WHAT TO KNOW BEFORE GETTING STEM CELLS

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Aspen Integrative Medicine

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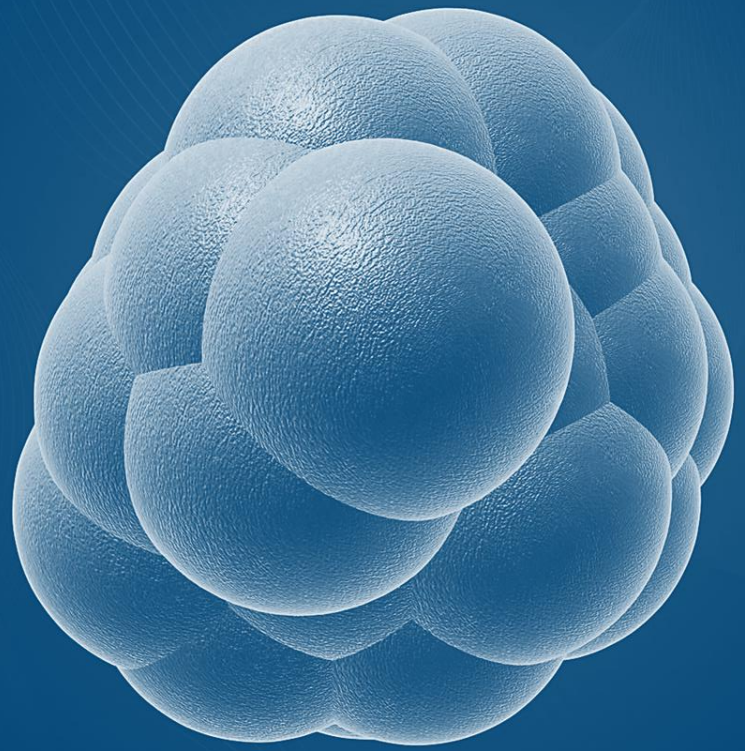
Disclaimer:
I have no material interest or investment in any stem cell companies or products.



I. Introduction to stem cells

II. Mesenchymal stem cells

III. Pluripotent stem cells

IV. Making a stem cell decision

Embryonic Stem Cells	Adult Stem Cells
Derived from the fetus	Derived from bone, adipose, or blood
Requires special regulatory approval	Requires physician expertise and quality control
Mostly used for research purposes	Mostly used for regenerative and cosmetic purposes
Not readily available	Readily available
Expensive	Less expensive
Not autologous	Autologous use is permitted in US

Derived from the embryo

Forms all cell types in the body

Used for early embryonic development

"Baby cells" (from a zygote) that becomes a pluripotent cell

Derived from the embryo or blood

Forms all cell types in the body except the embryo or the placenta

Does not have a specialized trajectory of development

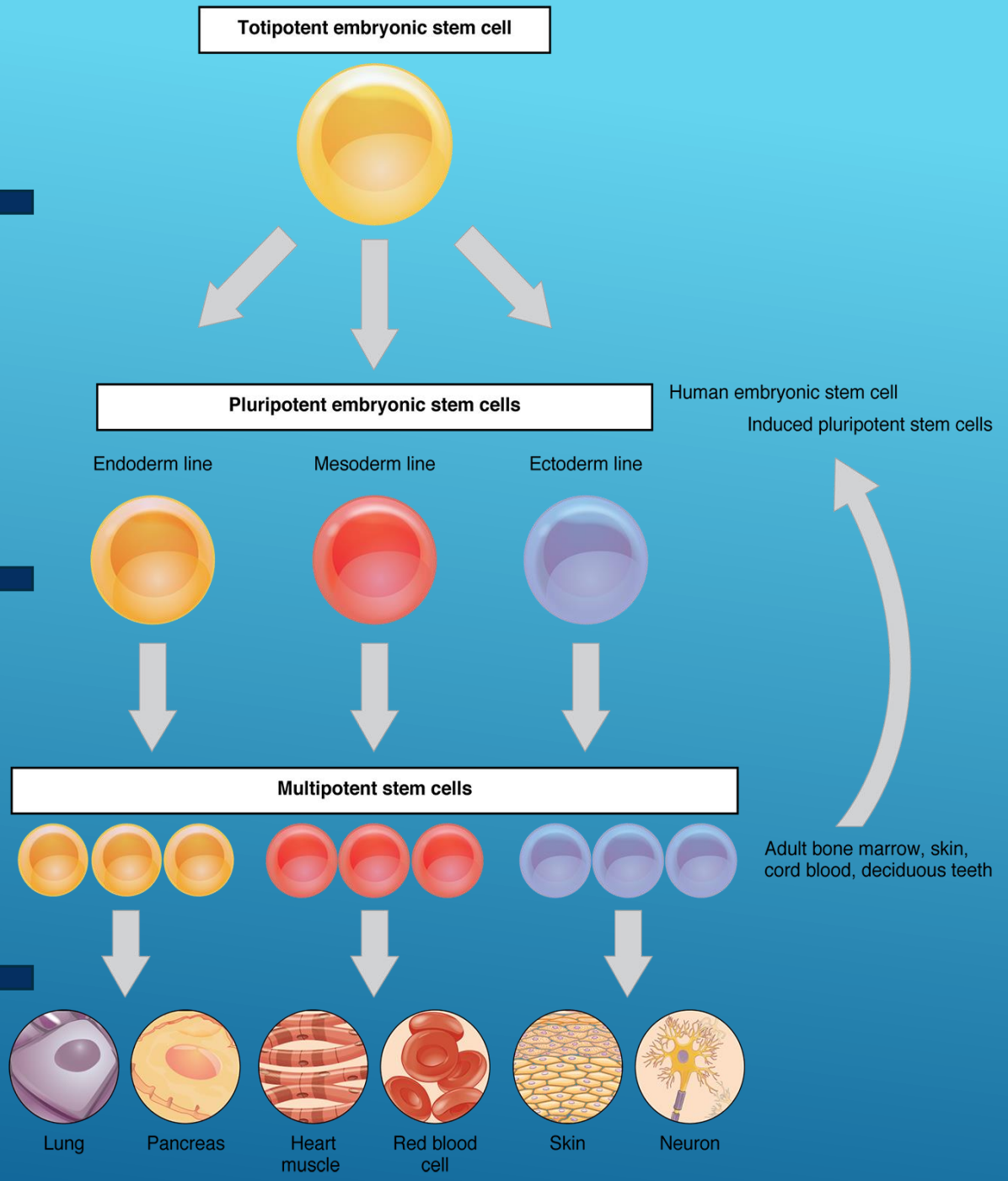
"Youthful cell" with great ability to differentiate into other cell types

