WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 15, 1365-1384. Review Article ISSN 2277–7105

"EXOSOMES AS MULTIFUNCTIONAL NANOCARRIERS: INTEGRATIVE ROLES IN CANCER, CARDIOVASCULAR DISEASE, AND CIRCADIAN REGULATION"

Sweta Thakur[1], Sheetal Sharma[2*], Bhartendu Sharma[3], Vikramjeet Singh[4], Swati Sharma[5] and Priyanka Kumari

[1]Research Scholar, School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Baddi, Solan - 173205, Himachal Pradesh, India.
[2,4]Assistant Professor, School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Baddi, Solan - 173205, Himachal Pradesh, India.
[3]Associate Professor, School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Baddi, Solan - 173205, Himachal Pradesh, India.

Article Received on
20 June 2025,
Revised on 10 July 2025,
Accepted on 30 July 2025
DOI: 10.20959/wjpr202515-37769

***Corresponding Author**
Sheetal Sharma
Assistant Professor, School of Pharmacy and Emerging Sciences, Baddi University of Emerging Sciences and Technology, Baddi, Solan - 173205, Himachal Pradesh, India.

ABSTRACT

Exosomes are small extracellular vesicles that range from 30 to 150 nanometers in size. They have emerged as essential mediators of intercellular communication, transporting bioactive molecules such as proteins, lipids, and nucleic acids between cells. Once thought to be mere cellular waste, exosomes are now recognized for their significant roles in various physiological and pathological processes, including cancer, cardiovascular diseases (CVDs), and the regulation of circadian rhythms. Their ability to cross biological barriers, maintain stability in circulation, and deliver molecular cargo precisely to target sites makes them promising candidates for both diagnostics and therapeutics. In cancer, exosomes function as non-invasive liquid biopsies that reflect the genetic and proteomic profiles of tumor cells. This capability allows for early detection and the development of personalized treatment strategies. Furthermore, exosomes show great potential as vehicles for the targeted delivery of chemotherapeutic agents, although challenges remain regarding standardization, scalability, and the heterogeneity of isolation methods in clinical applications. In the context of cardiovascular disorders, exosomes released from cardiomyocytes, endothelial cells, and stem cells play

critical roles in regulating inflammation, oxidative stress, and endothelial function—key factors in the pathogenesis of CVD. Their potential as diagnostic and therapeutic tools is actively being investigated, particularly focusing on their use as biomarkers and drug delivery systems in innovative treatment approaches. Emerging evidence also highlights the dynamic relationship between circadian rhythms and exosomal activity. Disruptions in circadian cycles can alter the secretion patterns and molecular content of exosomes, which in turn affects gene expression and metabolic pathways. Exosomes may even contribute to maintaining circadian homeostasis, suggesting new opportunities for chronotherapy and time-based disease management. This review provides a comprehensive overview of recent advances in exosome research, examining their diverse roles in cancer, cardiovascular disease, and circadian biology. It also highlights ongoing technological innovations aimed at overcoming current limitations, paving the way for the clinical application of exosomes in precision medicine.

KEYWORDS: Exosomes, Cancer Diagnosis, Cardiovascular Diseases, Circadian Rhythms, Liquid Biopsy, Drug Delivery, Precision Medicine.

INTRODUCTION

Exosomes are nanosized extracellular vesicles (EVs) with a diameter ranging from 30 to 150 nm. They are naturally secreted by cells as part of normal cellular activity. Initially considered waste, exosomes are now recognized for their essential role in intercellular communication. They transport various biomolecules, including proteins, lipids, DNA, and non-coding RNAs, and play a significant role in processes such as immune regulation, apoptosis, angiogenesis, and tumor progression.[1] Exosomes are part of a broader category of extracellular vesicles that include microvesicles and apoptotic bodies, and they are distinguished by their size and mode of biogenesis. Exosome formation occurs through endosomal pathways, primarily via ESCRT-dependent mechanisms that involve the sequential assembly of ESCRT complexes (0, I, II, III), or through ESCRT-independent routes involving tetraspanins (e.g., CD63, CD81) and ceramide. These vesicles can be found in various biofluids, such as blood, urine, saliva, cerebrospinal fluid, and breast milk, making them accessible for non-invasive diagnostics.[2] In liquid biopsies, exosomes serve as carriers of disease-specific biomarkers, reflecting the physiological state of tissues such as the brain, liver, kidneys, and heart. Their diagnostic potential is particularly valuable in cancer, as they can transport tumor-derived signatures including miRNAs and surface proteins like PSA,

CEA, and CA19.9. Advances in genomic technologies now enable the detection of exosomal DNA and RNA, supporting early diagnosis and disease monitoring.[3]

Historical Background of Exosome Discovery

The term "exosome" was originally used in the 1980s. In 1983, two landmark studies by Pan and Johnstone demonstrated the release of transferrin receptors in small vesicles (~50 nm) from reticulocytes (immature red blood cells) during their maturation. Initially thought to be a cellular waste disposal mechanism, further studies in the 1990s and early 2000s revealed that exosomes play active roles in intercellular communication, immune response, and disease progression. Since then, exosomes have gained significant attention in diagnostics, therapeutics, and nanomedicine.[4]

Importance in Cell–Cell Communication

Exosomes serve as natural messengers in cell–cell communication by transferring their cargo—proteins, lipids, and genetic material (mRNA, miRNA, lncRNA)—to recipient cells.[5] This process influences various physiological and pathological processes, including:

- Immune modulation
- Tumor growth and metastasis
- Angiogenesis
- Neurodegeneration
- Inflammation

