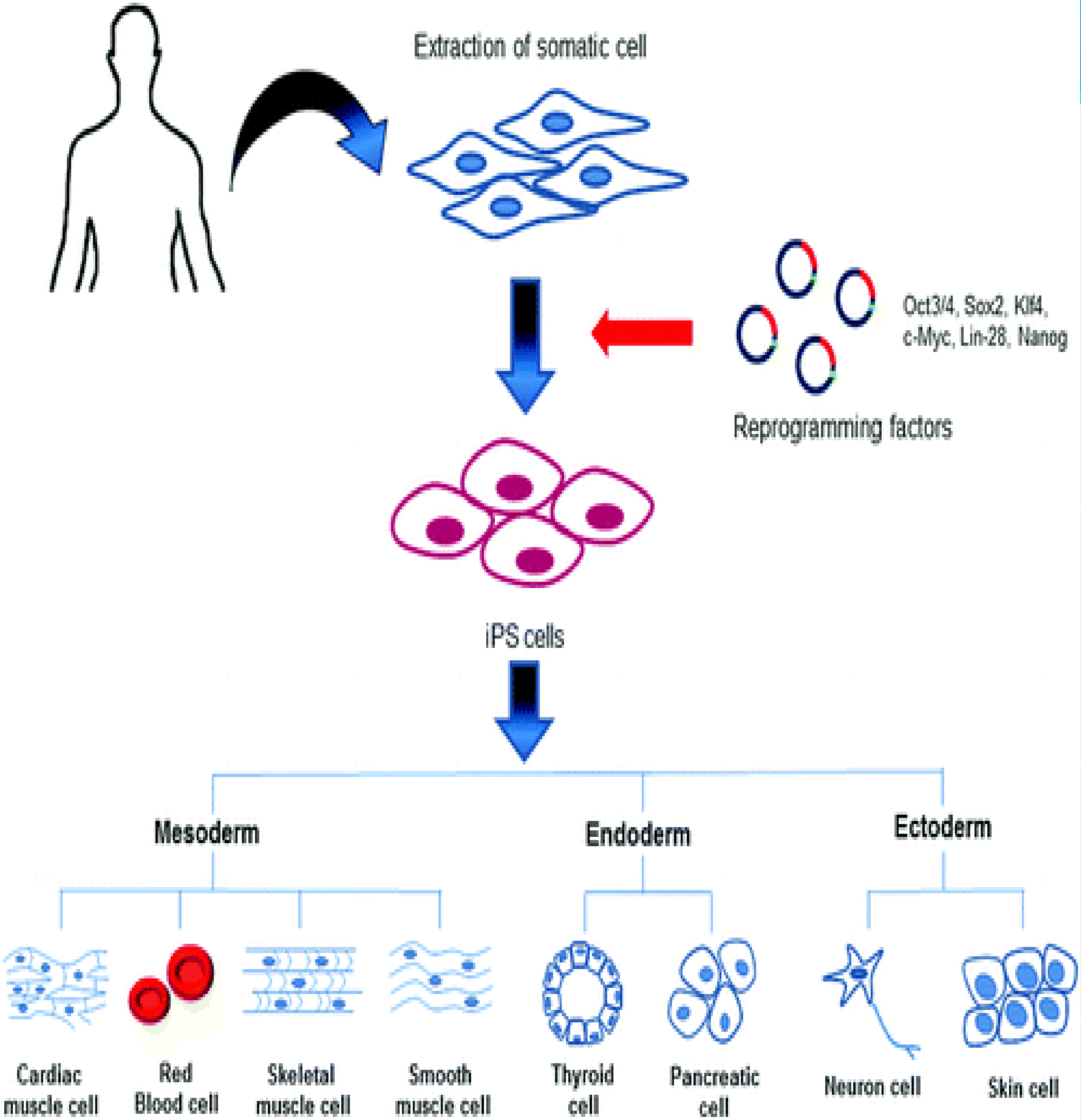
Making induced pluripotent stem (iPS) cells is a bit like turning back time. Scientists add particular genes to cells from the body to make them behave like embryonic stem cells. Genes give cells instructions about how to behave. So, this process is a bit like changing the instructions in a computer programme to make the computer do a new task. Scientists call the process they use to make iPS cells '**genetic reprogramming**'.

Why are they exciting?

Researchers hope that one day they might be able to use iPS cells to help treat diseases like Parkinson's or Alzheimer's. They hope to:

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, e.g. certain brain cells. These cells would be made from the patient's own skin cells so the body would not reject them.

There is a long way to go before scientists can do this, but iPS cells are an exciting discovery.



Extraction of somatic cell
Oct3/4, Sox2, Klf4,
c-Myc, Lin-28, Nanog
Reprogramming factors
iPS cells
Mesoderm
Endoderm
Ectoderm
Cardiac muscle cell
Red Blood cell
Skeletal muscle cell
Smooth muscle cell
Thyroid cell
Pancreatic cell
Neuron cell
Skin cell

Induced pluripotent stem cell

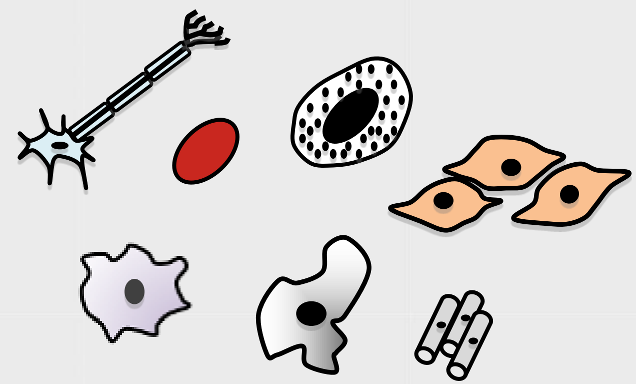
'genetic reprogramming'
= add certain genes to the cell

cell from the body

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

differentiation

culture iPS cells in the lab



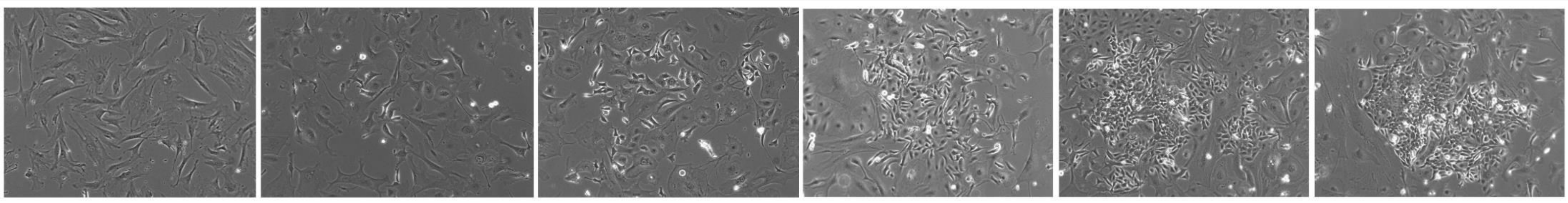
all possible types of specialized cells

Advantage: no need for embryos!

