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Faculty of Medical Engineering

Regenerative medicine with stem cells

Regenerative medicine

Regenerative medicine is an emerging field that combines **tissue engineering** with stem cells, engineering, materials methods, and suitable biochemical and physicochemical factors to restore, maintain, improve or replace different types of biological tissues.

Regenerative medicine often involves the use of **stem cells** or **stem cells placed on tissue scaffolds**

It was categorized as a sub-field of biomaterials, but now it can be considered as a field in its own.

Types of Regenerative medicine :

- cells,
- induction of regeneration by biomolecules,
- cells + matrix approach (often referred to as a scaffold).

Regenerative medicine- key points

• A variety of stem cells;

• An engineered three-dimensional microenvironment supports cellular expansion;

•Differentiation into functional tissue (scaffold);

•Manufacturing processes which can facilitate tissue assembly;

•Its successes are predominantly limited to thin or avascular structures;

•Advances are being realized generating clinical translatability while surgeons should become familiar with these emerging technologies.

Why is Regenerative medicine necessary?

- Most tissues cannot regenerate when injured or diseased.
- Even tissues that can regenerate spontaneously may not completely do so in large defects (e.g., bone).
- Replacement of tissue with permanent implants is greatly limited.

Stem cells

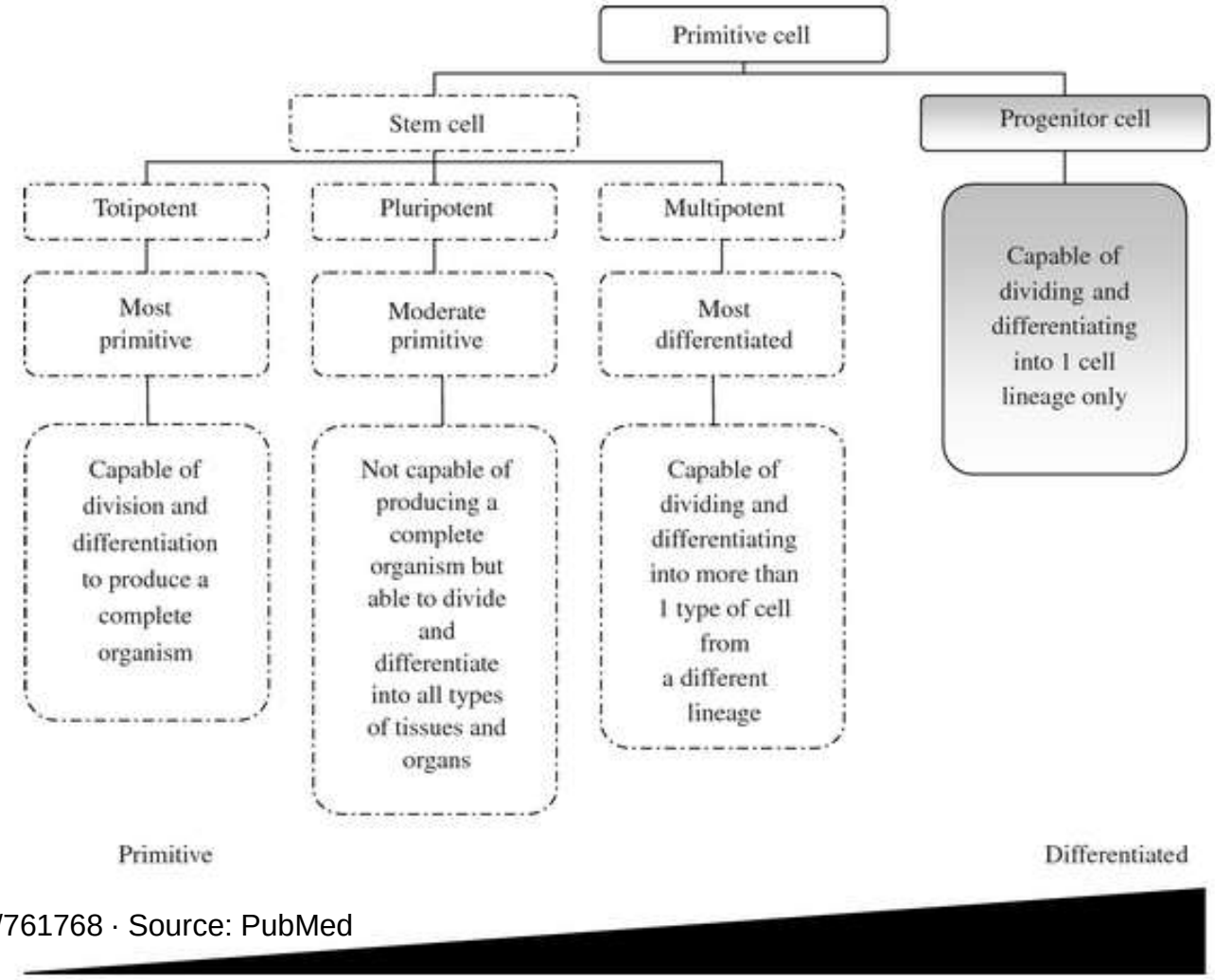
Totipotent Stem Cells

Pluripotent Stem Cells

Multi-, Oligo-, or Unipotent Stem Cells

Progenitor Cells
Precursor / blast Cells

Stem cells



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Regenerative medicine

Types of Regenerative medicine :

