STEM CELLS

Dr. Monika Nema

INTRODUCTION

- Stem cells are one of the most fascinating areas of biology today.

  Stem cells are a special kind of cell that have the ability to divide indefinitely and have the potential to give rise to specialized cells (that is, any cell of the body).

Stem Cell Characteristics

- **‘Blank cells’ (unspecialized)**
- **Capable of dividing and renewing themselves for long periods of time (proliferation and renewal)**
- **Have the potential to give rise to specialized cell types (differentiation)**
- **Plasticity**

Unique properties of all stem cells

1] Stem cells are **unspecialized**

One of the fundamental properties of stem cells is that it does not have any tissue specific structures that allow it to perform specialized function.

2] **Proliferation**

They are capable of dividing and renewing themselves for indefinite periods

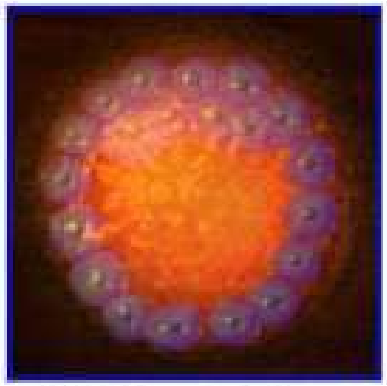
3]**Differentiation**

They can give rise to specialized tissue. Under certain **physiological** and **experimental conditions** unspecialized cell can give rise to specialized cells such as including heart muscle cells, blood cells or nerve cells required to repair damaged or depleted adult cell population or tissue.

4]**Plasticity**

Stem cell from one tissue may be able to give rise to cell types of completely different tissue , a phenomenon known as plasticity. e.g. Blood cells becoming neuron, liver cells producing insulin and haematopoietic stem cells, developing into heart muscle.

Stem cell timeline



Single Cell Embryo

Totipotent

5-7 Day Embryo

Embryonic Stem (ES) Cells

Pluripotent

Infant

Adult

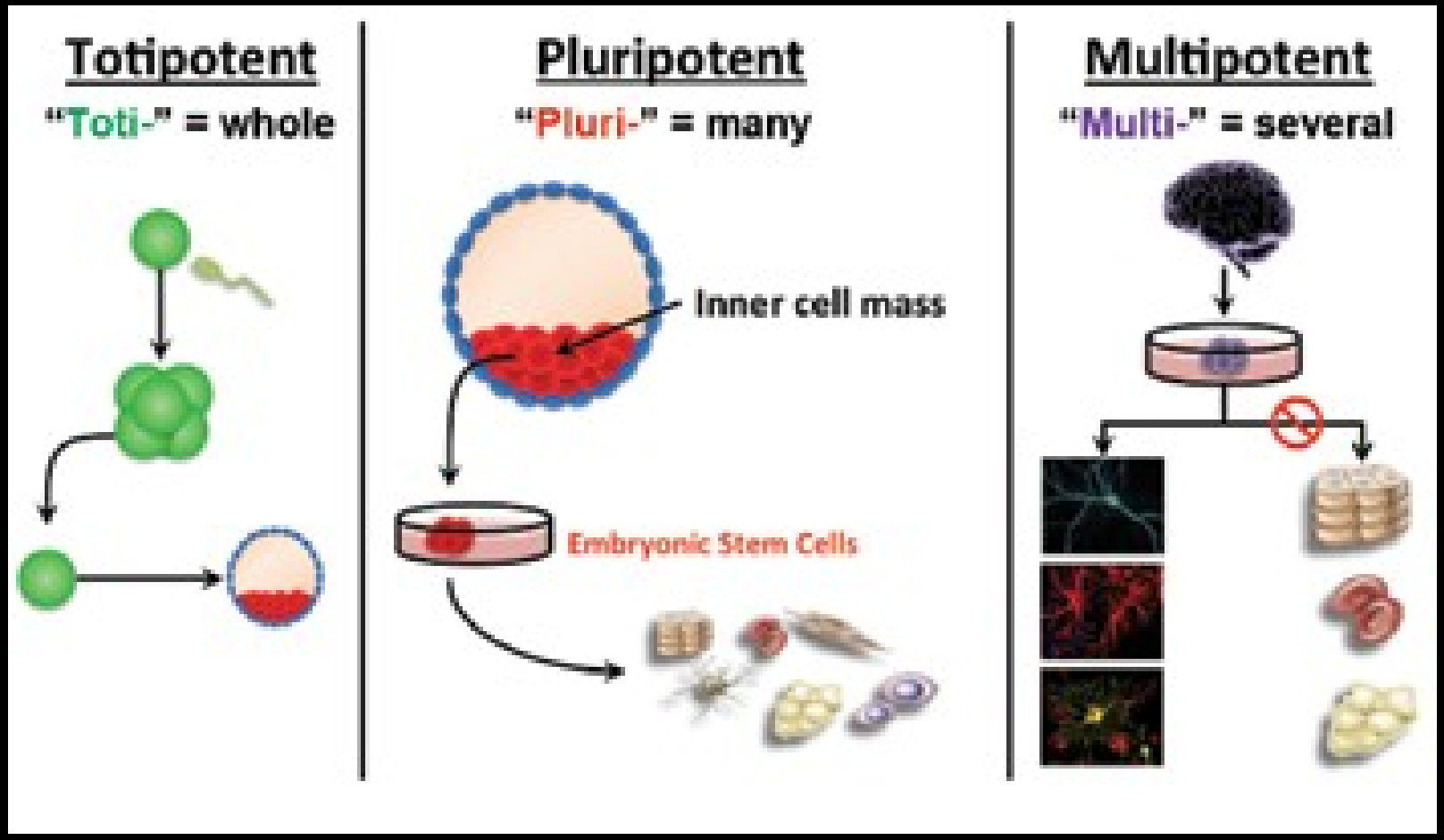
"Adult" Stem Cells

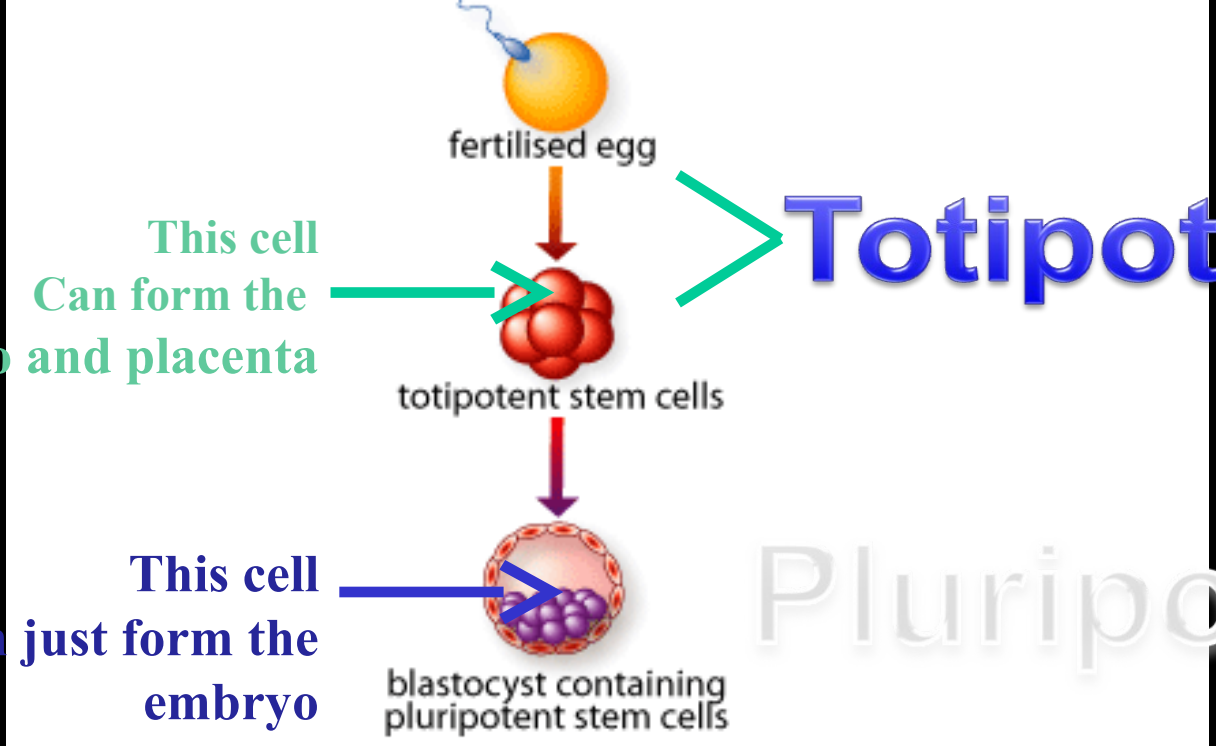
Multipotent

Cord Blood Stem Cells

Placental Stem Cells

Multipotent


Totipotent
"Toti-" = whole
Pluripotent
"Pluri-" = many
Inner cell mass
Embryonic Stem Cells
Multipotent
"Multi-" = several

