Medicon Medical Sciences
Volume 9 Issue 3 September 2025
Article Type: Review Article
ISSN: 2972-2721

Exploring The Potential of Extracellular Vesicles from Oral Biofluid as Biomarker for Periodontal and Peri-implant Diseases: Narrative Review

Siti Zakiyah Muharam[1]*, Mohd Faizal Hafez Hidayat[1] and Norhayati Liaqat Ali Khan[2,3]

[1]Centre for Periodontology Studies, Faculty of Dentistry, Universiti Teknologi MARA, Sungai Buloh Campus, 47000 Sungai Buloh, Selangor, Malaysia

[2]Centre of Preclinical Science Studies, Faculty of Dentistry, Universiti Teknologi MARA, Sungai Buloh Campus, 47000 Sungai Buloh, Selangor, Malaysia

[3]Cardiovascular Advancement and Research Excellence Institute (CARE Institute), Universiti Teknologi MARA, Sungai Buloh Campus, 47000 Sungai Buloh, Selangor, Malaysia

***Corresponding Author:** Mohd Faizal Hafez Hidayat, Faculty of Dentistry, Universiti Teknologi MARA (UiTM), Sungai Buloh Campus, Selangor Branch, 47000, Jalan Hospital, Sungai Buloh, Selangor, Malaysia.

Received: August 6, 2025; **Published:** September 02, 2025

DOI: 10.55162/MCMS.09.311

Abstract

Background: Current clinical diagnosis of periodontitis and peri-implantitis relies on clinical parameters and radiographic analysis, which are prone to errors and may not consistently diagnose these conditions. Biomarkers, detectable in bodily fluids such as in saliva, gingival crevicular fluid (GCF), or peri-implant crevicular fluid (PICF), offer promising avenues for non-invasive examination of immunological markers for dental disease. EVs possess a remarkable potential as carriers of diagnostic information due to their ability to transport diverse cargoes such as circular RNAs, mRNAs, miRNAs, DNA, lipids, and proteins. ***Aim:*** This review aims to identify the most appropriate oral biofluid (GCF, PICF, or saliva) for isolating EVs as diagnostic markers for periodontal and peri-implant diseases, to determine the optimal EV isolation methods and to identify and characterise EV-derived biomarkers in these oral fluids. ***Methodology:*** Scopus, Pubmed and Web of Sciences databases were searched for available literature with the keywords; (extracellular vesicles OR exosomes) AND (crevicular fluid OR saliva) AND (periodontitis OR peri-implantitis OR peri-implant diseases) spanning from 2014 to May 2025. ***Results:*** A total of eleven articles were found on EVs derived from oral biofluids as biomarkers for periodontal and peri-implant diseases. Crevicular fluids offer site-specificity, enabling direct diagnosis for each tooth or implant. In contrast, saliva is easier and more comfortable to collect but may not provide an accurate reflection of the patient's periodontal condition. Precipitation-based methods are commonly used to isolate EVs, whereas the miRNeasy and Trizol protocols are commonly used for extracting miRNA from EVs. ***Conclusions:*** GCF and PICF emerge as the most suitable, site-specific oral biofluids for diagnosing periodontal and peri-implant diseases. A combination of EV isolation methods, such as size-exclusion chromatography (SEC) and precipitation-based techniques, can enhance EV purity. Notably, miRNAs within these EVs represent promising biomarkers for the early and accurate diagnosis of periodontal and peri-implant diseases.

Keywords: extracellular vesicles; exosomes; periodontitis; peri-implantitis; biomarkers; crevicular fluid; saliva

Abbreviations

PI: Peri-implantitis.
PIM: peri-implant mucositis.
PID: peri-implant diseases.
PD: periodontal diseases.
GCF: gingival crevicular fluid.
PICF: peri-implant crevicular fluid.
EVs: extracellular vesicles.
Exo: Exosome.
MVs: Microvesicles.
NTA: Nanoparticle Tracking Analysis.
TEM: Transmission Electron Microscopy.
WB: Western Blot.
rRNA: ribosomal RNA.
PPD: periodontal pocket depth.
BOP: bleeding on probing.
CAL: clinical attachment loss.
ELISA: Enzyme-Linked Immunosorbent Assay.
qPCR: Quantitative PCR.
AUC: area under curve.

Introduction

Periodontal and Peri-implant Diseases

Periodontitis is a chronic, complex inflammatory disease driven by an imbalanced plaque biofilm and characterized by the progressive destruction of tooth-supporting structures, including the gingiva, periodontal ligament, alveolar bone, and cementum (Kinane and Mark Bartold; Papapanou et al.). It is highly prevalent worldwide, affecting up to 50% of the global population (Kassebaum et al.). Although dental plaque is essential for disease initiation, individual susceptibility plays a crucial role in determining the extent and severity of the disease, influenced by environmental factors such as smoking, lifestyle, and diet, as well as genetic predispositions (Bartold and Van Dyke). The current understanding of periodontitis emphasizes the complex interplay between microbial dysbiosis, host inflammatory responses, tissue destruction, and modifying factors, including systemic diseases and genetic risk (Kinane and Mark Bartold).

Peri-implantitis also shows similar features. Peri-implant diseases affect the tissues around dental implants and are divided into two types: peri-implant mucositis, which is inflammation of the soft tissue only, and peri-implantitis, which includes both soft tissue inflammation and bone loss around the implant (Zitzmann and Berglundh). The prevalence of peri-implant diseases varies considerably, with peri-implant mucositis affecting approximately 43% of individuals (ranging from 19%-65%) and peri-implantitis affecting about 22% (ranging from 1%-47%) (Salvi et al.).

The progression of peri-implantitis is closely linked to bone remodelling, a dynamic process that involves osteoclast-mediated resorption of mineralized bone, followed by osteoblast-mediated formation of new bone matrix. This remodelling cycle has three sequential phases: resorption, where osteoclasts break down old bone; reversal, marked by the appearance of mononuclear cells on the bone surface; and formation, during which osteoblasts produce new bone to fully replace the resorbed bone (Hadjidakis and Androulakis). Clinically, peri-implantitis is typically diagnosed when there is a continuous bone loss of ≥2 mm around the dental implant one year after the prosthetic supra-structure is placed (Schwarz et al.).

