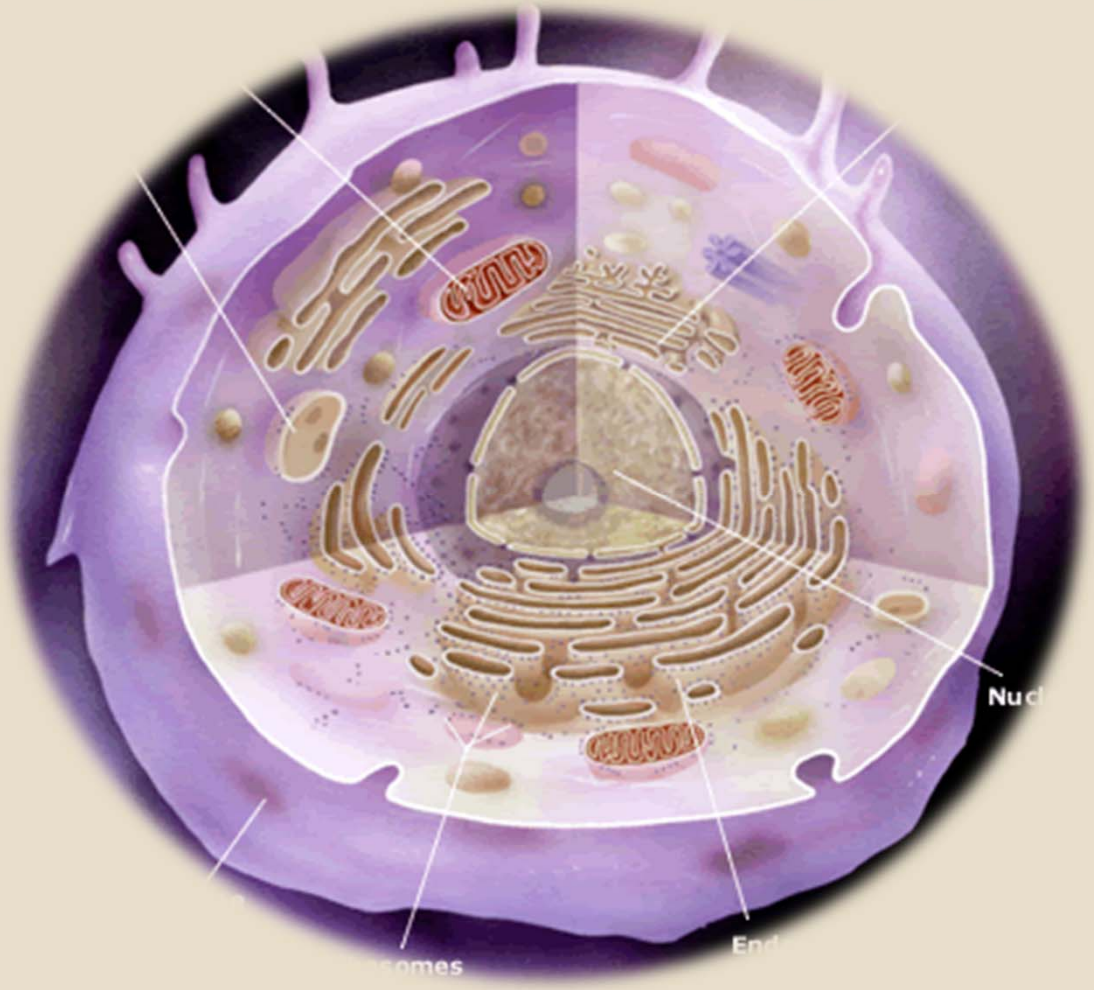
CELLULE STAMINALI MESENCHIMALI

04/01/2012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HELP!

Growth Factor

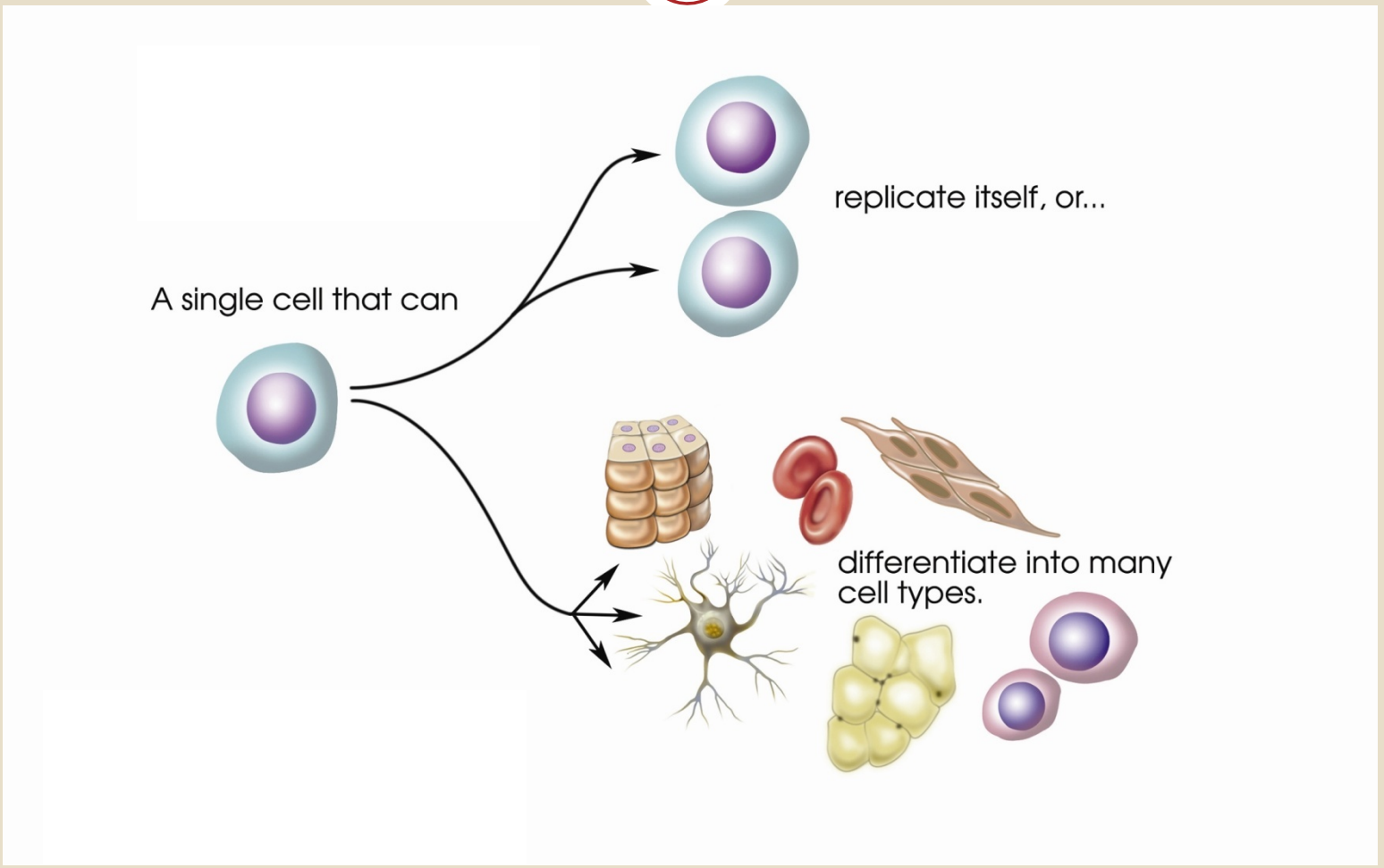
Bone Marrow

Stem Cells

Pheripheral Blood

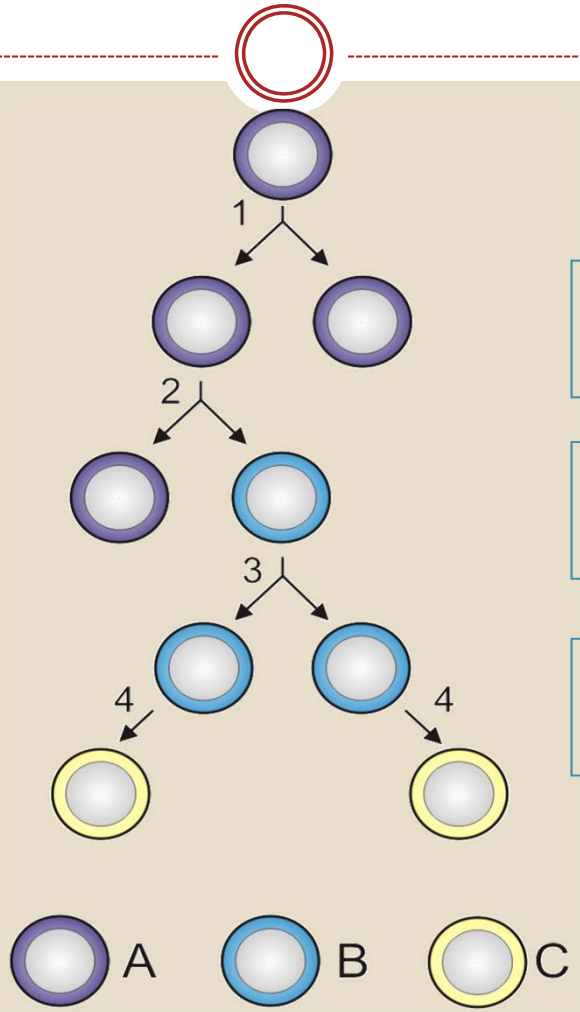
Biomaterials

What is a stem cell?