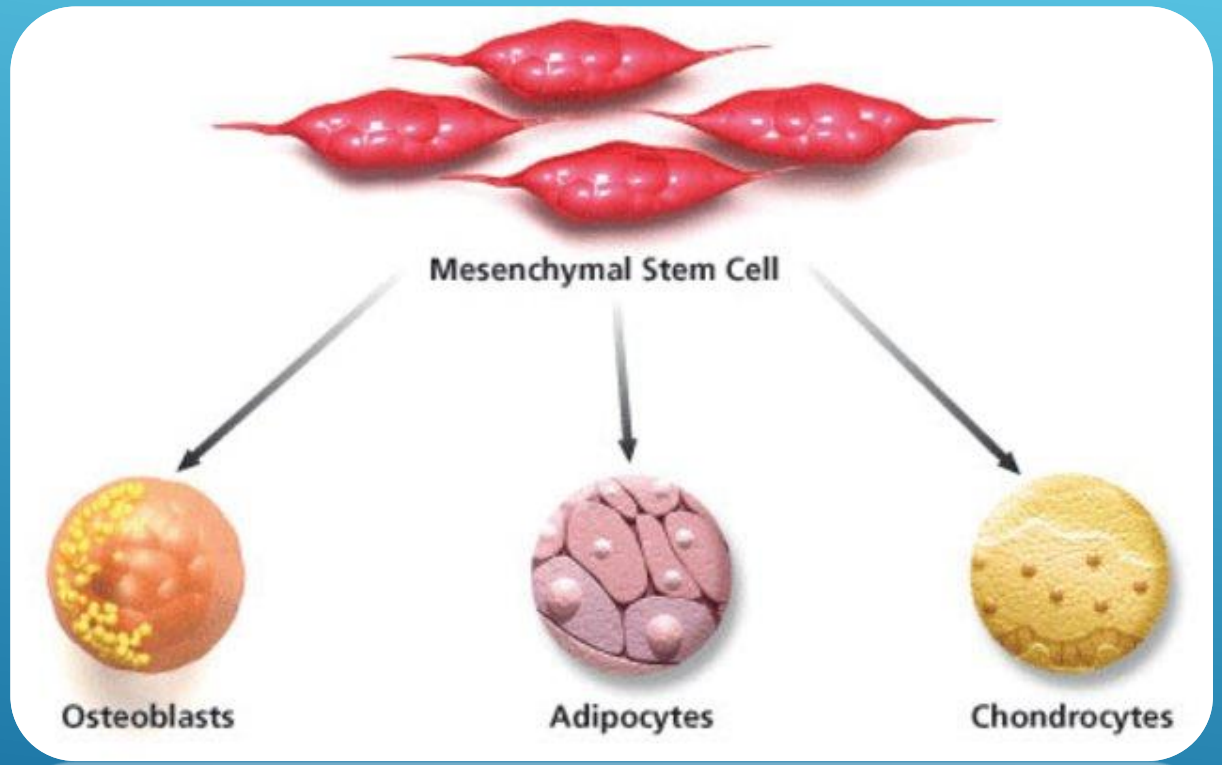
Derived from cord blood, blood, bone marrow, fat and muscle

Forms only cell types from the mesoderm

Has a development trajectory towards a specific type of cell

"Teenage cell" already differentiated into it's target adult cell type





MESENCHYMAL STEM CELLS (MSCs)

MESENCHYMAL STEM CELLS (MSCs)

Discovery

- Alexander Friedenstein discovered mesenchymal stem cells in mice (*Mus musculus*).
    - From 1966 through 1987, Friedenstein provided evidence that stem cells from bone marrow can differentiate into mesenchymal tissues
- Since then, the cell potency of mesenchymal stem cells differentiation has been a cause of debate
    - Are they truly multipotent or unipotent?

https://embryo.asu.edu/pages/mesenchyme

MESENCHYMAL STEM CELLS (MSCs)

Defined

- Mesenchyme: loose cells embedded in the extracellular matrix
  - a mesh of proteins and fluid that allows cells to migrate easily
- Directs development of morphological structures during the embryonic and fetal stages
  - connective tissues, bones, cartilage, lymphatic and circulatory systems
- Carry over 480 growth factors and are attracted to target tissues of inflammation
- Primarily isolated from fat or bone marrow through a time-insensitive, invasive process
  - Human fat (adipose tissue) has about 10x more stem cells than bone marrow

https://embryo.asu.edu/pages/mesenchyme

MESENCHYMAL STEM CELLS (MSCs)

How They Work

- Derived from pluripotent stem cells, have already partially differentiated, and they continue specializing as they develop
- Must be activated appropriately – often mixed with human plasma
- Considered *multipotent* because their specialization potential is *limited* to one or more cell lines
    - *Current research suggests multipotent cells are able to go beyond the boundaries of producing one specific cell type but do so infrequently and only under narrow conditions*

http://www.explorestemcells.co.uk/multipotentstemcells.html

MESENCHYMAL STEM CELLS (MSCs)

How They Work

- Modulate endogenous tissue and immune cells
- Actively interact with nearby cells
    - Observed benefits of MSC therapy may result from the relinquishment of their molecular contents upon administration
- Therapeutic effects are short-lived

  "Recent studies have suggested that less than 1% of systemically administered MSCs persist for longer than a week following injection."

