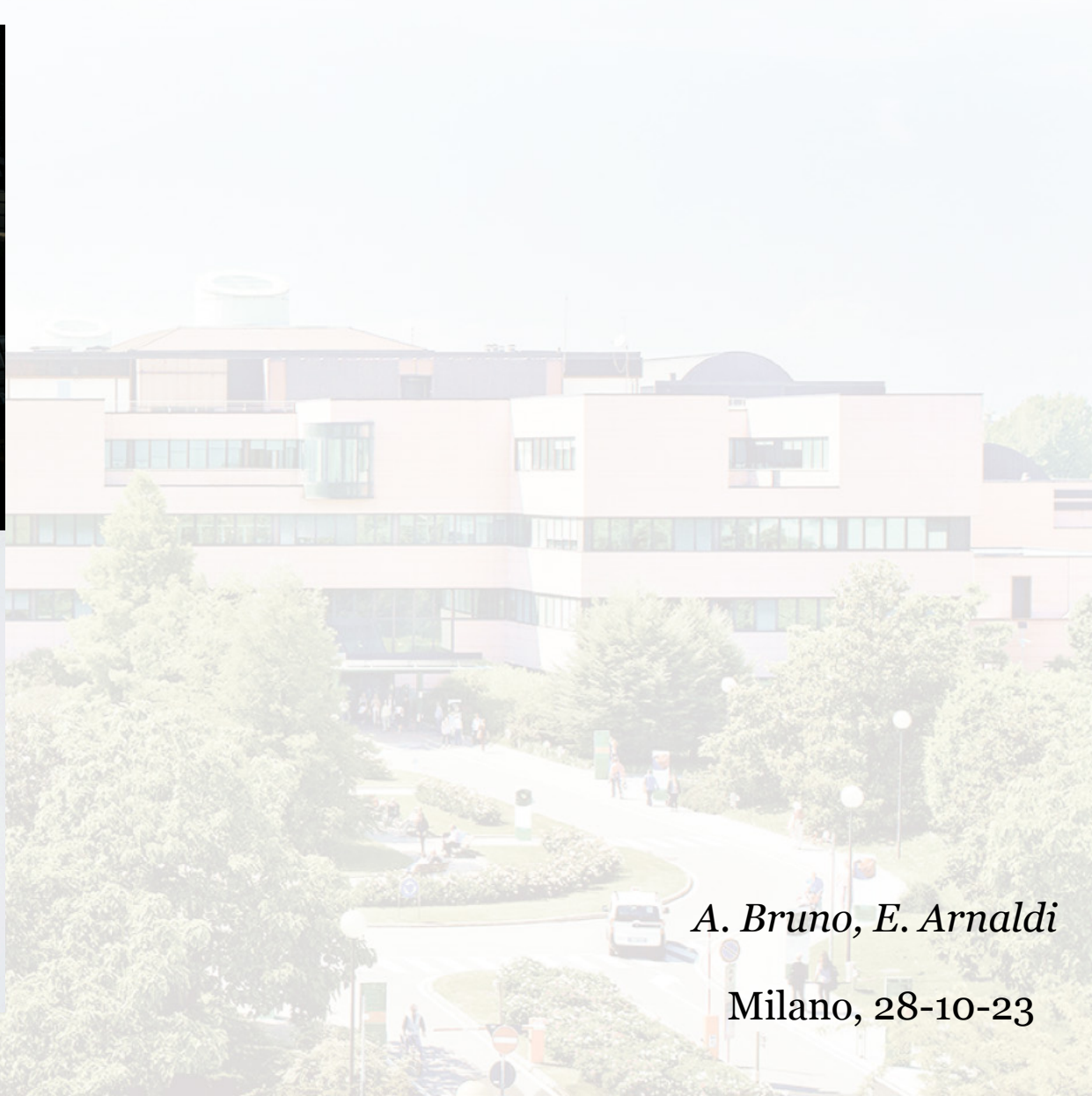
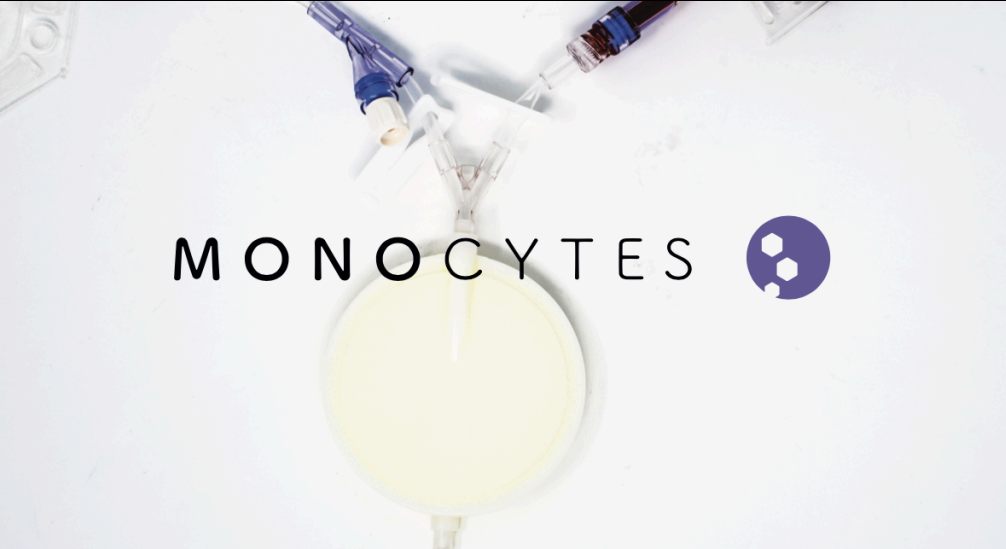


