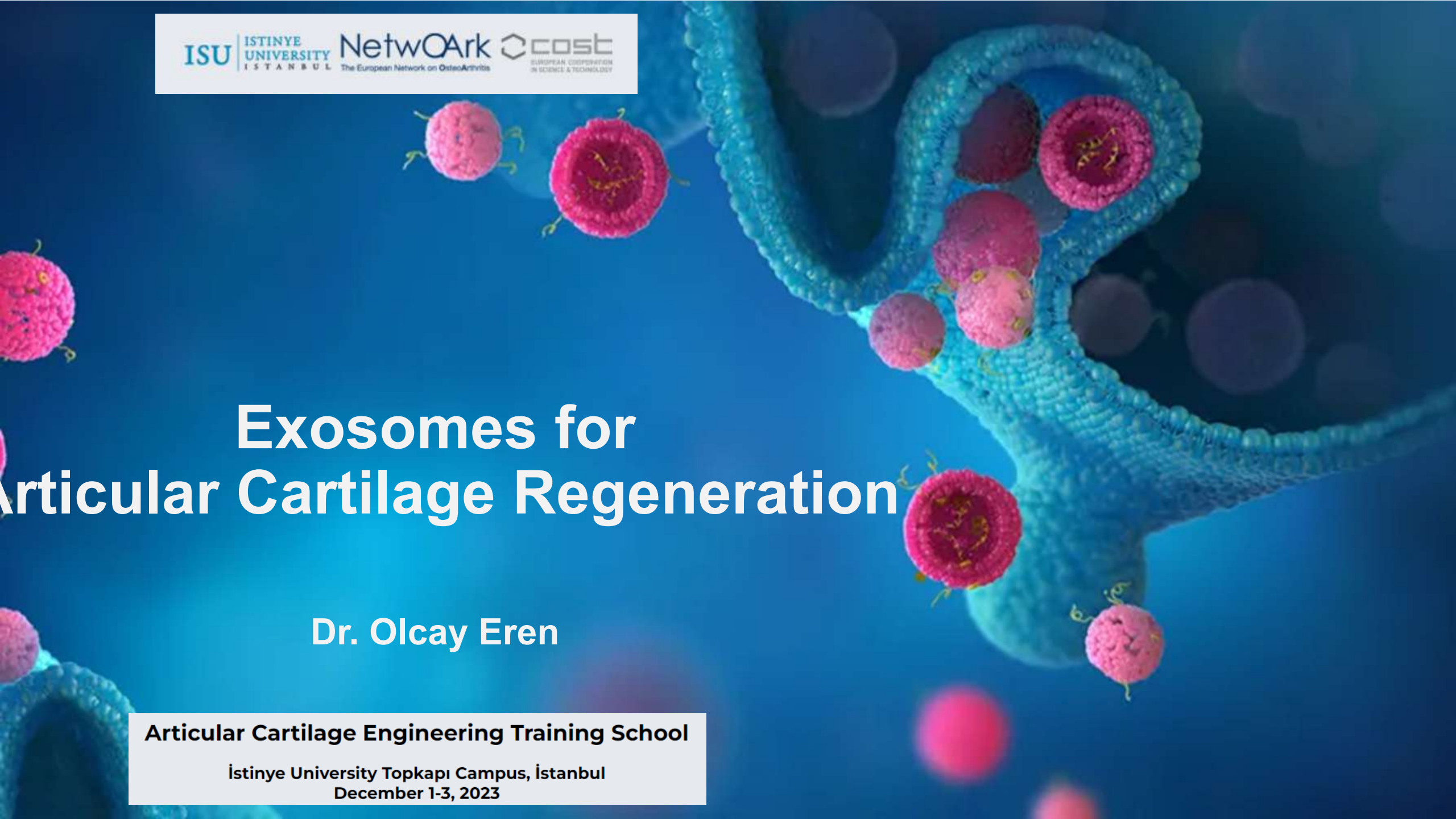


# Exosomes for Articular Cartilage Regeneration

Dr. Olcay Eren

**Articular Cartilage Engineering Training School**

İstinye University Topkapı Campus, İstanbul
December 1-3, 2023

**DISCLOSURE STATEMENT**

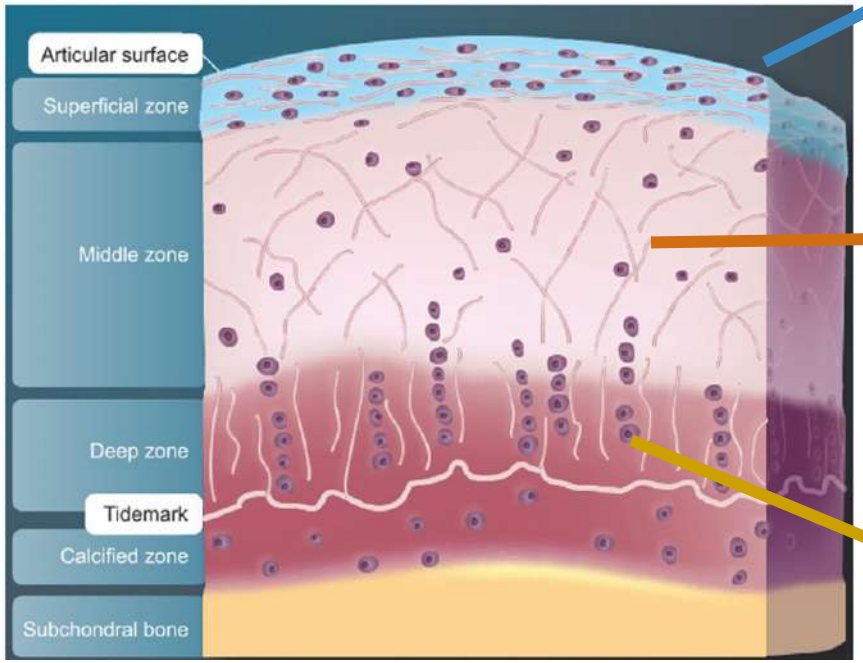
Dr. Olcay EREN

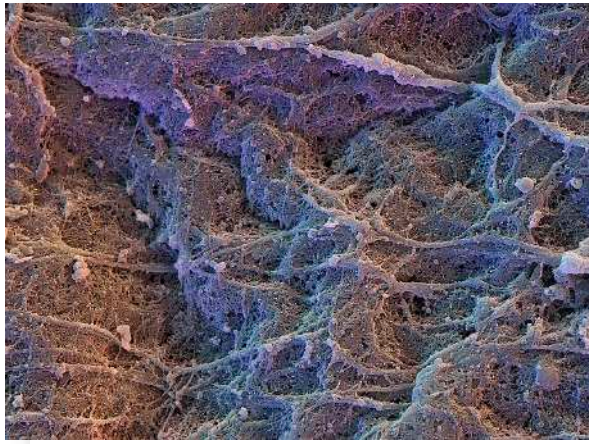
I have the following potential conflicts of interest to report:

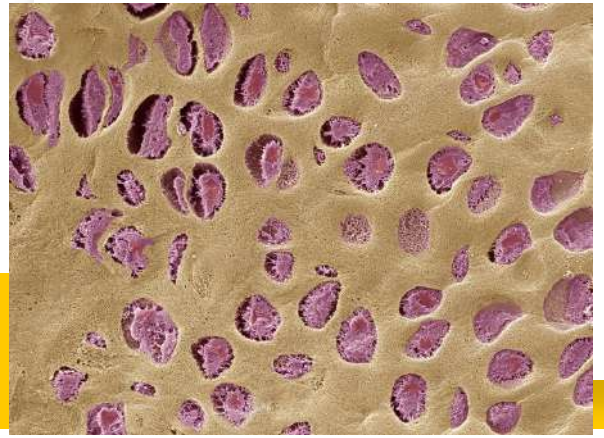
* Research Contracts
* Consulting
* Employment in the Industry
* Stockholder of a healthcare company
* Owner of a healthcare company

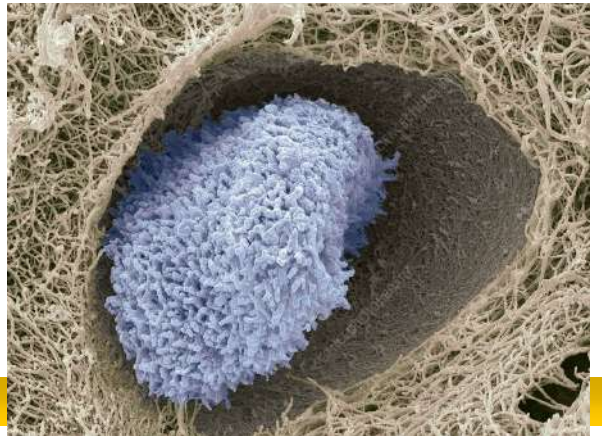
I declare that I have no potential conflict of interest.

# Cartilage



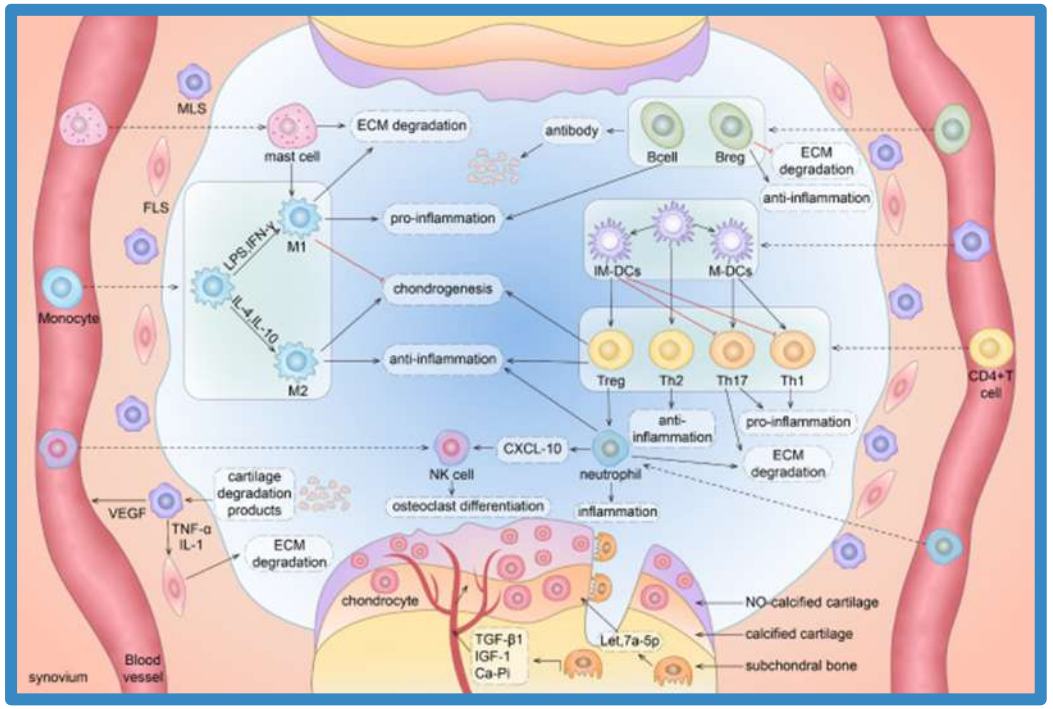




SEM images of hyaline cartilage

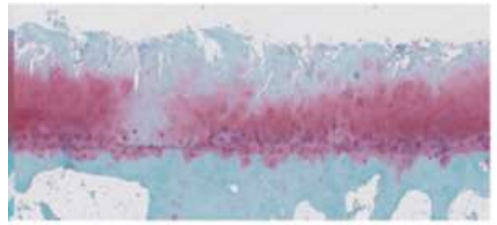
https://www.sciencephoto.com/media/303330/view

