

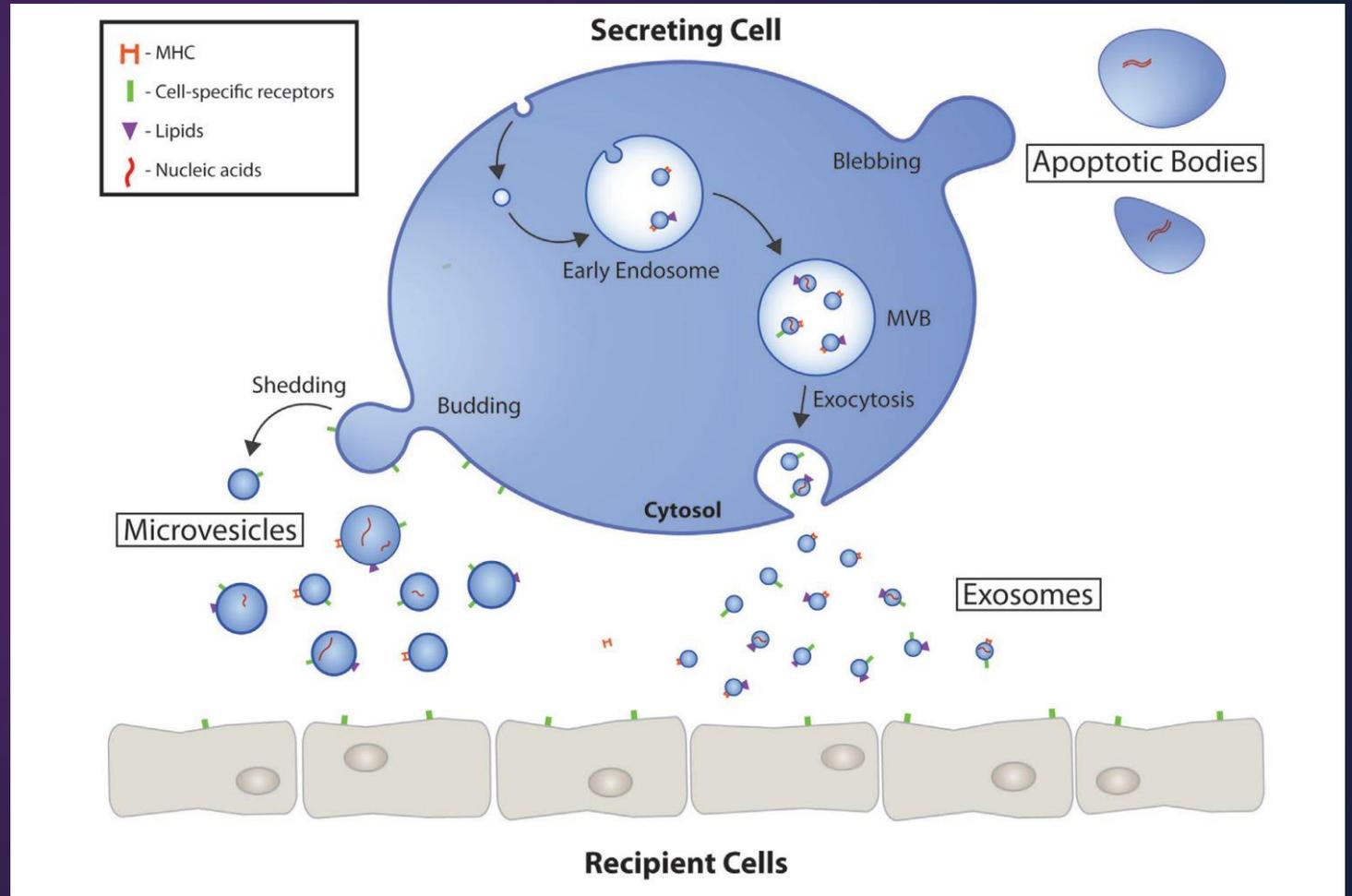
Exosomes: Biological Nanoparticles with Therapeutic and Diagnostic Potential



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Directed by: Dr. Elaheh Molaakbari

Origin and intracellular communication of extracellular vesicles (EVs)



Characteristics of



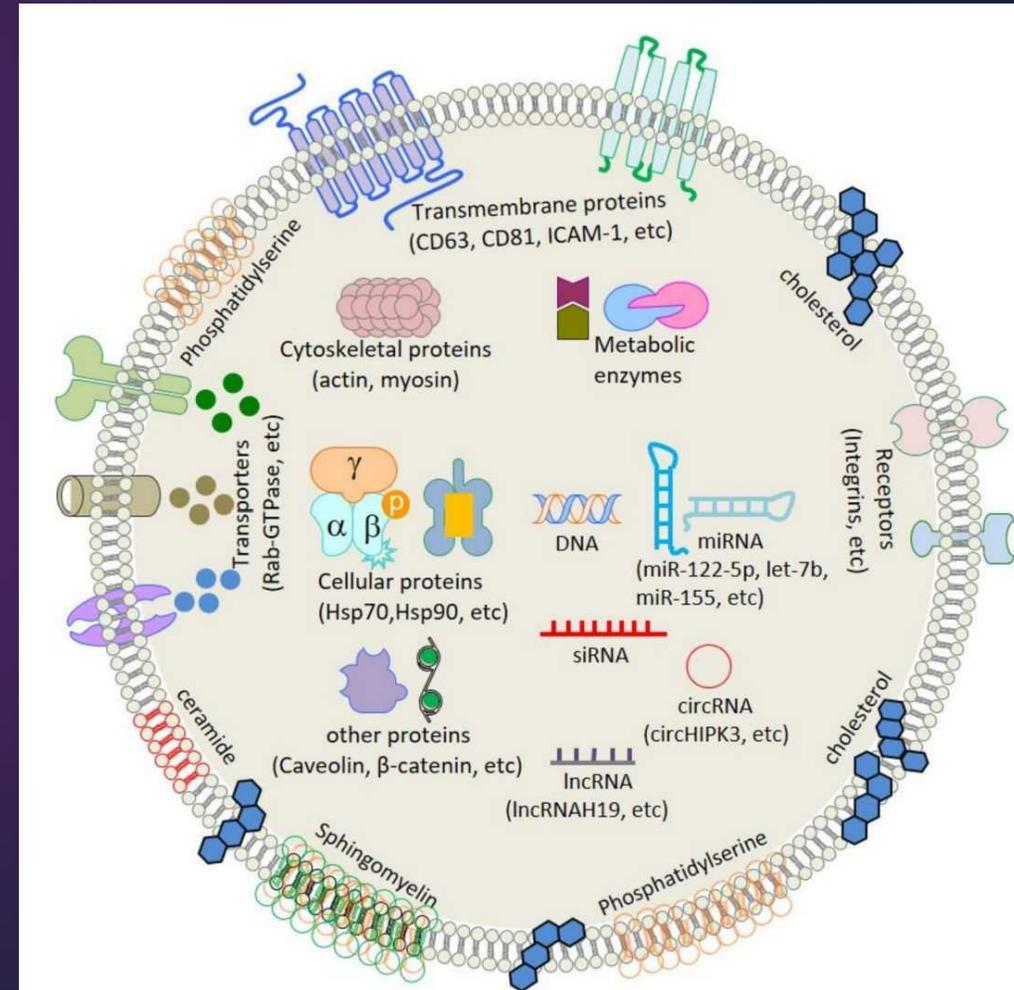
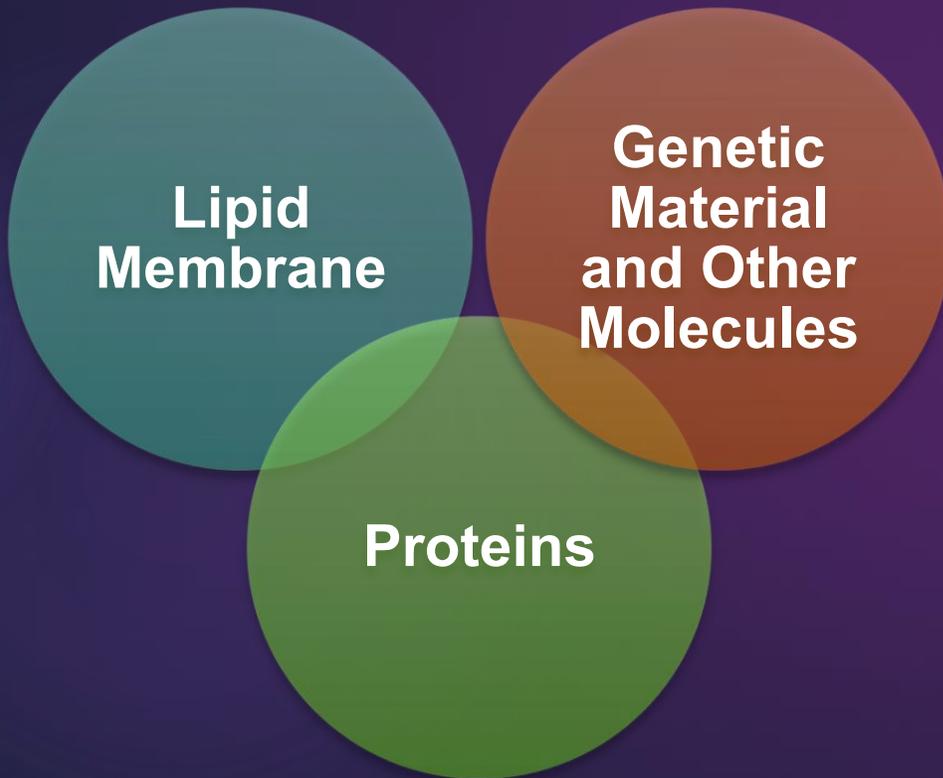
Extracellular vesicles	Exosomes	Microvesicles	Apoptotic bodies
Origin	Multivesicular bodies/Late endosome	Plasma membrane	Cells undergone apoptosis
Size	30-120 nm	100-1000 nm	1000-6000 nm
Mechanism of generation	Inward budding of multivesicular body	Outward budding of the plasma membrane	Cell shrinkage and segmentation
Pathway	ESCRT-dependent and ESCRT-independent Constitutive-dependent Stimuli-dependent	Ca ²⁺ -dependent Constitutive-dependent Stimuli-dependent	Apoptosis-dependent
Content	mRNA, miRNA, Proteins, lipids	mRNA, miRNA, proteins, lipids	proteins, nuclear segments, DNA, RNAs, cell organelles, lipids
Shape	Spheroid/cup-shaped	Irregular	Irregular
Function	Intercellular communication	Intercellular communication	Phagocytosis and recycling

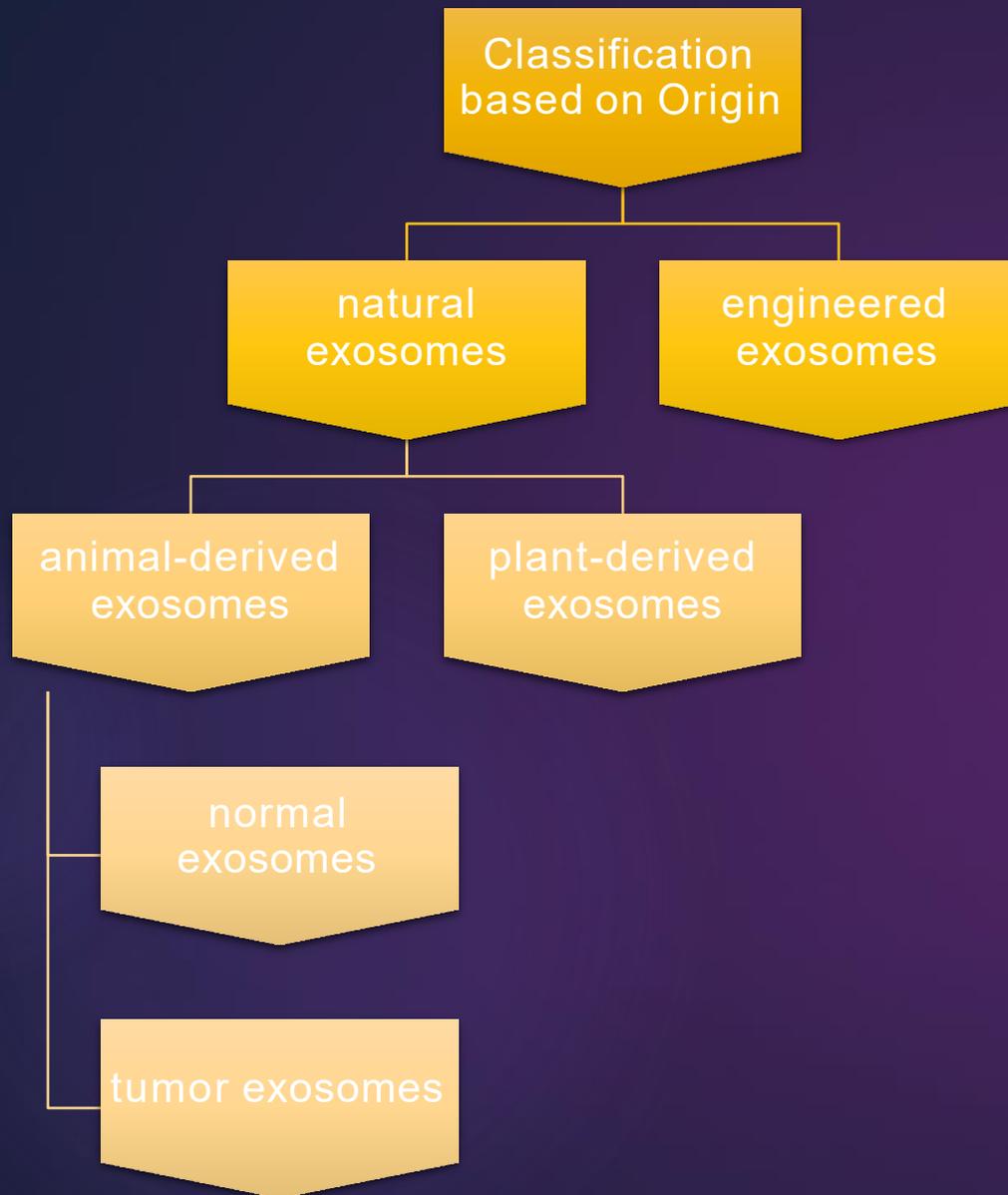
History of exosomes

- The first observation of exosomes was as early as in 1960s, while not much was known.
- More findings regarding to exosomes were obtained later in the 1980s.
- During a study of sheep reticulocyte maturation, the mechanism of exosome formation was revealed.
- The study showed that small vesicles were formed inside endosomes and then released into the extracellular environment during exocytosis.
- Years later, a study proposed that exosomes were small shuttles containing mRNAs and microRNAs (miRNAs) which enable remote genetic communication.
- Since then, exosome has unveiled a new paradigm in various therapeutic fields.

Structure of Exosomes

Exosomes are spherical vesicles with a lipid bilayer membrane derived from their parent cells. Their structure consists of the following components:





Natural Synthesis of Exosomes (Biogenesis)

Endocytosis:

- Plasma membrane invaginates → **early endosomes**.

Maturation:

- Early → **late endosomes**.

MVB Formation:

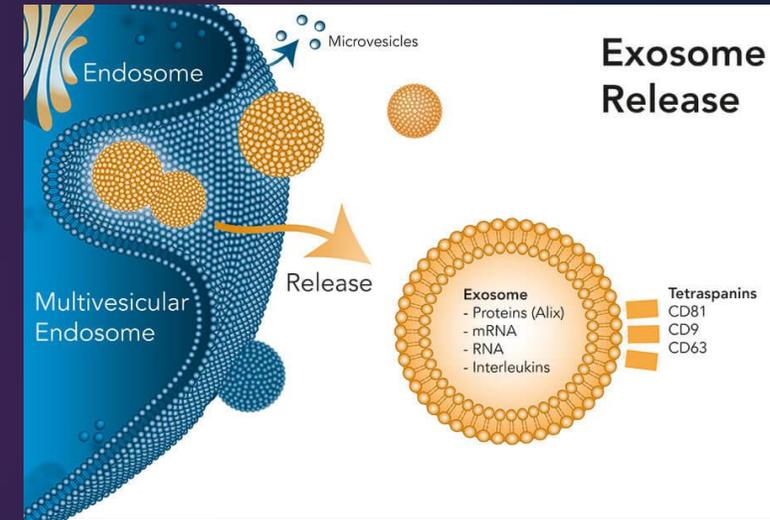
- Inward budding → intraluminal vesicles (ILVs) as **exosome precursors**.