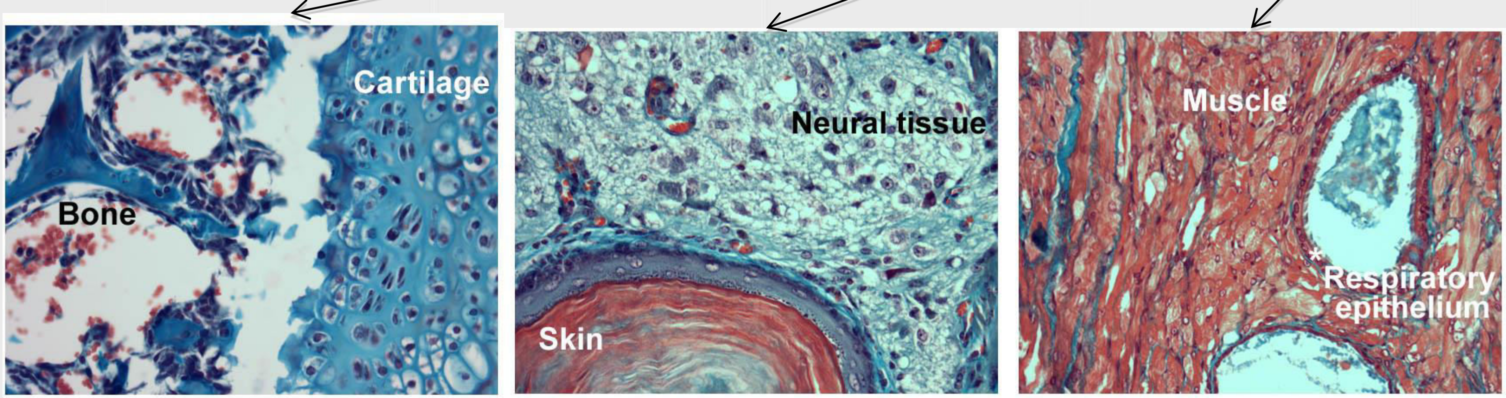
Induced pluripotent stem cell (Contd)