# Cartilage Homeostasis




Young normal

Old normal

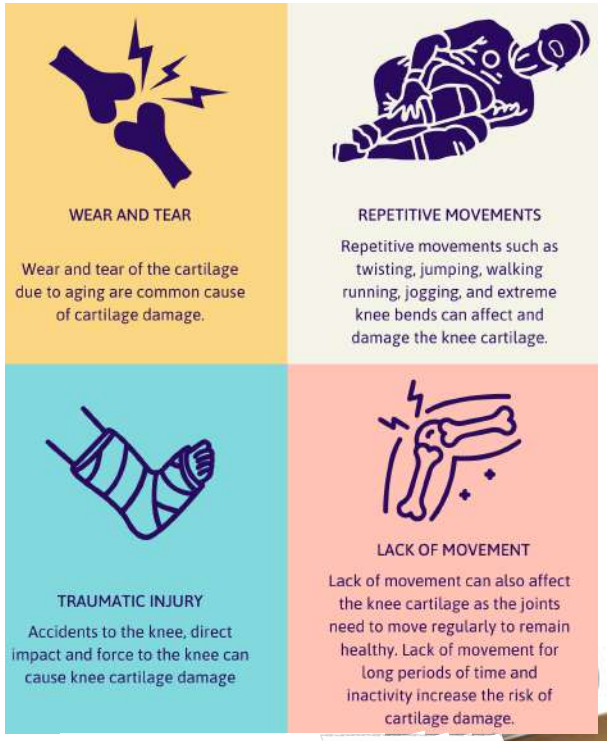


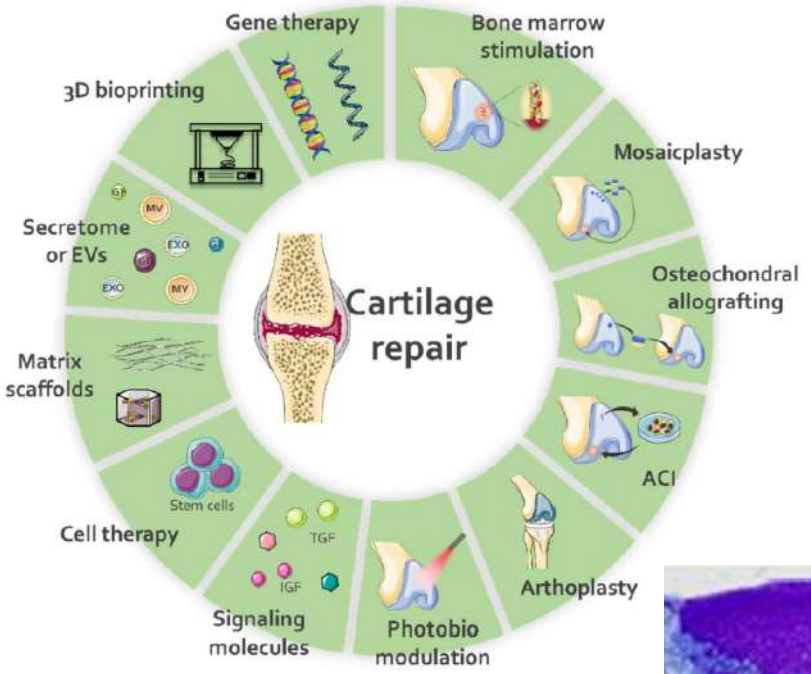
OA

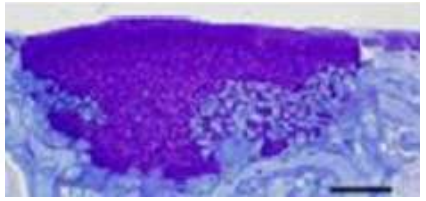
- **Cartilage homeostasis** can functionally be defined as the condition where a normal cartilage ECM composition deals *with mechanical stress without structural or cellular damage*

Lotz M, Loeser RF. Effects of aging on articular cartilage homeostasis. Bone. 2012 Aug;51(2):241-8. doi: 10.1016

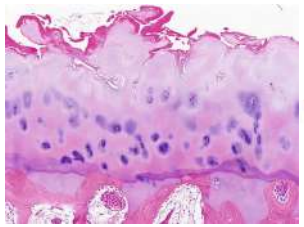
# Homeostatic Imbalance



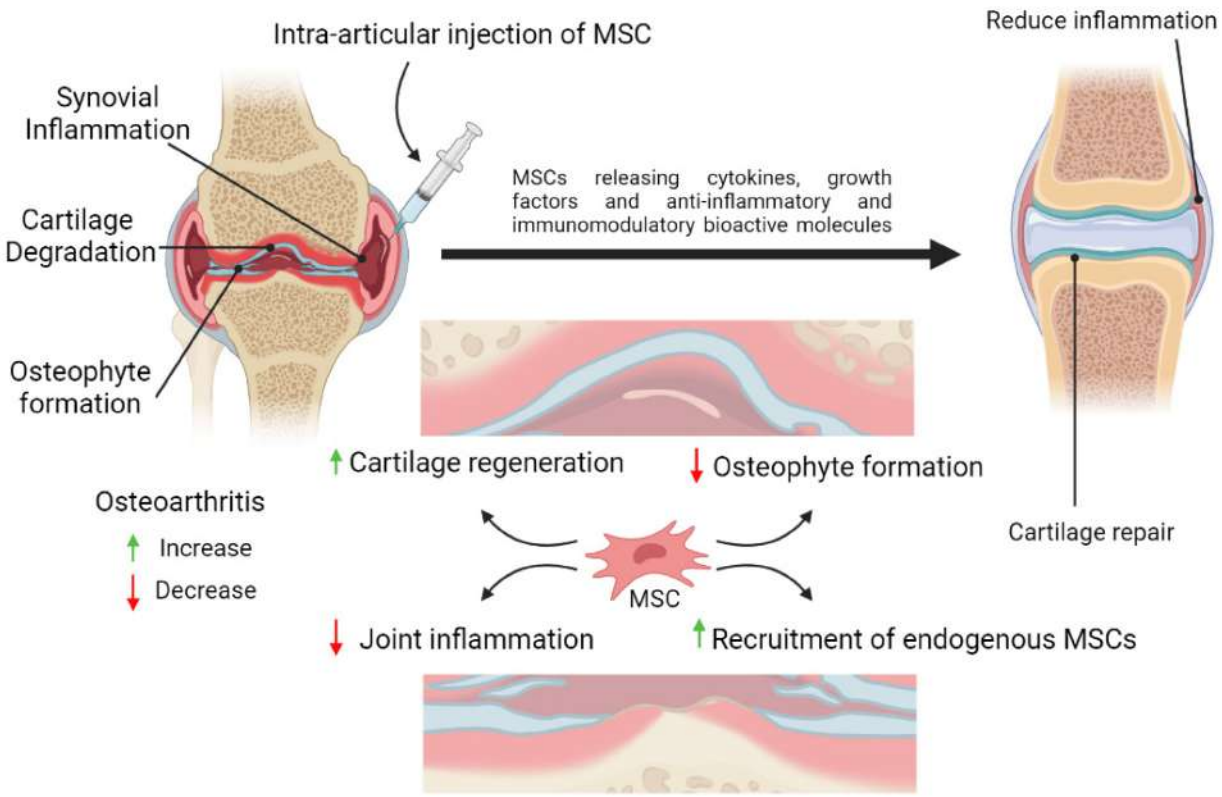
If this balance disrupted….

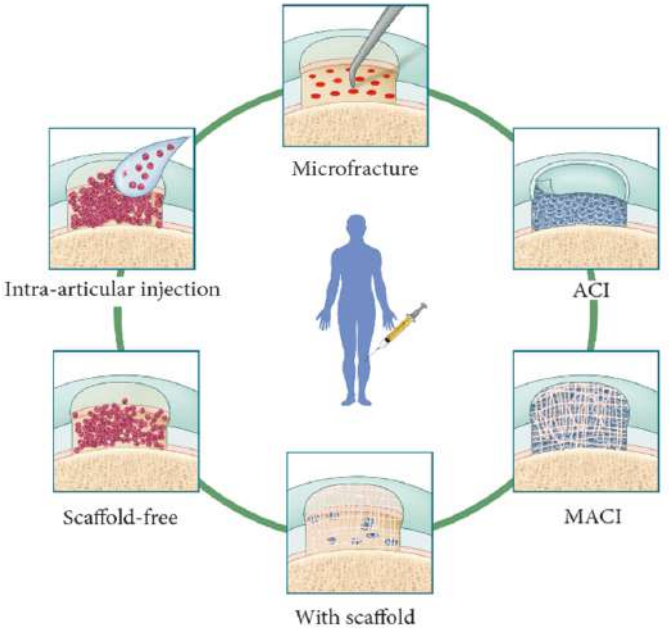


Cartilage, alone cannot provide adequate response

OrthoBiologics

# Treatment option(s)

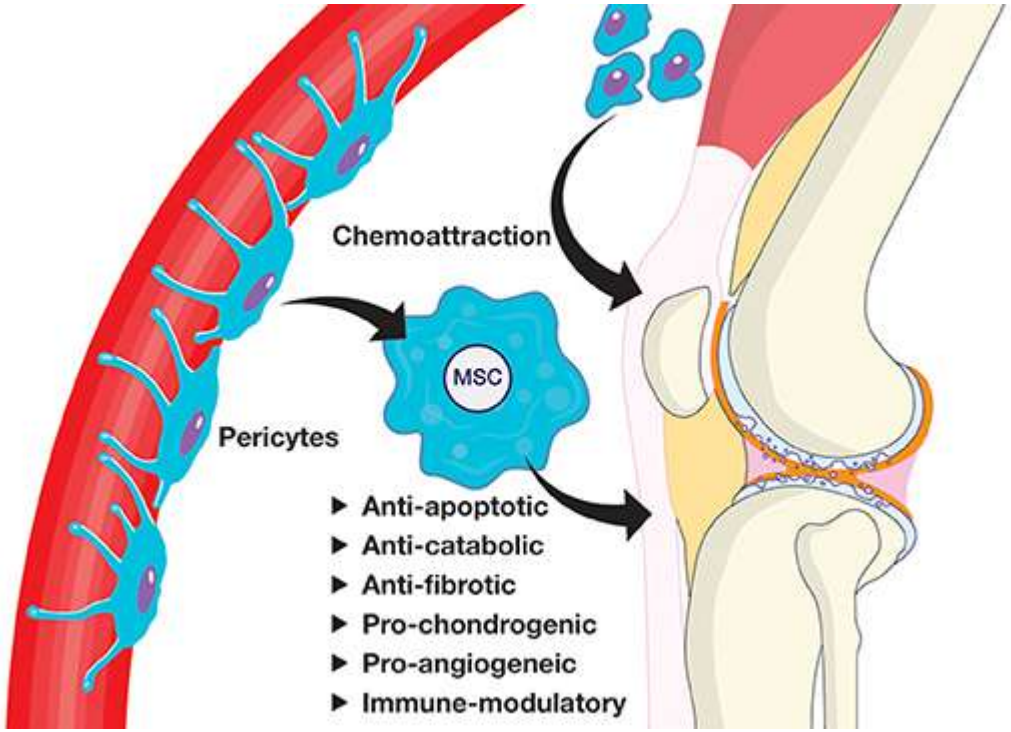



- There is no proven treatment method that can reverse the changes in oa or modify the proggresive feature of the disease
- Cartilage has no blood supply, avascular
- Orthobiological treatment methods to prevent cartilage damage have become more popular recently and studies on this subject continue at full speed……

Nurul, A.A.; Azlan, M.; Ahmad Mohd Zain, M.R.; Sebastian, A.A.; Fan, Y.Z.; Fauzi, M.B. Mesenchymal Stem Cells: Current Concepts in the Management of Inflammation in Osteoarthritis. Biomedicines **2021**, 9, 785

# Is stem cell therapy for arthritis safe and effective?




Transplantation of mesenchymal stem cells (MSCs), have been gradually applied for the prevention and treatment of OA

However, almost all MSCs have their own limitations, such as the loss of their availability due to the **increasing donor age**, **limited proliferation capacity** during in vitro expansion *(Siddappa et al., 2007)*

and strict regulatory requirements throughout the **isolation, collection, storage, and transportation process** *(Zhang et al., 2016)*

In addition, the use of live cells results in unavoidable safety issues such **as immune rejection** *(Zhang et al., 2013)*

**tumorigenesis** due to uncontrolled cell differentiation *(Nori et al., 2015)*

and the **inability to remove** the transplanted cells in case of adverse reactions *(Toh et al., 2017)*

Mancuso P, Raman S, Glynn A, Barry F and Murphy JM (2019) Mesenchymal Stem Cell Therapy for Osteoarthritis: The Critical Role of the Cell Secretome. *Front. Bioeng. Biotechnol*. 7:9

# Cell Free Therapy


Since the first therapy using stem cells has been developed in **1957**, only few stem cell-based therapies have entered the clinic