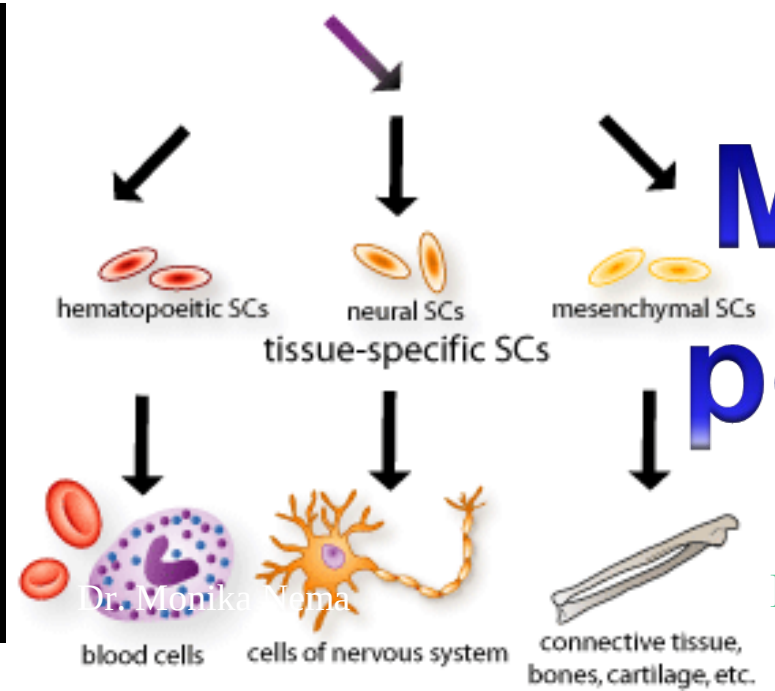


This cell
Can form the
Embryo and placenta

Totipotent

This cell
Can just form the
embryo

Pluripotent



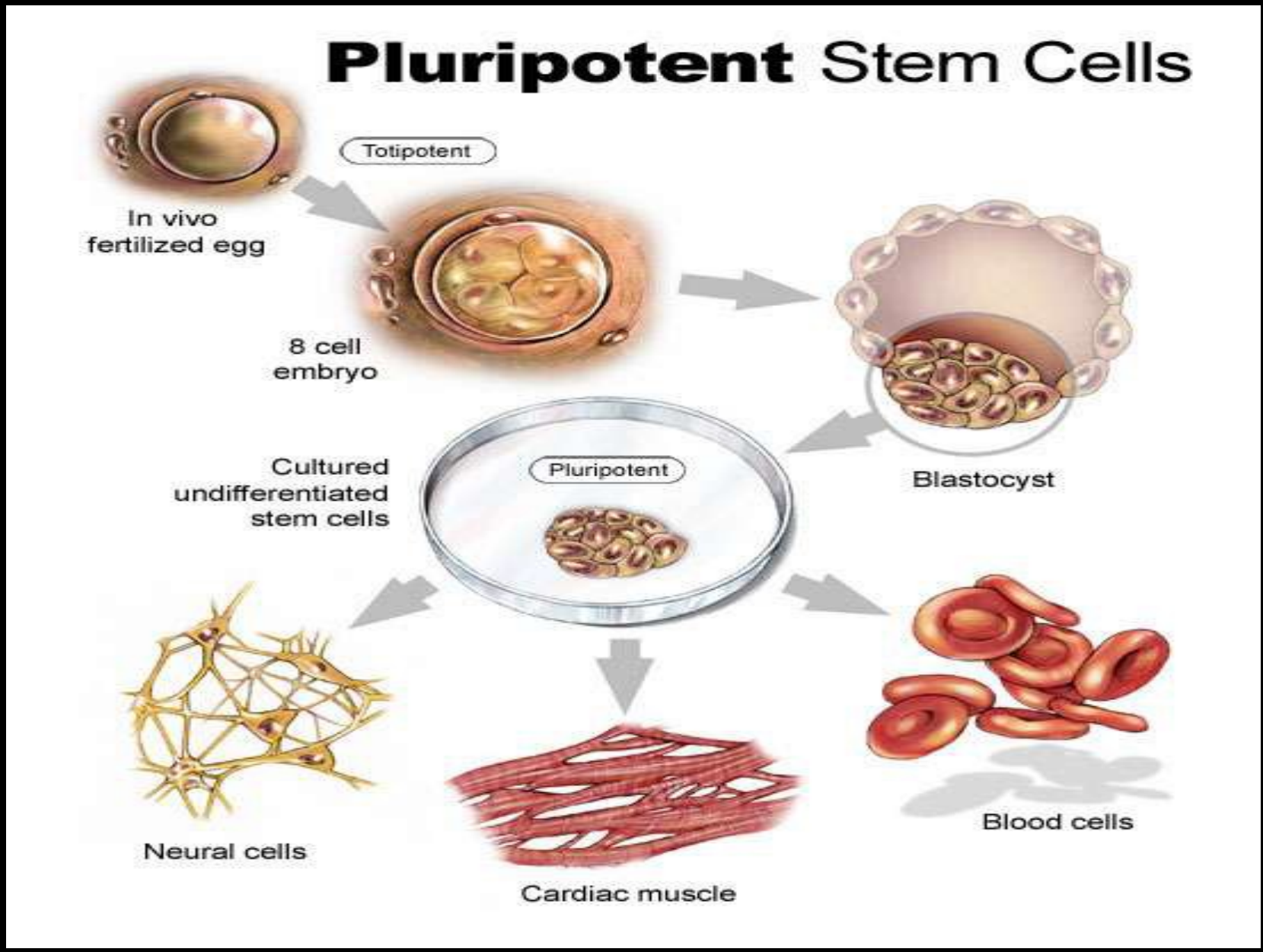
Multi-potent

Fully mature

Terminology

- **Totipotent cells.** These cells have the potential to become
  - any type in the adult body;
  - any cell of the extraembryonic membranes (e.g., placenta).
- The only totipotent cells are the **fertilized egg** and the first 4 or so cells produced by its cleavage (as shown by the ability of mammals to produce identical twins, triplets, etc.).

- **Pluripotent stem cells.** These are true stem cells, with the potential to make any differentiated cell in the body (but probably not those of the placenta which is derived from the trophoblast).

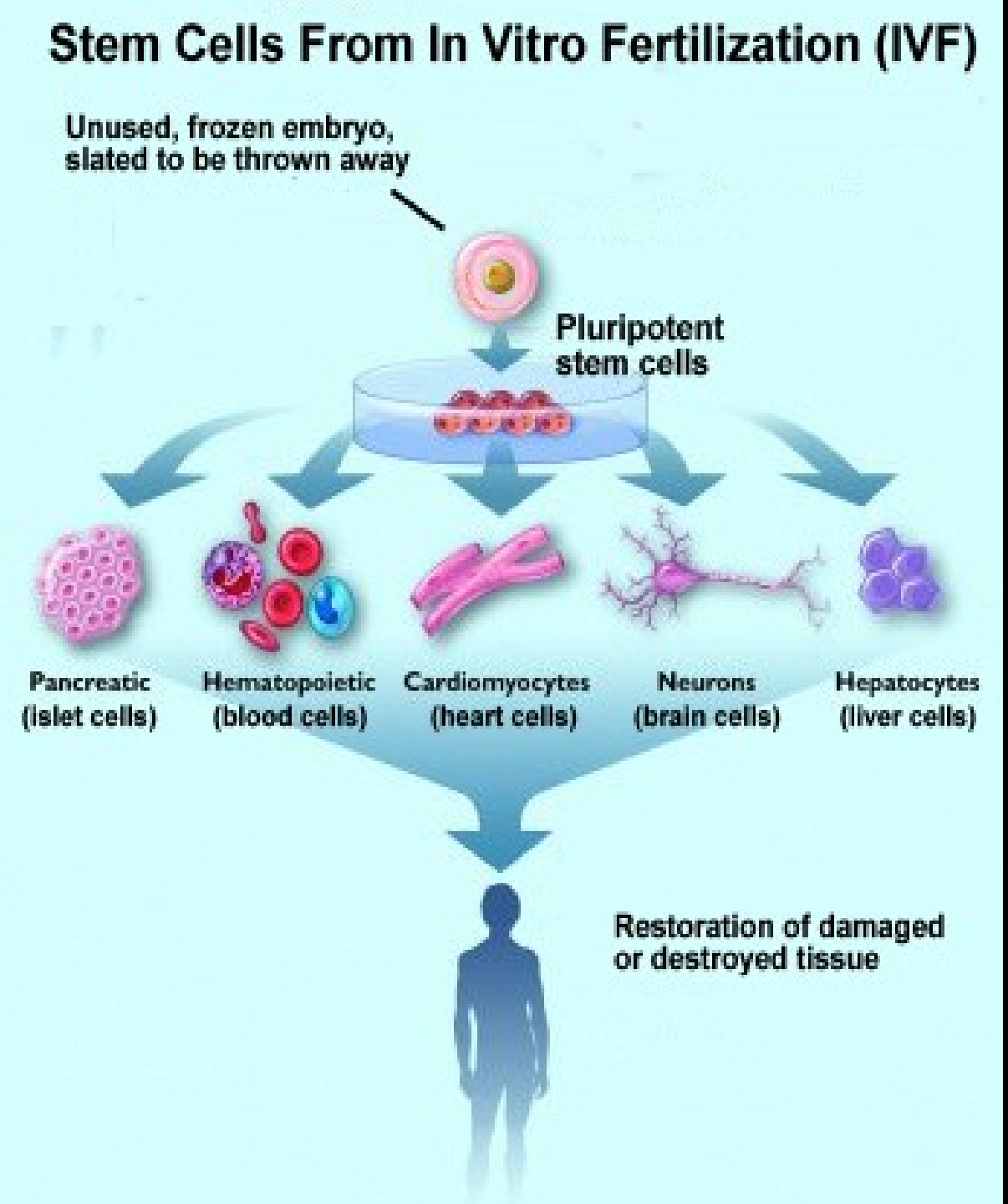

Pluripotent Stem Cells
Totipotent
In vivo fertilized egg
8 cell embryo
Blastocyst
Cultured undifferentiated stem cells
Pluripotent
Neural cells
Cardiac muscle
Blood cells

Two types of pluripotent stem cells have been found

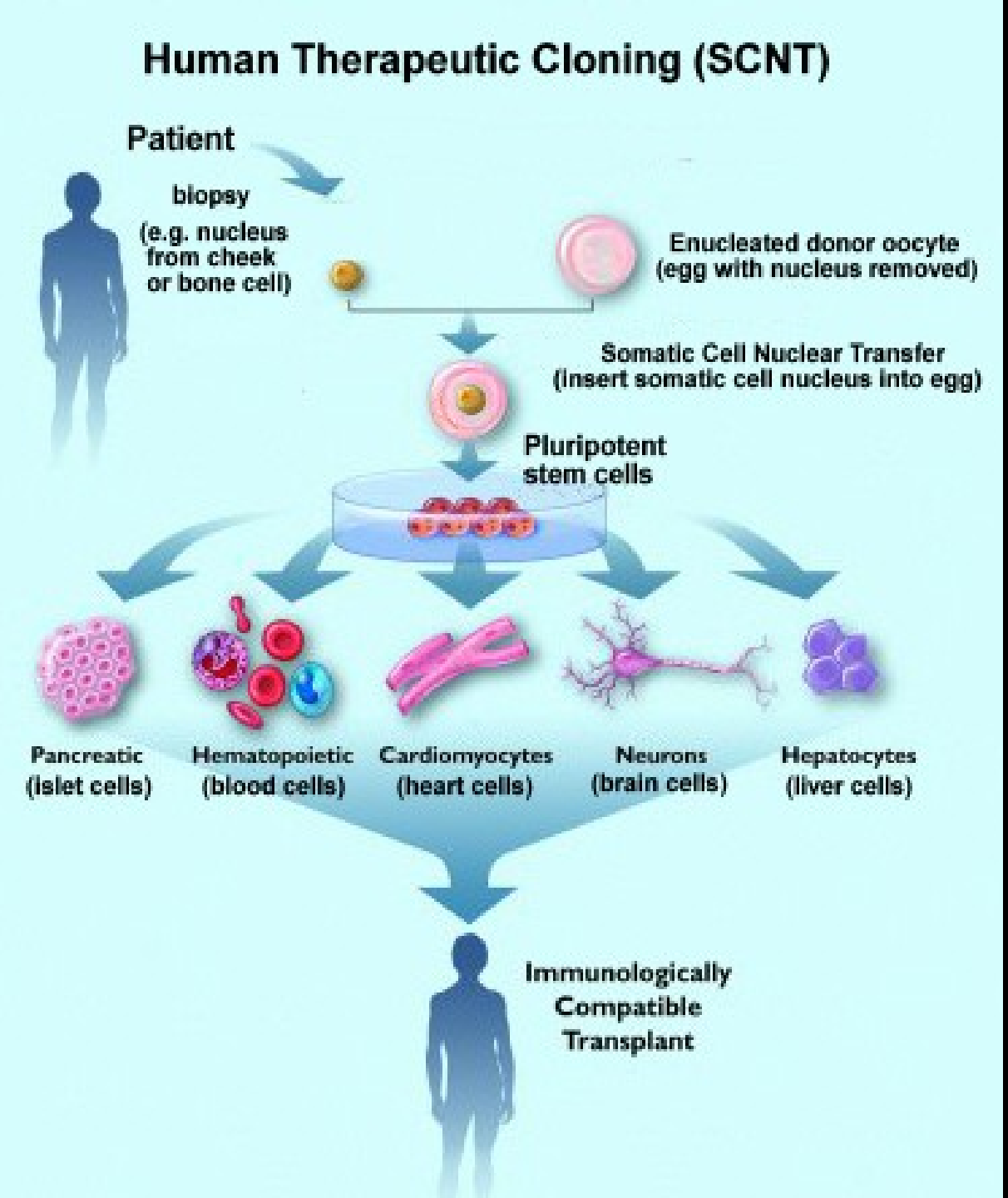
- **Embryonic Stem (ES) Cells.** These can be isolated from the **inner cell mass** (ICM) of the blastocyst — the stage of embryonic development when implantation occurs. For humans, excess embryos produced during in vitro fertilization (IVF) procedures are used.
- **Embryonic Germ (EG) Cells.** are derived from the part of a human embryo or foetus that will ultimately produce eggs or sperm (gametes).

These types of pluripotent stem cells

- can only be isolated from embryonic or fetal tissue;
- can be grown in culture, but only with special methods to prevent them from differentiating.



- Tens of thousands of frozen embryos are routinely destroyed when couples finish their treatment.
- These surplus embryos can be used to produce stem cells.
- Regenerative medical research aims to develop these cells into new, healthy tissue to heal severe illnesses.



