ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at:www.ijdsir.com

Volume – 8, Issue – 5, September – 2025, Page No. : 301 - 314

Exosomes: New Frontiers in Regenerative and Dental Medicine

[1]Dr Sandhya Jain, Professor, Department of Orthodontics, Government College of Dentistry, Indore

[2]Dr Ram Kishore Ratre, HOD & Professor, Department of Orthodontics, Government College of Dentistry, Indore

[3]Dr. U.R.Neelima, Juniour Resident, Department of Orthodontics, Government College of Dentistry, Indore

[4]Dr. Bharti Vyas, Juniour Resident, Department of Orthodontics, Government College of Dentistry, Indore

[5]Dr Vijay Bhan Singh, Juniour Resident, Department of Orthodontics, Government College of Dentistry, Indore

[6]Dr Akshata Pedamkar, Juniour Resident, Department of Orthodontics, Government College of Dentistry, Indore

Corresponding Author: Dr. U.R. Neelima, Juniour Resident, Department of Orthodontics, Government College of Dentistry, Indore.

Citation of this Article: Dr Sandhya Jain, Dr Ram Kishore Ratre, Dr. U.R. Neelima, Dr. Bharti Vyas, Dr Vijay Bhan Singh, Dr Akshata Pedamkar, "Exosomes: New Frontiers in Regenerative and Dental Medicine", IJDSIR- September – 2025, Volume – 8, Issue – 5, P. No. 301 – 314

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

A specialized subclass of extracellular vesicles, known as exosomes, has gained significant attention as key mediators of intercellular communication, influencing a wide range of physiological and pathological processes. These nanosized vesicles are actively secreted by almost all cell types and play crucial roles in transporting proteins, lipids, nucleic acids, and signaling molecules between cells. This review provides an overview of the classification, molecular composition, and intricate biogenesis of exosomes, while highlighting their essential functions in immune regulation, disease progression, and therapeutic delivery. Recent advances in isolation and characterization techniques have further expanded their translational potential, particularly in fields such as oncology, regenerative medicine, and dentistry. Notably, mesenchymal stem cell–derived exosomes exhibit remarkable regenerative capacity, with promising applications in periodontal regeneration, pulp therapy, and maintaining orthodontic stability. By offering novel, cell-free strategies for diagnostics and therapeutics, exosomes represent versatile and innovative tools that could revolutionize future biomedical research and clinical practice.

Keywords: Biogenisis, Exosome, Isolation, Multivesicular body, Regeneration

Introduction

In both prokaryotic and eukaryotic cells, extracellular vesicles (EVs) are released as a natural aspect of physiology and during acquired diseases. EVs fall into two general categories: exosomes and ectosomes. These vesicles, which are known as ectosomes, range in

diameter from 50 nm to 1 µm. They pinch off the plasma membrane's surface through outward budding[1]. Exosomes are phospholipid bilayer EVs that originate in the endosome and range in size from around 40 to 160 nm (average diameter of about 100 nm) [2]. Ceramides and cholesterol are among the lipid components found in their phospholipid bilayer membrane, which aids in exosome sorting, secretion, and communication with the host cell [3].

The presence of exosomes in extracellular space was identified as early as in late 1980s [4]. Exosomes were originally thought to be EVs used to eliminate undesirable cellular waste, but further research has shown that they are also crucial molecular mediators in cellular communication because of their capacity to move different nucleic acids, proteins, and metabolites throughout the body [5]. Interestingly, exosomes from cancer cells have been shown to promote angiogenesis, modulate the immune system and remodel the surrounding parenchymal tissue, all factors supporting tumor progression [6]. In contrast to microvesicles, which are created by the direct budding of the plasma membrane, exosomes are created by the endosome membrane invaginating and are released through the fusing of the plasma membrane and multivesicle bodies (MVB) [7]. Numerous cell and tissue types, including as macrophages, placental tissue, epithelial cells, endometrial cells, uterine cells, follicular fluid, embryos, oviductal epithelium, dendritic cells, cancer cells, and mesenchymal stem cells, produce exosomes. Their widespread presence in a variety of body fluids, including breast milk, amniotic fluid, plasma, saliva, semen, and cervical-vaginal fluid, indicates their potential use as biomarkers by showing the physiological condition of diverse donor cells [3].

The functional states of exosome origin cells can be estimated by analyzing the contents of easily accessible exosomes. This method establishes the basis for diagnosis based on exosomes. Exosomes also have the potential to be used as therapeutic tools in a number of areas, such as cancer treatment, medication delivery, and tissue regeneration, in addition to illness diagnosis [8].

Classification of extracellular vesicles

EVs are categorised according to the biogenesis mechanism they employ, such as autophagy, microvesicles, apoptosomes, and exosomes [9]. The classification is elaborated as follows [10].

1. **Classical EVs**

- **Exosomes (40–150 nm)**: Small EVs derived from the inward budding of Multivesicular endosomes (MVEs). Tetraspanins like CD63, CD9, and CD81 are usually expressed by them.
- **Microvesicles (150–1000 nm)**: Bud directly from the plasma membrane. Include classical microvesicles, large oncosomes (1–10 µm), and ARMMs (ARRDC1-mediated microvesicles).
- **Apoptotic bodies (1–5 µm)**: These are released during apoptosis and contain phosphatidylserine (PS), DNA, and cellular debris.