Cargo Sorting:

- Selective loading of proteins, RNAs, lipids (via **ESCRT-dependent or independent pathways**).

Exocytosis:

- MVBs fuse with plasma membrane → release ILVs as **exosomes**

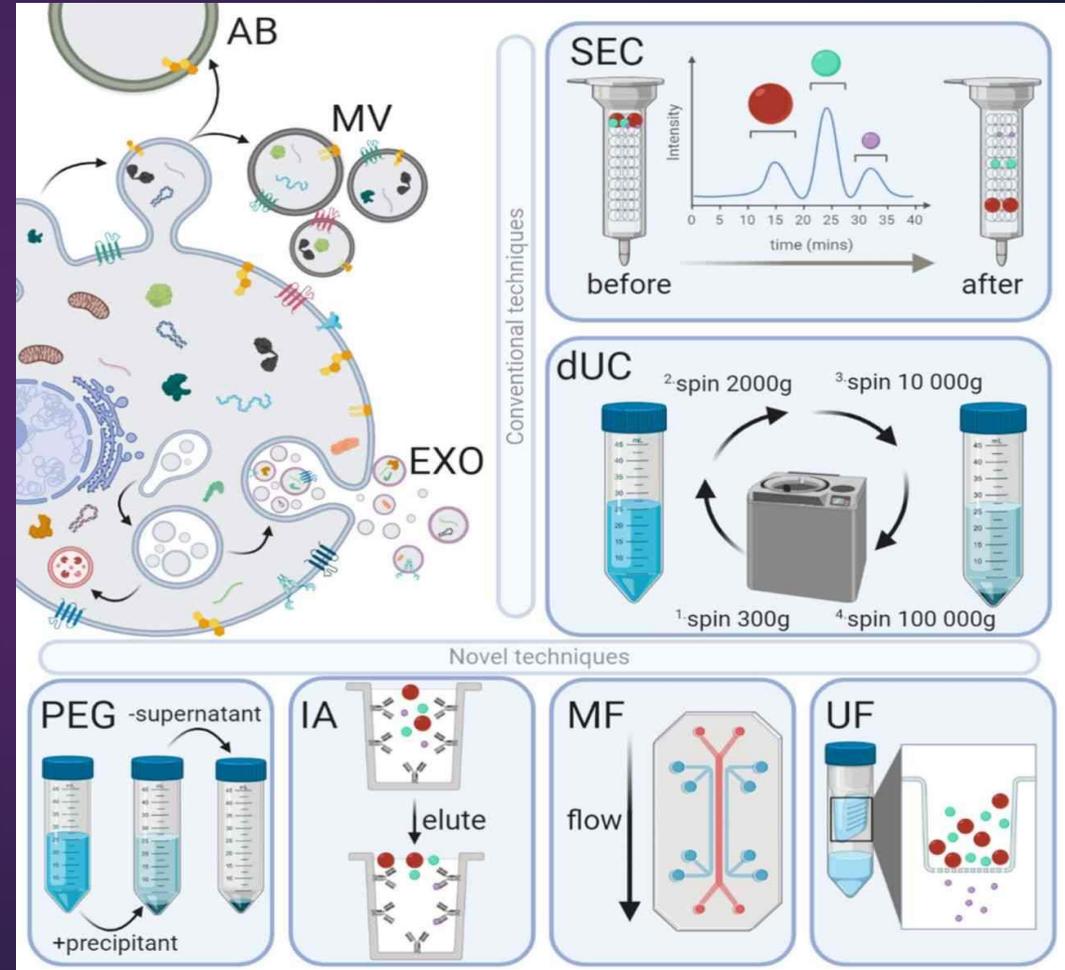


Artificial Synthesis Methods

- ▶ **To overcome limitations of natural production (e.g., low yield and heterogeneity), biomimetic or artificial exosomes are produced:**
 - **Cell-Based Methods:** Cells are cultured, and exosomes are extracted, but to increase yield, techniques like extrusion (forcing cells through nanometer-sized filters) are used to produce exosome-like nanovesicles.
 - **Lipid Membrane Synthesis:** Involves artificial synthesis using liposomes fused with exosomal components. Techniques like liposome fusion, synthetic assembly, and programmed assembly create nanovesicles (NVs), exosome mimics (EMs), and hybrids. These methods offer higher yield, faster production, and similar chemical and biological properties (e.g., size, morphology, function) to natural exosomes.
- ▶ **These methods enable exosome engineering for targeted drug delivery, such as loading drugs or RNAs.**

Isolation Methods

- Ultracentrifugation
- Polymer Precipitation
- Size-Based Isolation
- Immunoaffinity Chromatography (IAC)
- Emerging Methods



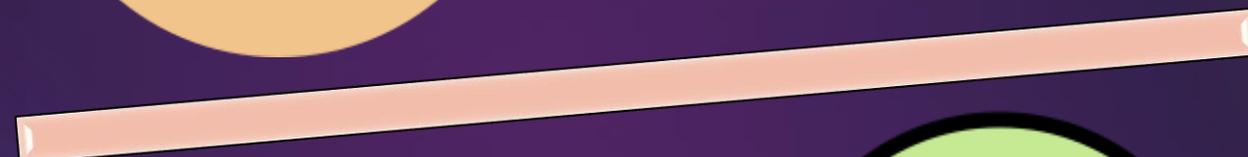
Role of Exosomes in the Body

- ▶ **Exosomes are involved in numerous biological processes, including:**
 1. **Intercellular Communication:** Exosomes transfer bioactive molecules between cells, regulating processes such as immune responses, tissue repair, and cell proliferation.
 2. **Role in Diseases:** Exosomes are implicated in diseases such as cancer, neurodegenerative disorders (e.g., Alzheimer's, Parkinson's), and cardiovascular diseases. For instance, exosomes secreted by cancer cells can facilitate metastasis.
 3. **Role in the Immune System:** Exosomes can enhance or suppress immune responses, balancing pro-inflammatory and anti-inflammatory responses.