Their ability to cross biological barriers, such as the blood–brain barrier, further enhances their role in systemic communication and makes them vital players in disease mechanisms and therapeutic interventions.[6]

Why Exosomes are Considered Promising Nanocarriers

Exosomes have emerged as **natural, smart delivery vehicles** due to their intrinsic biological advantages.[7] The key features that make them suitable for drug delivery and targeted therapy include:

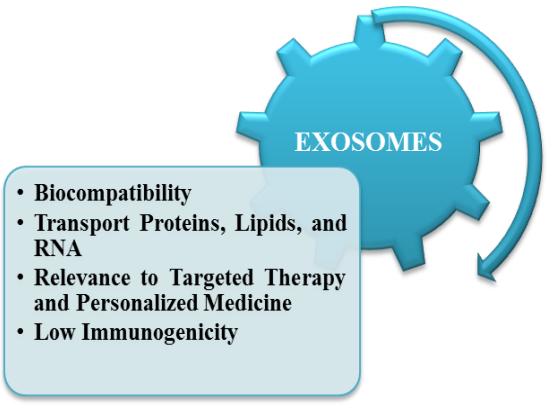

Fig. 1: Exosomes as a Promising Nanocarriers.

1. Biocompatibility

Exosomes are derived from the body's own cells, making them inherently **biocompatible** and safe. Unlike synthetic nanoparticles, they do not induce significant cytotoxicity or systemic toxicity upon administration.

2. Ability to Transport Proteins, Lipids, and RNA

Exosomes encapsulate and **protect their cargo** from enzymatic degradation in the extracellular environment. They can efficiently carry:

- Therapeutic **proteins** (e.g., enzymes, antibodies)
- Bioactive **lipids**
- Genetic materials such as **siRNA, miRNA, and mRNA**

These properties enable **gene therapy**, **protein replacement therapy**, and **small molecule delivery** in a natural form.

3. Low Immunogenicity

Due to their endogenous origin, exosomes elicit **minimal immune response**, even in repeated dosing. This low immunogenic profile is particularly beneficial for **chronic diseases**, **cancer therapy**, and **autoimmune disorders**.[8]

4. Relevance to Targeted Therapy and Personalized Medicine

Exosomes naturally express **surface markers** (e.g., tetraspanins like CD9, CD63, CD81) and **adhesion molecules** that facilitate **targeted delivery** to specific tissues or cell types. Furthermore, they can be **engineered** or **loaded with therapeutic agents** for precision medicine.

Their role in **personalized medicine** is growing, particularly in:

- **Oncology**: delivering chemotherapeutics, RNA interference agents, or tumor antigens
- **Neurology**: transporting therapeutic genes or drugs across the blood-brain barrier
- **Cardiology**: facilitating cardiac regeneration by carrying cardioprotective factors.[9]

Biogenesis of Exosomes

Cell-to-cell communication and signaling are initiated and enhanced by chemical messengers, which are often packaged in extracellular vesicles (EVs). These vesicles can be easily modified to carry genetic materials such as messenger RNA (mRNA), microRNA (miRNA), non-coding RNAs, genomic DNA (gDNA), genetic lipids, and specific proteins. Among the different types of EVs, exosomes are a promising candidate for developing nanoparticles because of their unique characteristics.[10] Exosomes bud from the plasma membrane, typically ranging in size from 100 to 1000 nanometers. They are lipid bilayer vesicles derived from cellular membranes and carry bioactive cargo mainly composed of small genetic materials (like microRNAs) and peptides, as illustrated in (Fig. 2). Exosomes are released into systemic circulation as free-floating particles and possess the ability to adhere to other cells.[11]

