Stem cells in (regenerative) orthopedics

Between basic research and clinical practice in orthopedic surgery

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Orthopedic surgeon, scientific researcher in stem cells regenerative medicine and magnetic nanomaterials)

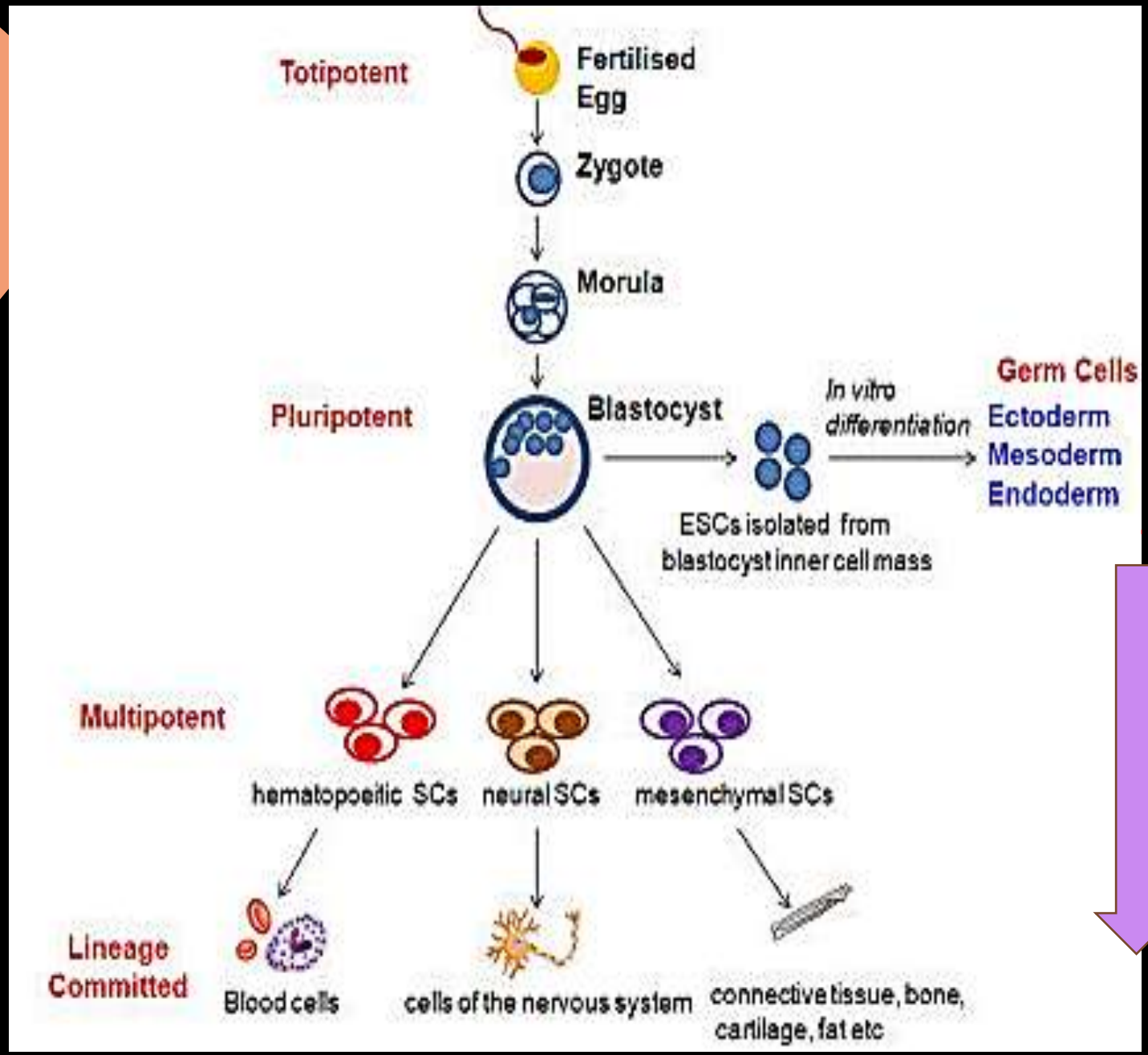
Outline

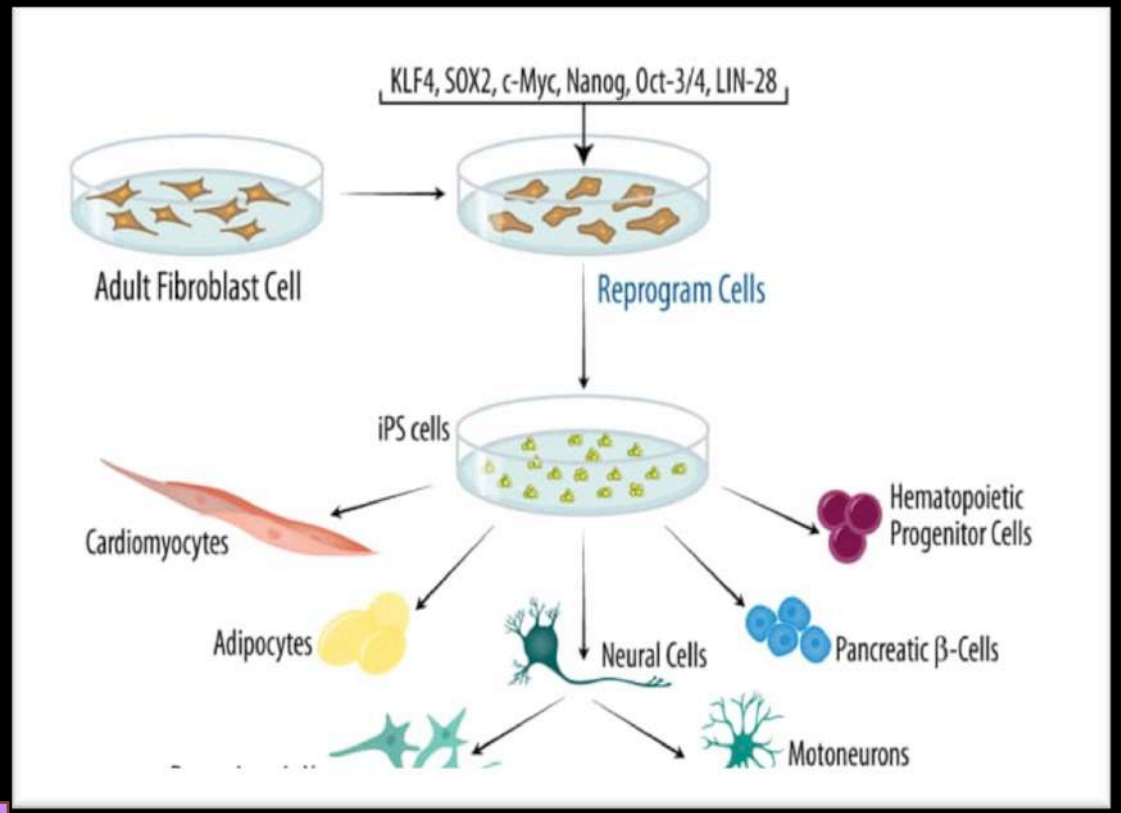
- Stem cell definition
- Type of stem cells
- Stem cells and regenerative medicine
- Stem cell in orthopedics-cell sources
- Current available orthobiologics and ATMPs

Stem cells

- Basic units of organism, organ and tissue development , maintenance &repair;
- A distinctive trait – asymmetric division & differentiation;
- A hierarchy based on their ability to give rise to progeny;
- Tremendous hope for harnessing their potential in preventing and treating diseases;
- Autologous /allogeneic/xenogeneic.

Hayes, M., Curley, G., Ansari, B. *et al.* Clinical review: Stem cell therapies for acute lung injury/acute respiratory distress syndrome - hope or hype?. *Crit Care* **16**, 205 (2012).