Both periodontitis and peri-implantitis are driven by a microbial challenge that triggers an exaggerated host inflammatory response, resulting in tissue breakdown and bone resorption (Bartold and Van Dyke). These diseases share similar multifactorial etiologies, influenced by site-specific factors, systemic health, environmental exposures, and genetic predispositions (Bartold and Van Dyke; Kinane and Mark Bartold). The key difference lies in the tissue interface: while periodontitis involves natural teeth and a periodontal ligament, peri-implantitis affects dental implants with a direct bone-to-implant contact (Araujo and Lindhe). Despite these differences, their shared pathogenesis underscores the importance of controlling microbial biofilms, modulating the host response, and assessing individual risk factors for effective prevention and management (Salvi et al.).

Current Clinical Periodontal and Peri-implant Disease

The 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions proposed a new classification and definition for periodontitis. This classification is based on the severity/complexity of management (Stages I, II, III, and IV), the rate of progression (Grades A, B, and C), and grade modifiers (Tonetti et al.). Given the multifactorial and complex nature of periodontal disease, accurate diagnosis can be particularly challenging, especially when trying to assess the disease's current activity or status in real-time. Traditionally, the diagnosis of chronic periodontitis (CP) has relied on conventional clinical assessments, including measurements of plaque accumulation, bleeding on probing (BOP), periodontal pocket depth (PPD), and alveolar bone loss (ABL) using radiographic imaging. However, the accuracy of these diagnostic tools can vary significantly depending on the clinician's skill and experience (Fujimori et al.).

Similarly, the diagnosis of peri-implantitis remains complex, often relying on clinical parameters such as radiographic evaluation and probing depths. Routine clinical examinations, including probing and visual inspections of dental implants, are crucial for identifying peri-implant diseases. However, the final diagnosis typically requires confirmation through persistent observations of progressive radiographic bone loss (Monje et al.). Given the inherent limitations and variability in the accuracy of these traditional diagnostic methods, there is a growing need to develop more reliable and non-invasive diagnostic tools, particularly those that leverage molecular techniques using oral biofluids (Bornes et al.).

Saliva or Gingival/Peri-implant Crevicular Fluid in periodontium and peri-implant mucosa

Biomarkers relevant to periodontal and peri-implant diseases can be identified in various oral biofluids, such as saliva, gingival crevicular fluid (GCF), and in the context of implants, peri-implant crevicular fluid (PICF) (Delucchi et al.). These oral biofluids are rich in biological molecules derived from both the host and the local microbiota, including inflammatory mediators, cytokines, leukocytes, enzymes, tissue breakdown products, genetic materials, circular RNAs, extracellular vesicles (EVs), and proteins, making them a promising candidate for non-invasive diagnostic tools (Han, Bartold, and Ivanovski). GCF, a fluid derived from serum and secreted in the gingival sulcus present in healthy gingival sulci and periodontally diseased pockets, containing a diverse array of cellular and molecular components from the host, as well as from the bacteria, fungi, and viruses residing in these niches (Chaparro Padilla et al.). Its dynamic composition and flow rate at specific sites can reflect changes in the local microenvironment, offering valuable insights into the disease status (Monteiro et al.).

The discovery of PICF by (Apse et al.) further expanded the diagnostic possibilities within the field. PICF, found in the peri-implant crevice, is site-specific and easily obtainable, providing a convenient, non-invasive source for evaluating immunological biomarkers (Jansson et al.). The volume of PICF often increases in cases of peri-implantitis, which enhances its diagnostic potential and allows for repeated assessments over time (Delucchi et al.).

Extracellular Vesicles (EVs) as biomarkers for Periodontal or Peri-implant Diseases

Over recent decades, EVs have garnered increasing attention in the medical field. These bilayered vesicles, released by various cell types into biological fluids, range in size from 30 nm to 5 μm and carry an array of biological cargo, including mRNAs, miRNAs, DNA,

circular RNAs, lipids, and proteins Van Niel et al., 2018). Functionally, EVs play critical roles in mediating cell-to-cell communication by delivering these biological molecules to recipient cells, where they can modulate cellular behaviour. Additionally, EVs carry ligands capable of activating cell surface receptors, such as bone-modulators RANKL and RANK, thereby stimulating specific pathways in target cells (Holliday et al.). These vesicles are essential not only for maintaining normal physiological processes but also for mediating pathological conditions (Van Niel et al.).

EVs serve as key carriers of microRNAs (miRNAs) and are commonly classified into three types: exosomes, microvesicles, and apoptotic bodies (Théry, Witwer, et al.). Notably, EVs released by diverse cell types are highly conserved, stable, and easily detectable in body fluids, making them particularly promising as biomarkers for various inflammatory diseases (Clark et al.). In the context of oral health, EVs with a bi-layered membrane and characteristic morphology (size < 200 nm) have been identified in GCF and PICF samples (Chaparro et al.; Chaparro Padilla et al.).

Despite their promising attributes as diagnostic biomarkers for periodontal and peri-implant diseases, research on EVs derived from oral biofluids in these specific contexts remains limited. Therefore, this review aims to identify the most appropriate oral biofluid (GCF, PICF, or saliva) for isolating EVs as diagnostic markers for periodontal and peri-implant diseases, to determine the optimal EV isolation methods and to identify and characterise EV-derived biomarkers in these oral fluids.