Applications

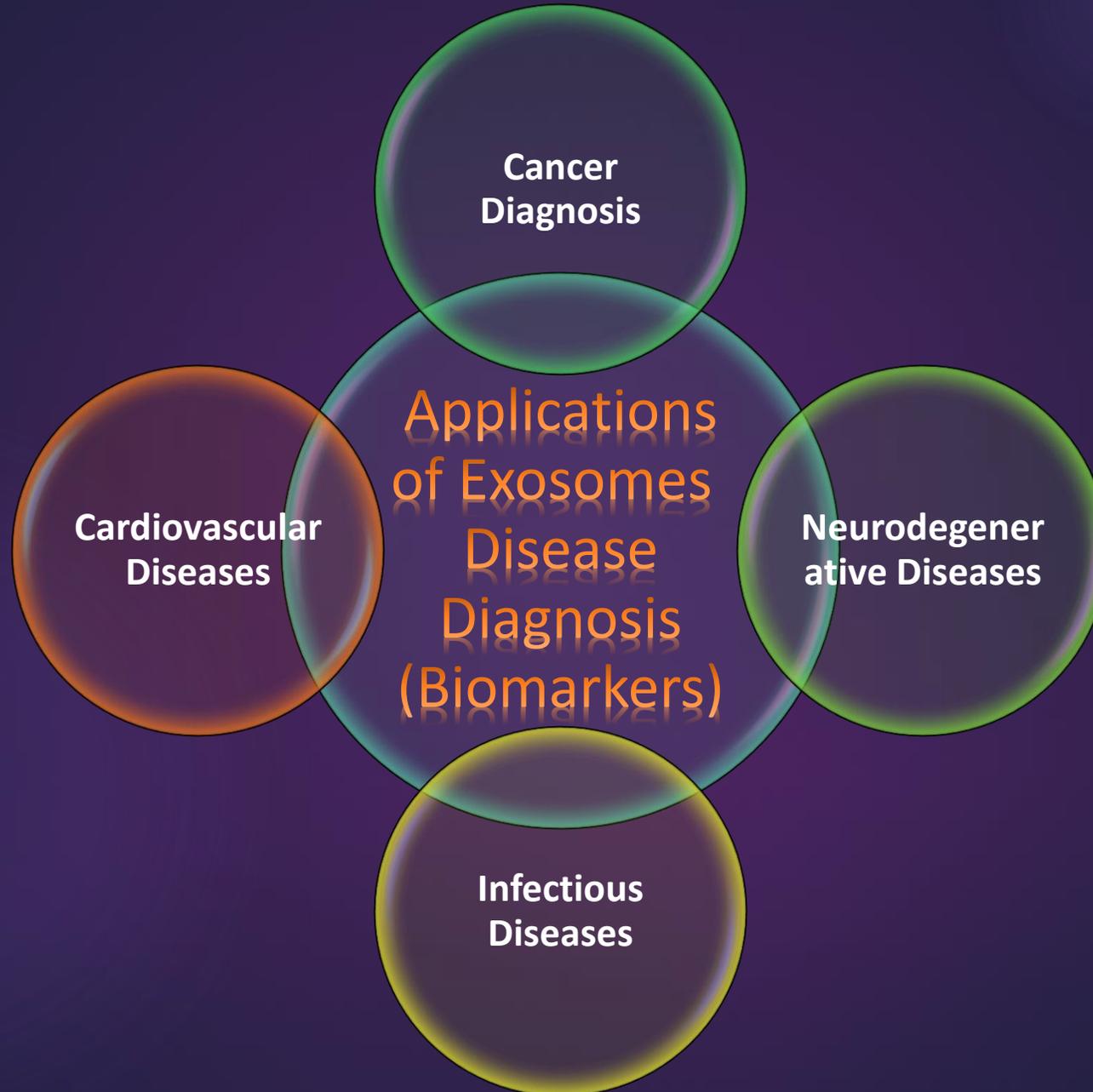


Disease
Diagnosis

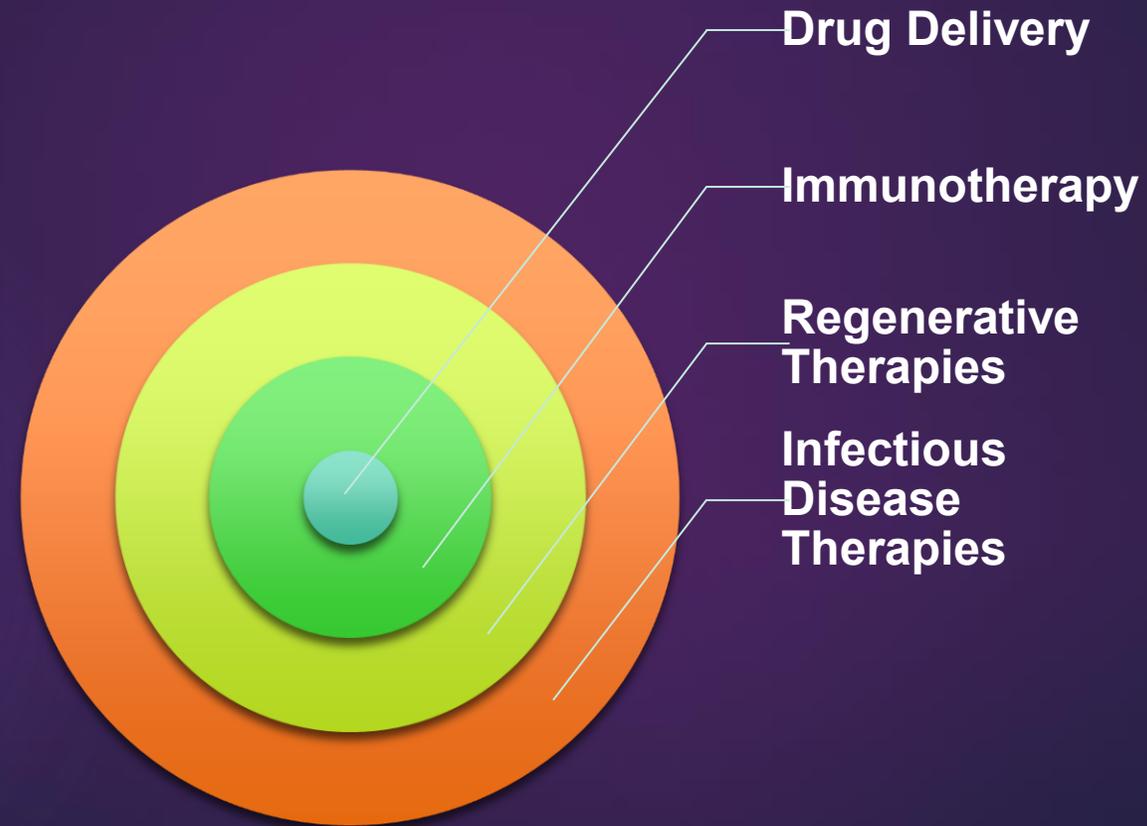


Disease
Treatment





Applications of Exosomes in Disease Treatment



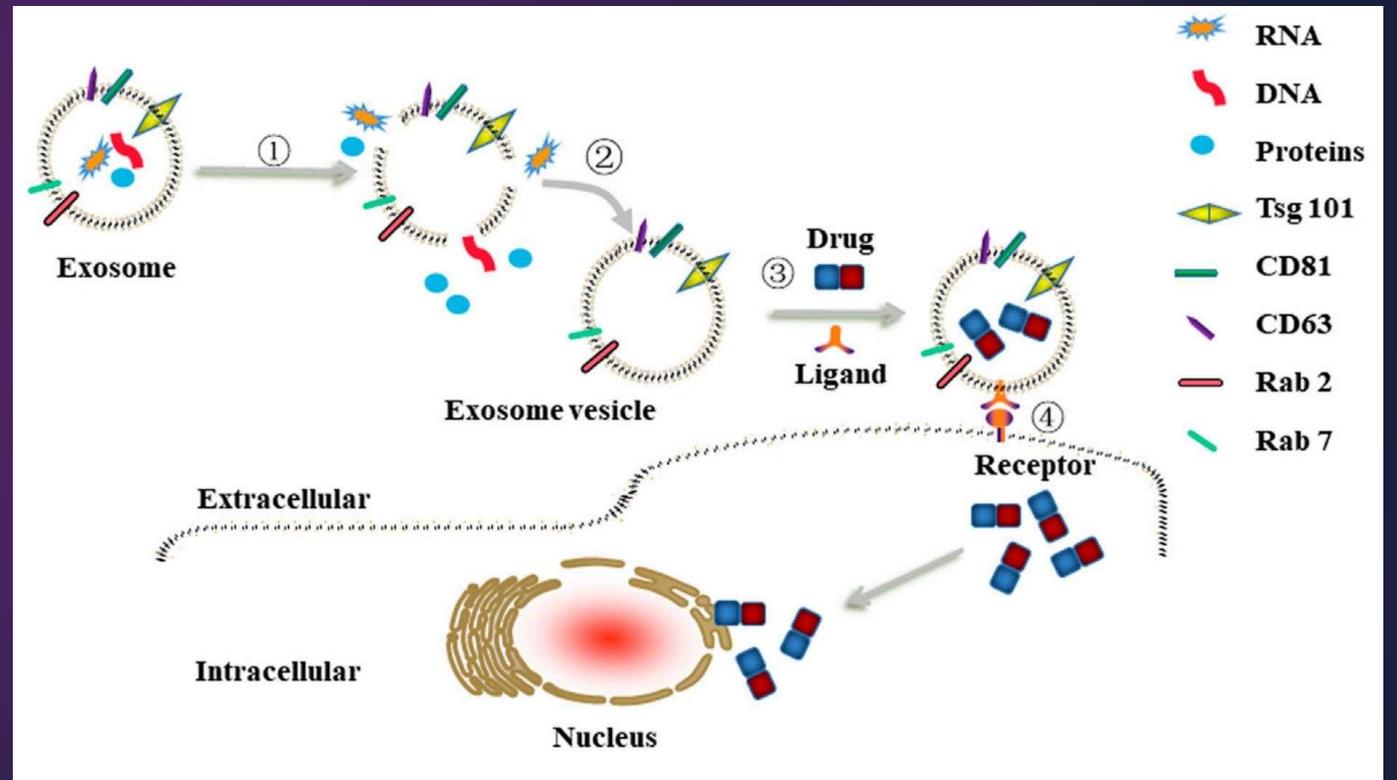
Common drug loading ways of exosomes



Passive Loading Methods

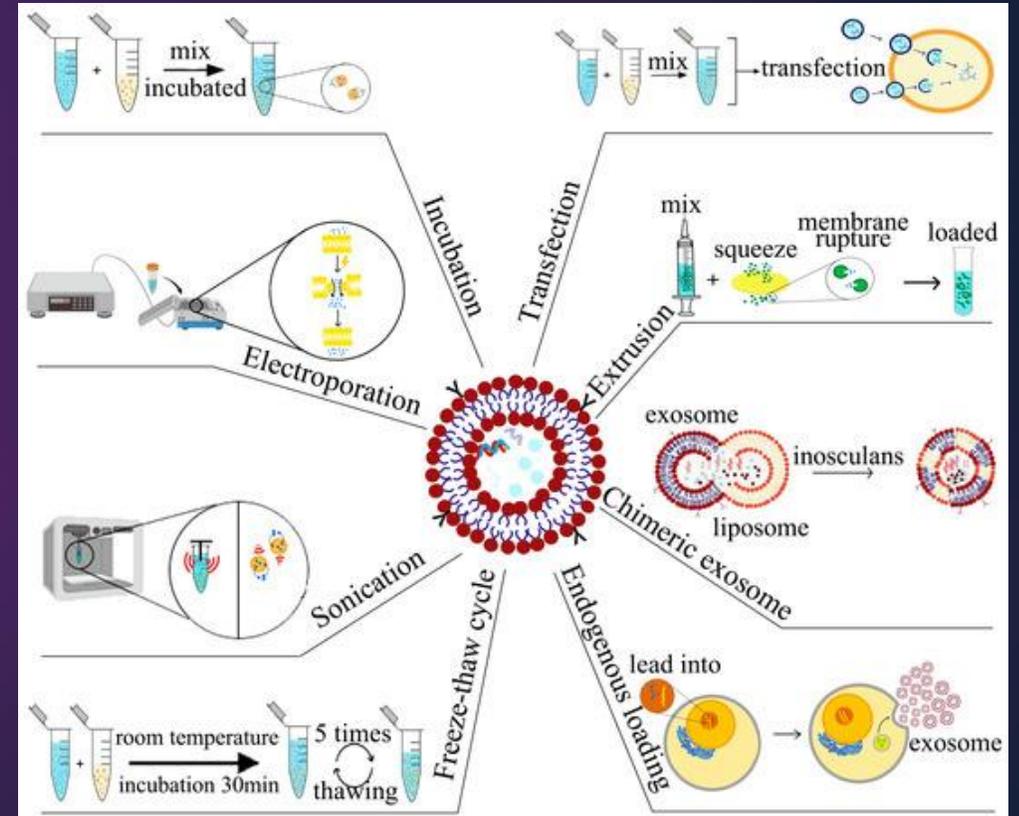
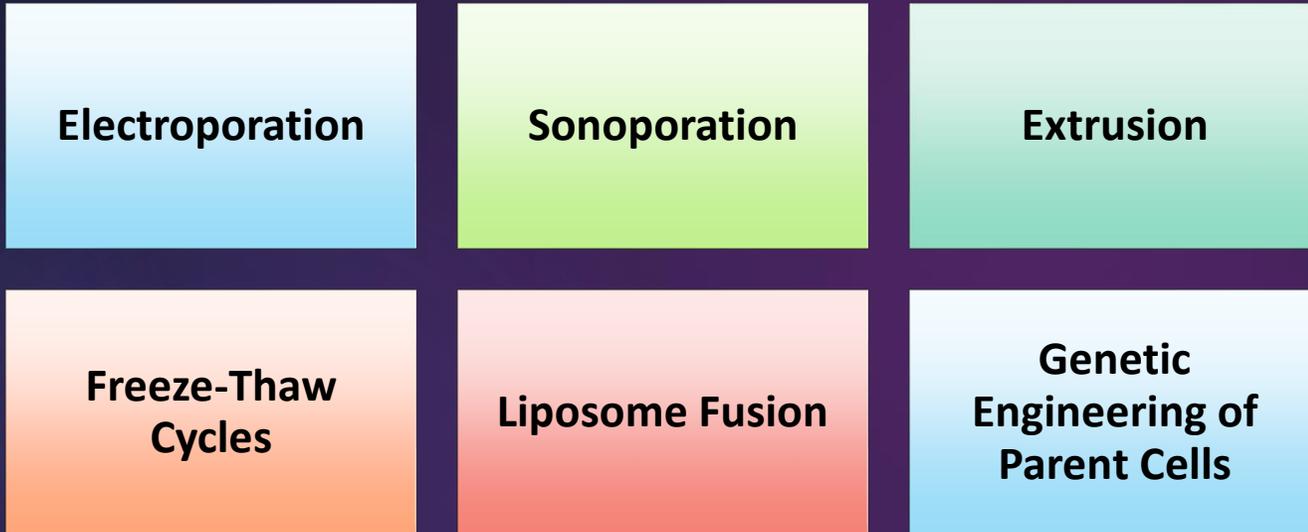
Simple Incubation

Incubation with Enhancers



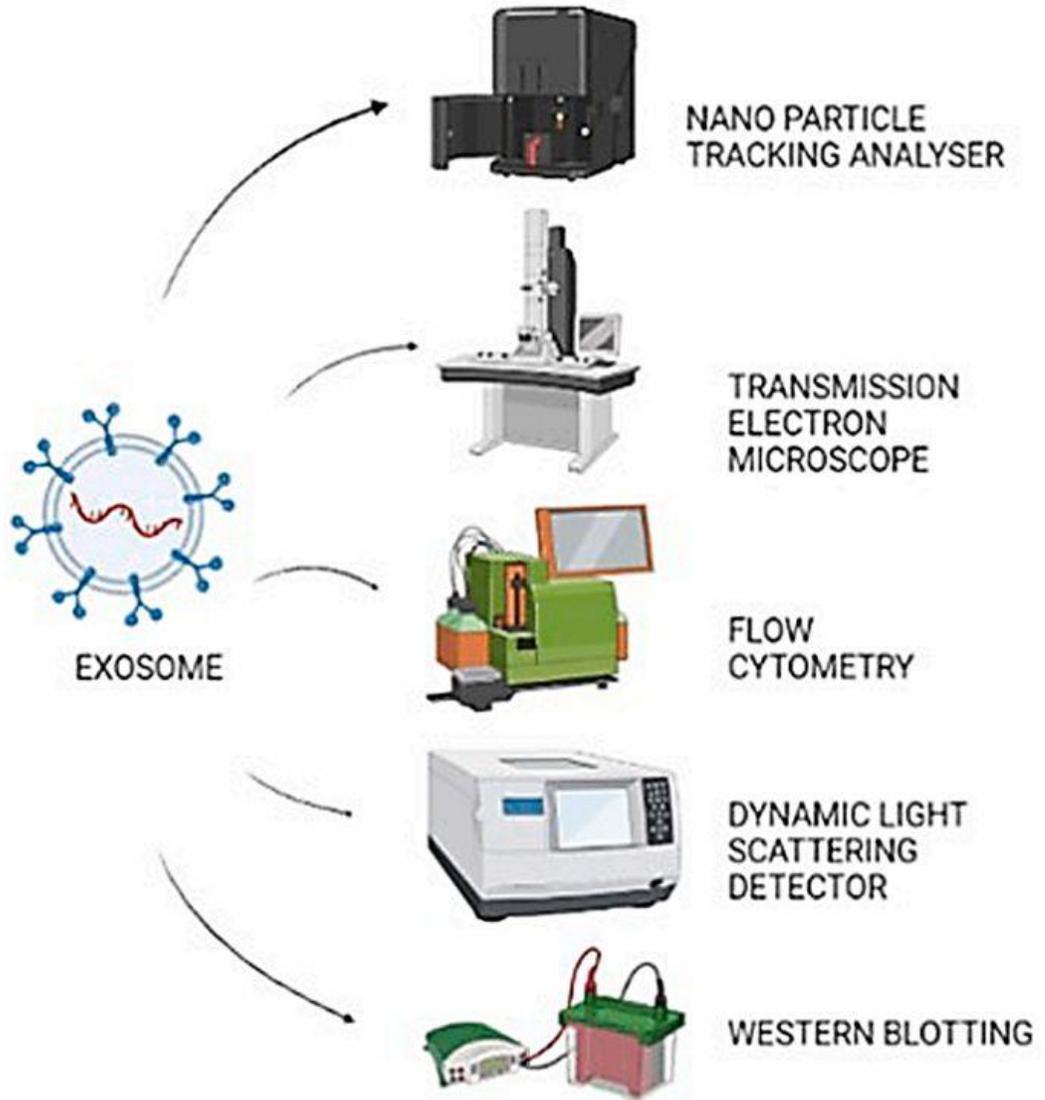
Active Loading Methods

- ▶ Active methods use external techniques to facilitate drug entry into exosomes, offering higher efficiency but increased complexity and cost.



The choice of loading method

- Drug Type: Hydrophobic drugs (e.g., curcumin) are suitable for simple incubation, while hydrophilic molecules (e.g., siRNA) require active methods like electroporation.
- Therapeutic Goal: Targeted therapies may require genetic engineering.
- Exosome Stability: Methods like freeze-thaw may damage the exosome membrane.
- Production Scale: Simpler methods like incubation are suitable for initial experiments, while active methods are more scalable for clinical use.
- ▶ Challenges
 - Loading Efficiency: Many methods have low efficiency, especially for large or hydrophilic molecules.
 - Exosome Integrity: Some methods (e.g., electroporation) may damage exosome structure or biological properties.
 - Standardization: Lack of standardized protocols for loading and efficiency assessment hinders commercialization.
 - Cost and Complexity: Active methods require advanced equipment and expertise.



The storage conditions for exosomes

Exosomes should generally be stored at low temperatures to preserve their integrity and biological activity.

It is essential to avoid storing exosomes at room temperature or higher temperatures for extended periods, as this leads to degradation of proteins and RNA contents.

Using cryoprotectants such as trehalose or dimethyl sulfoxide (DMSO) can help protect exosomes during freezing by preventing ice crystal formation and membrane damage.

Some studies suggest that storage at -20°C may also preserve exosomes reasonably well, but -80°C remains the preferred condition for maintaining exosome integrity over time.

For short-term storage (up to 24 hours), keeping exosomes at 4°C is suitable and maintains their concentration and protein levels.

For long-term storage (over a week to months), storing exosomes at -80°C is recommended as it best preserves their stability, contents, biodistribution, and functional properties.

Freeze-thaw cycles should be avoided since repeated freezing and thawing can damage exosomes and reduce their quality.



Advantages of exosome as a natural nano-carrier for therapeutic delivery

compartmentalization

Long circulation

High biocompatibility

Easily uptaken by cells

Cell/tissue specific targeting

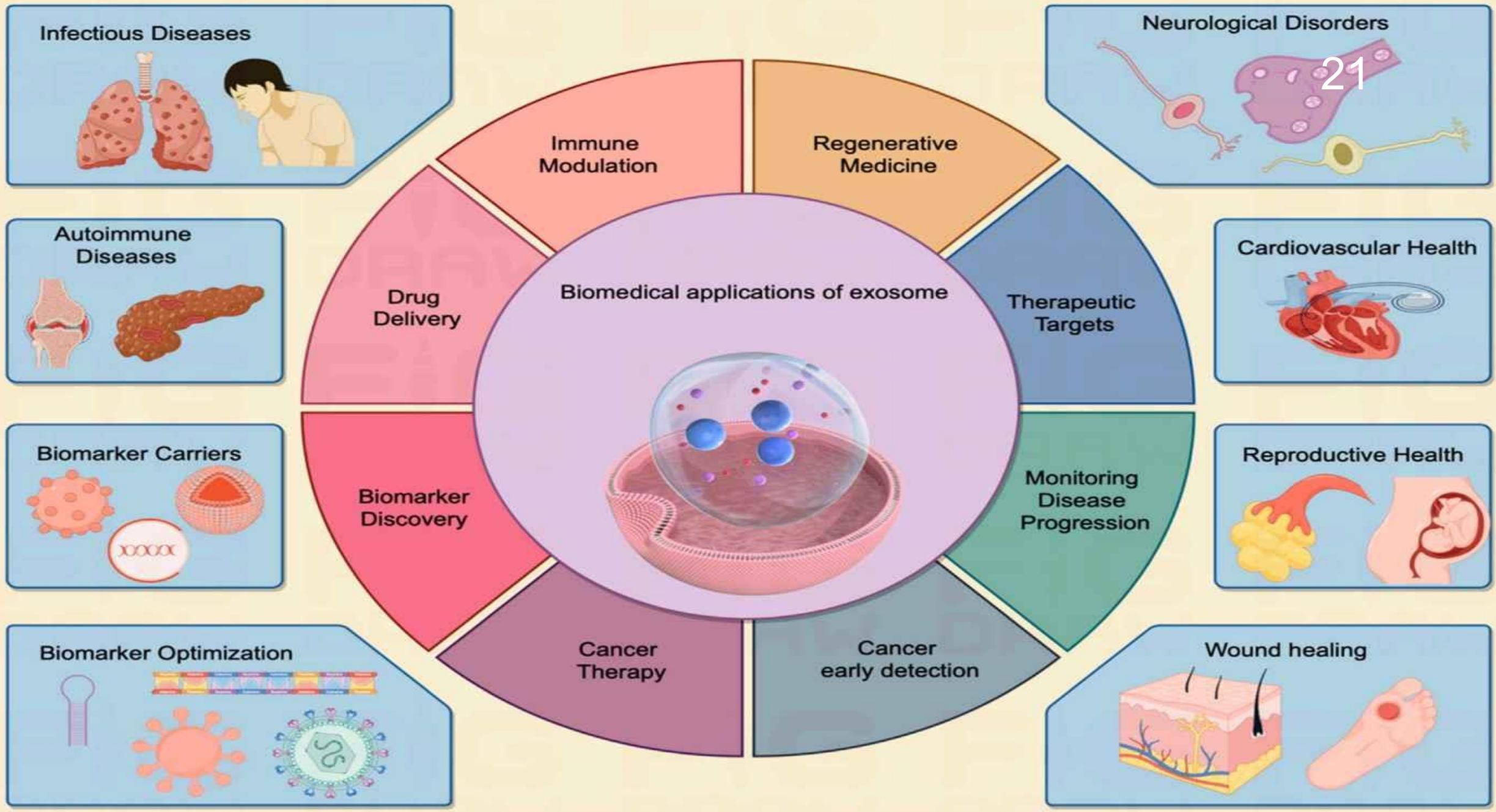
High penetration through difficult barriers