# HUMANITAS

RESEARCH HOSPITAL

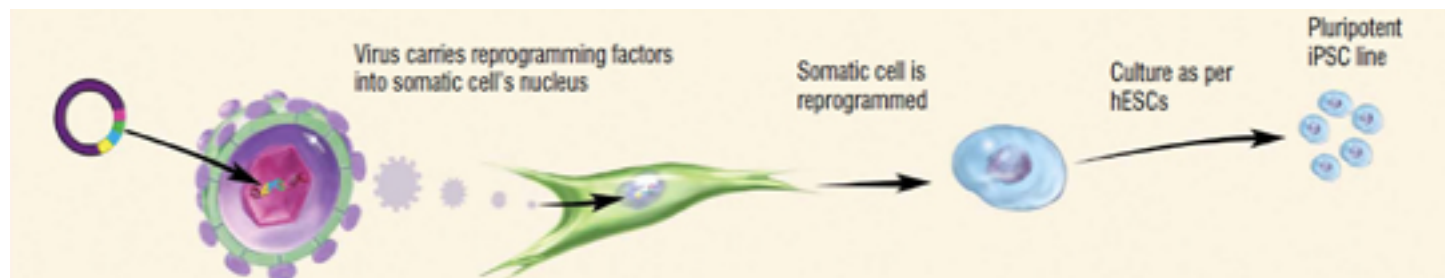


*A. Bruno, E. Arnaldi*

Milano, 28-10-23

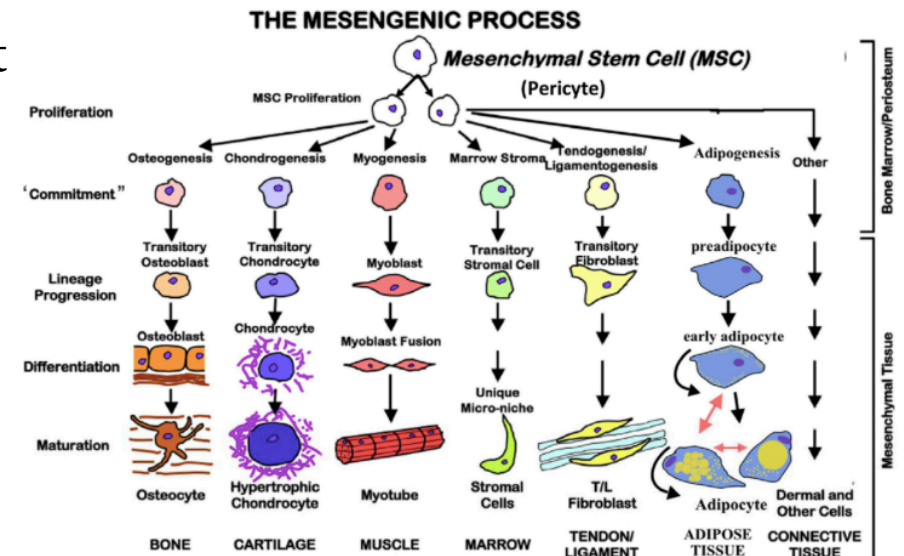
## WHAT DO YOU MEAN BY “STEM CELLS”?