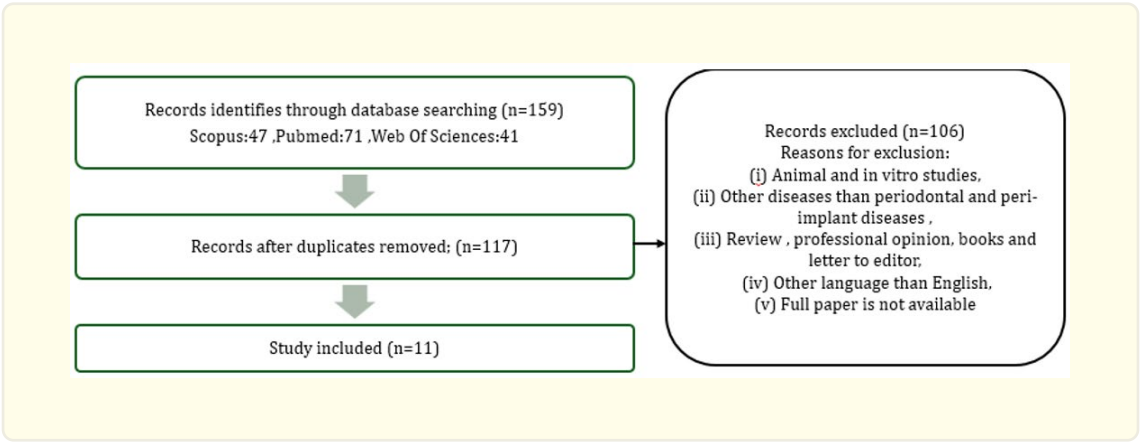
Materials and Methods

Search strategy and eligibility criteria

A literature search was performed in the Scopus, Pubmed and Web of Sciences database, for articles published up to May 2025 using Medical Subject Heading search terms + free text terms and in different combinations. The search strategy used a combination of keywords related to oral biofluid biomarkers and periodontal diseases/peri-implant diseases. The search string used were: (extracellular vesicles OR exosomes) AND (saliva OR crevicular fluid) AND (periodontitis OR periodontal diseases OR peri-implantitis OR peri-implant diseases) spanning from the year 2015 to May 2025.

Inclusion Criteria: (i) be written in the English language, (ii) be published in an international peer-reviewed journal, and (iii) be on humans. Exclusion criteria (i) animal and in vitro studies, (ii) studies that studied EVs from non-periodontal or peri-implant diseases, and iii) studies that studied EVs isolated from other sources than oral biofluids.

The search yields were transferred to the Mendeley Reference Manager. Citation tracking was completed for identified studies included in the refined library.



Results

Summary of the findings/search

This review covers only eleven studies that was conducted in Chile, Malaysia, Australia, China, Japan and Colombia between 2015 and May 2025 and was written in English. Table 1 presents comprehensive details for the listed research.

Reference	*Biofluid collection*	*Selection criteria*	*EV isolation method*	*EV characterisation*	*Key Findings*
(Chaparro Padilla et al.)	• Saliva:refrain from eating for 2h and drinking 30 min before saliva collection. passive unstimulated saliva by drooling method for 1 min • GCF samples were collected using Periopaper strips. All samples were collected from the mesiobuccal site of the sulcus/pocket.	• Gingival healthy or gingivitis (n=45) :PPD ≤ 3mm and by less than 10% bleeding on probing sites • Periodontitis stages II,III and IV (n=41):CAL ≥ 3mm with pocketing > 3mm detected at ≥ 2 teeth or interdental CAL detectable at ≥ 2 non-adjacent teeth, or buccal.	EVs from GCF isolated using a precipitation-based method	• NTA • TEM • WB	• The size/distribution (mode) of GCF-derived EVs in patients with periodontitis was 144.2nm and 160.35nm in healthy/gingivitis patients • TEM examination of materials precipitated by GCF showed the presence of EVs surrounded by a bi-layered lipid membrane that resembled the shape of EVs. • Compared to healthy / gingivitis subjects, the total concentration of GCF-EVs was considerably higher in participants with periodontitis. • positive markers by western blotting shows the identification of EVs/exosomal markers including the tetraspanin CD9 and the cytosolic markers TSG101 and Alix • The total concentration of tetraspanin CD63 was higher in periodontitis compare to healthy/gingivitis subjects
(Liu et al.)	Unstimulated saliva collected,Participants were instructed to fast and refrain from drinking and eating for at least one hour before saliva collection (from 8.00 am to 12.00 pm)	• Periodontal healthy (n=12):no history of periodontitis and PPD ≤ 3 mm. • Periodontitis(n=20): ≥ 30% of sites with PPD ≥ 3 mm, CAL ≥ 5 mm, and at least five sites with PPD ≥5 mm on at least three non-adjacent teeth, with radiographic evidence of bone loss extending from the root of the tooth by one-third or more.	• SEC • EXO-NET	• NTA • TEM • ELISA	• EXO-NET EVs exhibited higher EV-specific proteins and significantly elevated levels of EV surface markers (CD9, CD81, CD63) but contained less pathogenic DNA than SEC-EVs. • In periodontitis patients, EXO-NET EVs had increased levels of IL-6 and IL-8 and reduced levels of IL-10, in contrast to those from patients without periodontitis.