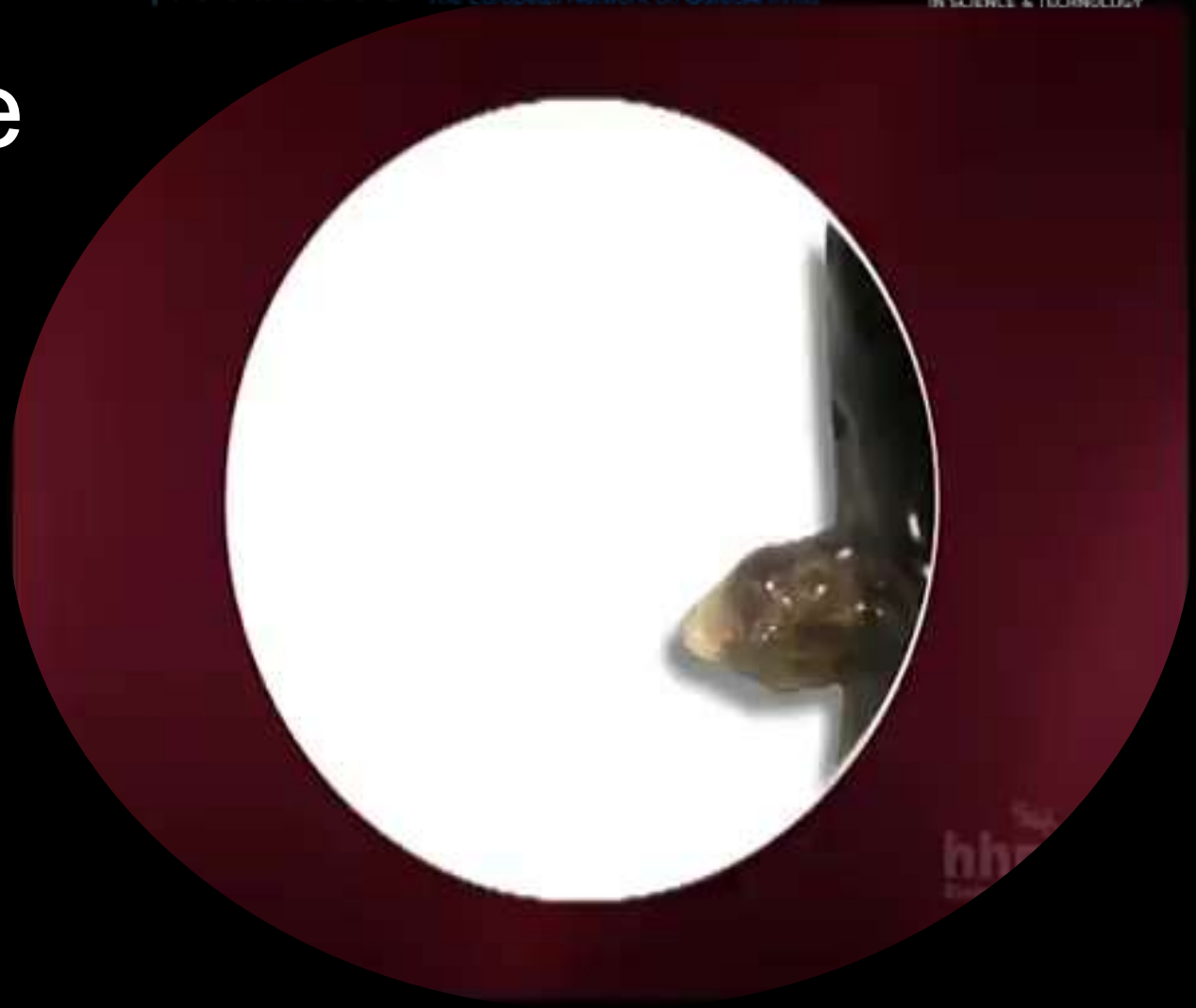


Totipotent
Pluripotent
Multipotent
Unipotent /oligopotent
Differentiated (lineage committed adult cells)

Stem cell hierarchy

Regenerative medicine

- “Restitutio ad integrum” anatomical, functional of diseased, degenerated, congenitally abnormal or absent , lost (to trauma or tumor) tissue, organs, systems –
- Leland Kaiser 1992, William Haseltine 1999