Easy to load therapeutics

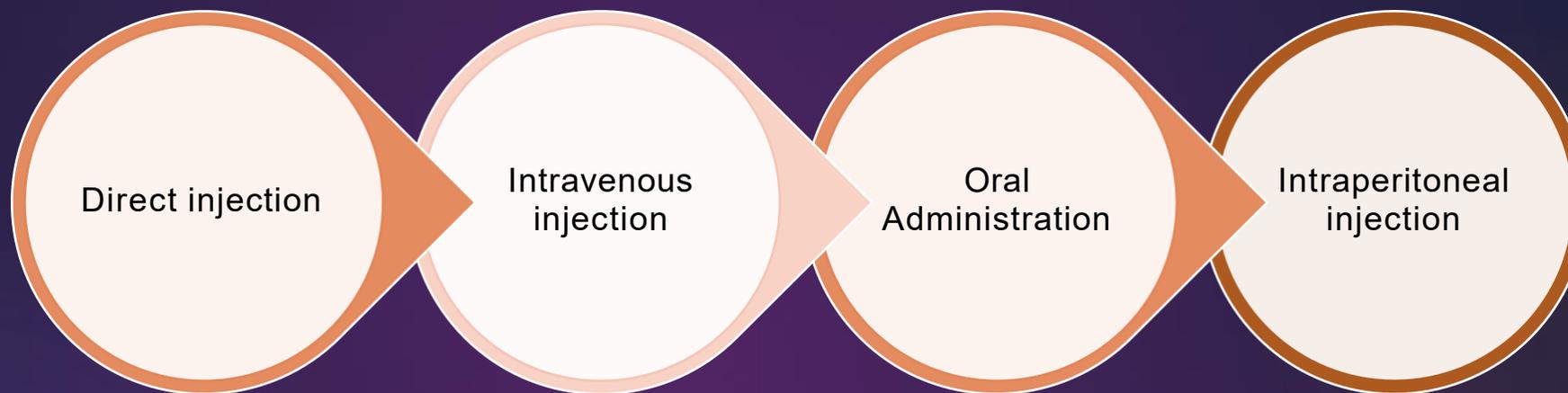
Non-immunogenic

Cargo protection from degradation

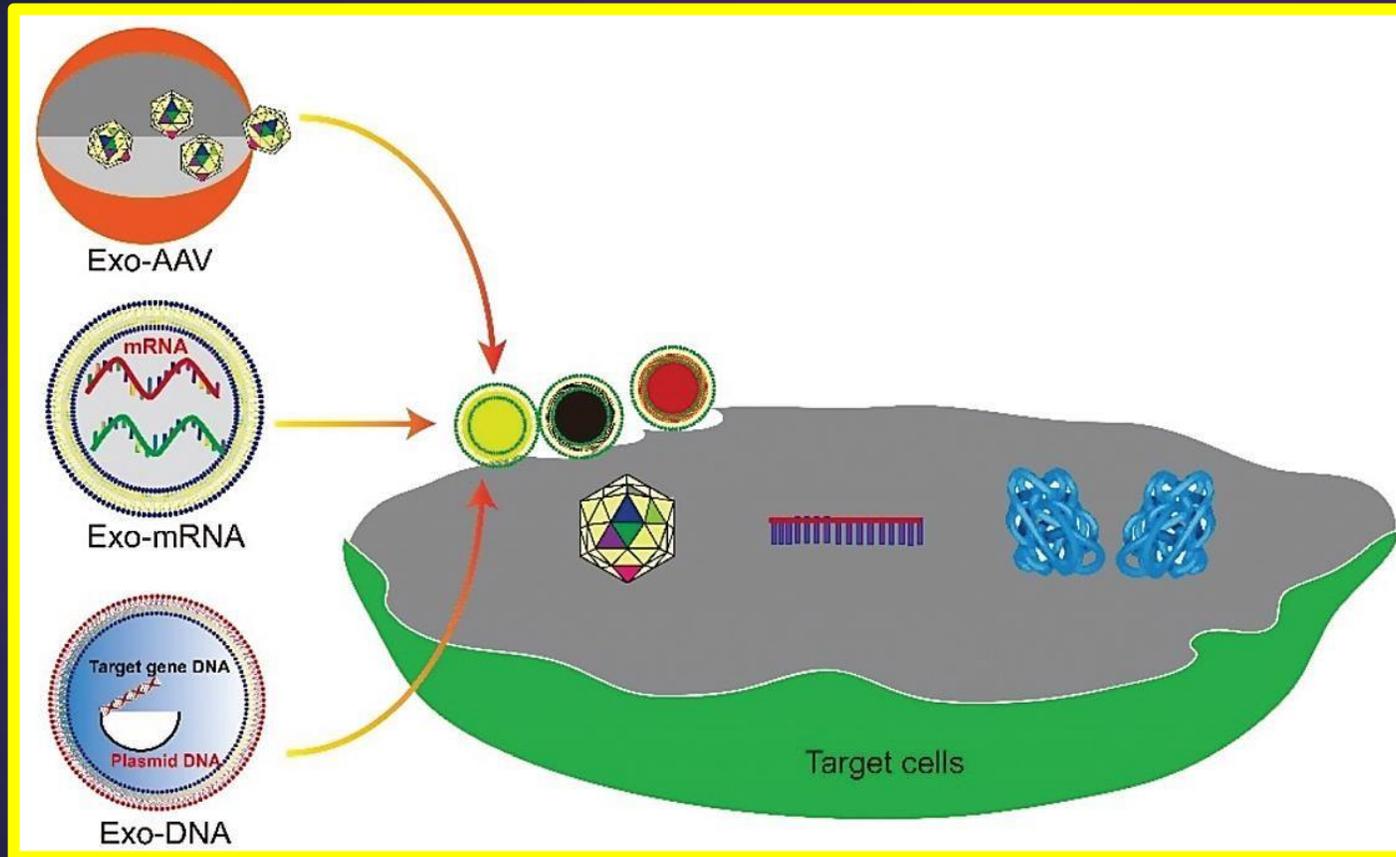
Easy to modify



exosome administration routes

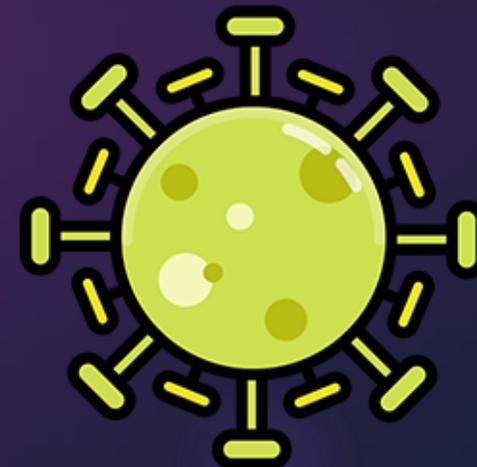
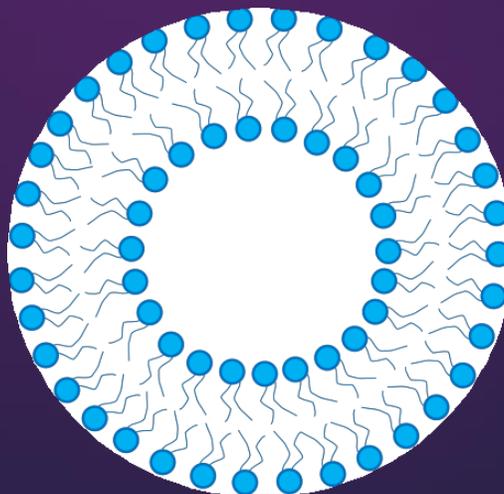
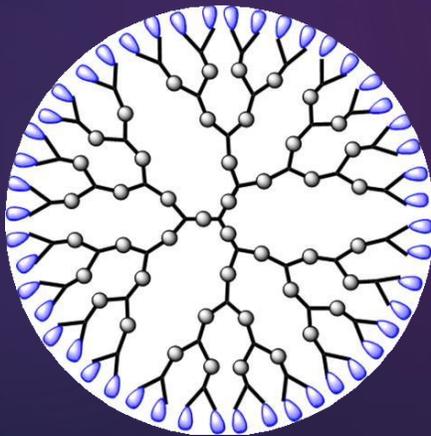


Schematic illustration of exosome-mediated gene delivery strategies to exert functional therapies by translating into proteins, RNA interference, genome editing, etc.



Comparison of the different delivery vehicles for the CRISPR/Cas9 gene editing system

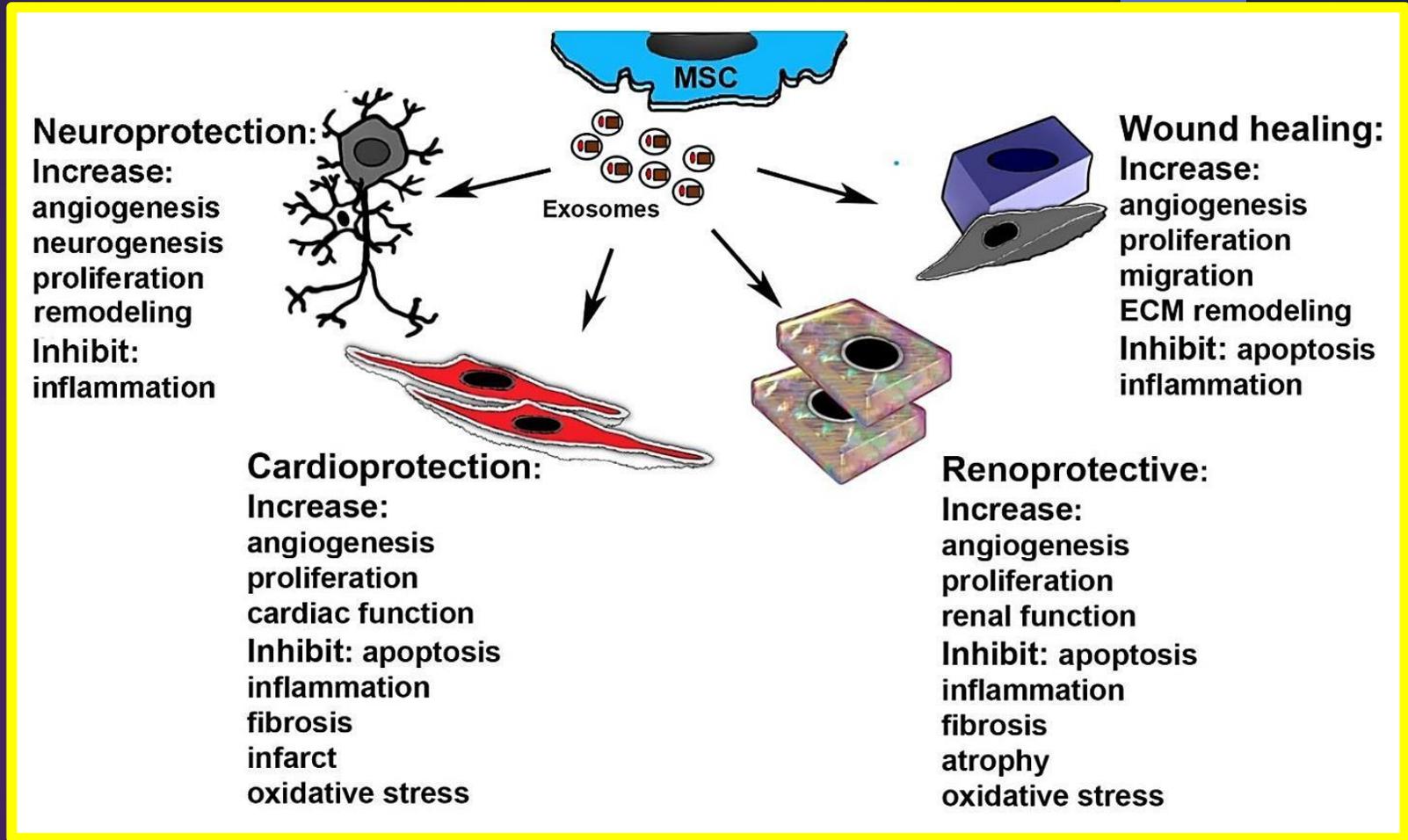
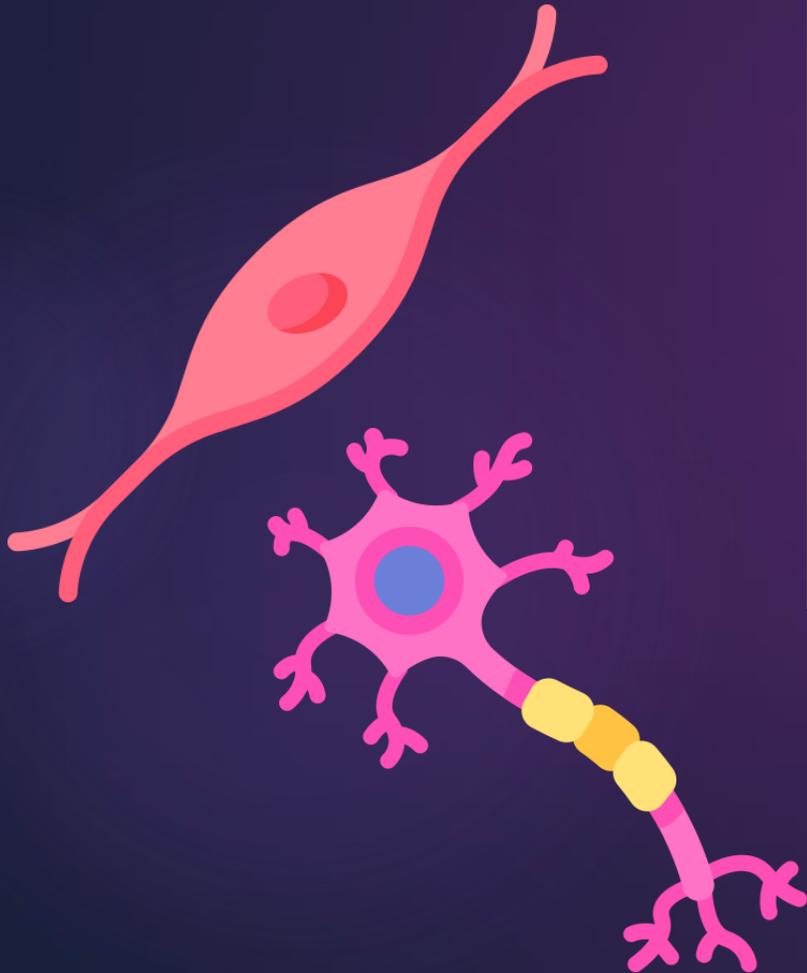
	exosome	Lipid nanoparticle	Polymer nanoparticle	dendrimer	Virus
Biocompatibility	Excellent	Low	May accumulate undesirably in the liver, spleen etc.	Non-toxic	Poor
Loading efficiency	High	N/A	N/A	N/A	High
Targeting engineerability	yes	Yes	yes	Yes	Yes



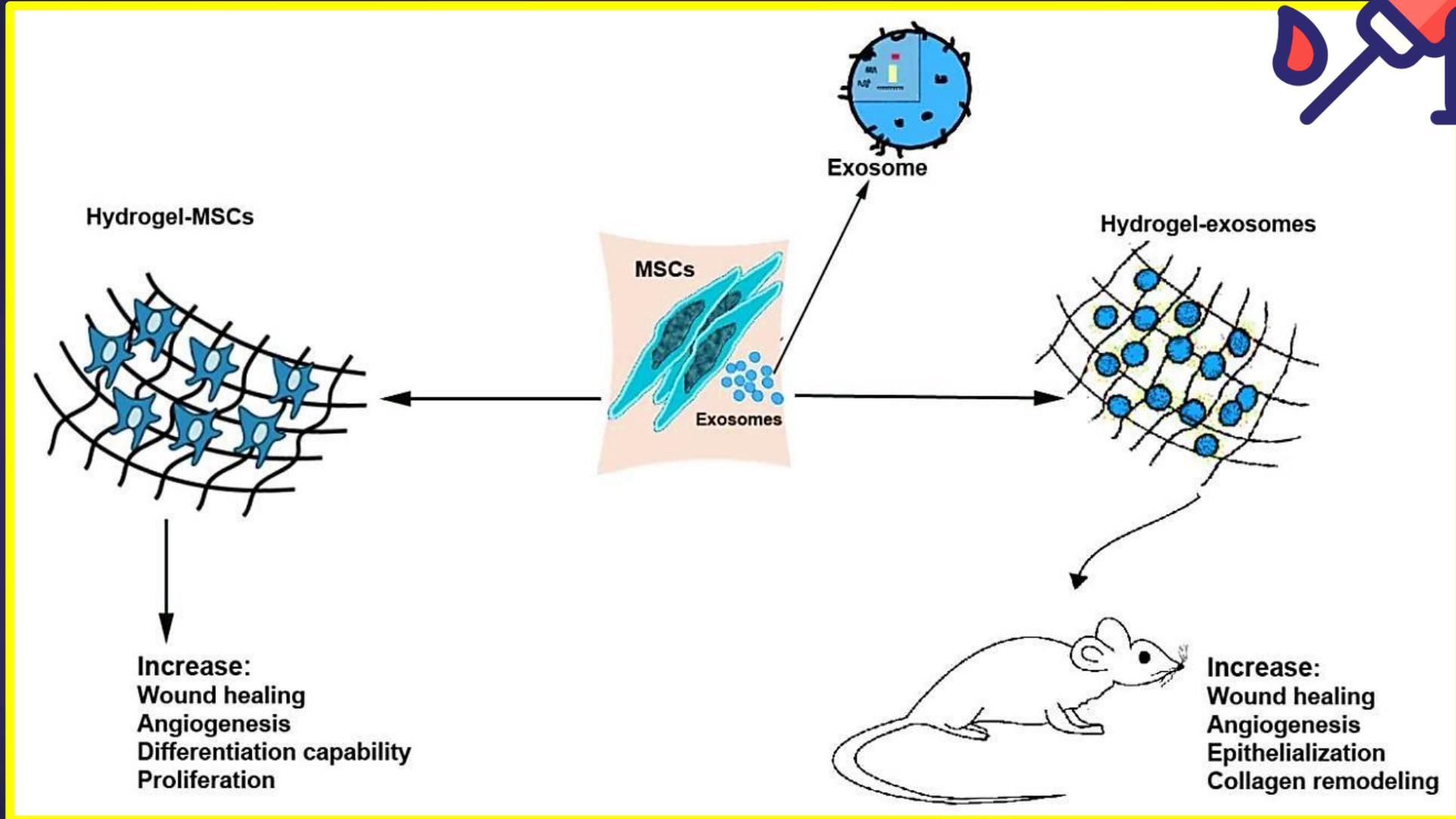
Tissue engineering & Regenerative medicine