(Yu et al.)	Unstimulated saliva collected using the spitting method,no food, drinks, or oral hygiene products are consumed for at least 1 hour before the collection.	• Periodontal healthy (n=30):PD <3mm,CAL <3mm and no radiographic evidence of alveolar bone breakdown;-gender unclear • Periodontitis(n=61):at least one single-root with CAL≥6mm and probing depth ≥5mm;gender unclear	Precipitation based method	• NTA • TEM • RT-qPCR • Anti-ALIX	• TEM: Showed spherical membrane-bound particles with diameters ranging from 30 to 100 nm. • NTA: Human EVs had an average diameter of 95nm. • Mean salivary EVs PD-L1 expression was approximately 10 times higher in periodontitis patients compared to matched control subjects (P < 0.001).
(Han, Bartold, Salomon, and Saso Ivanovski)	Unstimulated whole saliva was collected using the spitting method, with no food or drink consumed for 1 hour prior to collection.	• Healthy(n=10):no periodontal history, BOP<15%,PPD<3mm, 6 males,4 females, • Gingivitis (n=9):no periodontal pocket, PPD<3mm;BOP>30% sites; 7 males,2 female • Periodontitis stage III/IV (n=10):>30% of sites with PPD ≥ 5mm on at least 3 non-adjacent teeth ;7 males, 3 females • All three groups had no systemic disease	SEC	• NTA • TEM • WB • RT-qPCR	• Compared to miRNAs in whole saliva, three significantly elevated miRNAs (hsa-miR-140-5p, hsa-miR-146a-5p, and hsa-miR-628-5p) were uniquely detected in EVs in periodontitis, compared to healthy controls. These miRNAs demonstrated a strong diagnostic potential for periodontitis (AUC = 0.96). • The size of EVs was less than 200 nm. The particle numbers, size, and protein content of EVs were similar across healthy, gingivitis, and periodontitis groups.
(Han, Bartold, Salomon, and Sašo Ivanovski)	Unstimulated whole saliva was collected via the spitting method, with participants abstaining from food and drink for 1 hour before collection.	• Healthy(n=7):no periodontal history,BOP<15%,PPD<3mm, 4 males,3 females • Gingivitis (n=7) :no periodontal pocket, PPD<3mm;BOP>30% sites; 6 males,1 female • Periodontitis (n=8):>30% of sites with PPD ≥ 5mm on at least 3 non adjacent teeth ;5 males, 3 females • All three groups had no systemic disease	SEC	• NTA • TEM • WB • ELISA	• In periodontitis EVs, there was a significant increase in LPS+ OMVs, global 5mC methylation, and four periodontal pathogens (T. denticola, E. corrodens, P. gingivalis, and F. nucleatum) that secreted OMVs, compared to those from healthy individuals. • The size of salivary EVs was less than 200 nm, with sEV particle counts similar across healthy, gingivitis, and periodontitis groups. • The levels of CD9 in saliva and CD9+ EVs were comparable among healthy, gingivitis, and periodontitis groups.

(Tobón-Arroyave et al.)	Unstimulated saliva was collected using the spitting method before breakfast and before performing any dental hygiene procedures.	• Healthy (n=45);BOP<10% bleeding sites,PPD ≤3mm and absence of erythema,oedema and patients symptoms in the presence of reduced clinical attachment and bone leves;11 males,34 females • Periodontitis (n=104):interdental CAL detectable at ≥non adjacent teeth and buccal or lingual CAL ≥3mm with pocketing >3mm detectable at ≥2 teeth;42 males,62 females	Precipitation based method	• TEM • ELISA	• The size of EVs was not specified. • Significantly lower salivary levels of CD9 and CD81 EVs were observed in periodontitis patients compared to healthy controls. • A significant reduction in CD9 and CD81 EV subpopulations was found in stages II, III, and IV periodontitis patients compared to stage I patients. A similar trend was noted in Grade B and C compared to Grade A periodontitis.
(Nik Mohamed Kamal et al.)	Refrain from eating and drinking for 1 hour before the collection of unstimulated saliva and blood samples.	• Healthy(n=8) :PD <3 mm,BOP ≤20% and no radiographic evidence of alveolar bone loss (ABL) ;2 males,6 femaled • Chronic periodontitis (n=8):have eight sites in a different tooth with PD ≥ 5mm,BOP ≥ 20% and radiographic evidence of ABL ;1 male ,7 females	Ultracentrifugation method	• WB • TEM	• The size of EVs was unspecified. • Out of the 2549 exosomal miRNAs detected, 33 EVs miRNAs showed significant downregulation. • Among the 1995 EVs miRNAs detected, with 1985 being down-regulated and 10 up-regulated, differential expression was observed in chronic periodontitis (CP) EVs compared to healthy salivary EVs. • Plasma EVs containing hsa-miR-let-7d, hsa-miR-126-3p, and mir-199a-3p (AUC=1), as well as salivary EVs containing hsa-miR-125a-3p (AUC=1), demonstrate potential as markers for chronic periodontitis.
(Chaparro et al.)	Four PICF samples per implant site (facial, lingual, mesial and distal) were collected by placing papers strips into the peri-implant sulcus/pocket	• Peri-implant health (n=17):the absence of oedema, BOP, inflammation, and suppuration, as well as the lack of increasing probing depth and the absence of radiographic alveolar peri-implant bone loss, • Peri-implant mucositis (n=19):inflammation of the peri-implant soft tissues, along with intact peri-implant alveolar bone and BOP,	EVs from PICF isolated using a precipitation-based method	• NTA • TEM	• The size of PICF-EVs revealed the presence of EVs with a suitable size range (< 200 nm), which matched the definitions of MVs and Exo size. • TEM examination of materials precipitated by PICF showed the presence of EVs surrounded by a bi-layered lipid membrane that resembled the shape of EVs.

		swelling, and, in certain situations. • Peri-implantitis (n=18): progressive bone loss in addition to soft peri-implant tissue inflammation, similar to that of peri-implant mucositis.			• Compared to healthy and peri-implant mucositis implants, the total concentration of PICF-EVs was considerably higher in participants with peri-implantitis. • Compared to patients with peri-implant mucositis, patients with peri-implantitis exhibited considerably lower expression of miRNA-21-3p and miRNA-150-5p. • There were no variations in miR-26a expression across the various peri-implant diagnoses.
(Huang et al.)	Unstimulated whole saliva.Drink,eat and toothbrush refrained from midnight on the day before sampling	• Healthy(n=11) :PD ≤3 mm,BOP ≤20% and no obvious CAL;gender not stated • Severe periodontitis (n=11):PD ≥ 6mm,CAL ≥ 5mm,and bone loss extending to mid-third of the root and beyond;gender not stated	Precipitation based method	• TEM • WB	• The salivary EVs had a size of less than 200 nm, with particle numbers and sizes, as well as EV protein contents, being comparable across healthy individuals, gingivitis patients, and severe periodontitis patients. • Protein Composition: Severe periodontitis and healthy groups had 26 and 58 EVs proteins respectively. • GO Analysis: significant enrichment of innate immune response, cytolysis, and complement activation in the severe periodontitis group. • C6 Protein: Western Blot analysis revealed a marked increase in C6 protein levels in the EVs from the severe periodontitis group compared to the healthy group.
(Han, Lai, et al.)	Unstimulated whole saliva. Refrain from food and drink 1hour before sampling	• Healthy(n=5):no periodontal history,BOP<15%,PPD<4mm;4 males,1 females • Gingivitis (n=7) :no periodontal pocket, PPD<4mm;BOP>30% sites;4 males,3 females • Both groups had no systemic disease	• SEC • Ultracentrifugation	•TEM • NTA • WB	• The size of small EVs < 200 nm. The number of EV size, particles, and EV protein content were similar between the healthy and gingivitis groups. • SEC-EVs: had a higher particle yield and particle-to-protein ratio, which were comparable between the healthy and gingivitis groups.