- injection of stem cells,
- the induction of regeneration by biomolecules
- cells + matrix approach (often referred to as a scaffold).

- *directed differentiation* (pluripotent stem cells)
- *direct reprogramming* (somatic cell types)

Stem cells

Totipotent Stem Cells

Totipotent stem cells can produce all three primary germ cell layers, as well as, extraembryonic tissues, such as the placenta. They are only present in the first weeks of embryogenesis (zygote). They are unavailable for tissue engineering / regenerative medicine applications.

endoderm
Embryonic tissue that is the precursor of the gut and associated organs.

mesoderm
Embryonic tissue that is the precursor to muscle, connective tissue, skeleton and many of the internal organs.

ectoderm
Embryonic tissue that is the precursor of the epidermis and nervous system.

Stem cells

Pluripotent Stem Cells (ESCs)

•pluripotent stem cells are present in later embryogenesis

•they similarly can differentiate into all three germ layers

• they are unable to differentiate into the placenta

• represent the gold-standard in stem cell-based therapies

• ESCs spontaneously differentiate into embryonic bodies but can form teratomas during animal implantation.

“how much manipulation can be allowed until it is considered playing God and morally unacceptable?”

Stem cells

Induced pluripotent stem cells (iPSCs) (2006)

• ethical concerns of ESCs were overcome by reprogramming fully differentiated fibroblasts into induced pluripotent stem cells (iPSCs) using transcription factors Oct3/4, Sox2, c-Myc, and Klf4

• iPSCs seem to offer the benefit of pluripotency without the ethical concerns associated with ESCs

•significant similarities with regard to and differentiation potentials

•costs, ethical concerns, reproducibility.