Salamander Limb Regeneration — HHMI BioInteractive Video - YouTube

Regenerative medicine - stem cell-based therapies-

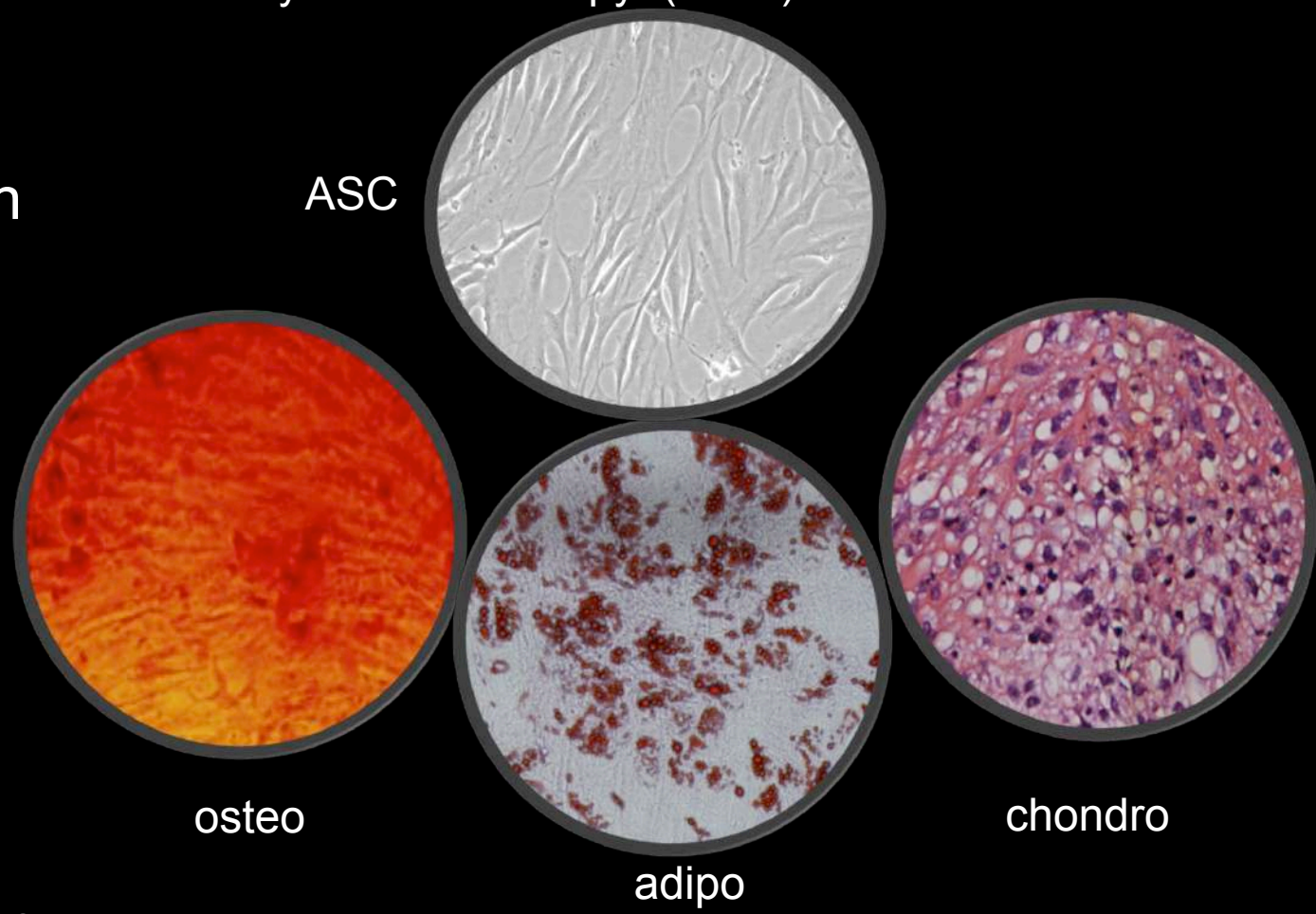
- Tissue engineering (classical “triad” cells, scaffolds, growth factors to in vitro or ex vivo engineer tissues, organs/additive manufacturing)- direct role of stem cells
- Cell therapy – (stem) cell suspension delivered locally, regionally or systemically; functional tissue regeneration- cytokines, growth factors, metabolic tissue modulation, stimulation of local progenitor cell types, vasoactive,– anti-inflammatory & immune modulation role
- Gene therapy (Cell& gene therapy); correction of (mostly) monogenic inherited diseases
- Drug Delivery Devices/Systems –DDD/S- stem cell targeting role