At least **200 clinical trials** have been registered in *ClinicalTrials.gov* investigating exosome-based therapies for a variety of diseases, including respiratory diseases, infectious diseases, and cancer

Among those trials, 31 apply exosomes derived from stem cells, primarily mesenchymal stem cells from different tissues, which are tested as an **alternative to mesenchymal stem cell therapy**

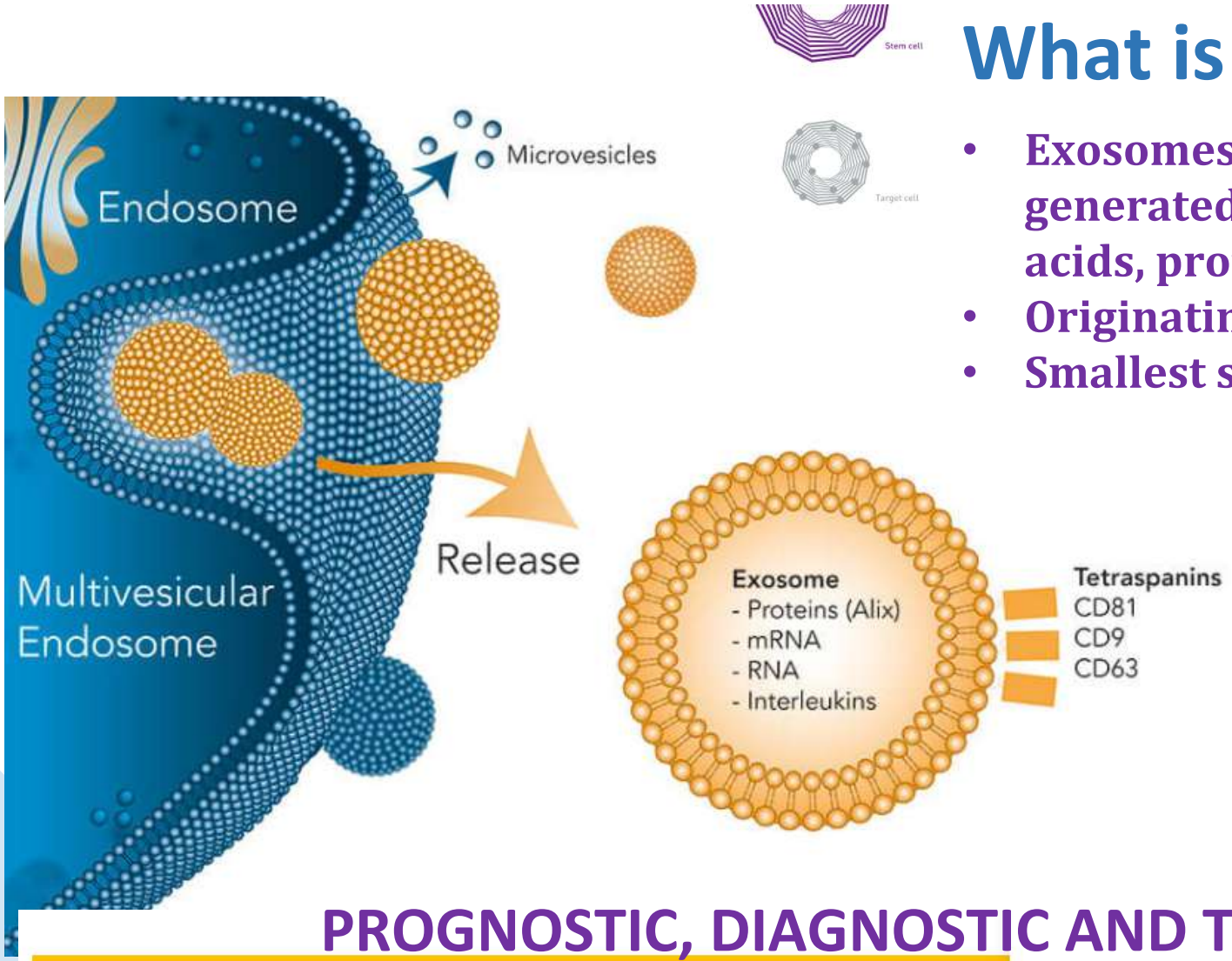
These pre-clinical and clinical investigations suggest that exosomes released by stem cells have a therapeutic effects of their donor cells without the drawbacks inherent to stem cell therapy

| Exosome therapy | Stem cell therapy |
|---|---|
| • Small size<br>• Minimal risk of immune response and tumour formation<br>• Stable for long-term storage and transportation<br>• No ethical issues<br>• Multiple delivery routes<br>• Can be engineered to deliver drug cargos | • Easy to isolate and expand at a large scale<br>• Multilineage differentiation potential<br>• Long-term engraftment<br>• Extensive pre-clinical and clinical study results<br>• Well established FDA guidelines |
| • Difficult to upscale manufacturing and purification<br>• Batch-to-batch variation<br>• No compatible GMP facility<br>• No established regulations and standards | • **Oncological complications**<br>• Fusion toxicity<br>• Immunogenicity<br>• Harsh storage and transportation conditions<br>• Ethical issues |

Zhang, K., Cheng, K. Stem cell-derived exosome versus stem cell therapy. *Nat Rev Bioeng* (2023). https://doi.org/10.1038/s44222-023-00064-2

Anisimov, S. V., Morizane, A. & Correia, A. S. Risks and mechanisms of oncological disease following stem cell transplantation. Stem Cell Rev. Rep. 6, 411–424 (2010)

# What is EXOSOME?




- **Exosomes are extracellular vesicles generated by all cells and they carry nucleic acids, proteins, lipids, and metabolites**
- **Originating from the cell membrane**
- **Smallest subgroup of extracellular vesicles**

**BIOACTIVE ENVELOPE**

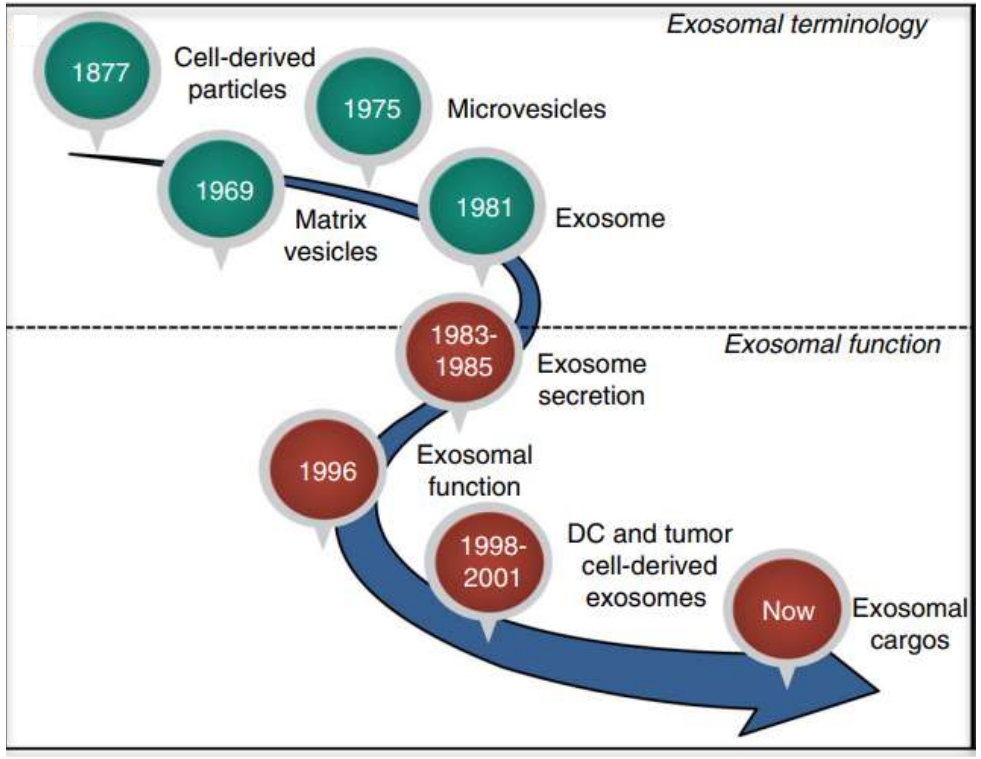
**It has many important roles, such as**

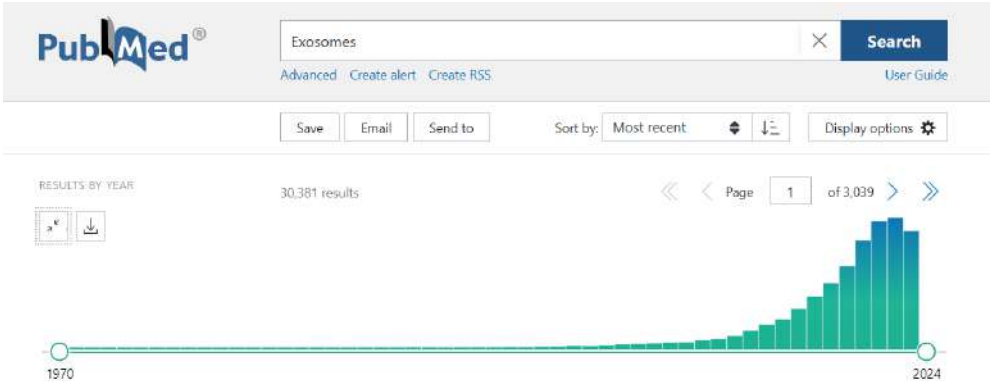
- **REGULATING the IMMUNOLOGICAL RESPONSE**
- **INTERCELL COMMUNICATION**
- **SIGNAL TRANSMISSION**
- **GENETIC MATERIAL TRANSFER**

## PROGNOSTIC, DIAGNOSTIC AND THERAPEUTIC VALUES

Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478):eaau6977.

# History of exosomes




In 1987, ***Johnstone et al.*** observed that exosome release during reticulocyte maturation was associated with **plasma membrane activities**

***Raposo et al***. later found that exosomes played an important role in **antigen presentation and T cell activation**

In 2007, ***Valadi et al***. found that **mRNA and microRNA** can be sent to other cells by exosomes, indicating that exosomes may mediate intercellular communication by delivering nucleic acids

Thereafter, an increasing number of studies have shown that exosomes play important physiological and pathological roles by mediating **cell–cell communication**

Gao, M., Gao, W., Papadimitriou, J.M. *et al.* Exosomes—the enigmatic regulators of bone homeostasis. *Bone Res* **6**, 36 (2018)

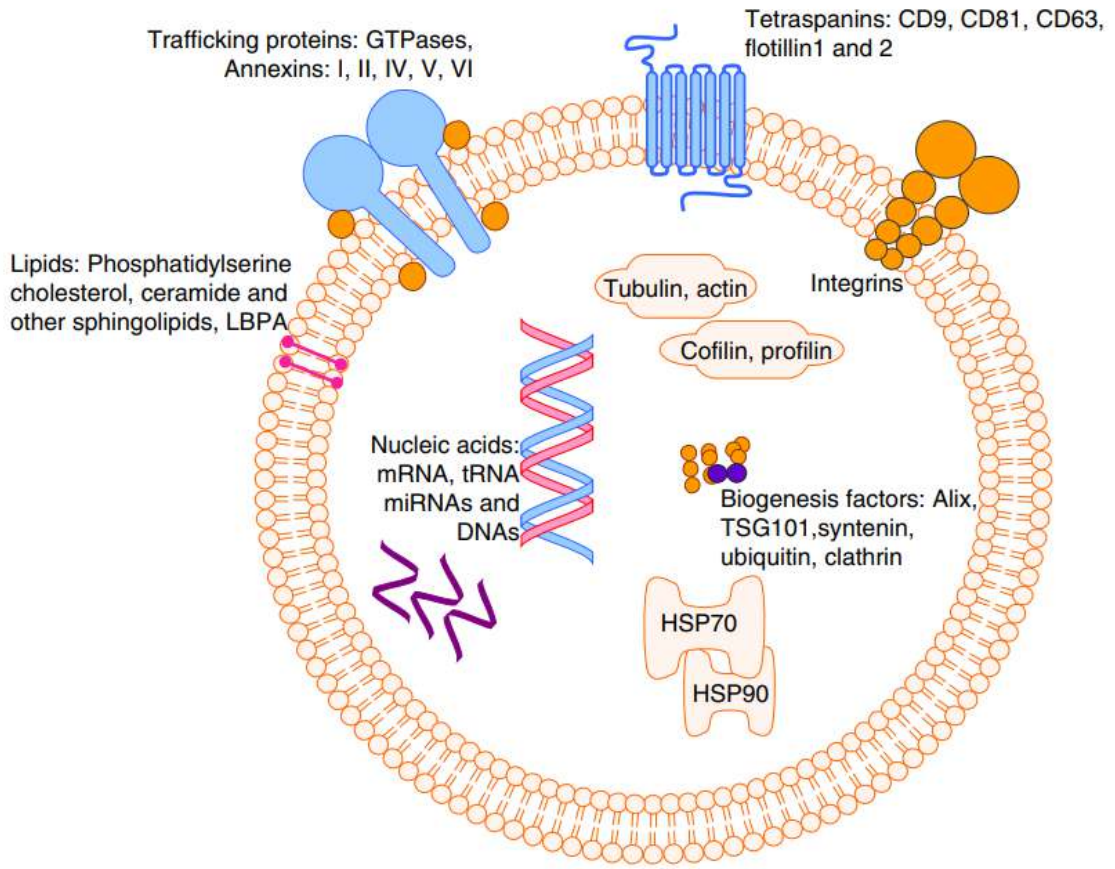
# Composition of exosomes

Exosomes have defined as **40-100** nm diameter extracellular vesicles

Exos  contain biosynthetic antibodies (**Alix and TSG101**) involved in MVBs, **cholesterol, ceramide, phosphoglyceride** that provides structural stability,