2. **Autophagic EVs**

- Originating from amphisomes (fusion of autophagosomes and endosomes), these vesicles contain autophagy-related markers (e.g., LC3B-PE, p62) and may carry nuclear and cytosolic components such as dsDNA and histones.

3. **Stressed EVs (Stressomes)**

- Released in response to cellular stress or damage (e.g., heat, hypoxia). They are Enriched in heat shock proteins (HSP90, HSPs) and may arise via plasma membrane shedding or autophagy-related pathways.

4. **Matrix Vesicles**

- Found embedded within or coated by extracellular matrix (ECM). These vesicles often carry matrix-related proteins (e.g., fibronectin, proteoglycans) and matrix metalloproteinases (MMPs), contributing to ECM remodeling, immune modulation, and metastatic niche formation.

5. **Non-Vesicular Nanoparticles**

- Includes exomeres (~35–50 nm), which are non-membranous particles containing molecules like ST6Gal-I and HSP90. Also includes vaults (e.g., MVP-containing structures) and histone-associated particles, often released during stress or cell death.

Composition of exosomes

Based on their structural composition, exosomes include a wide range of biomolecules both inside and outside of them, such as receptors, transcription factors, enzymes, extracellular matrix proteins, lipids, and nucleic acids [11]. The tetraspanin family (CD9, CD63, and CD81), endosomal sorting complex required for transport (ESCRT) proteins (Alix, TSG101), integrins, heat shock proteins (Hsp), actin and flotillins [12]. CAMs, integrins, tetraspanins, MHC class I, II, and transferrin receptors are the primary membrane-bound and cytosolic proteins included in exosomes. Proteins like Alix, heat shock proteins like Hsc70 and Hsc90, cytoskeleton proteins like actin, myosin, and tubulin, and fusion and transferring proteins like Rab2, Rab7, flotillin, and annexin are all examples of this. Sphingomyelin, cholesterol, and ceramides are lipid components found in the stiff bilayer membrane of exosomes that affect cargo sorting, exosome secretion, shape, and signalling [13]. Exosome composition includes a complex of nucleic acids, including DNA, mRNA, and noncoding RNA species. MicroRNAs (miRs) are one of the most abundant RNA species in exosomes [14]. According to Valadi et al. even though the RNA seems to have primarily broken down into pieces smaller than 200 nt, there must still be some full-length molecules present because the recovered RNA could be utilised to create recognisable full length proteins through the use of an in vitro translation system [15]. Additionally, exosomes have saccharide groups on their outside. These were shown to be richer in complex N-linked glycans, polylactosamine, mannose, and α-2,6 sialic acid, according to a recent study by Batista et al. [16]. Exosomes have a particular lipid makeup and are higher in cholesterol, phosphatidyl-serine, ceramide, and sphingomyelin [17].

Biogenesis of exosome

Exosome biogenesis can be divided into four stages, Multivesicular body (MVB) formation, MVB transport, MVB-plasma membrane fusion, and cargo sorting to MVBs. Exosome heterogeneity results from a variety of processes that mediate each stage of exosome synthesis and vary greatly according on cargo, cell type, and microenvironment. In particular, distinct mechanisms are not mutually exclusive, but can be employed by the same MVB of cargo can adopt different mechanism to mediate exosome sorting. Importantly, cancer cells can exploit multiple strategies to modulate exosome biogenesis and change the composition and function of exosomes, thereby favouring the release of tumor-promoting exosomes [18]. The similar type exosomes are constitutively from from late endosomes, which are formed by inward budding of the limited multivesicular body (MVB) membrane. The development of intraluminal vesicles (ILVs) within big MVBs is the result of late endosomal membrane invasion. During this process, cytosolic components are absorbed and confined within the ILVs, while some proteins are integrated into the invaginating membrane. When ILVs fuse with the plasma membrane, the majority are discharged into the

extracellular space, where they are known as "exosomes". As an alternative, these elements are transported to lysosomes for deterioration. When prepared artificially, canonical exosomes have a specific biconcave or cup-like form, however when viewed under transmission electron microscopy, they seem spheroid in solution. Their densities on sucrose gradients usually range from 1.13 g/mL (exosomes derived from B cells) to 1.19 g/mL (exosomes derived from epithelial cells) [19].

The best known mechanism for formation of MVBs and ILVs is driven by the endosomal sorting complex required for transport (ESCRT), which carries approximately thirty proteins that aggregates into four complexes (ESCRT-0, -I, -II and -III) with associated proteins (VPS4, VTA1, ALIX also called PDCD6IP) conserved from yeast to mammals. The ESCRT-0 complex identifies and captures ubiquitinated transmembrane proteins within the endosomal membrane. The main function of the ESCRT-I and ESCRT-II complexes, on the other hand, is to stretch the membrane in order to develop buds that store the sorted cargo. Following this, ESCRT-III components facilitate the final scission of the vesicle. ESCRT-0 is composed of HRS (hepatocyte growth factor-regulated tyrosine kinase substrate, officially known as HGS), which links to monoubiquitinated cargo proteins and combine with STAM (signal transducing adaptor molecule, the other ESCRT-0 component) and Eps15 and clathrin (two non-ESCRT proteins). HRS recruits TSG101 (Tumor Susceptibility Gene 101) of the ESCRT-I complex, and ESCRT-I is then involved in the recruitment of ESCRT-III via ESCRT-II or ALIX. The final step of ESCRT machinery disassembly and recycling relies on its interaction with the AAA-ATPase VPS4. While the processes governing the incorporation of soluble cytosolic proteins into ILVs remain poorly understood ,a role for HSC70 has been proposed recently : The chaperone binds to soluble cytosolic proteins containing a KFERQ sequence and to PS on the MVB outer membrane and thus enters ILVs formed in a TSG101- and VPS4-dependent manner [20].