					• Gingivitis-UC-EVs: UC-EVs from the gingivitis group were increased compared to the healthy group. There were no significant differences in the oral bacterial genomic DNA, or DNA methylation of gene promoters and size of EVs for five cytokines between the UC-EVs and SEC-EVs in both health conditions.
(Yamaguchi et al.)	Unstimulated saliva. Refrain from using oral hygiene products for 1 hour before sampling	• Stage III and IV periodontitis (n=30):interdental CAL ≥5 mm, PD ≥6 mm, and radiographic bone loss extending to the mid-third of root and beyond	Precipitation based method	• WB • RT-PCR	• C6 Expression: Patients with increased C6 expression following Initial Periodontal Therapy (IPT) exhibited significantly higher levels of periodontal inflamed surface area (PISA), miR-142, and miR-144 both before and after IPT compared to those with decreased C6 expression after IPT. • CD81 Expression: Those with decreased or unchanged CD81 expression post-IPT had significantly higher probing depth (PD), clinical attachment loss (CAL), and PISA before IPT than after IPT. • TSG101 Expression: Patients showing decreased or unchanged TSG101 expression following IPT had notably higher PD before IPT than after IPT. • HSP70 Expression: Patients with increased HSP70 expression after IPT had significantly higher PD and PISA both before and after IPT compared to patients with unchanged HSP70 levels post-IPT. • miRNA Correlation: The expression levels of miR-142, miR-144, miR-200b, and miR-223 varied in relation to changes in the levels of C6, CD81, TSG101, and HSP70 in the extracellular vesicles (EVs) of periodontitis patients before and after IPT.

Table 1: Oral biofluid-derived EVs in Periodontitis and Peri-Implantitis.

The most appropriate oral biofluid (GCF, PICF, or saliva) for isolating EVs as diagnostic markers for periodontal and peri-implant diseases

In these review, unstimulated saliva was the most commonly used biofluid for EV isolation, while crevicular fluid (CF) samples were limited to just two investigations (Chaparro Padilla et al.; Chaparro et al.). The collection of CF involved the placement of paper strips into the gingival sulcus or peri-implant sulcus/pocket for 30 seconds, and the small volume collected in these studies may have influenced the yield and quality of isolated EVs.

Chaparro Padilla and colleagues found that while significant differences in EV concentrations and size distributions were observed in GCF between healthy and diseased states, no such differences were apparent in saliva samples (Chaparro Padilla et al.). Moreover, EVs populations in saliva did not significantly correlate with clinical periodontal parameters, whereas strong correlations were noted in GCF samples. These observations highlight the potential of GCF and PICF as more robust biofluids for EVs-based analyses in periodontal and peri-implant disease research.

However, challenges remain in comparing EVs across these fluids. For instance, the actual volume of GCF or PICF collected on paper strips is often undisclosed, complicating direct comparisons with saliva-derived EVs. Standardizing the volume of fluid collected, optimizing collection techniques, and carefully selecting the appropriate method for EV isolation are critical for improving reproducibility and ensuring the accuracy of biomarker discovery (Théry, Witwer, et al.).

Overall, this review suggests that GCF and PICF, due to their site-specific and inflammation-responsive nature, are superior to saliva as sources of EVs for diagnosing periodontitis and peri-implantitis. Nevertheless, standardising sample collection and isolation methods, and validating these findings in larger, well-designed studies are fundamental requirements to fully harness the diagnostic potential of EVs in oral biofluids.

To determine the optimal EVs isolation methods

In two studies by (Chaparro et al.) and (Chaparro Padilla et al.), EVs from crevicular fluid (CF) were isolated using a precipitation-based method, which appears to be particularly well-suited for isolating EVs from CF when working with small biofluid sample volumes. Conversely, EVs from saliva samples were isolated using a combination of ultracentrifugation, precipitation-based methods, or size-exclusion chromatography (SEC), which provided more flexibility in handling larger volumes (Chaparro Padilla et al.; Han, Lai, et al.; Han, Bartold, Salomon, and Sašo Ivanovski; Han, Bartold, Salomon, and Saso Ivanovski; Huang et al.; Nik Mohamed Kamal and Shahidan; Tobón-Arroyave et al.; Yamaguchi et al.; Yu et al.).

Han and colleagues found that EVs isolated using SEC yielded more particles and exhibited a higher particle-to-protein ratio compared to those obtained using UC. Additionally, UC-isolated EVs showed lower levels of DNA methylation for TNF-α in salivary EVs compared to SEC-isolated EVs, with methylation undetectable in seven participants, suggesting that SEC may be more effective for obtaining representative EV samples for methylation analysis (Han, Lai, et al.). Furthermore, Han and colleagues observed that SEC-isolated samples contained higher concentrations of small EVs compared to large EVs, aligning with SEC's recognized ability to isolate EVs with high purity and functional integrity (Böing et al.).

A study by Liu et al. reported that EXO-NET, an immunoaffinity-based method using magnetic beads, yielded EVs from periodontitis samples with higher purity and significantly greater protein concentrations, two to three times higher compared to SEC-derived EVs. These EXO-NET-isolated EVs also exhibited significantly higher surface marker expression and substantially lower levels of periodontal pathogen DNA, indicating that EXO-NET can enrich host-derived EVs while minimising microbial DNA contamination. This suggests that EXO-NET is a promising technique for obtaining highly purified saliva-derived EVs with reduced bacterial impurities.

To identify and characterise EV-derived biomarkers in these oral fluids

Characterisation of EVs was consistently performed using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and western blotting (WB), enabling a thorough evaluation of their morphology, size, concentration, and surface markers.

