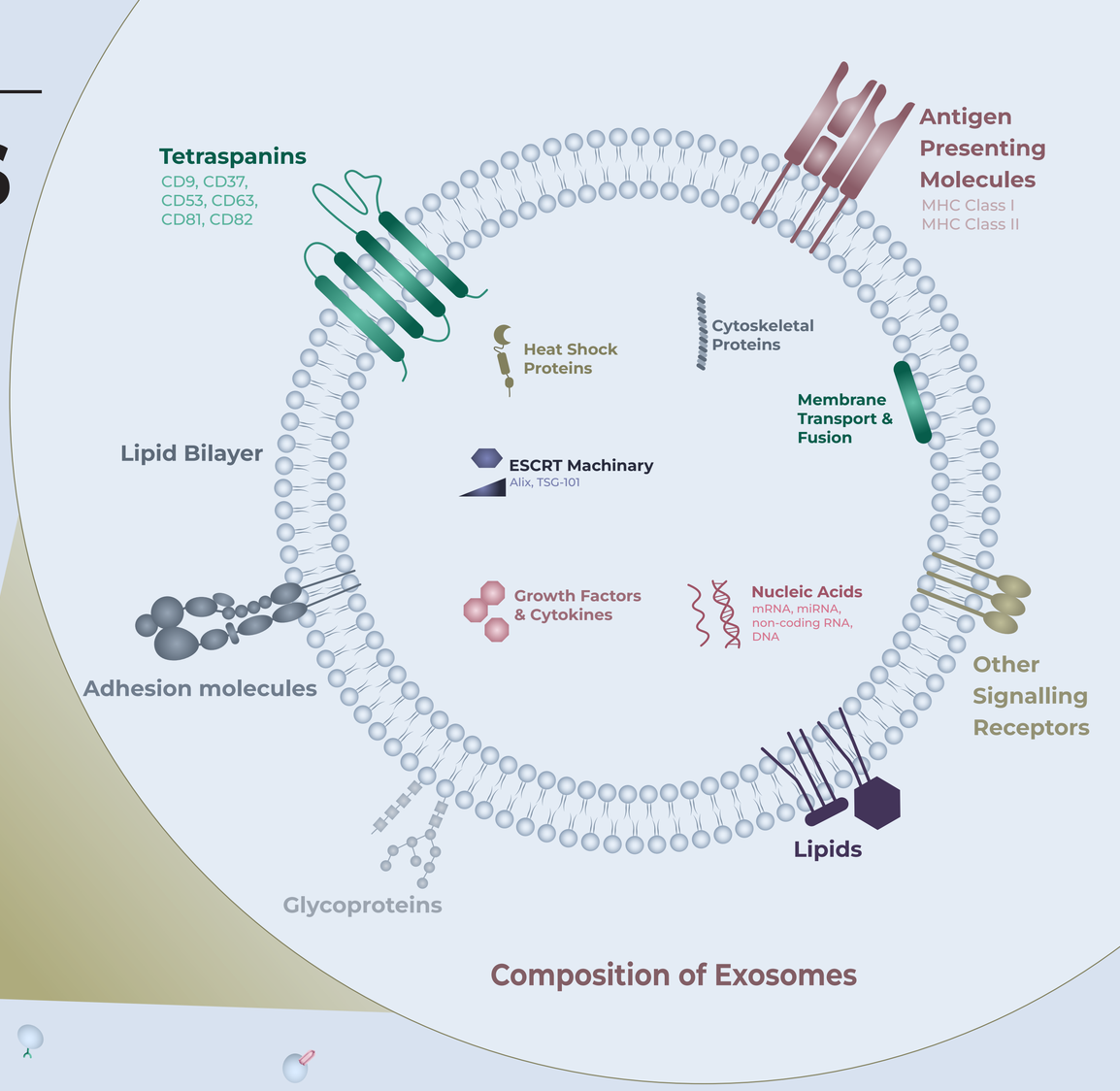
Understanding Exosomes

What is Exosome?