Exos also carry functional **mRNAs and miRNAs** that can be transferred between cells

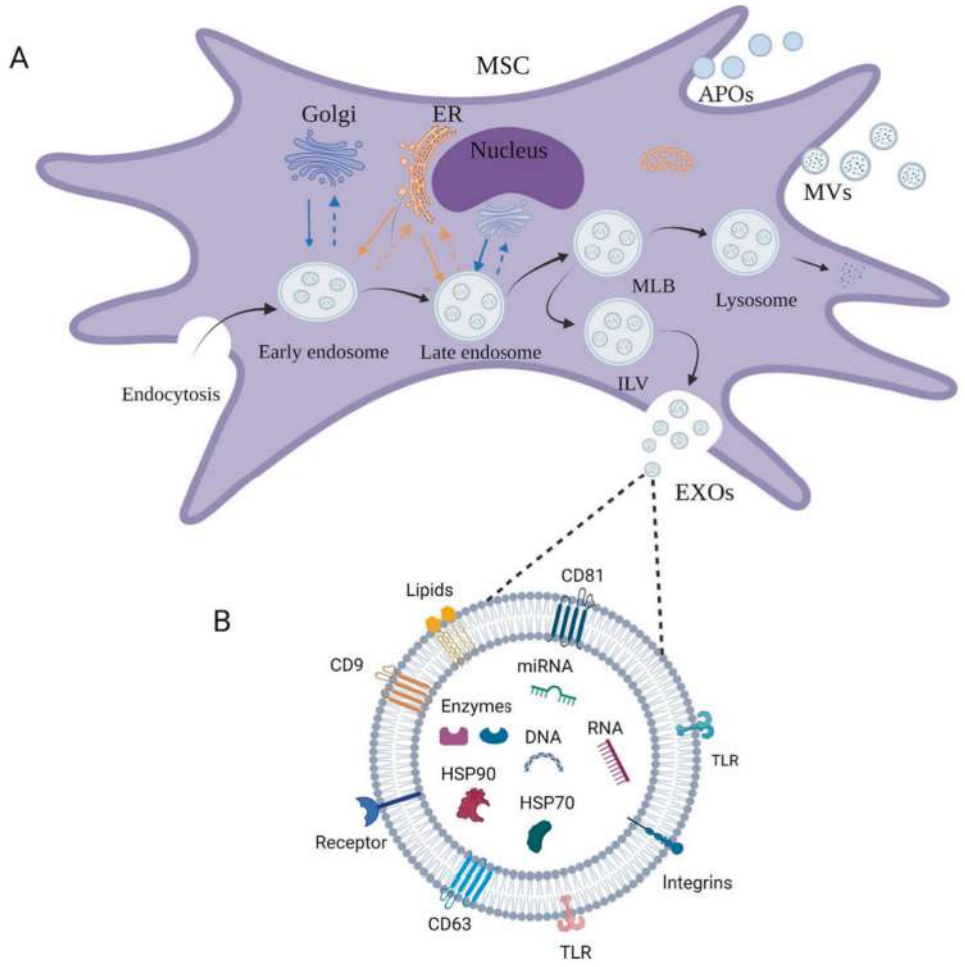
Typically, exosomes contain a group of common membrane and cytoplasmic proteins, including membrane transport and fusion proteins (**Rab GTPases, annexin, integrin, and fibronectin**), tetraspanins (**CD9, CD63, CD81, and CD82**),
heat shock proteins (**Hsp20 and Hsp27**), and lipid-related proteins




The function and biological characteristic of exosomes are determined by their specific contents ---Among the exosomal components**, lipids, proteins, and nucleic acids** are three main cargos which determine the specificity of exosomes

Kou, M., Huang, L., Yang, J. et al. Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: a next generation therapeutic tool?.
Cell Death Dis 13, 580 (2022)

# Difference-Variations

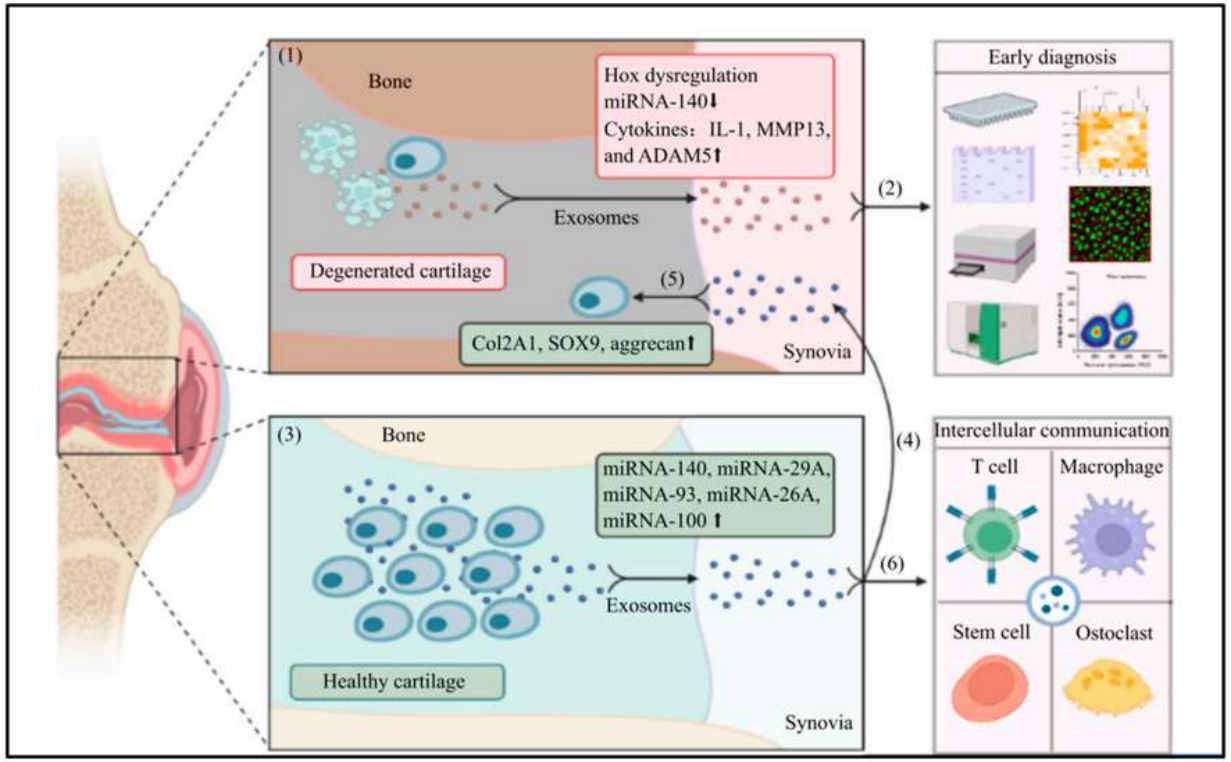



| Composition of exosomes | Classification | Examples | Functions |
|---|---|---|---|
| Proteins | Surface proteins and tetraspanins | CD9, CD63, CD81, CD82, Alix, TSG101 | Organizing membranes into tetraspanin-enriched domains (TEMs)and contributing to exosome binding to target cells |
| | ESCRT-related proteins | Alix, TSG101 | Controllers of exosome secretion via regulating ESCRT machinery |
| | Heat-shock proteins | Hsp70, Hsp90 | Exosome formation or externalization during the maturation. |
| | Rab GTPases proteins | Rab27a, Rab27b<br>Rab35 | Involving in MVBs interaction with the plasma membrane |
| | Annexins | Annexins I, II, IV, V, and VII | Membrane transport/trafficking |
| | Phospholipase | Phospholipase D | Regulating exosome secretion via hydrolyzation of phosphatidylcholine |
| | Cytosolic proteins | β-catenin and<br>Elongation factor-1α | Signal transduction and protein translation |
| Lipids | Glycerophospholipids | Phosphatidylserine | The activator of negative charge and the recruiter of signalling proteins |
| | | Phosphatidylglycerol | Involved in transbilayer transport mechanism |
| | Sphingolipids | Sphingomyelin | Involved in exosomal membrane construction and cargo sorting |
| | Sterol lipids | Oxysterol | Involved in membrane contact between intracellular secretory vesicles and the plasma membrane |
| | Neutral lipids | Ceramide | Triggering an exosome biogenesis pathway independent of the ESCRT machinery |
| | | Free cholesterol | Regulating the biogenesis and cellular trafficking in endosomes |
| | Polyglycerophospholipid | BisMonoacylglyceroPhosphate (BMP) | Involved in MVB formation and ILV biogenesis |
| Nucleic acids | mRNA | CD2AP mRNA (Kidney disease) | Being biomarkers related to podocyte damage |
| | | GSTπ1, MGMT, APNG, ERCC1, ERCC2, MVP, ABCC3, CASP8 and IGFBP2 (Tumor) | Involved in drug resistance of tumors |
| | miRNA | miR-223 (Tumor) | Promoting cancer invasion |
| | | miR-146a (Cardiovascular disease) | Mediating regenerative function of cardiosphere |
| | | miR-155 (Asthma) | Relating to the development of inflammatory infiltration into the lung and to airway remodelling |
| | DNA | Double-stranded DNA (Tumors) | Identifying mutations present in parental tumor cells |
| | | Double-stranded DNA (Tumor) | Carrying mutations identical from partental cells |

Ni, Z., Zhou, S., Li, S. et al. Exosomes: roles and therapeutic potential in osteoarthritis. Bone Res 8, 25 (2020).

# Cartilage Tissue-Exosomes

- The mechanisms of exosomes for cartilage repair include the *regulating immune response, inhibiting chondrocytes apoptosis, inhibiting matrix degradation, promoting the proliferation and chondrogenesis of in situ stem cells, as well as the directional migration to an injury site*





- Zhang S, Chuah SJ, Lai RC, Hui JHP, Lim SK, Toh WS. MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity. Biomaterials. 2018 Feb;156:16-27.
- Liu Y, Wang M, Luo Y, Liang Q, Yu Y, Chen F, Yao J. Enhancing Stem Cell Therapy for Cartilage Repair in Osteoarthritis-A Hydrogel Focused Approach. Gels. 2021 Dec 14;7(4):263.