Recent studies have shown that the formation of MVBs and ILVs is not exclusively dependent on the ESCRT complex. Instead, emerging evidence highlights the presence of ESCRT-independent pathways involved in exosome formation.

Lipids also carry a important role in exosome biogenesis. Exosomes are particularly enriched in cholesterol, phosphatidylcholine, phosphatidylserine, sphingomyelin, and ceramide—each contributing uniquely to the processes of exosome formation and uptake, ultimately influencing recipient cells. Ceramide, in particular, has been identified as a key factor in exosome biogenesis. Furthermore, studies suggest that some cargo proteins can be sorted into ILVs through lipid-dependent, ESCRT-independent mechanisms. For instance, proteolipid protein (PLP), a major membrane protein in the central nervous system, shows a preference for endosomal sorting in oligodendrocytes even when key ESCRT components like TSG101, ALIX, or HRS are absent. This sorting capability remains intact despite siRNA-induced depletion or the expression of dominant-negative VPS433.

Alongside PLP, the enzyme neutral sphingomyelinase 2 (nSMase 2), which converts sphingomyelin into ceramide within endosomes, acts as a key promoter of ILV and exosome formation. Ceramide's role within MVBs is further enhanced by various supporting pathways that amplify exosome biogenesis. One such pathway involves the autophagy related protein LC3 (microtubule-associated protein 1A/1B-light chain 3), which helps recruit factors associated with nSMase (FAN), an

activator of nSMase, to endosomal membranes. This recruitment facilitates ceramide-driven ILV formation. It has also been demonstrated that RAB31 activation surges the formation of exosomes and the integration of EGFR into exosomes produced from cancer cells. Notably, this enhancement appears to occur via a ceramide-dependent ILV formation pathway, highlighting the potential importance of ILVs in exosome generation by cancer cells.

Tetraspanins are integral membrane proteins commonly enriched in small extracellular vesicles (EVs) and play a key role in exosome biogenesis. They interact with integrins and other membrane-associated proteins to organize highly structured tetraspanin-enriched microdomains (TEMs) at the cell surface. Classic tetraspanins such as CD63, CD81, and CD9 are well-known exosomal markers that not only participate in exosome formation but also act as molecular organizers, directing the sorting of other TEM-associated proteins into exosomes. For instance, the melanocyte-specific glycoprotein (PMEL) is prisicely sorted into ILVs through interaction with CD63. Likewise, CD9 facilitates the inclusion of membrane metalloproteinase CD10 into ILVs, while CD9 and CD82 cooperate with E-cadherin to promote the exosomal release of β-catenin. Despite these insights, studies aiming to fully clarify the complex role of tetraspanins in exosome biogenesis have produced inconsistent findings.

The influence of tetraspanins on exosome biogenesis can vary depending on the specific tetraspanin involved. In some cases, their role may be redundant, meaning that the absence of a particular tetraspanin has little effect on the overall process. Conversely, disrupting TEMs by depleting one tetraspanin can lead to changes in the localization or protein–protein interactions of other tetraspanins, potentially resulting in increased exosome production. Moreover, inhibiting exosome formation at certain membrane sites, such as the endosomal membrane, might enhance exosome generation at alternative locations like the plasma membrane. This dynamic can lead to an overall rise in exosome release. Therefore, careful consideration is required when categorizing exosomes based solely on the presence or absence of specific tetraspanins within a heterogeneous population of small EVs [21].

Measuring the rate of exosome production is complicated by the dynamic nature of both exosome release and the uptake of external exosomes by cells. Time-lapse studies on single human cells, using tetraspanin antibody-based capture systems, have shown differing net exosome production rates between noncancerous cells and MVBs. These differences can influence the total fluid and solid content, especially when isolation methods also capture other extracellular vesicles (EVs). Advanced fractionation techniques have identified that exosomes themselves can be subdivided into size-based subpopulations, with size variations affecting cargo composition. Both the microenvironment and intrinsic cell biology shape the molecular makeup and markers of exosomes. Exosomes has a variety of molecules, including membrane, cytosolic, nuclear, and extracellular matrix proteins, as well as metabolites and nucleic acids like mRNA, non-coding RNAs, and DNA. Importantly, exosomal cargo is not uniformly distributed; for example, miRNA levels can vary significantly between individual exosomes. Proteomic studies of EVs have highlighted this marker heterogeneity, raising caution about relying solely on marker based purification methods. Despite this, the proteome of exosomes, such as those from breast cancer cells can reflect the epithelial or mesenchymal nature of their source cells. Moreover, specific proteins and nucleic acids tend to be enriched in exosomes compared to their parent cells,

indicating the presence of selective protein-sorting mechanisms during exosome formation and cargo loading[2].