Extracellular Vesicles (EVs) are lipid-bound vesicles secreted by cells into the extracellular space. The three main types of EVs are:

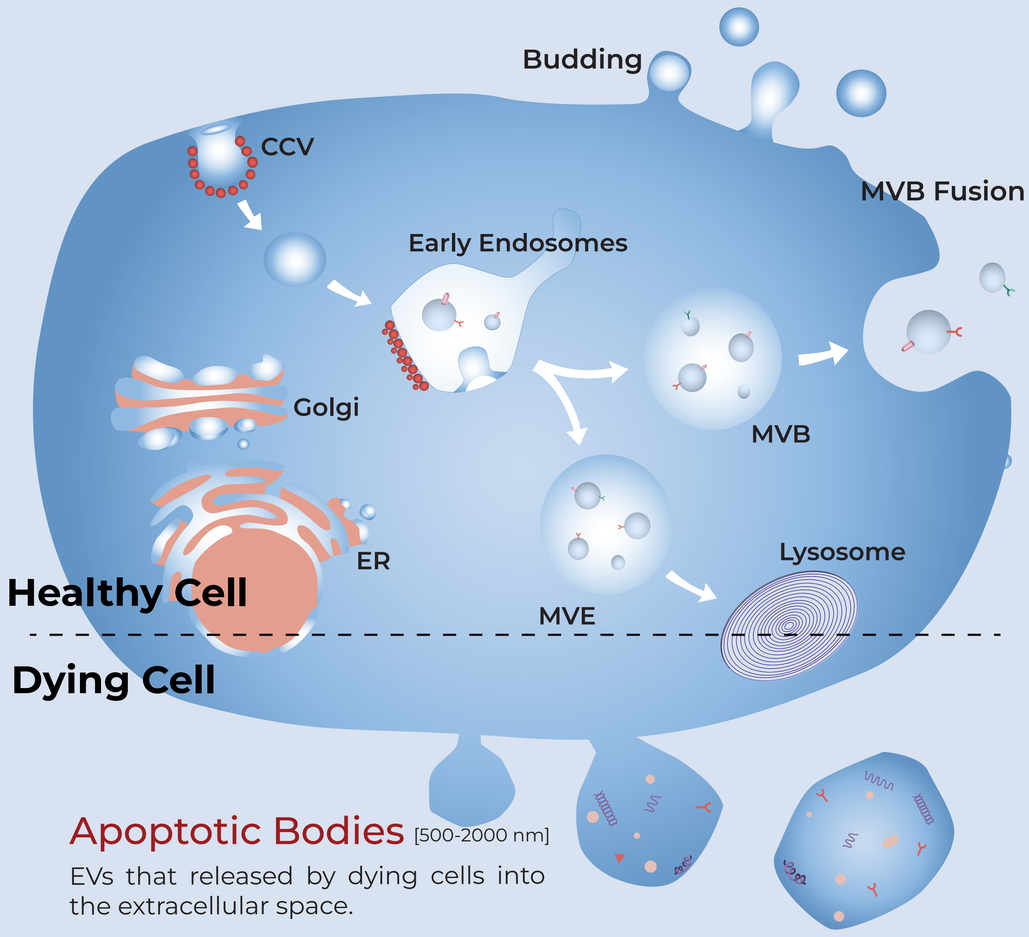
- Exosomes
- Microvesicles
- Apototic Bodies