NTA

Despite methodological advances, the findings consistently indicate that EVs concentrations in crevicular fluid are higher in periodontitis and peri-implantitis patients compared to healthy or gingivitis subjects (Chaparro et al.; Chaparro Padilla et al.). In periodontitis- and peri-implantitis-affected sites, the concentration of these EVs (including microvesicles and exosomes) is significantly higher than in healthy gingiva and implants (Chaparro Padilla et al.; Chaparro et al.). However, two studies did not provide size characterisation of the EVs (Tobón-Arroyave et al.; Nik Mohamed Kamal et al.).

TEM

Transmission electron microscopy (TEM) analysis of precipitated samples from oral biofluids consistently revealed the presence of extracellular vesicles (EVs) encapsulated by a distinct bilayered lipid membrane, confirming their characteristic morphology (Figure 1) (Chaparro et al.; Chaparro Padilla et al.; Han, Lai, et al.; Han, Bartold, Salomon, and Sašo Ivanovski; Yu et al.).Across these studies, the size of EVs derived from oral biofluids was within a suitable range (Chaparro Padilla et al.; Chaparro et al.; Han, Bartold, Salomon, and Sašo Ivanovski; Han, Bartold, Salomon, and Saso Ivanovski; Yu et al.; Nik Mohamed Kamal et al.).

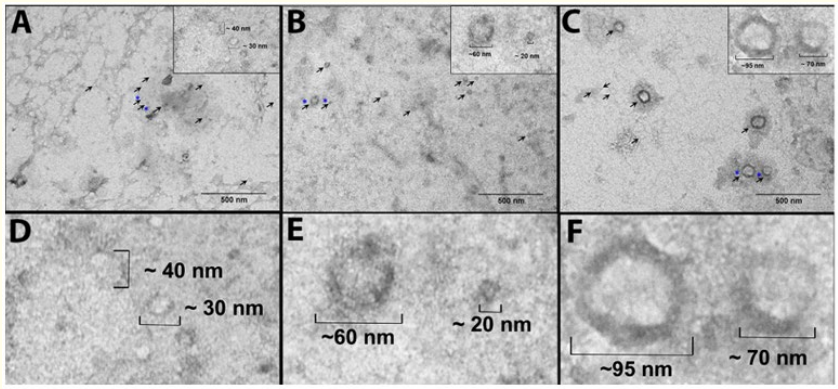


Figure 1: Morphology of EVs observed by TEM of PICF-EVs in healthy implants (A and D), peri-implant mucositis (B and E), and peri-implantitis implants (C and F). (Adapted from (Chaparro et al.).

EV cargoes

Salivary extracellular vesicles (EVs) carrying hsa-miR-126-3p (AUC = 1) have emerged as promising biomarkers for periodontitis (Nik Mohamed Kamal et al.). Similarly, significant upregulation of miRNAs such as hsa-miR-140-5p, hsa-miR-146a-5p, and hsa-miR-628-5p has been observed in salivary EVs from periodontitis patients compared to healthy individuals (Han, Lai, et al.). In addition, elevated levels of PD-L1 mRNA have been detected in salivary EVs from affected patients, potentially reflecting a regulatory mechanism aimed at limiting tissue damage in severe periodontitis (Yu et al.).

Interestingly, no notable differences were reported in EV size, presence of oral bacterial genomic DNA, or methylation status of cytokine promoters (IL-6, TNF-α, IL-1, IL-8, and IL-10) between ultracentrifugation-derived (UC-EVs) and size-exclusion chromatography-derived EVs (SEC-EVs) (Han, Lai, et al.). The expression of miR-142, miR-143, and miR-223 in salivary EVs further underscores their role in mediating inflammatory responses in periodontitis (Yamaguchi et al.). Moreover, Chaparro et al. found that miRNA-21-3p and miRNA-150-5p were significantly downregulated in peri-implantitis compared to peri-implant mucositis. These miRNAs, known to promote osteogenesis and bone formation in mesenchymal stem cells, may also play protective, anti-inflammatory roles in periodontal inflammation.

Nik Mohamed Kamal et al. also identified hsa-miR-125a-3p as a potential inflammatory biomarker, with significantly altered expression between chronic periodontitis and healthy samples (Nik Mohamed Kamal et al.). Among the miRNAs associated with periodontitis, hsa-miR-140-5p has been implicated in regulating cell proliferation, apoptosis, senescence, and inflammation; hsa-miR-146a-5p mitigates IL-1-induced inflammation by inhibiting IL-6 and IL-1 production (Buragaite-Staponkiene et al.) ; and miR-628-5p suppresses cellular proliferation, migration, invasion, and mesenchymal marker expression while promoting apoptosis (Wang et al.). Despite these insights, to date no studies have specifically explored EV-associated miRNAs in oral biofluids that are directly linked to the promotion of osteoclastogenesis or bone resorption in periodontitis or peri-implant diseases.

Western blotting

EVs surface markers, such as tetraspanin proteins (CD9, CD81, and CD63), along with RNA and miRNA, originate from parent cells and provide valuable insights into local and systemic health. Elevated levels of CD9 and CD63 were observed in gingival crevicular fluid (GCF) samples from periodontitis patients compared to those from healthy or gingivitis individuals (Chaparro Padilla et al.). In contrast, CD9 levels in saliva and CD9+ EVs showed no significant differences across healthy, gingivitis, and periodontitis groups (Han, Bartold, Salomon, and Sašo Ivanovski). A notable decline in CD9 and CD81 EV subpopulations was reported in Stages II, III, and IV periodontitis compared to Stage I (Tobón-Arroyave et al.), while C6 protein levels were significantly increased in EVs from severe periodontitis patients compared to healthy controls (Huang et al.).