genetic reprogramming

cell from the body (skin) → pluripotent stem cell



differentiation

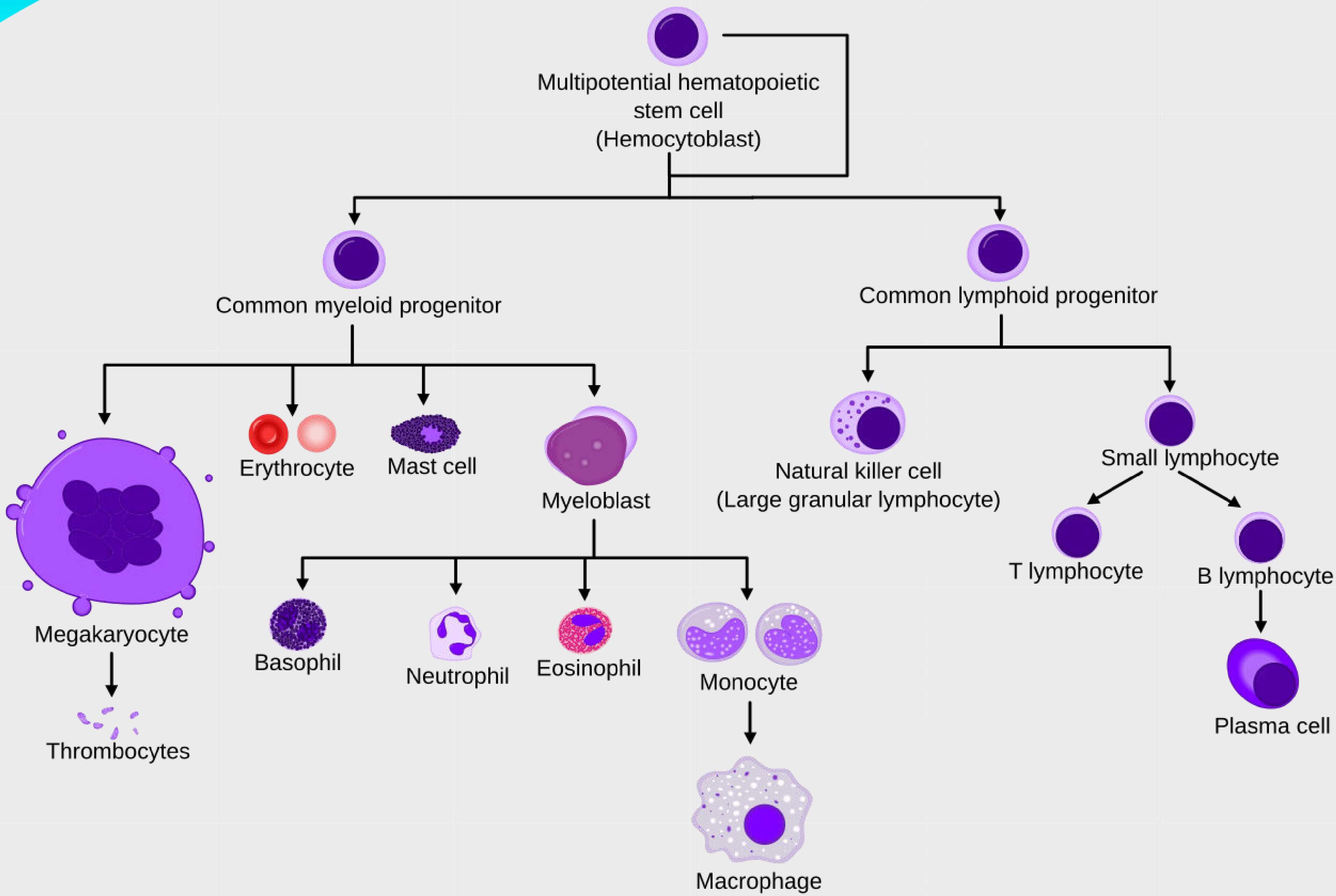


Multipotent stem cells

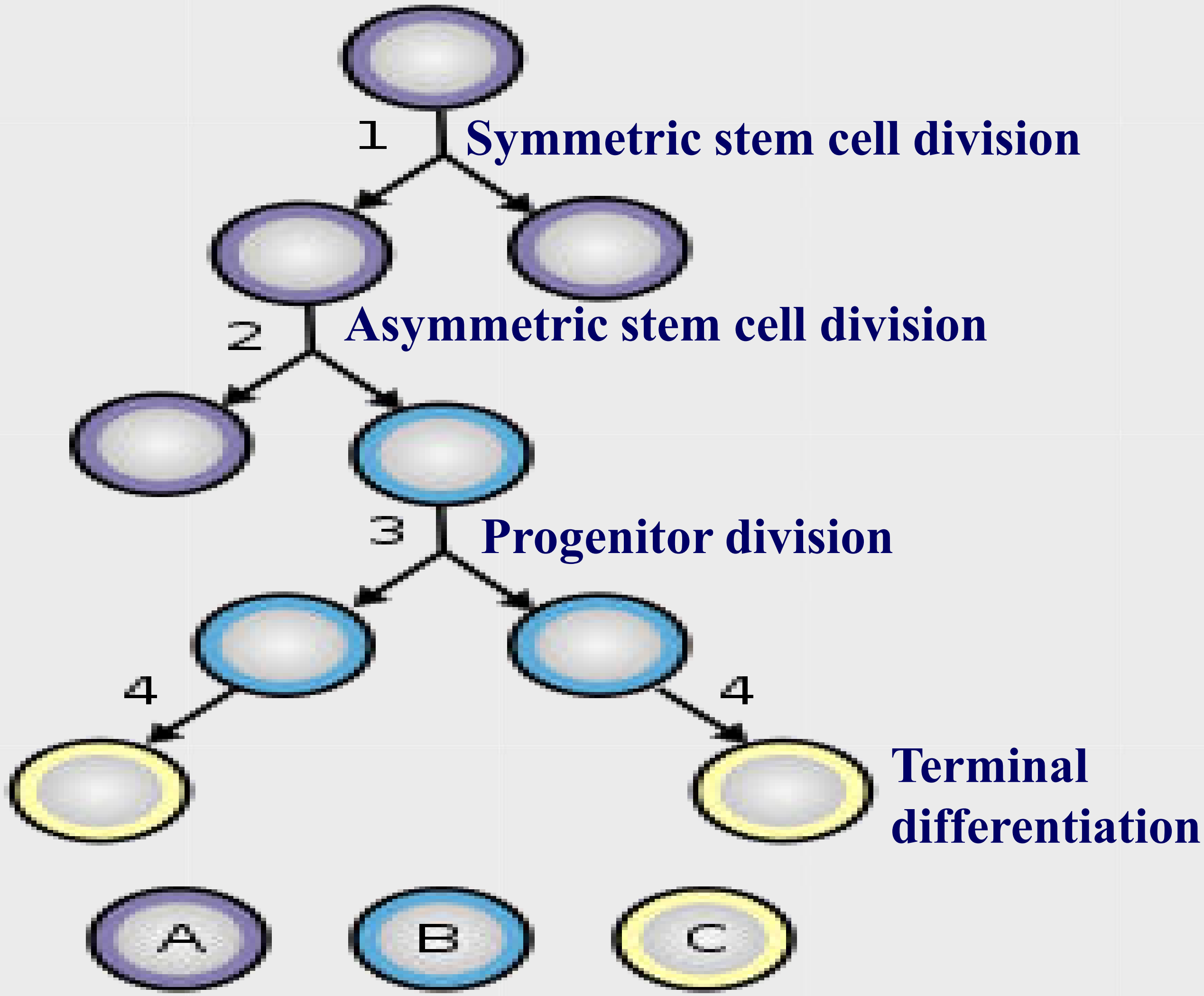
- **Multipotent stem cells have a narrower spectrum of differentiation than PSCs**, but they can specialize in discrete cells of specific cell lineages, e.g.,
  - **Haematopoietic stem cell (HSC)**, which can develop into several types of blood cells. After differentiation, a haematopoietic stem cell becomes an **oligopotent cell**. Its differentiation abilities are then restricted to cells of its lineage.
  - However, some multipotent cells are capable of conversion into unrelated cell types, which suggests naming them pluripotent cells.
- **Oligopotent stem cells** can differentiate into several cell types.
  - E.g., A myeloid stem cell, which can divide into white blood cells but not red blood cells.