Composition of Exosomes

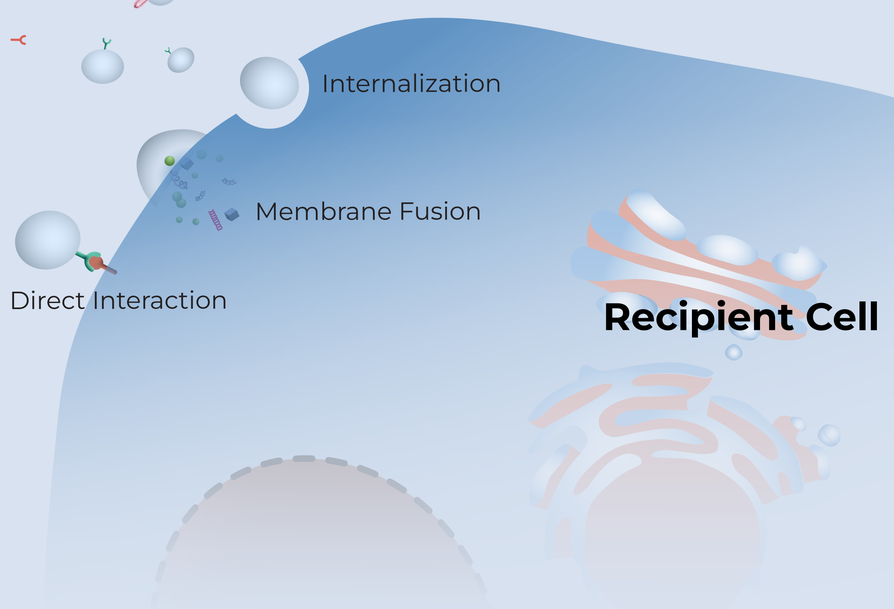
Microvesicles [50-1000 nm]

Extracellular vesicles that forms by directly outward budding, or pinching, of the cell's plasma membrane.



Exosomes [30-150 nm]

Intraluminal vesicles that are enclosed within a single outer membrane, and are secreted by all cell types.



Apoptotic Bodies [500-2000 nm]

EVs that released by dying cells into the extracellular space.

EV Guidance in Standarization of Protocols & Reporting

-- MISEV Guidelines

The Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines were first released in 2014 by the **International Society For Extracellular Vesicles (ISEV)** to provide guidance in standardization of protocols and reporting in the extracellular vesicle field.

The guideline was updated with broad community input in 2018 as MISEV 2018, with 318 authors. The MISEV 2018 updated the topics in

1. Nomenclature
2. Collection and Preprocessing
3. EV separation and concentration
4. EV characterization
5. Functional studies
6. Reporting

**Refer to the Complete MISEV2018 Guidelines in https://www.tandfonline.com/doi/full/10.1080/20013078.2018.1535750

MISEV 2018 is now the field-leading consensus document on the best practices and reporting of EV research.

Clinical Trials using Exosomes

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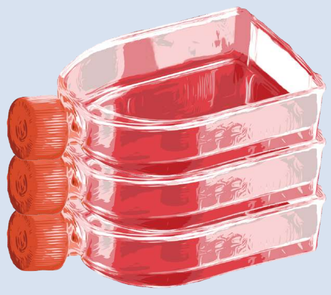
Bioprocessing

Mesenchymal Stem Cell (MSC)-derived Exosomes

Bioprocessing is defined as any process that uses complete living cells or their components (e.g. bacteria, enzymes, chloroplasts) to obtain desired value-added products.



Low-Speed Centrifugation

Cell Lysates

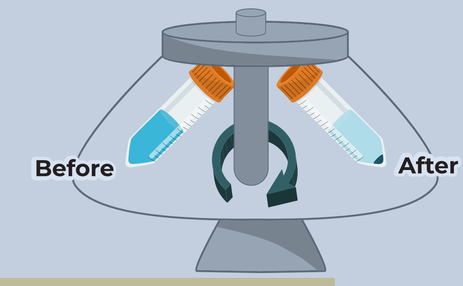
Conditioned Medium (CM)

1 MSC Culture

Exosomes are released by cells into biological fluids in vivo and cell culture conditioned media in vitro.