Discussion

EVs from Saliva or Gingival/Peri-implant crevicular fluid

The use of oral biofluids, particularly saliva and crevicular fluid (CF), for isolating extracellular vesicles (EVs) in periodontal and peri-implant disease diagnostics has been steadily gaining attention. Saliva, a hypotonic solution primarily composed of water (~99%) and organic molecules (~1%), contains a variety of components including DNA, RNA, EVs, proteins, microbiota, and constituents from gingival crevicular fluid (GCF), serum, salivary gland secretions, and oral mucosal exudates. Produced largely by the salivary glands, it plays a pivotal role in maintaining oral health through its antibacterial properties and capacity to cleanse the mouth. In these studies, unstimulated saliva, collected without any masticatory, gustatory, or mechanical stimuli, was predominantly used (Chaparro Padilla et al.; Han, Bartold, Salomon, and Sašo Ivanovski; Han, Bartold, Salomon, and Saso Ivanovski; Han, Lai, et al.; Huang et al.; Liu et al.; Nik Mohamed Kamal et al.; Tobón-Arroyave et al.; Yamaguchi et al.; Yu et al.).

Gingival crevicular fluid (GCF), found within the gingival sulcus, is a fluid that originates as a serum transudate in health but becomes an inflammatory exudate during periodontal inflammation, reflecting the host's immune response (Offenbacher et al.). Both GCF and peri-implant crevicular fluid (PICF) reflect the local inflammatory status of the periodontal and peri-implant tissues. While GCF collection is more technically challenging and yields smaller volumes compared to saliva, it provides site-specific information that can directly correlate with periodontal disease activity (Saito et al.). This contrasts with saliva, which, despite being easier and more comfortable to collect, may not reliably represent localised periodontal conditions (Saito et al.).

Supporting this, (Han, Bartold, Salomon, and Saso Ivanovski) observed that the particle number, size, and protein content of salivary EVs were similar across healthy, gingivitis, and periodontitis groups, likely due to the non-site-specific nature of saliva. Conversely, studies by Chaparro and coworkers showed that participants with periodontitis or peri-implantitis had significantly higher total concentrations of EVs in GCF or PICF compared to healthy or mucositis subjects (Chaparro Padilla et al.; Chaparro et al.).These findings reinforce the notion that site-specific fluids like GCF and PICF better reflect the underlying inflammatory environment and thus may offer more accurate insights for disease diagnosis.

Extracellular Vesicles (EVs)

Oral biofluid samples contain EVs encased in a bilayer membrane, consistent with their size and morphology (less than 200 nm) (Chaparro et al.; Chaparro Padilla et al.; Han, Bartold, Salomon, and Sašo Ivanovski; Han, Bartold, Salomon, and Saso Ivanovski; Nik Mohamed Kamal et al.; Yu et al.). EVs are the smallest nanoparticles mediating cellular communication and molecular transport between different cell types (Théry, Ostrowski, et al.). Previously considered waste products, EVs are now recognised as active biological agents that reflect cellular states and mediate intercellular communication by carrying cell-specific proteins, mRNA, microRNAs, and other molecules (Théry, Ostrowski, et al.; Yuana et al.). These vesicles vary in biogenesis, release mechanisms, biophysical properties, size, and surface markers (Théry, Ostrowski, et al.) . The International Society for Extracellular Vesicles defines EVs as lipid bilayer-bound particles naturally released by cells, incapable of replication and lacking a functional nucleus. EV subtypes include exosomes (originating from endosomes) and microvesicles or ectosomes (derived from the plasma membrane). They influence target cells via paracrine or endocrine pathways and play crucial roles in immune responses and the pathogenesis of various chronic inflammatory, autoimmune, and infectious diseases (Yuana et al.). Consequently, EVs are increasingly considered potential diagnostic and therapeutic tools for numerous diseases and are found abundantly in plasma, urine, saliva, breast milk, and other body fluids (Théry, Ostrowski, et al.).

Extraction method of miRNA from Extracellular Vesicles

Although miRNAs initially encapsulated within EVs remain inactive, they can become active once the EVs are transfected into neighbouring cells, suggesting that miRNAs within EVs play an important role in intercellular communication (Kosaka et al.). The miRNA expression profile within EVs holds significant therapeutic potential and could serve as a diagnostic or prognostic tool (Turchinovich et al.). These miRNAs are short, non-coding RNA molecules comprising approximately 18-22 nucleotides (Menini et al.). Recognised as gene regulators and epigenetic mechanisms, miRNAs orchestrate a range of biological processes, including cell proliferation, programmed cell death, and tumorigenesis (Wu et al.). They have been implicated in various inflammatory conditions, such as periodontitis, rheumatoid arthritis, and osteoporosis (Asa'ad et al.), as well as potentially in peri-implant diseases. More recently, miRNAs have been identified as crucial regulators of bone homeostasis, influencing the commitment of stem cells to the osteogenic lineage and affecting osteoclastogenesis or bone-resorbing pathways (Asa'ad et al.). Consequently, miRNAs could contribute to mediating crestal bone loss, a defining feature of peri-implantitis.

In terms of experimental methods, Han and colleagues isolated total RNA from EV particles using the Trizol method (Han, Bartold, Salomon, and Saso Ivanovski), while Yamaguchi and colleagues employed the Trizol RNA Isolation method as well (Yamaguchi et al.) . Kamal and coworkers used the miRNeasy micro kit (Qiagen, United States) to extract total RNA (Nik Mohamed Kamal et al.). For miRNA expression analysis, Chaparro and colleagues extracted total miRNA directly from four PICF paper strips according to the miRNeasy protocol (Chaparro et al.). Overall, this review summarises that the Trizol and miRNeasy protocols are the most commonly employed methods for miRNA extraction from EVs.

Isolation Method of Extracellular Vesicles

Directly comparing the precipitation-based method with SEC is challenging due to differences in collection methods and sample amounts used in the reviewed studies. The precipitation-based method works by concentrating biological components until they exceed their solubility threshold, causing them to precipitate out of solution. Chaparro and colleagues reported that, using this meth-

od, medium and large EVs were more concentrated in gingival crevicular fluid (GCF) compared to small EVs (Chaparro Padilla et al.). ExoQuick-TC™, a precipitation reagent, is also particularly effective for isolating EVs from small saliva samples (Huang et al.). While precipitation-based methods can yield high amounts of EVs, they often co-precipitate proteins, DNA, and RNA, which can compromise the purity of the EVs and limit their therapeutic applications (Hammerschmidt et al.; Karttunen et al.).