- Limited in numbers – unlikely that true MSCs circulate peripherally (~0.01% of mononuclear bone marrow cells)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3759519/

MESENCHYMAL STEM CELLS (MSCs)

Clinical Indications

- Allogenic bone marrow transplants (from the same species to another of the same species) clinically used since the 1980s
- Autologous fat and bone marrow transplantations (from the same individual back to same individual) can be used to support target tissues that are not from the same cell type
    - E.g., injecting fat MSCs into joints orthopedically to support the growth of new cartilage
    - **These MSC's do not develop into new cartilage cells** – they provide growth factors, reduction in inflammation, and immune modulation that may support joint health
    - They are already on a development trajectory and their effects on unique target tissues are mostly paracrine (effecting nearby cells)

MESENCHYMAL STEM CELLS (MSCs)

Clinical Indications

- Outside the US, MSCs can be cultured for several weeks to build up the cell counts
  - The idea is that with more MSCs, more target tissues will benefit
  - **Challenge**: they grow older the more times that they replicate so they are less effective

- **MSCs are generally best used for transplantation into similar tissues from which they derive**
  - E.g., MSCs from fat are best transplanted into areas in need of fat replacement

  (breast augmentation, subcutaneous fat areas of the body - facial, lip, buttocks transplants)

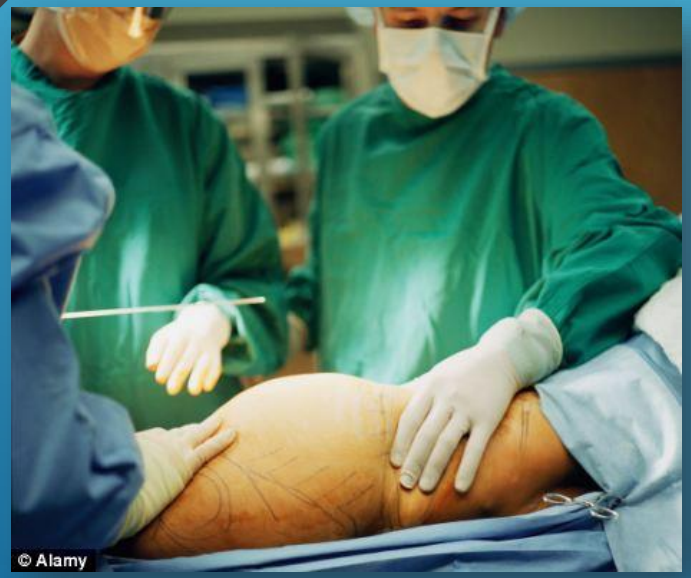
- Most effective clinical use of MSCs:
  - Same tissue transplantation (bone marrow to bone marrow, fat to fat)
  - Joint conditions (if related to an autoimmune or systemic inflammation)
  - Autoimmune disorders and systemic inflammatory conditions (see table on next slide)

Animal studies of diseases shown to respond to administration of mesenchymal stem cells

Disease	Animal model(s)	Method of administration	Evidence of MSC efficacy
Acute renal failure	Rodent Cisplatin Ischemia/reperfusion	Intravenous infusion	Decreased serum creatinine Decreased apoptosis Increased epithelial proliferation Suppression of proinflammatory cytokine gene expression
Myocardial infarction	Rodent LAD ligation Pig Temporary LAD occlusion	Intravenous infusion Intramyocardial transplantation	Reduction in scar formation Improvement in cardiac function Differentiation of MSCs into functioning myocardium
Type I diabetes mellitus	Rodent NOD mice Streptozotocin	Intravenous infusion	Partial restoration of glucose management Reduction in anti-insulin T cells Prevention of $FOXP3^{+}$ cell apoptosis
Graft-versus-host disease	Rodent HLA-mismatched bone marrow transplantation	Intravenous infusion Intravenous coinfusion with bone marrow transplant	Increased survival

MESENCHYMAL STEM CELLS (MSCs)

Dangers and Side Effects



- Harvesting of bone marrow and fat MSCs is unpleasant for the patient
- There is a limited number of times one can extract and use fat MSCs
- Many patients have to repeat the procedure to gain significant benefit
    - MSCs reduce inflammation for a time period of 6 months to 2 years but have limited regenerative benefits
- Are generally designed to affect one germ layer and tissues derived from that mesodermal layer - and under most conditions are unipotent (have the capacity to differentiate into only one cell type)

MESENCHYMAL STEM CELLS (MSCs)