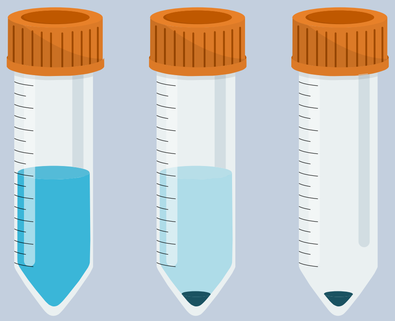
2 Exosome Isolation

Based on size and affinity of exosomes, different isolation strategies can be used to isolate them from CM.



Ultracentrifugation

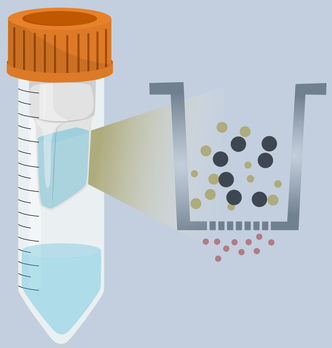
High-speed centrifugation is used to purify exosomes from other cell components and contaminants. After purification of the potential contaminants, the remaining supernatant is centrifuged at 100,000g to pellet exosomes.



Precipitation by PEG

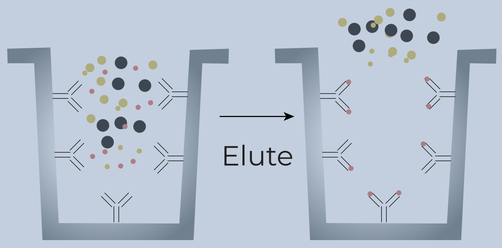
The addition of PEG solution could wrap the exosomes together, thereby forming exosome aggregates which could be easily precipitated by low-speed centrifugation.

Ultrafiltration

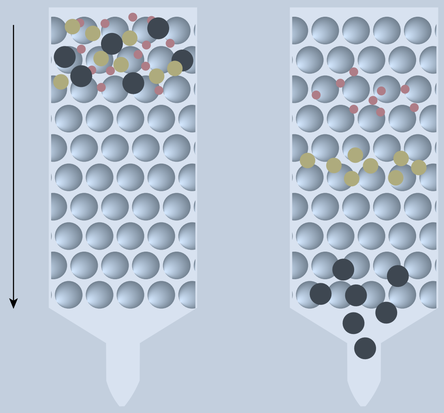


Ultrafiltration uses an ultrafine nano-membrane with different filter sizes to isolate exosomes from cell culture medium. The medium is purified through 1000-nm filter and 50-kD cut-off to remove other cell components and finally collect the exosomes <200 nm via a 200-nm filter.

Immunoaffinity Capture

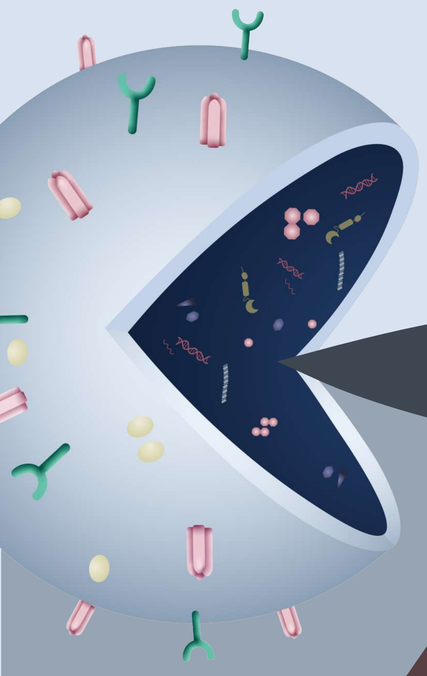


This technique relies on recognition of antigens exposed on the surface of an exosome by specific antibodies covalently bound to magnetic microsphere.