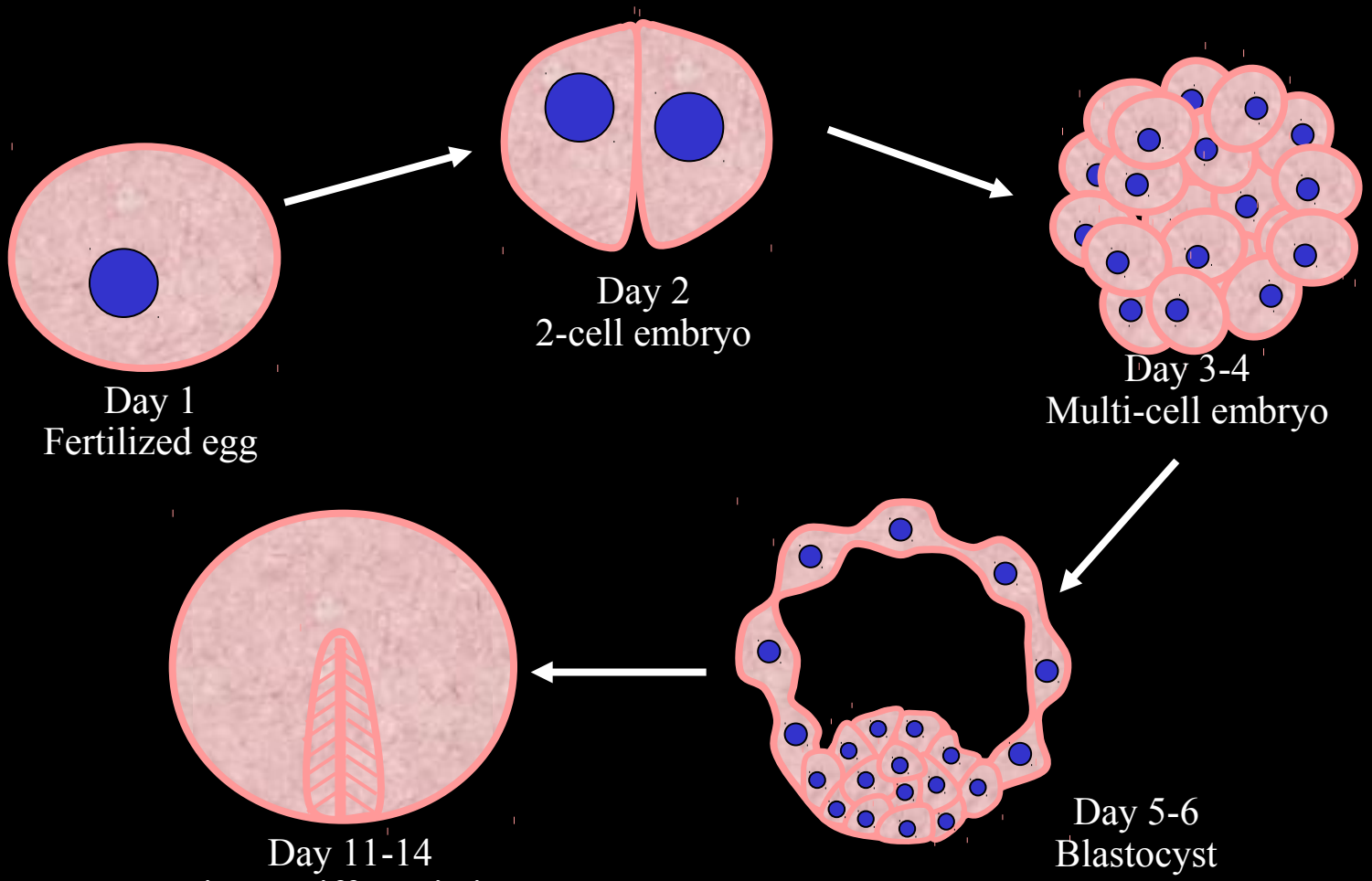
Somatic Cell Nuclear Transfer

- 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.

How are embryonic stem cells harvested?

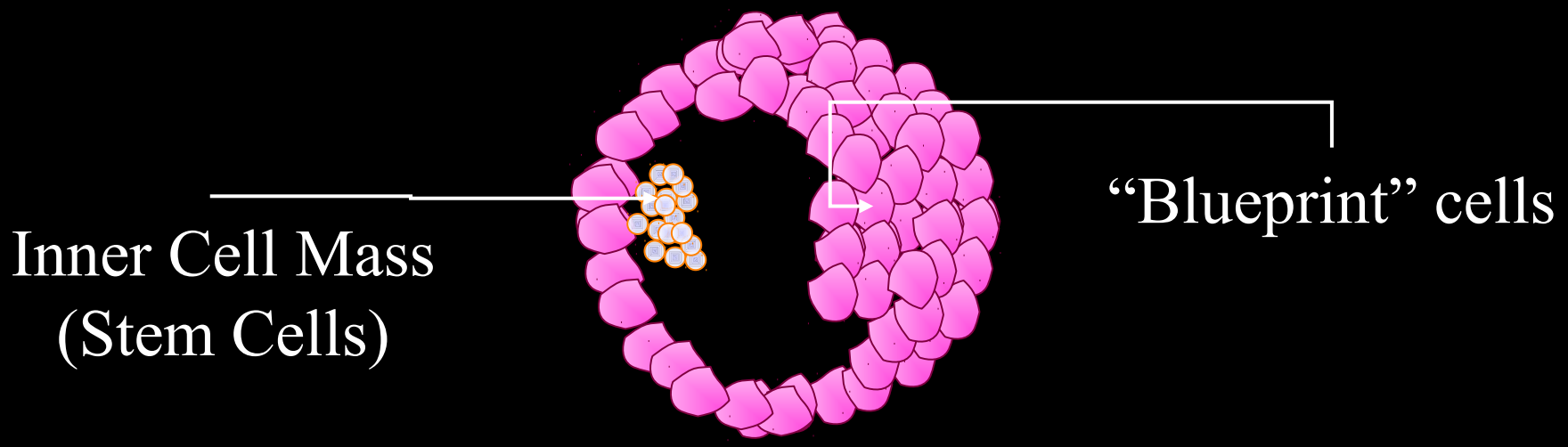
- Growing cells in the laboratory is called as cell culture.
- Human ES cells are derived from 4-5 day old blastocyst
- Blastocyst structures include:
  - Trophoblast: outer layer of cells that surrounds the blastocyst & forms the placenta
  - Blastocoel: (“blastoseel”) the hollow cavity inside the blastocyst that will form body cavity
  - Inner cell mass: a group of approx. 30 cells at one end of the blastocoel:
    - Forms 3 germ layers that form all embryonic tissues (endoderm, mesoderm, ectoderm)

Stages of Embryogenesis



A primer on Human Embryonic Stem Cells

Blastocyst -
from In Vitro Fertilization Clinic



A Blastocyst is a hollow ball of cells with
a small clump of stem cells inside

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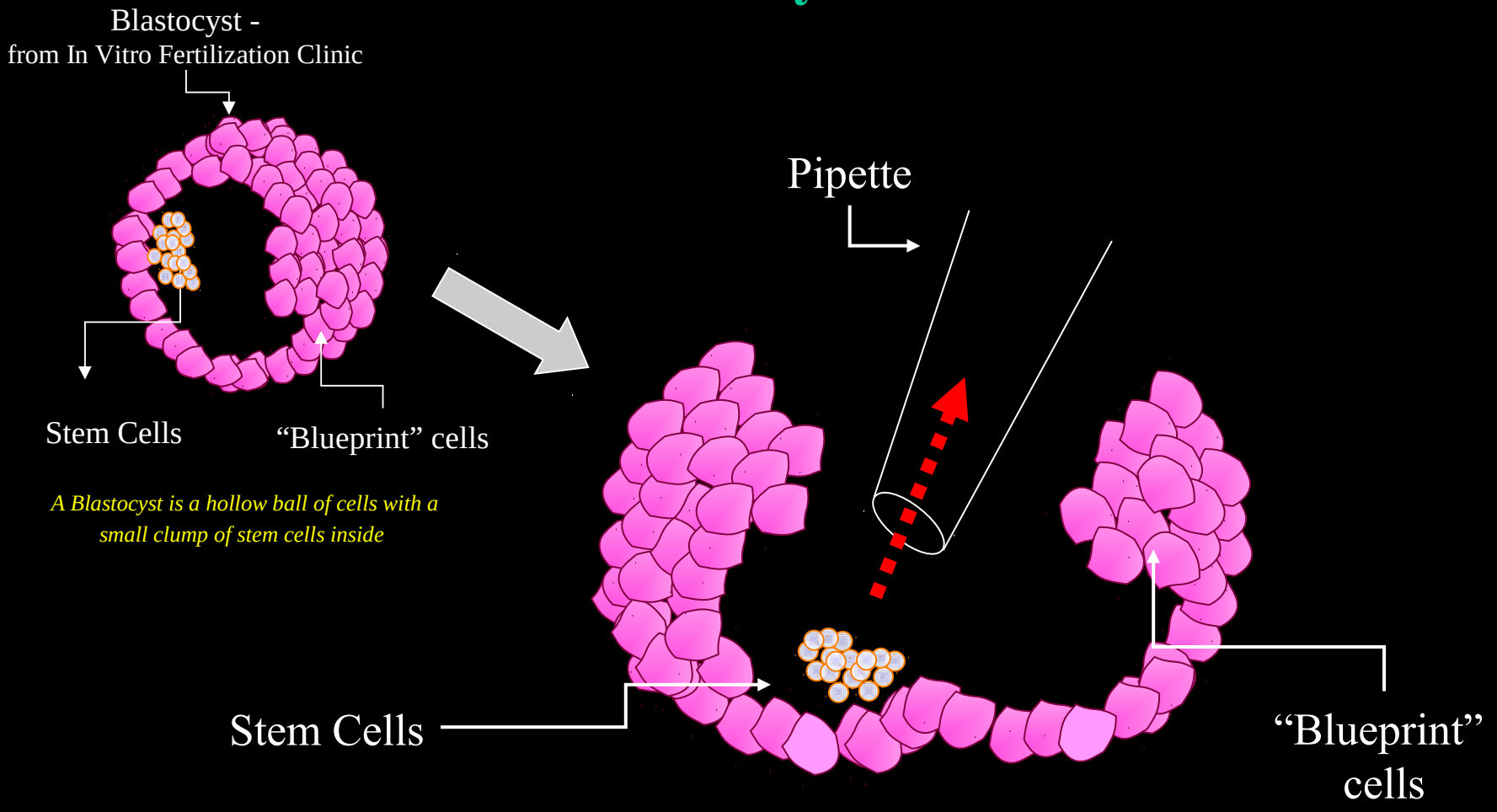
Human Embryonic Stem Cells



To remove the stem cells, the Blastocyst is opened and the stem cells removed with a pipette