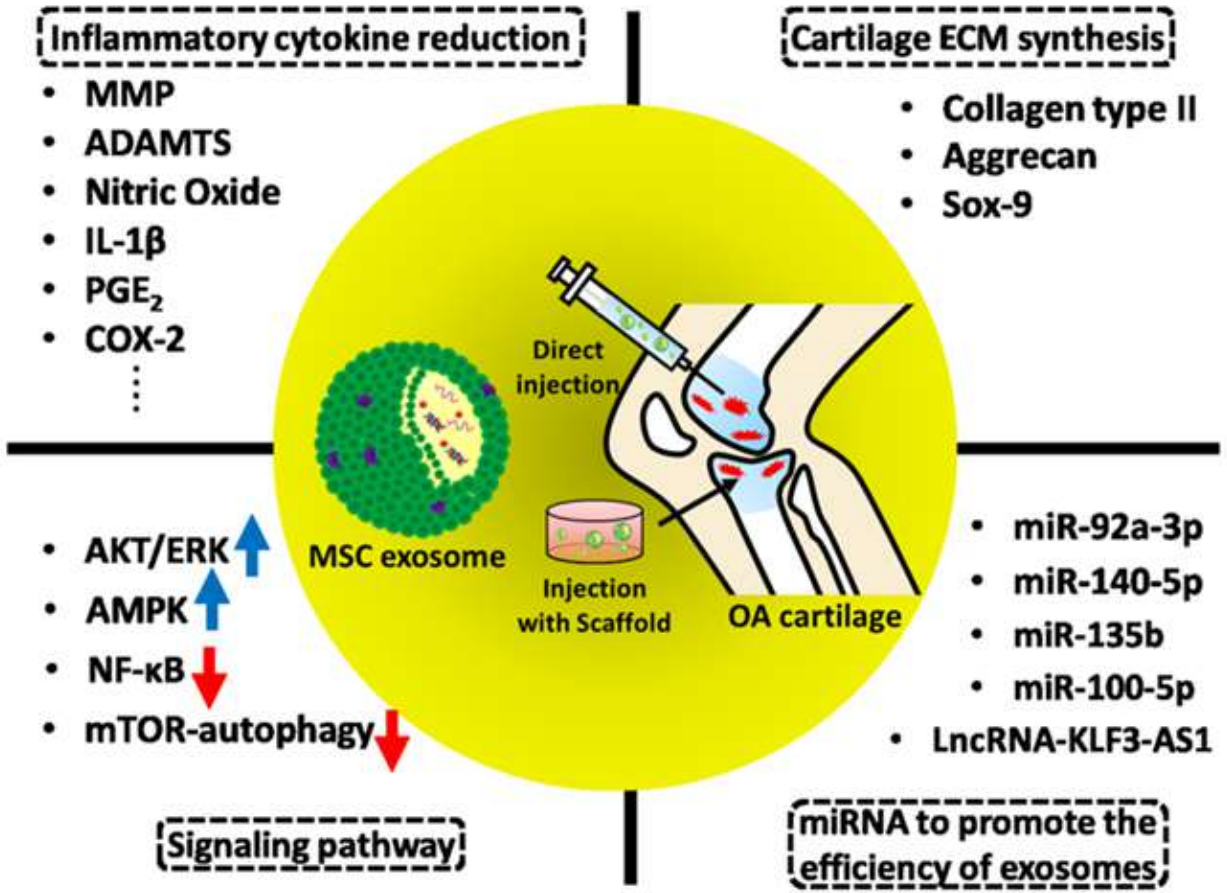
# Multidimensionally ?

It has **multidimensionally therapeutic effects** on the progression of OA, as they envelop various bioactive substances, e.g., *cytokines, growth factors, and RNA*, which can be directly transferred in neighboring cells through membrane fusion and then modulate the signal transduction to promote the cell proliferation, differentiation, and matrix formation

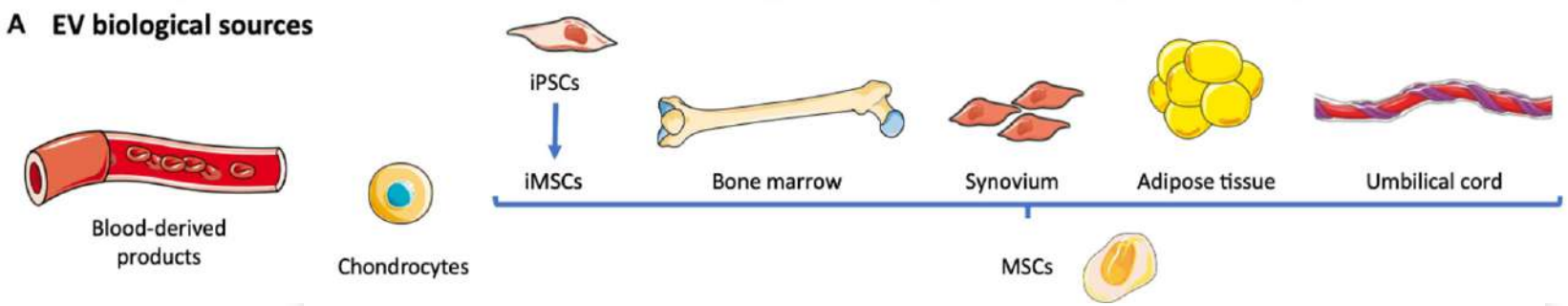
***(Lee et al., 2023)***

Exosome-based treatment can function as an effective therapy to modulate the pathological damage of cartilage for OA patients

***(Xian Bo et al., 2022)***

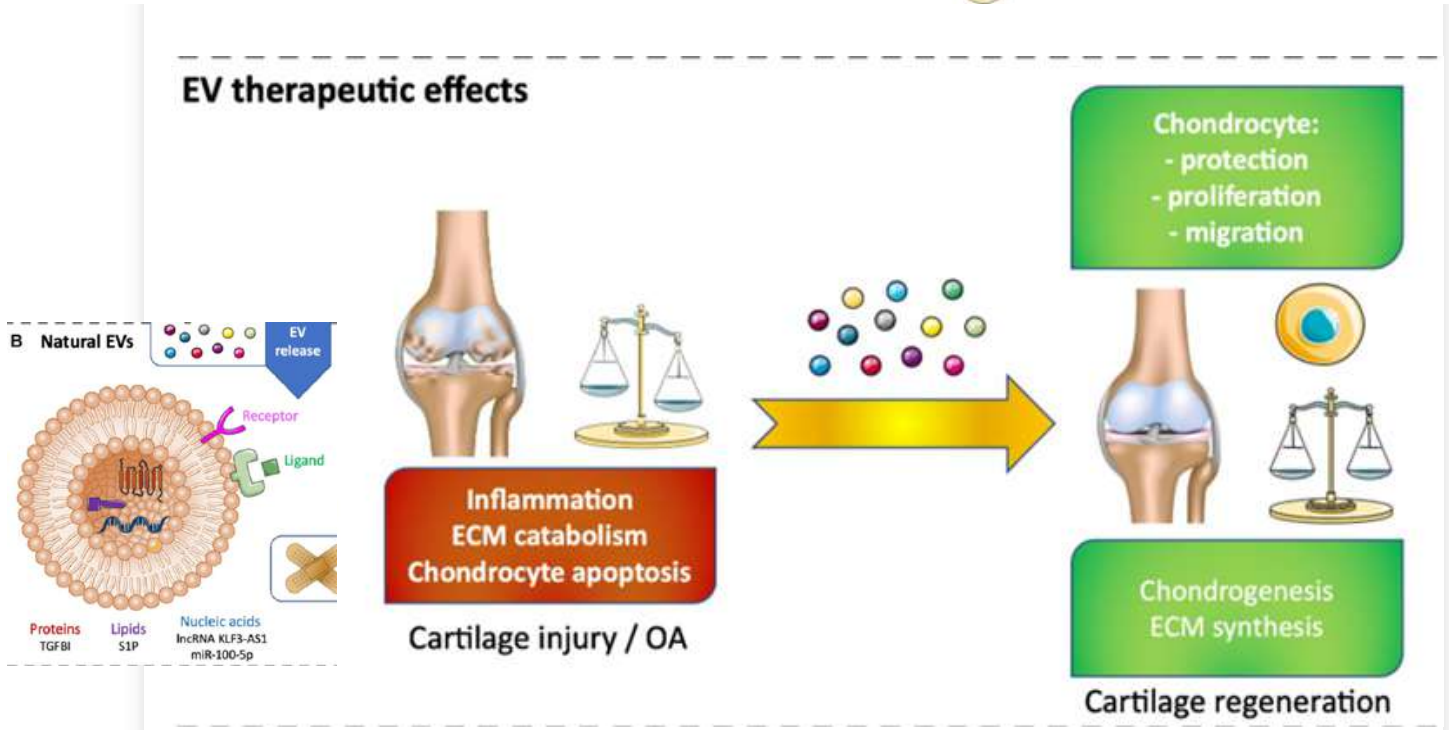


# Original cells?




The therapeutic effects of exosome on OA is basically related to its original cells, e.g., bone mesenchymal stem cells **(BMSCs**), adipose tissue mesenchymal stem cells (**AMSCs**), synovial mesenchymal stem cells (**SMSCs**), and embryonic stem cells (**ESCs)**,

Because these cells exhibit different cellular activities and biological responses to the different stages of OA




*Asghar et al., 2020; Fan et al., 2022*

Fan, W. J., Liu, D., Pan, L. Y., Wang, W. Y., Ding, Y. L., Zhang, Y. Y., et al. (2022).
Exosomes in osteoarthritis: Updated insights on pathogenesis, diagnosis, and treatment. Front. Cell Dev. Biol. 10, 949690.

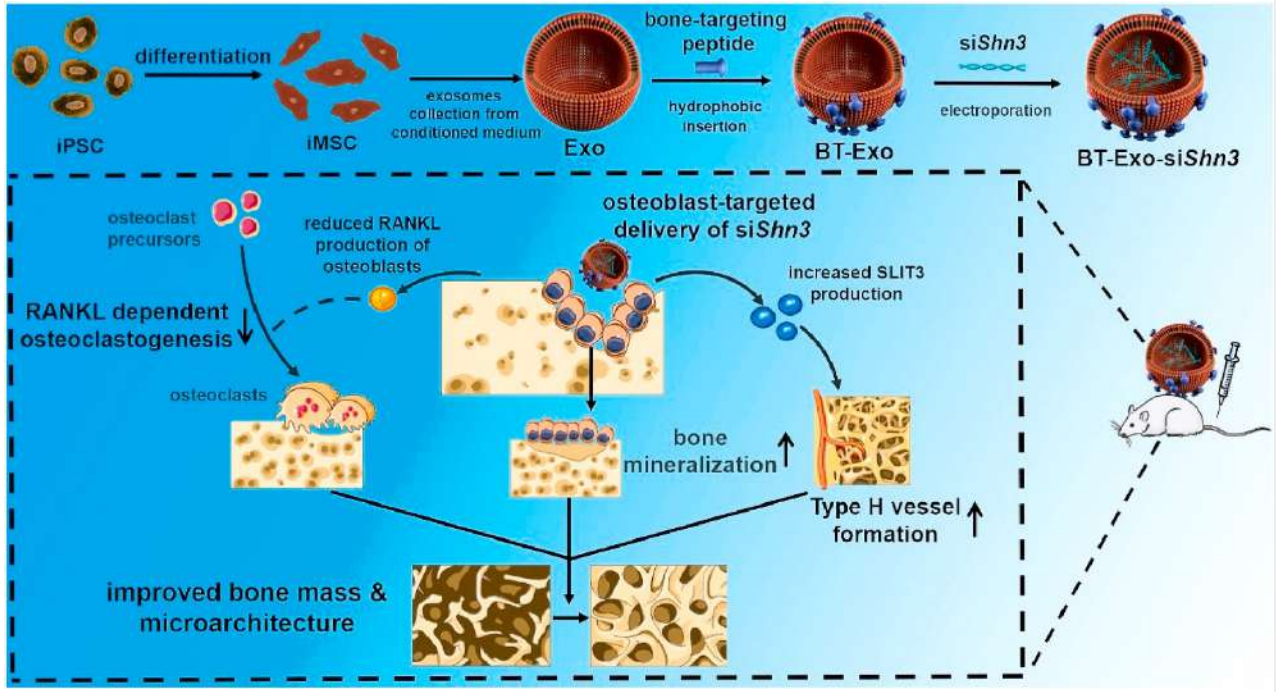
# How?