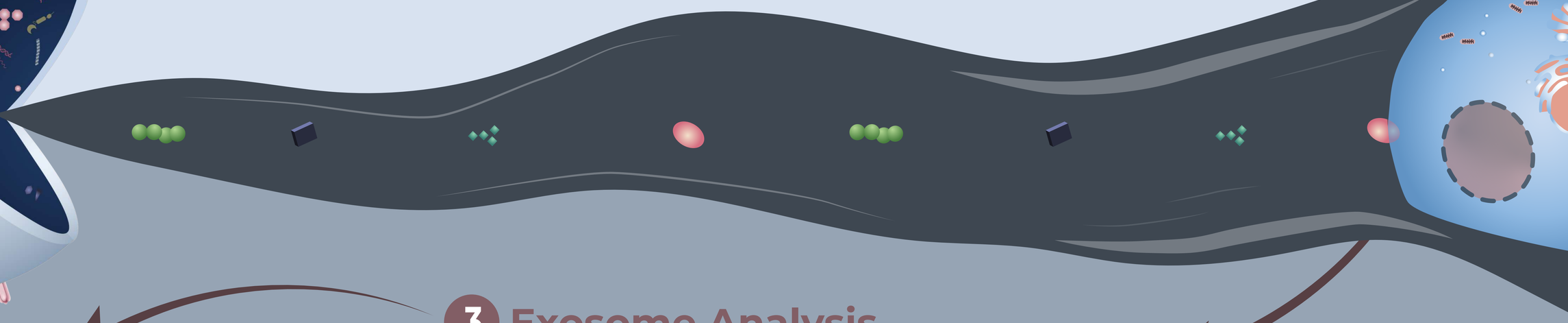
Size Exclusion Chromatography

Size Exclusion Chromatography (SEC) purification uses porous resin to separate molecules by size. The particles with smaller radius, enter into the pores for longer traffic distance, will elute later than the larger particles.



Considerations for the Selection of Exosome Isolation Method

- The Exosome Recovery Rate/Yield
- The Purity of the Obtained Exosomes
- Time & Labor Cost



3 Exosome Analysis

To analyse and verify the exosome identity. Generally, the specific markers on the exosomes will be used to identify the exosome.

Western Blot

Exosomes isolated from a variety of sources are enriched with a number of proteins that differ from the originating cell and other types of vesicles. Identifying these proteins in a sample is indicative of the isolated vesicles being exosomes.

Electron Microscope

Due to the small sizes of exosomes, electron microscope is the common method to study exosome morphology. Regular Transmission EM can be used to:

1. Validate the existence of exosomes
2. Access the quality of exosomes
3. Study the morphology of exosomes

Nanoparticle Tracking Analysis

NTA is used for the measurement of exosome concentration and size distribution. It has better reproducibility as compared to TEM and flow cytometry.

Flow Cytometry

Prior to detection, exosomes are attached to beads (as the exosomes size fall below the resolution limit). Flow cytometry can be used to detect specific membrane proteins/markers.

4 Preservation of Exosomes

- **Cryopreservation**

  It is the storage of biological materials at very low temperature, -80 °C for exosomes. Anti-freezes are added to prevent the formation of ice crystals, and imbalance of osmosis during freezing process.

- **Freeze Dry**

  The sample is freezed into its solid state and the ice from the samples is sublimated in the form of water vapour under vacuum. Freeze-drying can maintain the sample's original activity and reduce the damage to biological materials. The sample can be easily kept in a constant storable state and can be reconstituted by simply adding water.

- **Spray Dry**

  The sample is atomized in the drying room, then the moisture quickly vaporizes in contact with the hot air to obtain dry powders. Compared to freeze-drying, spray-drying is a continuous process, which can realize one-step milling and is expandable.