Stem cells

mesenchymal

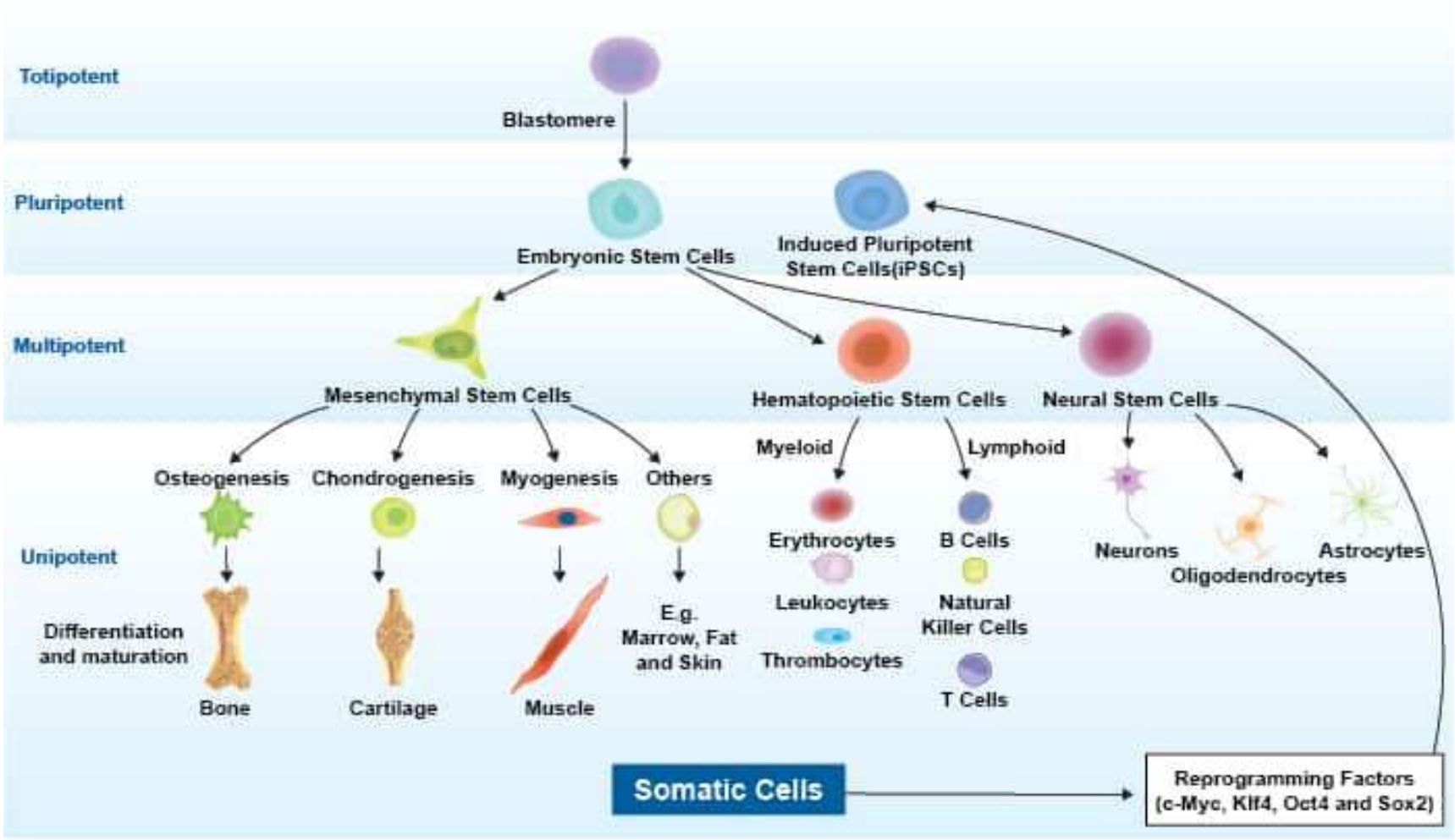
Multi-, Oligo-, or Unipotent Stem Cells hematopoietic

Adult stem cells (ASC) are typically described as unipotent, oligopotent, or multipotent cells

These stem cells have the capacity for self-renewal and to develop into individual or multiple cell types.

Disadvantages of adult stem cells are focused on the decrease in pluripotency along with the lack of continued self-renewal or cellular cross-contamination.

Stem cells



https://www.novusbio.com/research-areas/stem-cells

Stem cells

Stem cells	Description
Totipotent stem cells	• Gives rise to all three germ layers as well as extraembryonic tissues • Available only during early embryogenesis • Not accessible for engineering applications
Pluripotent stem cells	• Gives rise to all three germ layers • Can be induced from fully differentiated cells via reprogramming • Gold standard for stem cell-based therapies • Widely investigated across a multitude of tissue types
Multi-, Oligo-, or Unipotent stem cells	• Present postnatally • Identified in many tissues • Limited in differentiation potential • Can be easily accessible (i.e., adipose) • Widely investigated in regenerative engineering applications