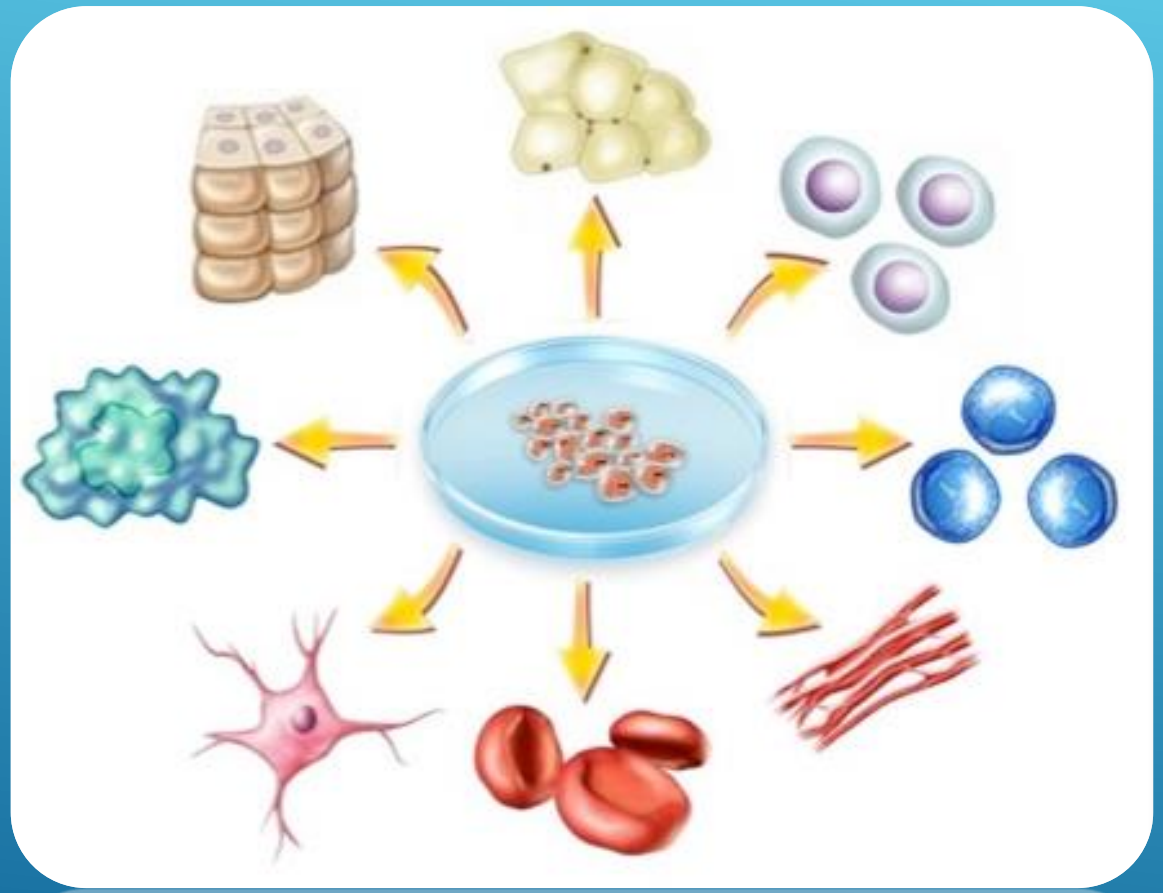
Dangers and Side Effects

- Because of the immunomodulatory effects of these MSCs, they predispose the patients more infections or even cancer
- After MSC infusions were used to treat nine patients suffering from GvHD, three developed viral infections
    - Immunosuppression by the MSCs had caused a reduction of immuno-surveillance to viruses

https://www.ncbi.nlm.nih.gov/pubmed/16604097

- MSCs, when administered in rats, can engraft in the renal tubules and mal-differentiate into adipocytes that hinder normal function of the kidney and lead to chronic kidney disease

https://www.ncbi.nlm.nih.gov/pubmed/17460140



PLURIPOTENT STEM CELLS

PLURIPOTENT STEM CELLS

Discovery

- Henry Young et al. (2004) demonstrated connective tissue (including blood) contains reserve precursor cells
  - Reserve precursor cells consist of: tissue-specific progenitor cells, germ-layer lineage stem cells, and **pluripotent stem cells**
    - Tissue-specific progenitor cells can be unipotent or multipotent
    - Progenitor cells can only double 50–70 times while germ-layer lineage stem cells and **pluripotent stem cells** have a much greater lifespan
    - Pluripotent stem cells were thought only to exist in embryonic stem cells until Dr. Young's discovery of them in the peripheral blood in the late 20th century

PLURIPOTENT STEM CELLS

Defined

- Precursor cells can be
  - Tissue-specific progenitor cells
  - Lineage-committed (ectodermal, mesodermal, and endodermal) germ-layer lineage stem cells
  - Lineage-uncommitted **pluripotent epiblastic-like stem cells**
- What we are interested in today are the lineage uncommitted pluripotent stem cells (some researchers call these cells, blastomere-like stem cells)

https://mail.google.com/mail/u/0/#search/henry+young+/13b421977b3e5c33?projector=1

PLURIPOTENT STEM CELLS

How They Work

Understanding lineage uncommitted pluripotent stem cells requires an understanding of the germ layers