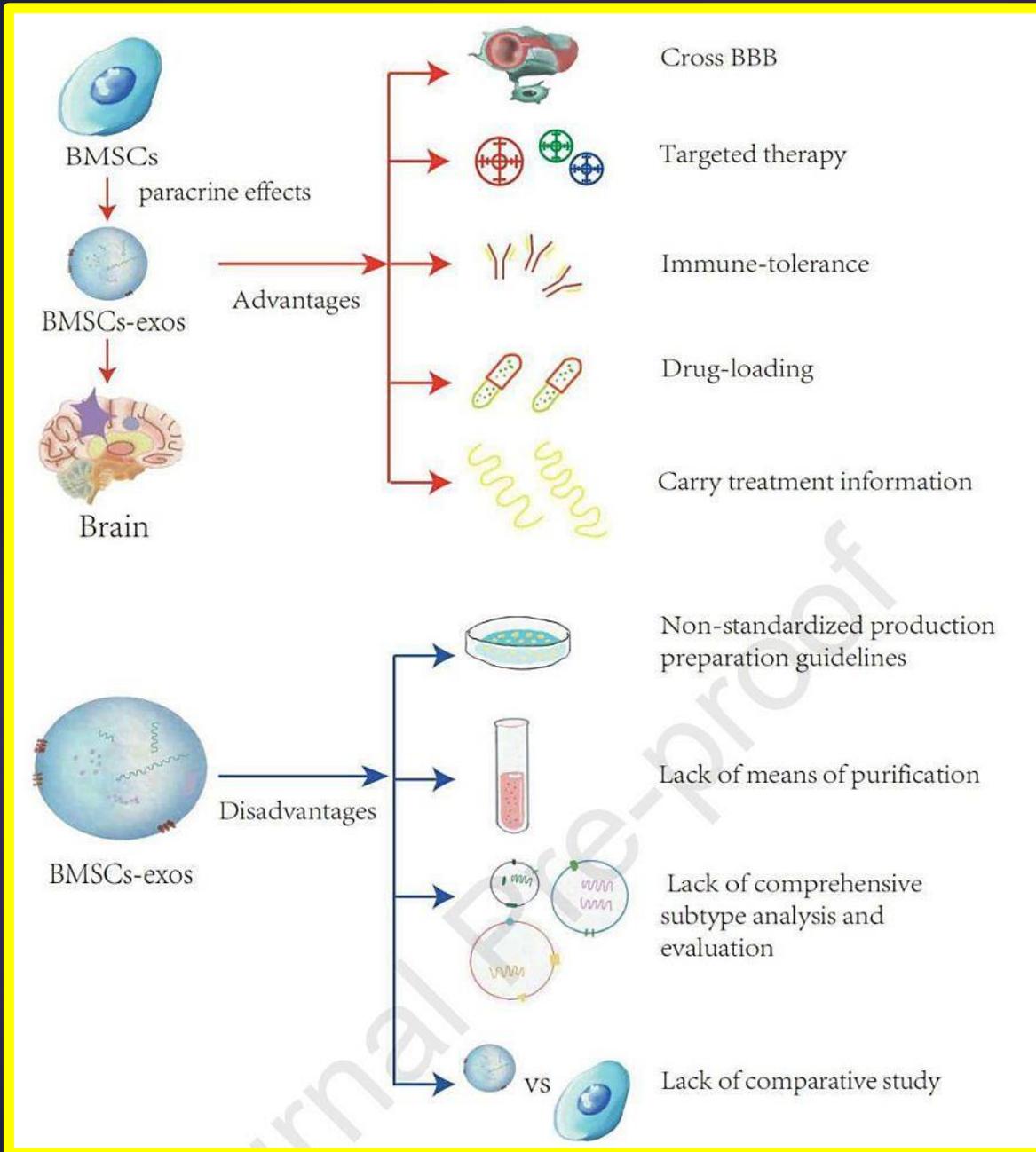


Schematic diagram of therapeutic effects of mesenchymal stem cell (MSCs) derived exosome in different diseases

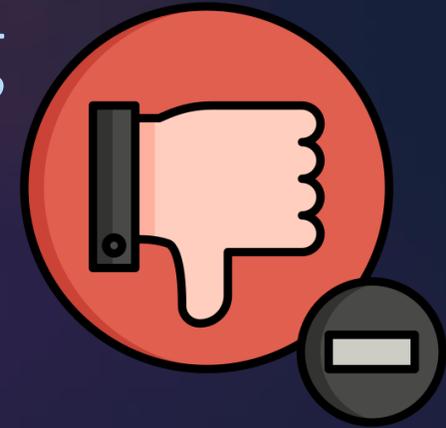


The encapsulation of mesenchymal derived exosomes into chitosan/silk hydrogel for the treatment of diabetic diseases

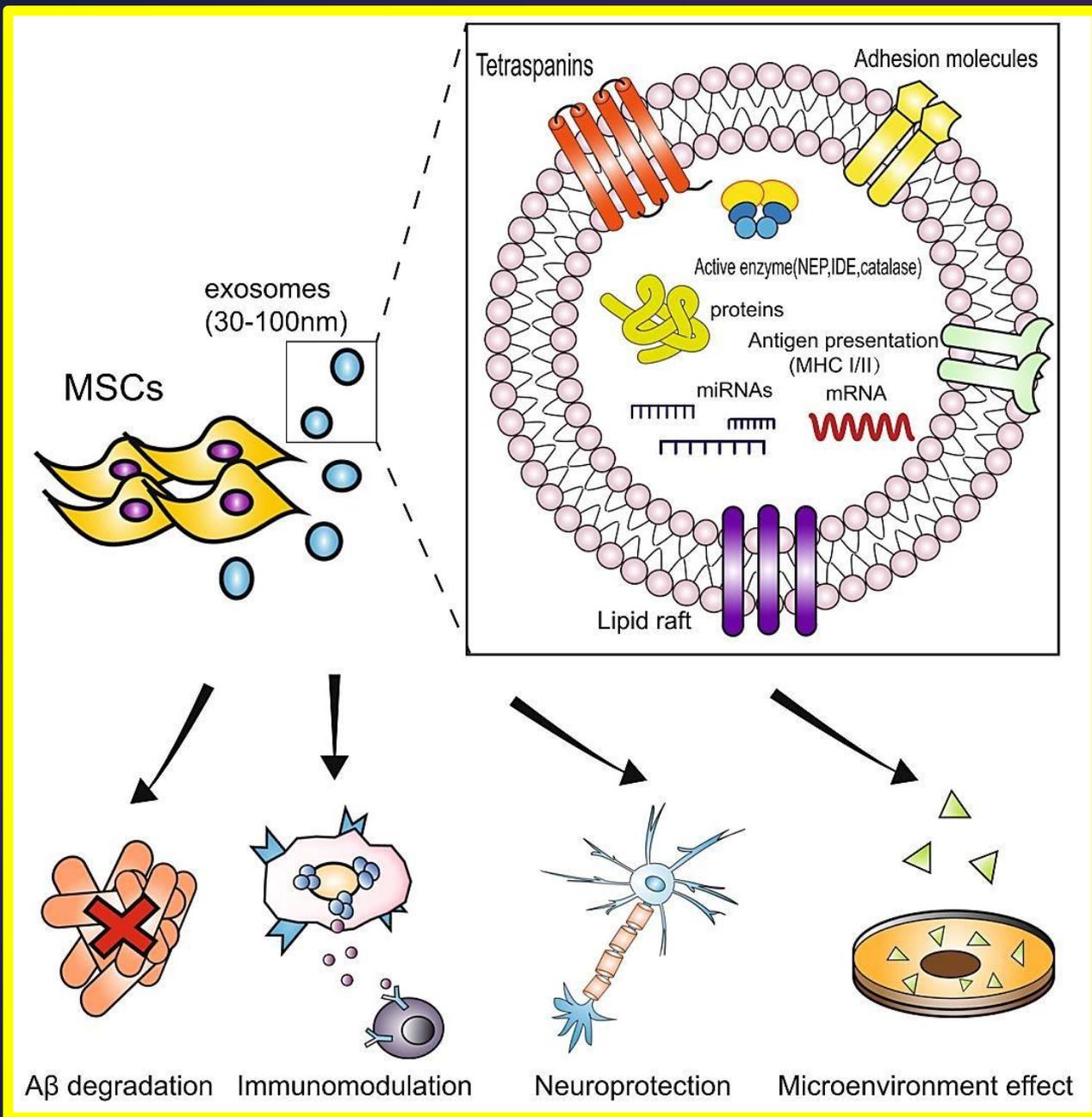




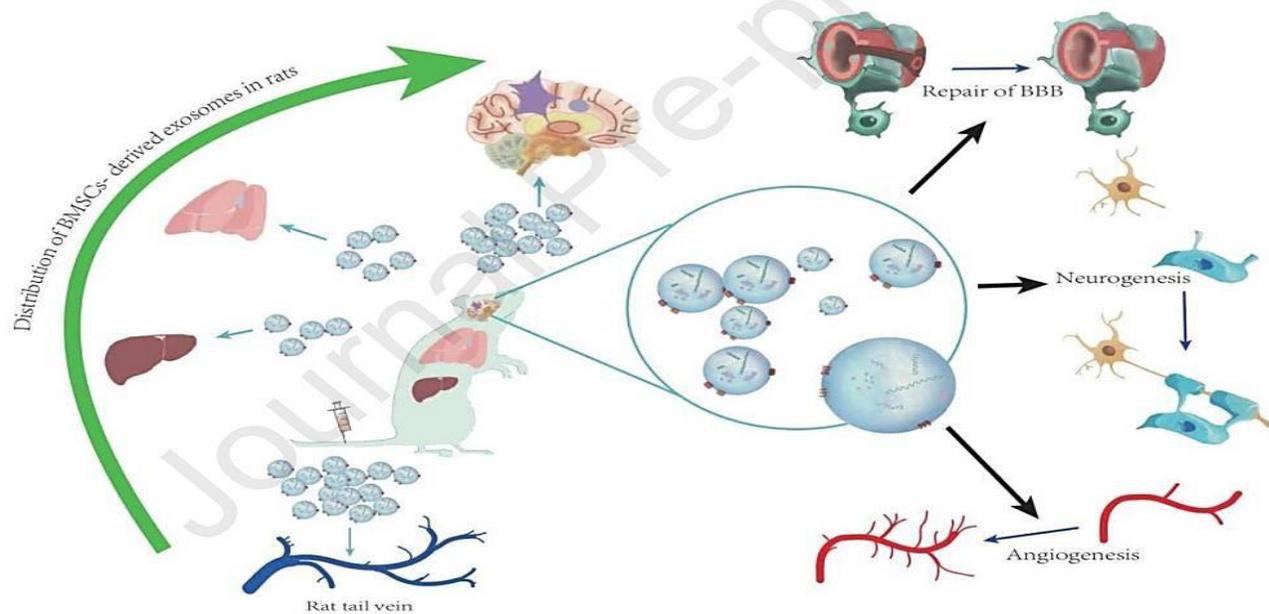
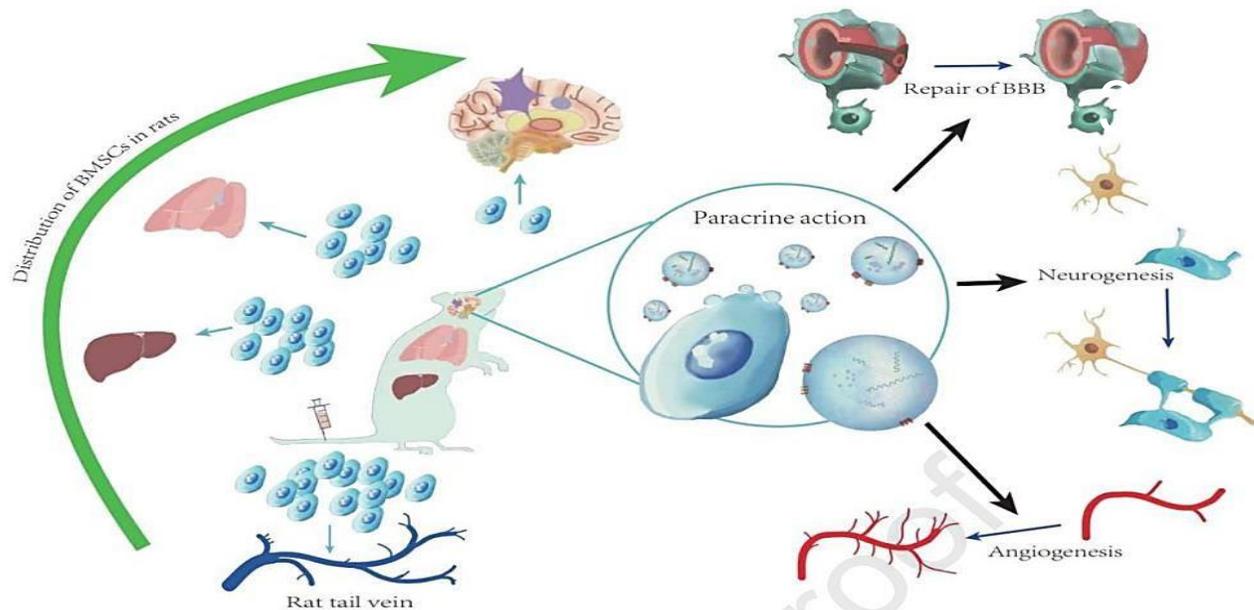
Advantages and disadvantages of BMSCs-exos



Therapeutic properties of MSC-exos in AD



A face-to-face comparison between stem cell therapy and exosome therapy in preclinical rat models