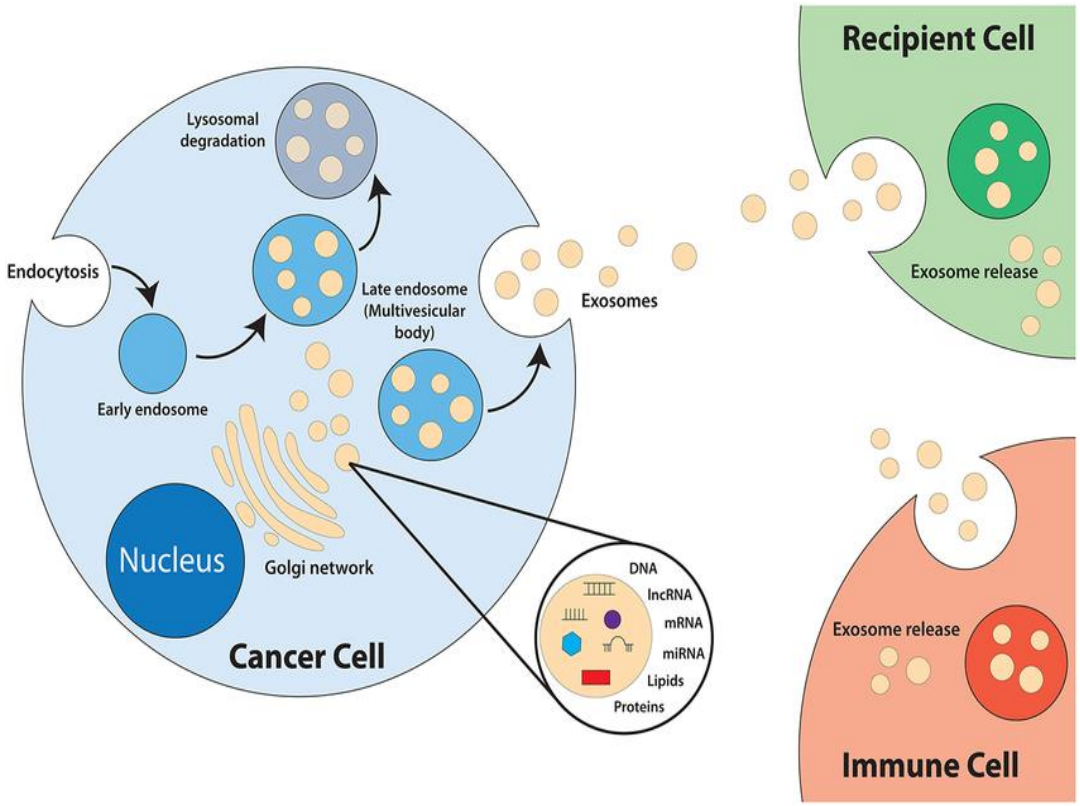

Fig. 2: Biogenesis of Exosomes.

Composition of Exosomes

Exosomes are composed of various proteins, including Major Histocompatibility Complex (MHC)-II, integrins, clusters of differentiation (CD), tetraspanins, heat shock proteins (HSP), and Ras-related proteins (RAB), as illustrated in Figure 3. They also contain a diverse array of lipids, such as sphingomyelins and cholesterol, as well as various genetic materials, including nucleic acids like miRNA, mRNA, and non-coding RNAs (Figure 3). When exosomes fuse with neighboring cells, they release their contents into these recipient cells, initiating fundamental changes in gene expression that can have both positive and negative effects. Because exosomes carry genetic variants from their parent cells, they provide a snapshot of the environmental influences on the existing cell's health and origin. As a result of their unique ability to transport signaling molecules and genetic information, exosomes have proven to be effective biomarker proteins for the early diagnosis of various disorders, including cancer and neurodegenerative diseases. In the future, they may also facilitate the monitoring and prognosis of these conditions.[12]

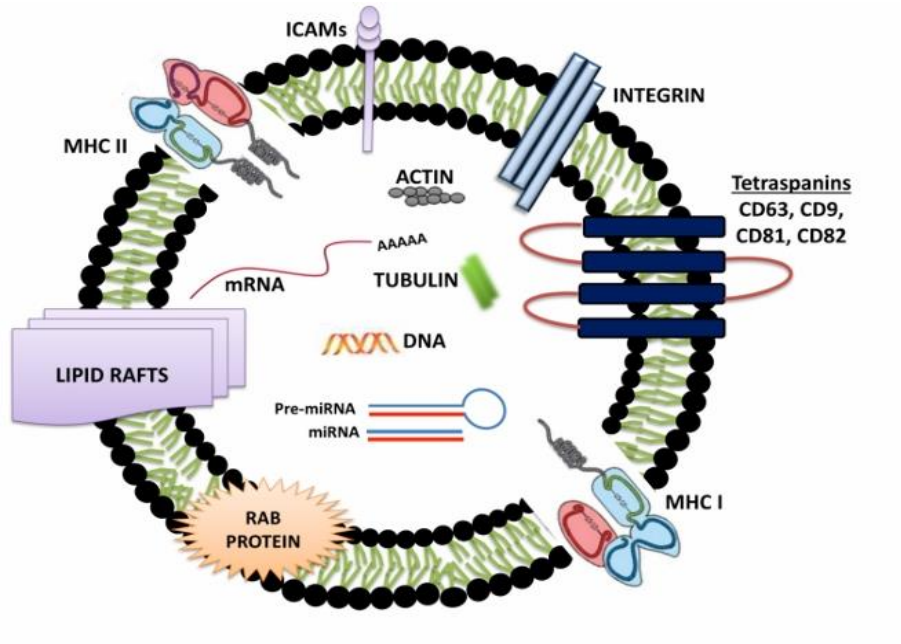

Fig. 3: Composition of Exosomes.

Structure of Exosomes

Exosomes possess a spherical, nanoscale architecture with a lipid bilayer membrane that closely resembles the plasma membrane of the parent cell. This bilayer is crucial for maintaining the structural integrity of exosomes and for protecting their internal cargo from enzymatic degradation in the extracellular environment.[13]

1. Lipid Bilayer

The exosomal membrane is primarily composed of phospholipids, cholesterol, sphingomyelin, and ceramide—components that contribute to membrane rigidity and stability.

The presence of lipid rafts—microdomains rich in cholesterol and sphingolipids—supports selective cargo sorting and signal transduction.

2. Surface Proteins and Targeting Ligands

The exosome surface is decorated with various membrane-bound proteins, many of which serve as targeting ligands or cell-specific markers, including:

- Tetraspanins (CD9, CD63, CD81): structural markers involved in exosome formation and fusion
- Integrins and Adhesion Molecules: help in directing exosomes to specific target cells or tissues
- MHC I and II: involved in antigen presentation, particularly in immune cell-derived exosomes
- Heat shock proteins (Hsp70, Hsp90): facilitate protein folding and protect against stress conditions

These surface proteins play critical roles in target cell recognition, membrane fusion, and uptake via receptor-mediated endocytosis, making exosomes highly suitable for targeted drug delivery.