Tissue derived stem cells for musculoskeletal regeneration –mesenchymal stem cells- MSC

Bone marrow- BM-MSCs
Adipose tissue ASC
Peripheral blood PbMSC
Perichondrium PcMSC
Wharton jelly & placenta MSC WJMSC

Freshly isolated
Culture expanded

Minimal criteria for defining MSCs International Society for Cell Therapy (ISCT) criteria



"Surface markers CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR" Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8(4):315-7. doi:SVF, CD45-CD235a-CD31-CD34+. Bourin P, Bunnell BA, Casteilla L, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy*. 2013;15(6):641-648. doi:10.1016/j.jcyt.2013.02.006

Orthopedic Surgeon

Quod Natura relinquit imperfectum, Ars perfici/what nature left imperfect, (Medical) Art can make it perfect / Paracelsus

orthobiologics

- **Ortho + Biologics are biological substance(s) made from naturally occurring materials in the body; treatments aimed at restoring "musculoskeletal function" by harnessing the natural healing processes of the body"'**
- Ortho + Biologics are not: a "cure-all"; a way to restore worn-out tissues in the mature patients, eventually, target full tissue regeneration.
- Not routinely recognized by health insurance, and therefore not always reimbursed. (Bravo D, Jazrawi L, Cardone DA, Virk M, Passias PG, Einhorn TA, Leucht P. Orthobiologics A Comprehensive Review of the Current Evidence and Use in Orthopedic Subspecialties. Bull Hosp Jt Dis (2013). 2018 Dec;76(4):223-231. PMID: 31513506.)

The art of healing is like any other art; it is made up of many elements, and no one yet become a master in all of it.“ Ibn Sina

Orthobiologics- levels of complexity

- Marshal Urist 1964 – bone morphogenetic protein (BMP) –bone and fracture healing, nonunion cure;
  - Ist **generation – hyaluronic acid visco-supplementation (HA);**
  - IInd **generation – PRP (platelet rich plasma)**
  - IIIrd **generation – stem & cell therapies**

(Sampson, Mendelbaum, Current Reviews in Musculoskeletal Medicine, 2008. 1(3-4): p. 165-174)

Bone marrow aspirate ➡ BMAC

Knee Surgery, Sports Traumatology, Arthroscopy (2023) 31:2140–2151
https://doi.org/10.1007/s00167-022-07153-6

SPORTS TRAUMATOLOGY

Bone marrow aspirate concentrate quality is affected by age and harvest site

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Received: 22 June 2022 / Accepted: 30 August 2022 / Published online: 26 September 2022

Abstract

Purpose To compare the number and properties of bone marrow stromal cells (BMSCs) collected from bone marrow aspirate concentrate (BMAC) obtained from different harvest sites and from patients of different ages.

Methods BMAC was obtained from two groups of patients based on age ($n = 10$ per group): 19.0 ± 2.7 years for the younger and 56.8 ± 12.5 for the older group. In the latter, BMAC was obtained from both iliac crest and proximal tibia for a donor-matched analysis. Mononucleated cell count and CFU-F assay were performed, together with phenotype characterization of BMSCs from iliac crest and proximal tibia, the study of chondrogenic and osteogenic differentiation capacity, histological staining and spectrophotometric quantification, and the analysis of mRNAs expression.

Results Cells derived from iliac crest and proximal tibia showed the same phenotypic pattern at flow cytometry, as well as similar chondrogenic and osteogenic potential. However, a significantly higher number of mononuclear cells per ml was observed in younger patients ($3.8 \pm 1.8 \times 10^7$) compared to older patients ($1.2 \pm 0.8 \times 10^7$) ($p < 0.0005$). The latter yield, obtained from the iliac crest, was significantly higher than resulting from the BMAC harvested from the proximal tibia in the same group of patients ($0.3 \pm 0.2 \times 10^7$, $p < 0.0005$). This result was confirmed by the CFU-F analysis at day 10 (15.9 ± 19.4 vs 0.6 ± 1.0, $p = 0.001$) and day-20 (21.7 ± 23.0 vs 2.9 ± 4.2, $p = 0.006$).