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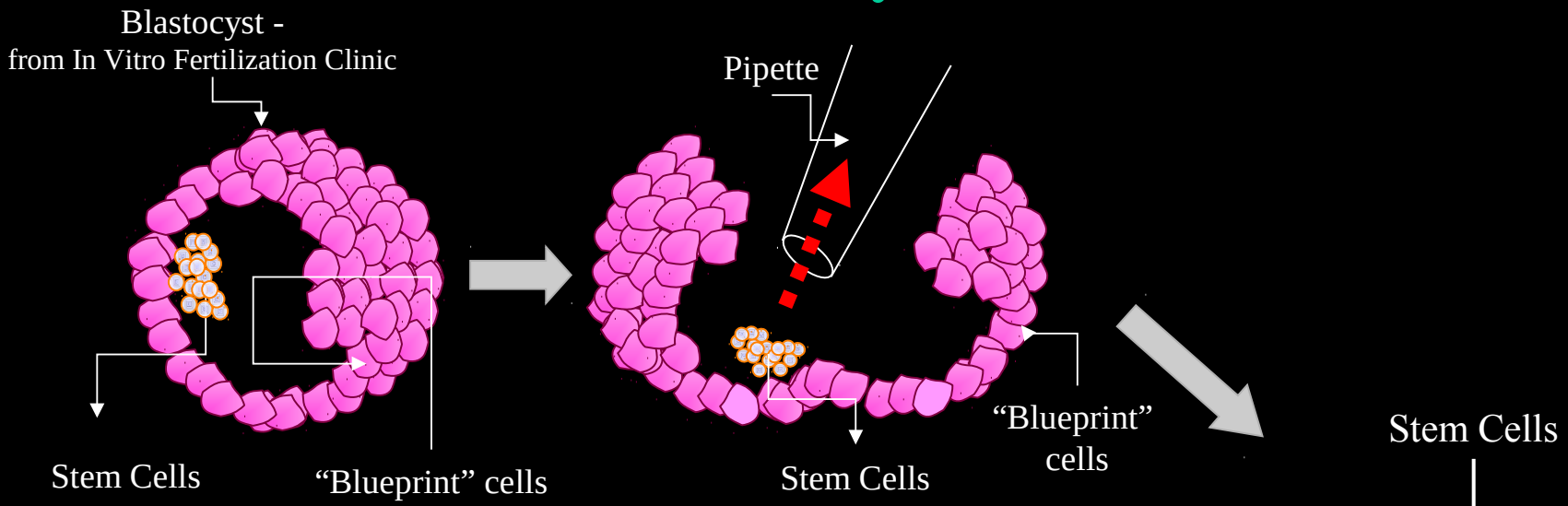
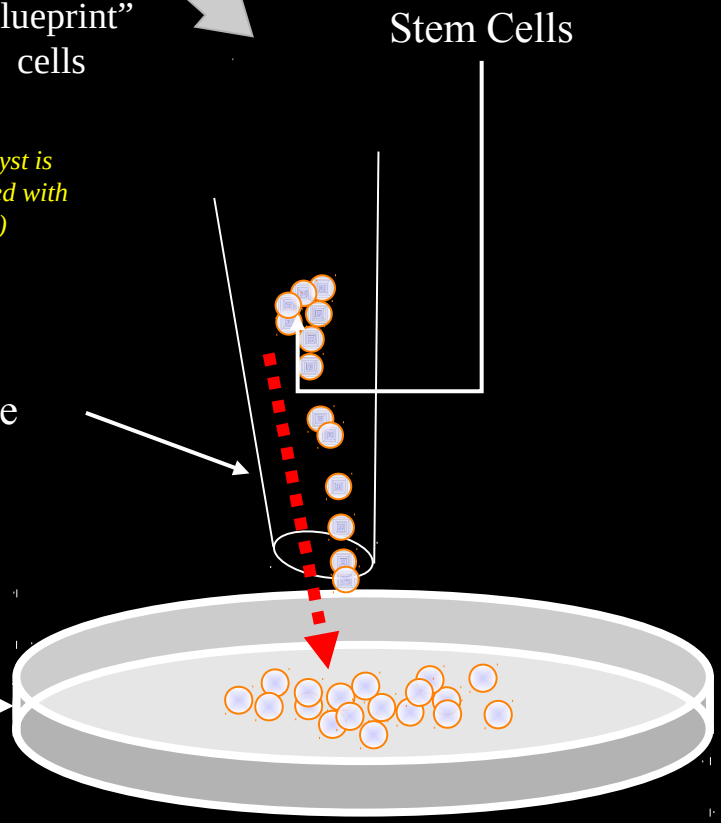
Human Embryonic Stem Cells



A Blastocyst is a hollow ball of cells with a small clump of stem cells inside

To remove the stem cells, the Blastocyst is broken open and the stem cells removed with a pipette(an ultra thin glass tube)

The stem cells are placed in a dish and are fed and cared for (each blastocyst = 1 stem cell line)



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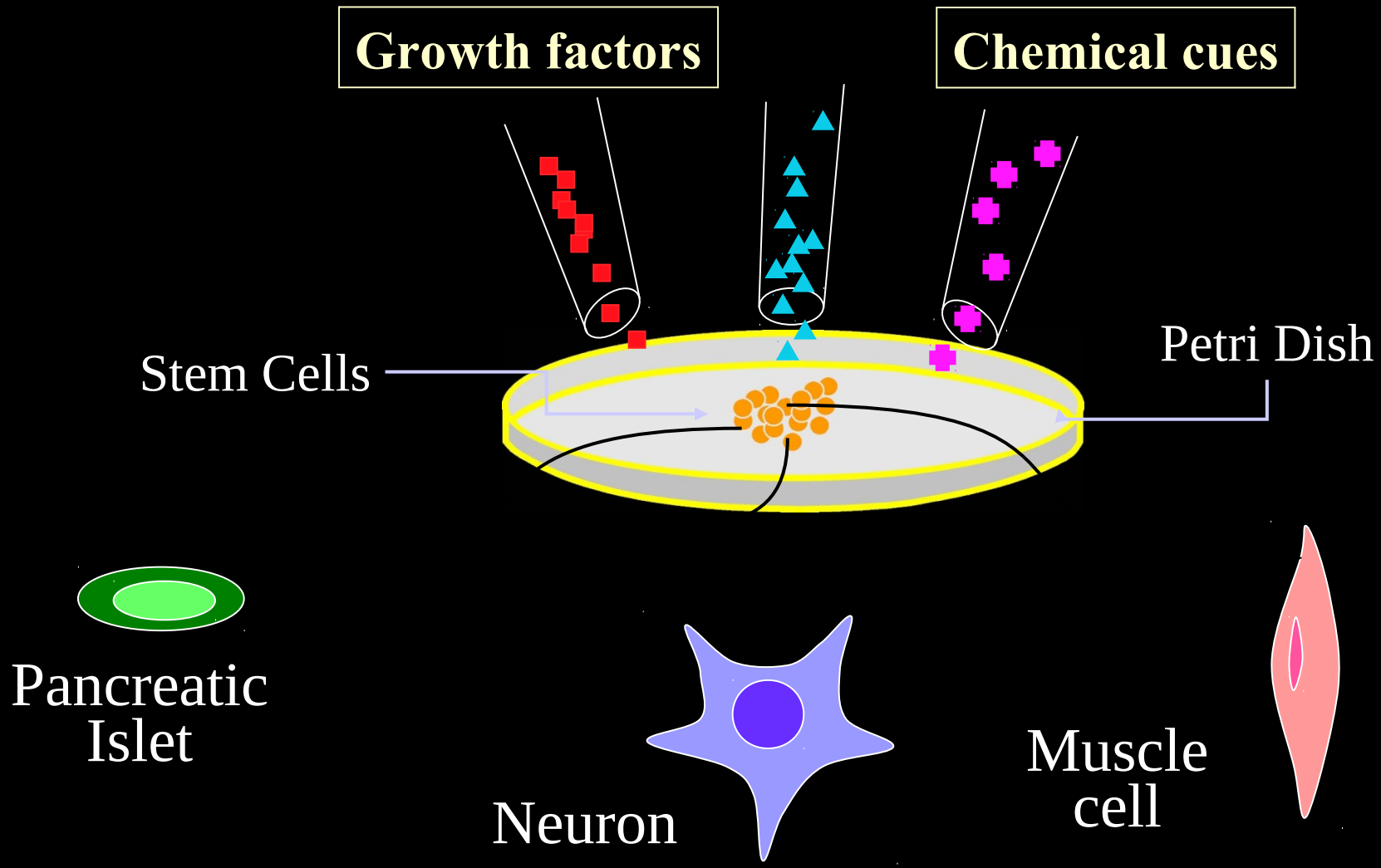


Different chemicals / molecules are added to the stem cells to make them become specific types of cells.

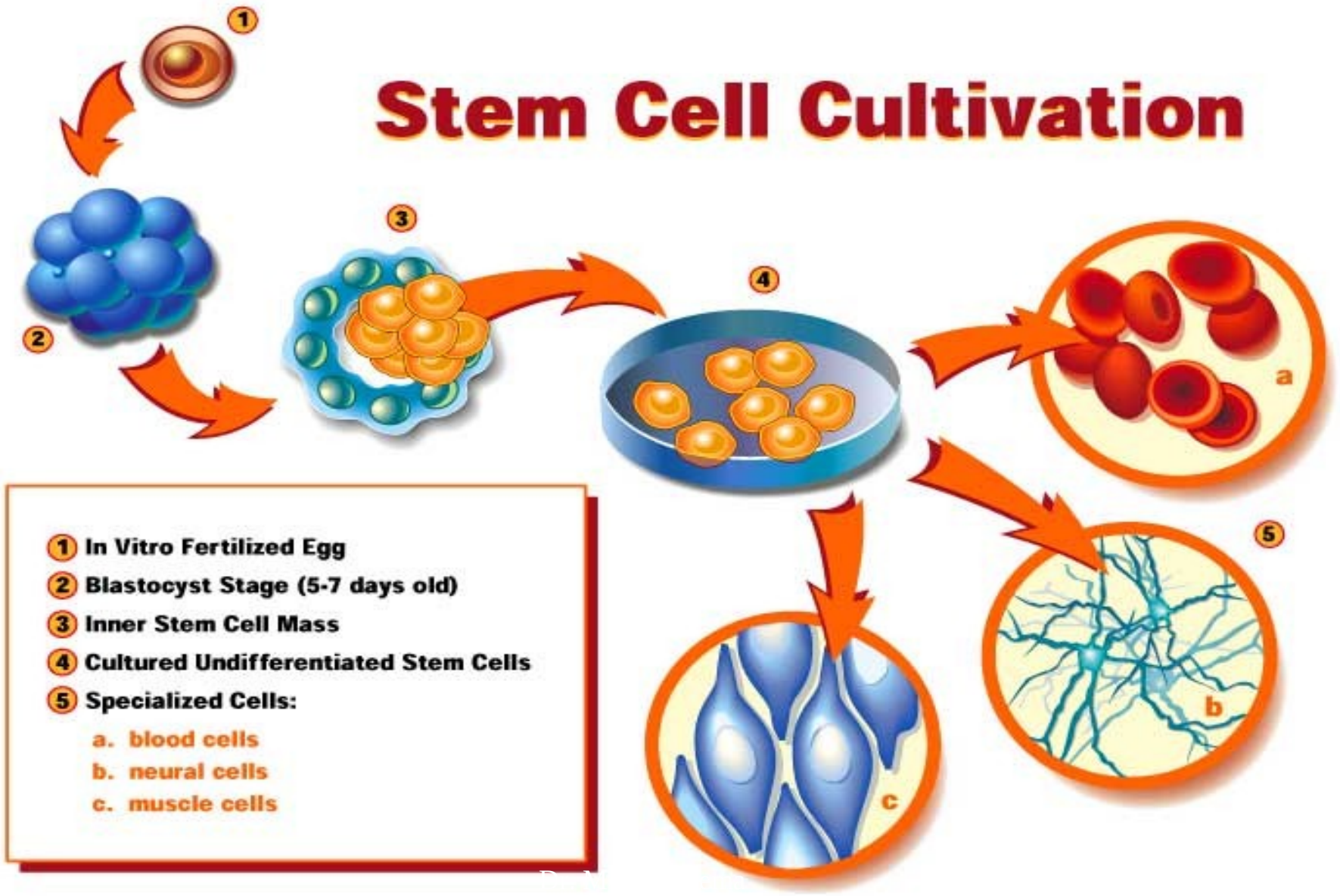
Cell Culture Techniques for ESC

- Isolate & transfer of inner cell mass into plastic culture dish that contains culture medium
- Cells divide and spread over the dish
- Inner surface of culture dish is typically coated with mouse embryonic skin cells that have been treated so they will not divide

- This coating is called a FEEDER LAYER
  - Feeder cells provide ES cells with a sticky surface for attachment
  - Feeder cells release nutrients
- Recent discovery: methods for growing embryonic stem cells without mouse feeder cells
  - Significance – eliminate infection by viruses or other mouse molecules
- ES cells are removed gently and plated into several different culture plates before crowding occurs

- Over the course of several days the inner cell mass proliferate and begin to crowd the culture dish.
- They are then gently removed and plated into several fresh culture dishes.
  - The process of plating the cells is repeated several times and for many months and is called subculturing .
  - Each cycle of subculturing is referred to as a passage.
- After six months or more the inner cell mass yield millions of embryonic stem cells. These cells are pluripotent and appear genetically normal and are referred to as an embryonic stem cell line.

