Professor of PM&R, MD.
Istanbul University, Faculty of Medicine

Head of Department of Physical Medicine and Rehabilitation

Head of Division of Pain Medicine

Vice President of Turkish Society of Physical Medicine and Rehabilitation

General Secretary of Turkish Association of Manual Medicine

General Secretary of Association of Complementary Medicine TETAD
Certified Prolo-therapist
Certified Ozone-therapist

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Multidisciplinary International Positive Ageing Group

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PER LA GESTIONE UNIFICATA E INTERDISCIPLINARE DEL DOLORE MUSCOLO-SCHELETRICO E DELL'ALGODISTROFIA
EHOA Working Group

A member of
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European Consensus Group of Viscosupplemantation

A member of
International Symposium Intra Articular Treatment
Scientific Board

A member of
FEDERAZIONE MEDICO SPORTIVA ITALIANA
Scientific Board of Intra-articular Therapy in Sports Medicine

A member of
McMaster University
MORE™

A member of
SIR Società Italiana Reumatologia
ESKOA

A delegate of
FIMM
International Federation for Manual/ Musculoskeletal Medicine

The Horizon of Exosome IA Administration

Rudolph Virchow

Omnis Cellula E Cellula

Every cell originates from another cell similar to itself.

Bucharest 2-4 October 2025

Agenda

- Introduction to Exosome Biology
- Methods of Obtaining Exosomes
- Clinical Applications of Exosomes
- Future Perspectives

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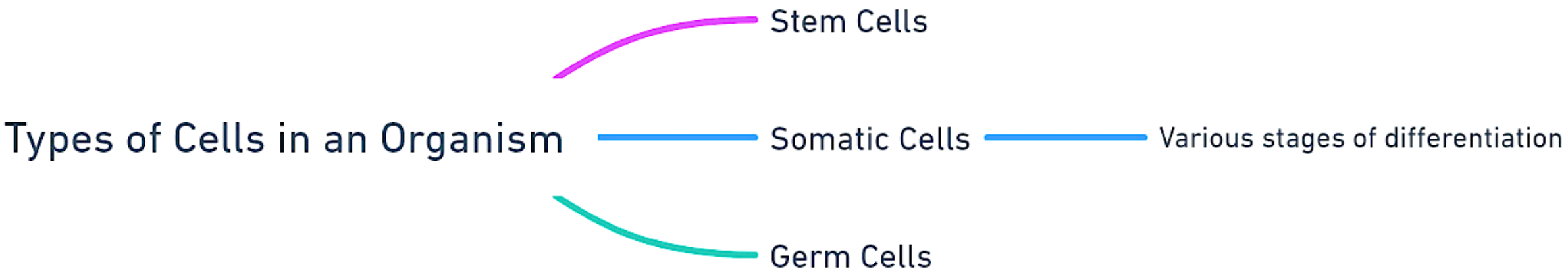
Regenerative Medicine / Orthobiologic Treatments

- This approach is based on the potential of biological materials and cell-based therapies to promote tissue healing and regeneration.
- The potential of orthobiologics is transforming the landscape of medical treatment, with the potential to significantly enhance patient outcomes.
- In recent years, stem cell applications have emerged as a prominent area of cell-based therapy.

Types of Cells



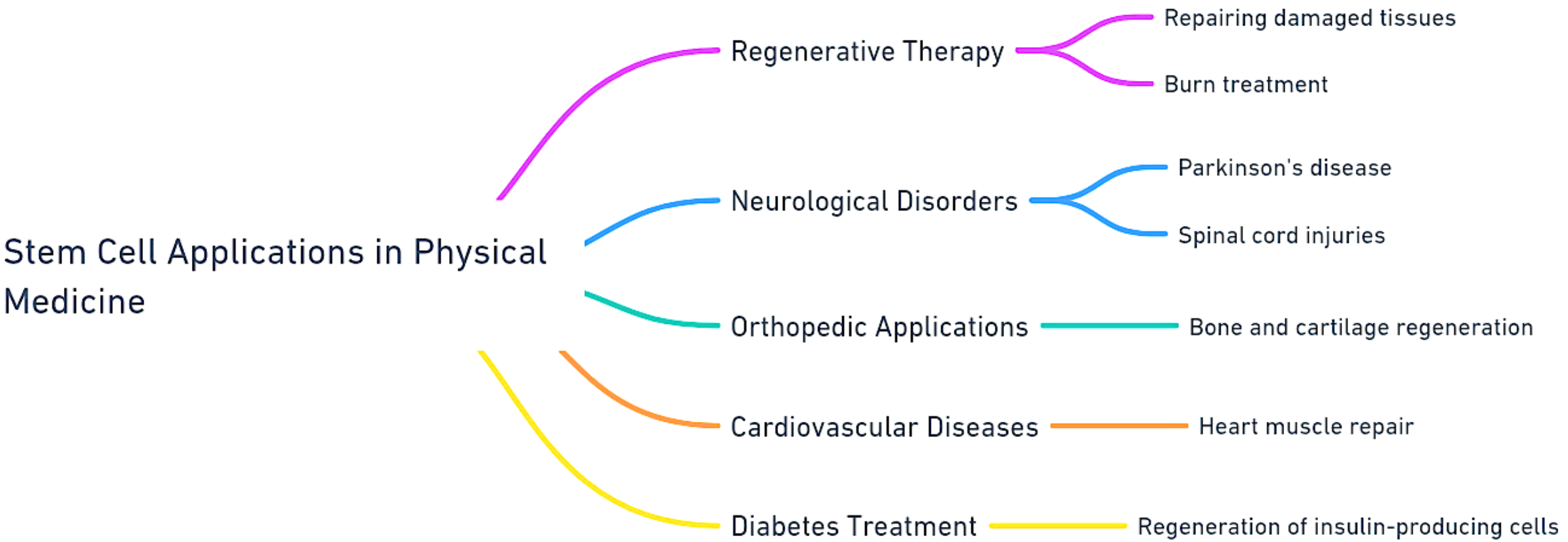
Definition of Stem Cell

1- Self-renewal

2- Differentiation into various cell types (potency), pluripotency

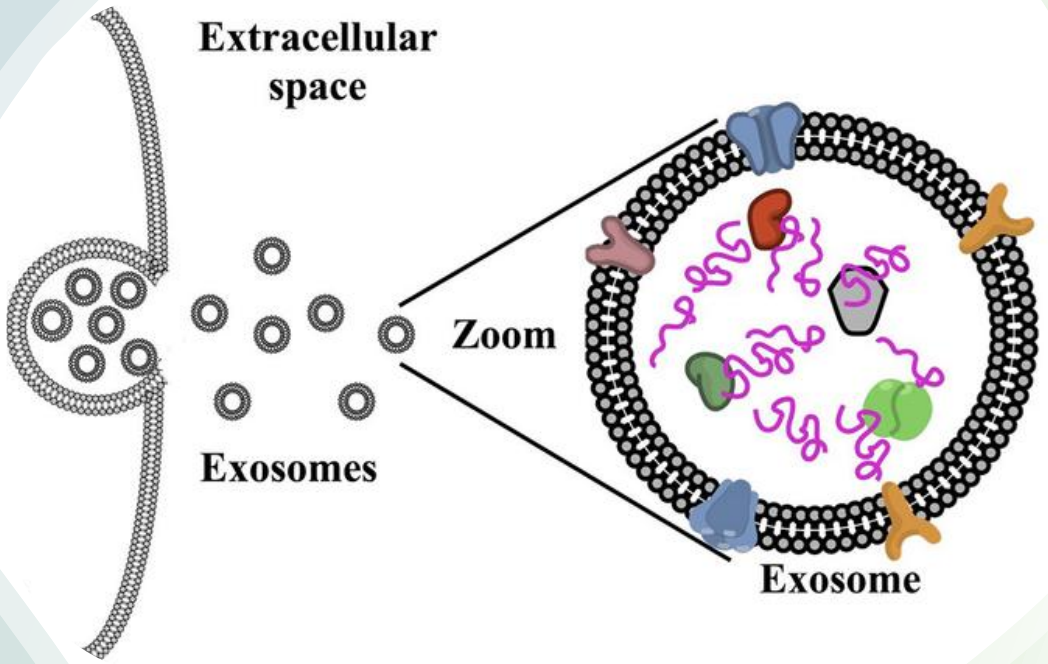
3- Colony formation

Stem Cell Applications

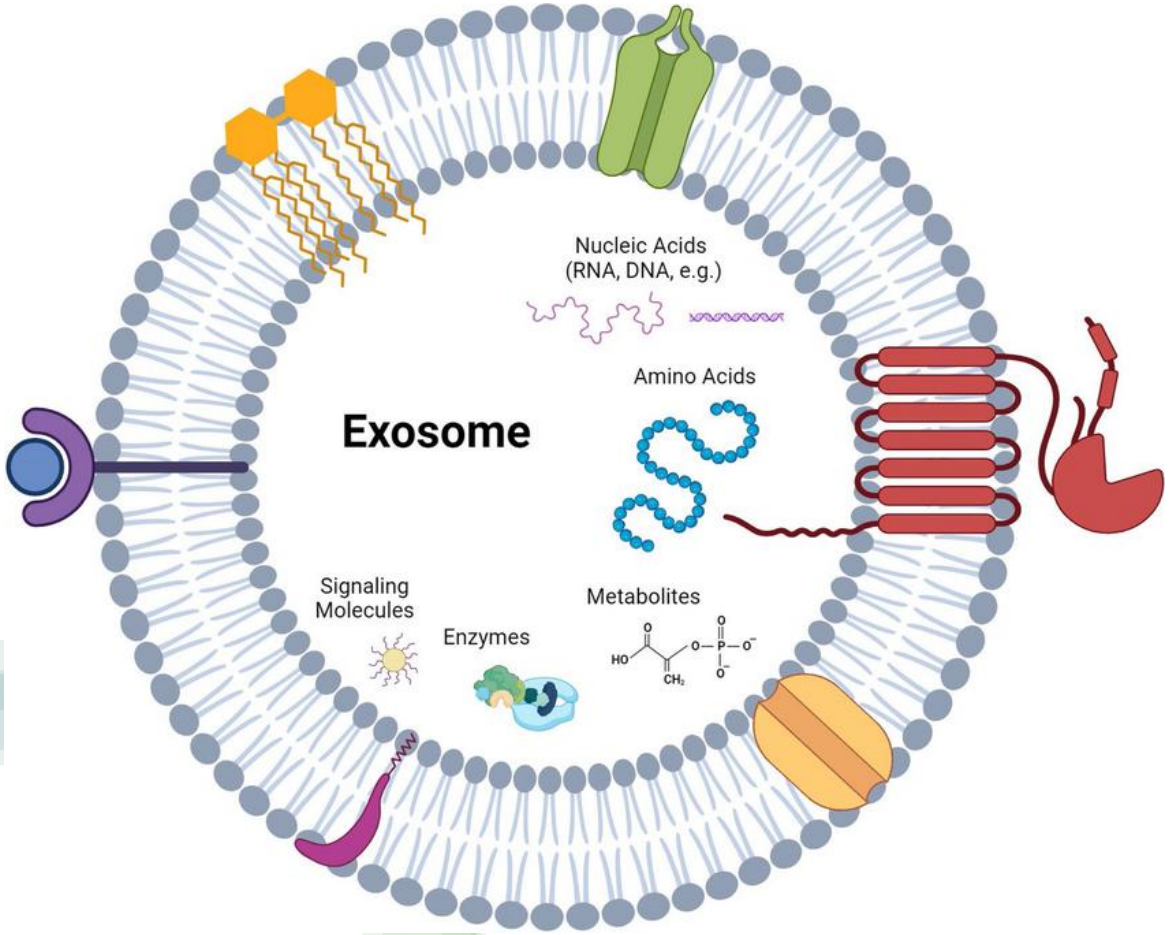


What is an Exosome?