3. Internal Cargo

Inside the exosome, a diverse range of biologically active molecules is encapsulated:

- Proteins: enzymes, cytokines, signaling molecules
- Nucleic acids: mRNA, miRNA, lncRNA, DNA fragments
- Lipids: bioactive molecules that also contribute to signal transduction.[14]

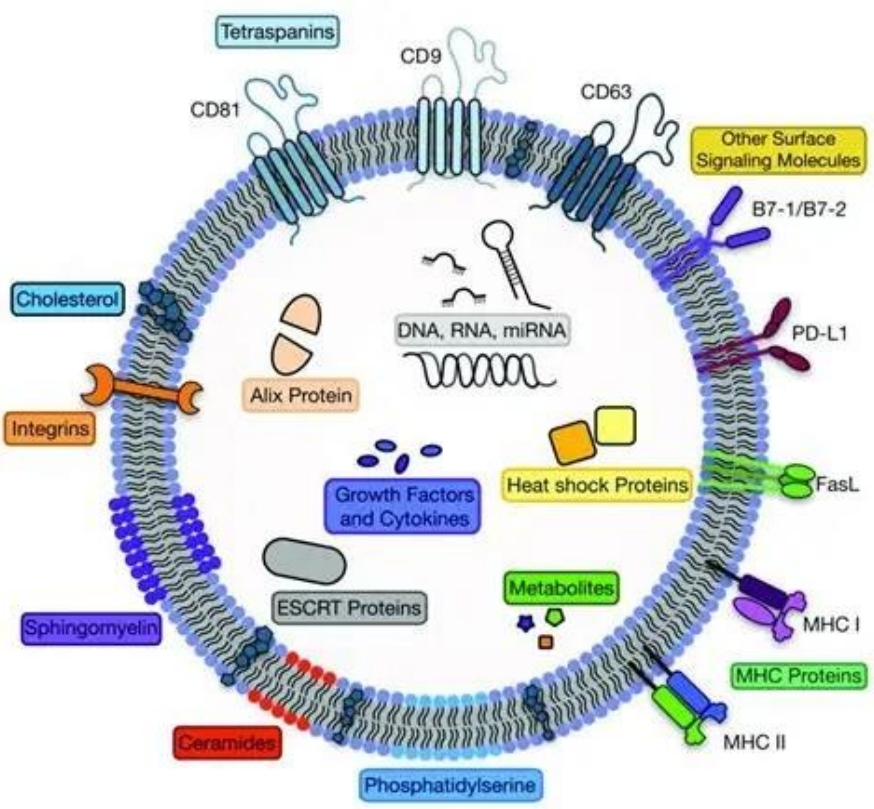

Fig. 4: Structure of Exosomes.

Exosomes in Cancer Therapy

Exosomes are small vesicles that are secreted by cells, including tumor cells, under physiological conditions. However, tumor cells are particularly prolific producers of exosomes, possibly due to the hypoxic (low oxygen) environments they often inhabit. Stressed tumor cells tend to produce more exosomes as a response to their unfavorable conditions.[15]

Tumor-derived exosomes (TEXs) carry a range of molecules and factors that can transfer information from the parent tumor cell to other cells both within and outside the tumor microenvironment. TEXs exhibit distinct properties compared to exosomes produced by normal cells and have been implicated in both immune suppression and immune activation.

Studies have shown that exosome fractions obtained from the plasma of cancer patients are enriched with various immunosuppressive molecules. These include death receptor ligands such as FasL, PD-L1, and TRAIL, as well as inhibitory cytokines like IL-10 and TGF-β1, along with PGE2. Unlike exosomes from normal cells, those derived from tumors can induce apoptosis (programmed cell death) in activated CD8+ T cells, promote the differentiation and

function of regulatory T cells (Treg), and disrupt dendritic cell (DC) differentiation, thereby favoring the expansion of myeloid-derived suppressor cells (MDSCs). Importantly, TEXs not only carry immunosuppressive factors but also tumor-associated antigens, a variety of co-stimulatory proteins, and major histocompatibility complex (MHC) molecules. This dual signaling capability, both immunostimulatory and immunoinhibitory, positions TEXs as potential biomarkers for cancer progression and immune response to tumors. In addition to their role in modulating the immune response, TEXs mediate various pro-tumorigenic effects. They promote tumor growth, sustain autocrine loops, and alter the functions of stromal cells. A considerable amount of literature describes TEXs as carriers of oncogenic signals or active oncogenes, indicating their role in neoplastic transformation.[16]

Thus, due to their ability to facilitate tumor growth, TEXs, also referred to as "oncosomes," may serve as promising diagnostic tools or therapeutic targets in cancer treatment.

Functional Diversity and Naming

Exosomes are incredibly versatile and have been referred to by various names based on their biological origin and functional roles:

1. Oncosomes

- **Definition**: Exosomes released by tumor cells that promote oncogenic activity.
- **Functions**: Facilitate cancer cell communication, angiogenesis, immune evasion, metastasis, and drug resistance.

2. Tolerosomes

- **Definition**: Exosomes involved in **immune tolerance**.
- **Functions**
  - Secreted by antigen-presenting cells (APCs).
  - Carry MHC molecules and immunosuppressive agents (e.g., IL-10, TGF-β).
  - Promote peripheral tolerance, especially in autoimmune diseases and transplantation.

3. Argosomes

- **Definition**: Exosomes associated with **morphogen transport** during embryonic development.
- **Functions**
  - Carry signaling molecules (e.g., Wnt, Hedgehog) involved in tissue patterning.
  - Facilitate gradient formation during organogenesis.

4. Other Nomenclature Based on Function

- **Dexosomes** – Exosomes derived from dendritic cells, involved in immune stimulation.
- **Texosomes** – Exosomes derived from tumor cells.
- **Myelinosomes** – Associated with neurodegenerative diseases like multiple sclerosis.
- **Cardiosomes** – Exosomes secreted by cardiac cells involved in heart function and injury response.[17]

These classifications emphasize the wide-ranging physiological and pathological activities mediated by exosomes.