Stem Cell Cultivation

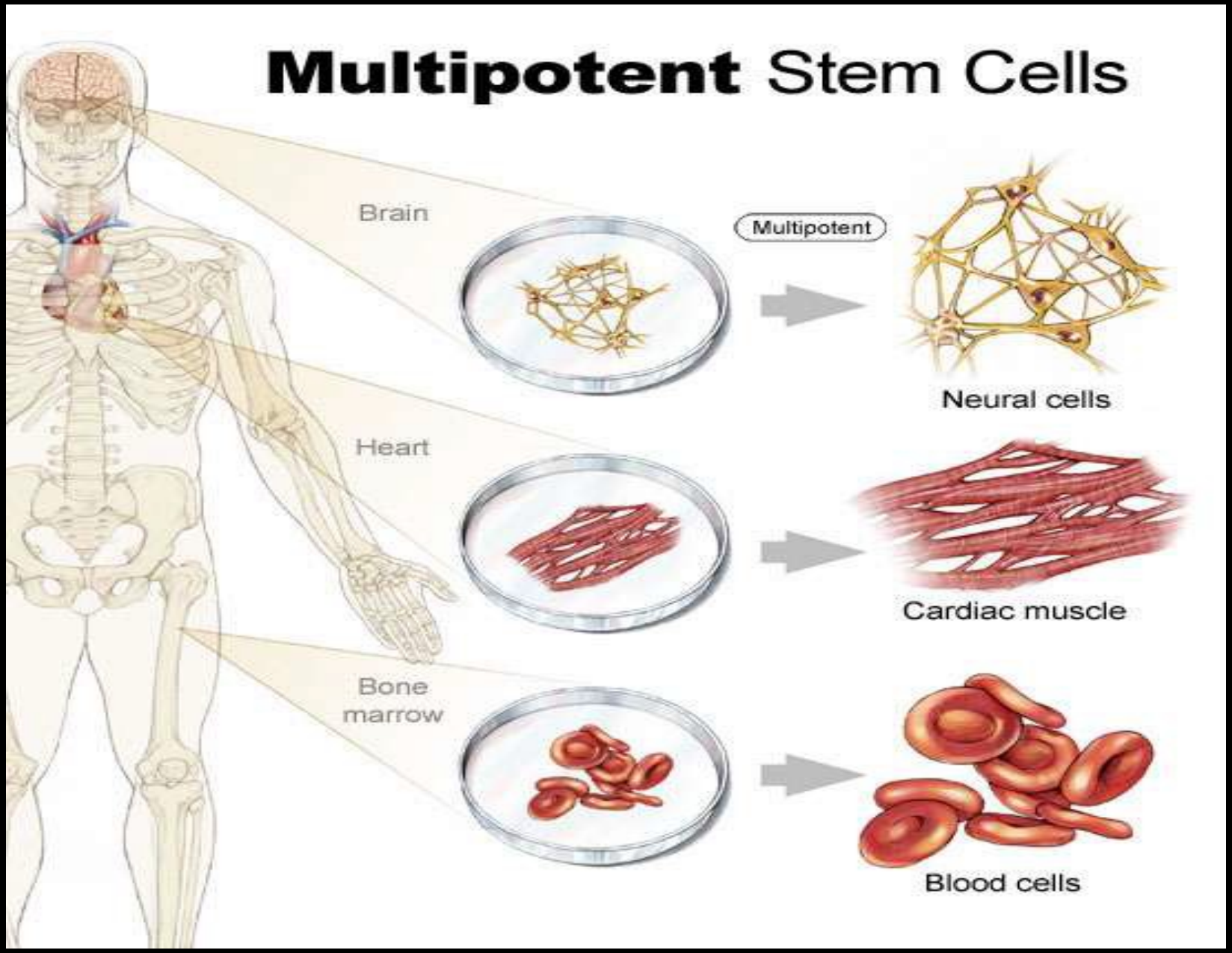


1. In Vitro Fertilized Egg
2. Blastocyst Stage (5-7 days old)
3. Inner Stem Cell Mass
4. Cultured Undifferentiated Stem Cells
5. Specialized Cells:
   a. blood cells
   b. neural cells
   c. muscle cells

http://www.news.wisc.edu/packages/stemcells/illustration.html Images depict stem cell research at the University of Wisconsin Madison.

Multipotent stem cells

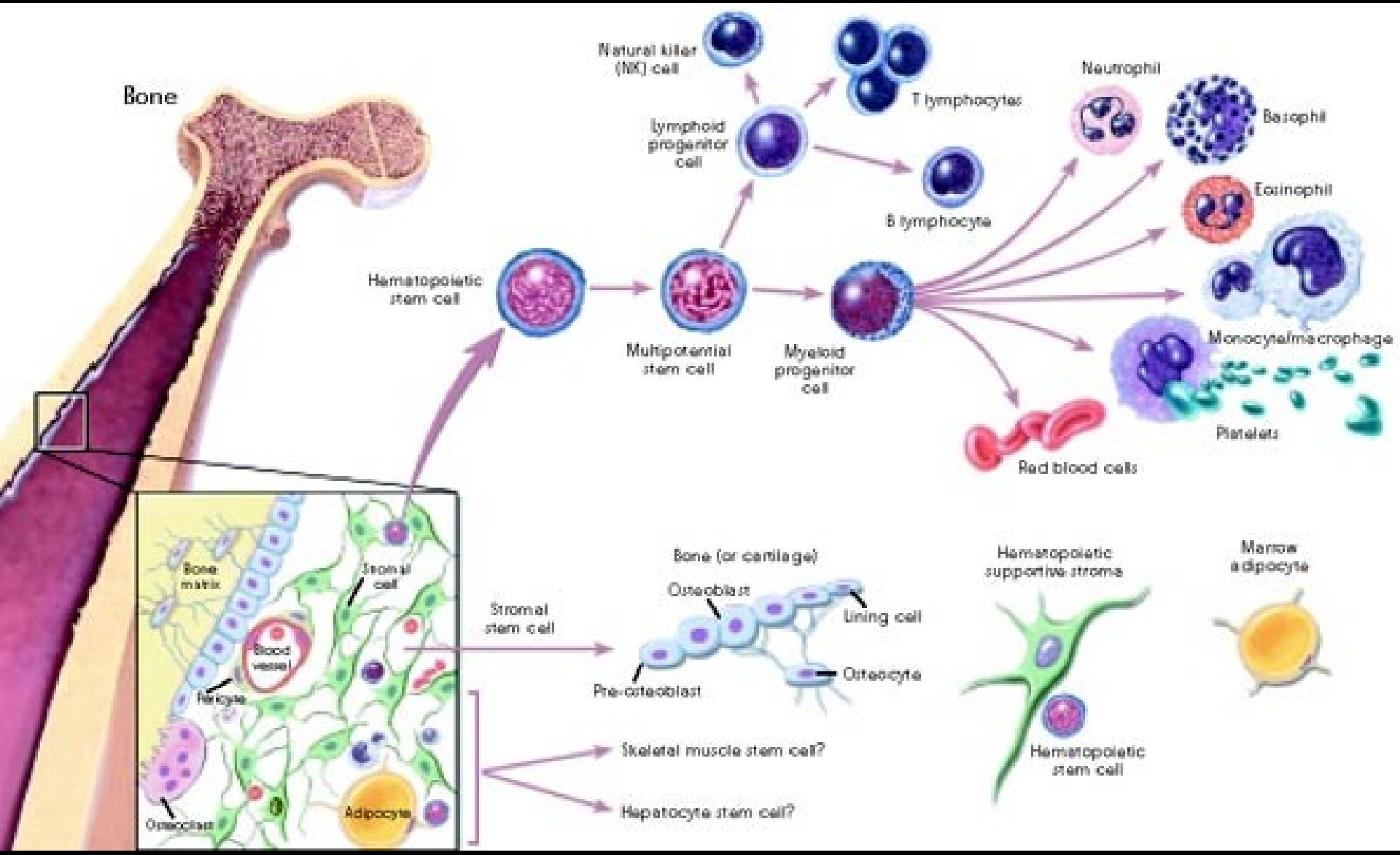
- These are true stem cells but can only differentiate into a limited number of types.
  - For example, the bone marrow contains multipotent stem cells that give rise to all the cells of the blood but not to other types of cells.
- Multipotent stem cells are found in adult animals; perhaps most organs in the body (e.g., brain, liver) contain them where they can replace dead or damaged cells.
- These **adult stem cells** may also be the cells that — when one accumulates sufficient mutations — produce a clone of cancer cells.

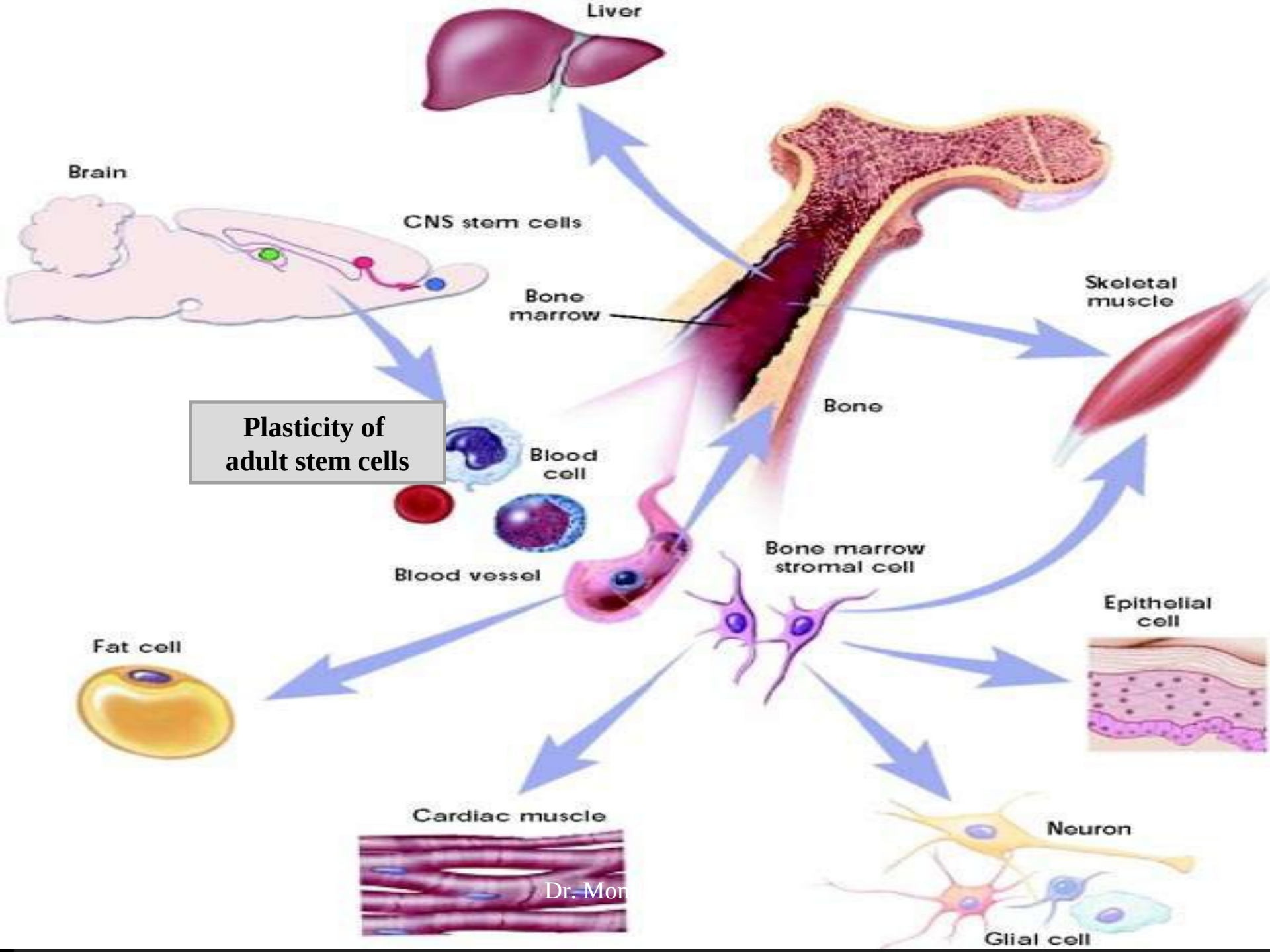

Multipotent Stem Cells
Brain
Multipotent
Neural cells
Heart
Cardiac muscle
Bone marrow
Blood cells