Isolation of exosomes

Despite their crucial role in prior detection and therapy, exosomes are difficult to isolate due to their small size (30–150 nm), low density (1.13–1.19 g/ml), and their existance alongside other similar components (e.g., cell fragments, proteins) in the body fluids, which cause major challenges for their isolation. Additionally, the biological properties of exosomes can be influenced by the separation techniques used. Therefore, establishing standardized isolation and quantification methods is essential for advancing both exosome research and their clinical applications [22].

The standard protocol for exosome isolation involves ultracentrifugation, frequently combined with sucrose density gradients or sucrose cushions to separate and float the low-density exosomes. Isolation of membrane vesicles by sequential differential centrifugations is sometimes difficult due to the possibility of overlapping size propotions with other microvesicles or macromolecular complexes. Moreover, centrifugation-based pelleting may be inadequate for effectively separating vesicles according to their size. However, sequential centrifugations, when linked with sucrose gradient ultracentrifugation, can give a high enrichment of exosomes [23].

SEC (size exclusion chromatography), sequential filtration, and ultrafiltration are the three primary types of size-based methods. Ultrafiltration, characterized by a 10–100 kDa molecular weight cut-off (MWCO), is commonly used as a first step to concentrate exosomes from large volumes of original material into small-volume samples that can be used in subsequent purification procedures. The sequential filtration process is typically carried out in three distinct steps. In the first step, cells and cellular debris are purified; next, free proteins are decreased, and the samples are concentrated; finally, exosomes are sorted using filters with specific, defined pore sizes. Compared to centrifugation and filtration techniques, SEC offers several advantages, such as high reproducibility, cost-effectiveness, and preservation of exosome integrity. Notably, it is also well-suited for isolating exosomes from serum and plasma. An advanced ultrafiltration, sequential centrifugal ultrafiltration (SCUF) approach has also been used to obtain highly pure exosomes and to sieve out MVs from a human colon cancer cell line [24]. Another approach is Immuno-affinity-based approaches. Exosomes can be isolated through immunoaffinity capture when they display specific target proteins or membrane components that are unique to, or predominantly expressed on, their surface without soluble equivalents in surrounding fluids. Over the years, several exosomal markers have been identified, including lysosome-associated membrane protein-2B, transmembrane proteins, heat shock proteins, platelet-derived growth factor receptors, fusion proteins such as flotillins, annexins, GTPases, as well as lipid-associated proteins and phospholipases. Various commercial products utilize these markers for selective exosome isolation, such as the Exosome-Human CD63 Isolation Reagent (Thermo Fisher), Exosome Isolation Kit CD81/CD63 (Miltenyi Biotec), and the Exosome Isolation and Analysis Kit (Abcam), which focuses transmembrane proteins like Rab5, CD81, CD63, CD9, CD82, annexin, and Alix. Immunoaffinity capture serves as a powerful approach to separate exosomes into specific populations based on their biomarker profiles [25].

The precipitation method isolates exosomes by inducing their aggregation using precipitating agents. This process involves combining the sample with a precipitant, allowing it to incubate, and then collecting the aggregated vesicles through low-speed centrifugation

(around 1500 g). The resulting pellet is then resuspended in a smaller volume of buffer for further analysis. Polyethylene glycol (PEG), a highly hydrophilic polymer, is the most commonly used precipitating agent. PEG has long been employed for precipitating viruses, nucleic acids, and other biomolecules, though the exact mechanisms behind PEG-induced precipitation remain not fully understood. This method offers advantages such as simplicity, speed, the ability to process multiple samples simultaneously, and minimal sample loss. Currently, PEG-based precipitation is the second most widely used technique for exosome isolation, following ultracentrifugation. Usually, 8–12% of 6-kDa PEG and 8–10% of 8-kDa PEG are used for exosome isolation[26]. Microfluidic-based platforms have been modified recently to indicate uses in the biological applications and diagnosis/treatment fields. Microfluidic devices are specifically well-suited for exosome isolation in therapeutic applications due to their inbuilt advantages, including laminar flow, low sample consumption, ease of handling, high surface-to-volume ratio, and rapid processing time. These devices are typically made from materials such as polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), glass, paper, silicon, and metals. Among these, PDMS is the most widely used material because of its desirable characteristics, including optical transparency, biocompatibility, cost-efficiency, and flexibility. Microfluidic isolation techniques are broadly divided into two types: passive and active methods. Active methods utilize external forces, such as acoustic waves, magnetic fields, or electric fields for separation, whereas passive methods rely on intrinsic physical properties and do not require external energy input. While active methods offer improved isolation efficiency and higher throughput, they also introduce greater system complexity and increased operational costs. Various microfluidic-based exosome isolation techniques have been developed, each with its own set of strengths and limitations. [27]. Neither ultracentrifugation nor precipitation allows enrichment for exosome subtypes. To date, immunoaffinity capture remains the only method for isolation of exosome subgroups. By coating magnetic beads with specific antibodies, exosomes with those surface antigens are able to be captured. CD9, CD63, and CD81 are the most common markers and are expressed on nearly all exosomes. Specific markers can also be utilized to selectively isolate exosomes derived from particular cell types. For example, chondroitin sulfate peptidoglycan 4 antibody-coated beads have been used to capture melanoma cell-derived exosomes , while magnetic beads coated with CD56 or CD171 antibodies have been used to capture neuronal cell derived exosomes [28].