Key Biological Functions of Exosomes

1. Molecular Communication

- Exosomes mediate **cell-to-cell communication** by transporting:
  - **Proteins**: enzymes, receptors, and signaling molecules.
  - **Lipids**: sphingomyelin, ceramides (involved in vesicle formation and signaling).
  - **mRNAs and microRNAs**: regulate gene expression in recipient cells.
  - **DNA fragments**: possibly contributing to horizontal gene transfer.
- **Cell-Specific Targeting**
  - Exosomal surface markers determine interaction specificity (e.g., integrins, tetraspanins like CD9, CD63, CD81).

2. Modulation of Recipient Cell Function

- Upon uptake, exosomal cargo influences:
  - **Gene expression**: Activation or silencing of pathways.
  - **Protein synthesis**: Through mRNA or microRNA transfer.
  - **Phenotypic reprogramming**: Especially in immune cells, stromal cells, and stem cells.

3. Exosome Secretion by Various Cell Types

- Exosomes are secreted by:
  - **Immune cells**: T cells, B cells, macrophages, dendritic cells.
  - **Non-immune cells**: Fibroblasts, endothelial cells, epithelial cells.
  - **Stem cells**: Especially mesenchymal stem cells (MSC) with regenerative properties.
  - **Neurons**: Involved in neuro-signaling and disease propagation.
  - **Cancer cells (TEX)**: Exhibit pro-tumorigenic effects.[18]

Role of Exosomes in Cancer

1 Tumor Microenvironment and Metastasis

- **TEX prepare pre-metastatic niches**
  - Educate stromal cells to support tumor cell colonization.
  - Alter extracellular matrix (ECM) components via MMPs (matrix metalloproteinases).
  - Recruit bone marrow–derived cells and immune suppressor cells.

2 Immune Suppression

- TEX inhibit anti-tumor immunity by:
  - **Reducing NK cell cytotoxicity** (via downregulation of NKG2D).
  - **Inducing apoptosis** of activated cytotoxic T cells.
  - **Triggering expansion of Treg cells** (regulatory T cells).
  - **Modulating antigen-presenting cells (APCs)** to become tolerogenic.

3 Angiogenesis and Drug Resistance

- Exosomes stimulate **blood vessel formation** by:
  - Releasing VEGF, FGF, and angiopoietins.
  - Carrying miRNAs (e.g., miR-210, miR-23a) that promote angiogenesis.
- **Drug Resistance Mechanisms**
  - Sequestration and efflux of chemotherapeutic drugs.
  - Transfer of **drug-resistance genes** (e.g., MDR1).
  - Delivery of **anti-apoptotic proteins** and miRNAs to sensitive tumor cells.
  - Inhibiting caspase activation in recipient cells.[19]

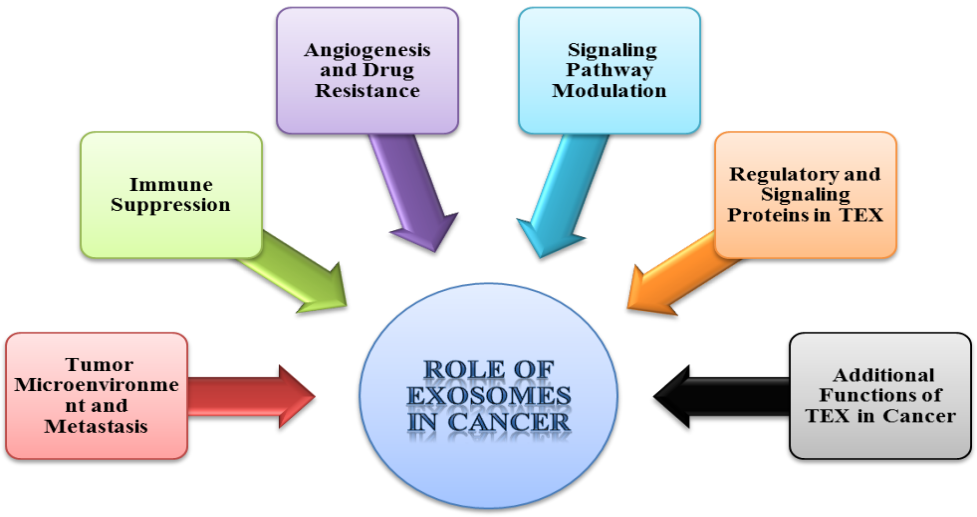

Fig. 5: Role of Exosomes In Cancer.

4 Signaling Pathway Modulation

- TEX modulate multiple cancer-related pathways:
  - **TGF-β**: Promotes epithelial-to-mesenchymal transition (EMT) and immunosuppression.
  - **Wnt/β-catenin**: Enhances proliferation and stemness of tumor cells.
  - **Notch**: Influences angiogenesis, cell fate, and tumor aggressiveness.
  - **PI3K/AKT/mTOR**: Promotes cell survival, growth, and metabolism.
  - **MAPK and ERK**: Stimulates proliferation and migration.

5 Regulatory and Signaling Proteins in TEX

- **p53**: Regulates secretion of exosomes under cellular stress or DNA damage.
- **PTEN**: Delivered via TEX; modulates PI3K/AKT signaling in target cells.
- **HSP70 and HSP90**: Heat shock proteins found in TEX, promoting immune evasion and survival.
- **Integrins**: Dictate tissue-specific metastasis by guiding exosomes to target organs.