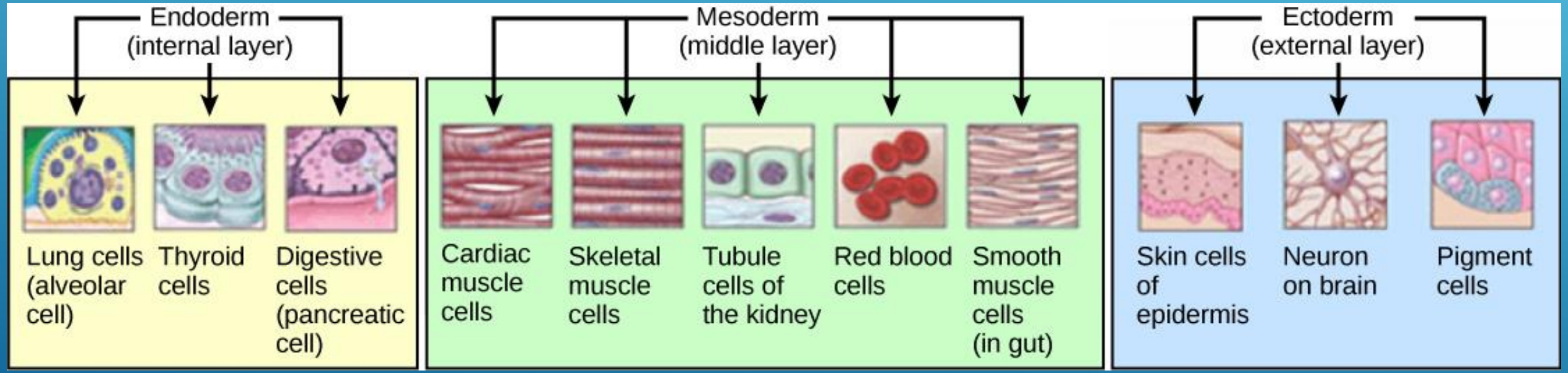


Table 5
Induction of Phenotypic Expression in Native and Induced Adult Precursor Stem Cell Lines

Phenotypic markers	ELSCs	EctoGLLSCs	MGLLSCs	EndoGLLSCs	PanPCs	3D-ILS
Embryonic						
Alkaline phosphatase	+	–	–	–	–	–
SSEA-1	+	–	–	–	–	–
SSEA-3	+	–	–	–	–	–
SSEA-4	+	–	–	–	–	–
CEA	+	–	–	–	–	–
HCEA	+	–	–	–	–	–
CD66e	+	–	–	–	–	–
CEA-CAM	+	–	–	–	–	–
Ectoderm						
Neuronal progen cells	+	+	–	–	–	–
Neurons	+	+	–	–	–	–
Ganglia	+	+	–	–	–	–
Oligodendrocytes	+	+	–	–	–	–
Astrocytes	+	+	–	–	–	–
Radial glial cells	+	+	–	–	–	–
Keratinocytes	+	+	–	–	–	–
Mesoderm						
Skeletal muscle	+	–	+	–	–	–
Smooth muscle	+	–	+	–	–	–
Cardiac muscle	+	–	+	–	–	–
White fat	+	–	+	–	–	–
Brown fat	+	–	+	–	–	–
Hyaline cartilage	+	–	+	–	–	–
Articular cartilage	+	–	+	–	–	–
Elastic cartilage	+	–	+	–	–	–
Growth plate cartilage	+	–	+	–	–	
Fibrocartilage	+	–	+	–	–	–
Endochondral bone	+	–	+	–	–	–
Intramembrane bone	+	–	+	–	–	–
Tendon/ligament	+	–	+	–	–	
Dermis	+	–	+	–	–	–
Scar tissue	+	–	+	–	–	–
Endothelial cells	+	–	+	–		
Hematopoietic cells	+	–	+	–	–	–
Endoderm						
Endodermal progenitor cells	+	–	–	+	–	–
Gastrointestinal epithelium	+	–	–	+	–	–
Liver oval cells	+	–	–	+	–	–
Liver hepatocytes	+	–	–	+	–	–
Liver biliary cells	+	–	–	+	–	–
Liver canalicular cells	+	–	–	+	–	–
Pancreas progenitor cells	+	–	–	+	+	–
Pancreas ductal cells	+	–	–	+	+	+
Pancreatic β-cells	+	–	–	+	+	+
Pancreatic α-cells	+	–	–	+	+	+
Pancreatic δ-cells	+	–	–	+	+	+

PPELSCs, pluripotent epiblastic-like stem cells (isolated and cloned). EctoGLLSCs, ectodermal germ-layer lineage stem cells (induced). MGLLSCs, mesodermal germ-layer lineage stem cells (isolated and cloned). EndoGLLSCs, endodermal germ-layer lineage stem cells (induced). PanPCs, pancreatic progenitor cells (induced). 3D-ILS, 3D-islet-like structures (induced). CEA, carcinoembryonic antigen. HCEA, human carcinoembryonic antigen. CD66e, carcinoembryonic antigen. CEA-CAM, carcinoembryonic antigen-cell adhesion molecule *(29,48,49)*.

PLURIPOTENT STEM CELLS

How They Work

- Adult pluripotent stem cells can be induced to form cells from the three primary germ-layer lineages (i.e., ectoderm, mesoderm, and endoderm).
- Results from neuronal, hematopoietic, diabetic, chondrogenic, osteogenic, myogenic, and cardiogenic studies demonstrate that adult pluripotent stem cells can be induced to undergo directed lineage induction.
- The activation of quiescent precursor cells is a potential component of tissue restoration.
    - Quiescent stem cells also assist the tissue-committed progenitor cells in forming the missing tissues