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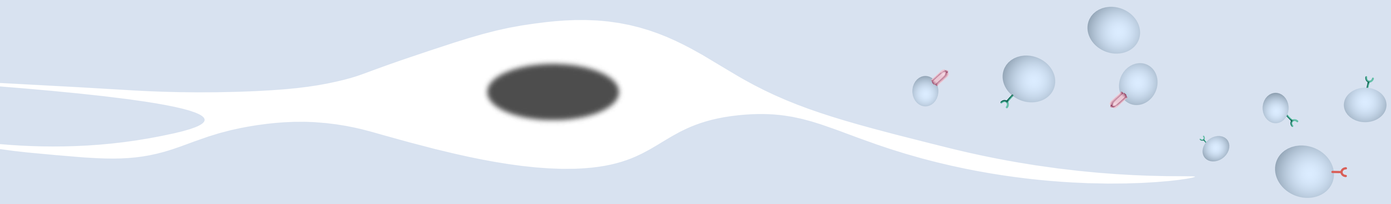
Mechanism of Actions

Mesenchymal Stem Cell (MSC)-derived Exosomes

MSC-exosomes elicit therapeutic activity by delivering their cargo of potentially therapeutic proteins and RNAs to the recipient cells. Their therapeutic potency is usually rationalized on the presence of a biologically relevant protein or RNA in the MSC-exosome.



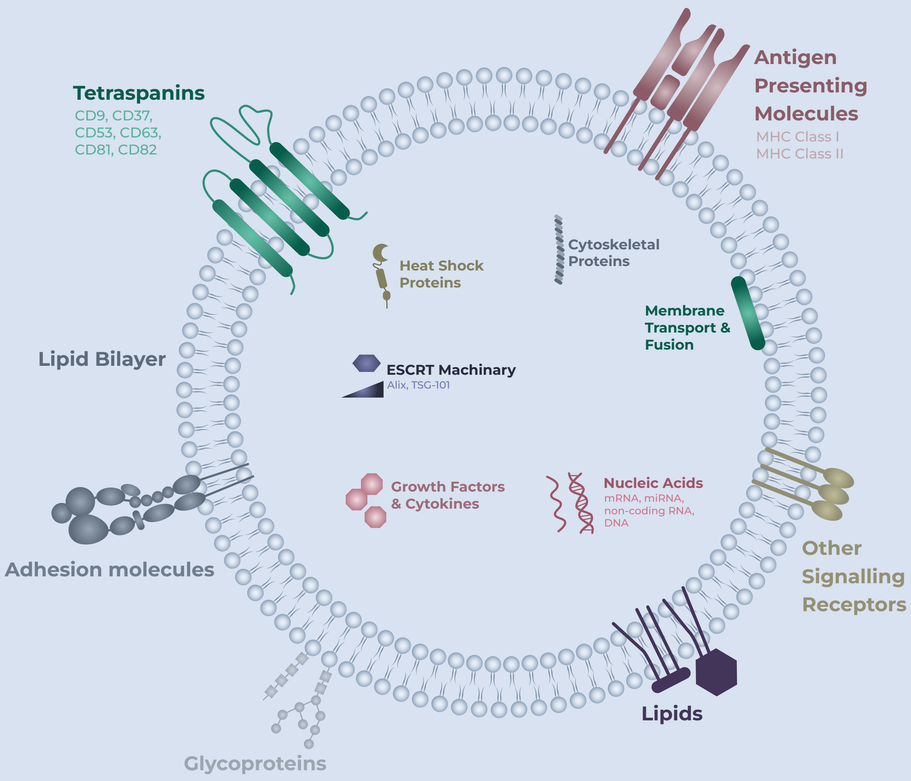
Mesenchymal Stem Cell (MSC)

MSCs are multipotent stem cells that are widely reported with their therapeutic efficacy against many diseases. MSCs possess various unique properties:

- homing to damaged tissues
- multilineage differentiation potential
- colony forming
- self-renewal abilities

MSC-exosomes

MSC-exosomes refer to EVs of 50-200 nm that are secreted by MSCs. They carry a rich diverse of proteome and RNA cargo to the recepient cells to modulate recepient cell biology **[Paracrine Effect]**.