6 Additional Functions of TEX in Cancer

- **Stemness Promotion**
  - TEX enhance cancer stem cell (CSC)-like traits by transferring pluripotency-related miRNAs.
- **ECM Remodeling**
  - Deliver enzymes like heparanase or MMPs to degrade extracellular matrix barriers.
- **Neurogenesis in Tumors**
  - Promote neurite outgrowth and innervation in tumors (important in prostate, head & neck cancers).
- **Therapy Response Modulation**
  - Predict patient response to chemotherapy or immunotherapy.
  - Serve as **liquid biopsy markers** for cancer detection and progression monitoring.[20]

Exosomes in Cardiovascular Disease

The Potential of Exosomes as Biomarkers in Cardiovascular Diseases

1. Diagnostic Potential of Exosomes

Exosomes reflect the state of disease through their contents, which include RNAs, proteins, and lipids. They are easily accessible in body fluids such as blood, making them ideal for non-invasive testing.[21]

1.1 Plasma Exosome Count

Variations in exosome concentration can indicate different stages of cardiovascular conditions. Elevated or reduced exosome counts may signal pathological changes within the cardiovascular system.

1.2 Exosomal miRNAs for Diagnosis

Specific microRNAs (miRNAs) found within exosomes can reflect cardiac tissue damage.

Example: Exosomal miR-208a correlates with myocardial injury more accurately than its circulating (free) form. This makes it useful for detecting coronary artery disease and monitoring post-surgical cardiac stress.

1.3 Endothelium-Derived Microvesicles

Different levels of endothelium-derived microvesicles have been observed in various conditions, such as:

- Stable angina
- First-time myocardial infarction
- Recurrent infarction

These variations can help differentiate between the stages or severity of cardiovascular events.[22]

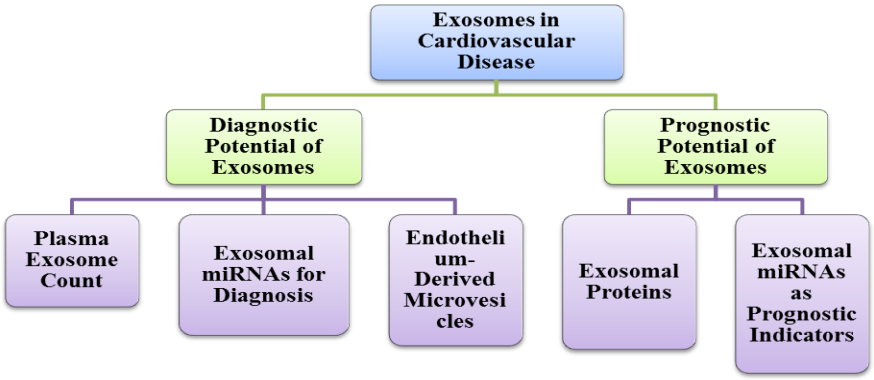

Fig. 6: Role of Exosomes in Cardiovascular Disease.

2. Prognostic Potential of Exosomes

Exosomes can help predict disease outcomes and monitor recovery.

2.1 Exosomal Proteins

Surface proteins, such as CD31 and others, may serve as indicators of adverse cardiac events. Although less studied than RNA content, this area shows promise for future research.

2.2 Exosomal miRNAs as Prognostic Indicators

Certain miRNAs in exosomes are found in elevated levels in damaged heart tissue.

Example: miR-34a is highly expressed after heart injury and is packaged into exosomes by cardiomyocytes and fibroblasts. These markers can be used to:

- Assess the severity of injury
- Monitor disease progression and response to therapy

3. Advantages of Exosomal Biomarkers

Exosomal biomarkers offer several advantages:

- High stability due to protection by a lipid bilayer
- More accurately reflect tissue-specific changes than free biomarkers
- Potential for real-time monitoring of disease progression and treatment response
- Non-invasive collection, particularly from blood, plasma, or urine.[23]

Emerging Role of Exosomes in Circadian Regulation

The **circadian cycle** is an internal biological clock that regulates physiological, metabolic, and behavioral processes in a roughly **24-hour cycle**, aligning them with the light-dark cycle of the environment.

- **Core Clock Components**

  The circadian rhythm is controlled by a **transcriptional–translational feedback loop (TTFL)** involving key clock genes and proteins:

  - **CLOCK** and **BMAL1**: Act as transcriptional activators.
  - **PER (Period)** and **CRY (Cryptochrome)**: Accumulate and inhibit CLOCK-BMAL1 activity in a negative feedback loop.

- **Main Oscillator: Suprachiasmatic Nucleus (SCN)**
  - Located in the hypothalamus, the **SCN** is the master clock that synchronizes peripheral clocks in other tissues.
  - It responds to **light signals** from the retina and sets the body's rhythm accordingly.

- **Peripheral Clocks**
  - Found in almost every cell and tissue (liver, ovary, pancreas, fat), these clocks regulate local functions.
  - They are **entrained** by the SCN and influenced by exosomes, metabolic cues, and hormonal signals.

- **Circadian-Controlled Processes**

  Includes:

  - Hormone secretion (e.g., melatonin at night, cortisol in the morning)
  - Sleep-wake cycle
  - Body temperature
  - Feeding-fasting behavior
  - Glucose metabolism and insulin sensitivity

- **Disruption of Circadian Cycle**
  - Causes: Shift work, stress, poor sleep hygiene, or genetic mutations in clock genes.
  - Consequences: Linked to metabolic disorders, PCOS, cancer, cardiovascular diseases, and mood disorders.[24]

Emerging Role of Exosomes in Circadian Regulation

1. Exosomes as Molecular Timekeepers

- Exosomes carry miRNAs, mRNAs, proteins, and lipids that can influence circadian rhythms.
- Circadian oscillations exist in exosomal cargo, indicating their secretion and content may follow a circadian pattern.