hematopoietic stem cell

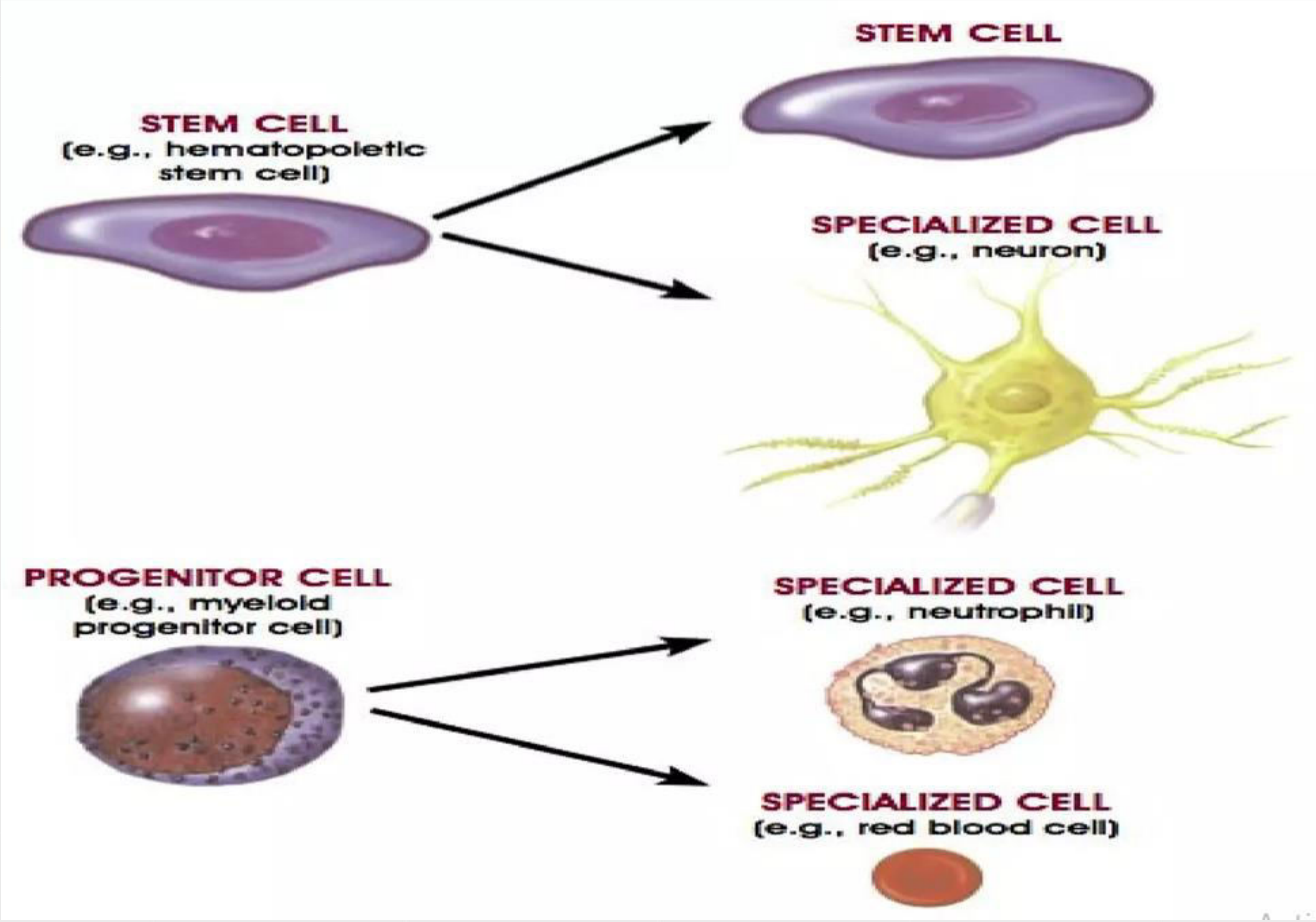
An immature cell that can develop into all types of blood cells, including white blood cells, red blood cells, and platelets. Hematopoietic stem cells are found in the peripheral blood and the bone marrow. Also called blood stem cell.


Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Megakaryocyte
Thrombocytes
Erythrocyte
Mast cell
Myeloblast
Basophil
Neutrophil
Eosinophil
Monocyte
Macrophage
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
T lymphocyte
B lymphocyte
Plasma cell

Stem cell division and Differentiation



Comparison of progenitor/precursor cell and Stem cell

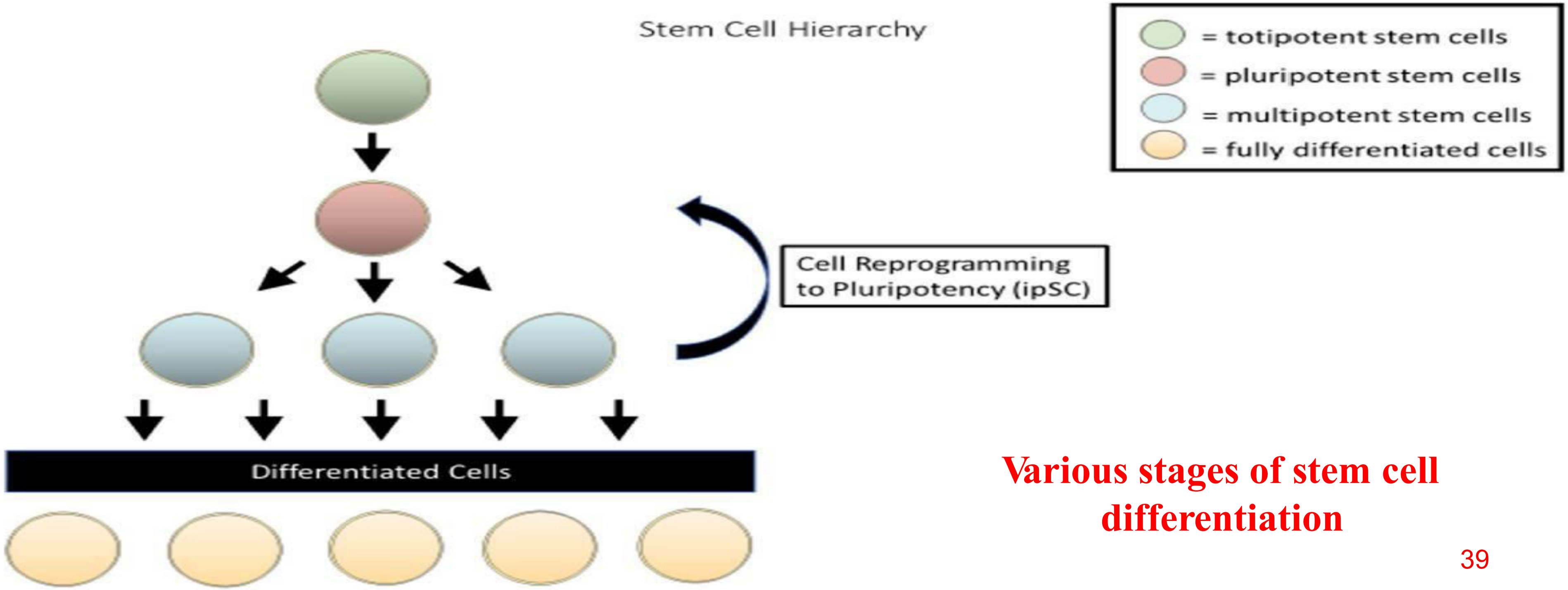


Difference between stem cell and progenitor cell

Stem cells	A progenitor cell
A stem cell is an unspecialized cell that develops into a variety of specialized cell types.	A progenitor cell is unspecialized that is capable of undergoing cell division and yielding two specialized cells.
a stem cell divides and gives rise to one additional stem cell and a specialized cell.	Example: a myeloid progenitor cell undergoing cell division to yield two specialized cells (a neutrophil and a red blood cell).
Example: a hematopoietic stem cell produce a second generation stem cell and a neuron.	