- Exosomes form the smallest known group of extracellular vesicles secreted by cells.
- They were first identified in 1981 by Trams and colleagues, observed in electron microscopy images of normal and cancer cells.
- In 1986, they were isolated by Johnstone and colleagues.
- ***Considered one of the most significant discoveries in cell biology over the past 30 years.***



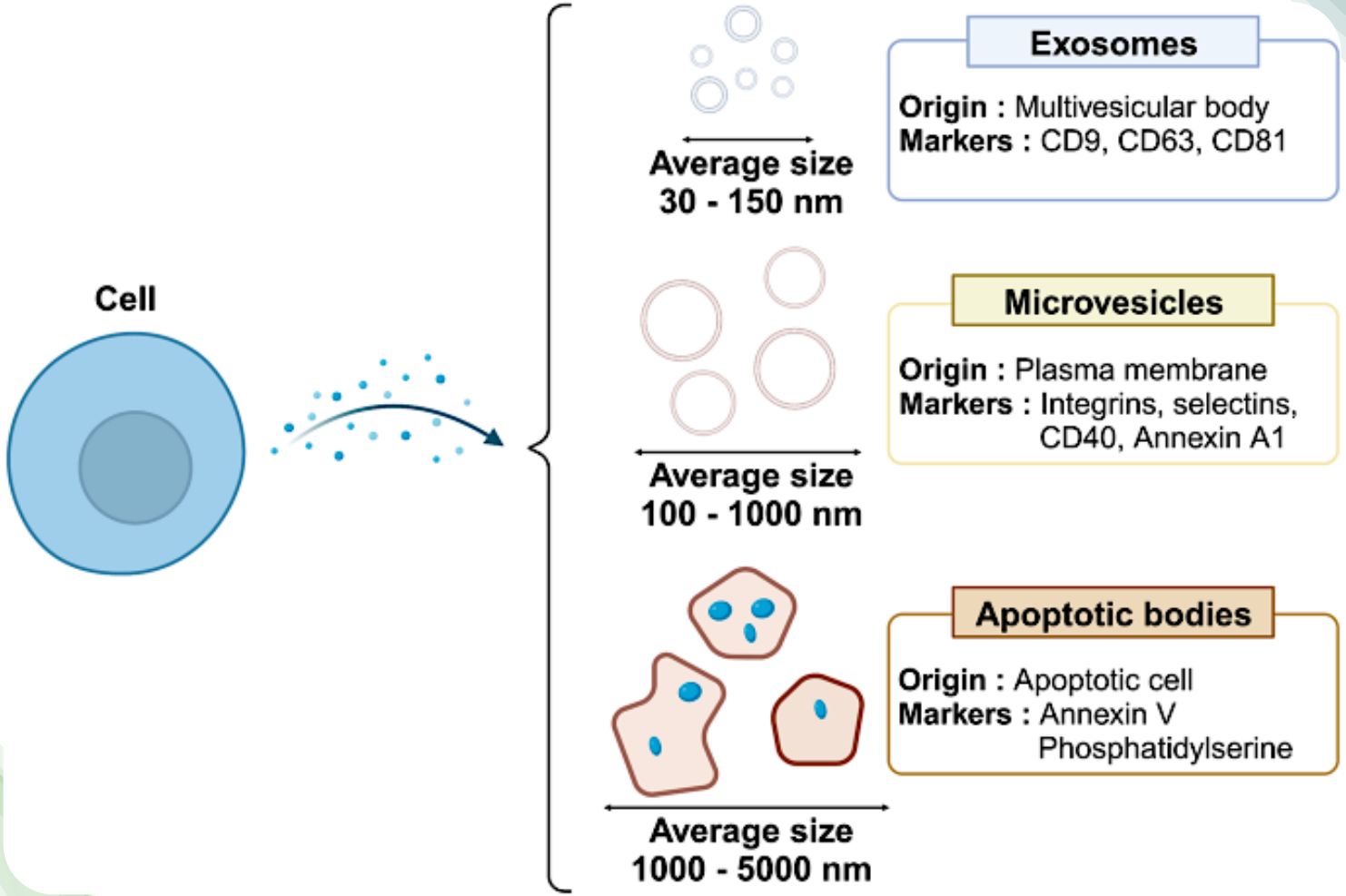
What is an Exosome?

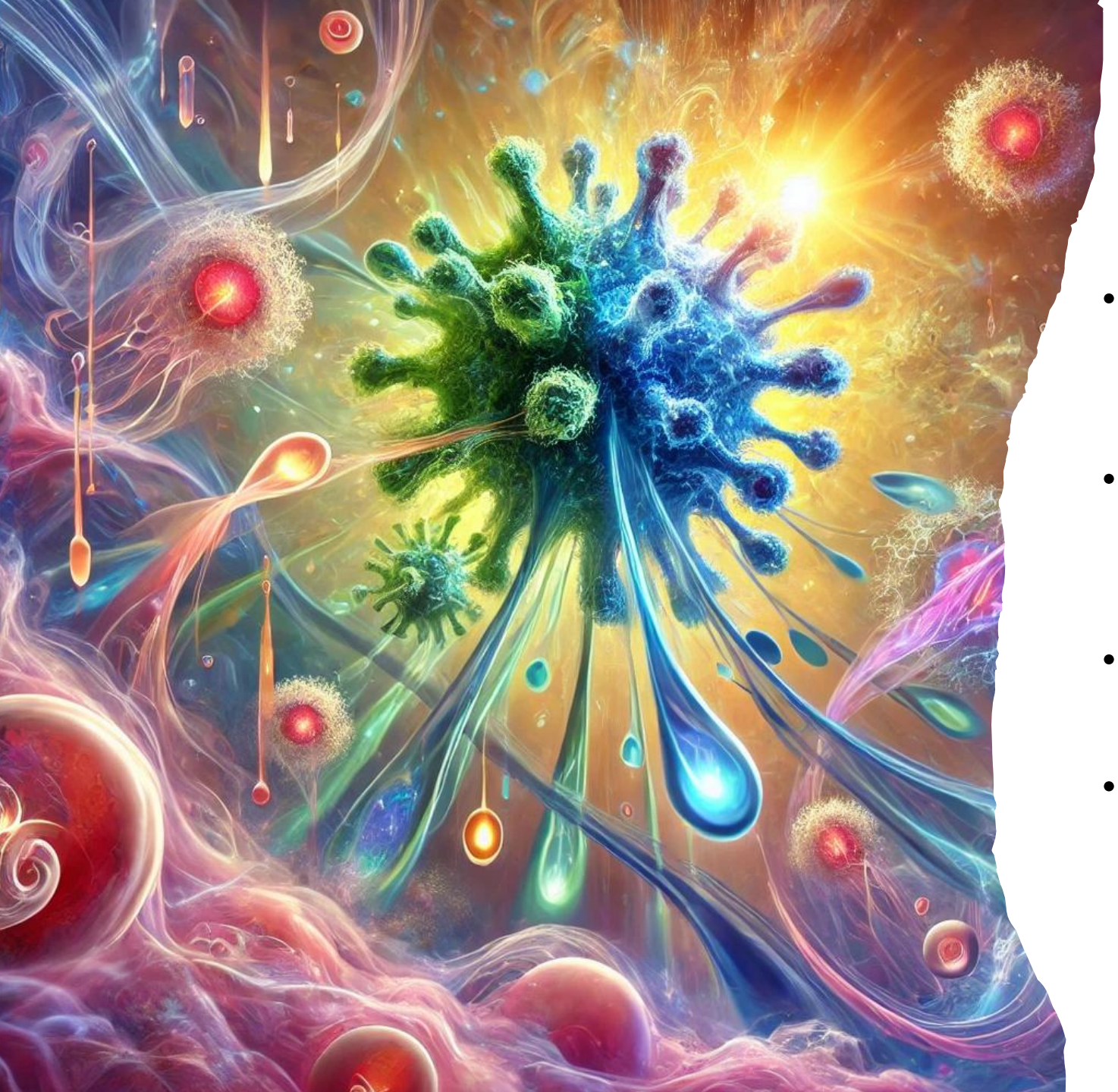


- Vesicles are 40-100 nm diameter and are surrounded by a double phospholipid layer.
- They are secreted by almost all cells in the body under both physiological and pathological conditions.
- Serve as ***key mediators*** of ***intercellular communication.***
- Ability to carry proteins, lipids, and nucleic acids such as miRNAs, mRNAs, DNAs, lncRNAs (long non-coding RNAs), and DNA fragments.
- Role in ***epigenetic regulation*** and ***disease modulation.***

Yang D, Zhang W, Zhang H, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics. 2020;10(8):3684-3707. Published 2020 Feb 19.

What is an Exosome?



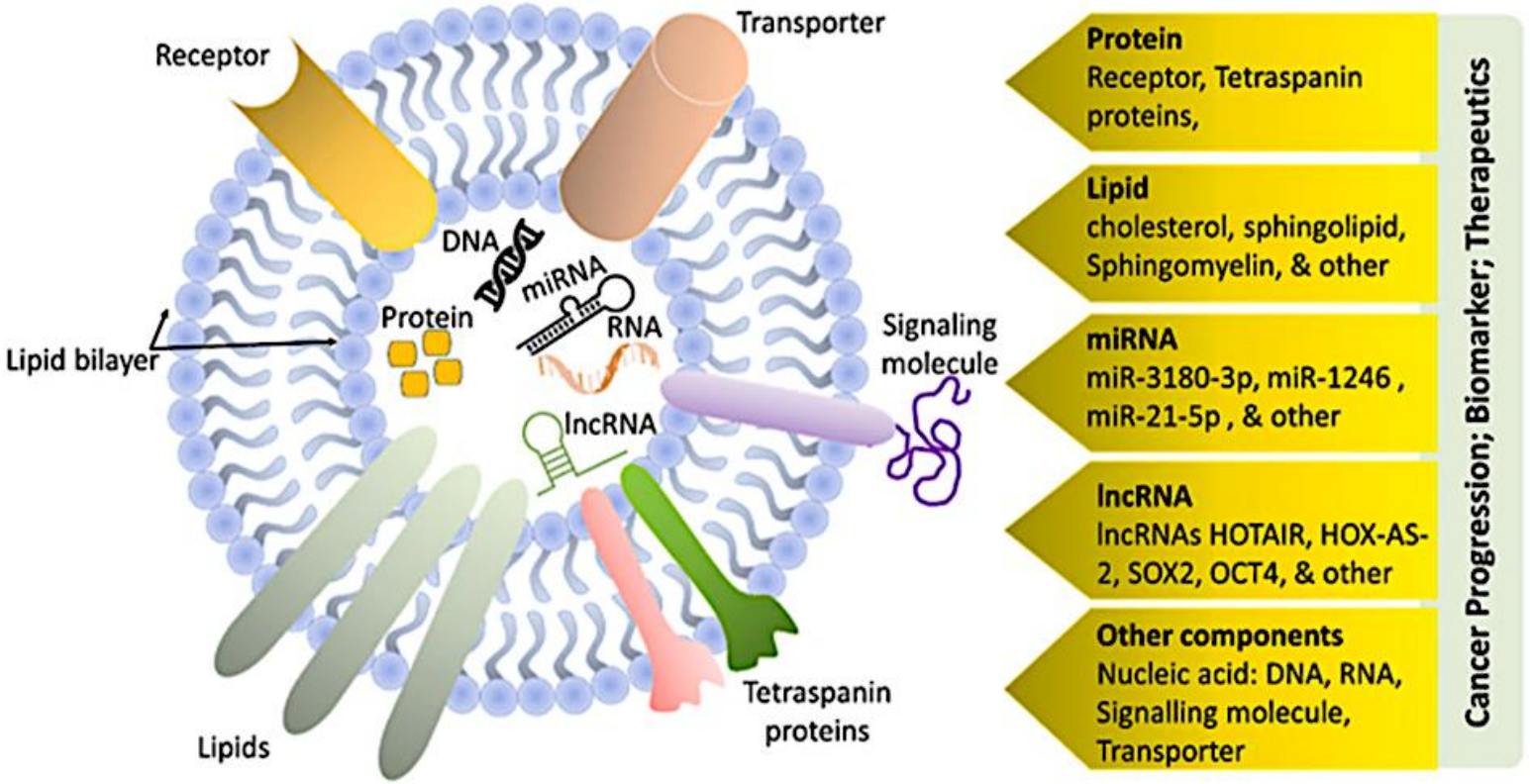


What is an Exosome?

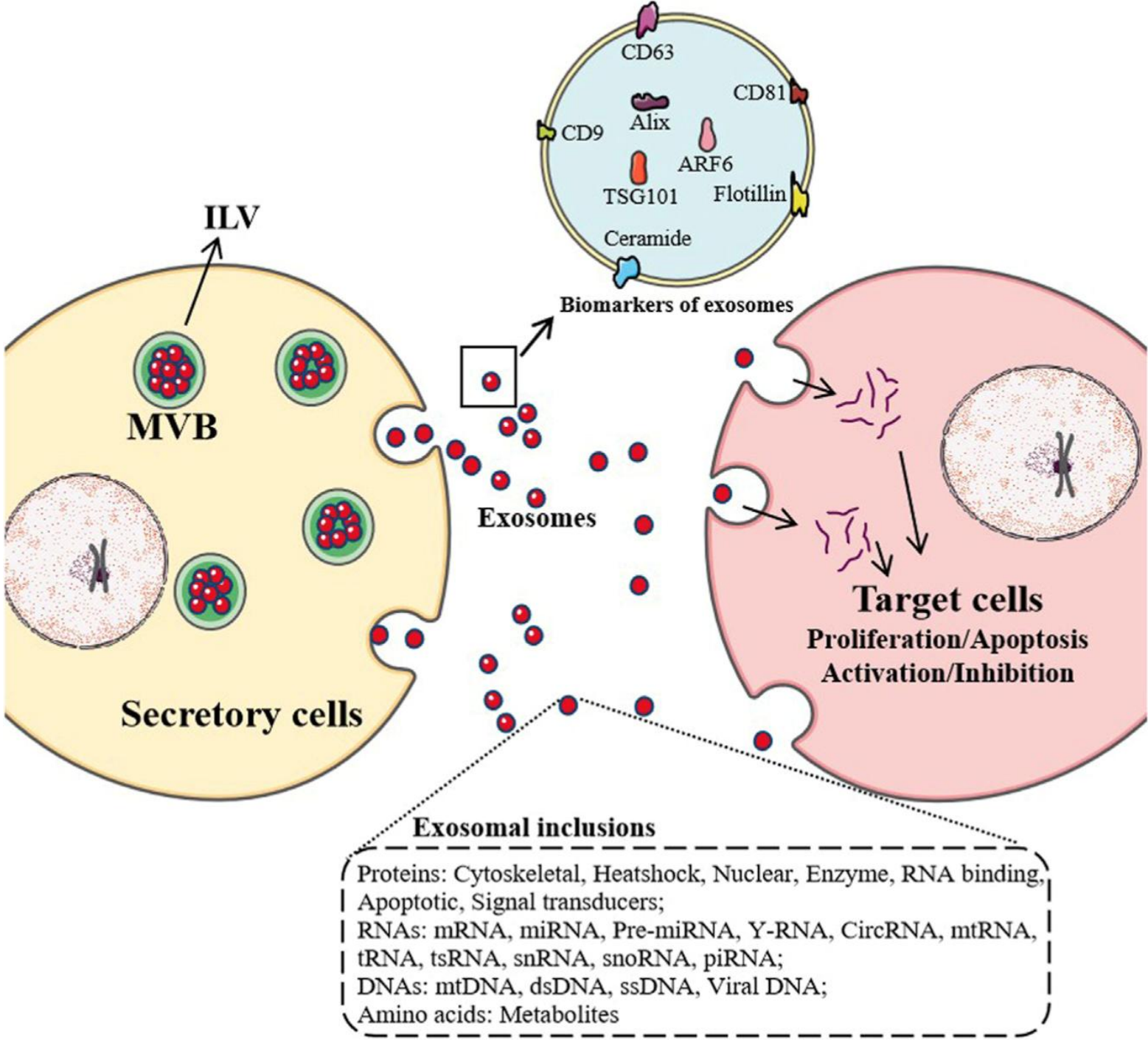
- Previously, they were thought to be solely responsible for removing waste from cells.
- Today we know that, they are vesicles that mediate the transport of biomolecules between cells.
- Gene expressions are regulated in the recipient cell through these biomolecules.
- Exosomes play a role in various biological functions such as ***immune regulation***, ***cell differentiation***, ***intercellular communication***, and ***cell migration***.