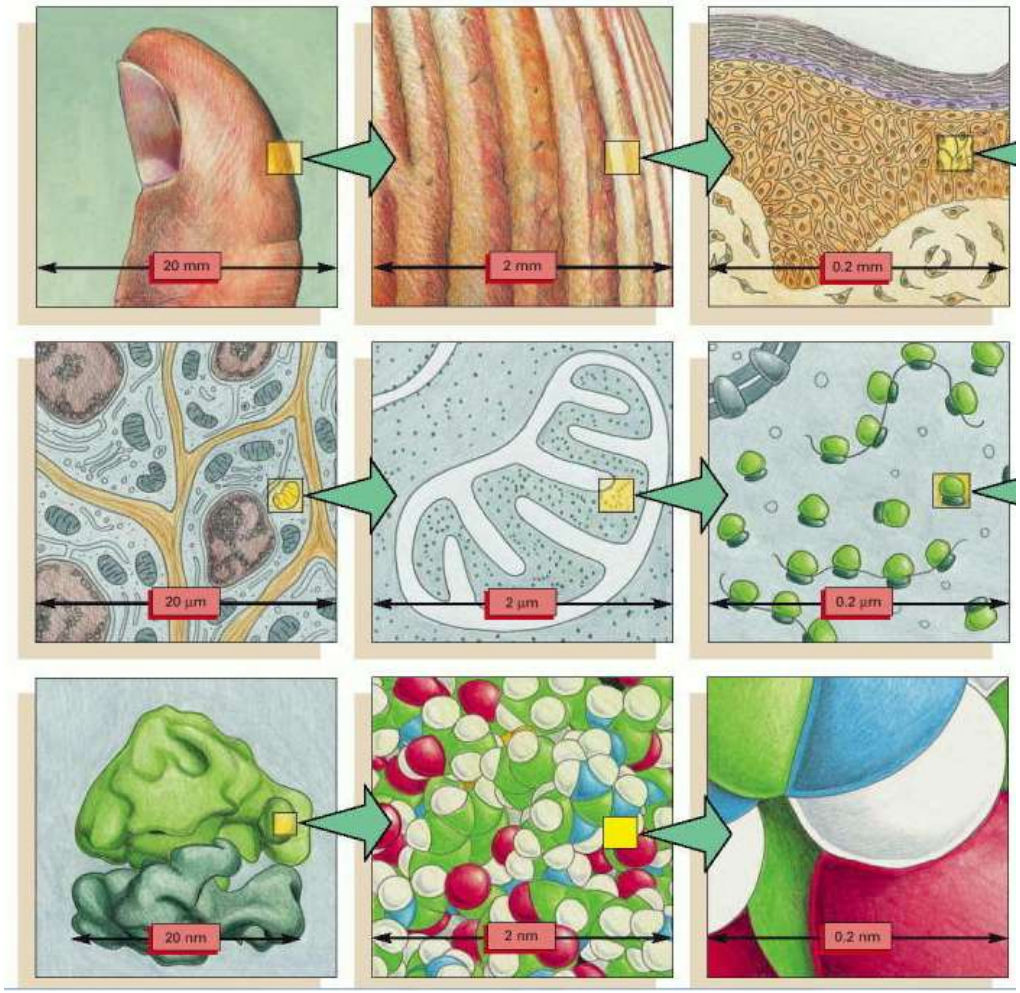
Goldenberg D, McLaughlin C, Koduru SV and Ravnic DJ (2021) Regenerative Engineering: Current Applications and Future Perspectives. Front. Surg. 8:731031. doi: 10.3389/fsurg.2021.731031

Key differences between 2D and 3D culture systems

2D culture	3D culture
Monolayer cell growth on plastic or glass	Natural cell growth on soft materials like collagen or other biomaterials
Easy fabrication	Additional expertise needed to make scaffolds and suspend cells
All cells equally exposed to nutrients and oxygen	Innermost cells may be deprived of nutrients and oxygen leading to necrosis
Gene expression profiles are dissimilar to *in vivo* tissues	Gene expression profiles are more like *in vivo* tissues
Are not predictive of the *in vivo* effectiveness/toxicity of drug treatments	Better predictors of *in vivo* drug effectiveness/toxicity

Goldenberg D, McLaughlin C, Koduru SV and Ravnic DJ (2021) Regenerative Engineering: Current Applications and Future Perspectives. Front. Surg. 8:731031. doi: 10.3389/fsurg.2021.731031