Biological functions of exosome

Since their discovery three decades ago, exosomes have been recognized for their crucial roles in various biological processes, including intercellular communication, immune regulation, stem cell development and differentiation, neuronal function, cell signaling, tissue regeneration, and viral replication [29]. Depending on their cell/tissue of origin many different functions have been attributed to exosomes.

Immune Response

Exosome content has been shown to be highly disease-specific, carrying information related to conditions such as cancer, viral infections, and neurodegenerative disorders, including Alzheimer's and prion diseases. Recent studies have also demonstrated that engineered exosomes can influence both innate and adaptive immune responses, highlighting their potential in the development of immunotherapies. Exosomes exert their immune functions primarily through the transfer and presentation of antigenic

peptides. Also, they can provide DNA to recipient cells, activating the cyclic GMP-AMP synthase (cGAS) and stimulation of interferon genes (STING) pathway. This innate immune mechanism detects cytosolic DNA, leading to the expression of inflammatory genes and the induction of a Type I interferon response. Furthermore, exosomes can regulate gene expression through miRNAs and activate various signalling pathways via their surface ligands [30]. Exosomes originated from various immune cells play a crucial role in mediating communication both among immune cells and between immune cells and target proteins. They contribute to the regulation of innate and adaptive immunity by facilitating antigen presentation and transporting immune-related substances such as cytotoxic proteins and inflammatory mediators. Innate immune cells—including macrophages, dendritic cells (DCs), natural killer (NK) cells, and granulocytes—can detect antigens through pattern recognition receptors (PRRs), thereby initiating immune responses. Exosomes can cause the polarization of macrophages, the regulation and expression of DCs, and the killing effect of NK cells. Adaptive immunity involves the activation, proliferation, and differentiation of T and B cells into effector cells following antigen stimulation, leading to a range of biological responses. During adaptive immune responses, exosomes can facilitate the spread of antigens or MHC-peptide complexes, enhancing the pool of dendritic cells involved in antigen presentation or directly interacting with memory T cells to modulate immune activity [31]. Exosomes released from macrophages infected by bacteria have been found to exert immunomodulatory effects, promoting the activation of macrophages and neutrophils. These exosomes stimulate the secretion of proinflammatory mediators, such as TNF-α and RANTES (regulated on activation, normal T cell expressed and secreted) and enhance the expression of inducible nitric oxide synthase (iNOS) [32]. Exosomes play a significant role in facilitating viral infections by spreading viral components and supporting viral persistence. This property also makes them promising carriers for delivering vectors containing specific genes to target cells. For example, exosome-associated adeno-associated viruses (AAVs) have been shown to efficiently transfer genetic material to immune cells [33].

Exosomes as a Biomarker Source

Exosomes play a crucial role in cancer progression by facilitating intercellular communication, enhancing metastatic potential, and contributing to the development of drug resistance. Importantly, exosomes are mostly always secreted by the cancer cells and are distributed widely in numerous body fluids. As a result, exosomes can be seen in body fluids, including blood, saliva, and urine.

Exosomal biomarkers have better performance in cancer diagnosis and prognosis than liquid biopsy used alone [34]. Exosomes play a crucial role in transferring cellular mRNAs and miRNAs among cells. Analyzing the horizontal transfer of genetic material through exosomes can provide valuable insights into cancer recurrence and help monitor treatment responses. A key advantage of using exosomes as biomarkers is their ability to be detected through non-invasive methods, eliminating the need for surgical procedures. This approach has already shown success in identifying cancers such as ovarian, lung, and pancreatic cancer through liquid biopsy. Additionally, exosomes demonstrate remarkable stability and specificity to target cells, maintaining their integrity even under harsh conditions, including multiple freeze-thaw cycles and pH fluctuations [30]. Because exosomes contain particular biomolecules, such as unique miRNA and protein fingerprints, they are attractive candidates for early cancer diagnosis. Analyzing exosomes isolated from bodily fluids through liquid biopsy provides a

minimally invasive alternative to conventional tissue biopsies. By identifying cancer-specific exosomal markers, researchers aim to develop non-invasive tests capable of detecting cancer in the earliest stages of the disease [35]. Although exosomes and their components are utilized as biomarkers, they currently cannot be used to accurately quantify disease severity. However, with continued research, exosomes hold potential to serve as valuable tools for diagnosis, prognosis, and therapeutic delivery in the future [30].

Exosomes as drug delivery vehicles

Exosomes are membrane vesicles at the nanoscale that have the exceptional capacity to target particular tissues or cells. By means of surface adhesion proteins, exosomes can regulate the horizontal transfer of genetic material, leading to alteration in the biological activity of recipient cells. Furthermore, exosomes have good host bio-distribution and biocompatibility and can be extracted from patient tissues or bodily fluids, which allows for diminished clearance by the mononuclear phagocyte system. As a result, the problem of immunogenicity can be avoided, and therapeutic medicines that are integrated can be administered without experiencing toxicity or fast clearance. Also Exosomes may be considered as appealing biological vesicles for the effective delivery of biological therapies to target cells across various biological barriers, in addition to their usage as therapeutic entities themselves [19].