- The contents of exosomes are closely related to the origin and pathophysiology of the cells that secrete them.
- Heat shock proteins, cytoskeletal proteins, and proteins from the tetraspanin family are found in all exosomes.
- There are also proteins specific to the cells from which they originate.

The Contents of Exosomes



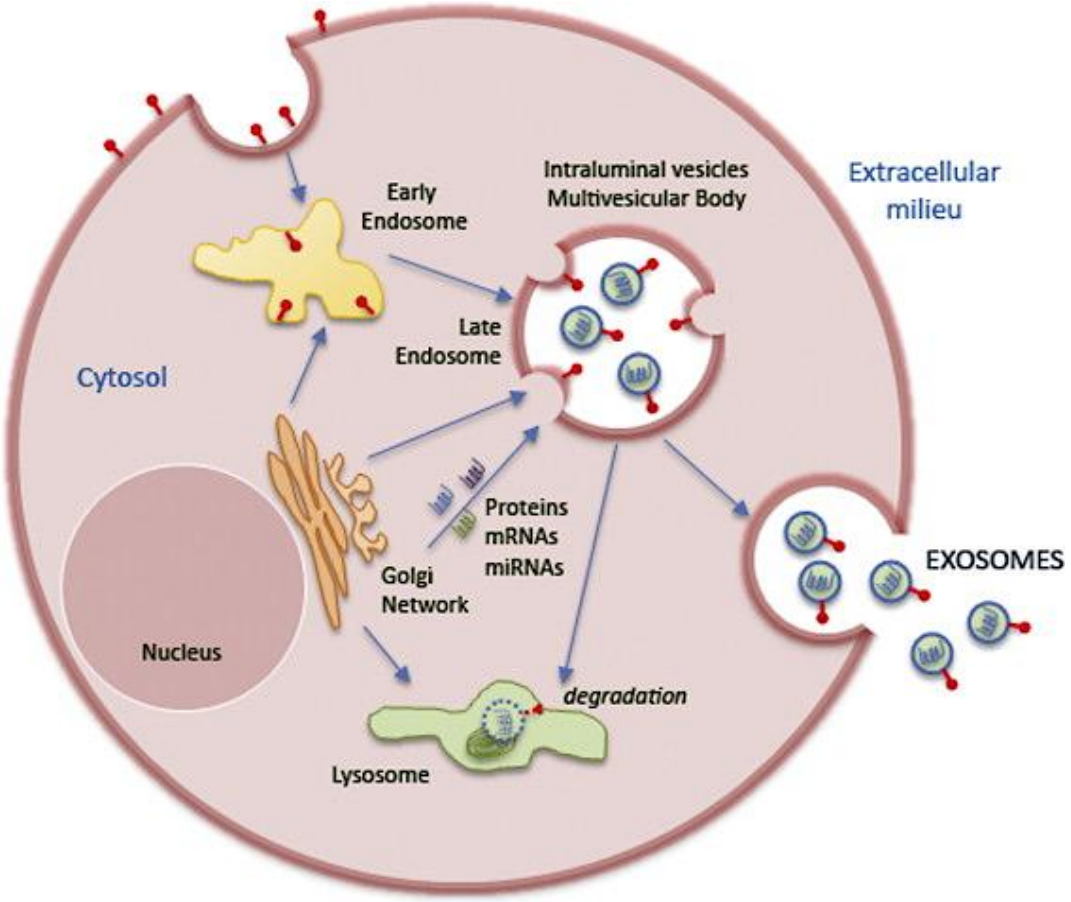
Maturation and secretion mechanisms of exosomes.



Miao C, Zhou W, Wang X and Fang J (2021) The Research Progress of Exosomes in Osteoarthritis, With Particular Emphasis on the Mediating Roles of miRNAs and lncRNAs. Front. Pharmacol. 12:685623.

Exosomal Biogenesis

1. **Origin from Endosomes**
    - Exosomes originate from **endosomes**, which are formed through the **indentation of the plasma membrane**.
2. **Role of Ceramide**
    - This indentation process is **induced by ceramide**, leading to the creation of early endosomes.
3. **Maturation to Late Endosome**
    - As early endosomes mature, they transition into **late endosomes**.
    - The endosome membrane undergoes **inward indentations**, forming multiple internal vesicles.



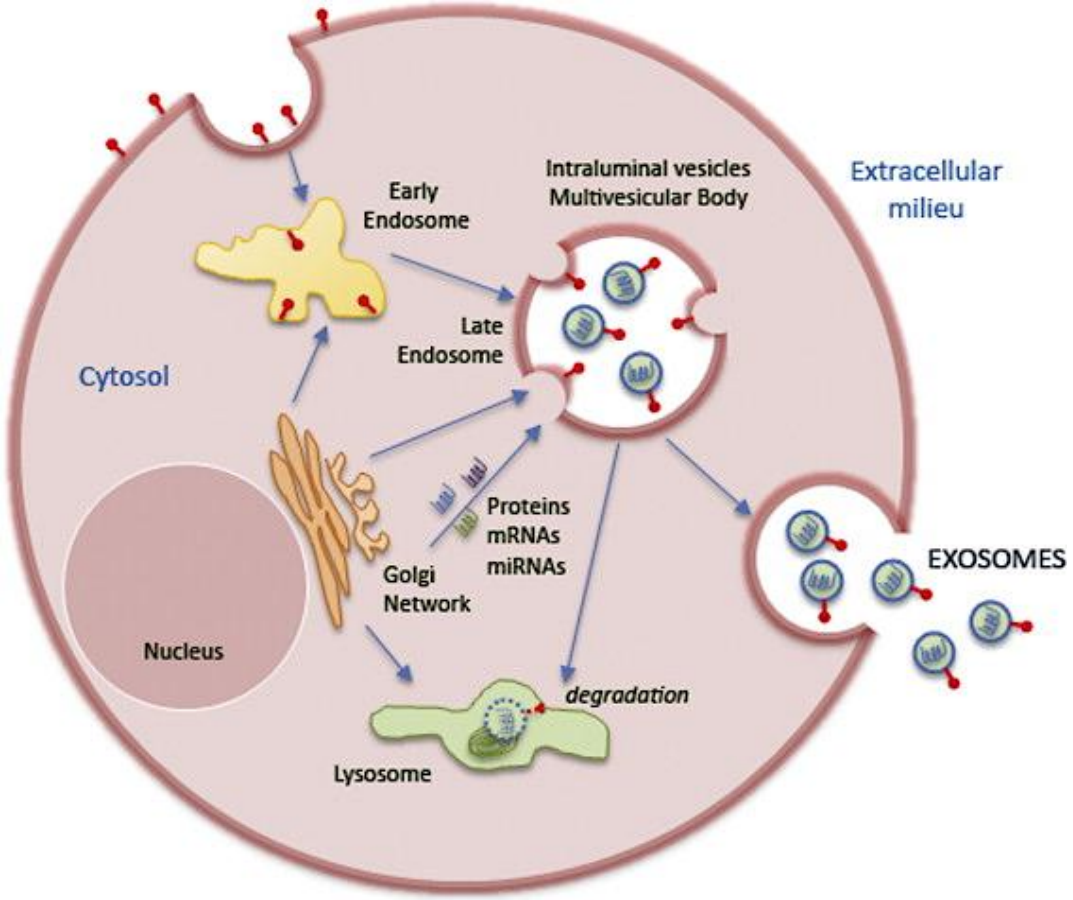
Exosomal Biogenesis

4. Formation of Multivesicular Bodies (MVBs)

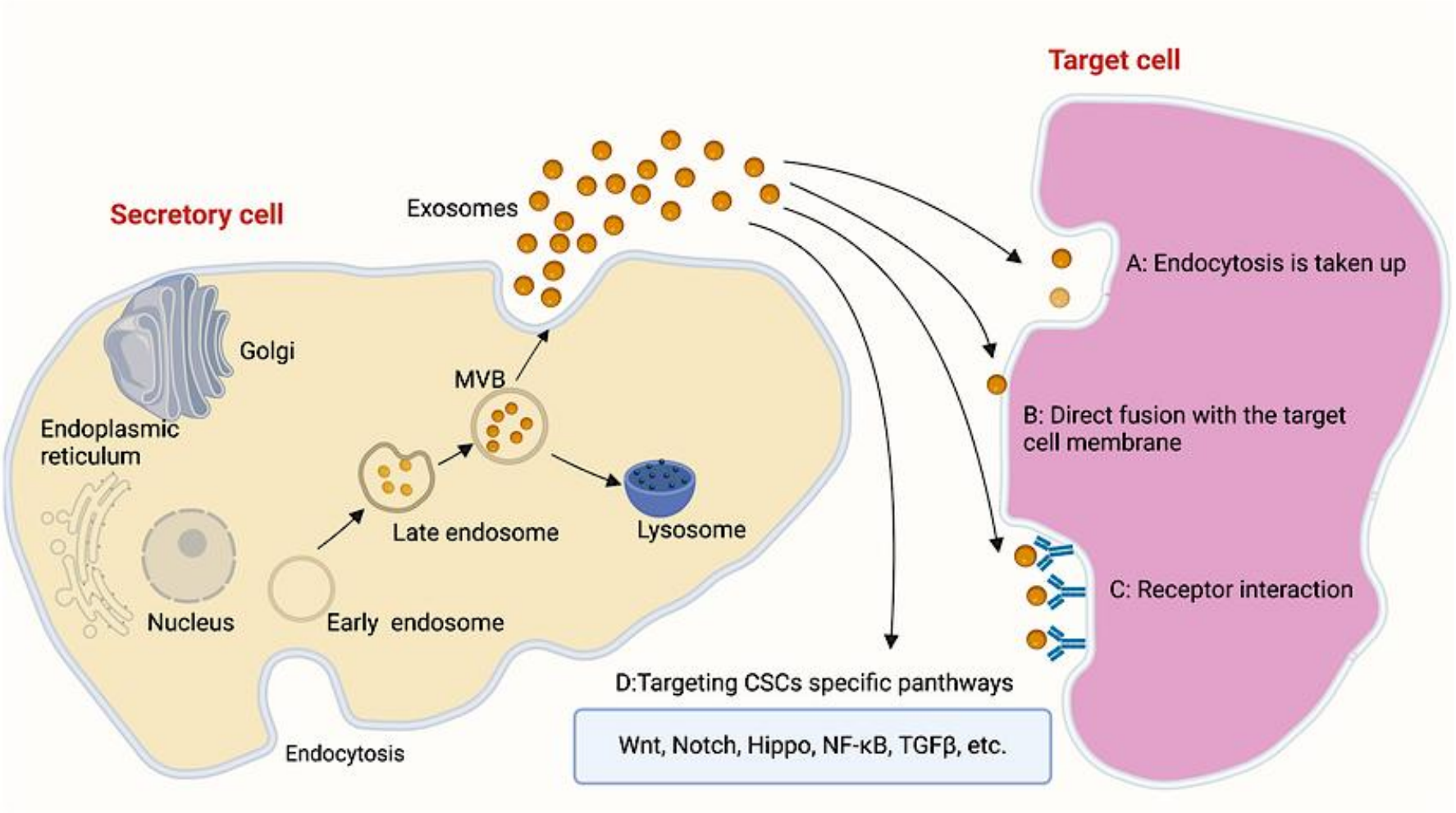
- At this stage, endosomes are termed **Multivesicular Bodies** containing numerous **intraluminal vesicles**.