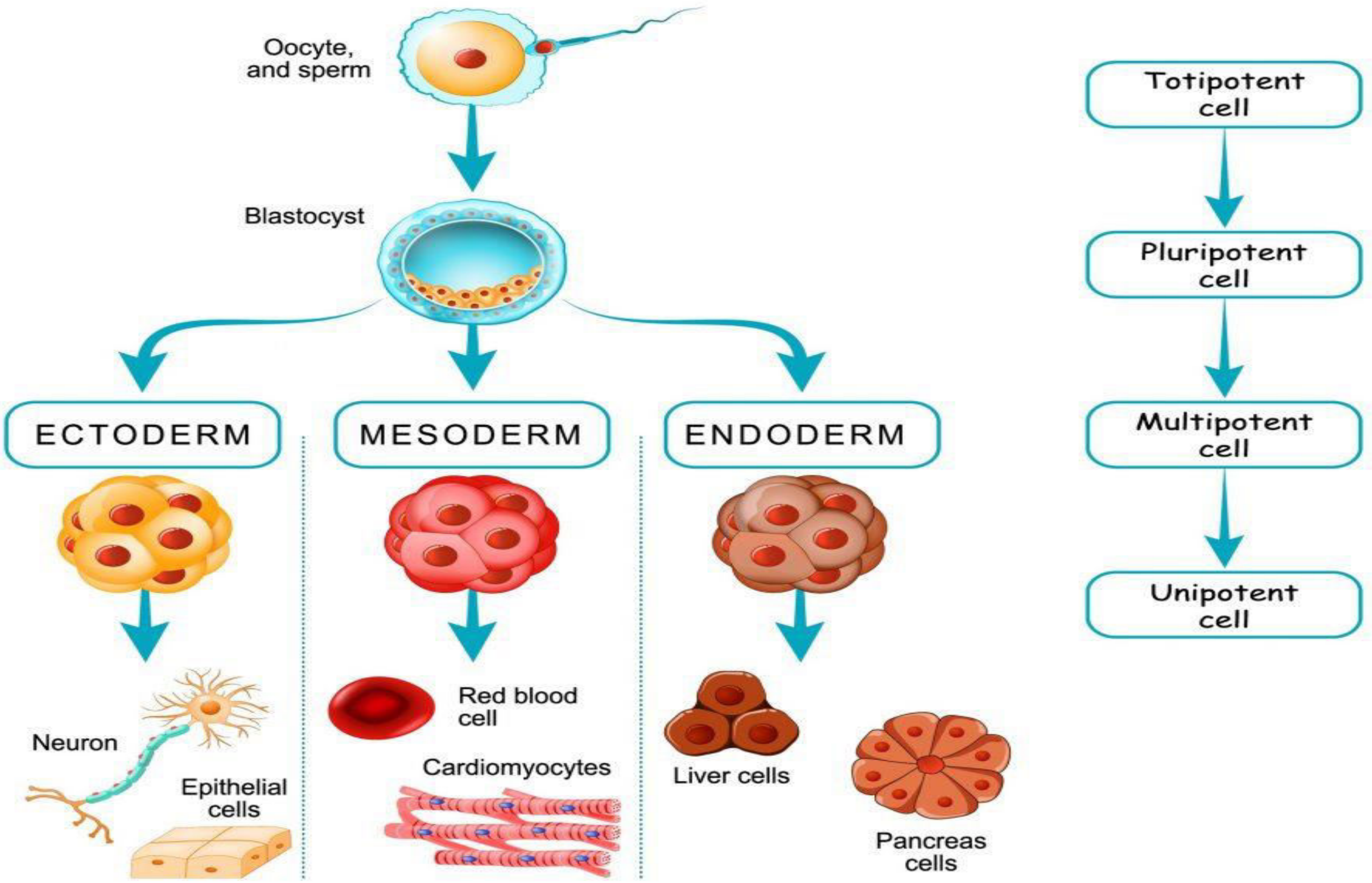
Unipotent stem cells

- **Unipotent stem cells are characterized by the narrowest differentiation capabilities and a special property of dividing repeatedly.**
- **Their feature makes them a promising candidate for therapeutic use in regenerative medicine. These cells are only able to form one cell type, e.g., dermatocytes.**