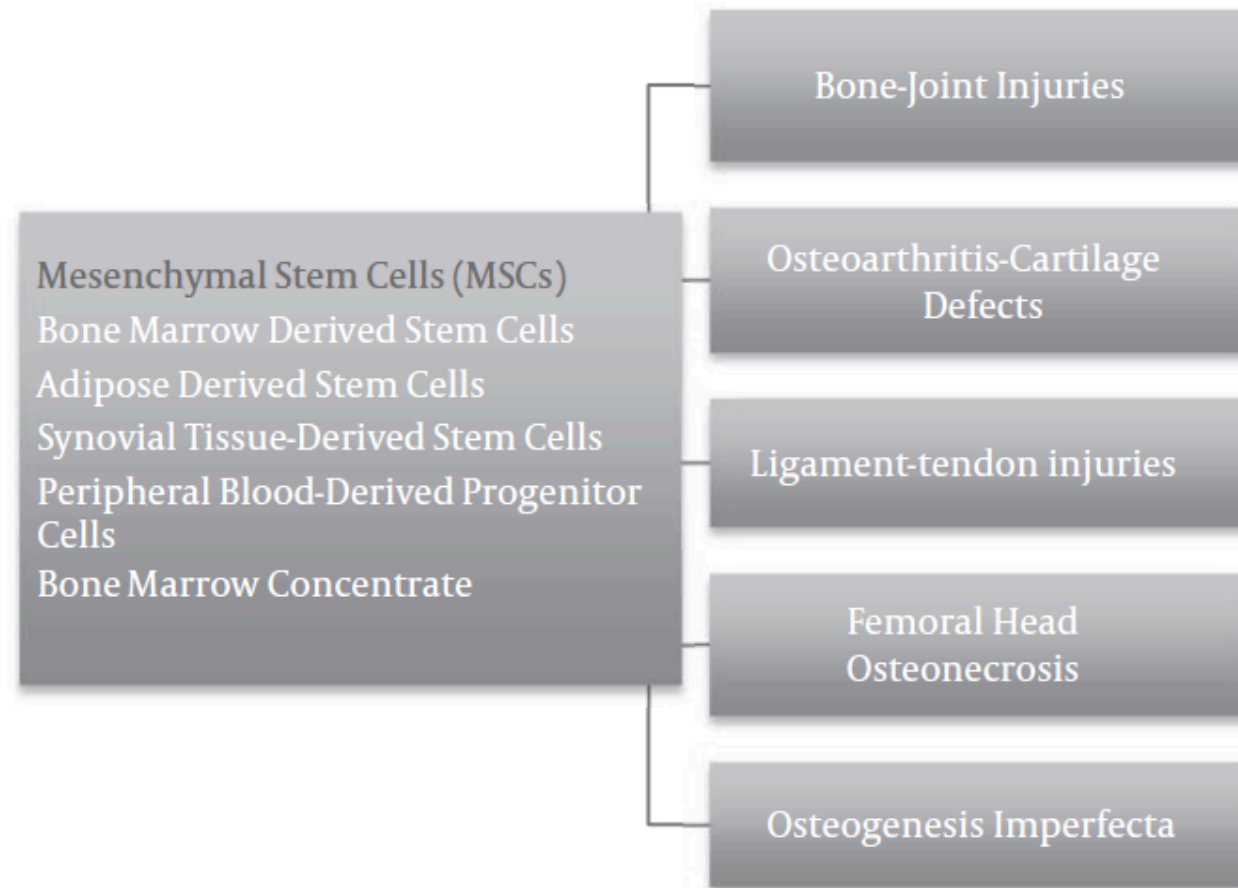
- Embryonic, fetal cartilage
- Cord blood derived
- Amniotic fluid derived
- Adult allogenic : cultures mesenchymal
- Adult autologous: adipose, BMAC, Peripheral blood, Synovial (SSCs), Fat - direct /cultured
- Adult autologous: induced pluripotent stem cells (iPSCs)



## WHY MSCs DERIVED FROM ADULT SOMATIC TISSUES?

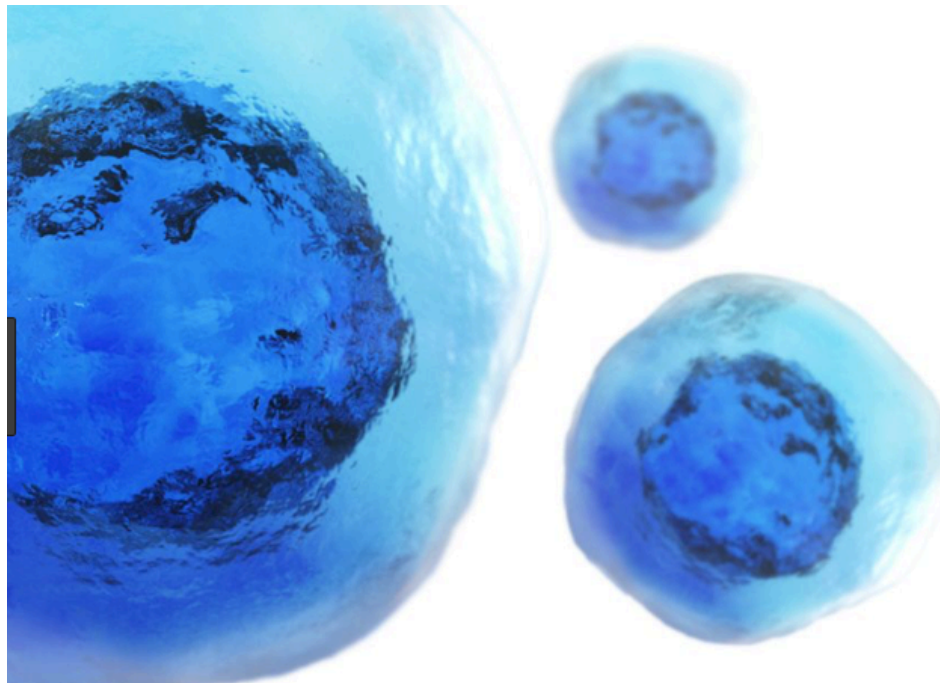
- Easily available
- Less ethical and safety concerns
- Proliferation capacity and differentiation potential (age-dependent)
- Can differentiate to chondrocytes
- Autologous minimise the immune response
- Moldable and adherent to fill cartilage defect
- One step surgery
- Reduced surgical time