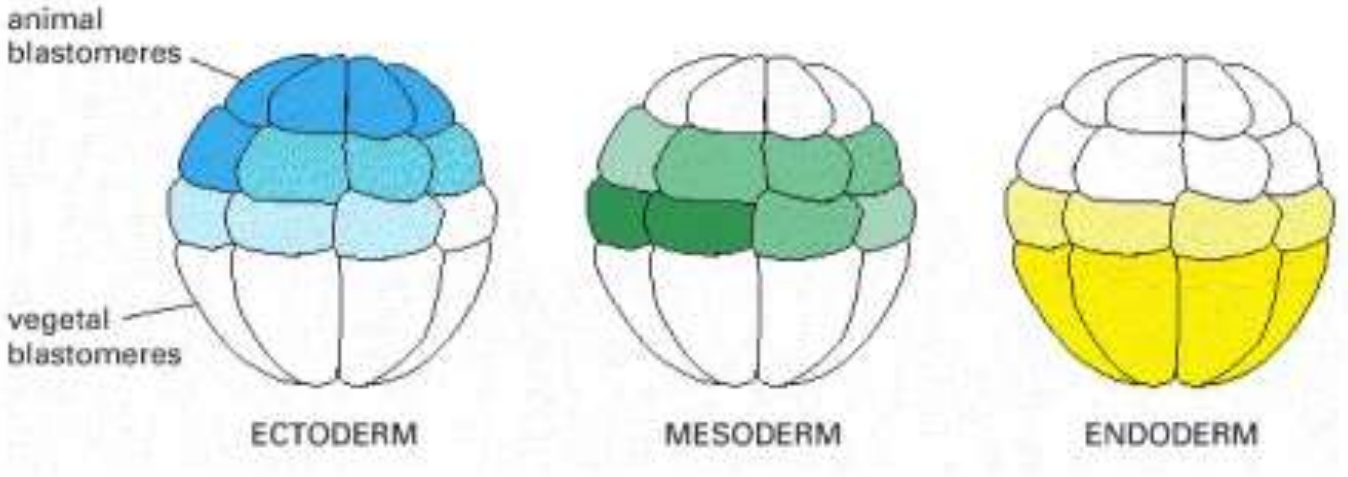
A sense of scale between living cells and atoms



Each diagram shows an image magnified by a factor of ten in an imaginary progression from a thumb, through skin cells, to a ribosome, to a cluster of atoms forming part of one of the many protein molecules in our body. Details of molecular structure, as shown in the last two panels, are beyond the power of the electron microscope.

Molecular Biology of the Cell. 4th edition.
Alberts B, Johnson A, Lewis J, et al.

The origins of the three germ layers can be traced back to distinct blastomeres of the embryo in its early cleavage stages



The endoderm derives from the most vegetal blastomeres, the ectoderm from the most animal, and the mesoderm from a middle set that contribute also to endoderm and ectoderm. The coloring in each picture is the more intense, the higher the proportion of cell progeny that will contribute to the given germ layer. (After L. Dale, *Curr. Biol.* 9:R812–R815, 1999.)

The rapid-cleaving animal pole contributes to the embryo proper, feeding from the yolk of the vegetal pole. https://www.rbmojournal.com/article/S1472-6483(10)61712-9/pdf