PLURIPOTENT STEM CELLS

How They Work

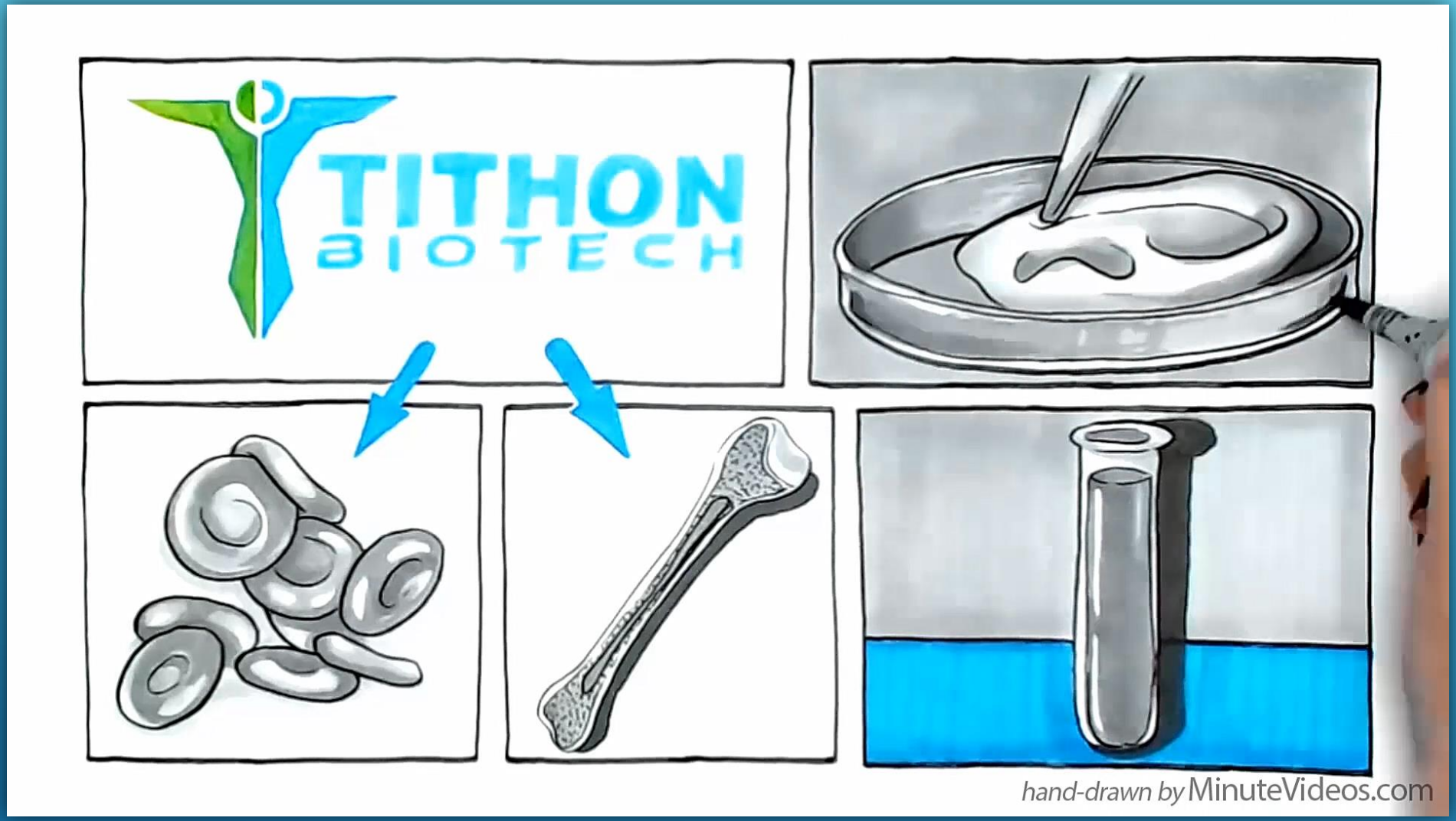
- Originate in bone marrow and present in peripheral blood
- Contain a unique marker that can be used to select them for both diagnostic and therapeutic procedures
- In abundance in peripheral blood and in reproductive tissue secretions

PLURIPOTENT STEM CELLS

Clinical Indications

- Lineage uncommitted pluripotent stem cells can be used to form any tissues in the endoderm, mesoderm, or ectoderm
- Treatment of a wide range of degenerative diseases in both humans and animals including, but not limited to:
    - **Diabetes, osteoarthritis, osteoporosis and Alzheimer's disease, to name a few, as well as regenerative applications associated with aging**
- TBI studies: When used in conjunction with hyperbaric oxygen therapy, intranasal and IV pluripotent stem cells (derived from blood plasma), after activation, have been shown, in case studies, to positively address post-concussive symptoms secondary to TBI: memory, sleep, mental fatigue, mental clarity, libido, motor function and balance.
- Could be shown useful in replacing bone marrow in post-cancer treatment