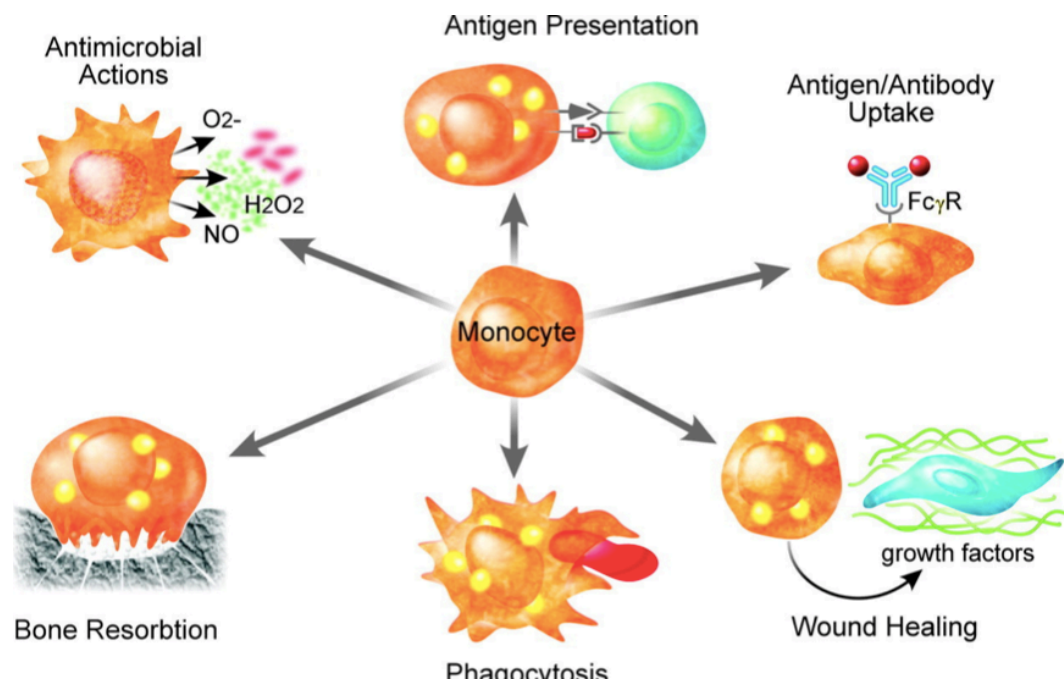
## WHERE ARE THEY USED?

- Office injection
- Surgery center
- Injection, arthroscopic application, arthrotomy applications
- Scaffolds



## Innate Immunity and Regeneration

- The immune system plays a critical role in tissue development, homeostasis and repair
- Monocytes and macrophages are the first to respond to a tissue damage
- Required to start regeneration process



## PBMNC (Peripheral Blood Mononuclear Cells)

- Angiogenesis (VEGF)
- Resident MSC activation (exosomes)
- Anti-inflammatory - immunomodulation (NFKB inhibition / macrophages M2 activation and shut down M1)