Adult Stem Cells

- An adult stem cell also called as somatic stem cells are an undifferentiated cells found among differentiated cells in a tissue or an organ which can renew itself and can differentiate to yield the major specialized cell type of the tissue or the organ.
- Primary role of adult stem cell is to maintain and repair the tissue in which they are found.
- Their origin is unknown
  - Haematopoietic stem cells – form all type of blood cells
  - Stromal cells – can generate cartilage, fat, and fibrous connective tissue
  - Brain stem cells – astrocytes , oligodendrocytes and neurons

Adult stem cells



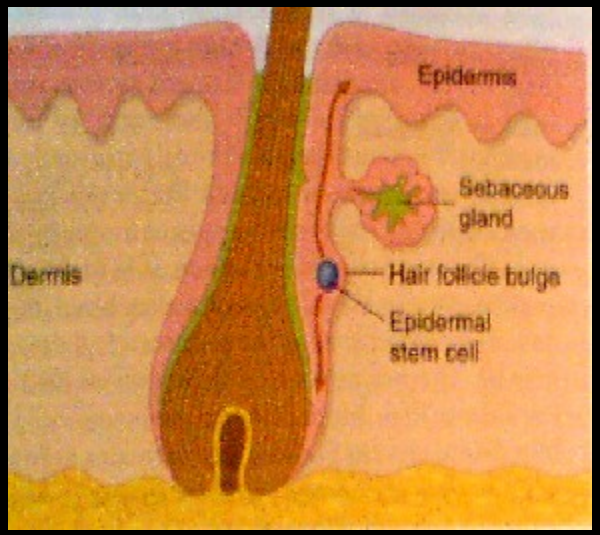

Liver
Brain
CNS stem cells
Bone marrow
Skeletal muscle
Bone
Plasticity of adult stem cells
Blood cell
Blood vessel
Bone marrow stromal cell
Epithelial cell
Fat cell
Cardiac muscle
Neuron
Glial cell

Adult stem cells

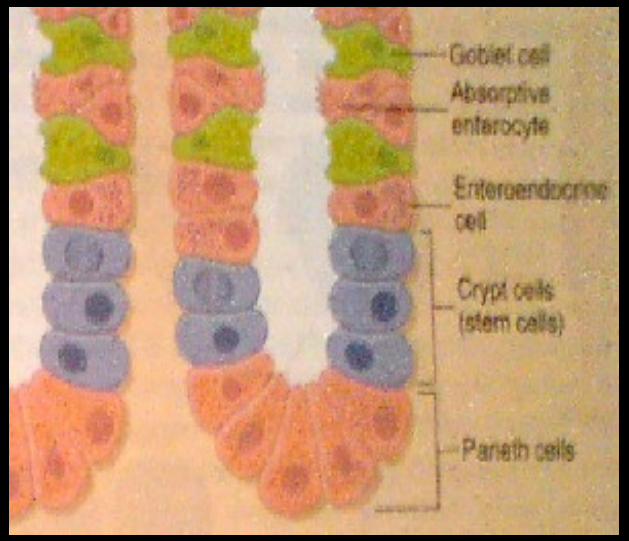
- Contrary to the ES cells, which are pluripotent, adult stem cells have a more restricted differentiation capacity and are usually lineage specific.
- Present in bone marrow and other tissues.
- Stem cells outside bone marrow are called as tissue stem cells.
- In the tissue, stem cells are located in the sites called niches.

Stem cells niches in various tissues

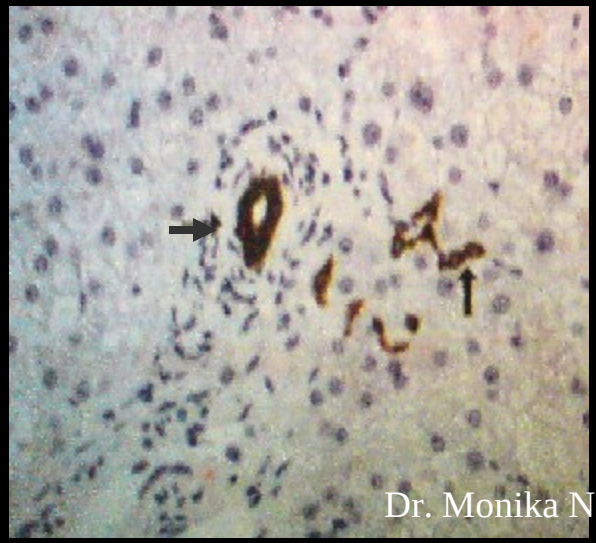
HAIR FOLLICLE



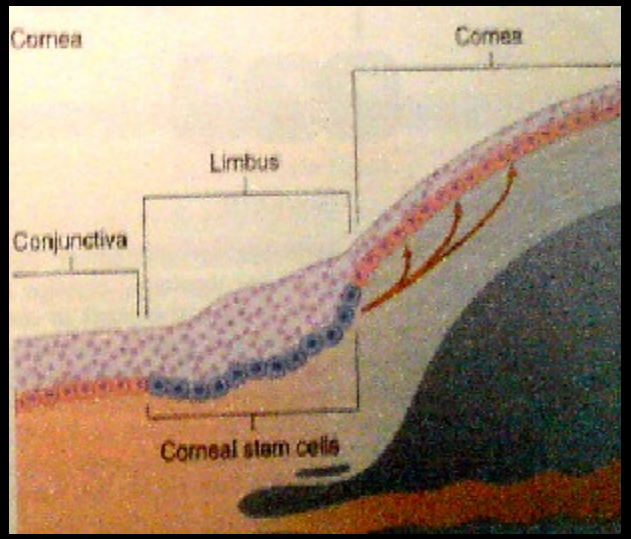
CRYPTS OF COLON



CANALS OF HERING



LIMBUS OF CORNIA



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Embryonic vs Adult Stem Cells

- Totipotent
  - Differentiation into ANY cell type
- Known Source
- Large numbers can be harvested from embryos
- May cause immune rejection
  - Rejection of ES cells by recipient has not been shown yet

- Multi or pluripotent
  - Differentiation into some cell types, limited outcomes
- Unknown source
- Limited numbers, more difficult to isolate
- Less likely to cause immune rejection, since the patient's own cells can be used

Cord Blood

- Umbilical cord blood is also known as placental blood.
- It is the blood that flows in the circulation of the developing fetus in the womb.
- After the baby’s birth, the left over blood in the umbilical cord and placenta is called cord blood.
- This blood is a rich source of stem cells.

Umbilical cord stem cells

- Umblical cord blood is a rich source of primitive stem cells as compared to adult stem cells. Therefore these are able to expand rapidly.
- Stored cord blood stem cells from a child is the perfect match for that child. This allows for an autologous transplant if needed, with no risk of Graft-vs- Host Disease(GVHD).
- Cord blood stem cells are a close match for siblings or family members in case of need, with low risk of GVHD.

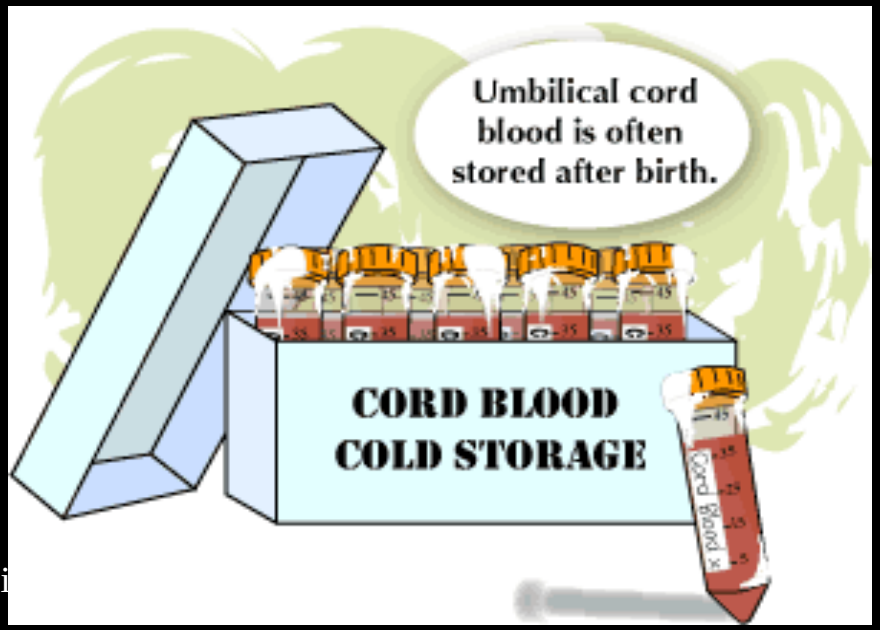
Making a decision to collect baby's cord blood stem cells.

- Yes, if there is a family history of malignant, benign or inherited disorders.
- Yes, even in the absence of "health risk factors", as there are potential benefits to family in the future.
- Yes, if the costs are affordable and this is something of value.
- Yes, it is the one chance to collect them.

Collecting cord blood stem cells

- This blood is collected by the physician after the baby is born and the cord is cut.
- It takes less than 5 minutes and there is no pain, harm or risk to mother or newborn.
- This cord blood containing the stem cells, is sent to a “Cord Blood Bank” either private or public where it is processed and the stem cells are preserved in liquid nitrogen.

- Immediately after a baby is delivered umbilical cord is clamped . After delivery of placenta , the placenta is placed on supporting frame , the cord is cleaned and needle is inserted into the umbilical vein . The umbilical cord blood is collected in a closed system and blood drained as a “standard gravity phlebotomy.”



- Second method involves collecting the cord blood while the placenta is still in the mother's womb.

  This method has theoretically two advantages

  - ✓ collection begins earlier before the blood has a chance to clot.
  - ✓ It uses the contraction of the uterus to enhance the blood drainage in addition to the gravity.

  Disadvantage: it is more intrusive and has the potential to interfere with after-delivery care for the mother and infant.

  - The cord blood collected from single placenta is called a cord blood unit ranging from 60-120 ml

Umbilical cord stem cells

- Adult stem cells of infant origin
- Greater compatibility
- Less expensive

Umbilical cord stem cells

Three important functions:

1. **Plasticity**: Potential to change into other cell types like nerve cells
2. **Homing:** To travel to the site of tissue damage
3. **Engraftment:** To unite with other tissues

Potential Uses of Stem Cells

- Basic research – clarification of complex events that occur during human development & understanding molecular basis of cancer
  - Molecular mechanisms for gene control
  - Role of signals in gene expression & differentiation of the stem cell
  - Stem cell theory of cancer

Potential uses

- Biotechnology (drug discovery & development) – stem cells can provide specific cell types to test new drugs
  - Safety testing of new drugs on differentiated cell lines.
  - Screening of potential drugs
    - Cancer cell lines are already being used to screen potential anti-tumor drugs
    - Availability of pluripotent stem cells would allow drug testing in a wider range of cell types & to reduce animal testing

Potential uses

- Cell based therapies:
  - Regenerative therapy to treat Parkinson's, Alzheimer's, ALS, spinal cord injury, stroke, severe burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis

Tissue Repair

- Regenerate spinal cord, heart tissue or any other major tissue in the body.