5. Exosome Release

- **MVBs fuse with the plasma membrane**, releasing **exosomes** outside the cell.



How Does Exosome Work?



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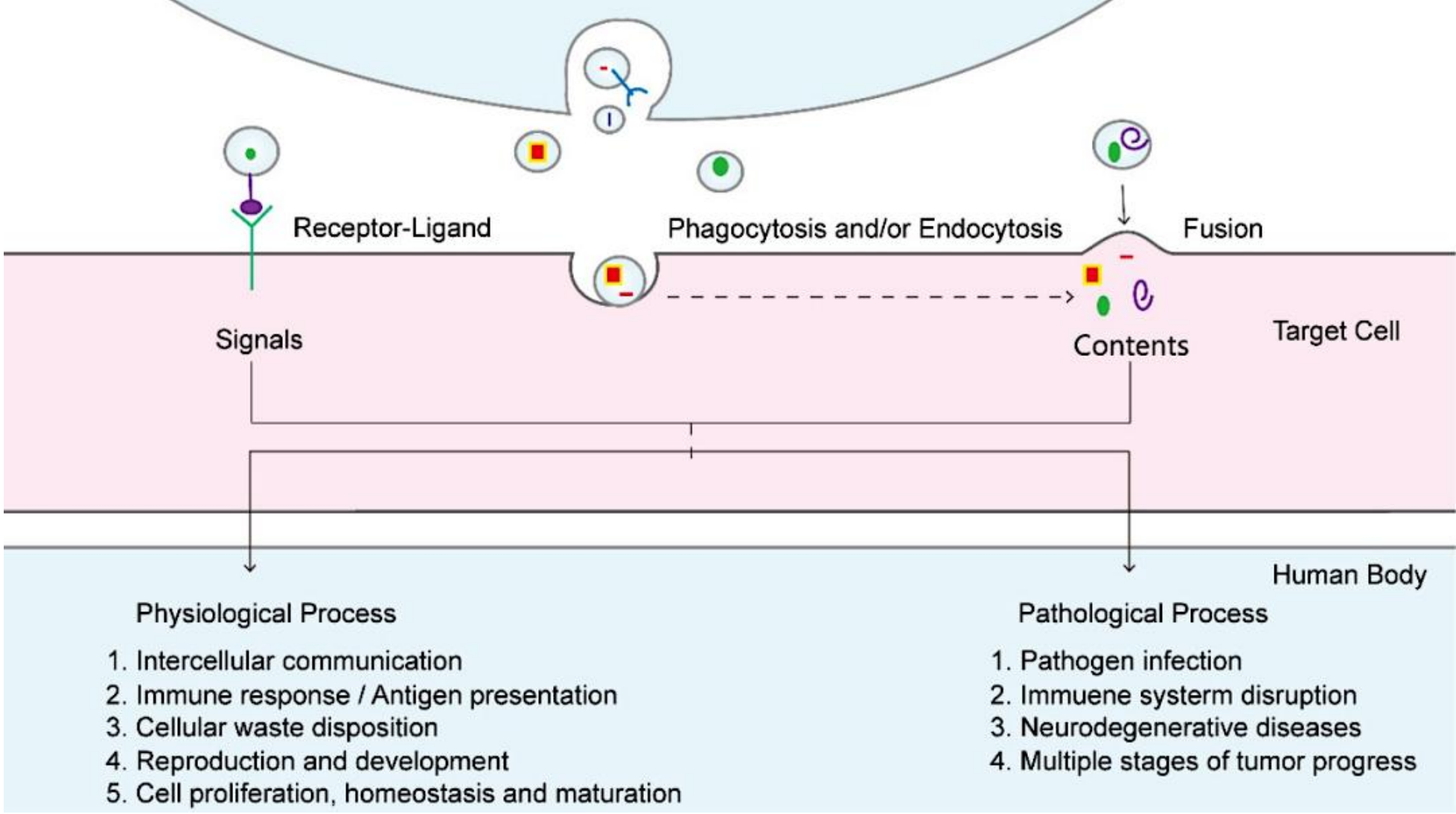

He C, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. Theranostics. 2018;8(1):237-255. Published 2018 Jan 1.

Bucharest 2-4 October 2025

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Sources of Exosomes

Body Fluids:

- Blood (serum and plasma)
- Urine
- Amniotic fluid
- Saliva
- Breast milk
- Cerebrospinal fluid

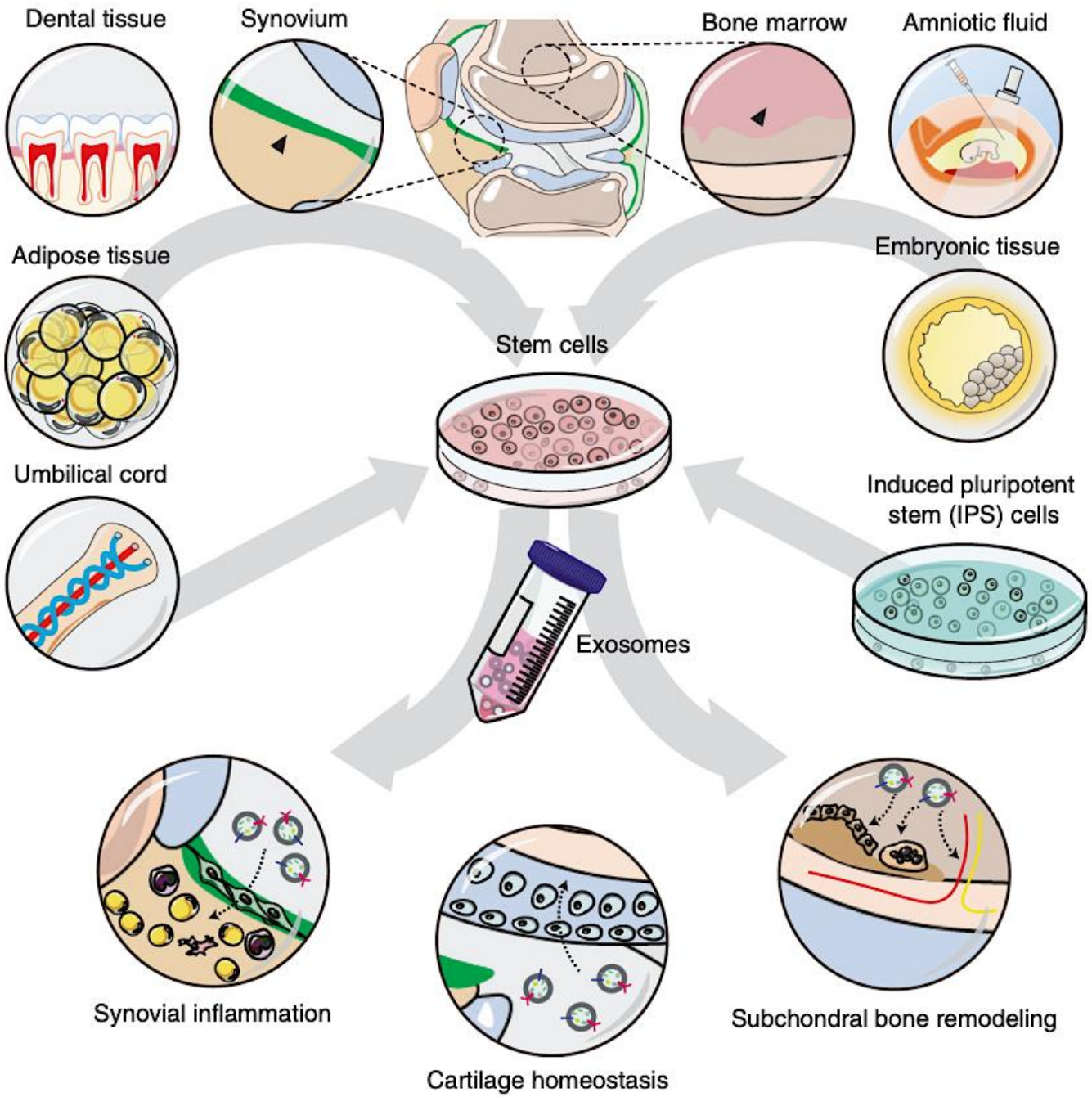
- Their widespread presence reflects their central role in maintaining ***cellular homeostasis*** and ***transmitting biological signals***.

Yang D, Zhang W, Zhang H, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics. 2020;10(8):3684-3707.

Sources of Exosomes

- **Stem cells** (e.g., mesenchymal stem cells)
- **Cell Culture Supernatants:** Exosomes are often obtained from cultured cells, where cells are grown and induced to release exosomes into the culture medium.
- **Cancer cell lines**
- **Immune cells** (e.g., dendritic cells and T cells)
- **Fibroblasts**
- **Tissues:** Exosomes can be isolated from tissue biopsies by applying specialized extraction techniques.
- **Synthetic Sources**

Sources of Exosomes



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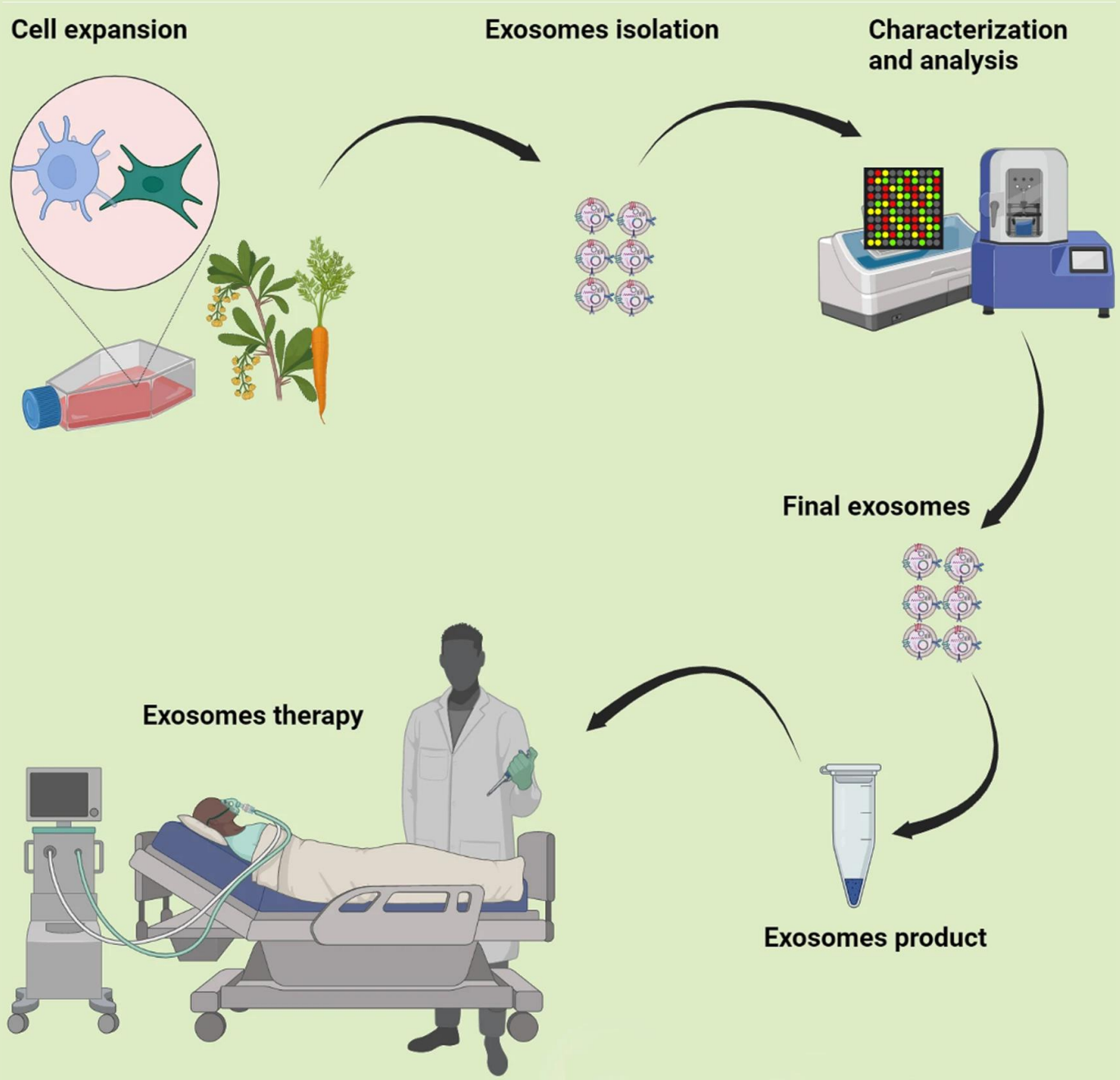


Manufacturing of Exosomes

- Exosomes are isolated using methods such as;
  - Ultracentrifugation
  - Size-exclusion chromatography
  - Precipitation kits
- Their source often determines their content and potential applications, especially in diagnostics, therapeutics, and cell communication studies.