TITHON BIOTECH TECHNOLOGY

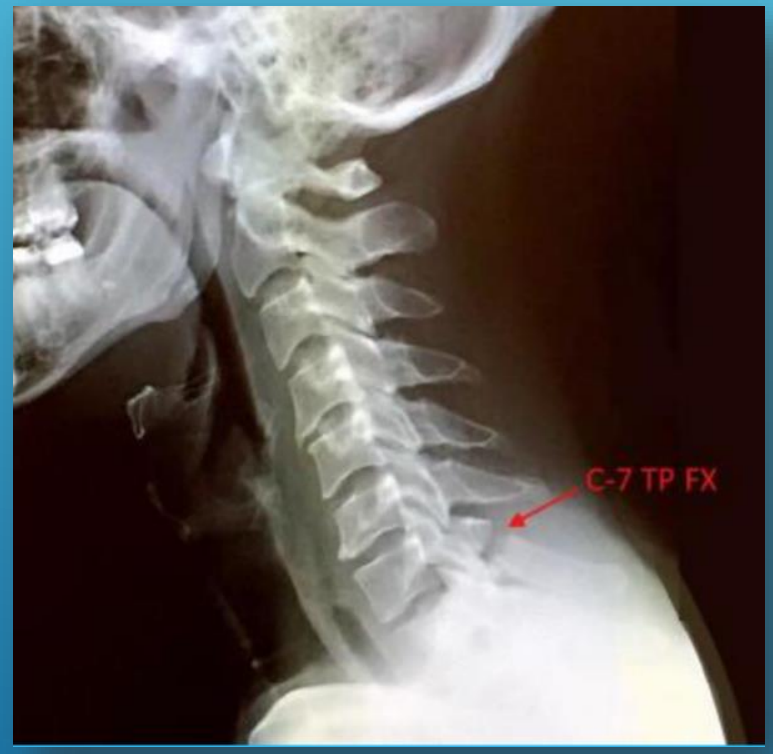


PLURIPOTENT STEM CELLS

Clinical Indications

Orthopedic Case

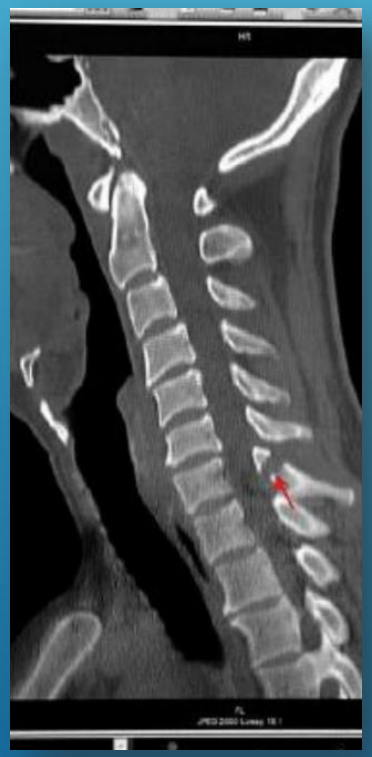
The following images are a compelling case of a post-traumatic, displaced (5mm) C-7 proximal spinal fracture, which had failed to heal 9 months post trauma (pseudoarthrosis).