Self renewal and differentiation

N.B: Self-regeneration is the ability of stem cells to divide and produce more stem cells. During early development, the cell division is symmetrical, when each cell divides to gives rise to daughter cells each with the same potential. Later in development, the cell divides asymmetrically with one of the daughter cells produced also a stem cell and the other a more differentiated cell.



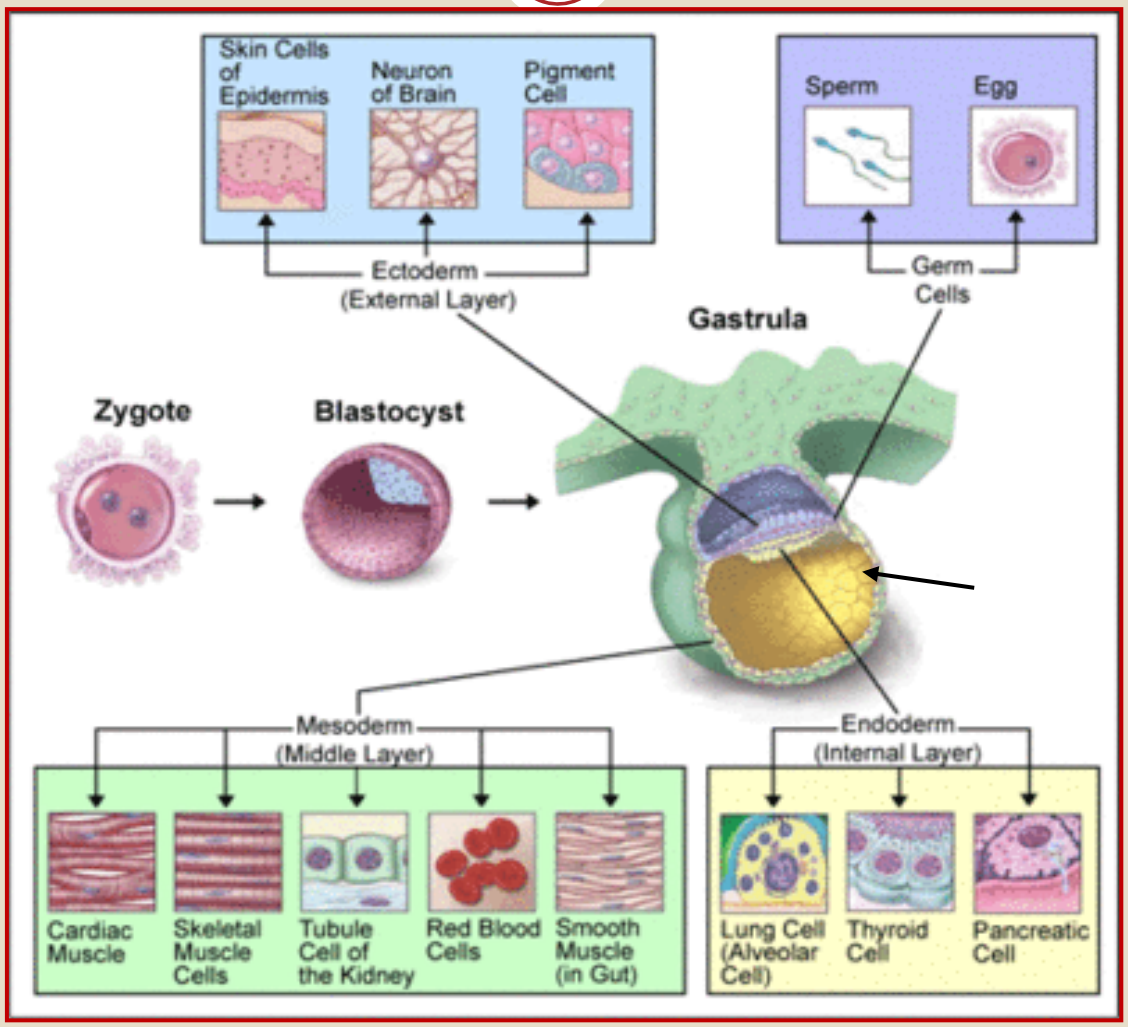
Symmetric stem cell divison

Asymmetric stem cell division

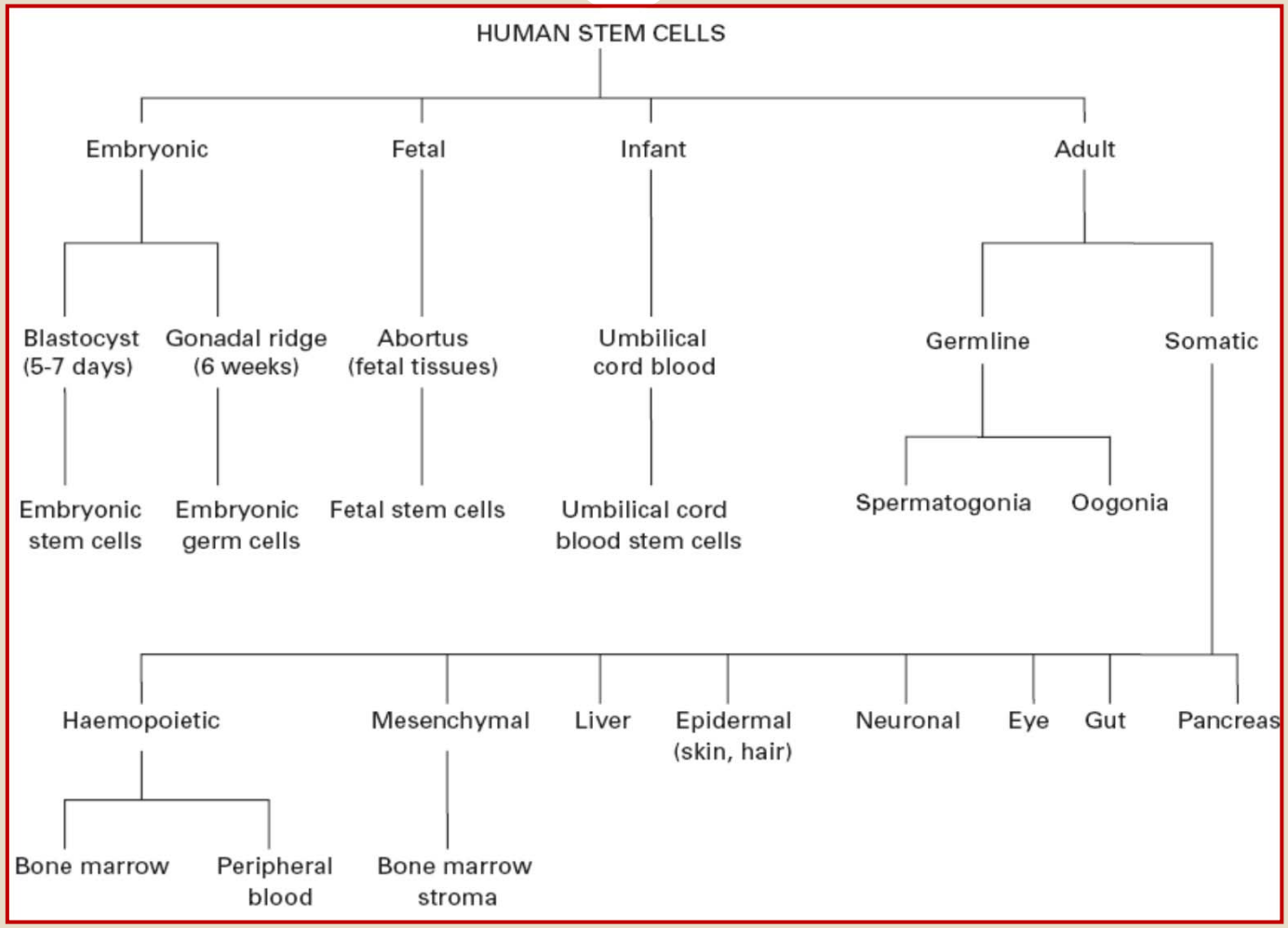
Progenitor cell division

Stem cell | **Progenitor cell** | **Differentiated cell**

Embryo development



Principal types of Stem Cells_Origin



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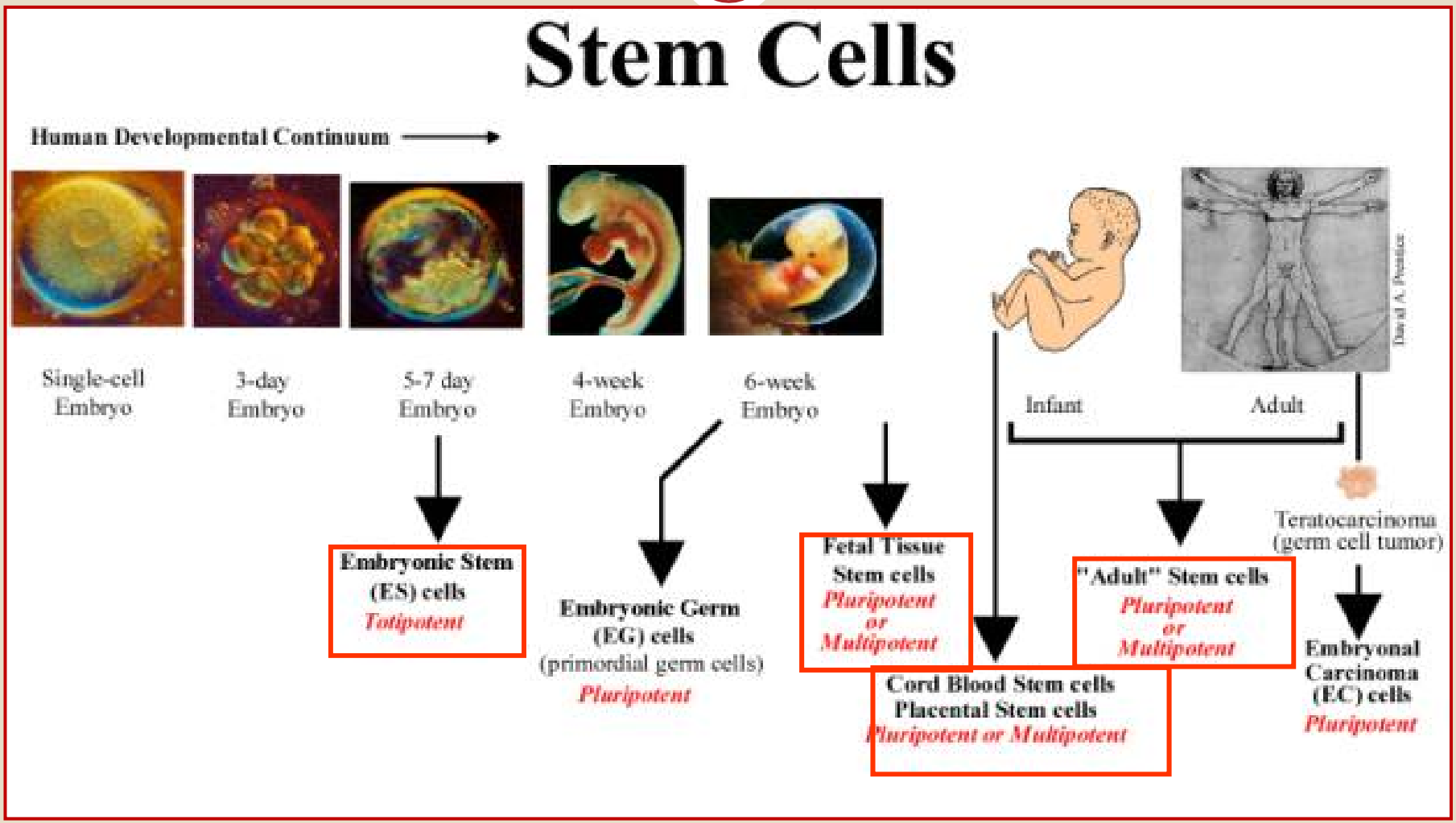