2. Circadian Control of Exosome Biogenesis and Secretion

- Clock genes such as **BMAL1**, **CLOCK**, and **PER/CRY** regulate intracellular processes that influence exosome formation.
- Rhythmic fluctuations in endosomal trafficking pathways and vesicle release have been observed in various cells.

3. miRNAs in Exosomes as Circadian Modulators

- Certain **miRNAs (e.g., miR-219, miR-132, miR-155)** within exosomes exhibit circadian expression.
- These miRNAs target core clock components and feedback loops, modulating the phase and amplitude of the circadian rhythm.

4. Intercellular Synchronization via Exosomes

- Exosomes can transfer circadian-related molecules between cells, helping to synchronize peripheral clocks with the central clock (SCN).

- Example: Exosomes from light-entrained cells can entrain recipient cells, suggesting exosomal roles in photoperiodic adaptation.

5. Impact on Hormonal Regulation

- The circadian system regulates hormone secretion (e.g., melatonin, cortisol), and exosomes influence hormone-sensitive tissues.
- Disruption in exosome-mediated signaling may contribute to circadian misalignment in endocrine disorders like PCOS.

6. Exosome-Mediated miRNA Crosstalk in PCOS and Circadian Disruption

- In PCOS, aberrant expression of exosomal miRNAs (e.g., **miR-21, miR-93**) affects insulin sensitivity and ovarian function.
- These miRNAs may also modulate circadian clock genes, potentially linking circadian disruption with PCOS pathogenesis.

7. Circadian Regulation and Metabolic Health via Exosomes

- Exosomes participate in regulating glucose metabolism and insulin signaling in a time-dependent manner.
- Altered exosome dynamics in circadian misalignment may worsen insulin resistance—a key feature of PCOS.

8. Therapeutic Potential

- Targeting exosomal miRNAs that interact with circadian genes presents a novel strategy to restore rhythm and metabolic homeostasis.
- Engineering circadian-tuned exosomes or miRNA-based therapies could support chrono-therapeutic interventions in PCOS.[25]

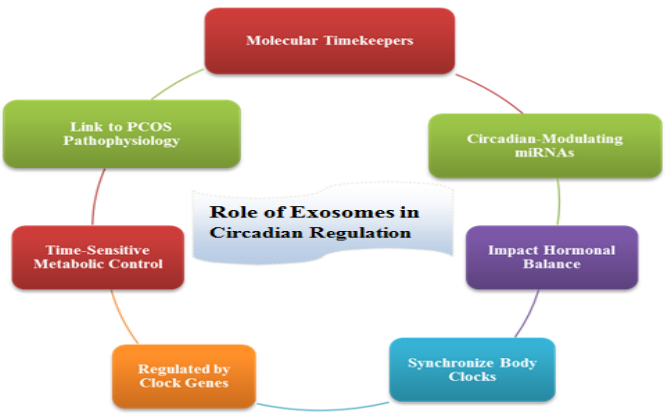

Fig. 7: Role of Exosomes in Circadian Regulation.

Challenges and Future Directions in Therapeutic Exosome Development[26, 27]

Theme	Current Challenges	Prospective Solutions / Future Directions
Isolation & Purification	• Labor-intensive ultracentrifugation • Contaminating proteins/lipoproteins• Poor scalability	• High-throughput SEC & TFF systems• Microfluidic immuno-affinity chips • Inline PAT (process analytical tech) for purity monitoring
Cargo Loading & Targeting	• Low encapsulation efficiency • Non-specific biodistribution	• Electroporation + transfection enhancers• Click-chemistry surface ligation of peptides/antibodies • Genetic engineering of donor cells for intrinsic tropism
Manufacturing & Stability	• Limited cell-culture yields • Loss of activity after storage	• Bioreactor-based hollow-fiber culture• Spray-drying/lyophilization with cryo-protectants • Robust release potency assays
Regulatory & Standardization	• Absence of harmonized GMP guidelines • Undefined critical quality attributes (CQAs)	• ISO/ICH guideline development for EV therapeutics • Multi-center reference standards and databases
Innovation Horizon	—	**Engineered exosomes:** ligand decoration, CRISPR cargos **Exosome mimetics:** membrane-coated nanoparticles **Personalized therapies:** autologous EV platforms **AI integration:** predictive QC and formulation design

CONCLUSION

Exosomes represent an emerging and rapidly developing frontier within the field of nanomedicine, functioning as natural, biocompatible, and multifunctional nanocarriers. Their intrinsic capability to transport a wide range of biological materials—including proteins, RNAs, and lipids—positions them as significant assets for diagnostic, prognostic, and targeted therapeutic applications. This review delineates the integrative roles of exosomes across critical pathological areas such as cancer, cardiovascular diseases, and circadian regulation, thereby emphasizing their therapeutic versatility and systemic relevance.

Despite the considerable promise of exosomes, several scientific and translational challenges persist. These challenges encompass efficient isolation, targeted delivery, scalable manufacturing, and regulatory standardization. Nevertheless, recent advancements in engineering strategies, synthetic mimetics, and artificial intelligence-assisted design are likely to address these barriers effectively. Through sustained research and innovation, exosome-based methodologies possess substantial potential to revolutionize the landscape of personalized and precision medicine.