For exosomes to serve effectively as drug delivery vehicles, therapeutic agents must be efficiently encapsulated within them. Active loading/encapsulation and passive loading/encapsulation are the two main methods used to insert therapeutic compounds into exosomes. Methods for passive drug loading are rather easy to understand. Either incubating the medication with donor cells or with exosomes will fulfil passive loading. When medications are incubated with exosomes, they diffuse into the exosomes following a concentration gradient; however, when donor cells are incubated, drugs are first administered to the cells, and the cells then release drug-loaded exosomes. The active drug loading approach makes it easier to load big molecules and provides increased drug loading efficiency. Techniques including sonication, extrusion, electroporation, or drug conjugation can all be used to achieve active drug loading. In the sonication method, the membrane integrity of exosomes is compromised to allow the drug to diffuse into exosomes without affecting membrane-bound proteins. Similarly, syringe-based lipid extruders facilitate membrane diffusion, which is disrupted by extrusion. In the electroporation method, exosomes are suspended in a conductive fluid and subjected to an electric field. The phospholipid bilayer is broken by the electric field, creating transient tiny holes that allow the medication to permeate into the exosomes. As an alternative, medications can be attached to the exosome surface by antibody binding or click chemistry. In contrast to antibody binding, which uses highly specific antibodies to connect to a specific antigen on the exosome surface, click chemistry involves directly attaching drug molecules to the surfaces of exosomes through covalent bonding. The loading capacities of the aforementioned drug loading techniques vary, and the best approach depends on the cargo's characteristics, including its molecular weight, hydrophilicity, and lipophilicity. Additionally, exosome membrane stability and integrity are crucial for drug distribution in addition to loading efficiency [36].

Exosomes in Dentistry

Exosomes have gained attention as promising tools in regenerative dentistry, owing to their crucial role in intercellular communication and promotion of tissue repair. Their ability to transfer bioactive molecules such as

proteins, lipids, and nucleic acids makes them valuable for dental tissue regeneration, immune modulation, and inflammation control.

Periodontal Regeneration

Periodontitis is a chronic inflammatory condition characterized by alveolar bone loss, with diabetes being a major contributing risk factor. Exosomes derived from mesenchymal stem cells (MSCs) have shown potential in promoting the regeneration of periodontal ligament, alveolar bone, and cementum by stimulating osteogenic differentiation and enhancing angiogenesis [37]. Albougha et al. in their study investigated the effects of exosomes from human periodontal ligament stem cells(HPDLSCs-Exo) on human osteoblast-like cells in vitro and on the healing of rat calvarial bone defects in vivo. They found that HPDLSCs-Exo promoted the migration and osteoblastic differentiation of human osteoblast-like cells and bone healing of rat calvarial bone defects. This suggests that HPDLSCs-derived exosomes (HPDLSCs-Exo) could serve as a promising therapeutic option for promoting bone healing in damaged periodontal tissues [38].

Dental Pulp Regeneration

Dental mesenchymal stem cells (MSCs) play a crucial role in the regeneration of dental pulp tissue. MSC-derived exosomes, as crucial biotransmitters in intercellular communication, have been shown to mimic the therapeutic effects of their parental cells. These exosomes offer greater stability, reduced immunogenicity, enhanced safety, and improved clinical efficacy, making them suitable candidates for pulp regeneration. Current research indicates that exosomes can stimulate the regeneration of dentin/pulp-like tissue in vivo, likely due to their ability to promote pulp angiogenesis, regulate the proliferation, migration, and differentiation of dental cells, and provide neuroprotective effects. The applications of exosomes in the treatment of pulp regeneration have great potential, and exosomes may become ideal therapeutic biomaterial in regenerative endodontics [39]. In their study, Chen et al. developed a "cell homing" model to study whether exosomes derived from dental pulp tissue (DPT-exos) could enhance dental pulp regeneration by recruiting stem cells from the apical dental papilla (SCAPs). They successfully isolated exosomes from both dental pulp tissue (DPT-exos) and dental pulp stem cells (DPC-exos) in swine. In vitro experiments showed that DPT-exos were more effective than DPC-exos in increasing the migration, proliferation, and differentiation of SCAPs. Additionally, in vivo studies showed that DPT-exos helped the recruitment of SCAPs, leading to the regeneration of dental pulp-like connective tissue enhanced with collagen, odontoblasts, and predentin like structures. Blood vessel growth was demonstrated by immunofluorescence. The study demonstrated the ability of DPT-exos to induce SCAPs to regenerate connective tissue similar to natural dental pulp. This technique has the potential for treating pulp deficiency caused by various pulp diseases [40].

Bone regeneration

Zhu et al. evaluated the effect of exosomes derived from pre-differentiated human alveolar bone-derived bone marrow mesenchymal stromal cells (AB-BMSCs), containing specific microRNAs, on bone regeneration. Exosomes collected from AB-BMSCs pre-differentiated for 0 and 7 days were cocultured with BMSCs in vitro to check their influence on the differentiation potential of BMSCs. In vitro experiments revealed that exosomes secreted by AB-BMSCs pre-differentiated for 7 days were effective at facilitating the osteogenic differentiation of BMSCs. Bioinformatic analysis of RNA sequencing data showed that osteogenic miRNAs were upregulated, while anti-osteogenic miRNAs were downregulated, activating the osteogenic IGF1R/PI3K/Akt signaling pathway. By

modifying exosomes with anti-miR-182–5p in addition to implantation of Poly L-lactic acid **(**PLLA) scaffolds, it was further proved that Exo-miR-182–5p-inhibitor- transfected BMSC-seeded PLLA scaffold efficiently promoted new bone formation. Therefore, the study demonstrated that osteogenic exosomes can be harvested from pre-differentiated AB-BMSCs and that gene modification within exosomes had excellent potential for bone regeneration strategies in the future [41].