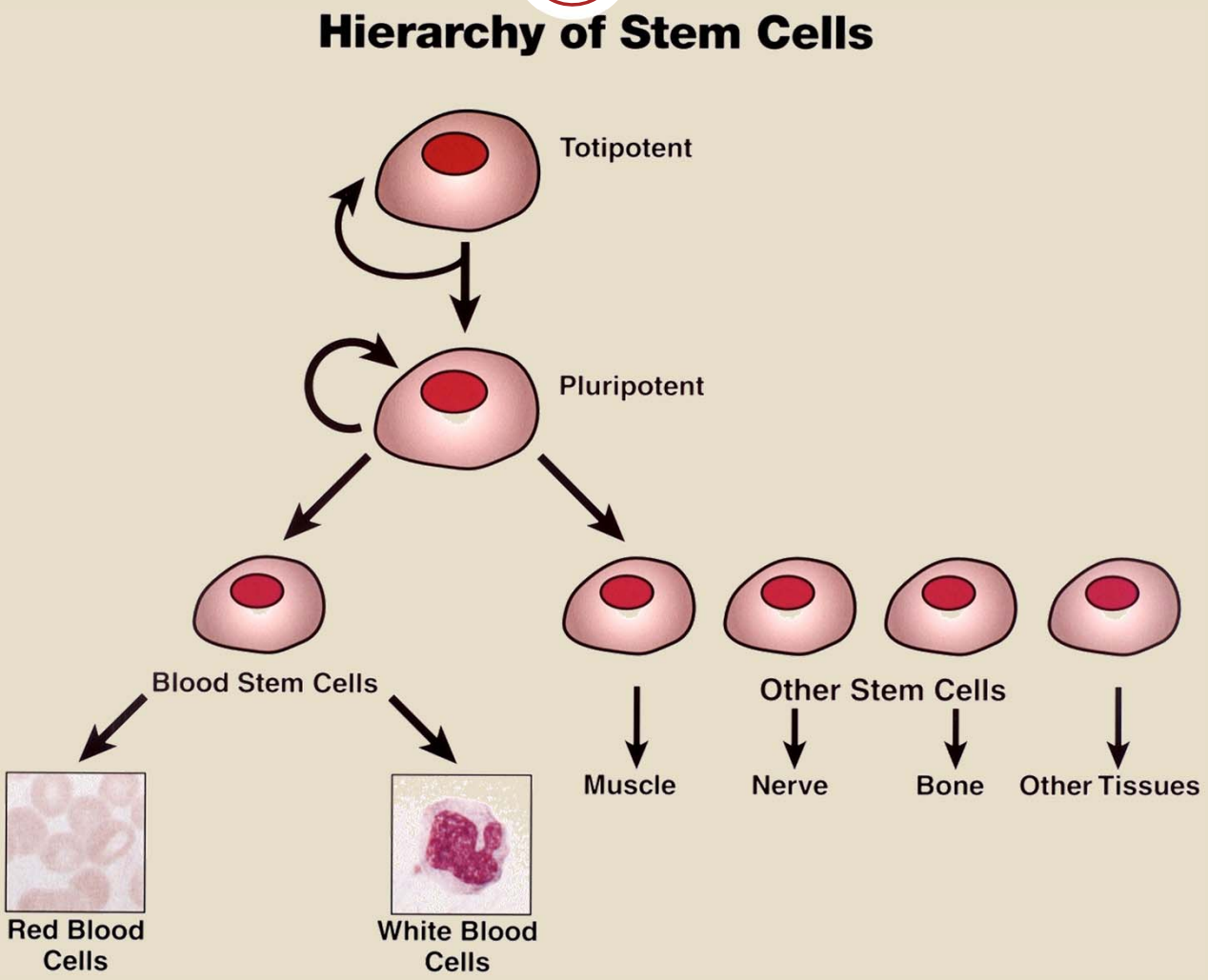


Principal types of stem cells_Potential

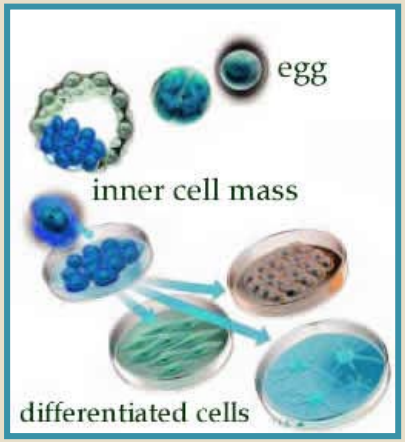
Differentiation Potential	Example of stem cell	Number of cell types	Cell types resulting from differentiation
Totipotential	Zygote (fertilized egg), blastomere	All	All cell types
Pluripotential	**Cultured human ES cells**	**All except cells of the embryonic membranes**	**Cells from all three germ layers**
Multipotential	**Hematopoietic cells**	**Many**	**skeletal muscle,cardiac muscle, liver cells, all blood cells**
Oligoptential	Myeloid precursor	Few	5 types of blood cells
	Mesenchymal progenitor cell	**Few**	**Cartilage cells, myocyte, bone-forming cells, fat cells, endothelial cells, neural cells**