RNA Cargo

MSC exosomal RNAs are mainly short RNAs of <300 nucleotides. MSCs secrete a select population of miRNAs in a regulated process, with only a fraction of the miRNAs identified in MSCs were secreted in MSC exosomes.

miRNAs are small, non-coding RNAs (22-26 nucleotides) that play a key role in gene expression. miRNAs work in a complex network in which each miRNAs controls hundreds of distinct target genes, while the expression of a single coding gene can be regulated by multiple miRNAs.

Proteome of Exosome

MSC-exosomes contain a large number of cytokines, chemokines, and trophic factors that regulate signaling pathways involved in cell growth, proliferation, survival, motility and immune response.

Mapping of the proteome to biological processes revealed that MSC exosome proteomes are involved in many key biological processes that are important in cellular communication, cellular structure, inflammation, exosome biogenesis, development, tissue repair and regeneration, and metabolism.

miRNA and Proteins as **Mediators** of MSC-Exosomes Therapeutic Efficacy

miRNA

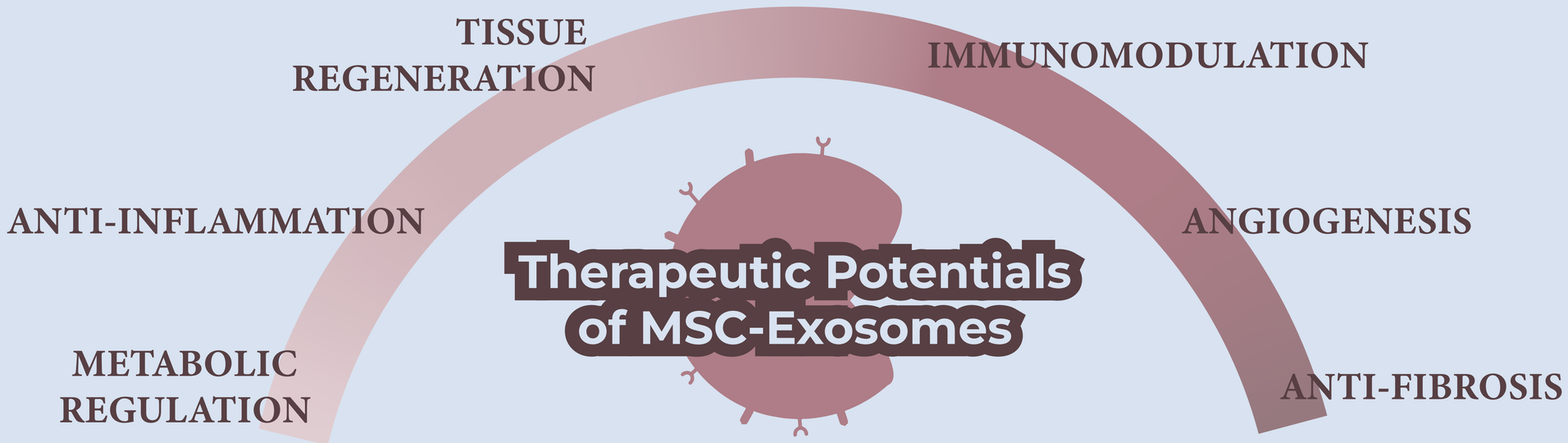
The wide repertoire of miRNAs in MSC-exosomes could conceivably provide an miRNA-based mechanism for the wide-ranging therapeutic effects of MSC secretion.

Regulation of Cell Growth and Survival	: miR-17-92; miR-19a, miR-21, miR-181-5p, miR-221
Regulation of Inflammation	: miR-24, miR-146a, miR-233
Regulation of Fibrosis	: miR-29
Regulation of Angiogenesis	: miR-494

Proteins

MSC-exosomes contain functional proteins related to anti-inflammation, tissue regeneration, and immunomodulation.

Anti-Inflammatory Cytokines	: IL-1RA, IL-4, IL-10, and transforming growth factor (TGF)-β
T Cell Regulation	: EDA-FN
Angiogenic Factors	: Angiogenin, hepatocyte growth factor (HGF)
Wound Repair	: Platelet-derived growth factor (PDGF)



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