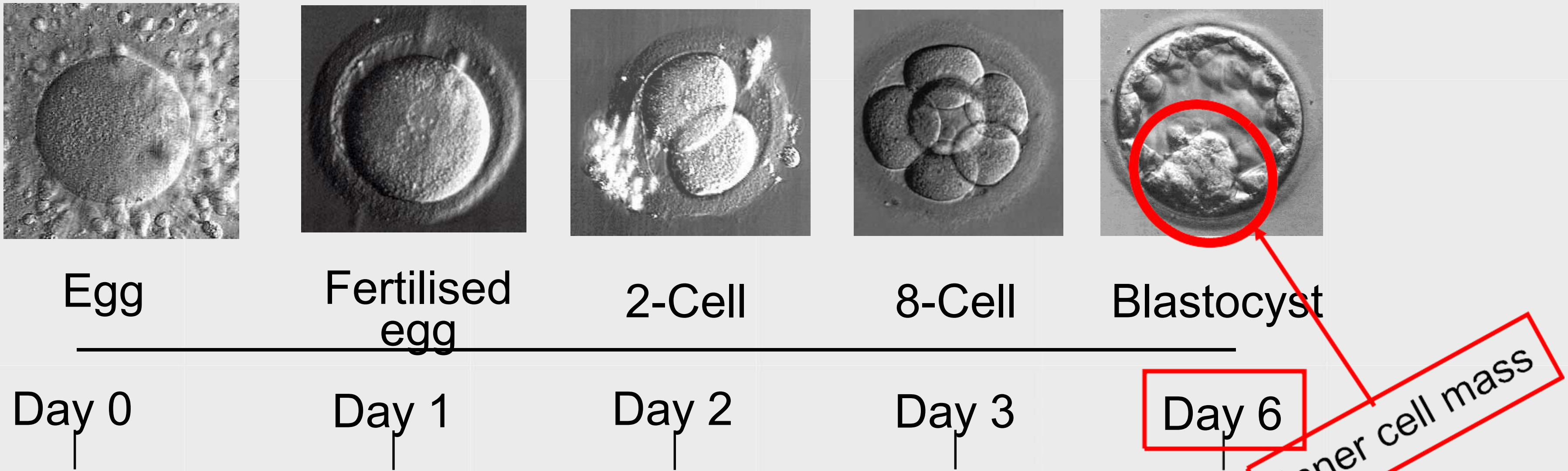


Various stages of stem cell differentiation

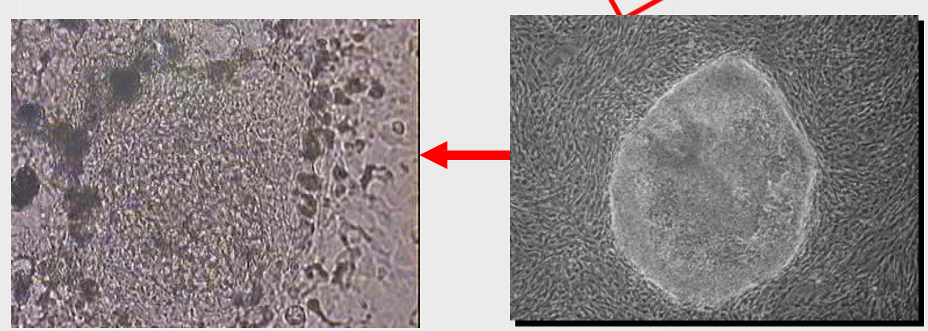
Cell potency



Embryonic stem cells



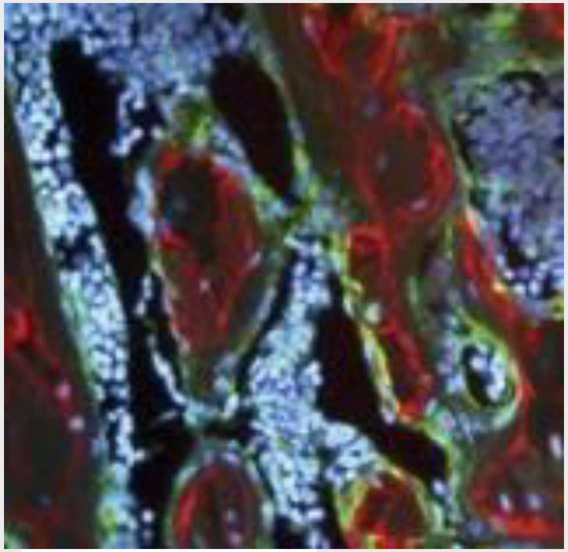
- Derived from embryos
- Can be grown indefinitely in the laboratory in an unspecialised state
- Retain ability to specialise into many different tissue types – know as pluripotent, express **pluripotency-based markers**
- Show high telomerase activity & Lack G1 checkpoint
- Tissue engineering and Regenerative Medicine



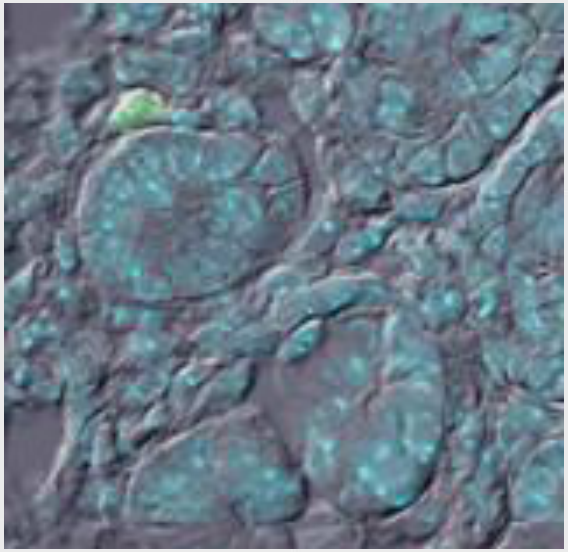
- Can restore function in <u>animal models following transplantation</u>

Adult/ Somatic/ Tissue stem cells

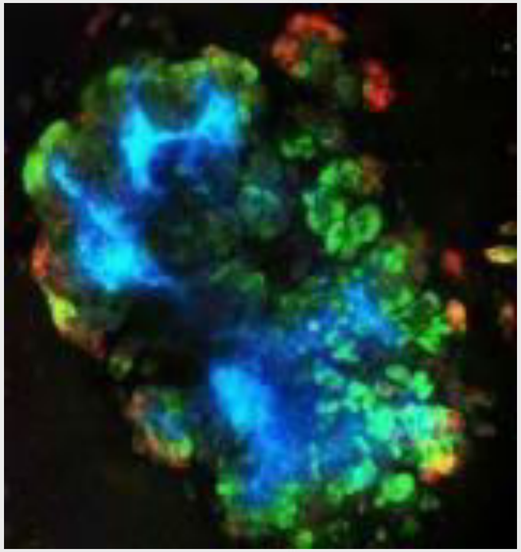
- **Multipotent Cells**- Group of heterogeneous multipotent cells
- **Regeneration-** Long-term self-renewal
- **Differentiation-** Give rise to mature cells having characteristic morphologies and specialized functions and Dispersed in tissues throughout the body
- **No ethical issues-** Unlike embryonic stem cells they do not represent any ethical concerns
- **Rare and hard to isolate & purify**
- **Origin of adult stem cells in tissues is yet not known**