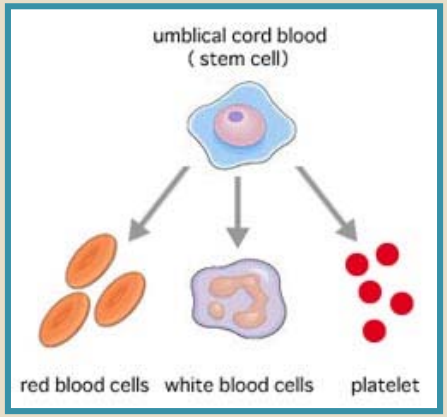
Stem cell potential

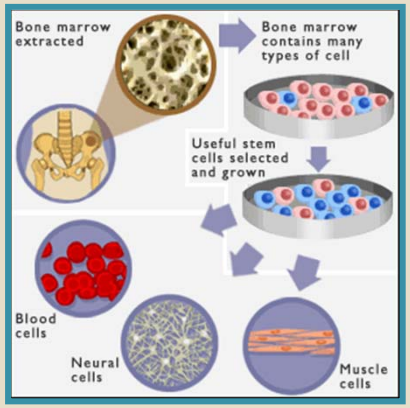


Principal types of stem cells

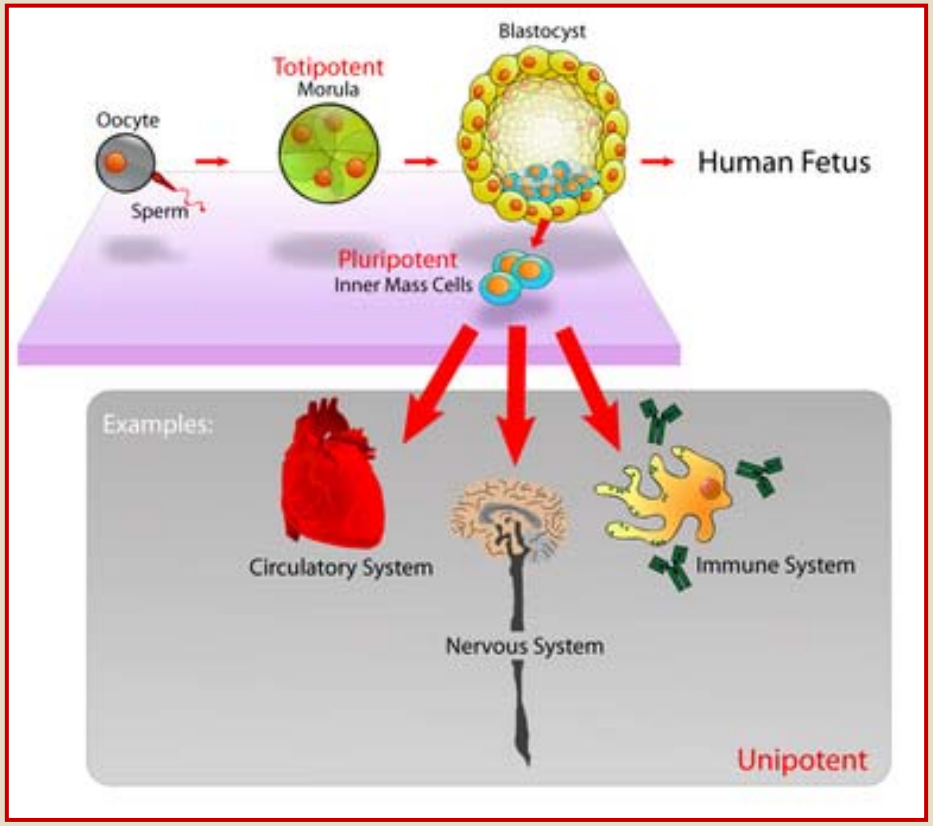
- Embryonic
- Fetal
- Placental or umbilical cord
- Adult







Embryonic Stem Cells 1



ES cells are pluripotent and give rise during development to all tissue of the three primary germ layers: ectoderm, endoderm and mesoderm.

They can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type.

Problems with therapeutic use of ES

- Diffiulty in controlling *in vitro* differentiation
- Possible rejection or immunological response
- Cancerogenity
- Ethical issues

Embryonic Stem Cells 2

Because of their unique combined abilities of unlimited expansion and pluripotency, theoretically embryonic stem cells are a potential source for regenerative medicine and tissue replacement after injury or disease.

To date, no approved medical treatments have been derived from embryonic stem cell research. This is not surprising considering that many nations, including Italy, currently have a moratorium on either ES cell research or the production of new ES cell lines.

Principal types of stem cells

- Embryonic
- Fetal
- Placental or umbilical cord
- Adult

Fetal Stem Cells (FSC)

• On day 49 (eight weeks after fertilization) the embryo becomes a fetus which contains stem cells. Fetal stem cells are also pluripotent.

• The difference between embryonic stem cells and fetal stem cells is the fetal stem cells have matured part of the way to mature cells (for example if it takes 20 maturation steps for an embryonic stem cell to turn into a mature skin cell, fetal skin cells are at step 10)

Problems with therapeutic use of FSC

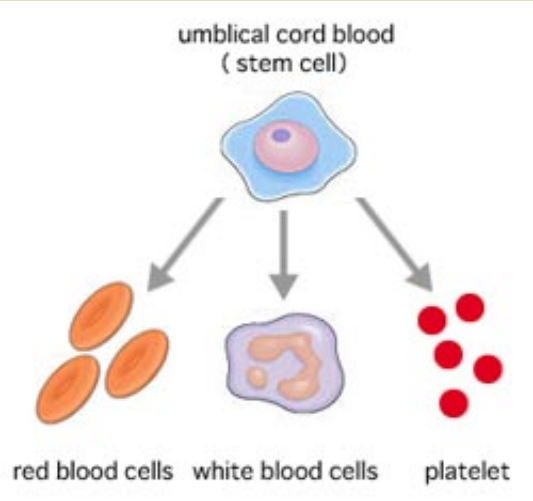
- The numbers of stem cells in fetal tissues may not be sufficient for the therapeutic needs of adults. Thus, methods need to be developed to greatly expand the supply of fetal stem cells
- Possible rejection or immunological response
- Cancerogenity
- Ethical issues

Principal types of stem cells

- Embryonic
- Fetal
- Placental or umbilical cord
- Adult

Umbilical Cord Stem Cells

- Cells in the umbilical cord are multipotent
- They can give rise to all the cells in normal bone marrow
- Therapies using cord blood cells is currently limited to treating diseases of the blood and immune systems.



Principal types of stem cells

- Embryonic
- Fetal
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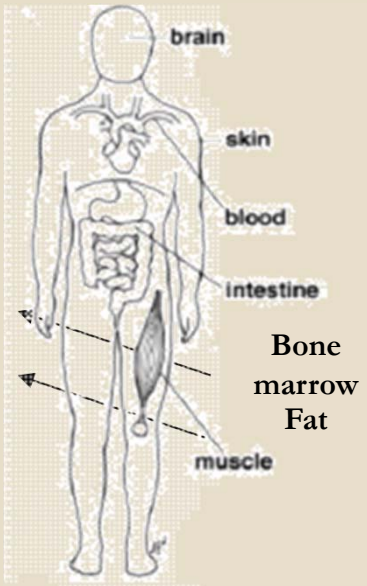
- Adult

Adult or Somatic Stem Cells

- They are undifferentiated cells found among differentiated cells in a tissue or organ
- They can renew itself and differentiate to yield the major specialized cell types of the tissue or organ, in order to maintain and repair the tissue in which they are found
- They may remain quiescent (non-dividing) for many years until they are activated by disease or tissue injury

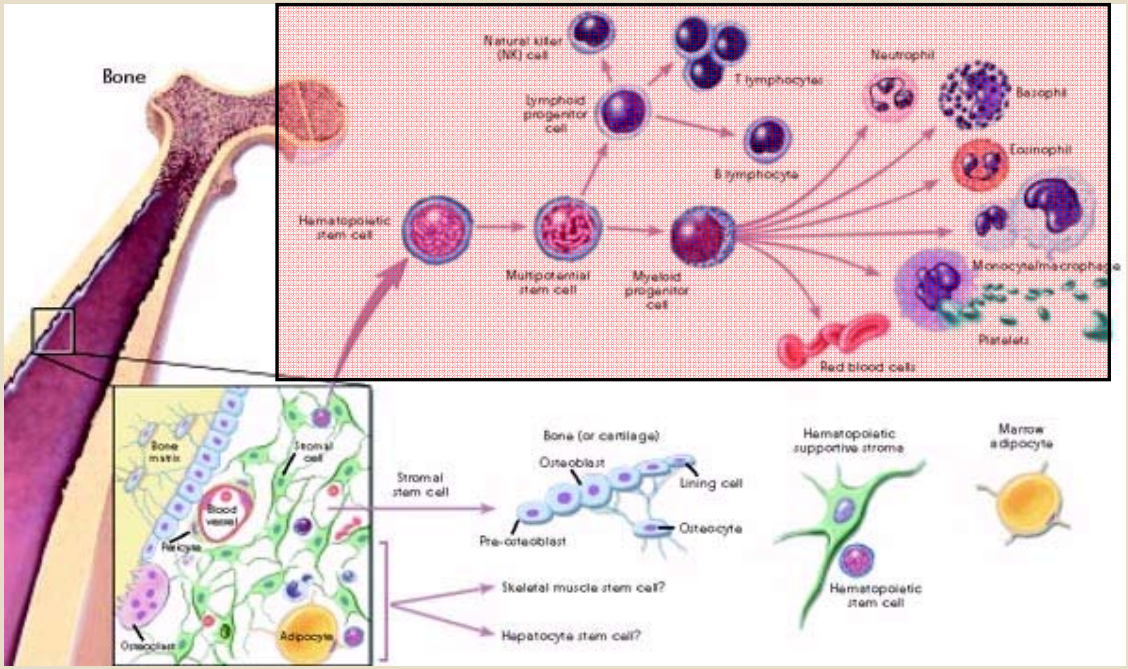
Adult stem cells have been identified in many organs and tissues (brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, adipose tissue, skin and liver), but there are a very small number of stem cells in each tissue



