RESEARCH PAPER

Saliva's secret weapon: understanding the multifaceted role of exosomes

Christina Amy Mathews[1]*, Anil Kumar Gujjari[2], Beena Sam Mathews[3], SubbaRao V Madhunapantula[4], Sowmya S[5], Sheela Kumar Gujjari[6]

[1*]PhD Research Scholar, JSS Dental College, Mysore
[2]Professor, Dept of Prosthodontics, JSS Dental College, Mysore
[3]Research Guide ,Obstetrics and Gynaecology, TMM Hospital, Tiruvalla
[4]Professor, Cellular and Molecular Biology, CEMR laboratory ,JSS Medical College
[5]Reader ,Dept of Prosthodontics, JSS Dental College, Mysore
[6]Professor ,Dept of Periodontology, JSS Dental College, Mysore

Abstract
Exosomes, nanoscale extracellular vesicles (EVs), are secreted by diverse cell types including mesenchymal stem cells, T lymphocytes, neurons, and mast cells. Saliva is among the body fluids that contain exosomes. Saliva is gaining prominence as a diagnostic biofluid due to its non-invasive and cost-effective collection. Salivary exosomes contribute significantly to maintaining oral homeostasis, modulating immune responses, and regulating the oral microbiome. Composed of proteins, lipids, RNA, and DNA, exosomes mediate intercellular communication and transport molecular cargo between cells. Due to their biological content and specific surface markers, salivary exosomes show strong potential in diagnostics, targeted drug delivery, immunomodulation, and tissue regeneration.This manuscript explores the structural components, biological functions, isolation techniques, and analytical methods associated with salivary exosomes, highlighting their role in oral and systemic health and their translational potential in clinical applications.

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1. Introduction

Exosomes are small, membrane-bound extracellular vesicles ranging from 30 to 150 nm in diameter and of endocytic origin. They are a subclass of extracellular vesicles (EVs) and differ from microvesicles (100–1000 nm, formed via plasma membrane budding) and apoptotic bodies (500 nm to 2 μm, derived from dying cells)[1,2,3]. First discovered by Pan and Johnstone in sheep reticulocytes, exosomes were initially considered cellular waste but are now recognized as crucial mediators of intercellular communication[4,5].

Exosomes are released into various biological fluids including blood, urine, cerebrospinal fluid, breast milk, and saliva[6]. Their cargo—proteins, lipids, RNA, and DNA—reflects the physiological or pathological status of their parent cells. The presence of cell-specific surface proteins (e.g., CD9, CD63, CD81, TSG101) distinguishes them from other vesicle types[7,8].

Saliva, a biofluid composed of 99% water and 1% solutes (proteins, salts), is secreted primarily by the parotid, submandibular, and sublingual glands[9]. These glands absorb biomolecules from the bloodstream via transcellular and paracellular pathways, incorporating them into saliva[10,11].

Therefore saliva is a good diagnostic option to detect any systemic diseases present in the body as the exosomal contents present in saliva is a representative of what is present in the rest of the body.

Salivary exosomes can diagnose and can be used for therapeutic purposes as it can transport biologically active molecules across intercellular boundaries. They serve as biomarkers for detecting various diseases. The various prominent functions they participate in are immune modulation, tissue repair, microbial defense [10].

2. Molecular Composition of Salivary Exosomes

Salivary exosomes are made of lipid bilayer membrane. Proteins, nucleic acids and signaling molecules are present in the lipid bi-layered membrane of Salivary exosomes which lead to their stability, specificity and biological functionality[6].

Table 1. Table showing the various proteins present in salivary exosomes and their functions

PROTEIN	FUNCTION
1.Membrane transport and fusion proteins	Exosome formation Cargo packaging Interaction with recepient cells[12]
2.Tetraspanins	Exosome biogenesis Cargo sorting, targeting and uptake[13,14]
3. Heat shock proteins	As molecular chaperones Assist in immune modulation[15]
4.Cytoskeletal proteins	Influence vesicle trafficking[16]
5.Alix	Promotes the formation of multivesicular

*Author for Correspondence: dr.christinaamy@gmail.com

	bodies (MVBs) cargo segregation and membrane restoration
6.Tumor susceptibility Gene 101	Plays a central role in endosomal sorting Regulation of cellular proliferation and tumor progression.
7.Transmembrane receptors	Facilitate recipient cells' absorption of particular receptors.
8.Lipid related proteins and phospholiases	Engage in signaling cascades and lipid metabolism[17].