Bone marrow

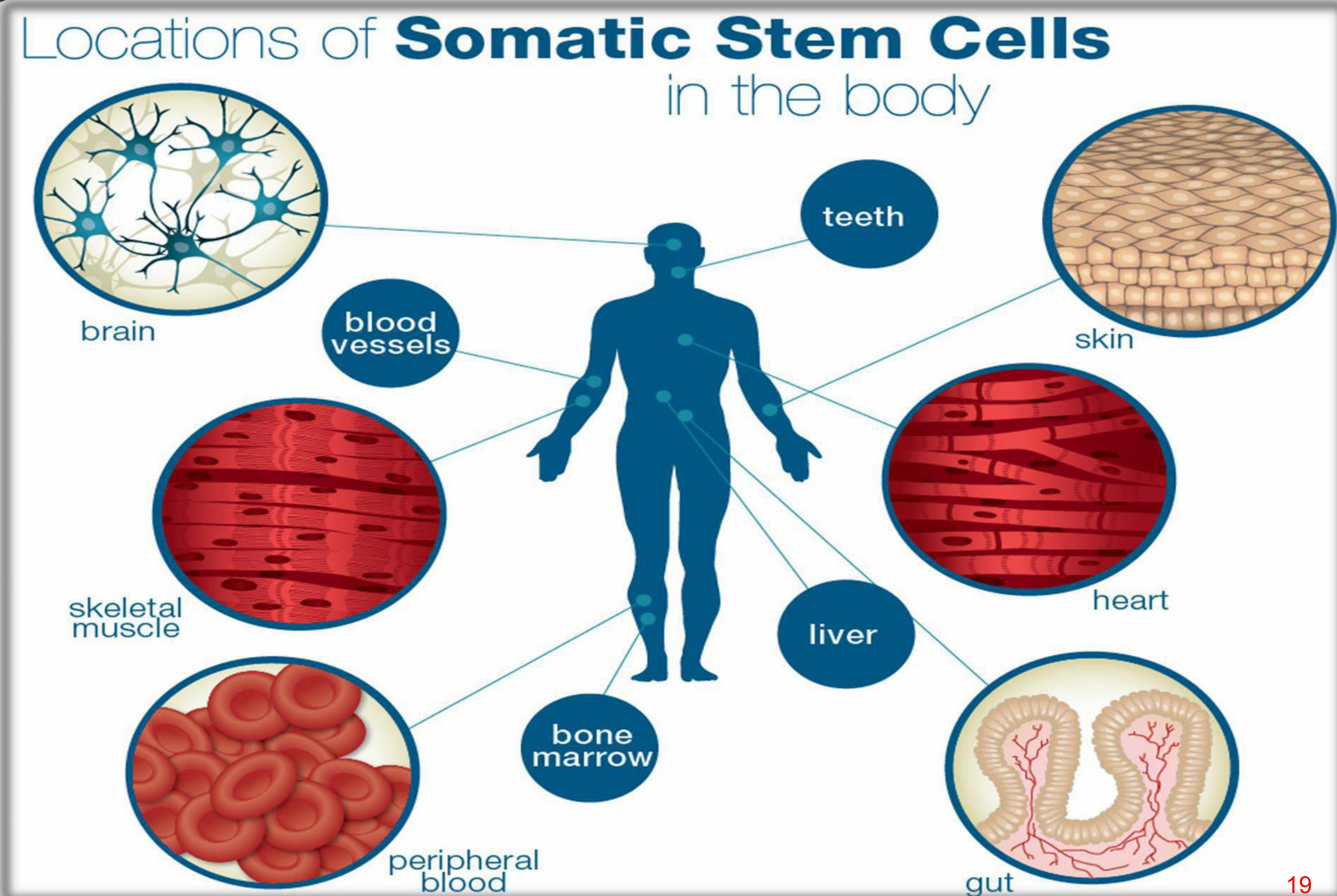


Kidney



Lung

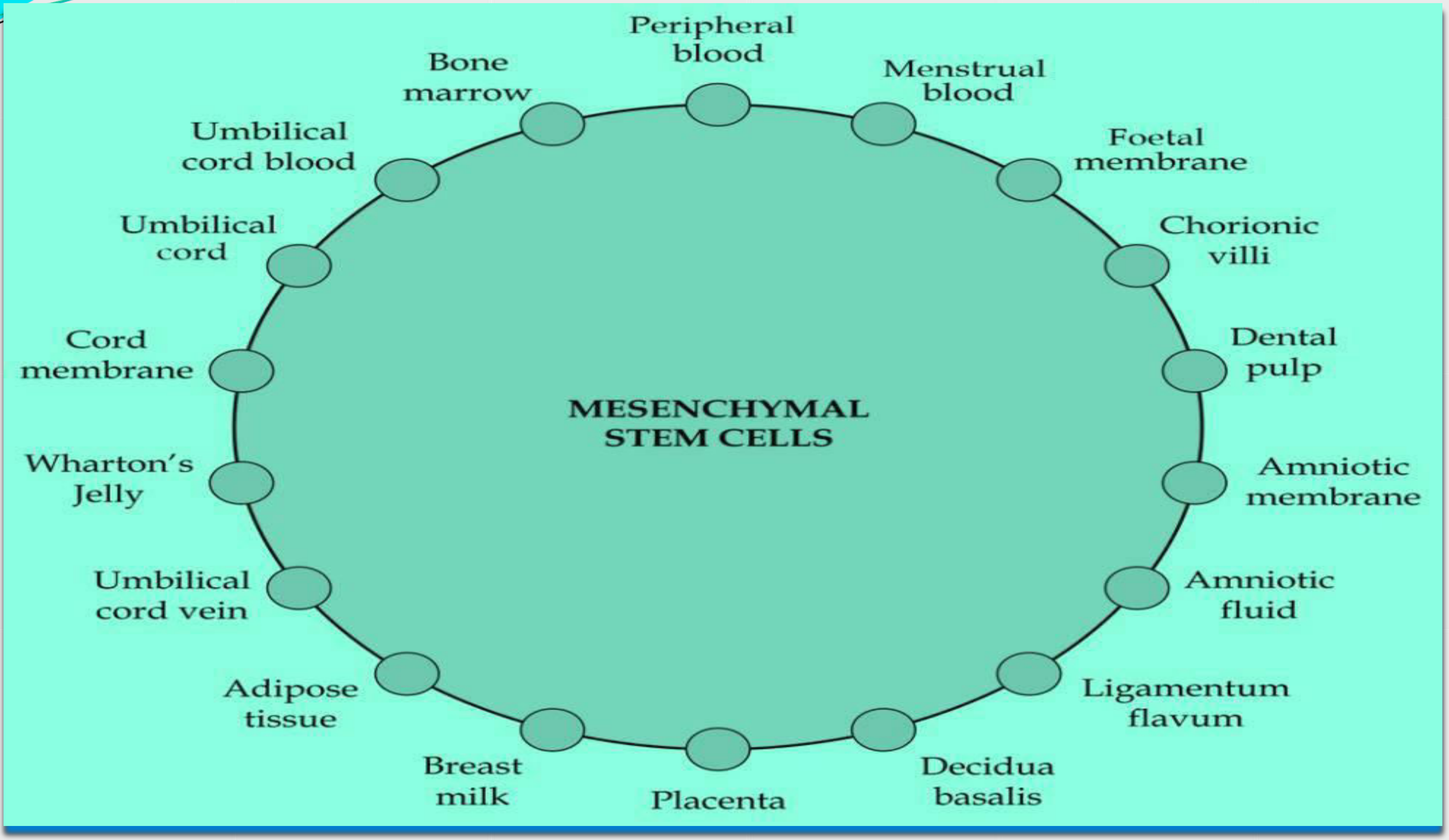
Source of adult stem cells



Mesenchymal stem cells/ Marrow Stromal cells

- Relative ease of isolation.
- Multipotent- The ability to differentiate into a wide variety of functional cell types of both Mesenchymal and Non-Mesenchymal origin.
- Their ability to be extensively expanded in culture without a loss of differentiative capacity.
- They are not only hypoimmunogenic, they also produce immuno-suppression upon transplantation.
- Their pronounced anti-inflammatory properties.
- Their ability to home to damaged tissues following in-vivo administration.
- The MSC capture and send molecular signals, which change the niche, either by modulating the immune system as providing mechanisms for tissue repair effectors, involving activation of cell homing, cell apoptosis, induction of the formation of new blood vessels, and the healing process.