Heart Disease

- Adult bone marrow stem cells injected into the hearts arteries are believed to improve cardiac function in victims of heart failure or heart attack.

Replace Skin

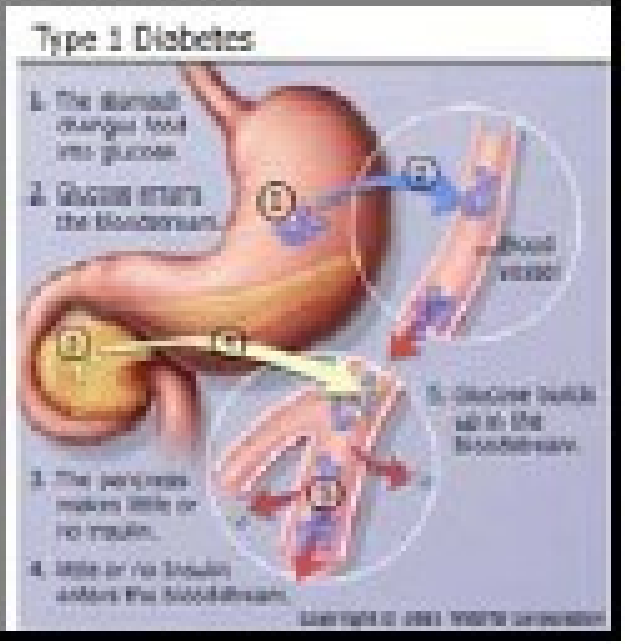
Rheumatoid Arthritis

- Adult stem cells may be helpful in jumpstarting repair of eroded cartilage.

A foot with painful, advanced rheumatoid arthritis

Type I Diabetes

- Pancreatic cells do not produce insulin.
- Basic research focused on understanding how embryonic stem cells might be trained to become pancreatic islets cells needed to secrete insulin.

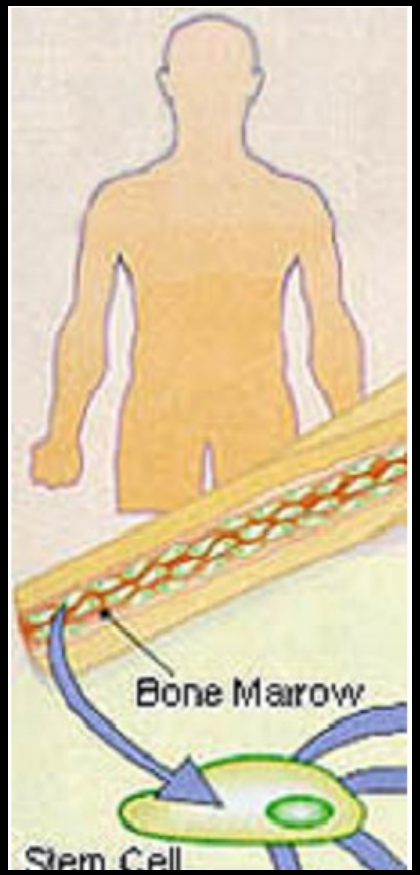


Potential uses

Stem cells in gene therapy

- Stem cells as vehicles after they have been genetically manipulated

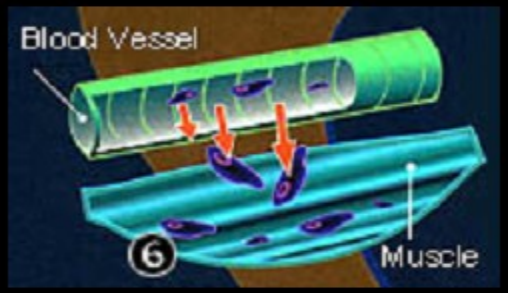
If scientists can figure out how stem cells "choose" among these "career" options, they might be able to direct them to become specific cell types and repair specific tissues

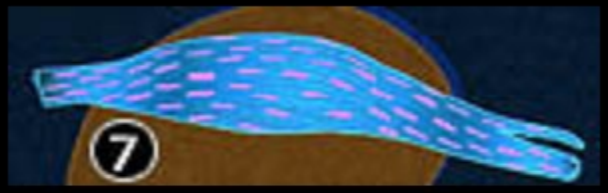


HOW STEM CELLS AND GENE THERAPY MIGHT WORK TOGETHER

1. A sample of bone marrow is removed.
2. Stem cells are isolated and allowed to multiply in culture.
3. Cells are treated with a modified virus containing a therapeutic gene

HOW STEM CELLS AND GENE THERAPY MIGHT WORK TOGETHER





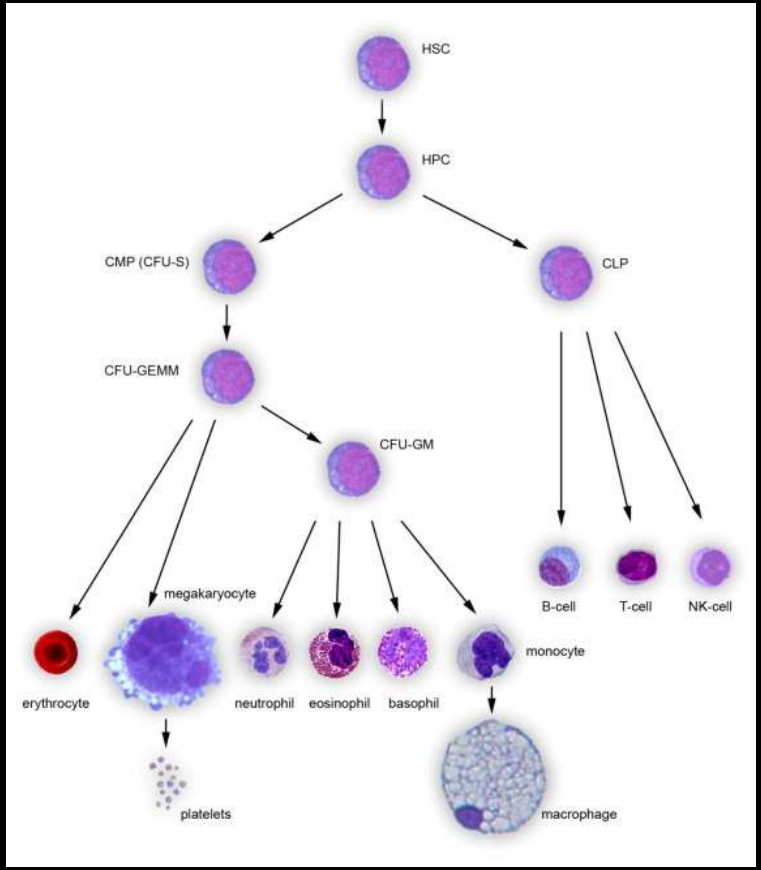
1. The virus is taken up by individual cells and the therapeutic gene goes into the cell's nucleus.
2. Treated ("corrected") cells are injected into the bloodstream.
3. Treated cells respond to injury signals from degenerating muscle or other tissues and migrate out of the bloodstream.
4. Treated cells patch damage and build healthy tissue

Haematopoietic stem cell transplantation

Best studied adult stem cells

Give rise to all blood cells

Well established clinical use

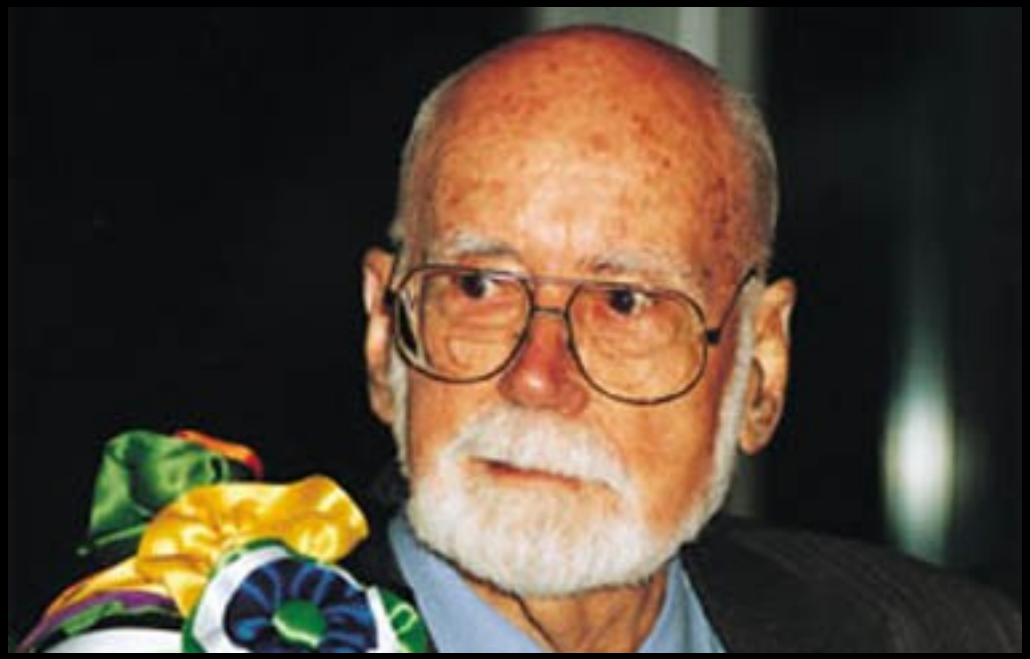


- **Definition**

  Any procedure where hematopoietic stem cells of any donor and any source are given to a recipient with intention of repopulating/replacing the hematopoietic system in total or in part.

The Nobel Prize, 1990

E. Donnall Thomas



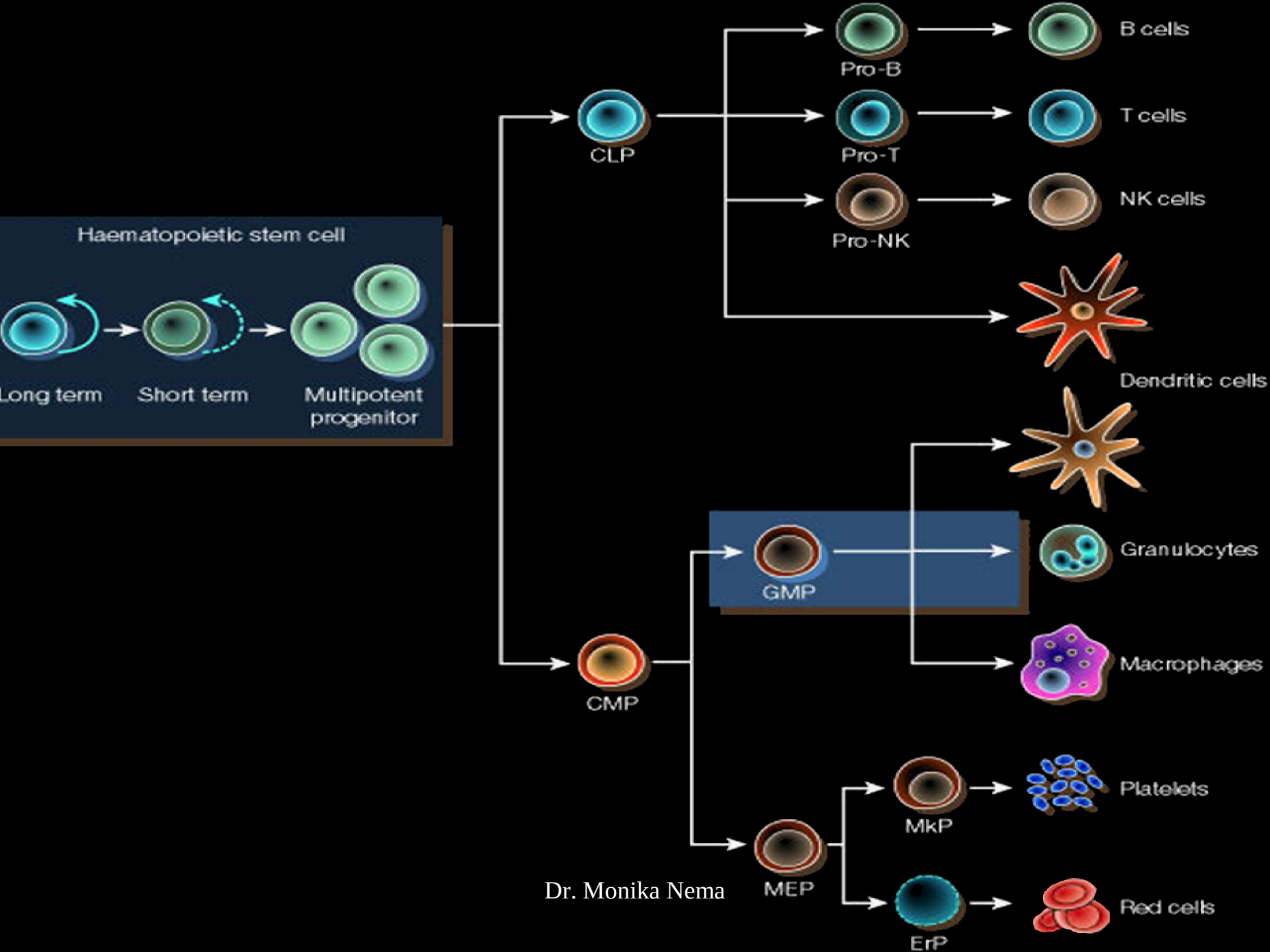
first succsessful HSCT in treatment of acute leukemias

Thomas ED, Lochte HL, Lu WC, Ferrebee JW. Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N. Engl. J. Med. 1957; 257: 491.