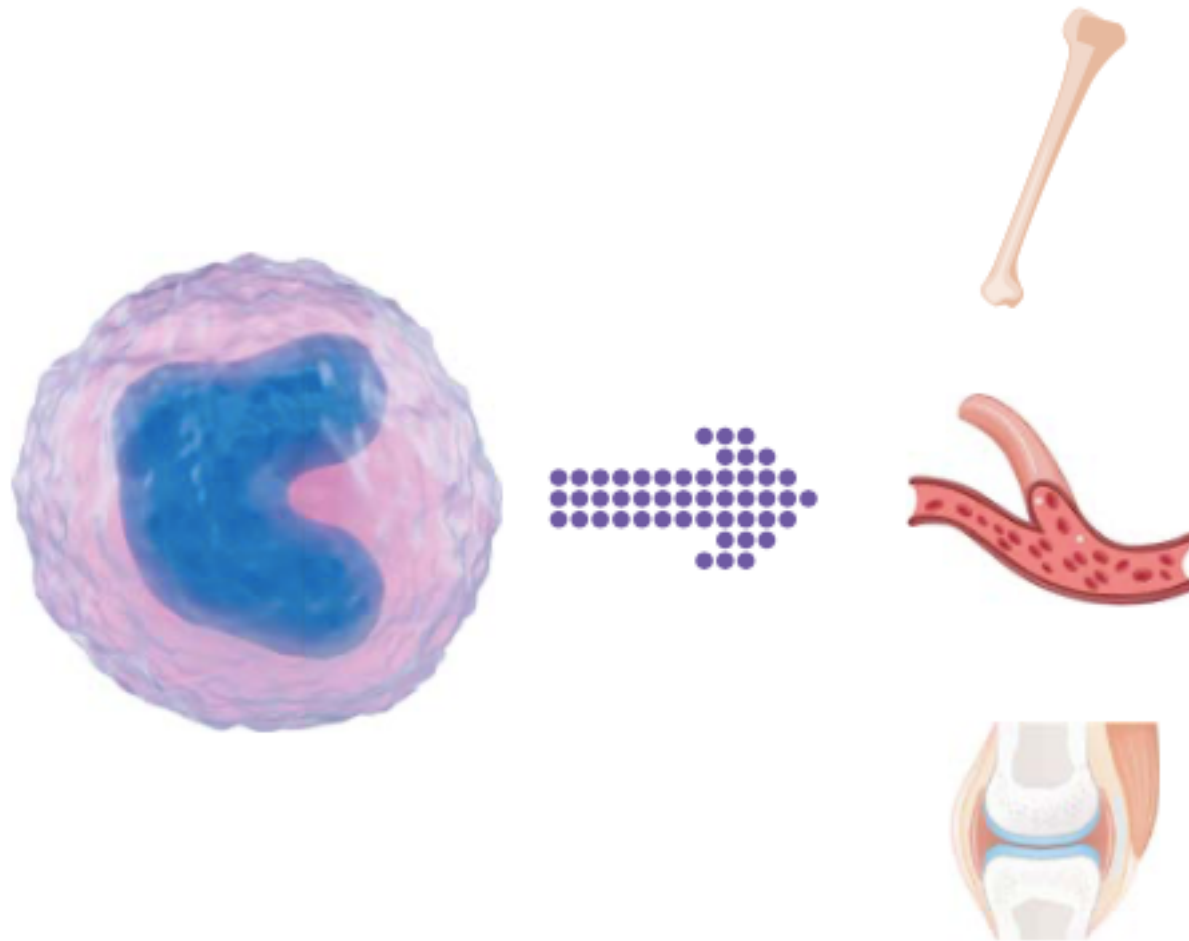


Hopper N, et al. Peripheral Blood Derived Mononuclear Cells Enhance osteoarthritic human chondrocyte migration. Arthritis research. 2015; 17:199

Hopper N, et al. Peripheral blood mononuclear cells enhance cartilage repair in in vivo osteochondral defect model. PLoS ONE 10, e0133937 (2015)

Hopper N, et al. Peripheral Blood Derived Mononuclear Cells Enhance the Migration and Chondrogenic Differentiation of Multipotent Mesenchymal Stromal Cells. Stem Cell international 2015 vol. 2015, Article ID 323454, 9 pages, 2015

N. Fahy et al Human osteoarthritic synovium impacts chondrogenic differentiation of mesenchymal stem cells via macrophage polarisation state Osteoarthritis and Cartilage 2014 22:8

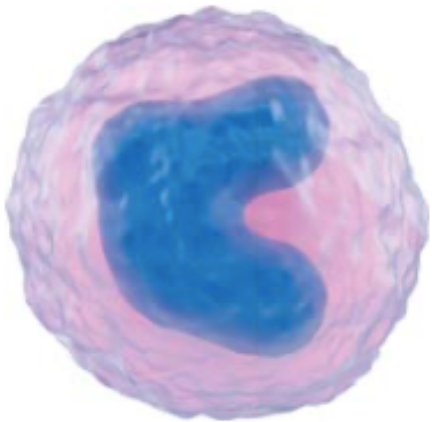


- Bone tissue: BMP2

- Angiogenesis (VEGF)

- Anti-inflammatory
- Immunomodulation
- NFKB inhibition /
- Macrophages M2 activation
- Shout down M1



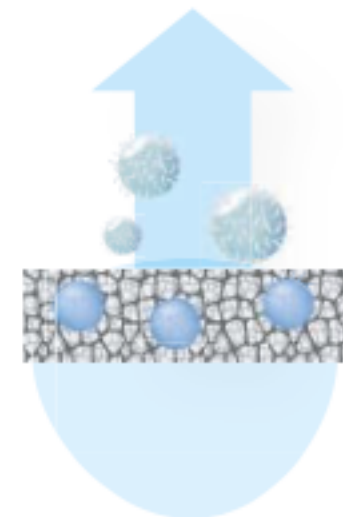
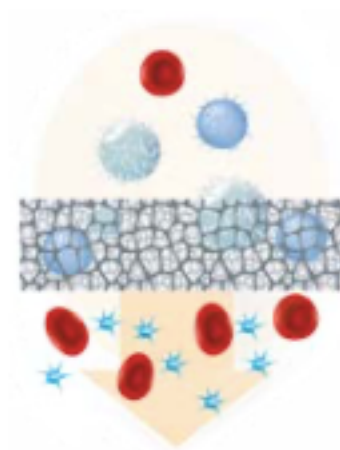
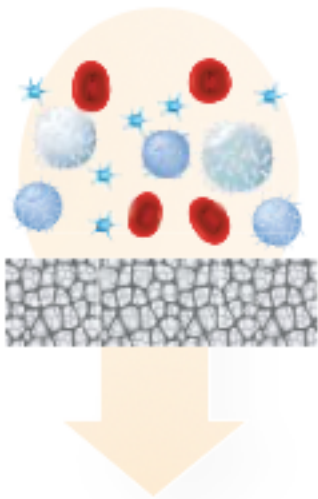