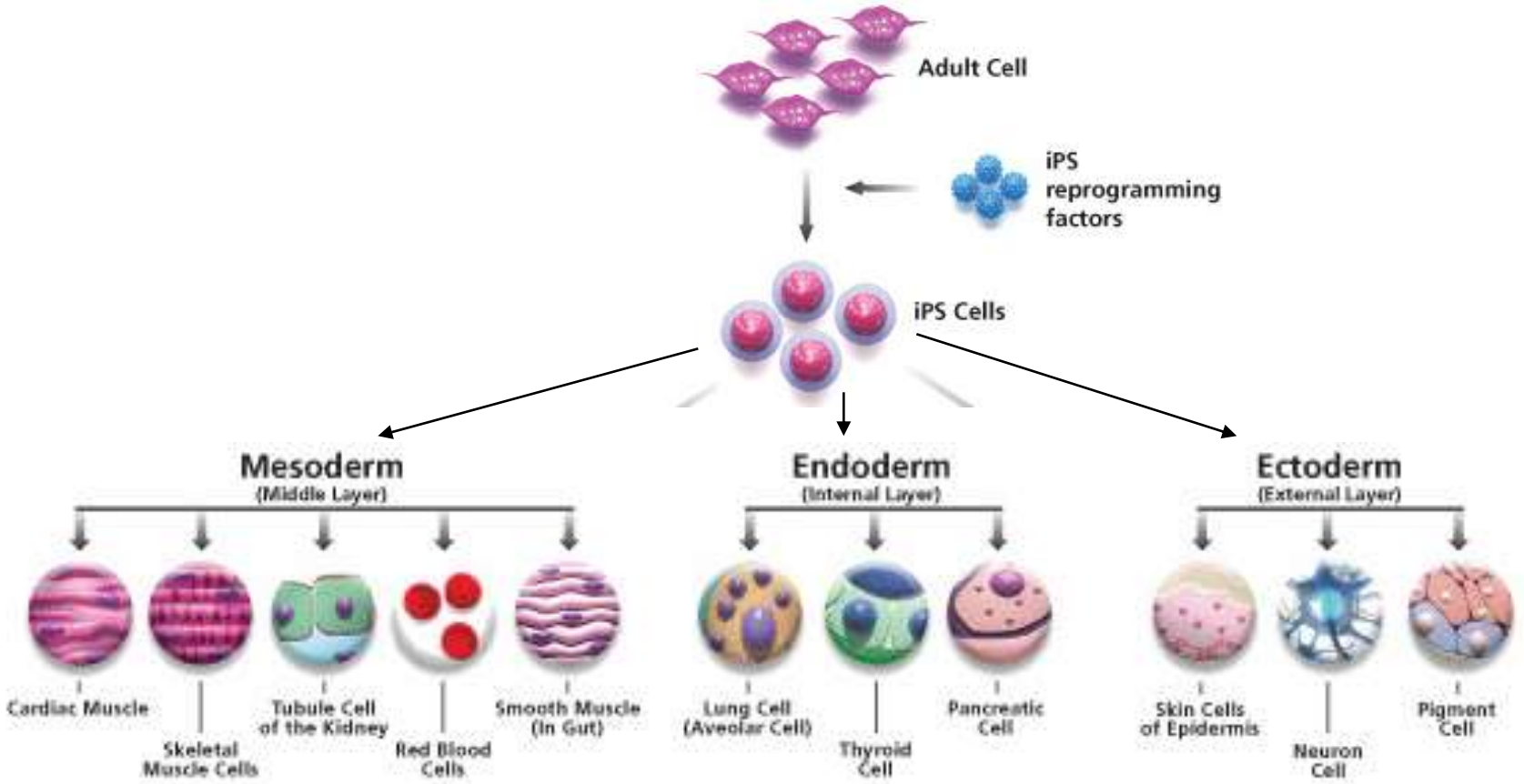
The chemicals that compose cells

Water, inorganic ions, and a large array of relatively small organic molecules (e.g., sugars, vitamins, fatty acids) account for 75 – 80 percent of living matter by weight.

The proteins accounts for approximately 20 percent of a cell's weight.
https://www.ncbi.nlm.nih.gov/books/NBK21473/