Conclusion Harvest site and age can affect the quality of BMAC. BMSCs obtained from iliac crest and proximal tibia present comparable mesenchymal markers expression as well as osteogenic and chondrogenic differentiation potential, but iliac crest BMAC presents a four times higher number of mononucleated cells with significantly higher clonogenic capacity compared to the tibia. BMAC of younger patients also had a three-time higher number of mononucleated cells. The identification of BMAC characteristics could help to optimize its preparation and to identify the most suitable indications for this orthobiologic treatment in the clinical practice.

Adipose tissue ➡ stromal vascular fraction (SVF)

Contents lists available at ScienceDirect

Journal of Clinical Orthopaedics and Trauma

journal homepage: www.elsevier.com/locate/jcot

Knee intra-articular administration of stromal vascular fraction obtained from adipose tissue: A systematic review

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ARTICLE INFO

Article history:
Received 16 November 2021
Received in revised form
7 January 2022
Accepted 13 January 2022
Available online 22 January 2022

Keywords:
Knee
ADSC
SVF
Osteoarthritis

ABSTRACT

Osteoarthritis is a debilitating chronic degenerative disease of cartilage joint surfaces and the knee is the weight-bearing joint most frequently plagued. Intra-articular cell therapies have recently emerged as a method to manage knee osteoarthritis. A literature search identifying all articles involving use of SVF to treat knee osteoarthritis was performed, consulting several databases. In conclusion, 24 clinical trials analysed report good to excellent clinical and radiographic results for the treatment of knee OA with the use of intraarticular administration of SVF.

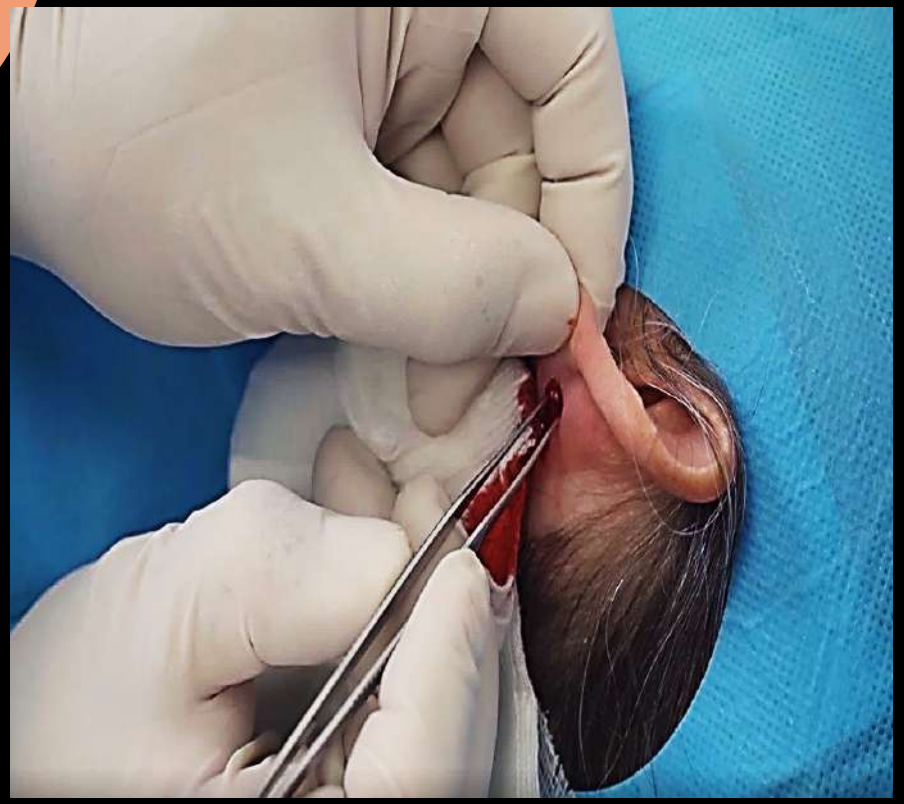
Peripheral blood ➡ Pb mesenchymal stem cells

Spectra Optia Apheresis System (terumobct.com)