Current Strategies for Exosome Separation

Isolation technique	Principle	Advantages	Disadvantages
Sequential ultracentrifugation	Particles have different density and size show different sediment speed under centrifugal force	• Low cost and • Low contamination risk with extra isolation reagents; •suitable for large volume preparation;	• High equipment requirement • Time consuming • Labor intensive • Potential mechanical damage due to high speed centrifugation • Protein aggregation • Not suitable for small volume diagnosis • Low portability
Gradient ultracentrifugation	After centrifugation in a dense medium, objects in a tube could stay in the position of the medium with similar density	• High purity of products • Allowing separation of subpopulation of exosomes	• Lower volume processability • High equipment requirement • Time consuming • Labor intensive • Potential mechanical damage due to high speed centrifugation • Not suitable for small volume diagnosis • Low portability
Ultrafiltration	Utilizing filter membrane with defined size-exclusion limit or molecular weight cut-off	• Low equipment cost • Fast procedure • good portability	• Moderate purity • Potential deterioration induced by shear stress • Possible loss due to clogging and membrane trapping
Size-exclusion chromatography	After adding to porous materials, substances eluted out in accordance with their particle size, with big particles eluted earlier	• High purity • Fast preparation • Keep native state of exosomes • Good reproducibility • Potential for both small and large sample capacity; • Capable of processing all type of samples	• Relatively high device costs • Additional method for exosome enrichment is required

Yang D, Zhang W, Zhang H, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics. 2020;10(8):3684-3707. Published 2020 Feb 19.

Current Strategies for Exosome Separation

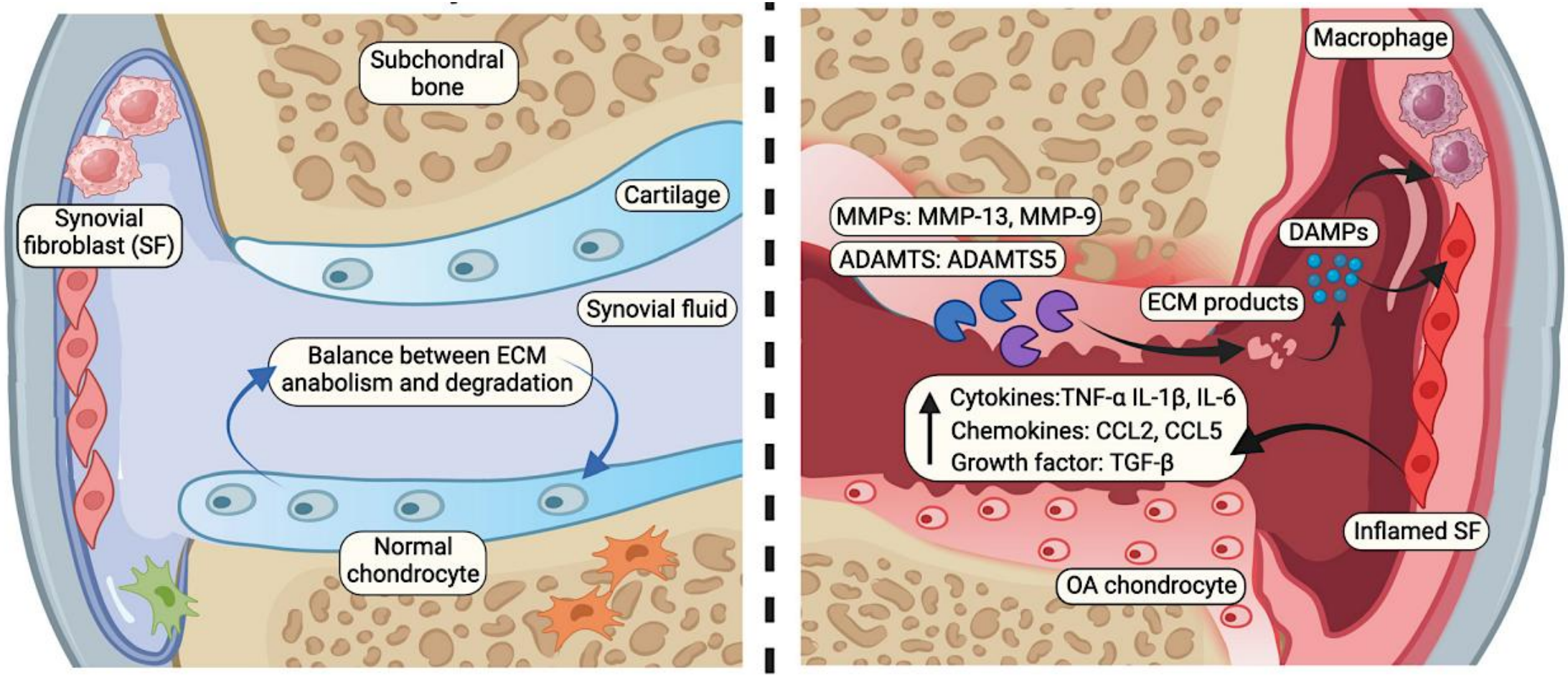
Isolation technique	Principle	Advantages	Disadvantages
Polymer Precipitation	High hydrophilic water-excluding polymers can alternate the solubility of exosomes	• Easy to use • Using ordinary equipment •Suitable for both small and large sample volume • High efficiency	• Contaminants of protein aggregates, other extracellular vesicles and polymeric contaminants • Extended processing time • Require complicated clean-up steps • Affecting downstream analysis and quantification
Immunoaffinity capture	Based on specific binding between exosome markers and immobilized antibodies (ligands)	• Suitable for separating exosomes of specific origin; • High-purity exosomes • Easy to use • No chemical contamination	• High-cost antibodies; • Exosome markers must be optimized • Low processing volume and yields • Extra step for exosome elution may damage native exosome structure
Microfluidics-based techniques	Based on different principles including immunoaffinity, size and density	• Highly efficient • Cost-effective • Portable • Easily automated & integrated with diagnosis	• Low sample capacity

Yang D, Zhang W, Zhang H, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics. 2020;10(8):3684-3707. Published 2020 Feb 19.

A comparison of the immunomodulatory effects of exosomes derived from various tissues and cells in healthy and osteoarthritic inflamed joint

Healty

Arthritic



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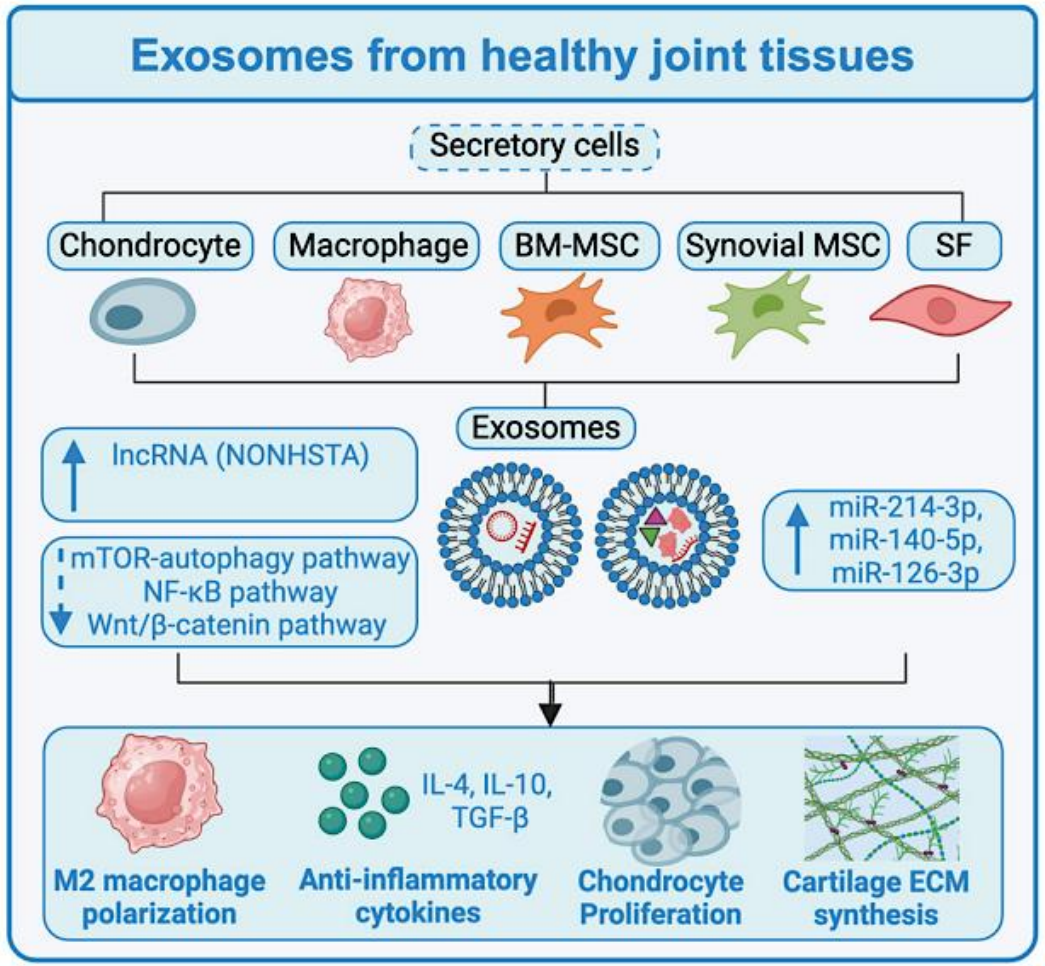
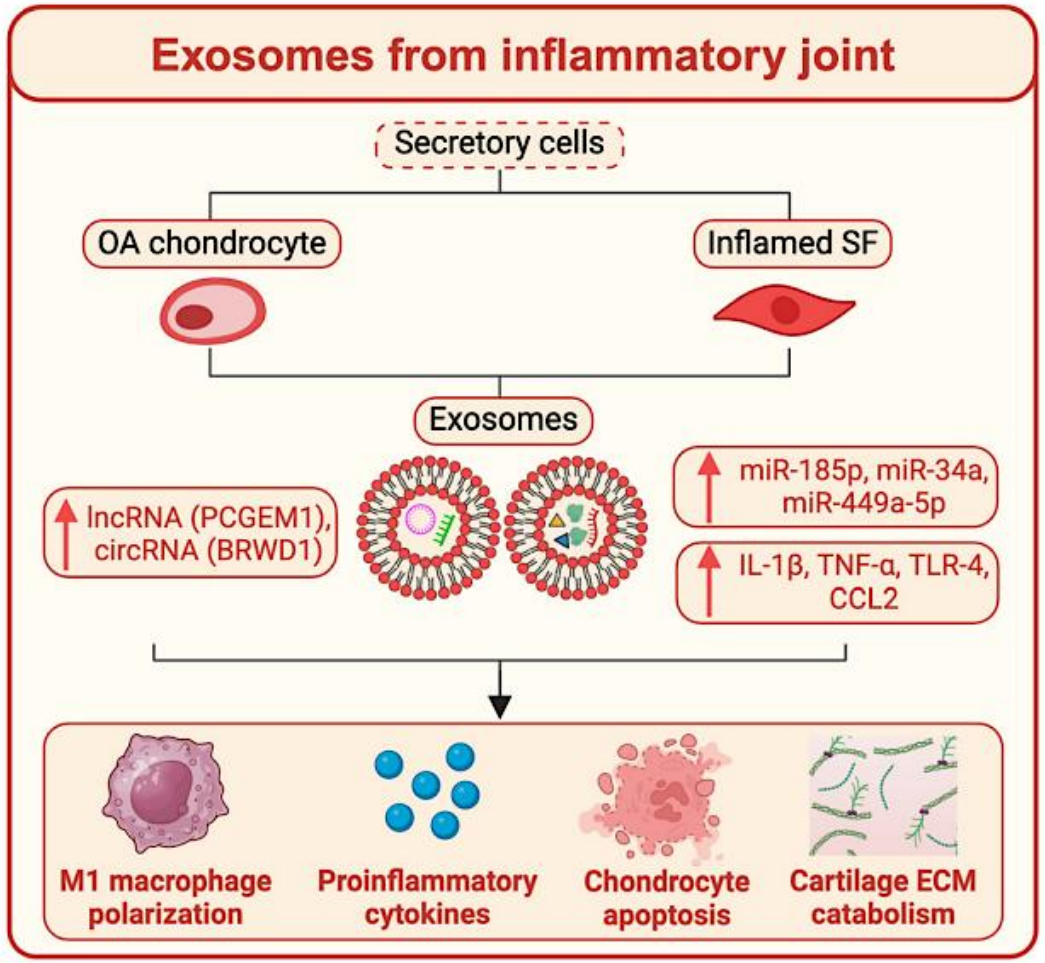


Arthritic



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Exosome-Based Therapies: Key Points

Rising Interest

- Preference for exosomes over stem cells due to similar therapeutic benefits.