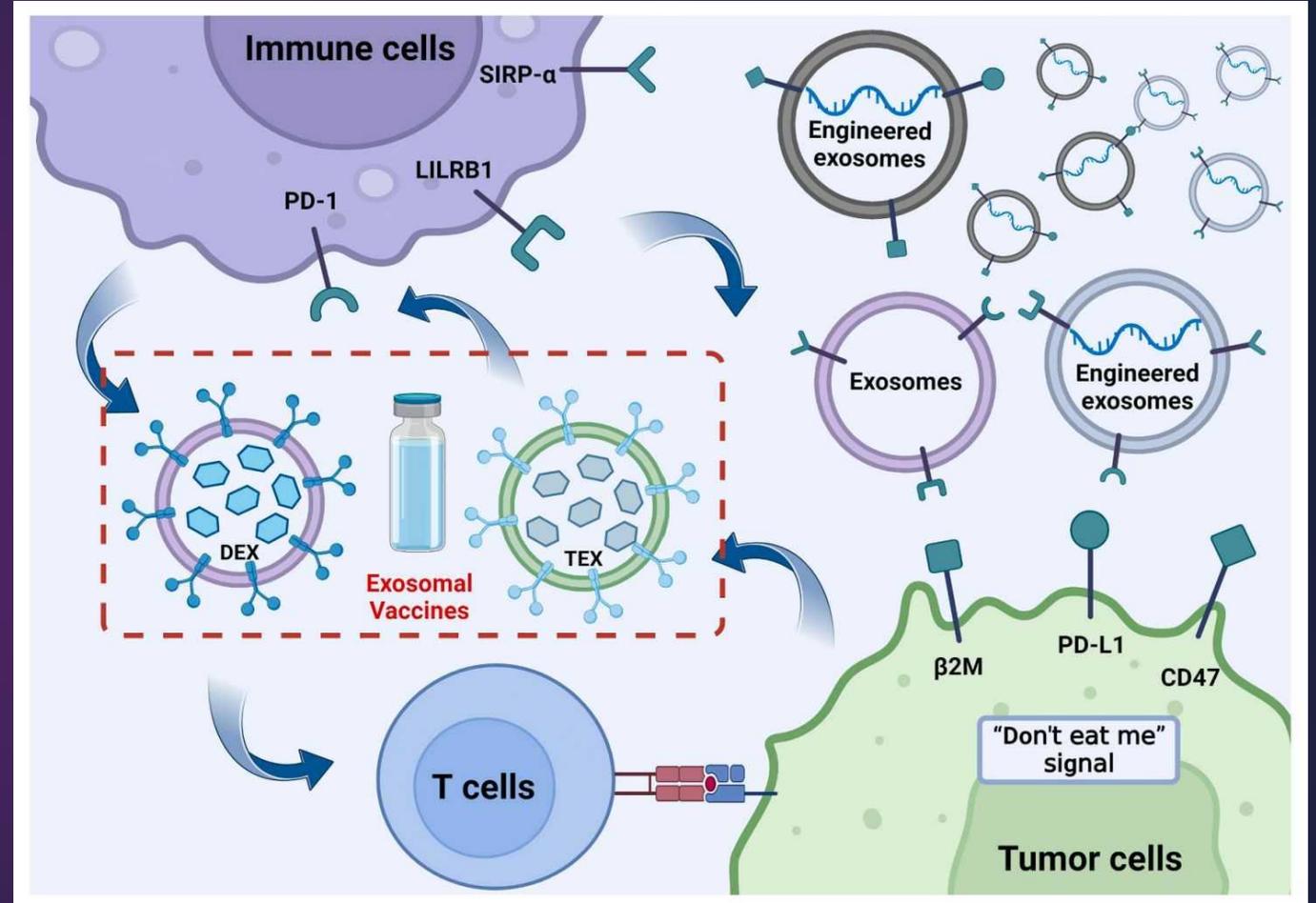
BMSCs BMSCs-exos Brain Lung Liver

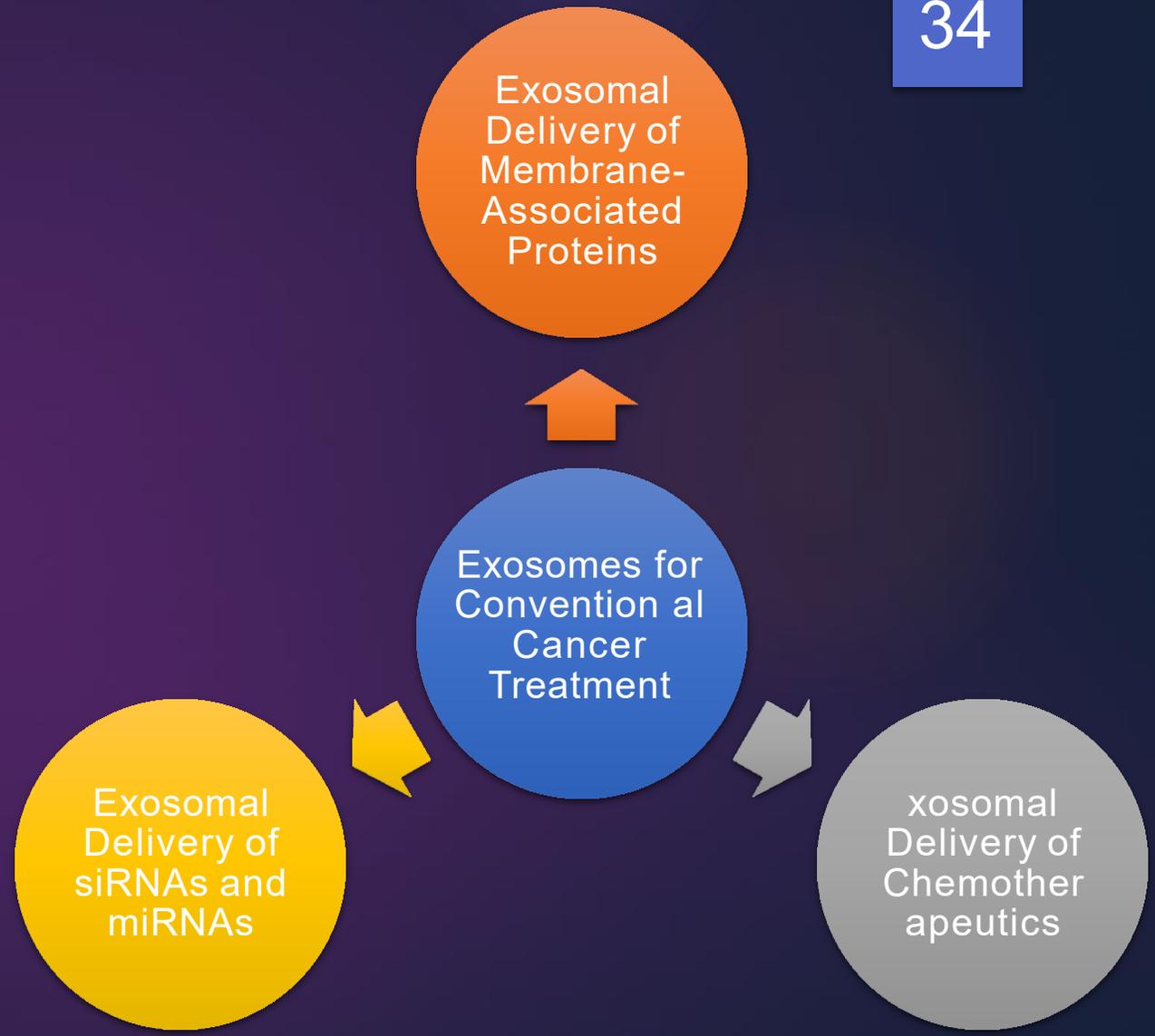
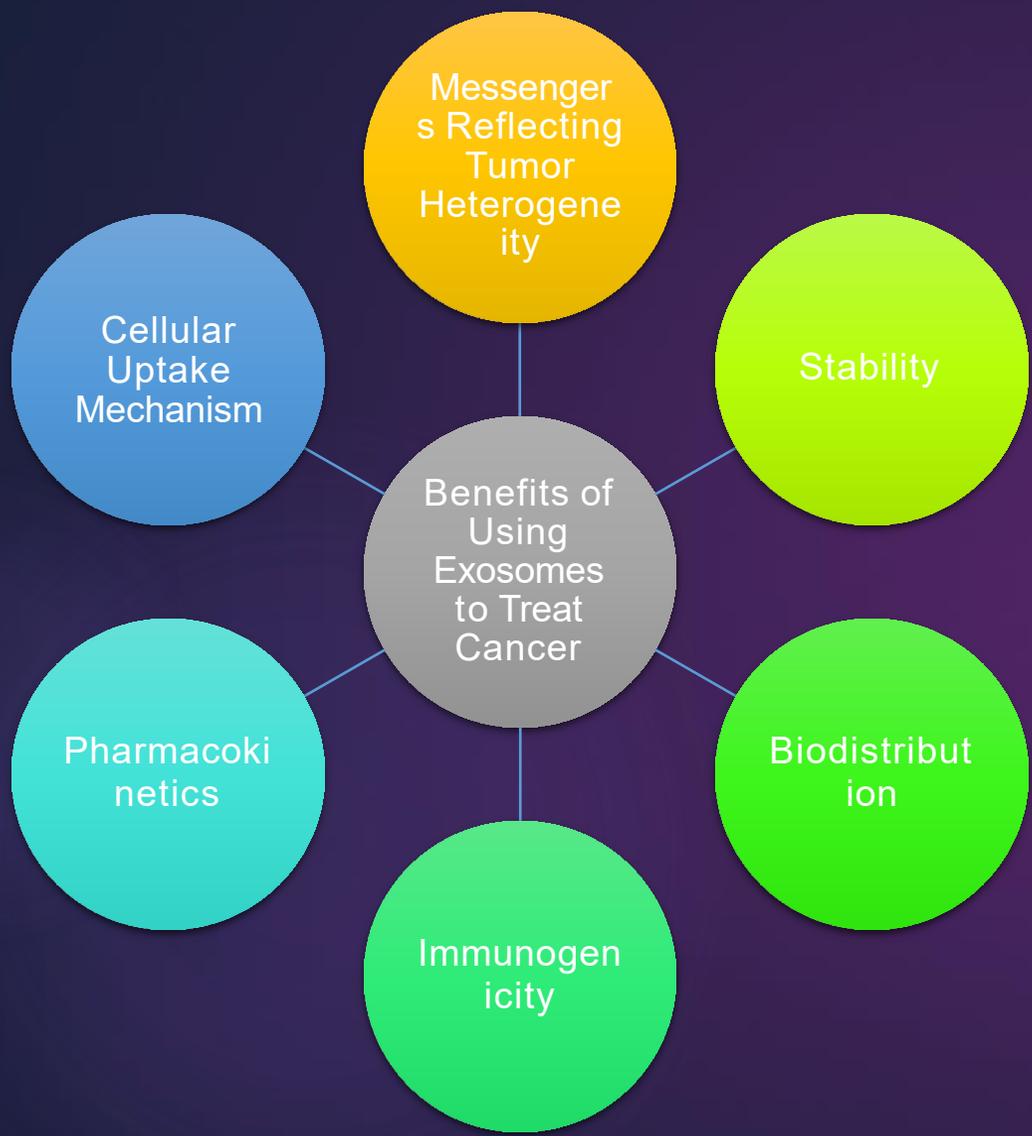
Cancer treatment



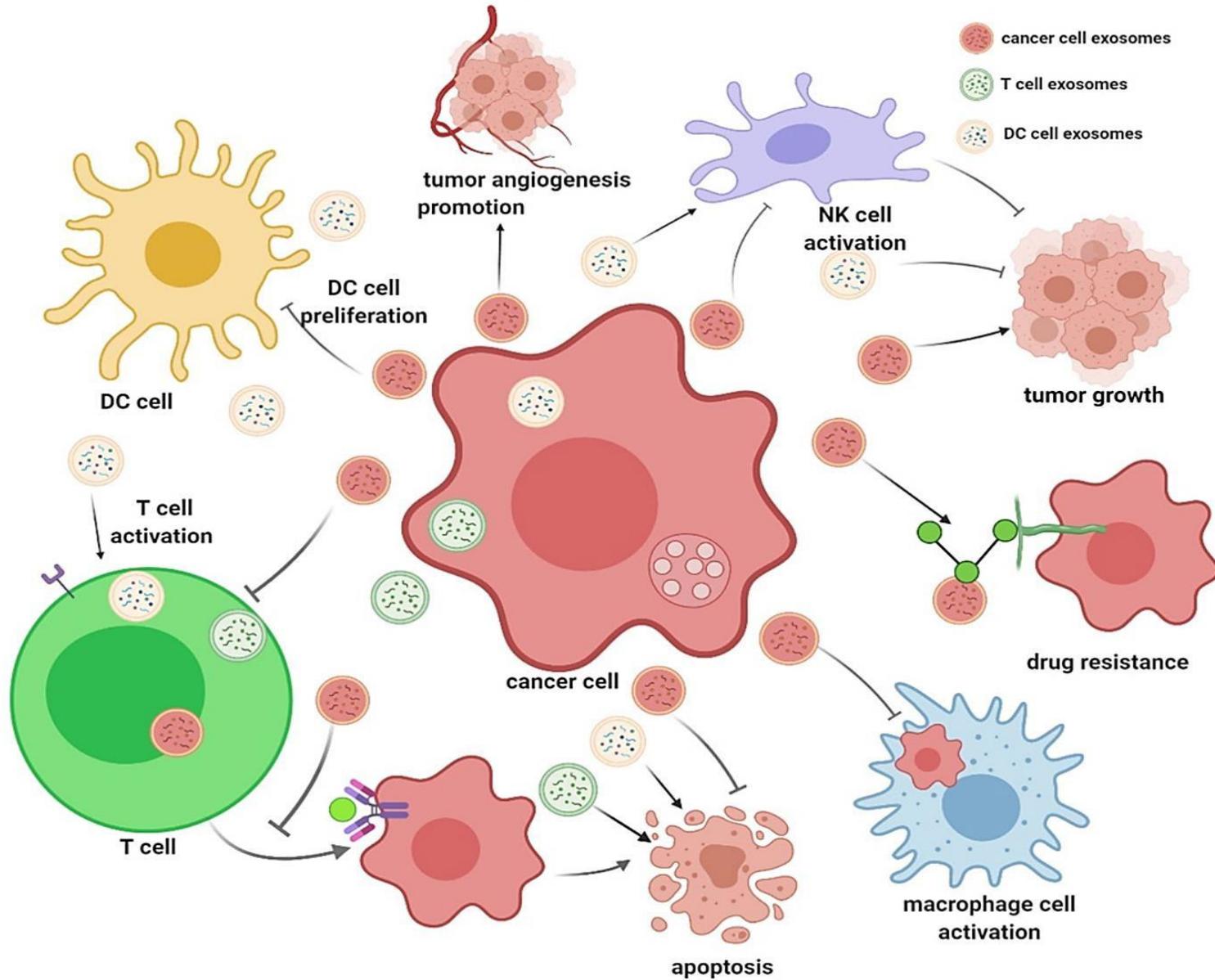
Cancer immunity cycle

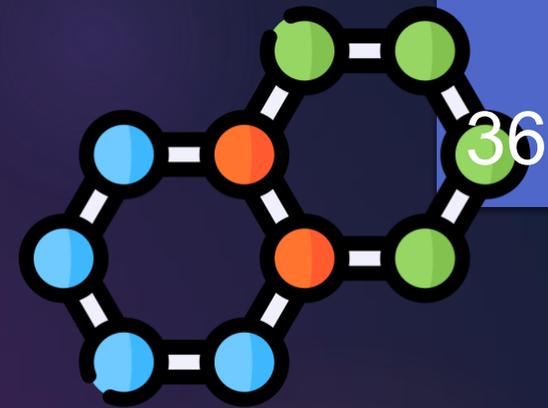
- Exo-DOX, exosome containing doxorubicin;
- Exo-PTX, paclitaxel-loaded exosome;
- Exo-OV, oncolytic virus-loaded exosome;
- TEX, tumor-derived exosome;
- DEX, dendritic cell-derived exosome;
- mVSVG-Exo, mVSVG protein expressing exosome;
- SIRP α -Exo, SIRP α expressing exosome;
- Exo-DNA, DNA-loaded exosome;
- Exo-STING, STING-loaded exosome;
- CD40L-Exo, CD40L expressing exosome;
- PH20-Exo, PH20 expressing exosome;
- CAR-Exo, CAR-T cell-derived exosome;
- SMART-Exo, synthetic multivalent antibodies expressing exosome;
- NK-Exo, NK cell-derived exosome;
- M1-Exo, M1-like macrophage-derived exosome.