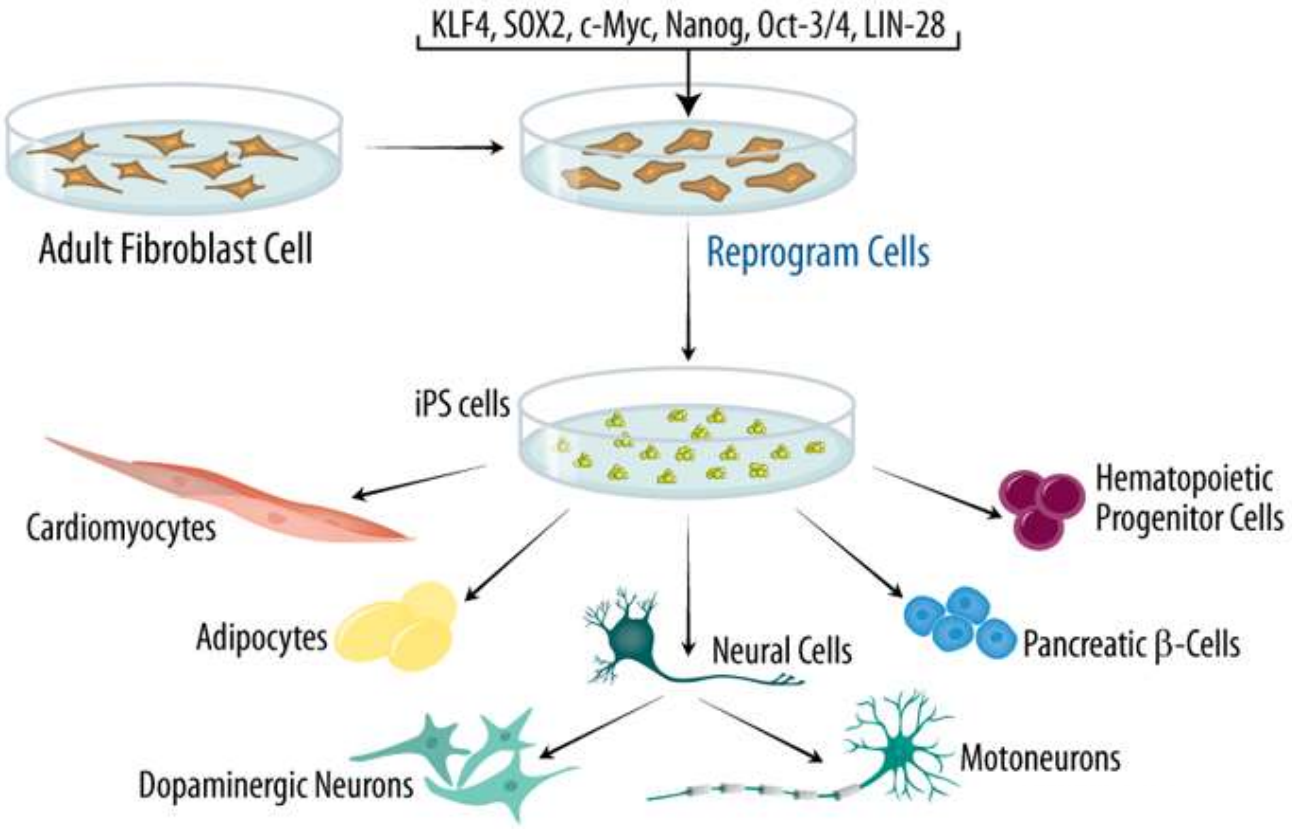
Water accounts for about 70% of a cell's weight, and most intracellular reactions occur in an aqueous environment.

Induced Pluripotent Stem Cell (iPSCs) Differentiation



Adult somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) with the over expression of key reprogramming genes (OCT4, KLF4, SOX2, cMYC, NANOG and LIN28).

Induced Pluripotent Stem Cell Differentiation (iPSCs)



Human Dermal Fibroblasts (HDF) are responsible for producing the extracellular matrix forming the connective tissue of the skin, and play a crucial role during wound healing.

Human iPSCs have the unique ability to differentiate into any cell type of the body including:
Ectodermal: Neuron, Astrocyte, Oligodendrocyte, Retinal Epithelial Cell (RPE), Epidermal, Hair and Keratinocytes.
Endodermal: Hepatocyte, Pancreatic β-islet Cell, Intestinal Epithelial Cell, Lung Alveolar Cells.
Mesodermal: Hematopoietic, Endothelial Cell, Cardiomyocyte, Smooth Muscle Cell, Skeletal Muscle Cell, Renal cell, Adipocyte, Chondrocyte and Osteocytes.

- Amabile G., Meissner A. (2009) Trends Mol. Med. **15** (2)**,** 59-68.
-https://www.rndsystems.com/resources/articles/differentiation-potential-induced-pluripotent-stem-cells

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