REFERENCES

1. Aryani, A., & Denecke, B. Exosomes as a nanodelivery system: a key to the future of neuromedicine?. Molecular neurobiology, 2016; 53(2): 818-834.
2. Kalluri, R., & LeBleu, V. S. The biology, function, and biomedical applications of exosomes. science, 2020; 367(6478): eaau6977.
3. Zou, Z., Li, H., Xu, G., Hu, Y., Zhang, W., & Tian, K. Current knowledge and future perspectives of exosomes as nanocarriers in diagnosis and treatment of diseases. International journal of nanomedicine, 2023; 4751-4778.
4. Schur, N., Samman, L., Shah, M., Dukharan, V., Stegura, C., Broughton, L., & Schlesinger, T. Exosomes: Historical evolution and emerging roles in dermatology. Journal of Cosmetic Dermatology, 2025; 24(1): e16769.
5. Wan, Z., Gao, X., Dong, Y., Zhao, Y., Chen, X., Yang, G., & Liu, L. Exosome-mediated cell-cell communication in tumor progression. American journal of cancer research, 2018; 8(9): 1661.
6. Record, M., Carayon, K., Poirot, M., & Silvente-Poirot, S. Exosomes as new vesicular lipid transporters involved in cell–cell communication and various pathophysiologies. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 2014; 1841(1): 108-120.
7. Amiri, A., Bagherifar, R., Ansari Dezfouli, E., Kiaie, S. H., Jafari, R., & Ramezani, R. Exosomes as bio-inspired nanocarriers for RNA delivery: preparation and applications. Journal of Translational Medicine, 2022; 20(1): 125.
8. Conigliaro, A., Fontana, S., Raimondo, S., & Alessandro, R. Exosomes: nanocarriers of biological messages. Exosomes in Cardiovascular Diseases: Biomarkers, Pathological and Therapeutic Effects, 2017; 23-43.
9. Li, T., Li, X., Han, G., Liang, M., Yang, Z., Zhang, C., & Yu, S. The therapeutic potential and clinical significance of exosomes as carriers of drug delivery system. Pharmaceutics, 2022; 15(1): 21.
10. Kowal, J., Tkach, M., & Théry, C. Biogenesis and secretion of exosomes. Current opinion in cell biology, 2014; 29: 116-125.
11. Théry, C., Zitvogel, L., & Amigorena, S. Exosomes: composition, biogenesis and function. Nature reviews immunology, 2002; 2(8): 569-579.
12. Rech, J., Getinger-Panek, A., Gałka, S., & Bednarek, I. Origin and composition of exosomes as crucial factors in designing drug delivery systems. Applied Sciences, 2022; 12(23): 12259.

13. Frydrychowicz, M., Kolecka-Bednarczyk, A., Madejczyk, M., Yasar, S., & Dworacki, G. Exosomes–structure, biogenesis and biological role in non-small-cell lung cancer. Scandinavian journal of immunology, 2015; 81(1): 2-10.
14. Agarwal, S., Agarwal, V., Agarwal, M., & Singh, M. Exosomes: structure, biogenesis, types and application in diagnosis and gene and drug delivery. Current Gene Therapy, 2020; 20(3): 195-206.
15. Kim, S. B. Function and therapeutic development of exosomes for cancer therapy. Archives of Pharmacal Research, 2022; 45(5): 295-308.
16. Czystowska-Kuzmicz, M., & Whiteside, T. L. The potential role of tumor-derived exosomes in diagnosis, prognosis, and response to therapy in cancer. Expert opinion on biological therapy, 2021; 21(2): 241-258.
17. Tkach, M., Kowal, J., & Théry, C. Why the need and how to approach the functional diversity of extracellular vesicles. Philosophical Transactions of the Royal Society B: Biological Sciences, 2018; 373(1737): 20160479.
18. Kalluri, R., & LeBleu, V. S. The biology, function, and biomedical applications of exosomes. science, 2020; 367(6478): eaau6977.
19. Paskeh, M. D. A., Entezari, M., Mirzaei, S., Zabolian, A., Saleki, H., Naghdi, M. J., & Ashrafizadeh, M. Emerging role of exosomes in cancer progression and tumor microenvironment remodeling. Journal of hematology & oncology, 2022; 15(1): 83.
20. Steinbichler, T. B., Dudás, J., Riechelmann, H., & Skvortsova, I. I. The role of exosomes in cancer metastasis. In Seminars in cancer biology. Academic Press, 2017, June; 44: 170-181.
21. Ailawadi, S., Wang, X., Gu, H., & Fan, G. C. Pathologic function and therapeutic potential of exosomes in cardiovascular disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 2015; 1852(1): 1-11.
22. Bei, Y., Yu, P., Cretoiu, D., Cretoiu, S. M., & Xiao, J. Exosomes-based biomarkers for the prognosis of cardiovascular diseases. Exosomes in Cardiovascular Diseases: Biomarkers, Pathological and Therapeutic Effects, 2017; 71-88.
23. Zarà, M., Amadio, P., Campodonico, J., Sandrini, L., & Barbieri, S. S. Exosomes in cardiovascular diseases. Diagnostics, 2020; 10(11): 943.
24. Chen, W. H., Huang, Q. Y., Wang, Z. Y., Zhuang, X. X., Lin, S., & Shi, Q. Y. Therapeutic potential of exosomes/miRNAs in polycystic ovary syndrome induced by the alteration of circadian rhythms. Frontiers in Endocrinology, 2022; 13: 918805.

25. Fekry, B., Ugartemendia, L., Esnaola, N. F., & Goetzl, L. Extracellular Vesicles, Circadian Rhythms, and Cancer: A Comprehensive Review with Emphasis on Hepatocellular Carcinoma. Cancers, 2024; 16(14): 2552.
26. Verma, N., & Arora, S. (2025). Navigating the Global Regulatory Landscape for Exosome-Based Therapeutics: Challenges, Strategies, and Future Directions.
27. Verma, N., & Arora, S. (2025). Navigating the Global Regulatory Landscape for Exosome-Based Therapeutics: Challenges, Strategies, and Future Directions.