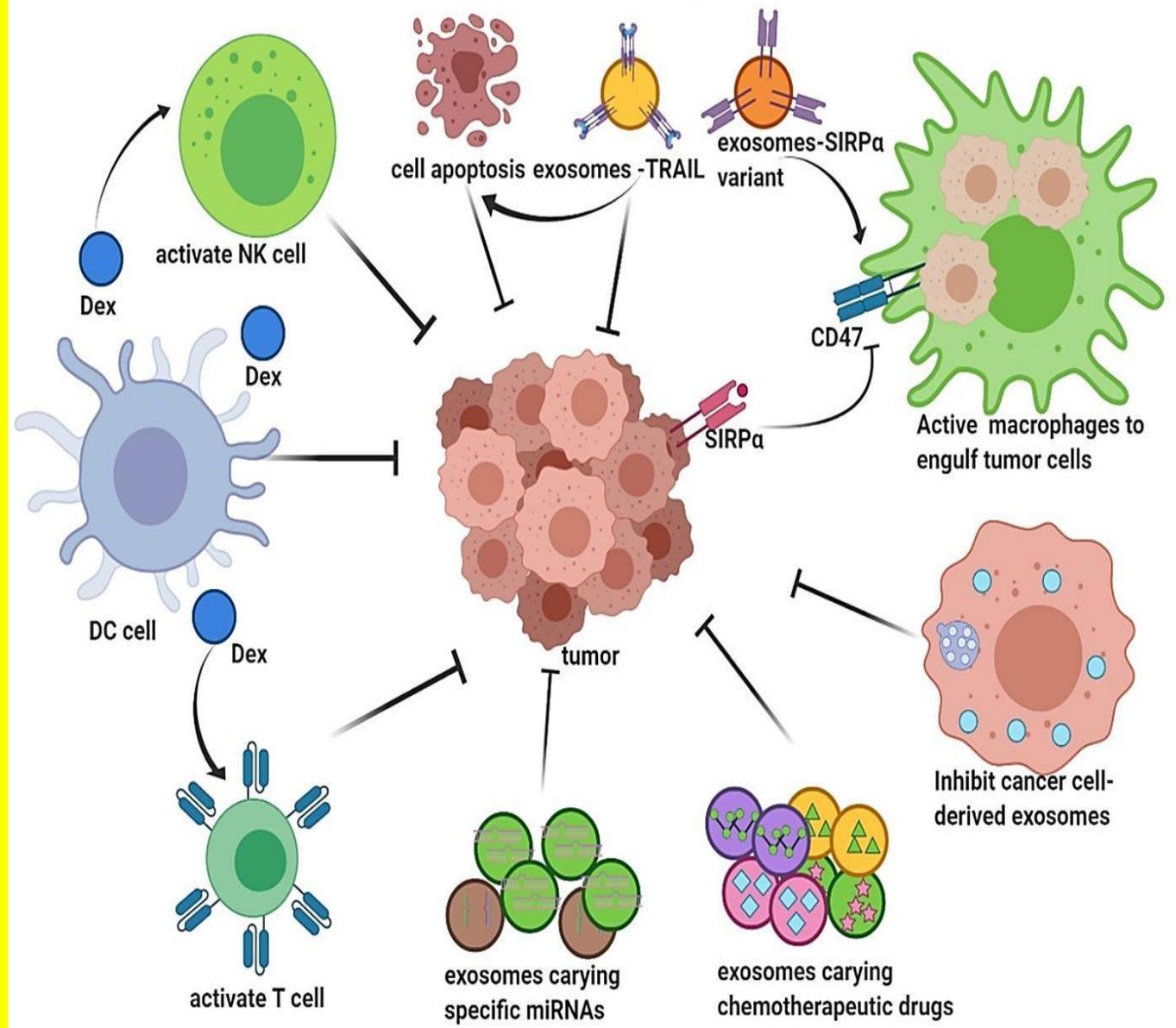


Dual role of exosomes in cancer progression





Exosome-Based Cancer Therapies



Exosome-based cancer therapies:

natural exosomes from some immune cells to suppress cancer cells

inhibition of cancer cells-derived exosomes

exosomes as gene carriers

exosomes as anti-cancer drug carriers

Exosome therapies in Ophthalmology



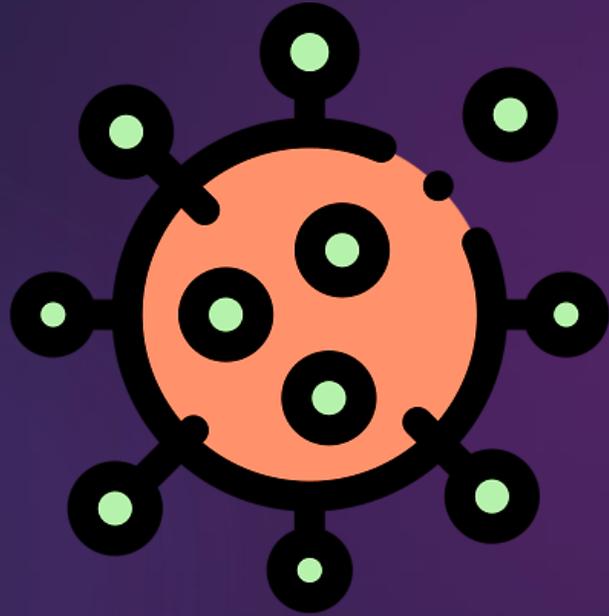


Exosome miRNA therapies in ophthalmology

Disease	References	Species	miRNAs	Therapeutic effects	Adverse effects
Glaucoma	Mead et al. (2018a)	Rodents	The neuroprotective effects might be related to the miRNAs from BMSC exosomes, namely MIR-100-5P, MIR-106A-5P, MIR-486-5P, MIR-144-5P, MIR-126-5P, MIR-100-5P	BMSC exosomes promoted neuroprotection of RGC and reduced degenerating axons in the optic nerve	Nil
	Mead et al. (2018b)	DBA/2J mice	The effects might be related to the miRNAs from BMSC exosomes	Human BMSC exosomes provided neuroprotection to the injured RGC and promoted RGC survival	Nil
	Mead et al. (2020)	Rats	The effects of MSC exosomes might be due to the modulation of their mRNA cargo and TNF α priming through increased levels of PEDF and VEGF-AA	MSC exosomes stimulated RGC survival resulting in neuroprotective effects in rats and human RGC	Nil
Dry eye disease	Weng et al. (2012)	Humans	The precise regulation role of MSCs has not been fully defined	MSCs can suppress the inflammatory and fibrous processes in dry eye in cGVHD by targeting specific CD8 ⁺ CD28 ⁻ T cells	Nil
Retinal ischaemia	Mosseiev et al. (2017)	C57BL/6 mice	The effects of human MSC might be due to the paracrine factors and miRNAs	Human MSC exosomes reduced retinal thinning and neovascularization	Nil
	Hajrasouliha et al. (2013)	C57BL/6 mice	The antiangiogenic effects of RAC exosomes might be caused by their multiple molecules (proteins, lipids, mRNA, miRNA)	Exosomes from mice RAC reduced choroidal neovascularization	Nil
	Ma et al. (2020)	Sprague-Dawley rats	The effects of MSC exosomes might be due to certain mRNAs, miRNAs and proteins	MSC exosomes prevented photoreceptor apoptosis post-retinal detachment, promoted anti-inflammatory effects and suppressed inflammatory cytokine induction	Nil
Diabetic retinopathy	Safwat et al. (2018)	Rabbits	The regenerative changes in the retina might be associated with the increased expression of miRNA-222	Adipose MSC exosomes induced repair of diabetic retinal degeneration and mediated tissue repair by transporting specific miRNAs	Nil
	Zhang et al. (2019)	Rats	miRNA-126 expression in MSC exosomes might reduce retinal inflammation by downregulating the high-mobility group box 1 (HMGB1) pathway degeneration (AMD)	MSC exosomes reduced the hyperglycaemia-induced retinal inflammation	Nil
Age-related macular Wet AMD	Nil		Potential increased wet AMD and choroidal neovascularization	Elbay et al. 2019	Humans
Uveitis	Bai et al. (2017)	Lewis rats	The effects of human MSC exosomes might be due to their cargo which is abundant in proteins and RNAs	Human MSC exosomes inhibited the autoimmune response, protected the retinal structure and rescued retinal function	Nil
	Shigemoto-Kuroda et al. (2017)	Rats	The therapeutic effects of MSCs in suppressing inflammation correlated with the TSG-6 mRNA level in MSCs	Human MSCs from different donors varied in their therapeutic effects in suppressing inflammation <i>in vivo</i> , and some MSCs failed to have any positive therapeutic effects	Nil
Optic neuropathy	Mead and Tomarev (2017)	Sprague-Dawley rats	The therapeutic effects might be caused by specific miRNAs, namely miR-17-92	BMSC exosomes promoted neuroprotection and neurogenesis of RGC. The expression of phosphatase	Nil
	Pan et al. (2019)	Wistar rats	The most abundant miRNAs in human UMSC exosomes are miRNA-21-5p, miRNA-125b-5p, miRNA-23a-3p, miRNA-100-5p and let-7f-5p	and tensin homolog (PTEN), an important suppressor of RGC axonal growth and survival, was downregulated UMSC exosomes promoted RGC survival and glial cell activation	Nil

BMSC = bone marrow stem cells; MSC = mesenchymal stem cells; RAC = retinal astroglial cells; RGC = retinal ganglion cells; UMSC = umbilical-cord mesenchymal stem cells.

Covid-19



COVID19

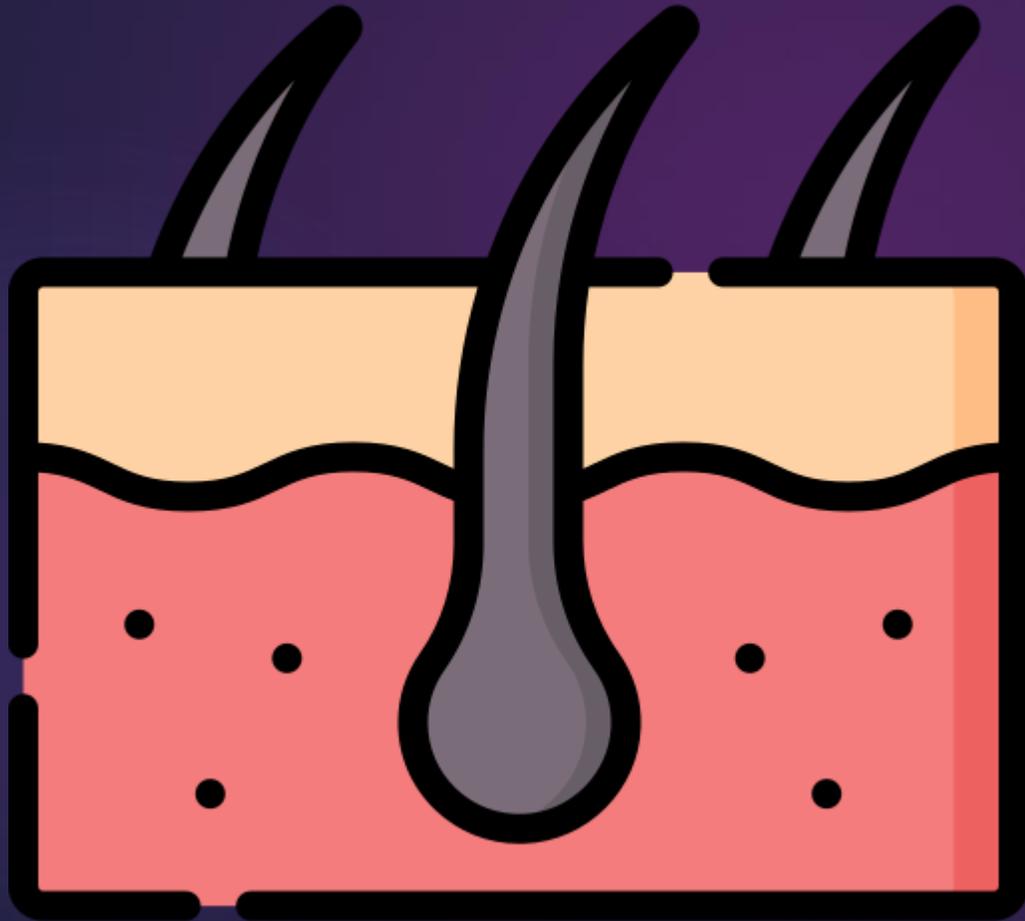
Clinical trials registered on ClinicalTrials.gov till 16 July 2020 utilizing Exosomes for the treatment of COVID-19

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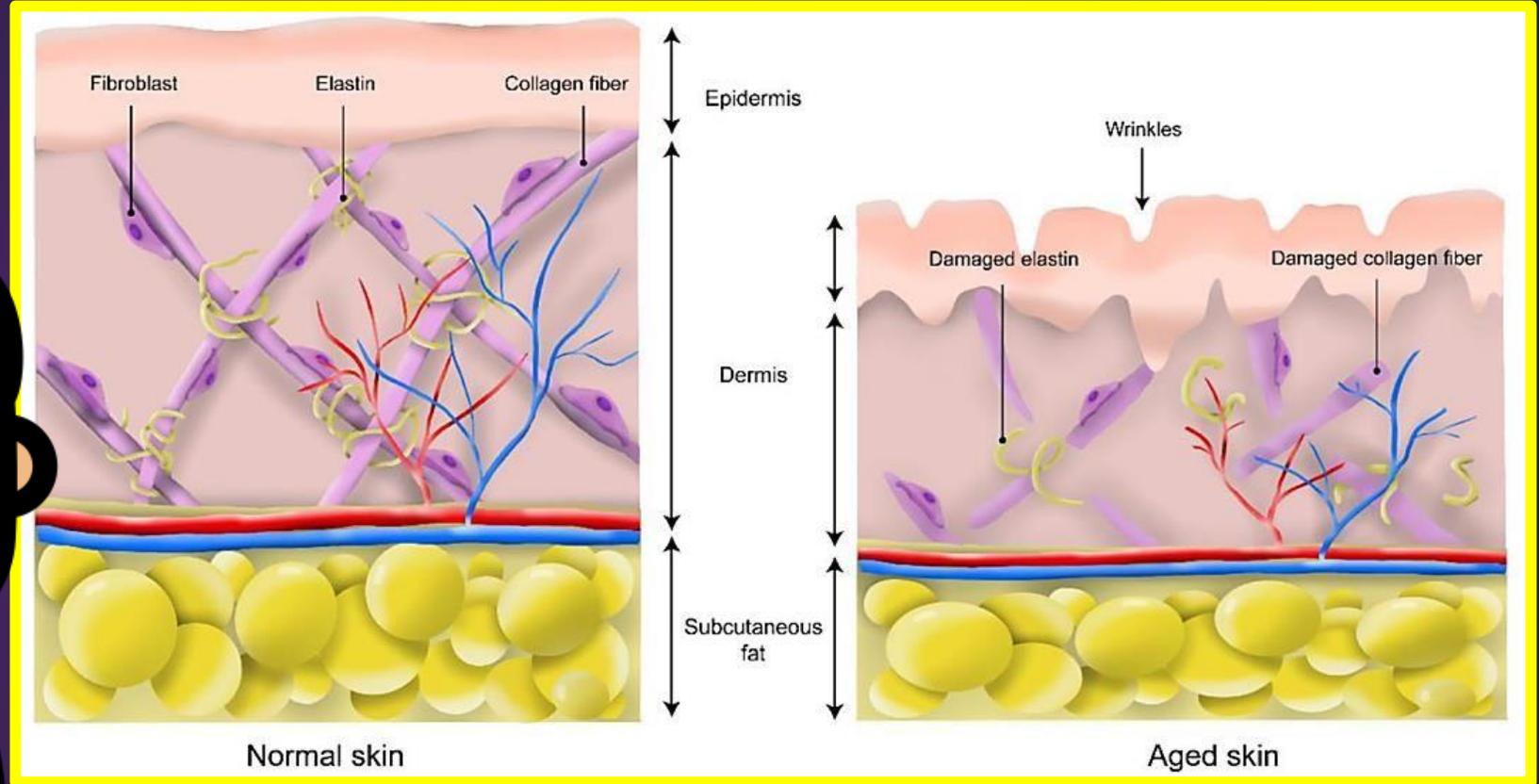
Study identifier	Exosome source	Study Phase; estimated enrollment (N)	Primary outcome measure(s)	Recruitment status
NCT04389385	COVID-19 specific T cell	Phase I; N=60	Adverse and severe adverse reaction (Time frame: 28 days); efficacy assessment (Time frame: 28 days); rate of recovery without mechanical ventilator (Time frame: 28 days)	Active, not recruiting
NCT04384445	Human amniotic fluid	Phase I/II; N=20	Incidence of any infusion associated adverse events (Time frame: 60 days); incidence of severe adverse events (Time frame: 60 days)	Not yet recruiting
NCT04276987	Allogenic adipose mesenchymal stem cells	Phase I; N=30	Adverse reaction and severe adverse reaction (Time frame: up to 28 days); time to clinical improvement (Time frame: up to 28 days)	Not yet recruiting