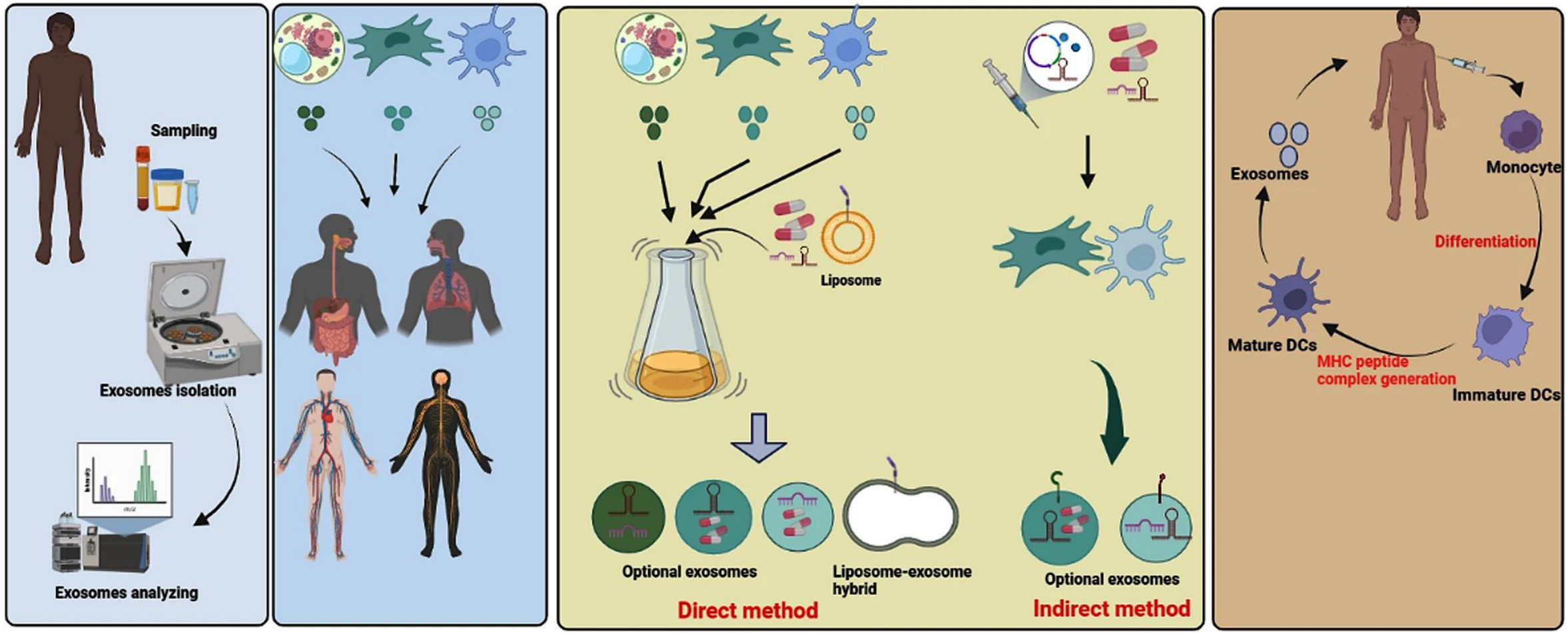
Therapeutic Potential

- Known to aid tissue recovery.

Enhancement Strategies

- ***Preconditioning*** of stem cells.
- ***Genetic modification*** and ***use of biomaterials***.

Clinical Application of Exosomes

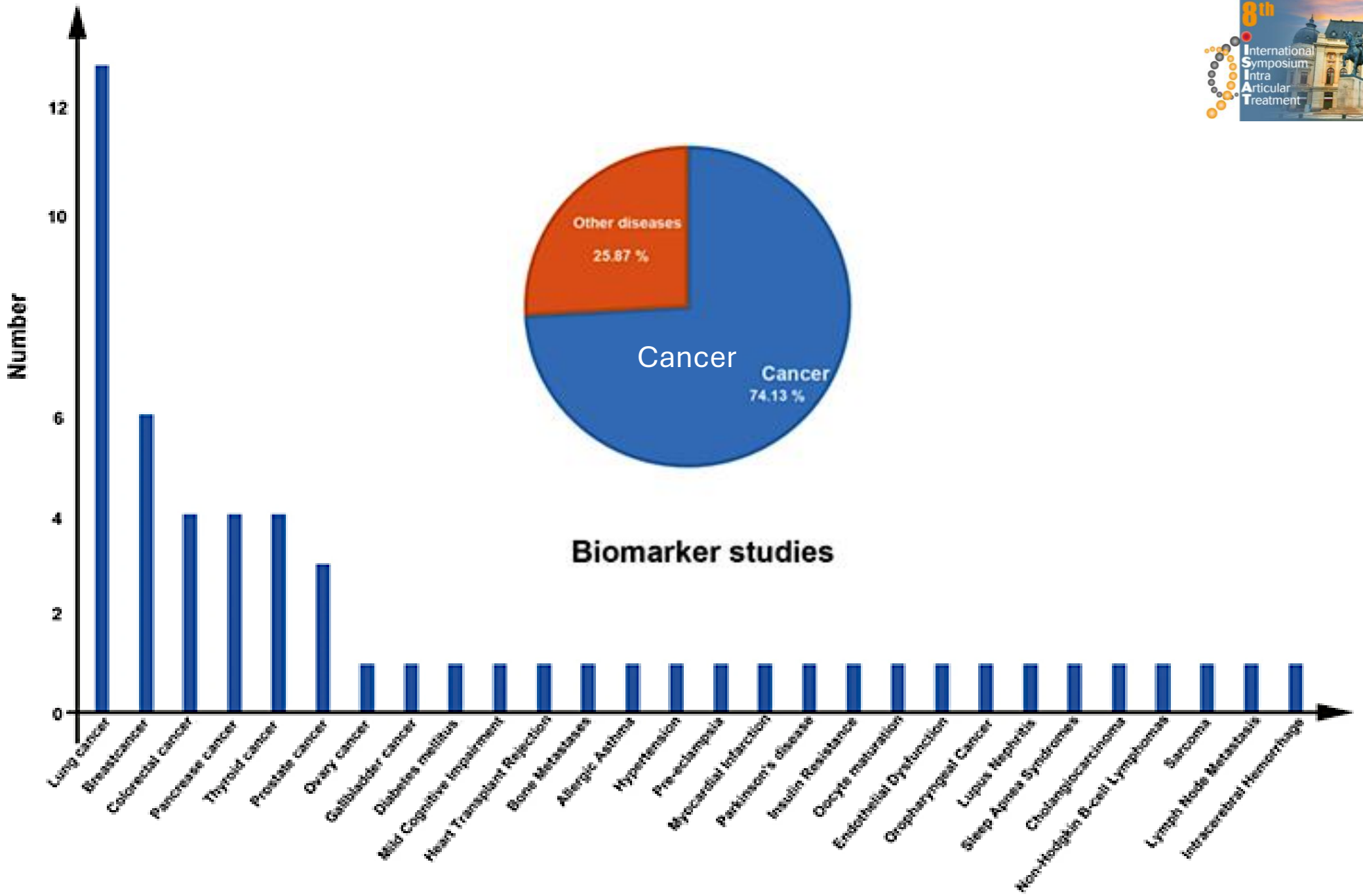


Biomarker

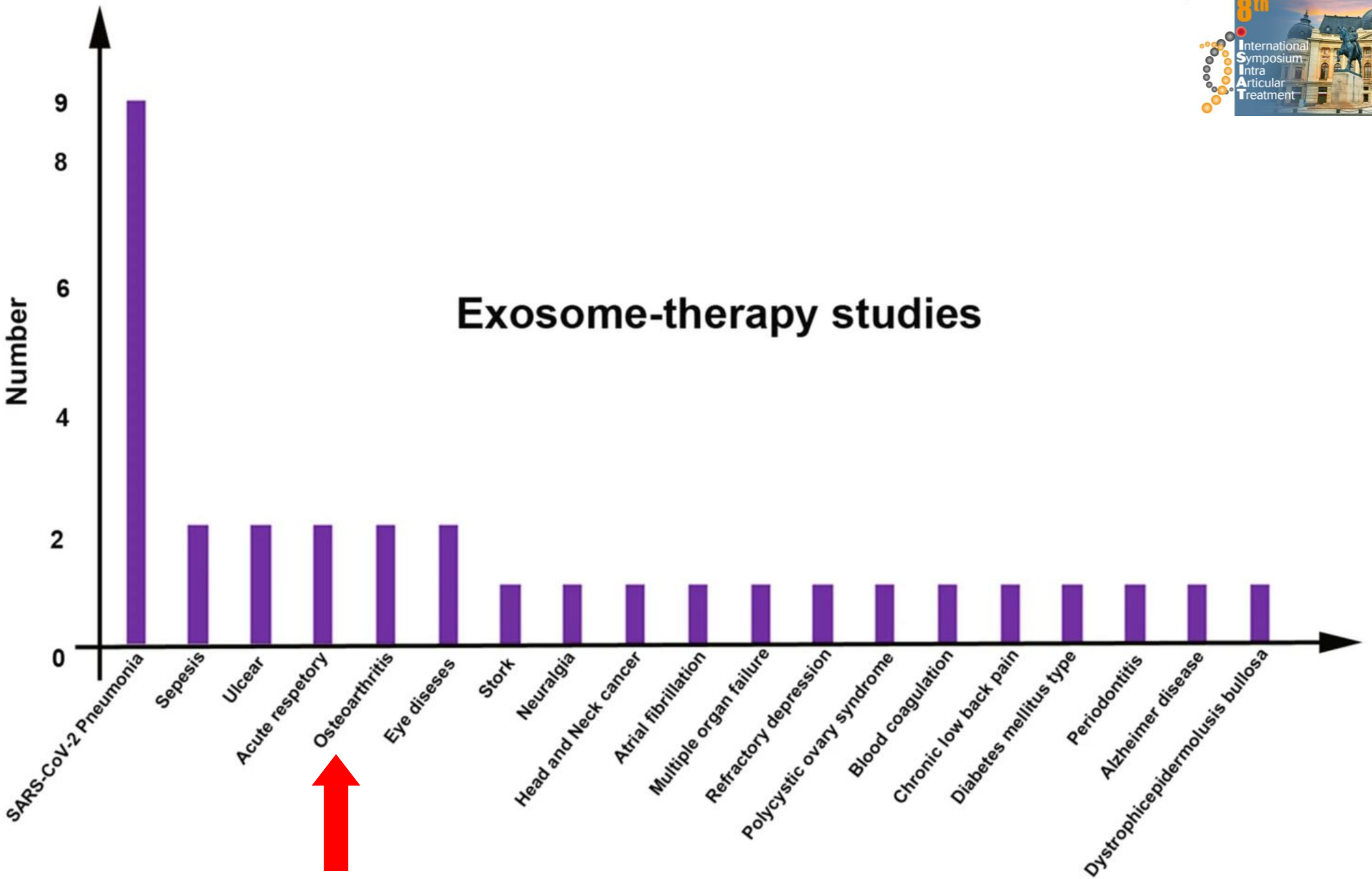
Exosome-therapy

Exosome drug delivery system

Exosome-vaccine


Number
12
10
6
4
2
0
Other diseases
25.87 %
Cancer
Cancer
74.13 %
Biomarker studies
Lung cancer
Breast cancer
Colorectal cancer
Pancreas cancer
Thyroid cancer
Prostate cancer
Ovary cancer
Gallbladder cancer
Diabetes mellitus
Mild Cognitive impairment
Heart Transplant Rejection
Bone Metastases
Allergic Asthma
Hypertension
Pre-eclampsia
Myocardial infarction
Parkinson's disease
Insulin Resistance
Oocyte maturation
Endothelial Dysfunction
Oropharyngeal Cancer
Lupus Nephritis
Sleep Apnea Syndromes
Cholangiocarcinoma
Non-Hodgkin B-cell Lymphomas
Sarcoma
Lymph Node Metastasis
Intracerebral Hemorrhage

Exosome-therapy studies



Advantages of Exosome-Based Therapeutics

- **Precision Targeting:** Ability to target diseased tissues specifically.
- **Reduced Immunogenicity:** Lower chance of immune rejection than whole-cell therapies.
- **Non-Tumorigenic:** Safer, as they lack replicative potential.
- **Stability and Storage:** Easier storage and handling than live cells.

Key Questions in Exosome Therapy Development

- **What Are the Mechanisms of Action?**
  - How do exosomes deliver therapeutic effects at the molecular level?
- **How Do We Ensure Consistent Efficacy?**
  - Variability in exosome quality based on source, isolation methods.
- **What is the Optimal Dosage?**
  - Lack of standardized dosing due to variable effects.

Challenges in Clinical Translation

- **Manufacturing & Standardization:**
  - Difficulties in large-scale production and maintaining consistency.
- **Purity and Safety Concerns:**
  - Risk of contamination and unintended immune reactions.
- **Regulatory Issues:**
  - Complex regulatory pathways due to limited clinical precedents for exosome-based therapies.

Key Applications of Exosome-Based Therapies

- **Oncology:** Targeted drug delivery, immune modulation, inhibition of tumor growth.
- **Neurology:** Potential for neuroprotection, repairing neural injuries, treating conditions like Alzheimer's and Parkinson's.
- **Cardiology:** Angiogenesis promotion, repair of cardiac cells post-myocardial infarction.
- **Wound Healing and Regenerative Medicine:** Accelerated healing through stimulation of tissue repair.

The Role of Exosomes in Musculoskeletal Diseases

1. Lysyl Oxidases and Extracellular Matrix Stabilization

2. Role in Inflammatory Environments:

E.g., chondrocytes treated with IL-1β, which mimics the OA environment, release EVs that increase the production of MMP13 by synovial fibroblasts.