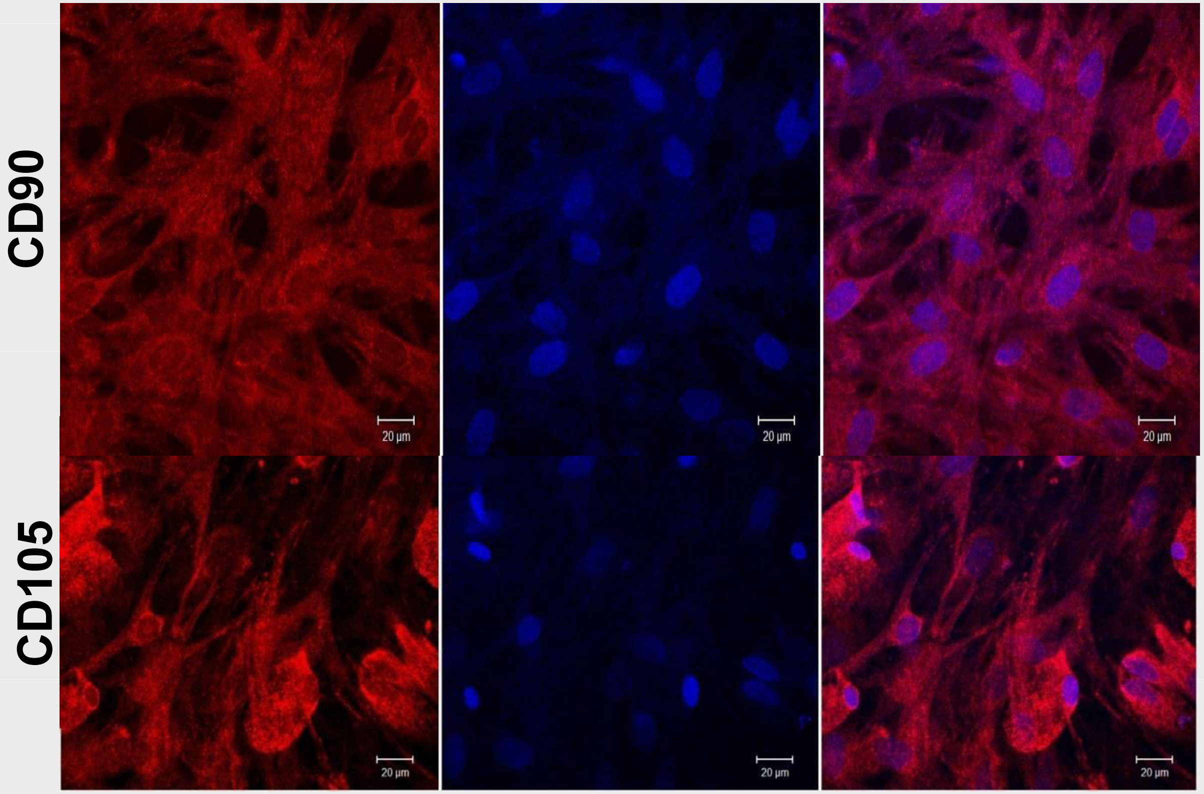
Source of Mesenchymal Stem Cells



MSCs can be derived from several adult or infant tissues

Source DOI: 10.5772/17588

Characterization of MSCs from Bone Marrow by Confocal Microscopy



MSCs were double stained in blue with DAPI nuclear stain CD90, CD105 antigens. The bright red cells indicate positivity. Scale bar = 20 µm

Table 2.1 Human MSC source, cell surface markers, and expansion media with serum supplements

Source	Method of isolation	Media	Serum supplement	Cell surface markers: Positive	Cell surface markers: Negative
Bone marrow	Ficoll density gradient method	DMEM	FBS	CD73, CD90, CD105, STRO-1	CD14, CD34, CD45, HLA-DR
	Novel marrow filter device	DMEM-F12			
		ADMEM			
Adipose tissue	Digestion method	DMEM	FBS	CD73, CD090, CD29, CD44, CD71, CD105, CD13, CD166, STRO-1	CD14, CD31, CD34, CD45
	Membrane filtration method	DMEM-LG	FCS		
Amniotic fluid and membrane	Density gradient method	α-MEM	FBS	CD29, CD44, CD90, CD105, CD, SH2, SH3, HLA-DR	CD10, CD14, CD34, HLA-DR
	Digestion method	DMEM/F12			
Dental tissues	Digestion method	α-MEM	FCS	CD29, CD44, CD90, CD105	CD14, CD34, CD45
		MEM	FBS		
Endometrium	Digestion method	DMEM-F12	FCS	CD73, CD90, CD105, CD146	CD34, CD45
Limb bud	Digestion method	DMEM-LG	FBS	CD13, CD29, CD90, CD105, CD106	CD3, CD4, CD14, CD15, CD34, CD45, HLA-DR
Peripheral blood	Ficoll density gradient	α-MEM	NBCS	CD44, CD90, CD105, HLA-ABC	CD45, CD133
Placenta and fetal membrane	Digestion method	DMEM-LG	FBS	CD29, CD73, CD90, CD105	CD34, CD45
Salivary gland	Digestion method (Ringer solution)	DMEM	FCS	CD13, CD29, CD44, CD90, STRO-1	CD34, CD45
Skin and foreskin	Digestion method	DMEM-HG DMEM DMEM-F12	FBS	CD44, CD73, CD90, CD105, CD166, SSEA-4, vimentin	CD34, CD45, HLA-DR

Clinical applications of MSCs in Degenerative, Inflammatory or Autoimmune Chronic Diseases

- **Muscular dystrophies**
- **Cardiovascular diseases**
- **Fulminant hepatic failure**
- **Rheumatoid arthritis**
- **Systemic lupus rythematosus**
- **Amyotrophic lateral sclerosis (ALS)**
- **Type 1 diabetes**
- **Multiple Sclerosis**
- **Parkinson’s disease**
- **Alzheimer disease**