Peripheral blood derived MSCs Pb-MSCs
Mononuclear cells from blood

Cartilage repair augmentarion
Osteoarthritis,
Tibial osteotomies (Saw, K., Anz, A., Merican, S., Tay, Y., Ragavana
idu, K., Jee, C. S. Y., et al. (2011). Articular Cartilage Regeneration With Autologous Periphe
ral Blood Progenitor Cells and Hyaluronic Acid After Arthroscopic Subchondral Drilling: A Report of 5 Cases With Histology. Arthroscopy: J. Arthroscopic Related Surg. 27, 493–506. doi: 10.1016/j.arthro.2010.11.054

Perichondrium derived progenitor cells



Autologous homologous cartilage progenitor therapy

Chondrocytes and perichondrium progenitor stem cell suspension cytokines,growth factors

Ceccarelli G, Gentile P, Marcarelli M, et al. In Vitro and In Vivo Studies of Alar-Nasal Cartila ge Using Autologous Micro-Grafts: The Use of the Rigenera® Protocol in the Treatment of an Osteochondral Lesion of the Nose. Pharmaceuticals (Basel). 2017; 10(2):53. Published 2017 Jun 13. doi:10.3390/ph10020053

Used for
knee, hip, finger
OA up to 3KL

Human cells, tissues, and cellular and tissue-based products (HCT/Ps)-FDA

HCT/Ps = most novel orthopaedic regenerative products and are defined as products containing or consisting of huma cells or tissues that are intended for implantation, transplantation, infusion, or transfer into human recipient **RMAT** (2016) is a special designation for products intended to treat serious or life-threatening conditions. This pathway mandates preliminary evidence that the product may address unmet clinical needs.

Category	FDA HCT/P oversight	Examples
Lowest risk	Not required	PRP, BMAC, Fat graft
Low risk	361	DMB, cellular bone matrix, chondrocytes SVF mechanically processed
Higher risk	351	Cultured chondrocytes, amniotic products, cultured mesenchymal cells, enzymatically processed SVF

Murray, I. R. (2022). Regulatory and Ethical Aspects of Orthobiologic Therapies. ***Orthopaedic journal of sports medicine, 10***(11),. /doi.org/10.1177/23259671221101626

Advanced Therapy Medicinal Products ATMPs -EMA

European Community (EC) No. 1394/ 2007 and the Human Cells and Tissues Directive (2004/ 23/EC).23 define advanced therapy medicinal products **ATMPs to** include gene therapy products, somatic cell products, or tissue engineered products that are more than minimally manipulated or used in a nonhomologous fashion. The "hospital exemption" clause within EC No. 1394/2007 allows nonroutine administration of ATMPs manufactured and prescribed within the same member state to individual patients.