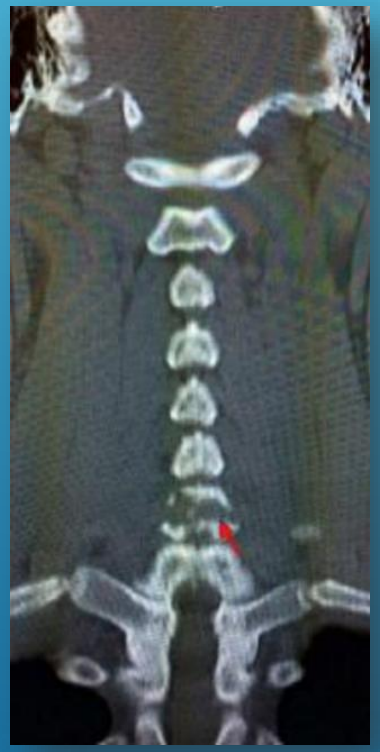


PLURIPOTENT STEM CELLS

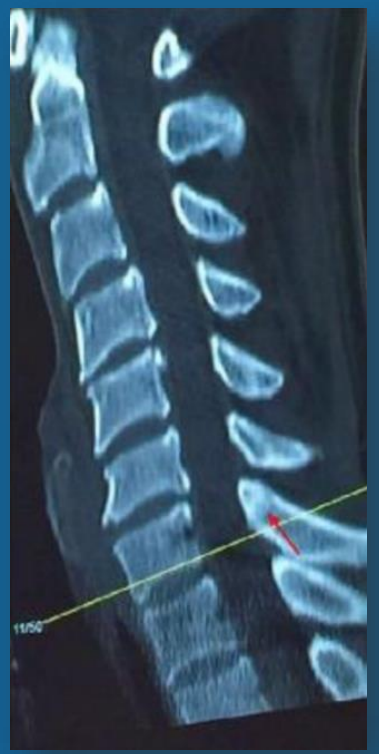
Clinical Indications

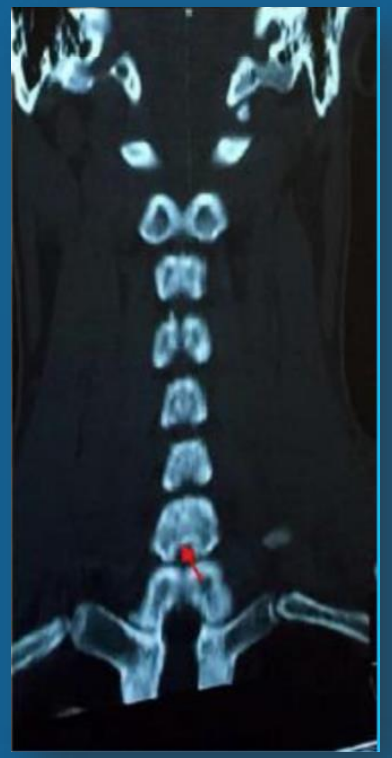
Pre-treatment





Post-treatment



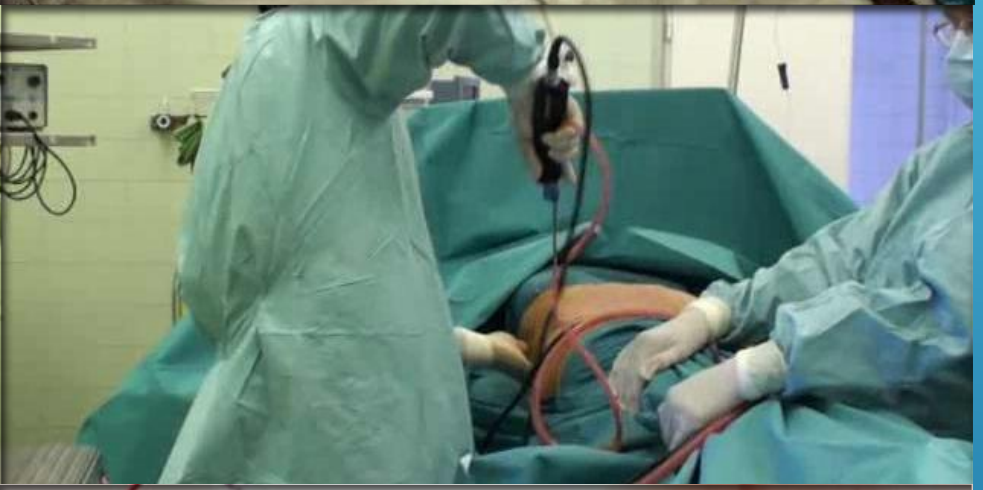


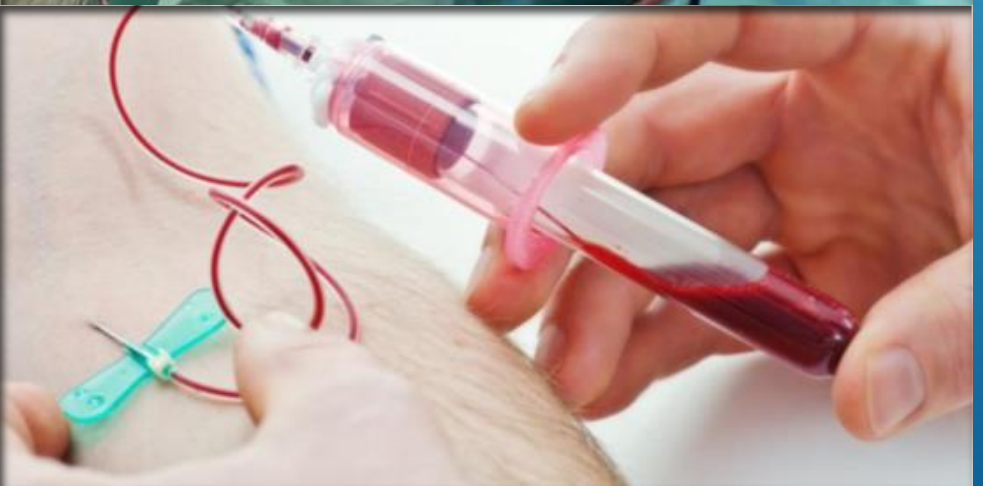
4 months post-treatment of PBSC-PRP, the fracture is fully healed

PLURIPOTENT STEM CELLS

Clinical Indications

- As featured on previous slides, what is most surprising is the fact that the new bone formation had to fill a 5mm displaced gap.
- If it had been a non-displaced fracture, one could make a case for a delayed spontaneously healed fracture. In this case, that would be a difficult argument to support.
- Similar cases have been observed, however, radiographically this one seems like a great visual way to show the osteoinductive properties of PBD-PSC's in the preparation.





MAKING A STEM CELL DECISION

Bone Marrow

Cost: $3,000 - $10,000

Recovery time: One Month

Adipose (Fat)

Cost: $6,000 - $15,000

Recovery time: One Month

Blood Based

Cost: $3,500 - $4,000

Recovery time: Less than a week

Aspen Integrative Medicine

970-927-0308

aspenintegrativemedicine.com

TBI Therapy

303-447-1257

tbitherapy.com

Q & A

aspenintegrativemedicine.com/what-to-know-stem-cells

REFERENCES

Cell Applications. https://www.cellapplications.com/stem-0

Cellular Differentiation. http://oerpub.github.io/epubjs-demo-book/content/m46036.xhtml

Kunter, U., Rong, S., Boor, P., Eitner, F., Müller-Newen, G., Djuric, Z., ... & Milovanceva-Popovska, M. (2007). Mesenchymal stem cells prevent progressive experimental renal failure but maldifferentiate into glomerular adipocytes. *Journal of the American Society of Nephrology*, *18*(6), 1754-1764.

MacCord, K. (2012). Mesenchyme. *The Embryo Project Encyclopedia.* Retrieved from https://embryo.asu.edu/pages/mesenchyme

Mesenchymal Stem Cell Reagents. Retrieved from http://www.sigmaaldrich.com/life-science/cell-biology/cell-biology-products.html?TablePage=22692887

Murnaghan, I. (2016). Multipotent stem cells. *Explore Stem Cells.* Retrieved from http://www.explorestemcells.co.uk/multipotentstemcells.html

Parekkadan B, Milwid JM. Mesenchymal Stem Cells as Therapeutics. *Annual review of biomedical engineering*. 2010;12:87-117. doi:10.1146/annurev-bioeng-070909-105309.

Stout, C. L., Ashley, D. W., Morgan, J. H., Long, G. F., Collins, J. A., Limnios, J. I., ... & Young, H. E. (2007). Primitive stem cells residing in the skeletal muscle of adult pigs are mobilized into the peripheral blood after trauma. *The American Surgeon*, *73*(11), 1106-1110.

Sundin, M., Örvell, C., Rasmusson, I., Sundberg, B., Ringden, O., & Le Blanc, K. (2006). Mesenchymal stem cells are susceptible to human herpesviruses, but viral DNA cannot be detected in the healthy seropositive individual. *Bone marrow transplantation*, *37*(11), 1051-1059.

Tithon Biotech (n.d.). Minutevideo retrieved from http://mv.pac.io/post/55b2e2b41e1818650cc7b872

Tithon Human Sciences (2015). Peripheral blood derived pluripotent stem cell technology. Rhttp://aspenintegrativemedicine.com/wp-content/uploads/Tithon-Human-Sciences-Ortho-Case-Study.pdf

Young, H. E., Duplaa, C., Romero-Ramos, M., Chesselet, M. F., Vourc'h, P., Yost, M. J., ... & Tamura-Ninomiya, S. (2004). Adult reserve stem cells and their potential for tissue engineering. *Cell biochemistry and biophysics*, *40*(1), 1-80.

Young, H. E., & Black, A. C. (2004). Adult stem cells. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology*, *276*(1), 75-102.