Orthodontic stability

Orthodontic relapse affects over 70% of patients, and current retention methods are limited in preventing it. Mesenchymal stem cell-derived exosomes (MSC-Exo) offer a promising solution by regulating periodontal bone remodeling, including osteoclast activity, osteoblast differentiation, and ligament function. Exosome-loaded hydrogels enable controlled release, enhancing bone regeneration. MSC-Exo-based therapies may provide an effective strategy to improve tooth stability and prevent relapse [42].

Accelerating Orthodontic tooth movement

Exosomes, have been shown to enhance alveolar bone remodeling during orthodontic tooth movement (OTM). In a rat model, exosome-enriched medium significantly increased tooth movement, osteoclast activity, and bone resorption, while reducing inflammation and root resorption. ELISA results confirmed elevated RANKL and reduced OPG levels, indicating accelerated bone remodelling suggesting exosomes may accelerate OTM and offer a novel adjunctive strategy in orthodontics [43].

Conclusion

Exosomes represent a promising frontier in biomedical research due to their unique ability to mediate intercellular communication and deliver bioactive molecules. Their roles in diagnostics, immune modulation, and targeted drug delivery offer novel, non-invasive strategies for disease management. In dentistry, exosome-based approaches show potential for regenerating periodontal and pulp tissues and enhancing orthodontic outcomes. Continued research into exosome biology, standardization of isolation techniques, and clinical translation will unlock their full therapeutic potential

Reference

1. Kong Q, Wang Y, Jiang N, Wang Y, Wang R, Hu X, et al. Exosomes as Promising Therapeutic Tools for Regenerative Endodontic Therapy. Biomolecules. 2024 Mar 11;14(3):330.
2. Kalluri R, LeBleu VS. The biology **,** function **,** and biomedical applications of exosomes. Science. 2020 Feb 7;367(6478):eaau6977.
3. Colombo M, Moita C, Van Niel G, Kowal J, Vigneron J, Benaroch P, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. Journal of Cell Science. 2013 Jan 1;jcs.128868.
4. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem. 1987 Jul 5;262(19):9412–20.
5. Statello L, Maugeri M, Garre E, Nawaz M, Wahlgren J, Papadimitriou A, et al. Identification of RNA-binding proteins in exosomes capable of interacting with different types of RNA: RBP-facilitated transport of RNAs into exosomes. Gerber AP, editor. PLoS ONE. 2018 Apr 24;13(4):e0195969.
6. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. Cancer Cell. 2016 Dec;30(6):836–48.

7. Van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018 Apr;19(4):213–28.
8. Basu J, Ludlow JW. Exosomes for repair, regeneration and rejuvenation. Expert Opinion on Biological Therapy. 2016 Apr 2;16(4):489–506.
9. Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, et al. Reassessment of Exosome Composition. Cell. 2019 Apr;177(2):428-445.e18.
10. Sheta M, Taha EA, Lu Y, Eguchi T. Extracellular Vesicles: New Classification and Tumor Immunosuppression. Biology (Basel). 2023 Jan 10;12(1):110.
11. Mathivanan S, Ji H, Simpson RJ. Exosomes: Extracellular organelles important in intercellular communication. Journal of Proteomics. 2010 Sep;73(10):1907–20.
12. Garcia NA, Ontoria-Oviedo I, González-King H, Diez-Juan A, Sepúlveda P. Glucose Starvation in Cardiomyocytes Enhances Exosome Secretion and Promotes Angiogenesis in Endothelial Cells. Busson P, editor. PLoS ONE. 2015 Sep 22;10(9):e0138849.
13. Skryabin GO, Komelkov AV, Savelyeva EE, Tchevkina EM. Lipid Rafts in Exosome Biogenesis. Biochemistry Moscow. 2020 Feb;85(2):177–91.
14. Huang X, Yuan T, Tschannen M, Sun Z, Jacob H, Du M, et al. Characterization of human plasma-derived exosomal RNAs by deep sequencing. BMC Genomics. 2013 Dec;14(1):319.
15. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007 Jun;9(6):654–9.
16. Batista BS, Eng WS, Pilobello KT, Hendricks-Muñoz KD, Mahal LK. Identification of a Conserved Glycan Signature for Microvesicles. J Proteome Res. 2011 Oct 7;10(10):4624–33.
17. Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. Current Opinion in Cell Biology. 2014 Aug;29:116–25.
18. Han QF, Li WJ, Hu KS, Gao J, Zhai WL, Yang JH, et al. Exosome biogenesis: machinery, regulation, and therapeutic implications in cancer. Mol Cancer. 2022 Nov 1;21(1):207.
19. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci. 2019 Dec;9(1):19.
20. Colombo M, Raposo G, Théry C. Biogenesis, Secretion, and Intercellular Interactions of Exosomes and Other Extracellular Vesicles. Annu Rev Cell Dev Biol. 2014 Oct 11;30(1):255–89.
21. Lee YJ, Shin KJ, Chae YC. Regulation of cargo selection in exosome biogenesis and its biomedical applications in cancer. Exp Mol Med. 2024 Apr 5;56(4):877–89.
22. Chen J, Li P, Zhang T, Xu Z, Huang X, Wang R, et al. Review on Strategies and Technologies for Exosome Isolation and Purification. Front Bioeng Biotechnol. 2021;9:811971.
23. Vlassov AV, Magdaleno S, Setterquist R, Conrad R. Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochimica et Biophysica Acta (BBA) - General Subjects. 2012 Jul;1820(7):940–8.
24. Zhu L, Sun HT, Wang S, Huang SL, Zheng Y, Wang CQ, et al. Isolation and characterization of exosomes for cancer research. J Hematol Oncol. 2020 Dec;13(1):152.