Adult (or Somatic) Stem Cells

- Hematopoietic Stem Cells
- Stromal or Mesenchymal Stem Cells
- MAPCs
- Neural Stem Cells
- Muscle Derived Stem Cells
- Pancreatic Stem Cells
- Hepatic Stem Cells
- Epithelial Stem Cells

Hematopoietic Stem Cells



Hematopoietic Stem Cells

- Circulating stem cells in the blood
- All hematopoietic cells derived from this small population of primitive cells

Characterized by:

- **Unlimited Self Renewal**
  - High self-renewal capacity
  - Give rise to identical daughter stem cells
  - CD 34+ve cell surface marker
- **Pluripotent / multipotential**
  - Able to differentiate into committed progenitor cells
  - Capacity to give rise to all lineages of blood cells
  - Acquisition of specific growth factor receptors
  - Myeloid Progenitor stem cell: committed progenitor of RBCs, WBCs and PLTs
  - Lymphoid Progenitor stem cell: committed progenitor of B and T cell lymphocytes

Hematopoietic Stem Cells

- Used for treating a variety of malignant and nonmalignant hematological diseases (cancers, autoimmune disorders…)

Bone Marrow Transplant (BMT)

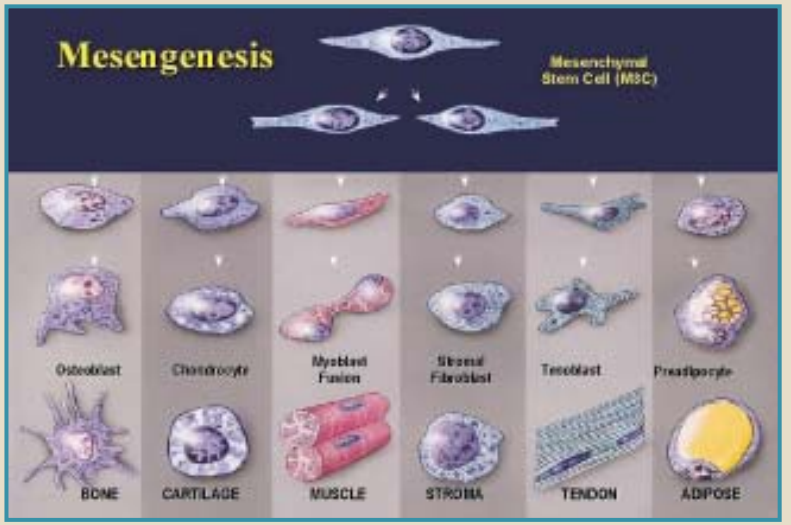
Mesenchymal Stem Cells (MSC)

Isolated from bone marrow, adipose tissue, umbelical cord blood, dental pulp, periosteum, trabecular bone, peripheral blood, skeletal muscles

Can be expanded in culture through many generations retaining their undifferentiated phenotype

Capacity to differentiate into cells of connective tissue lineages (bone, cartilage, fat, muscle, tendon, endothelium

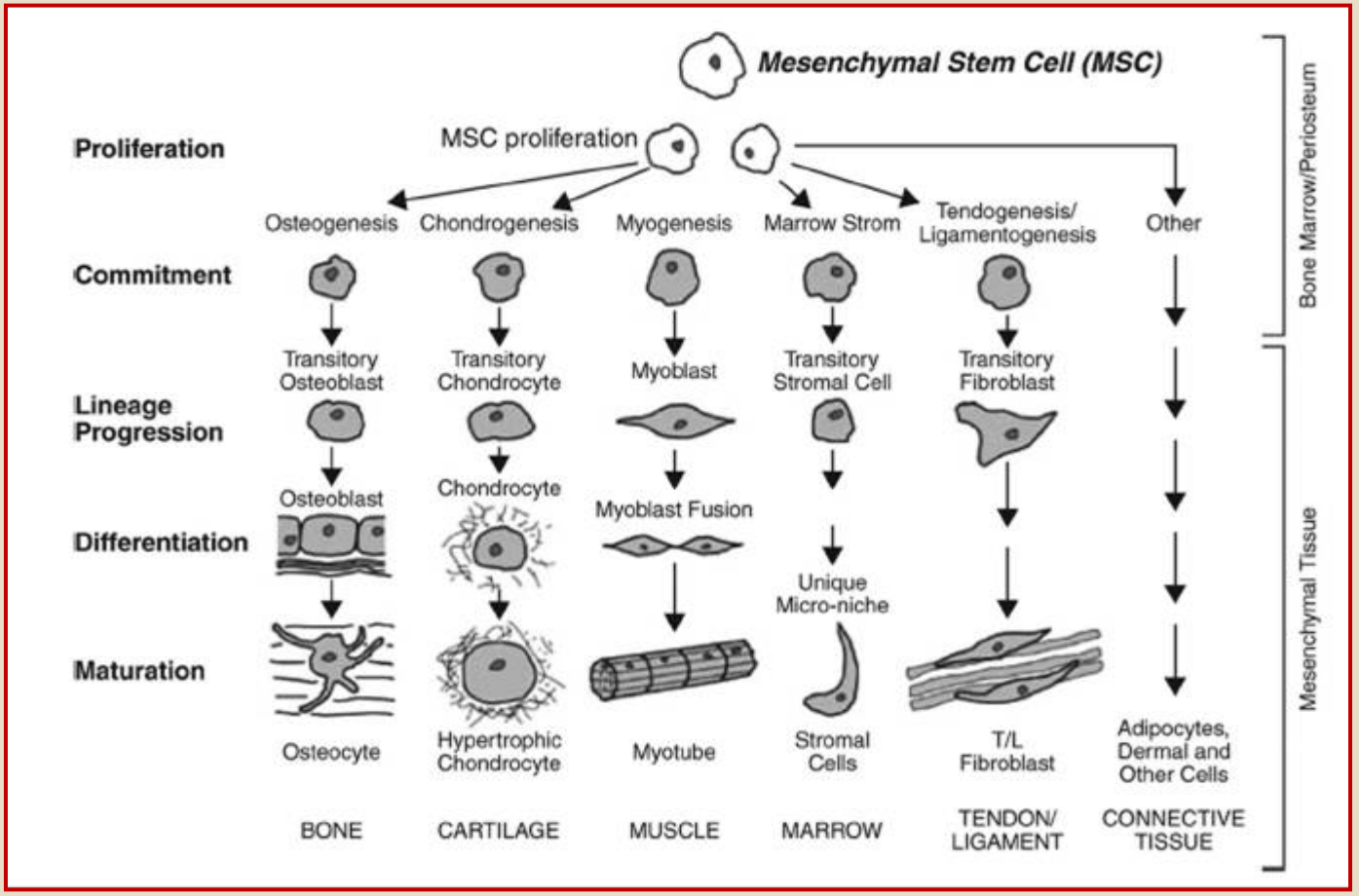
Low immunogenicity (do not express MHC class II, and low level of MHC class I) and they show immunosuppressive properties both in vitro and in vivo