## Indication

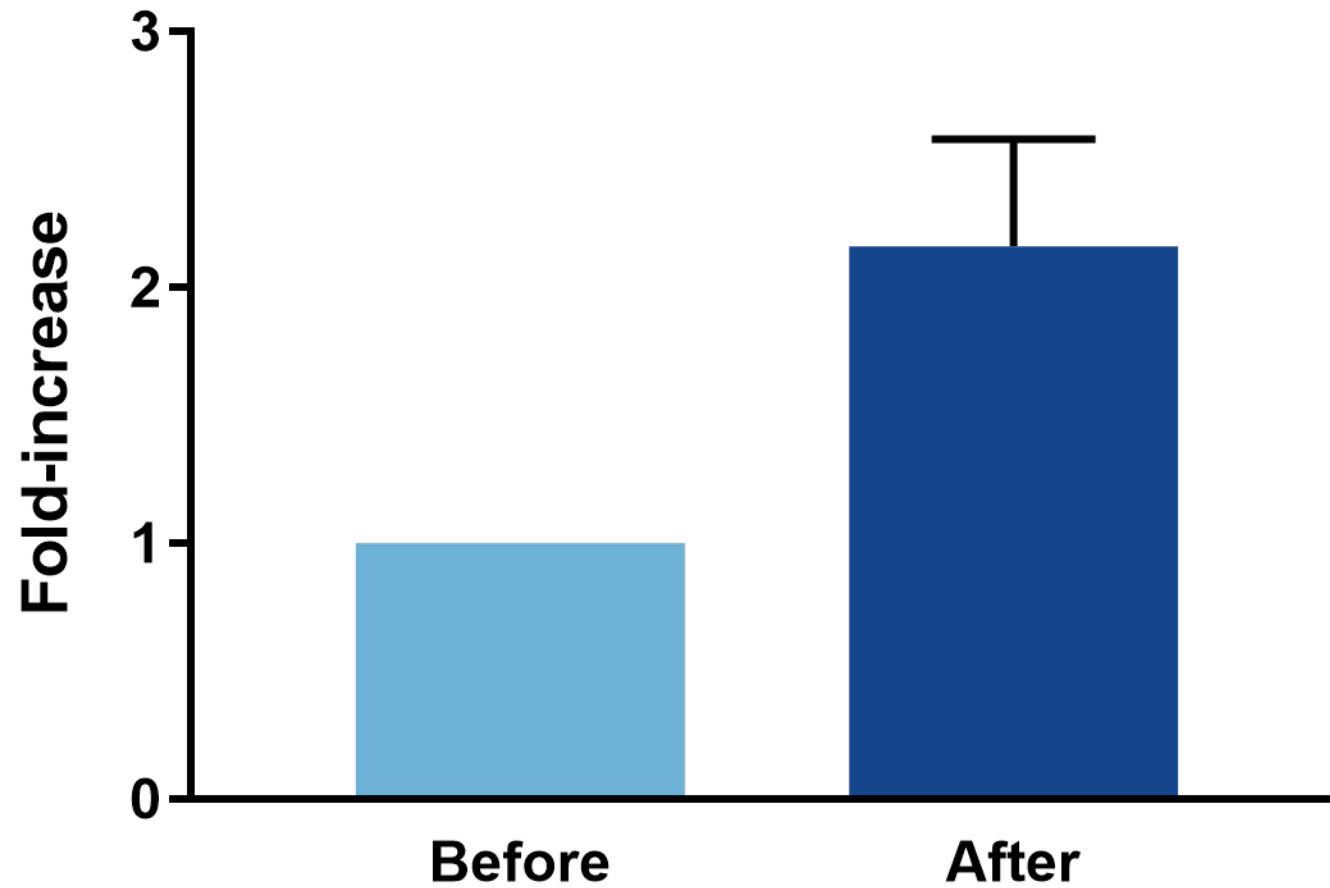
- **Orthopedics:** infiltration / scaffold enrichment
- **Vascular surgery:** perilesional /intramuscular infiltration
- **Wound Healing:** infiltration /advanced dressing