25. Gorgzadeh A, Nazari A, Ali Ehsan Ismaeel A, Safarzadeh D, Hassan JAK, Mohammadzadehsaliani S, et al. A state-of-the-art review of the recent advances in exosome isolation and detection methods in viral infection. Virol J. 2024 Jan 30;21(1):34.
26. Yakubovich EI, Polischouk AG, Evtushenko VI. Principles and Problems of Exosome Isolation from Biological Fluids. Biochem Moscow Suppl Ser A. 2022 Jun;16(2):115–26.
27. Shirejini SZ, Inci F. The Yin and Yang of exosome isolation methods: conventional practice, microfluidics, and commercial kits. Biotechnology Advances. 2022 Jan;54:107814.
28. Zhou M, Weber SR, Zhao Y, Chen H, Sundstrom JM. Methods for exosome isolation and characterization. In: Exosomes [Internet]. Elsevier; 2020 [cited 2024 Oct 27]. p. 23–38. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978012816053400002X
29. H. Rashed M, Bayraktar E, K. Helal G, Abd-Ellah M, Amero P, Chavez-Reyes A, et al. Exosomes: From Garbage Bins to Promising Therapeutic Targets. IJMS. 2017 Mar 2;18(3):538.
30. Sanyal K, Banerjee AG. Exosomes: A Brief Review of Biology and Medical Applications. SSRN Journal [Internet]. 2024 [cited 2024 Nov 22]; Available from: https://www.ssrn.com/abstract=4687014
31. Zou J, Peng H, Liu Y. The Roles of Exosomes in Immunoregulation and Autoimmune Thyroid Diseases. Front Immunol. 2021 Nov 15;12:757674.
32. Bhatnagar S, Schorey JS. Exosomes Released from Infected Macrophages Contain Mycobacterium avium Glycopeptidolipids and Are Proinflammatory. Journal of Biological Chemistry. 2007 Aug;282(35):25779–89.
33. Breuer CB, Hanlon KS, Natasan J shree, Volak A, Meliani A, Mingozzi F, et al. In vivo engineering of lymphocytes after systemic exosome-associated AAV delivery. Sci Rep. 2020 Mar 11;10(1):4544.
34. Wong CH, Chen YC. Clinical significance of exosomes as potential biomarkers in cancer. WJCC. 2019 Jan 26;7(2):171–90.
35. Umeche IE, Olaniyan MF. Exosomes: emerging biomarkers unveiling cellular mysteries—a narrative review. You J, editor. J Bio-X Res. 2023 Sep 30;06(03):104–15.
36. Patil SM, Sawant SS, Kunda NK. Exosomes as drug delivery systems: A brief overview and progress update. European Journal of Pharmaceutics and Biopharmaceutics. 2020 Sep;154:259–69.
37. Lu J, Yu N, Liu Q, Xie Y, Zhen L. Periodontal Ligament Stem Cell Exosomes Key to Regulate Periodontal Regeneration by miR-31-5p in Mice Model. Int J Nanomedicine. 2023;18:5327–42.
38. Albougha MS, Sugii H, Adachi O, Mardini B, Soeno S, Hamano S, et al. Exosomes from Human Periodontal Ligament Stem Cells Promote Differentiation of Osteoblast-like Cells and Bone Healing in Rat Calvarial Bone. Biomolecules. 2024 Nov 17;14(11):1455.
39. Yu S, Chen H, Gao B. Potential Therapeutic Effects of Exosomes in Regenerative Endodontics. Archives of Oral Biology. 2020 Dec;120:104946.
40. Chen Y, Ma Y, Yang X, Chen J, Yang B, Tian W. The Application of Pulp Tissue Derived-Exosomes in Pulp Regeneration: A Novel Cell-Homing Approach. IJN. 2022 Jan;Volume 17:465–76.
41. Zhu Q, Tang Y, Zhou T, Yang L, Zhang G, Meng Y, et al. Exosomes derived from mesenchymal stromal cells promote bone regeneration by delivering miR-

182–5p-inhibitor. Pharmacological Research. 2023 Jun;192:106798.

42. Peng B, Wang L, Han G, Cheng Y. Mesenchymal stem cell-derived exosomes: a potential cell-free therapy for orthodontic tooth stability management. Stem Cell Res Ther [Internet]. 2024 Oct 1 [cited 2025 Jul 15];15(1). Available from: https://stemcellres.biomedcentral.com/articles/10.1186/s13287-024-03962-3
43. Zwiri A, Alam MK, Hajeer MY, Alserhani EDM, Alanazi DA, Alanazi JM, et al. Exploring the Role of Exosomes in Accelerating Orthodontic Tooth Movement. Journal of Pharmacy and Bioallied Sciences. 2025 Jun;17(Suppl 2):S1267–9.