3. Role in Bone Remodeling and Mineralization:

In addition to participating in bone synthesis and resorption, extracellular vesicles actively contribute to the regulation of ECM mineralization. They play a crucial role in initiating mineralization at the growth plate during endochondral ossification.

The Role of Exosomes in Musculoskeletal Diseases

4. Angiogenic Effects

Angiogenesis is defined as the formation of new blood vessels through sprouting and internal growth from the existing vascular network and is critical for the regeneration of vascularized tissues.

This process includes the proliferation and migration of endothelial cells and the stabilization of newly formed vessels by mural cells (e.g., pericytes).

The Role of Exosomes in Musculoskeletal Diseases

5. Immunomodulatory Effects

They can contribute to both the activation and suppression of immune responses. EVs derived from bone marrow MSCs (BMSCs) have been extensively studied due to the immunomodulatory capacities of MSCs. EVs can be used as a potent non-cellular immunomodulatory agent without the challenges of cell transplantation.

Molecular Mechanisms of Action

- The therapeutic activity of exosomes is mediated by multiple intracellular pathways, including p38 , ERK, AKT, HDAC3, and NF-κB.
- Through the delivery of microRNAs, exosomes regulate pro-inflammatory cytokines such as interleukin-6 (IL-6), while simultaneously modulating matrix metalloproteinases like MMP-13, a key driver of cartilage destruction.

Wang Y, Kong Y, Du J, et al. Injection of human umbilical cord mesenchymal stem cells exosomes for the treatment of knee osteoarthritis: from preclinical to clinical research. J Transl Med. 2025;23(1):641. Published 2025 Jun 11.

Human Umbilical Cord MSC-Derived Exosomes

- Human umbilical cord MSCs (hUC-MSCs) represent an advantageous source of therapeutic exosomes due to their accessibility and potent biological activity.
- hUC-MSC-derived exosomes mobilize chondrocytes to damaged cartilage, inhibit apoptosis, and promote regeneration through mechanisms including suppression of MMP13 and ADAMTS5

Wang Y, Kong Y, Du J, et al. Injection of human umbilical cord mesenchymal stem cells exosomes for the treatment of knee osteoarthritis: from preclinical to clinical research. J Transl Med. 2025;23(1):641. Published 2025 Jun 11.

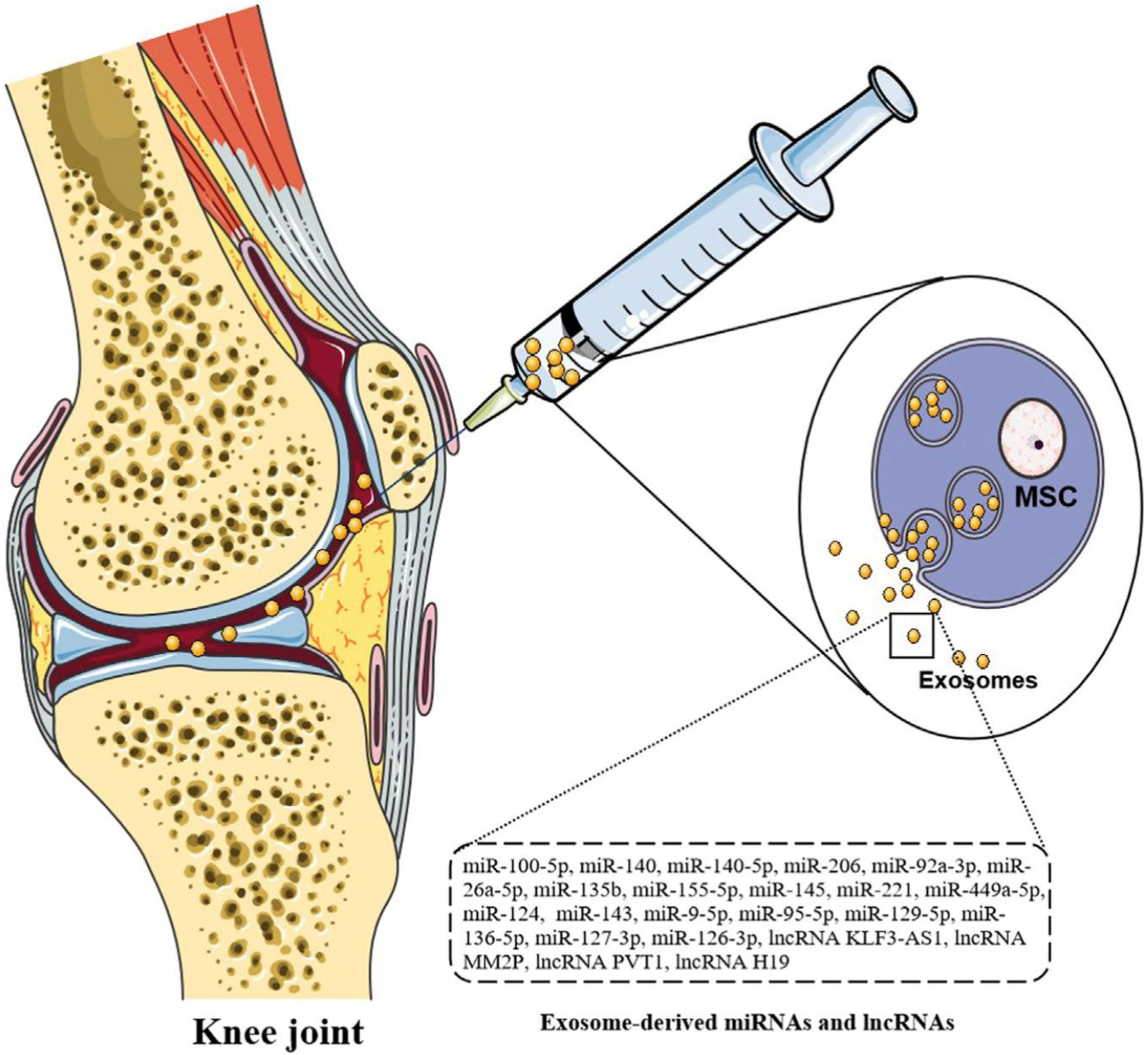
MSC-Derived Exosomes in OA Therapy

- Mesenchymal stem cell (MSC) - derived exosomes have demonstrated therapeutic potential.
  - Modulating inflammatory responses
  - Promoting anabolic metabolism of chondrocytes
  - Enhancing cartilage repair
- They represent a promising avenue for OA treatment.

Liu B, Xian Y, Shen T, et al. Targeted Blockage of Pathological Extracellular Vesicles and Particles From Fibroblast-Like Synoviocytes for Osteoarthritis Relief: Proteomic Analysis and Cellular Effect. J Extracell Vesicles. 2025;14(9):e70162.

Bone Marrow MSC-Derived Exosomes

Direct injection of exosomes carrying ***miRNAs*** and ***lncRNAs*** can significantly down-regulate arthritis score, ***inhibit fibroblast-like synoviocytes proliferation and invasion***, and reduce inflamatory response and joint damage.



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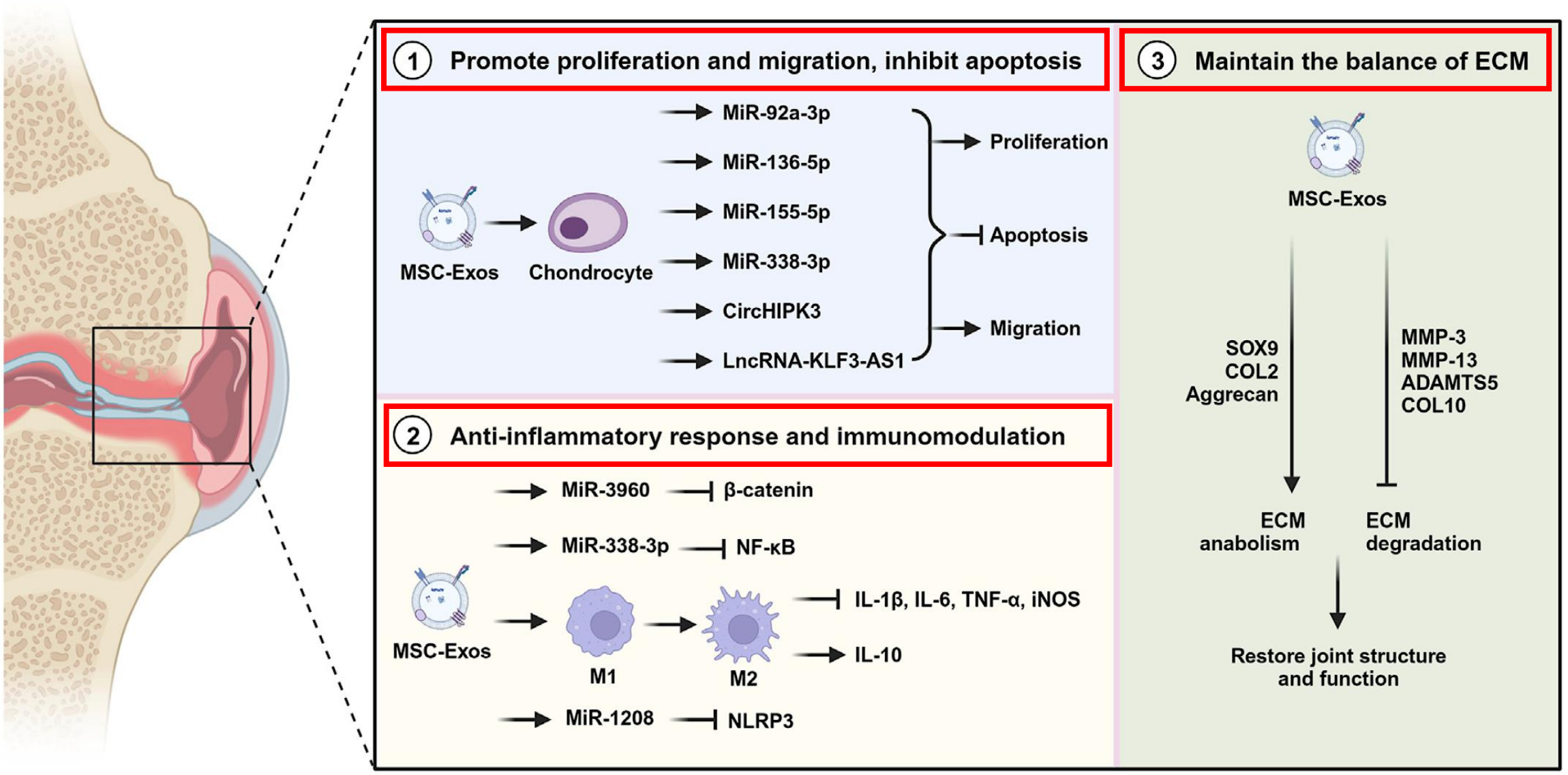
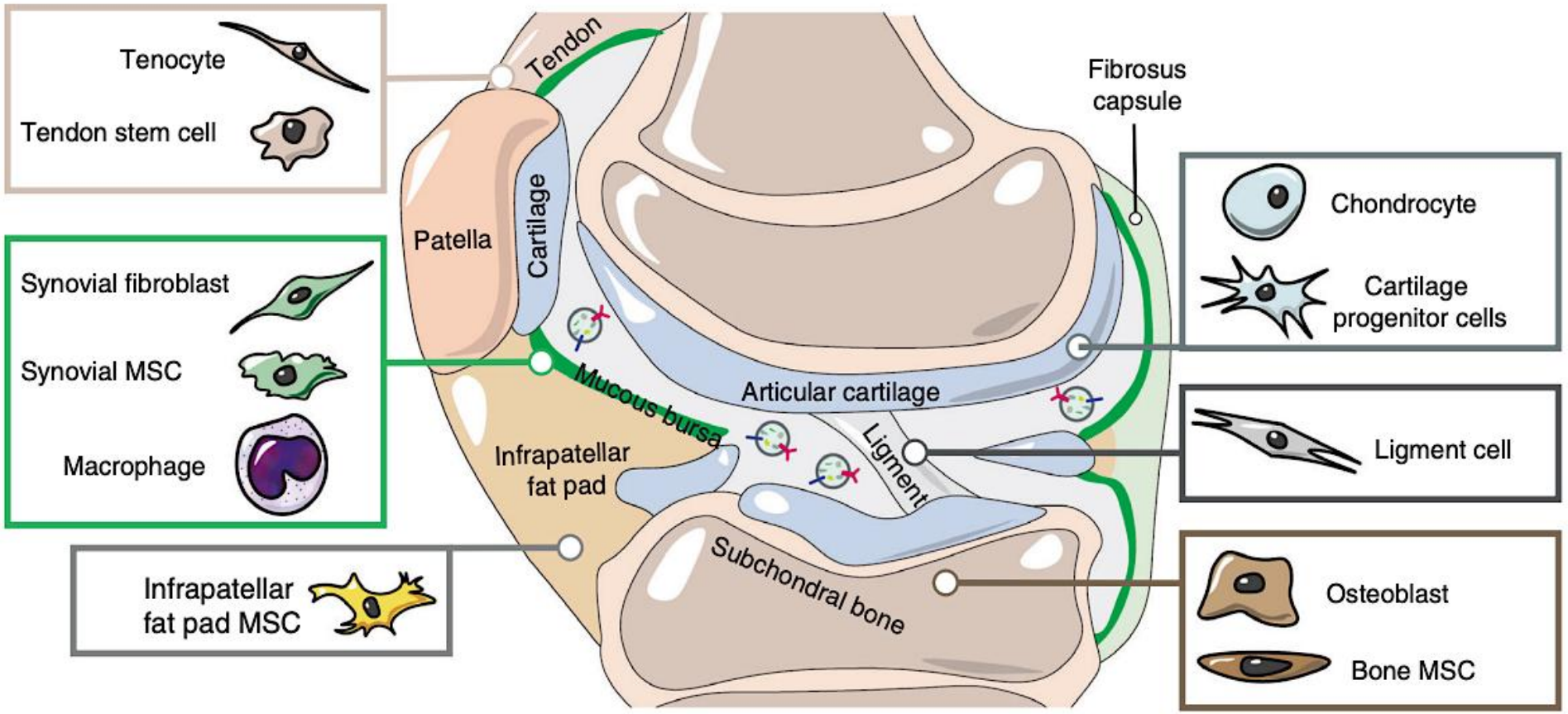
The Potential Mechanisms of MSC-Exosomes for OA Treatment.