Extracellular macromolecules and hydrophilic proteins are present in salivary exosomes which distinguishes them from the plasma exosomes which are more hydrophobic. Sphingomyelin, cholestrol, phosphatidylserine and gangliosides are present in the lipid membrane which improves their vesicle stability and fusion potential[18,19,20].

3. Biological Functions

3.1 Intercellular Communication

In order for oral cavity cells to communicate with one another, salivary exosomes are essential.The recipient cells may internalize them by direct membrane fusion, endocytosis, or receptor-ligand interaction after they are released . After being absorbed, exosomal components including proteins, mRNAs, and microRNAs affect cellular activity, signal transmission, and gene expression[21].

By attaching to target mRNAs and inhibiting protein translation, microRNAs (miRNAs) found in salivary exosomes perform a crucial regulatory role. Immune responses, inflammation, tissue remodeling, and even tumor suppression or growth can all be impacted by this regulation.

3.2 Molecular Transport

Biologically active substances are transported via exosomes. These include lipids, peptides, cytokines, and nucleic acids that affect recipient cells' biological reactions. Endocytosis or direct fusion with the plasma membrane are two possible ways for the transfer to take place. Maintaining tissue homeostasis and coordinating intricate cellular responses under stress or illness depend on this kind of molecular transport[21].

4. Physiological Roles in the Oral Cavity

4.1 Immune Modulation

Immunomodulatory chemicals such as cytokines, chemokines, and miRNAs are found in salivary exosomes. These can modify inflammatory reactions and affect the function of immune cells including T and B cells. It has been demonstrated that miRNAs inhibit the expression of pro-inflammatory genes, supporting immunological homeostasis in the oral environment.

4.2 Tissue Repair and Regeneration

Growth factors including epidermal growth factor (EGF) and transforming growth factor-beta (TGF-β) are found in exosomes and aid in wound healing, cellular division, and proliferation. Their promise as a treatment for mucosal injuries and periodontal disease is highlighted by their role in tissue restoration[22,23].

4.3 Antimicrobial Defense

Antimicrobial peptides, such as defensins and cathelicidins, which have direct antibacterial actions, may be present in salivary exosomes. They also carry regulatory RNAs that affect pathogen identification and host-microbiome interactions.

5. Isolation and Characterization of Salivary Exosomes

5.1 Isolation Techniques

Exosomes are separated from saliva using two main techniques:

(a)Ultracentrifugation

- Debris is eliminated during the first centrifugation at 12,000 × g.
- After being diluted with PBS, the supernatant is ultracentrifuged for three hours at 4°C at 120,000 × g.
- The pellet is kept at -70°C after being resuspended in PBS[24].

(b) Polymer-Based Precipitation (e.g., ExoQuick™)

- ExoQuick reagent is combined with saliva and incubated for 6–24 hours at 4°C.
- An exosomal pellet is produced by centrifugation at 1500 × g, which is then purified and preserved[22].

5.2 Morphological Assessment

Transmission electron microscopy (TEM):

The spherical shape and bilayer membrane structure of exosomes, which normally range between 30 and 120 nm, are shown using TEM.

Atomic force microscopy (AFM):

High-resolution topography imaging and particle size distribution are provided by AFM, which may differentiate between healthy and pathological conditions.

Immuno-Electron Microscopy (Immuno-EM): This technique combines TEM with antibody labeling to identify certain exosomal proteins and provide information on their molecular makeup[25,26].

6. Molecular Analysis

6.1 ELISA (Enzyme-Linked Immunosorbent Assay)

Certain exosomal proteins can be quantitatively detected using ELISA. This approach permits:

- Comparison between healthy and disease states,
- Identification of disease biomarkers,

- High-sensitivity profiling of low-abundance proteins in complex samples[27].

6.2 Western Blotting

Western blotting is employed to confirm the presence and expression levels of exosomal markers and disease-related proteins. This approach permits:

- Semi-quantitative protein data,
- Identification of diagnostic biomarkers,
- Functional insights into exosome biology[24].

7. Conclusion

A potential area in targeted therapies and non-invasive diagnostics is represented by salivary exosomes. They play a crucial role in immunological modulation, tissue regeneration, microbial defense, and oral health maintenance due to their rich molecular cargo, stability, and intercellular communication function. They can also be easily, conveniently, and readily isolated from saliva, which makes them appropriate for regular clinical application.Salivary exosomes have the potential to greatly improve therapeutic delivery methods, personalized medicine, and early disease detection in both oral and systemic health contexts as our understanding of their biological functions and clinical significance grows.

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