Specific exosomes in the synovial fluid are involved in the expression of **collagen type II alpha 1 (Col2A1),** and **aggrecan (ACAN)** in articular cartilage thus alleviating the development of OA

*(Kato et al., 2014)*

Modification of bone targeting exosomes with siShn3 to **silence Shn3** in osteoblasts enhances new bone formation and inhibits osteoclasts formation by downregulated **RANKL and TRAP**

*(Cui et al., 2022)*




Cui, Y., Guo, Y., Kong, L., Shi, J., Liu, P., Li, R., et al. (2022). A bone-targeted engineered exosome platform delivering siRNA to treat osteoporosis. Bioact. Mater 10, 207–221

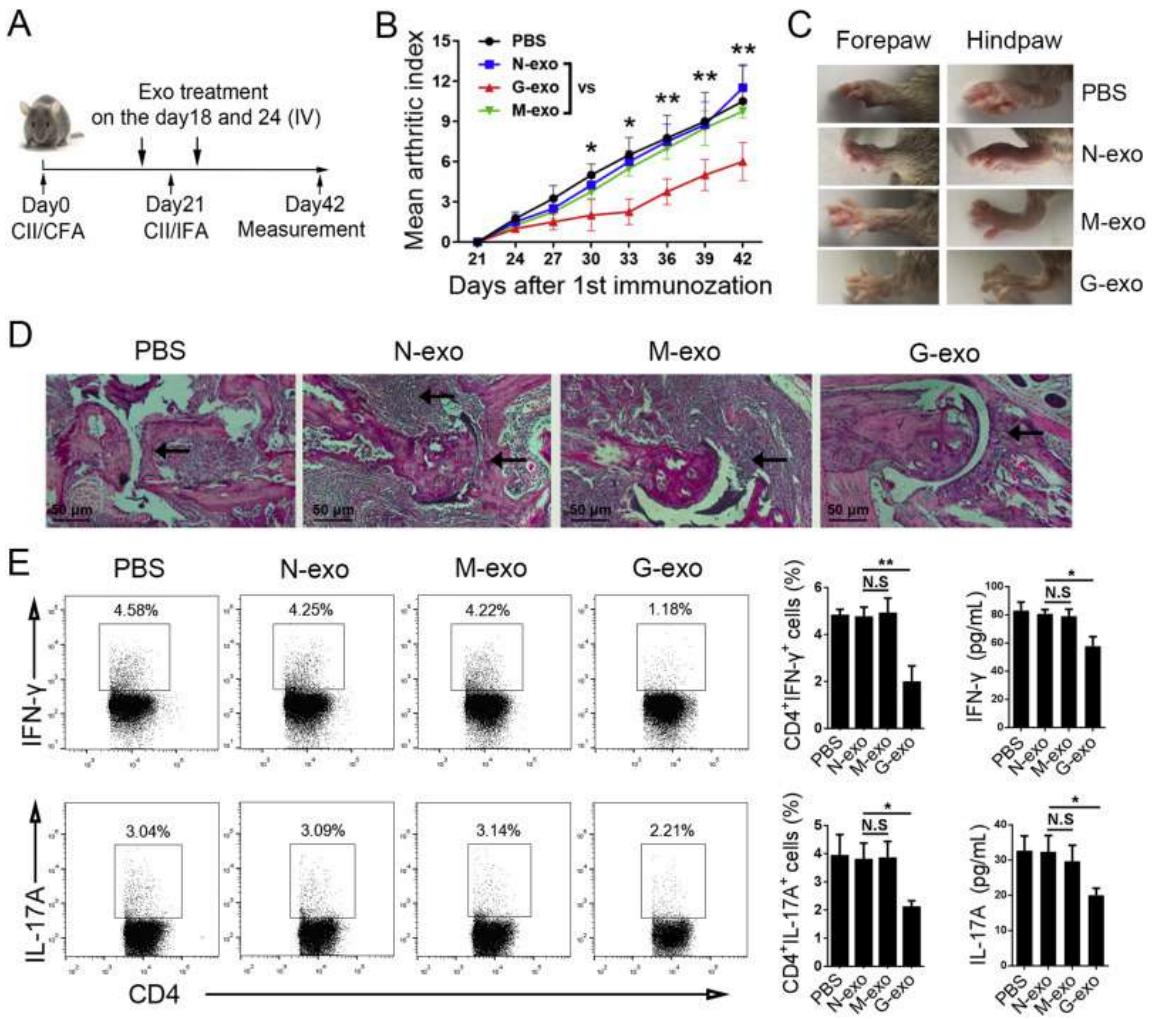
# How?

MSC-derived exosome has similar therapeutic effects with its original cells on the treatment of OA, **increasing the expression of chondrogenesis (Col2A1 and ACAN)** and **inhibiting catabolic enzyme (MMP-13 and ADAMTS5)**

*(Cosenza et al., 2017)*

Granulocytic-myeloid-derived suppressor cells (GMDSCs)-derived exosomal miRNAs **miRNA-29A-3 P** and **miRNA-93-5 P** can effectively reduce arthritis index, leukocyte infiltration, and cartilage destruction in an OA mouse model by inhibiting inflammatory responses of **T helper (Th1)** cells, and **Th17** cells

*(Zhu et al., 2019)*




Zhu, D., Tian, J., Wu, X., Li, M., Tang, X., Rui, K., et al. (2019). G-MDSC-derived exosomes attenuate collagen-induced arthritis by impairing Th1 and Th17 cell responses. Biochim. Biophys. Acta Mol. Basis Dis. 1865, 165540. doi:10.1016/j.bbadis. 2019.165540

# How?

Exosomes with a high expression of **miRNA-26a-5p** can alleviate the injury of synovial fibroblasts induced by **prostaglandin-endoperoxide synthase**

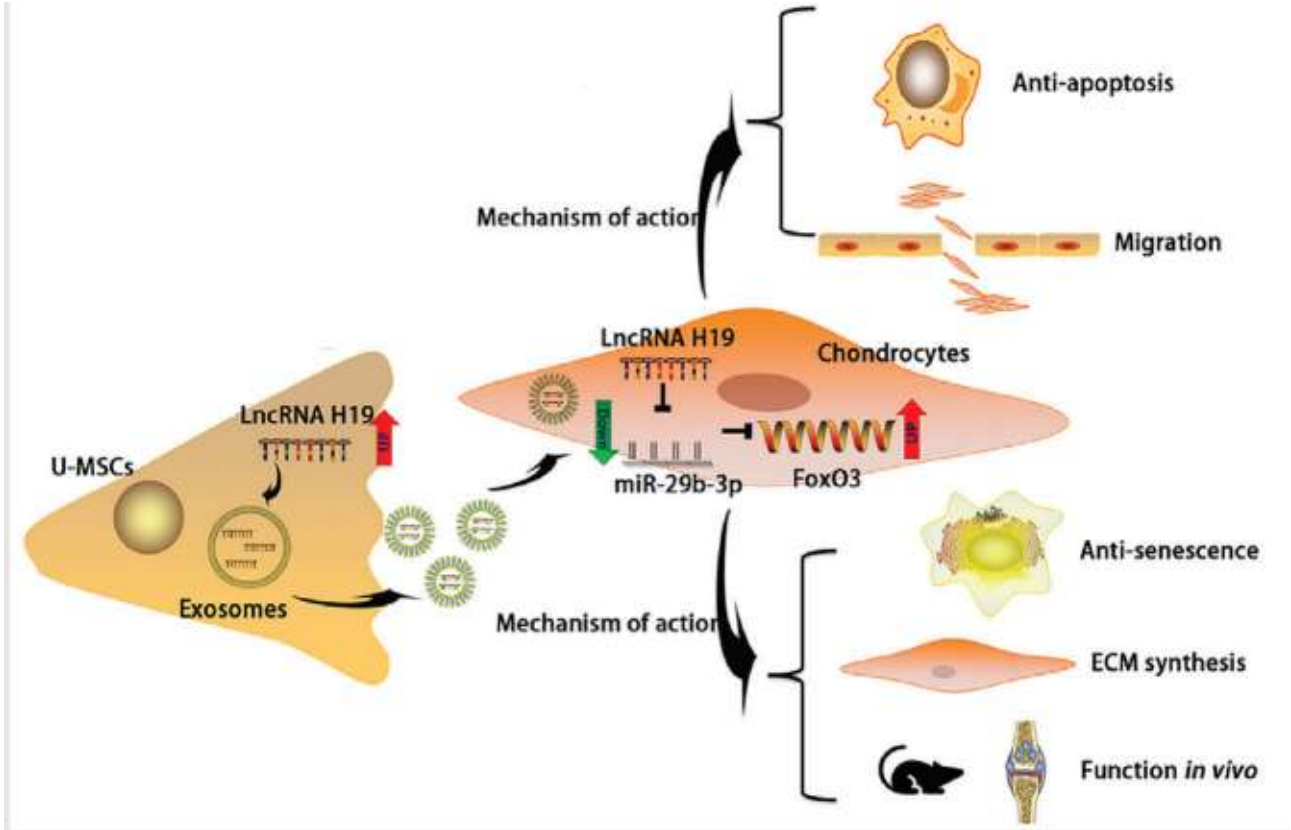
*(Rizzi et al., 2022*)

Exosomal **miRNA-100-5** P showed inhibitory effects on the mammalian target of rapamycin **(mTOR)** and inflammation thus having therapeutic effects on OA

*(Luo et al., 2019)*

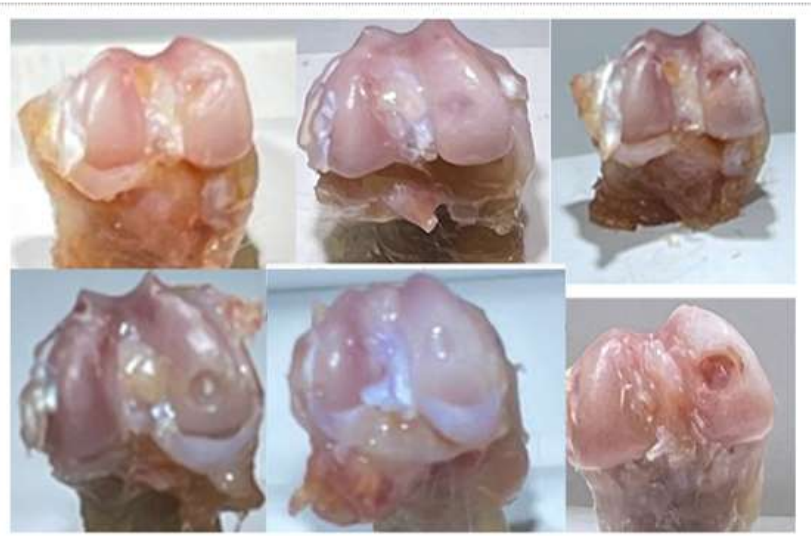
Likewise, exosomal **miR-92a-3p** can inhibit WNT family member 5 A **(WNT5a)** to relieve the cartilage damage caused by OA

*(Yan et al., 2021)*




Yan, L., Liu, G., and Wu, X. (2021). The umbilical cord mesenchymal stem cellderived exosomal lncRNA H19 improves osteochondral activity through miR-29b-3p/ FoxO3 axis. Clin. Transl. Med. 11, e255.