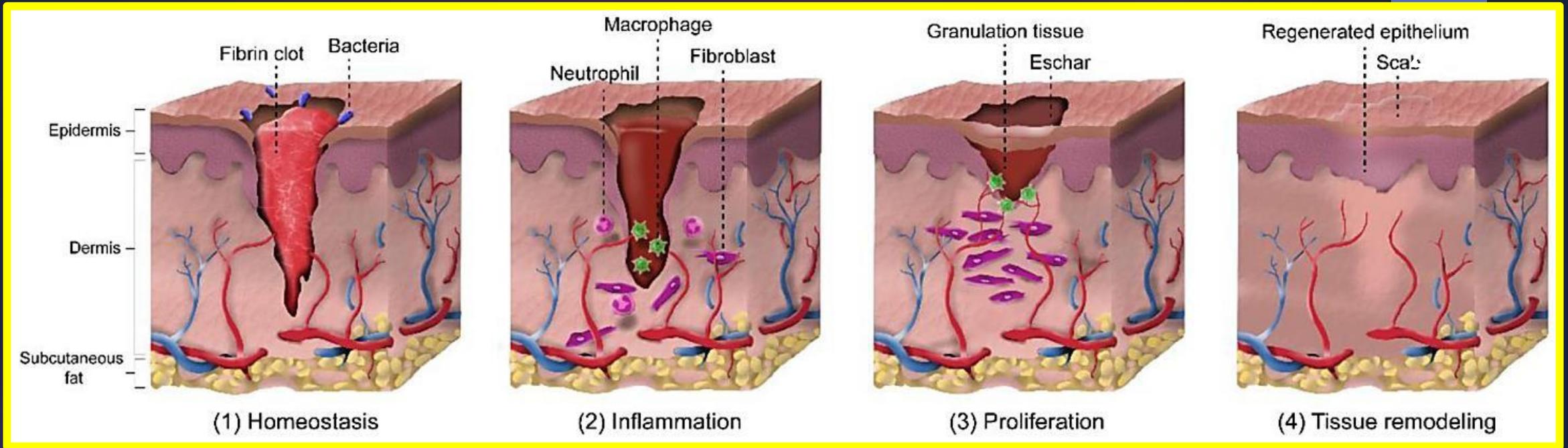


Skin treatment



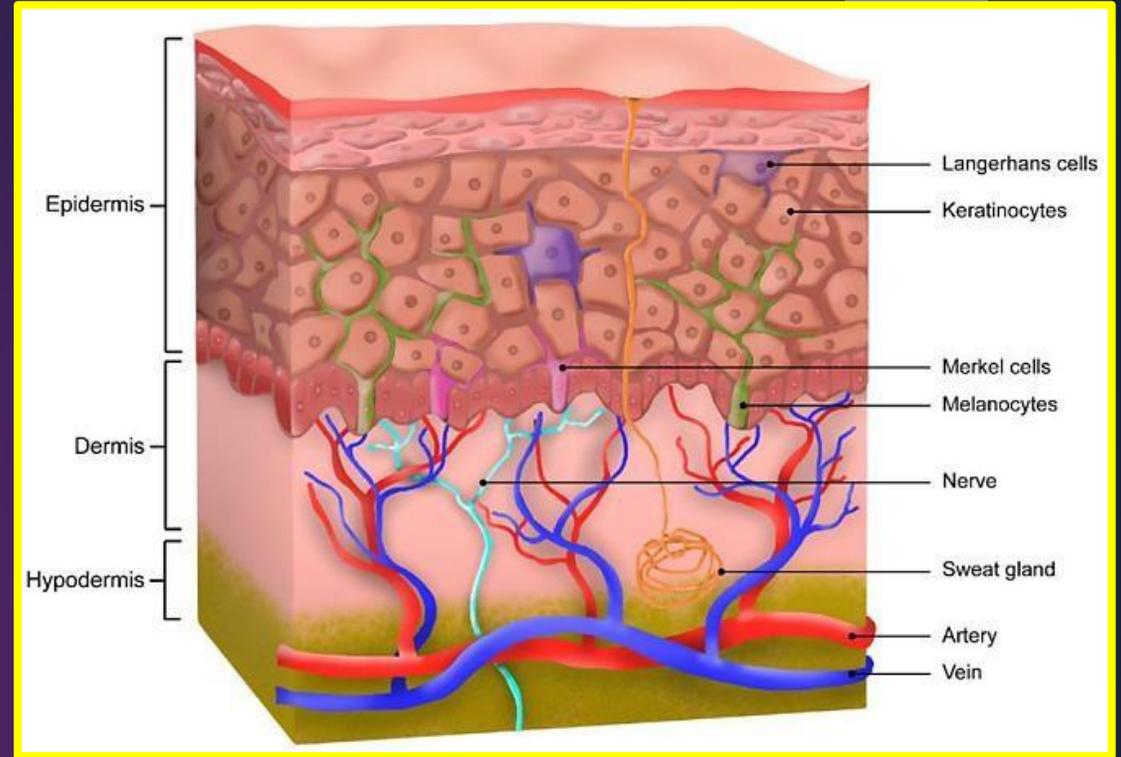
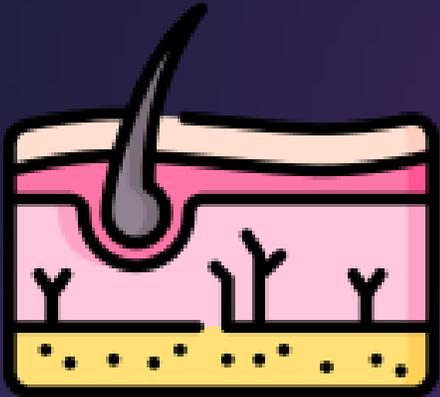
The structures of the skin before and after aging



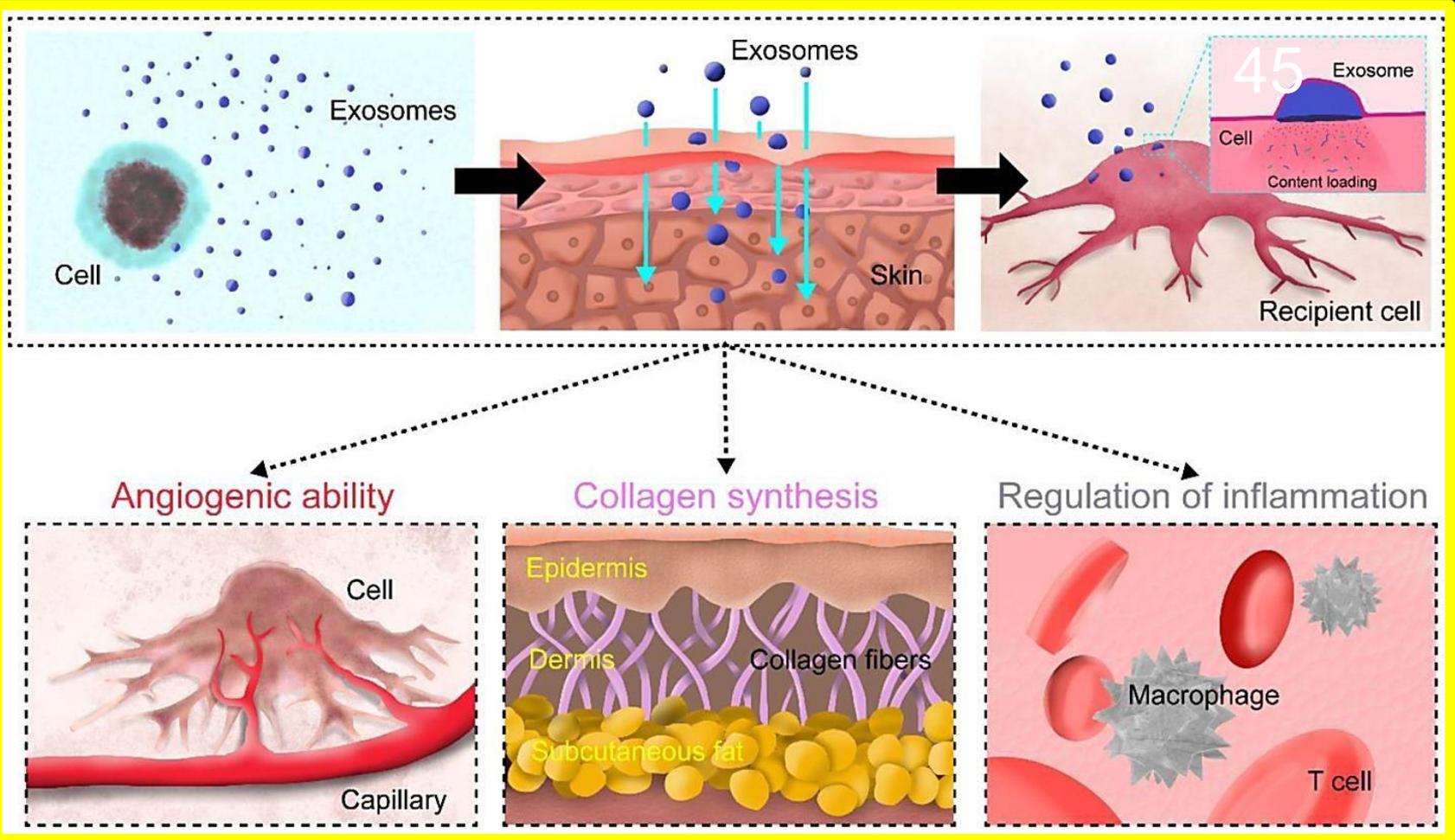


Wound healing process including the four stages

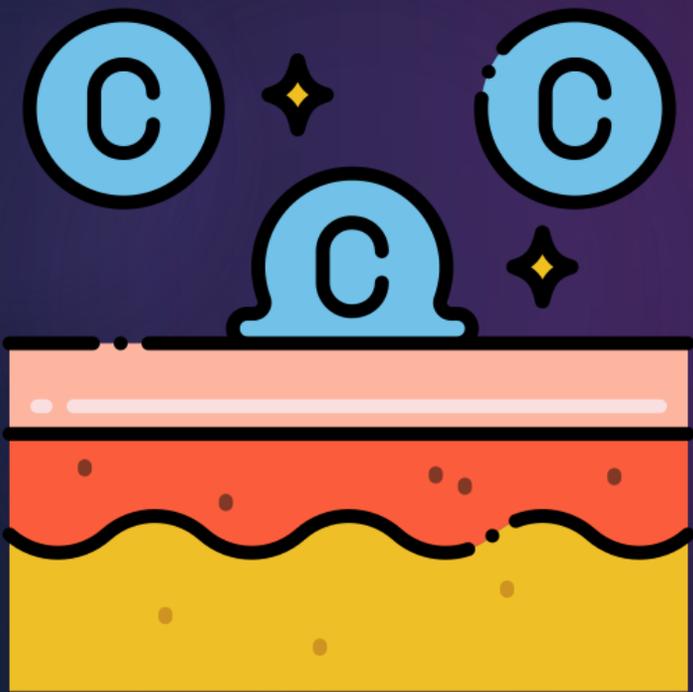
Skin treatment

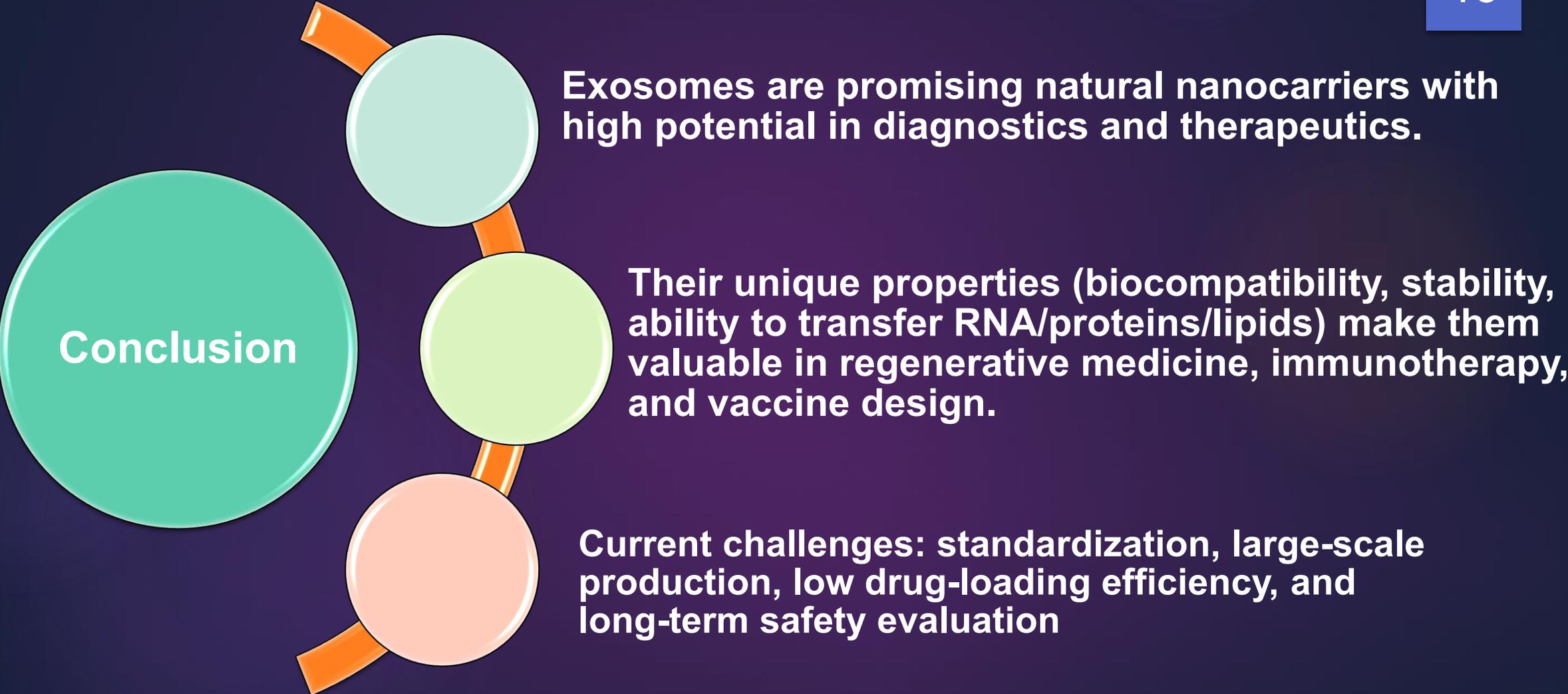


	Cells	Function
Epidermis	Keratinocytes Melanocytes Langerhans cells Merkel cells	Preservation of immune barrier Skin pigmentation Adaptive immune responses Mechanoreceptors
Dermis	Fibroblasts Mast cells	Synthesizing and depositing ECM component Immune and inflammatory responses
Hypodermis	Adipocytes	Energy storage, endocrine, nervous, and immune function



Schematic showing skin regenerative abilities of exosomes





Conclusion

Exosomes are promising natural nanocarriers with high potential in diagnostics and therapeutics.

Their unique properties (biocompatibility, stability, ability to transfer RNA/proteins/lipids) make them valuable in regenerative medicine, immunotherapy, and vaccine design.

Current challenges: standardization, large-scale production, low drug-loading efficiency, and long-term safety evaluation

Future Directions

Advanced **surface engineering** for targeted delivery.

More efficient **isolation and purification methods**.

Development of **next-generation exosome-based vaccines**.

Expanded **in vivo and clinical studies** to validate efficacy and safety.

Integration with **synthetic nanomaterials** for hybrid smart drug delivery systems

**THANK
YOU**