Luo D, Zhu H, Li S, Wang Z, Xiao J. Mesenchymal stem cell-derived exosomes as a promising cell-free therapy for knee osteoarthritis. Front Bioeng Biotechnol. 2024 Jan 16;12:1309946.

The Exosomes From Different Tissues of OA Joint and Their Potential Biological Effects



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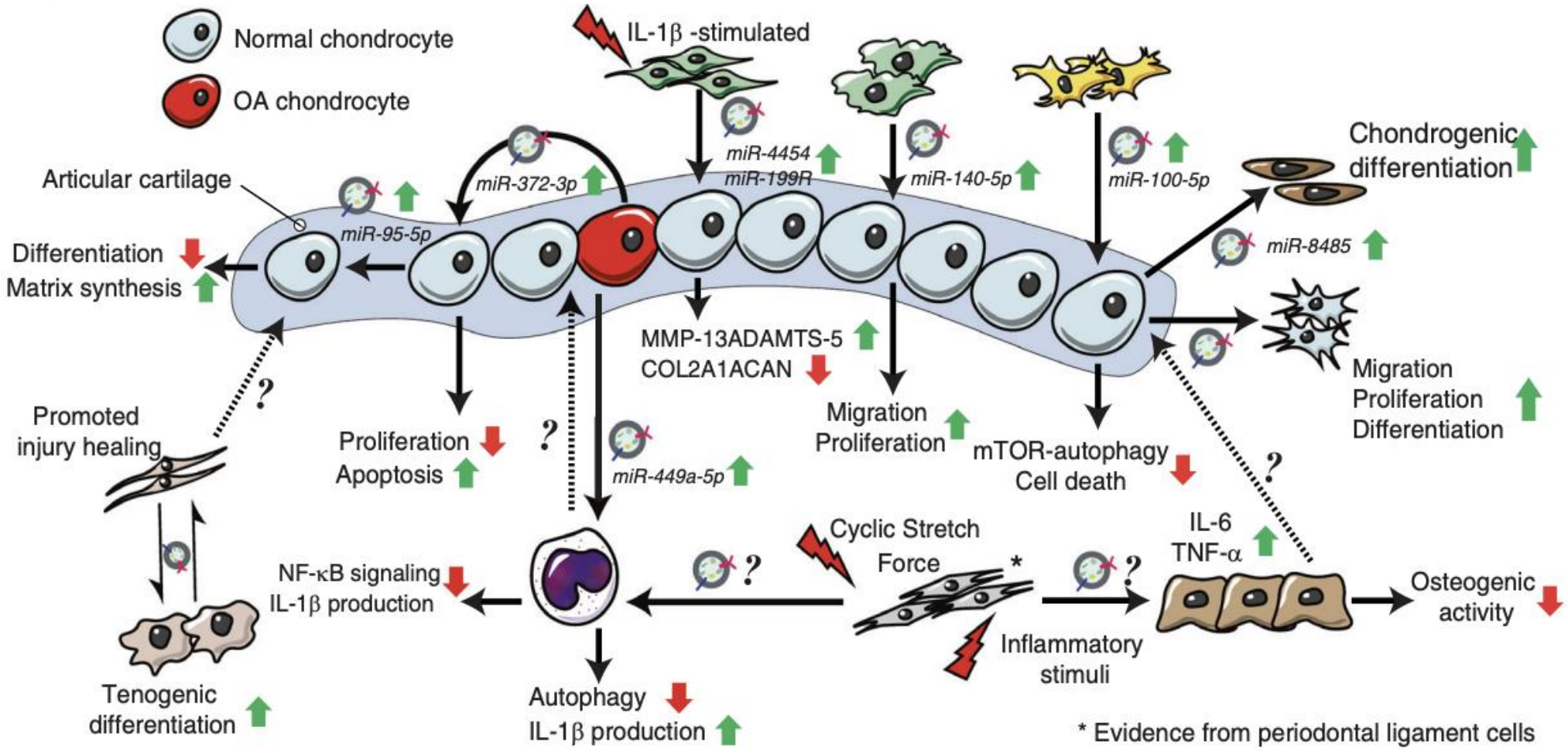

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Wang *et al. Journal of Translational Medicine* (2025) 23:641
https://doi.org/10.1186/s12967-025-06623-y

Journal of Translational Medicine

RESEARCH Open Access

Injection of human umbilical cord mesenchymal stem cells exosomes for the treatment of knee osteoarthritis: from preclinical to clinical research

Yuzhong Wang[1], Yajie Kong[1,3], Jiejie Du[5], Lifei Qi[5], Meiling Liu[1,3], Siyi Xie[1,7], Jianghui Hao[4], Ming Li[1,7], Shuxing Cao[1], Huixian Cui[3,7,8], Aijing Liu[6,3,7*], Jun Ma[3,7,8*] and Yongzhou Song[1,2,3,7,8*]

- **Efficacy**: Dose-dependent trends observed; ***high-dose group showed the most consistent and durable improvements*** in pain, stiffness, function, and MRI cartilage signals.
- **Safety**: Intra-articular hUC-MSC–exosome injections were well tolerated.
- **Limitations**: Small sample size, no placebo control, only K–L grade 2–3 patients.

Bolandnazar *et al. BMC Musculoskeletal Disorders* (2024) 25:856
https://doi.org/10.1186/s12891-024-07979-w

BMC Musculoskeletal Disorders

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Safety and efficacy of placental mesenchymal stromal cells-derived extracellular vesicles in knee osteoarthritis: a randomized, triple-blind, placebo-controlled clinical trial

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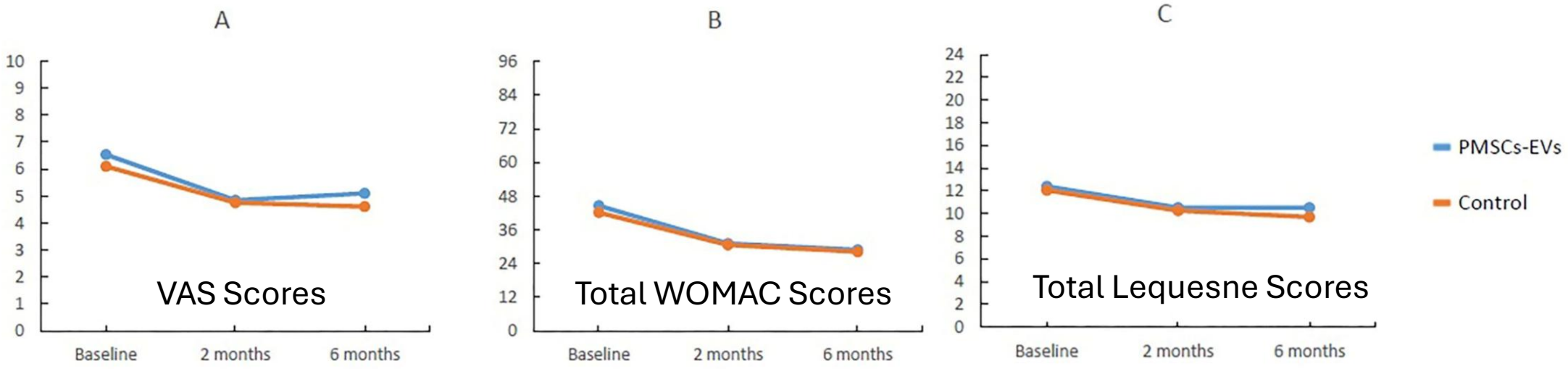
No different from placebo



Bucharest 2-4 October 2025

Agenda

- Introduction to Exosome Biology
- Methods of Obtaining Exosomes
- Clinical Applications of Exosomes
- Future Perspectives

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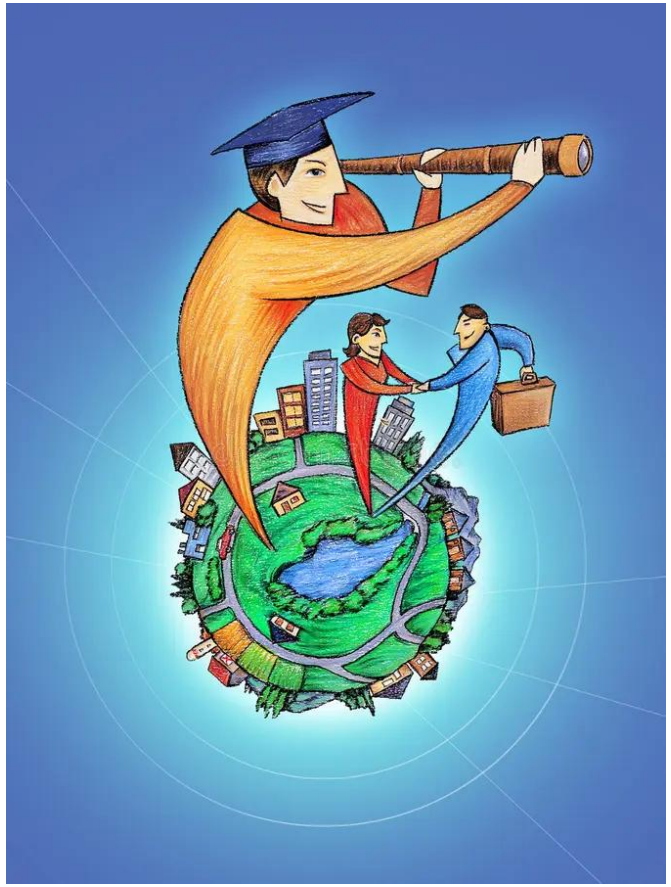
Ongoing / Recent Trials on Intra-Articular Exosomes for Knee OA

NCT / ID	*Title & Product*	*Sponsor / Country*	*Phase*	*Status (latest)*	*Design & Dose*	*Planned N*
NCT06431152	*Intra-articular Injection of UC-MSC Exosome in Knee OA* (UC-MSC sEVs)	Universidad de los Andes, **Chile**	I	**Recruiting** (May 2024)	Open-label, single IA dose; **dose-escalation** cohorts; primary focus: safety/feasibility; est. completion **Dec 31, 2025**	**12** CenterWatch
NCT05060107 (ExoOA-1)	*Intra-articular Injection of MSC-derived Exosomes in Knee OA* (XO-101)	Francisco Espinoza / Cells for Cells, **Chile**	I	**Active – Not Recruiting** (Sep 2021)	Phase 1 safety; IA injection of GMP sEVs; follow-up to 12 mo; listed completion Oct 2023	**10** CenterWatch
NCT06466850	*Mesenchymal Stem Cells Derived Exosomes in Osteoarthritis Patients*	Isfahan Univ. of Medical Sciences, **Iran**	I (per listing)	**Recruiting** (current as of Sep 2025)	IA MSC-exosomes; includes K-L grade 1–3; BMI window; detailed criteria published; completion not reported	— ClinConnect
NCT06937528	*Use of Extracellular Vesicles (EV) for Knee Osteoarthrosis*	University of Jordan, **Jordan**	I (dose-finding)	**Recruiting** (Apr 2025)	IA EVs; **dose-finding** for advanced K-L III–IV; adults 42–75	— CenterWatch+1

Future Perspectives in Exosome Research

- **Biological Engineering:** Enhancing exosomes with synthetic modifications.
- **Optimized Production:** Innovations in purification techniques.
- **Regulatory Frameworks:** Development of guidelines specific to exosome-based therapies for smoother clinical adoption.
- **Clinical Trials and Evidence Building:** Increasing number of trials to establish safety, efficacy, and therapeutic protocols.



Conclusion

- At present there are no well-designed phase II/III randomized controlled human trials proving the efficacy of exosomes (especially MSC-derived exosomes) as intra-articular injections for knee osteoarthritis or other musculoskeletal disorders.
- The available human data are very limited and mostly early-phase or observational.

İmportant!

- **Patient Safety**
  - Ensuring safe practices through GMP compliance.
- **Research Needs**
  - Comprehensive clinical trials for better regulation and patient safety.

diracoglu

Thank You!