# Where are we!

**32 rats**

G (1-2)Mammalian-(Adipose MSCs –foreskin MSCs) EXO

G (3-4)Plant-(pineapple-pumpkin) EXO

Interspecies interaction

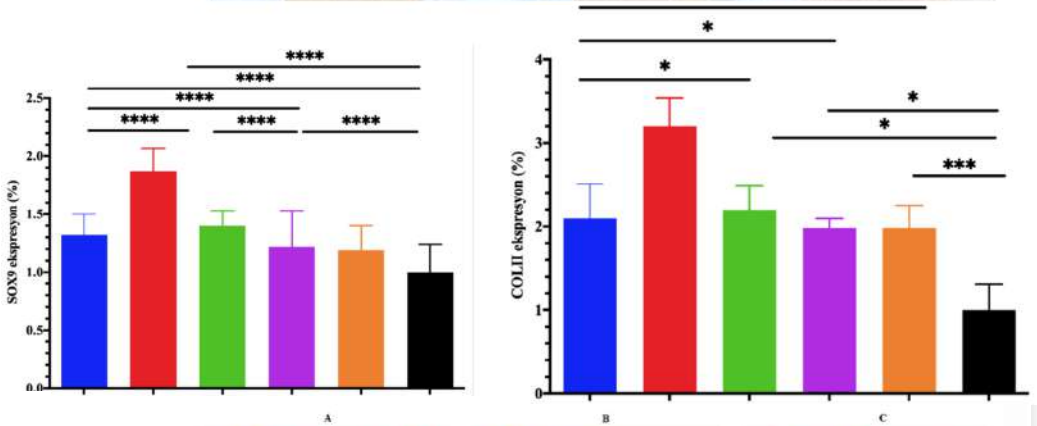
Compare the microfracture

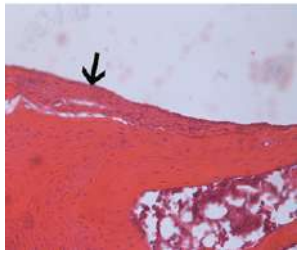
Reliability

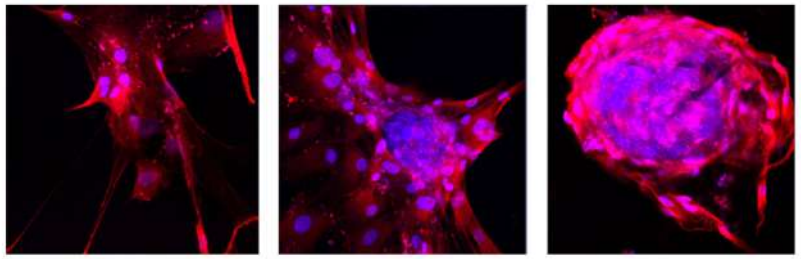
ECM---Col II-Agg- Sox9

Macroscopically, Histologically and Immunohistochemically

**SAFE and EFFECTIVE**



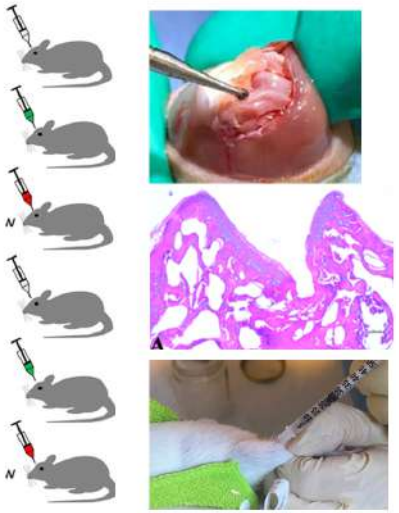


# Where are we!

## Methods and Materials

- Full-thickness cartilage defects were created in the trochlear grooves of both distal femurs (48 knees) of 24 adult rats
- The knees were randomly divided into six groups;

Group I: Control- saline,
Group II: **Wharton's jelly mesenchymal stem cell (MSC)**
Group III: **Wharton's jelly MSC-derived exosomes (EXO)**
Group IV: **Hyaluronic acid (HA)**
Group V: MSC and HA combination
Group VI: EXO and HA combination



A total of **3 intra-articular injections** were given to each rat at one-week intervals, starting 2 weeks after surgery

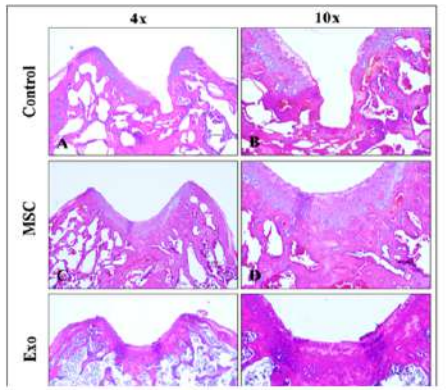
**4 weeks after the last injection**, all rats were euthanized and femurs were dissected for the evaluation

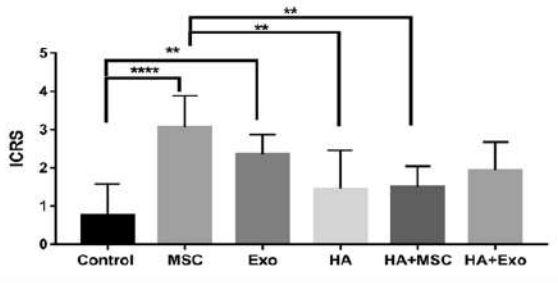
## Results

Macroscopic evaluations revealed that ICRS scores of the MSC group (8.2±0.7) were significant higher (P<0.05)

The MSC and EXO had a higher repair rate and a smoother surface.

The mean ICRS in the MSC, EXO, and HA groups were 8.2±0.7, 7.6±0.9 and 5.6±0.9



# Where are we!

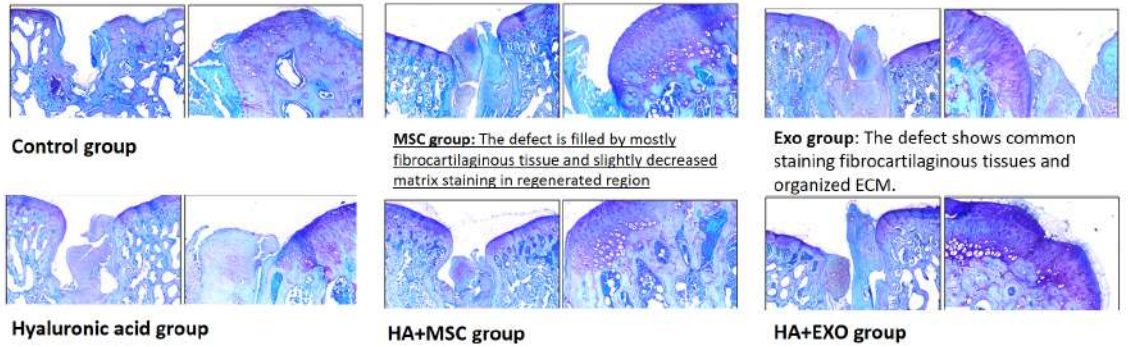
This study shows that **the efficacy of exosomes** in cartilage repair is as **Effective as stem cell therapies** and exosomes can be an alternative to cell-based therapies in the treatment of cartilage damage

However, **No significant effect of adding hyaluronic acid** to the use of stem cells and exosomes was found in the treatment of cartilage damage

Our findings provide a **Potentially effective therapeutic strategy** for treating osteochondral cartilage defect

## PROTEOGLICAN-EXTRACELLULAR MATRIX REGENERATION

**WHY IS IT IMPORTANT??? Water retention- Lubricant- Shock absorber-Material exchange-Growth factor binding**



**Control group**

**MSC group:** The defect is filled by mostly fibrocartilaginous tissue and slightly decreased matrix staining in regenerated region

**Exo group**: The defect shows common staining fibrocartilaginous tissues and organized ECM.

**Hyaluronic acid group**

**HA+MSC group**

**HA+EXO group**

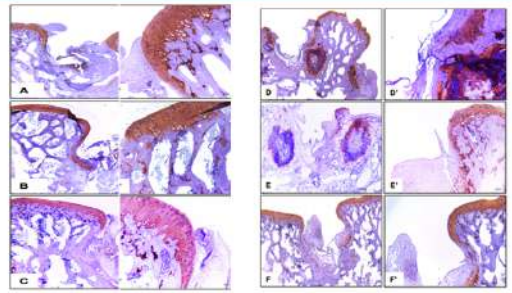
- In the treatment groups the regenerated tissue stained dark toluidine blue staining. Weak toluidine blue staining presents involvement of endochondral ossification and fibrotic tissue.
- Histological findings after toluidine blue staining in the 6 groups in the trochlear groove at 6 weeks after surgery

## Type II Collagen

- Type II collagen was orderly expressed through the all cartilage layer on the surface of trochlear region
- MSC and EXO group had a higher concentration of type II collagen expression in regenerated cartilage between the all groups.

Collagen type II represents 90–95% of the collagen in hyaline cartilage

Collagen type II is fibrillar network primarily responsible for the mechanical integrity of the tissue and plays a significant role in chondrocyte differentiation

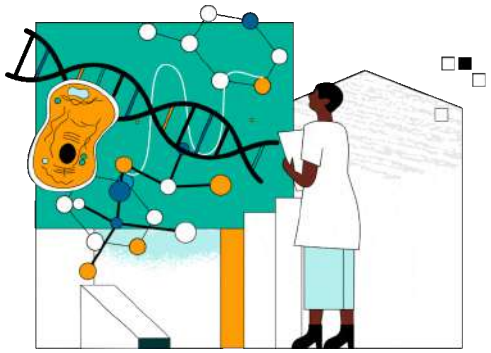


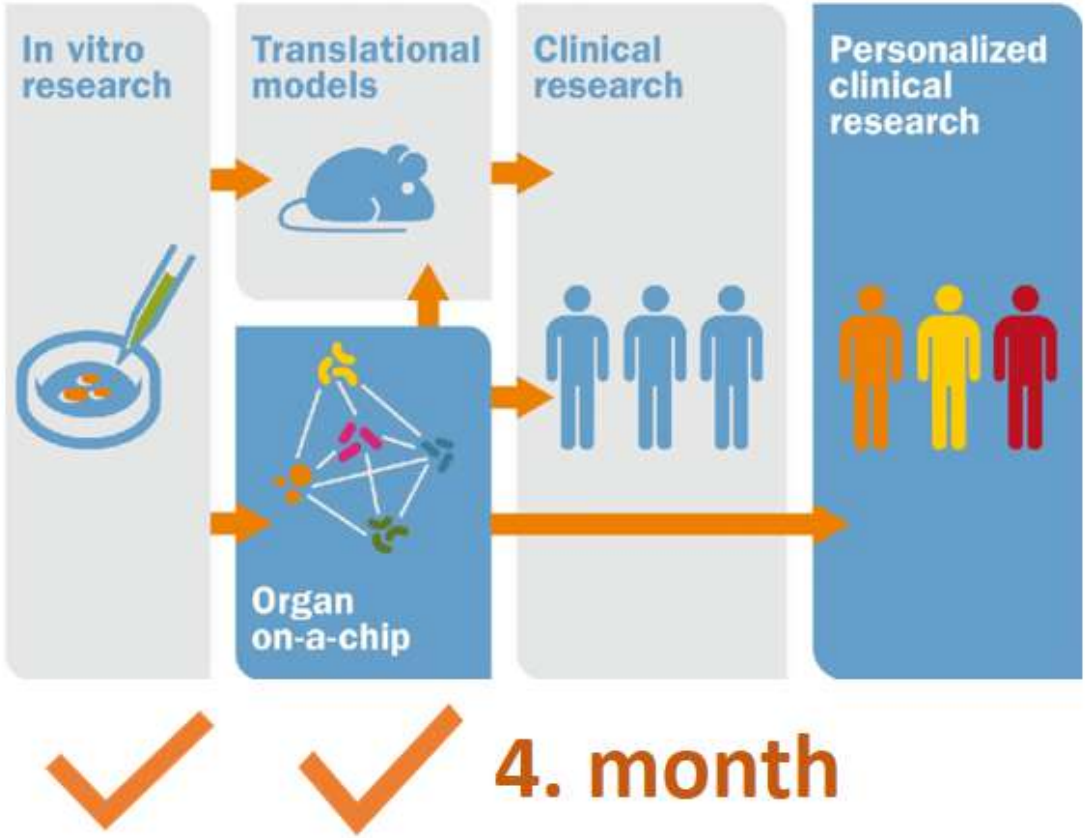
The microscopic appearance of type II collagen immunostaining of defects in the trochlear groove at 6 weeks after surgery.