## Monocytes

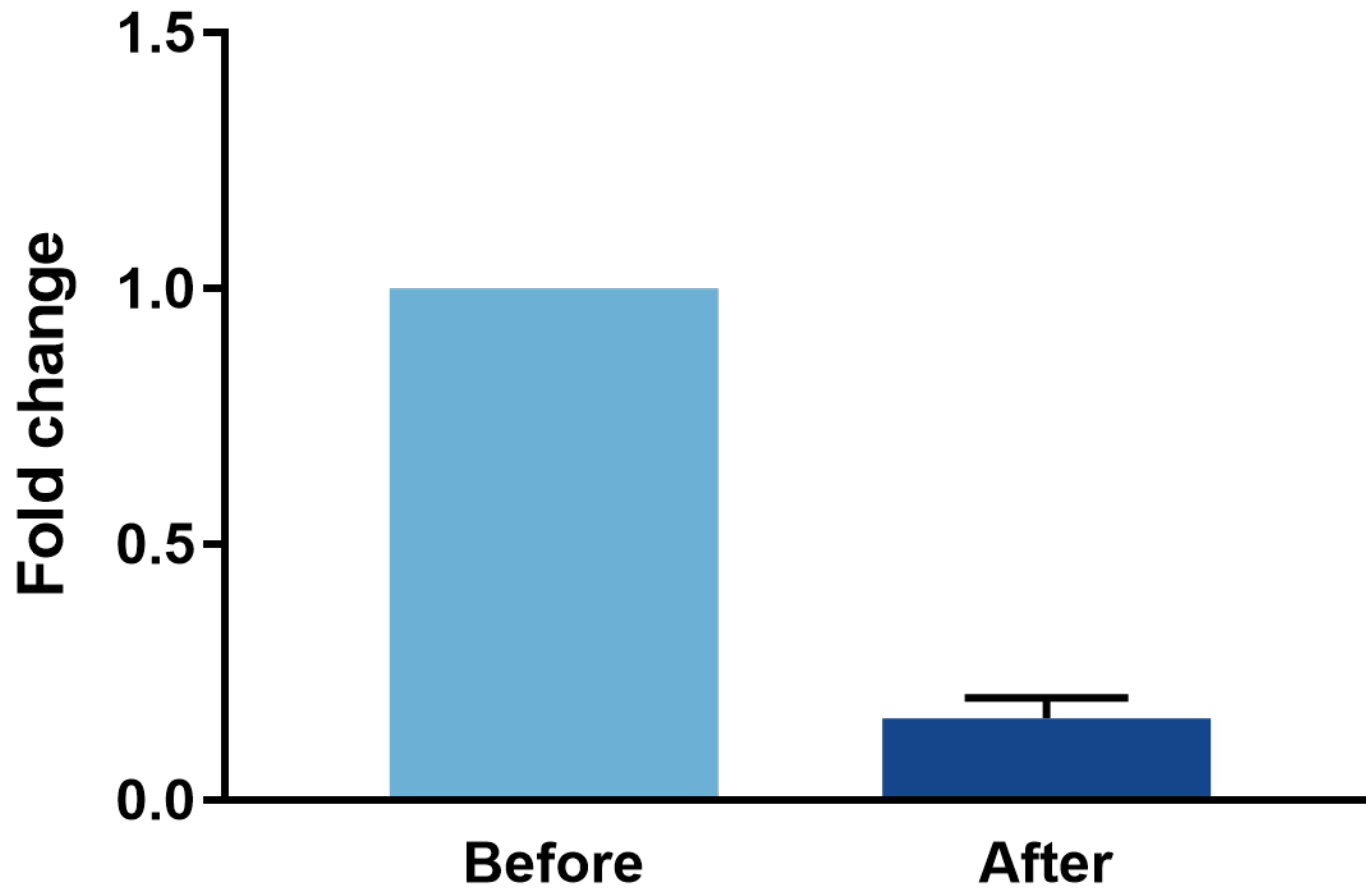
- **Selective filtration** of peripheral blood to obtain mononuclear cells
- **Filters blood through gravity** (avoiding centrifugation stress)
- Membrane selects cells by **dimensional filtration**
- Eliminating inflammatory cells es granulocytes



## Mononuclear cells enrichment



## Granulocytes depletion



## Monocytes

- Closed-loop system
- Centrifuge-independent procedure
- Single-shoot treatment
- Fast and easy



### 1 - Withdrawal

Open the kit on the sterile field and assemble it as illustrated

Load the withdrawal syringes with 10% of anticoagulant (ACD-A); e.g. 6 ml for 60 ml of blood.

Recommended withdrawal volume is 60-120 ml.

Cleanse the puncture area and aspirate the needed peripheral blood volume with the provided needle.

### 2 – Processing

Hang the kit on a stand, holding the upper bag **(A)** up.

Insert the withdrawn blood through the upper connector **(a)** to fill the upper bag **(A)** and open the clamps **(1 and 2)** to activate the circuit.

Wait until all the blood has passed through the filter.

Put 10 ml of saline solution from the same blood inlet **(a)** to wash the filter.

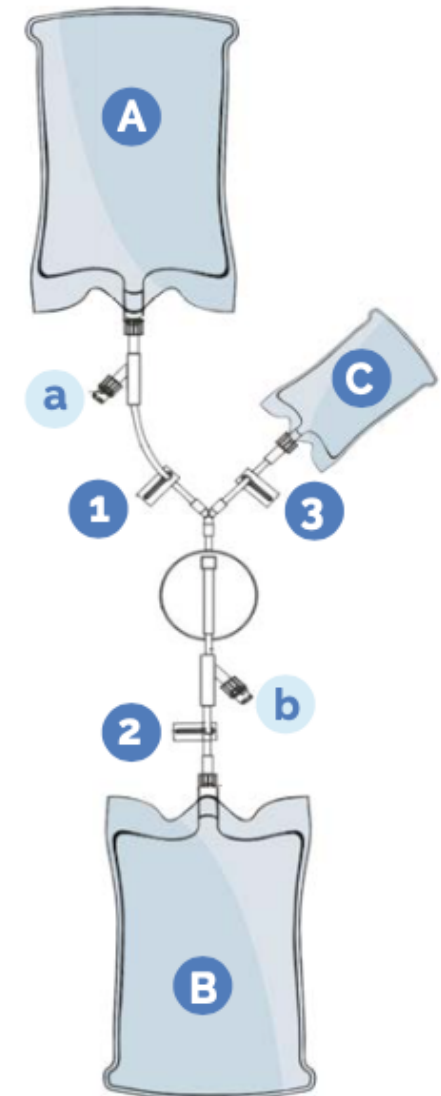
Now, waste is deposited in the lower bag **(B)** and cells are trapped inside the filter.