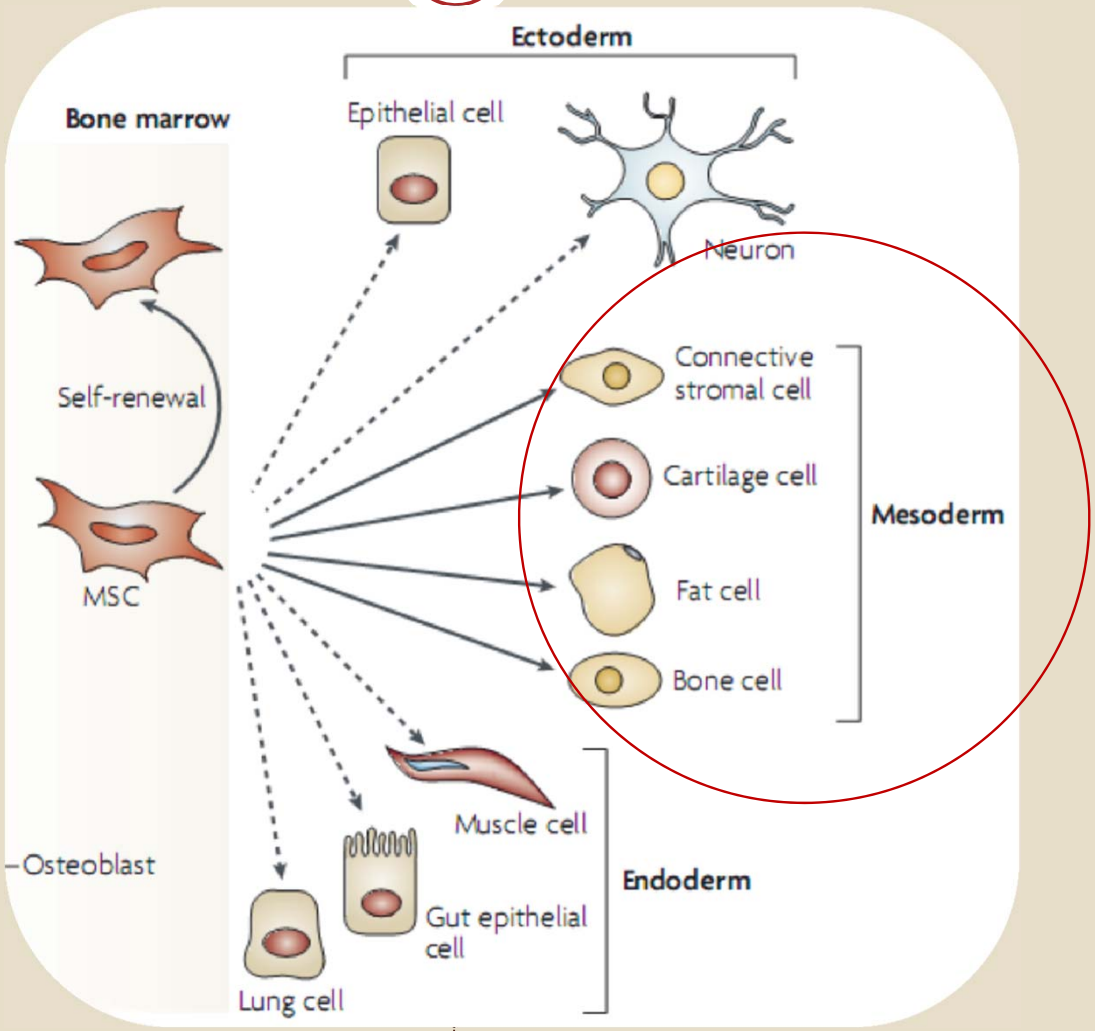
hASC pattern expression

CD 13	Aminopeptidase N, APN, gp150	+++
CD 29	VLA-1/4 subunit (Integrin β1 chain)	+
CD 44	H-CAM, Pgp-1, Hermes antigen (ECMR-III)	++
CD 49d	VLA-4 subunit (Integrin α4)	+
CD 54	InterCellular Adhesion Molecule, ICAM-1	+
CD 90	Thy-1	++
CD 105	Endoglin	++
CD 14	MO2 - LPS-receptor	--
CD 45	Leukocyte common antigen (LCA), T200	--
CD 71	T9 – Transferrin Receptor	-
CD 106	VCAM-1	--

The Mesengenic Process



Plasticity of MSC



Clinical application of MSC

- Dental
- Maxillo-facial
- Plastic and Reconstructive
- Cardio-Vascular Surgery
- NCS diseases
- Diabetes
- **Orthopaedics**

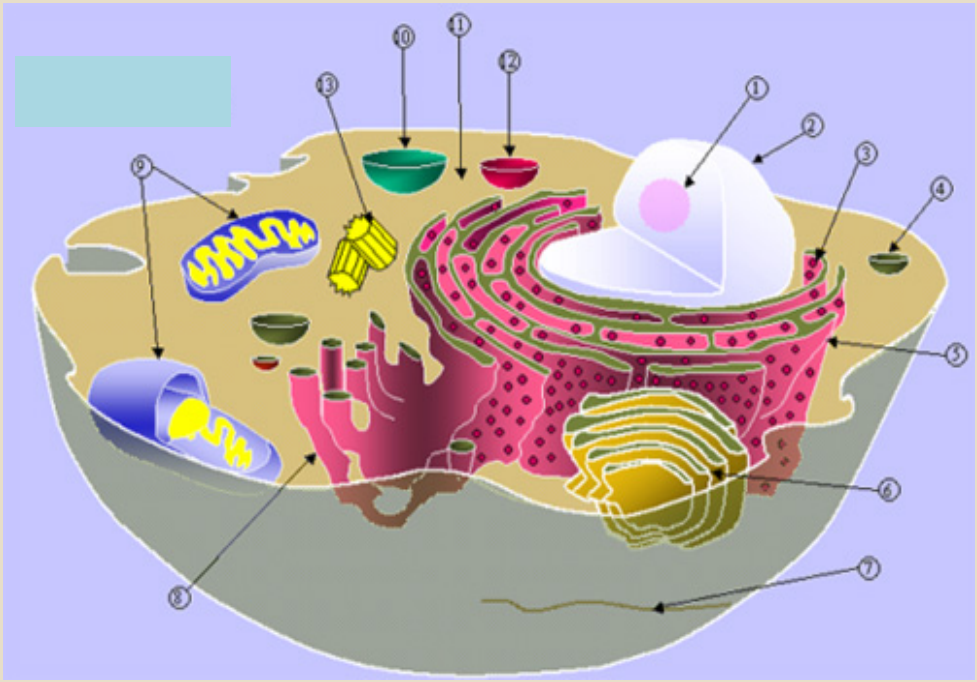
Application of MSC in orthopaedics

- Pseudoarthrosis, non union…., bone loss
- Focal Cartilage lesions (OCD)

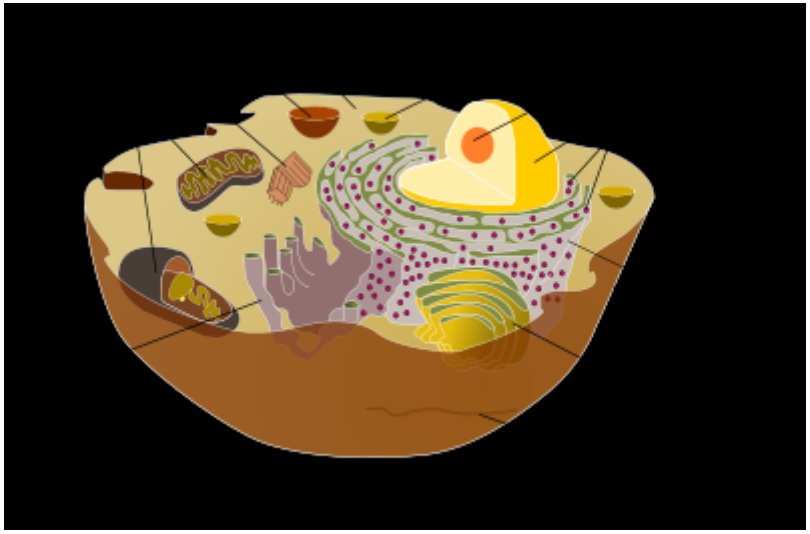
- Tendon rupture
- Ligament reconstructions
- Muscle

MSC structure

- MSC is a structurally normal cells (mononucleated) . They “talk” each other by means of the release of specific protein-



Cells are not growth factors!!!!!!!



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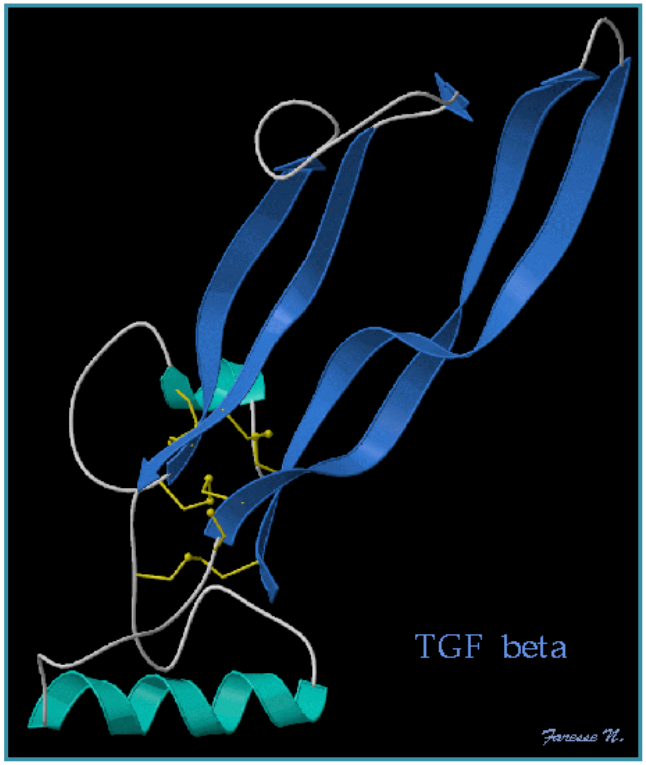