Haematopoietic stem cells

- It is defined as the cell with the ability to achieve long term reconstruction of both myeloid and lymphoid lineage.
- Features :-

1. Remarkable regenerative capacity
2. Ability to home to marrow spaces following IV injection. {mediated in par by interaction of selectin on bone marrow endothelium and integrin on early hematopoietic cells }
3. Ability to be cryopreserved.



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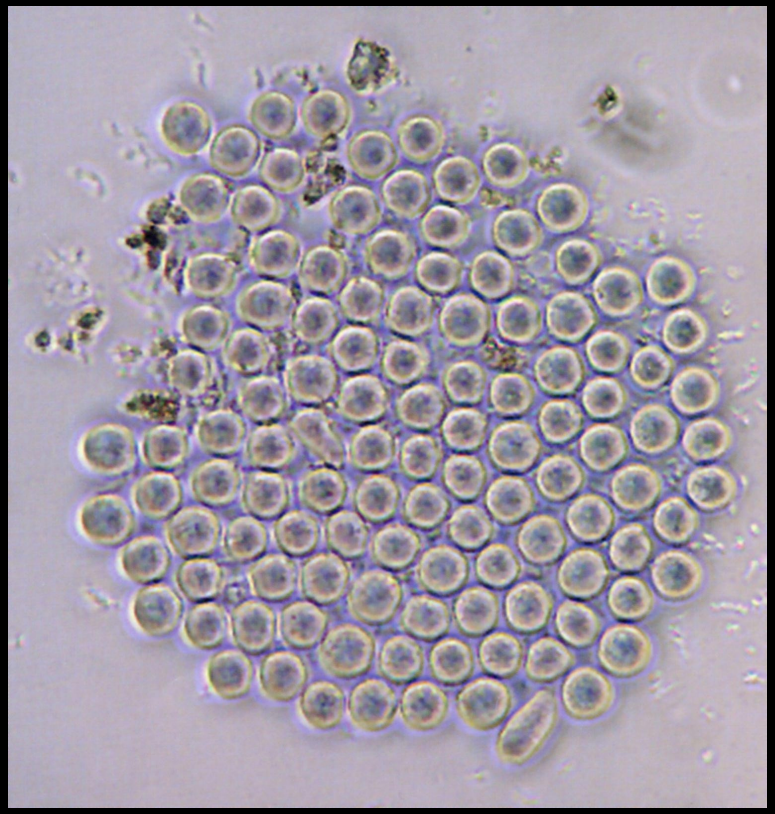
Hematopoietic stem cells

1 / 25 000 - 100 000 of bone marrow cells



Blood, 15 Jan 2004

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Charakteristic:

- CD34
- CD133
- Lin^-
- C-kit (CD117)
- BCRP

Basic Principle

- Replenish bone marrow cells eradicated by disease, chemotherapy, or radiation

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Sources of stem cells

- Bone marrow
- Peripheral blood
- Umbilical cord blood

BONE MARROW

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- Marrow is obtained by multiple aspirations from the posterior iliac crest under general or epidural anesthesia.
- For larger quantity anterior crest or sternum can be the site. Several skin puncture on each iliac crest and multiple bone puncture are usually required.
- Target volume is 10-15 mg/kg of recipient or donor weight , whichever is less.
- Marrow is collected in heparinized syringe and filtered through 0.3-0.2 mm screen to remove the fat and bony spicule.

- Further processing depend on the clinical situation, such as
  - Removal of RBCs to prevent hemolysis in ABO incompatible transplants {In BMSCT ABO mismatch is the most common}
  - Removal of immunocompetent donor T cells to prevent GVHD
  - Attempts to remove possible contamination of tumour cells in autologous transplantation.
- Marrow is usually transfused immediately after harvesting, but delay of upto 24 hrs may occur without adverse consequences.

Peripheral blood {PB }

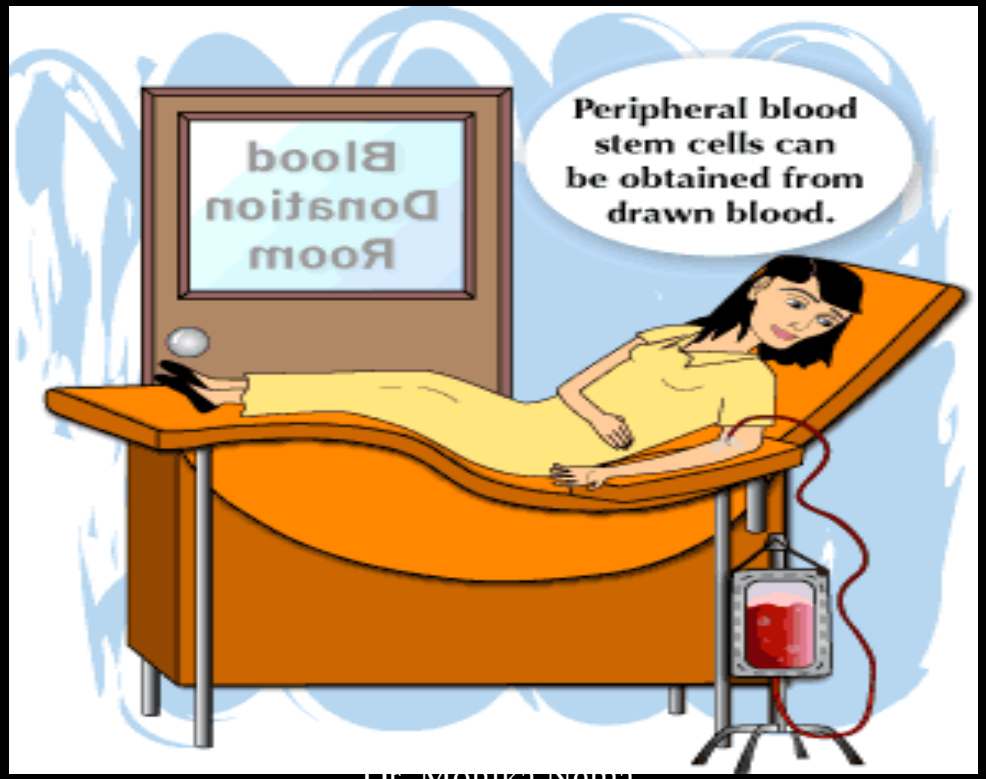
- These cells are now the most common source of stem cells for HSCT .
- Peripheral blood stem cells {PBSC} are collected by leokopheresis after the donor has been treated with hematopoietic growth factors or a combination of chemotherapy and growth factors. For pts. with malignancy cyclophosphamide based chemotherapy and G –CSF are used.
- The donors are typically treated with growth factor for 4-6 days, following which the stem cells are collected in one to two 4 hrs sessions.
- In autologous setting transplantation of >2.5 X 106 CD 34+ cells / kg leads to rapid and sustained engraftment.

APHERESIS

Dr. Monika Nema

Peripheral Blood Stem Cell Transplant

PBSCs are easier to collect than bone marrow stem cells, which must be extracted from within bones. This makes PBSCs a less invasive treatment option than bone marrow stem cells.



Cord Blood Collection

Dr. Monika Nema

Storage of haematopoietic stem cell

- Bone marrow cells can be cryopreserved for prolonged time. This is necessary for autologous HSC because the cells must be harvested months in advance of the transplant treatment.
- In allogenic transplants fresh HSC are preferred in order to avoid cell loss that might occur during the freezing and thawing process.
- The graft undergoes HLA typing, cell counts and testing for viruses.
- Allogenic cord blood is stored frozen at a cord blood bank because it is only obtainable at the time of child birth.
- To cryopreserved HSC a preservative (dimethyl sulfoxide)DMSO, must be added and cells must be cooled very slowly in a control rate freezer to prevent osmotic cellular injury during ice crystal formation.
- HSC may be stored for years in a cryofreezer.

- **Cellular characteristics of various sources of stem cells**

Cellular characteristic	source		
	Bone marrow	Peripheral blood	Cord blood
Stem cell content	adequate	Good	Low
Progenitor cell content	Adequate	High	Low
T-cell content	Low	High	Low , functionally immature
Risk of tumor cell contamination	High	Low	Not applicable

- **Cord blood progenitor cells have greater proliferative potential than that of peripheral blood and marrow progenitor cells.**

Clinical characteristics with various sources of stem cells

Cellular characteristic	source		
	Peripheral blood	Bone marrow	Cord blood
HLA Matching	Close matching required	Close matching required	Less restrictive than other
Engraftment	Fastest	Intermediate	Slowest
Risk of acute GVHD	Same as in bone marrow	Same as in peripheral blood	Lowest
Risk of chronic GVHD	Highest	Lower than peripheral blood	Lowest

Indication for HSCT

- **Neoplastic disorders**
  - Hematological malignancies
    - Lymphomas (Hodgkin and non-Hodgkin)
    - Leukemias (acute and chronic)
    - Multiple myeloma
    - MDS
  - Solid tumors
- **Non-neoplastic disorders**
  - Aplastic anemia
  - Autoimmune diseases
  - Immunodeficiency
  - Inborn errors of metabolism

Bone marrow transplantation unit

Dr. Monika Nema

Hematopoietic stem cell infusion

Dr. Monika Nema

ETHICS & POLITICS

Ethics

How far can we go with this?

Is it morally right?

Ethical issues concerning the use of stem cells

- There are some problematic issues relating to research on stem cells that mainly concern the origins and methods of stem cell production.
- Small numbers of adult stem cells can be found in the human body,
- The best sources of stem cells are fetuses and embryos.
- Fetal stem cell lines can be cultured from cells isolated from aborted fetuses.
- Stem cells from embryos can be isolated from 5–7 day-old blastocysts.
- The collection of stem cells of both fetal and embryonic origins involves destruction of the “donor” – the fetus or embryo – and this is ethically problematic

Key Ethical Issues

- The blastocyst used in stem cell research is microscopically small and has no nervous system. Does it count as a “person” who has a right to life?
- What do various religions say about when personhood begins? Does science have a view on this?
- In a society where citizens hold diverse religious views, how can we democratically make humane public policy?

Thank you

Are humans playing god?

Will embryo farms be around in the future?

Health care costs

- Who will pay for all this?

Will cloning factories produce human organs?