# Clinical translation of stem cell-derived exosomes

Clinical trials----345 studies

OA—CASE- CHILE

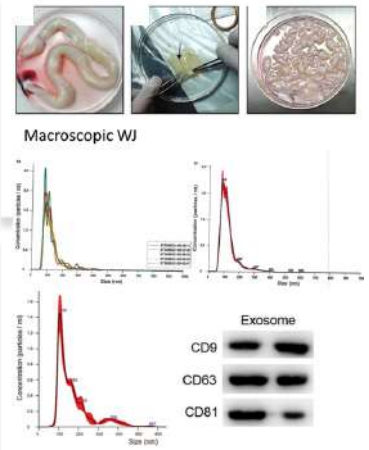


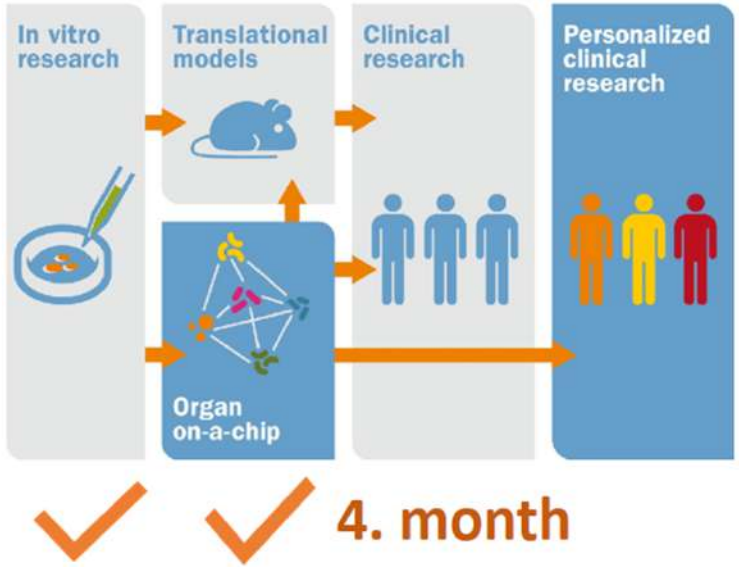
# Clinical translation of stem cell-derived exosomes

- **15 patients**;

  mean age 53.9y (50-61)

- 2 ml- 2x10$^{7}$ particule
- Wharton jelly derived MSC exosome
- Single injection
- 0. 3. 6. 12 . Months control
  - WOMAC
  - KOOS
  - SF-36
  - VAS


- Experienced radiologist
- 0. 3. 6. 12 months MRI and Cartialge Meeting
- MOCART scoring

## Inclusion Criteria

- Ages between 50-70 years old
- -Symptomatic knee osteoarthritis (VAS pain score >5)
- -Knee osteoarthritis degree Kellgren-Lawrance II-III
- -Chondromalacia I-III
- -Knee joint is stable (without ligament damage)
- - No History of Cancer

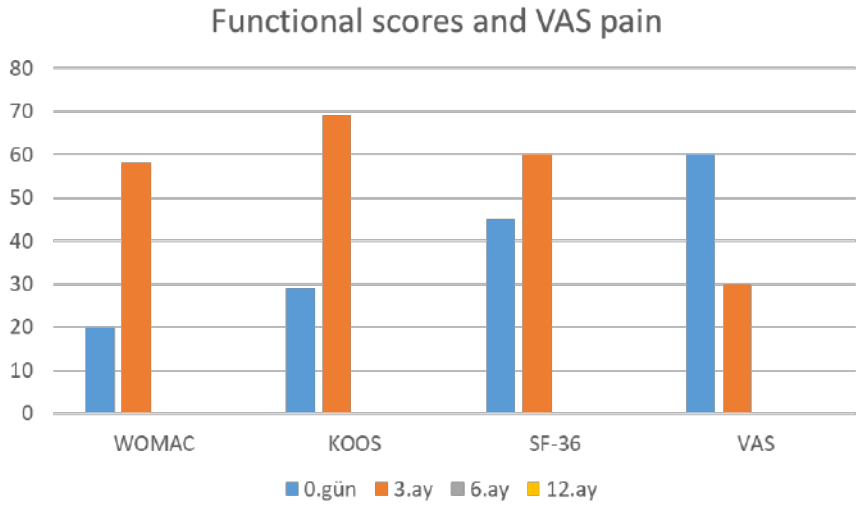
## Exclusion Criteria

- History of local infection in the knee
- History of corticosteroid or viscosupplementation injection
- Laxity or instability in the knee
- History of knee surgery up to 6 months ago
- History of knee trauma up to 3 months ago
- History of organ transplant or failure
- History of immunosuppressive drug use
- Past or ongoing tumor history
- History of serious neurological or psychiatric illness
- Patient who received local cortisone application
- BMI(body mass index)>30

# Clinical translation of stem cell-derived exosomes

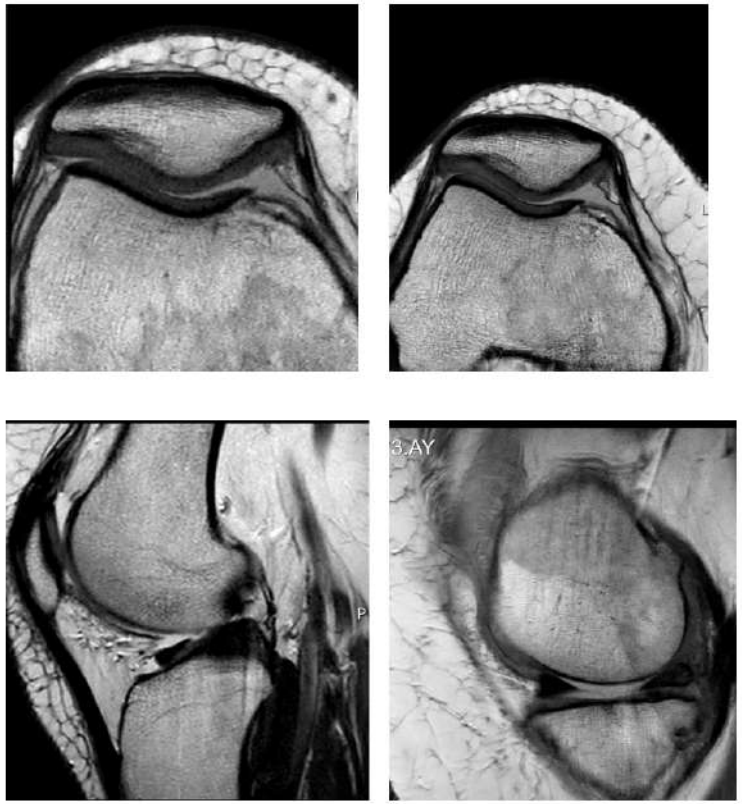
First 0-3 months

No side effects
Clinical scores better
Radiological evaluation ?

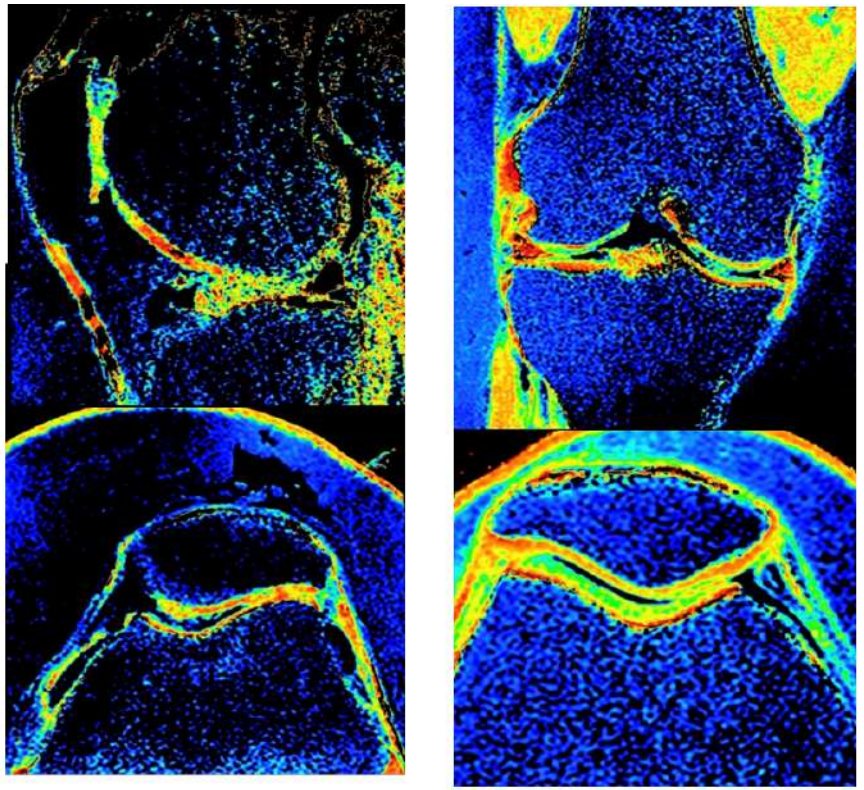




| | 0.day | 3.months | 6. months | 12.months |
|---|---|---|---|---|
| WOMAC | 20 | 58 | - | - |
| KOOS | 29 | 69 | - | - |
| SF-36 | 45 | 60 | - | - |
| VAS | 6 | 3 | - | - |

# Clinical translation of stem cell-derived exosomes



One patient synovial hypertrophy-
not clinically just radiologically



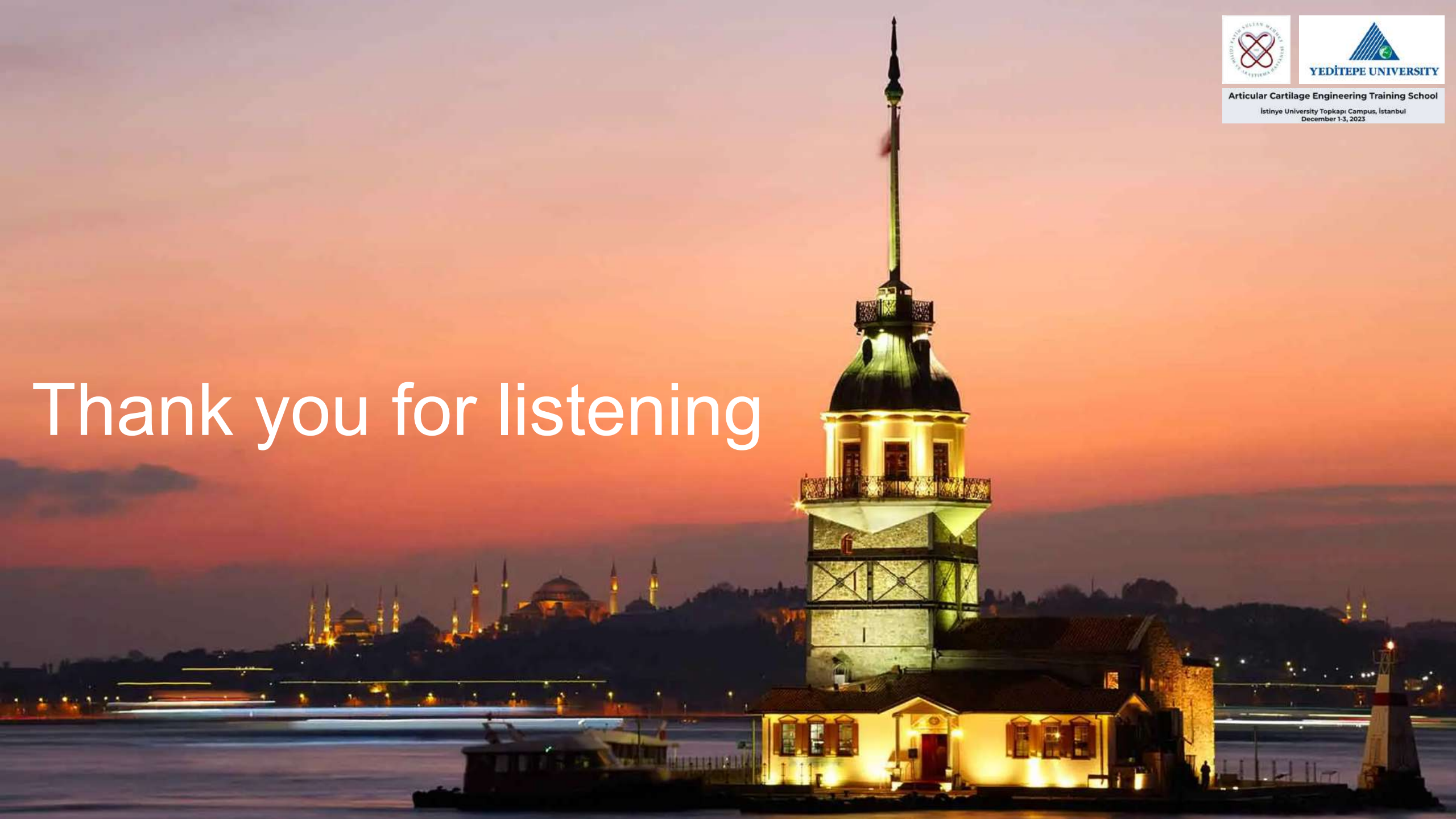
**MRI Cartilage mapping**

# Clinical translation of stem cell-derived exosomes

- According to global market reports,
  the global stem cell market is projected to reach **US$ 31.6 billion** by 2030, and
  the global exosome market is anticipated to reach **1.03 billion** by 2030

- First stem cell-related international standard **(ISO 24603)** was published in August 2022, which may also be referred to for exosome research to standardize upstream steps of exosome production for exosome therapy

- Exosome-based technology shall be the next-generation of **diagnosis modality and treatment for OA**


YEDİTEPE UNIVERSITY
Articular Cartilage Engineering Training School
İstinye University Topkapı Campus, İstanbul
December 1-3, 2023
Thank you for listening