Growth factors

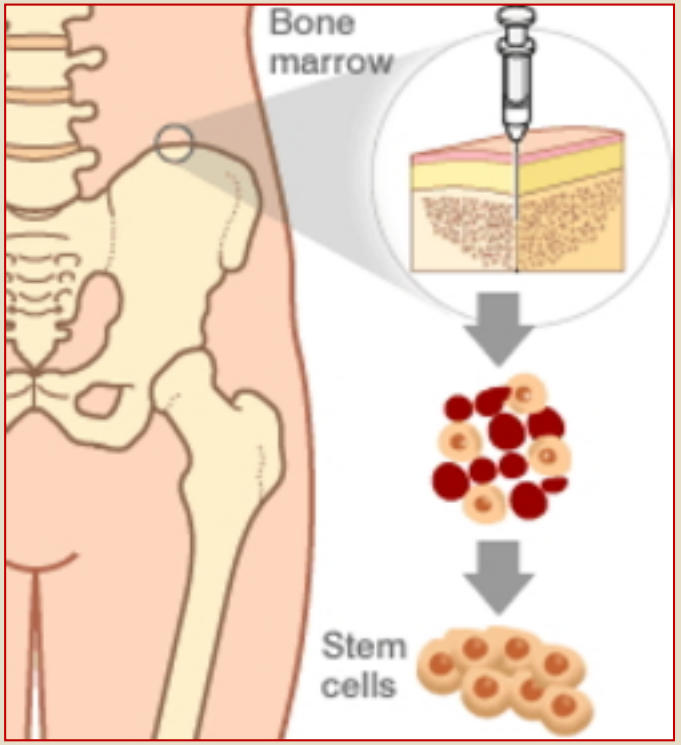
- Growth factors are proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and/or differentiation.
- Many growth factors are quite versatile, stimulating cellular division in numerous different cell types, while others are specific to a particular cell-type.
- They are fundamental in tissue engineering, because they are able to direct stem cell differentiation

Principal types of growth factors

Factor	Principal Source	Primary Activity	Comments
PDGF	platelets, endothelial cells, placenta	promotes proliferation of connective tissue, glial and smooth muscle cells	two different protein chains form 3 distinct dimer forms; AA, AB and BB
EGF	submaxillary gland, Brunners gland	promotes proliferation of mesenchymal, glial and epithelial cells	
TGF-a	common in transformed cells	may be important for normal wound healing	related to EGF
FGF	wide range of cells; protein is associated with the ECM	promotes proliferation of many cells; inhibits some stem cells; induces mesoderm to form in early embryos	at least 19 family members, 4 distinct receptors
NGF		promotes neurite outgrowth and neural cell survival	several related proteins first identified as proto-oncogenes; trkA (trackA), trkB, trkC
Erythropoietin	kidney	promotes proliferation and differentiation of erythrocytes	
TGF-b	activated TH_1 cells (T-helper) and natural killer (NK) cells	anti-inflammatory (suppresses cytokine production and class II MHC expression), promotes wound healing, inhibits macrophage and lymphocyte proliferation	at least 100 different family members, including BMPs
IGF-I	primarily liver	promotes proliferation of many cell types	related to IGF-II and proinsulin, also called Somatomedin C
IGF-II	variety of cells	promotes proliferation of many cell types primarily of fetal origin	related to IGF-I and proinsulin

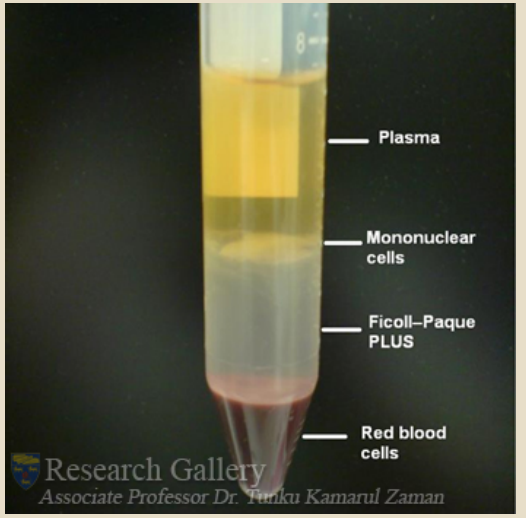
Source of MSC

- Bone marrow, adipose tissue, dental pulp…
- Microfractures allow to obtain progenitor cells contained in the blood clot



Intaoperative methods to collect MSC

- Mainly centrifugation or filtration of BM
- This techniques are a compromise between rapidity (to respect the operative time) and accuracy



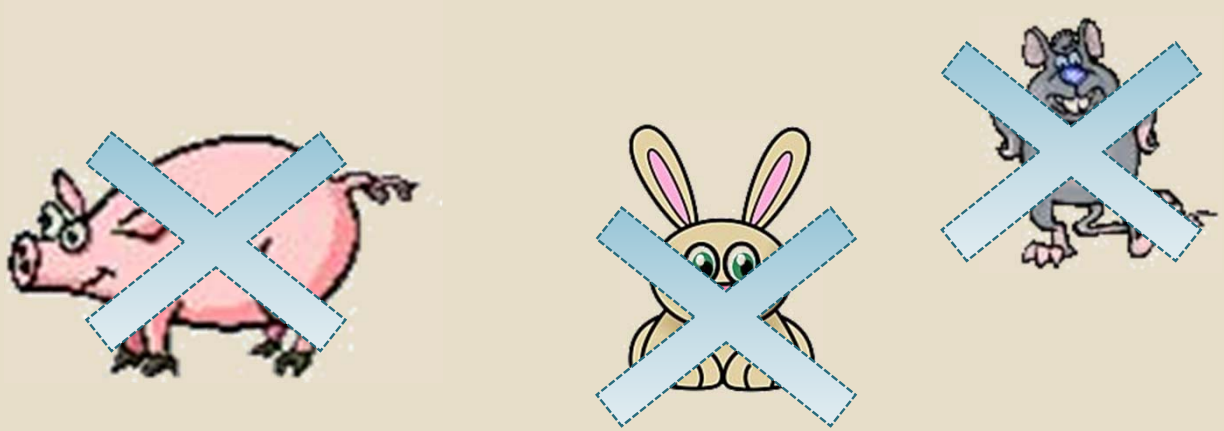
MSC in pre-clinical models

Table 1 | **Biological effects of MSCs in preclinical models of disease**

Disease	Species	Target organ	Mechanism of MSC effects	Route of MSC administration	Reference
Co-transplantation with human HSCs	Sheep	Haematopoietic organs	Support engraftment and increased haematopoiesis	Systemic	66
Myocardial infarction	Mouse	Heart	Generation of new myocytes and vascular structures	Local	68
Skin-graft rejection	Monkey	Skin	Inhibition of T cells	Systemic	17
Stroke	Rat	CNS	Release of trophic factors and induction of neurogenesis	Systemic	78
Melanoma	Mouse	Skin	Inhibition of tumour-specific T cells by $CD8^+$ T cells	Local	91
Acute renal failure	Rat	Kidney	Inhibition of pro-inflammatory cytokine production and induction of anti-apoptotic and trophic factors	Systemic	87
EAE	Mouse	CNS	Inhibition of myelin-specific T cells and induction of peripheral tolerance	Systemic	38
Diabetes	Mouse	Pancreas and renal glomeruli	Induction of local progenitors and inhibition of macrophage infiltration	Systemic	85
EAE	Mouse	CNS	Inhibition of production of myelin-specific antibodies and encephalitogenic cells; decreased axonal loss	Systemic	59
Rheumatoid arthritis	Mouse	Joint	Inhibition of T cell and of production of pro-inflammatory cytokines; induction of regulatory cells	Systemic	105
Retinal degeneration	Rat	Eye	Decreased retinal degeneration through anti-apoptotic and trophic molecules	Local	117
Acute lung injury	Mouse	Lung	Inhibition of production of pro-inflammatory cytokines	Systemic	88
Acute lung injury	Mouse	Lung	Inhibition of production of pro-inflammatory cytokines and increased production of IL-10	Local	118
Acute renal failure	Mouse	Kidney	Tubular-cell regeneration through IGF1 secretion	Systemic	119
Myocardial infarction	Rat	Heart	Anti-apoptotic and mitogenic effect by the WNT-related molecule SFRP2	Local	89
Hepatic failure	Rat	Liver	Inhibition of leukocyte invasionthrough the release of cytokines and chemokines	MSC-conditioned medium	120
Diabetes	Mouse	Pancreas	Induction of local progenitors and inhibition of β-cell-specific T cells	Systemic	86

MSC in clinical practice

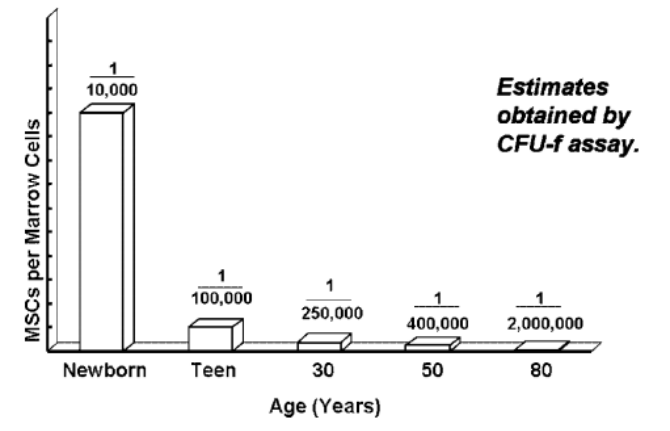
- Few in vitro studies, very few in vivo studies, none clinical study (above all in treatment of cartilage)