### 3 - Recovery

Close the clamps **(1 and 2)** to exclude the filter from the circuit.

Open the clamp on the side **(3)**. Aspirate 10 ml of saline solution with a new sterile syringe and connect it to the lower connector **(b)**.

Push the saline gently. Once the backwashing is done, recover the content of the collection bag **(C)** with a new syringe.



## CONCLUSIONS

- Regenerative potential
- Quick and safe procedure
- Reproducible
- Versatile

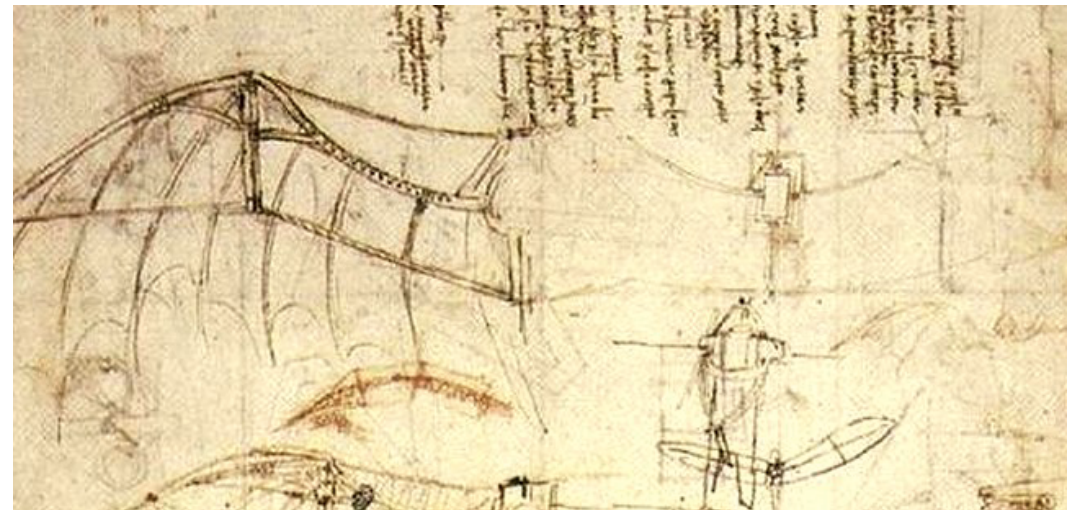


## CONCLUSIONS

- Few clinical studies with preliminary results
- Must compare apples to apples
- Critical science is needed to cut through hype
- Public is rapidly accepting and even demanding these treatments
- Empiric data and profit centers do not enhance science
- Prospective randomized studies at medium and long term

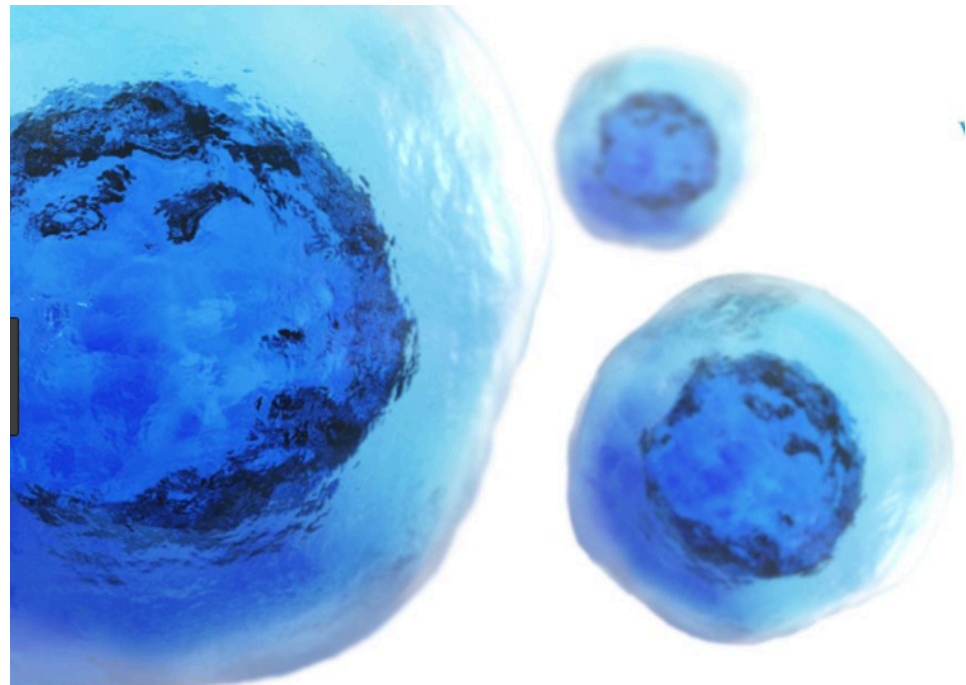


## DREAM BIG AND DEAR TO FAIL



**HUMANITAS**  
RESEARCH HOSPITAL

*DREAM, DARE AND DO*



*A. Bruno, E. Arnaldi*

**HUMANITAS**  
FONDAZIONE PER LA RICERCA

**HUMANITAS**  
FONDAZIONE PER LA RICERCA