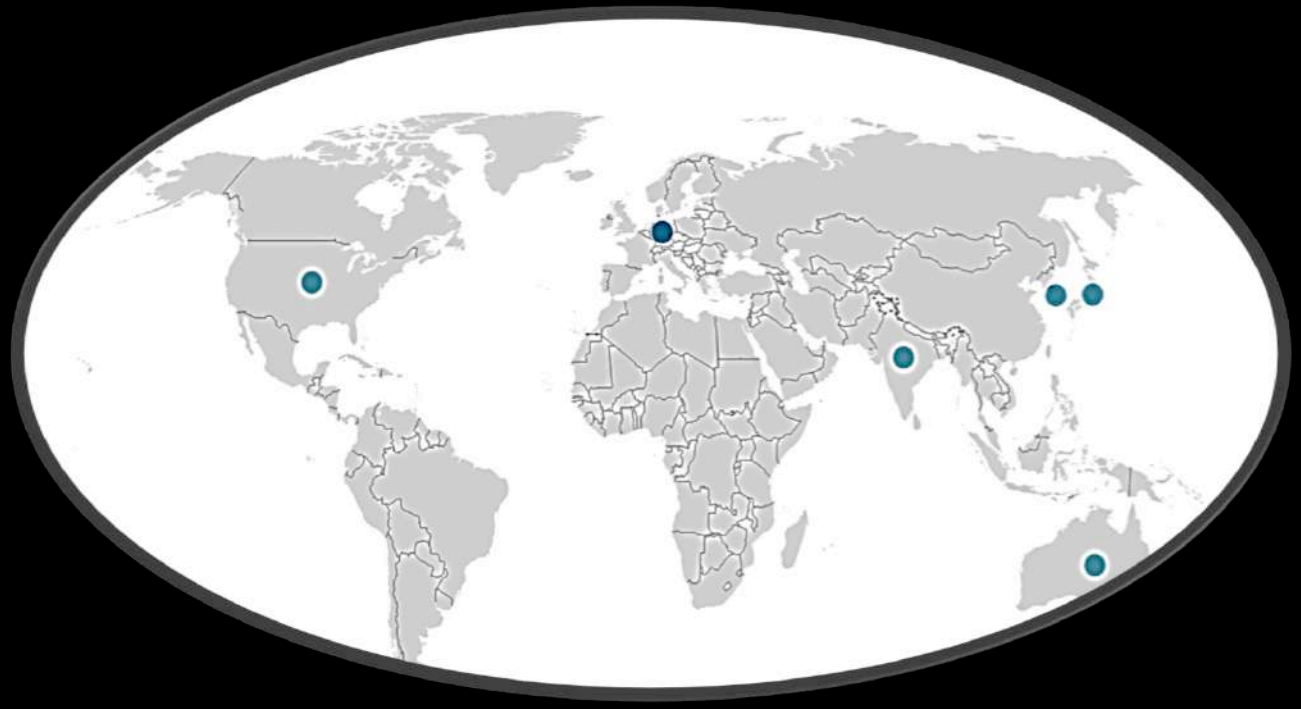
ATMP in Turkey = incorporated under the regulatory and registration of medicinal products for human use. Turkey keeps GMP and national regulation on medical devices updated as per the standards of the EMA and the FDA under Turkey Pharmaceuticals and Medical Devices Agency (TITCK) **Arcidiacono J. International Harmonization for Cell and Gene Therapy Products. Adv Exp Med Biol. 2023;1430:235-240. doi:10.1007/978-3-031-34567-8_14**

Global current availability of HCT/P 351/ ATMPs for orthopedics

- 19 cell therapy approved - 2 for orthopedic use Cartistem/ Republic of Korea for knee OA, Spherox/ EU for cartilage defects
- 22 Tissue engineered products (adult/stem cells) : 5 for orthopedics=
- 4 cartilage defects (JACC Japan, MACI –US, Novocart EU, Ortho_ACI Australia),1 Ossron – Korea, India bone defects

(
[Available Products - Alliance for Regenerative Medicine (alliancerm.org)_]

- Clinical trials going on - 109 for OA, 54 for cartilage defects (clinicaltrials.gov) 162 OA (https://trialsearch.who.int/)



South Korea	Cartistem	Cell therapy
Tuttingen Germany/EU	Spherox	
Japan	JACC	Tissue engineered
USA	MACI	
Tetlow Germany/EU	Novocart	
Australia	Ortho ACI	
India & South Korea	Ossron	

Stem cell-containing available therapeutics

Fresh extracted MSC suspension

- Autologous
- One step procedure (except Peripheral blood)
- Medical devices for closed tissue/ cell manipulation ;single use disposable kits
- Non reimbursed

MSC based products HTC/P ATMP

- Autologous/allogeneic
- One step or two step procedure
- GMP manufactured products/ jurisdiction dependent marketing authorization
- Product delivery
- Non reimbursed (exception ORTO-ACI Australia)

3 P Success triad with IIIrd generation orthobiologics

Patient profile
BMI, age
Comorbidities
Lifestyle
Profession
Leisure
Expectancies
MSJ disease stage
Rehabilitation

Point of Care
Clean room/
operating room
Basic equipment
Ultrasound
Rehabilitation facility

Physician
Education
Training
Frequent update
Analytical thinking
Procedure choice

- Autologous fresh stem cell suspension
- Minimally processed
- Used in same surgical/interventional procedure
- Results dependent on :cell and growth factor quantity& quality, stage of MSK disease, patient willingness to undergo rehabilitation, correct (targeted)delivery and procedure choice.

Acknowledgements

Financed by Ministry of Research, Innovation and Digitization, CNCS/CCCDI - UEFISCDI, project number ERANET-EURONANOMED-3-OASIs, within PNCDI III”.

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NetwOArk The European Network on OsteoArthritis

cost EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Teşekkürler
Thank you