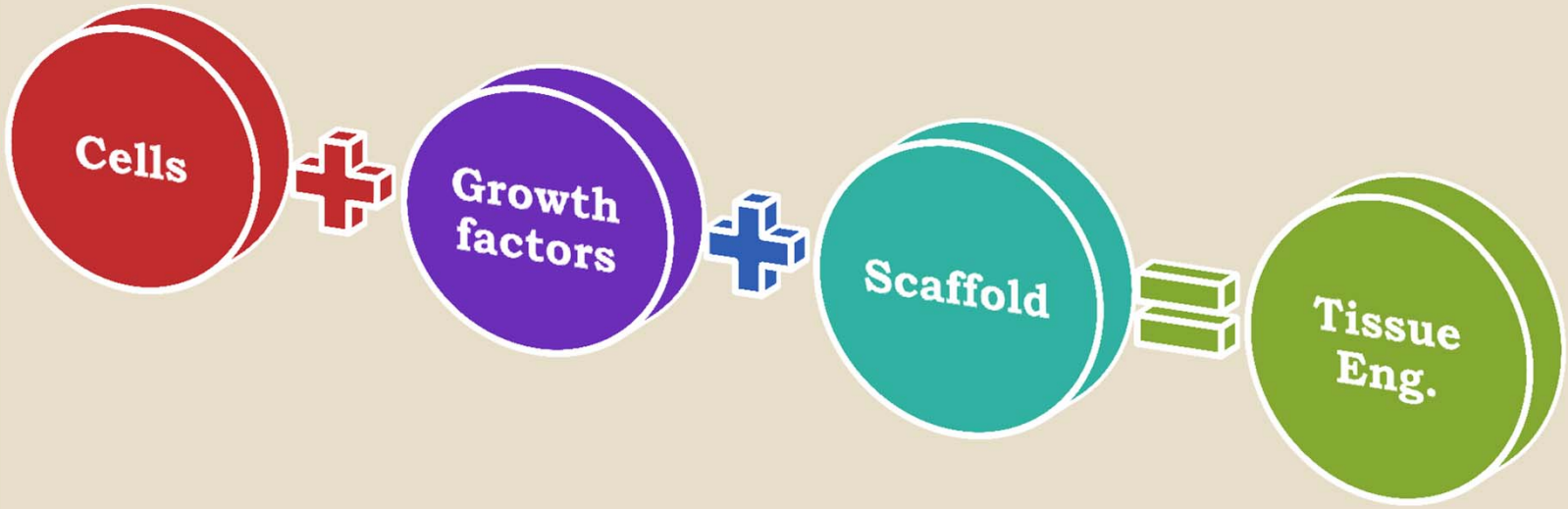
MSC are not able to do everything!!

- MSC can be very useful in orthopaedic applicaion, but they are not SUPER POWERFUL!!!

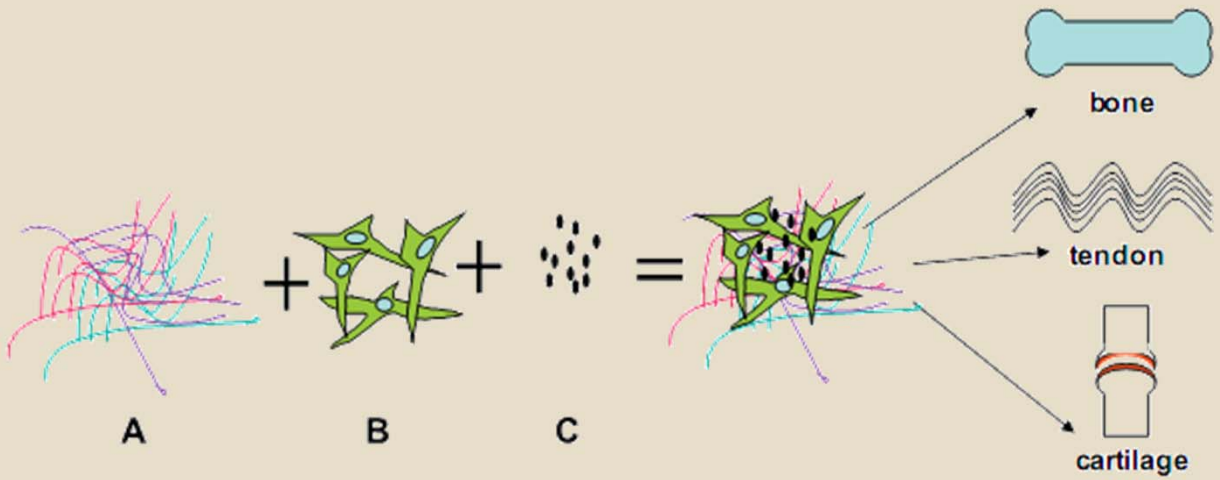
- They have to be used in the appropriate situation (remember the indicaton): younger rather than older....



MSC in tissue engineering application

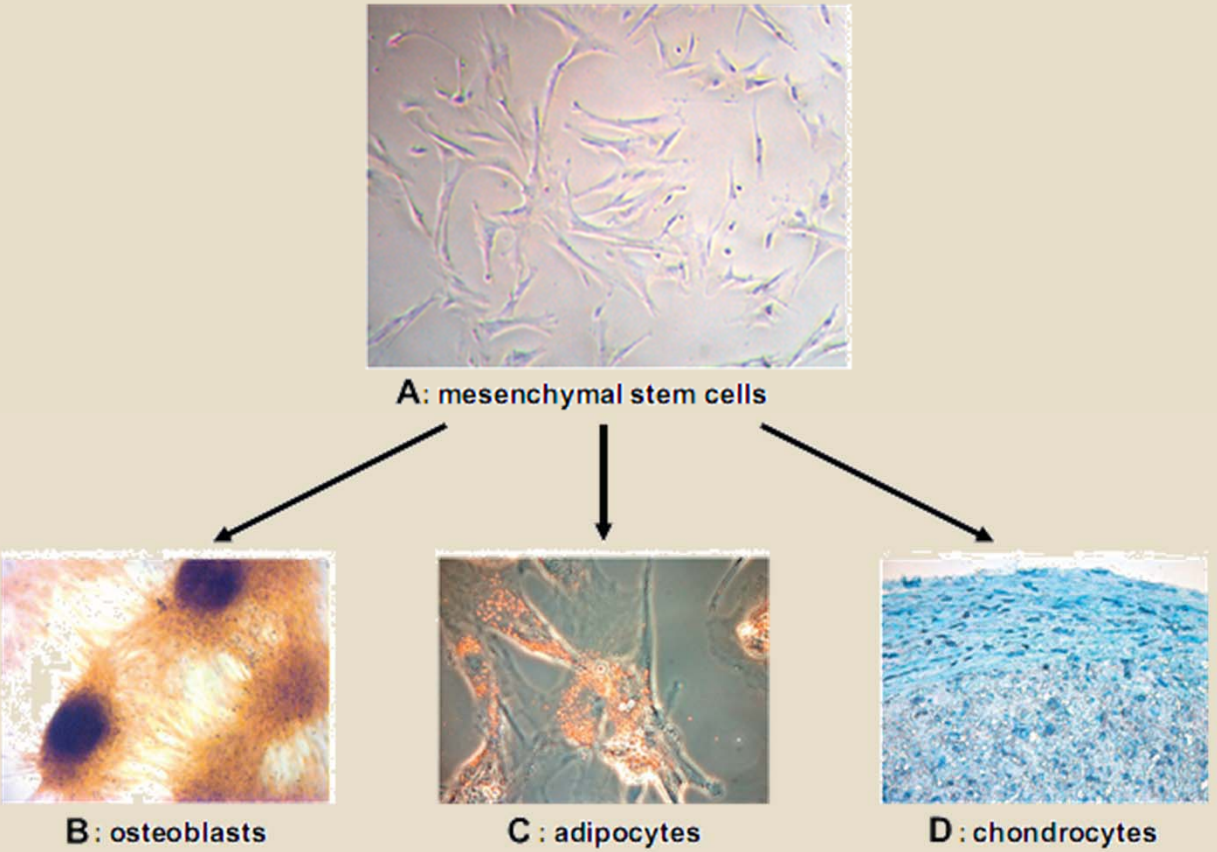


MSC in tissue engineering application



Very useful due to their availability and plasticity

MSC dfferentiation capacity



Considerations

- MSC could be also used pre-cultivated in vitro (like ACT), but still many studies are required.
- Safety: no definitive studies has been realized to exclude any problem related to the use of MSC.

How many cells? Which cells? Subpopulations? Pre-cultivated or not?

Development of a medical product

Preclinical Study

In vitro

Animal Models

Clinical Study

Phase I

Phase II

Phase III

Use