According to the updated MISEV 2023 guidelines, EV studies should evaluate three critical aspects: EV particle quantity, shape, and the presence of EV-enriched protein markers (Welsh et al.). Ultracentrifugation remains the gold standard for EV isolation, typically involving a sedimentation step at 100,000 g for 1.5 to 2 hours. Although SEC has emerged as an effective and time-efficient alternative, it cannot differentiate between similarly sized molecules such as EVs and some lipoproteins (Böing et al.).

In this review, among ten studies investigating salivary EVs, the precipitation-based method and SEC were the most commonly used, each employed in four studies (40%), followed by UC in two studies (20%). For GCF-EVs and PICF-EVs, only one study each was identified, both utilizing the precipitation-based method for EV isolation.

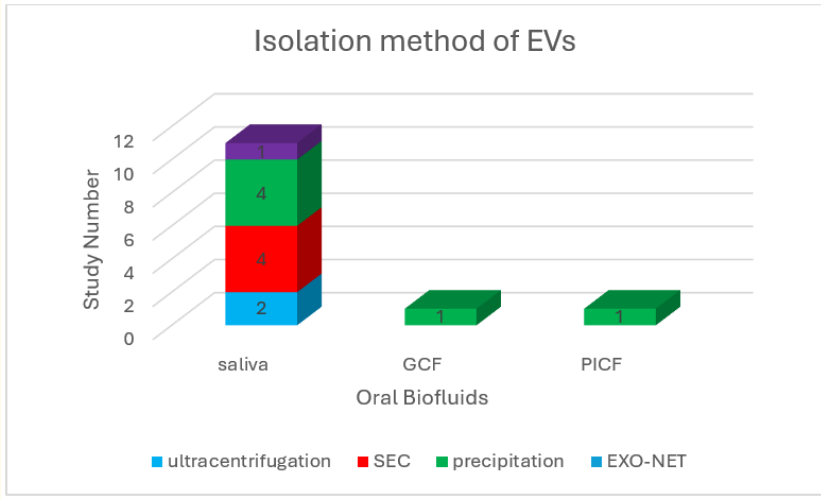


Figure 2: Different EVs isolation methods are used in EVs from oral biofluids in periodontitis and peri-implant diseases.

However, the small sample sizes, variations in EVs isolation methods, and differences in biofluid sources pose challenges to drawing firm conclusions. While these initial findings provide compelling evidence for the diagnostic utility of oral biofluid-derived EVs, further studies with standardised methodologies and larger cohorts are essential to establish robust EV-based biomarkers for periodontal and peri-implant diseases.

Limitation

A limitation of this review is the absence of specific information about the precise quantities of each sample, making it difficult to determine whether SEC methods are better suited for larger sample volumes and if the precipitation-based method is more appropriate for smaller volumes.

Conclusion

In conclusion, the miRNA cargo within EVs derived from oral biofluids presents promising biomarkers for diagnosing periodontal and peri-implant diseases. As miRNAs have emerged as key regulators of bone homeostasis, affecting osteoclastogenesis pathways, they may play a critical role in alveolar bone resorption, a hallmark of both periodontitis and peri-implantitis. For sampling, GCF or PICF is recommended over saliva, offering site-specific diagnostics for each tooth or implant. When it comes to EV isolation, combining methods like SEC and precipitation-based techniques can enhance purity and yield. The miRNeasy and Trizol protocols are commonly used for extracting miRNA from EVs, further supporting the consistency of these methods. Nonetheless, further studies are needed to fully explore the potential of extracellular vesicles as diagnostic markers in periodontal and peri-implant diseases.

Acknowledgement

Nil.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Apse Peter., et al. "Microbiota and Crevicular Fluid Collagenase Activity in the Osseointegrated Dental Implant Sulcus: A Comparison of Sites in Edentulous and Partially Edentulous Patients". Journal of Periodontology 24.2 (1989): 96-105.
2. Araújo Mauricio G and Jan Lindhe. "Peri-Implant Health". Clinical Oral Implants Research 28 Suppl.18 (2017): S249-S256.
3. Asa'ad Farah., et al. "Expression of MicroRNAs in Periodontal and Peri-Implant Diseases: A Systematic Review and Meta-Analysis". International Journal of Molecular Sciences 21.11 (2020): 1-14.
4. Bartold Peter Mark and Thomas E Van Dyke. "An Appraisal of the Role of Specific Bacteria in the Initial Pathogenesis of Periodontitis". Journal of Clinical Periodontology 46.1 (2019): 6-11.
5. Böing Anita N., et al. "Single-Step Isolation of Extracellular Vesicles by Size-Exclusion Chromatography". Journal of Extracellular Vesicles 3.1 (2014).
6. Bornes Rita., et al. "Peri-Implant Diseases Diagnosis, Prognosis and Dental Implant Monitoring: A Narrative Review of Novel Strategies and Clinical Impact". BMC Oral Health 23.1 (2023).
7. Buragaite-Staponkiene Benita., et al. "Gingival Tissue miRNA Expression Profiling and an Analysis of Periodontitis-Specific Circulating miRNAs". International Journal of Molecular Sciences 24.15 (2023).
8. Chaparro Alejandra., et al. "Diagnostic Potential of Peri-Implant Crevicular Fluid MicroRNA-21-3p and MicroRNA-150-5p and Extracellular Vesicles in Peri-Implant Diseases". Journal of Periodontology 92.6 (2021): 11-21.
9. Chaparro Padilla Alejandra., et al. "Molecular Signatures of Extracellular Vesicles in Oral Fluids of Periodontitis Patients". Oral Diseases 26.6 (2020): 1318-1325.

10. Clark Elizabeth A.., et al. "Concise Review: MicroRNA Function in Multipotent Mesenchymal Stromal Cells". Stem Cells 32.5 (2014): 1074-1082.
11. Delucchi Francesca., et al. "Biomarkers from Peri-Implant Crevicular Fluid (PICF) as Predictors of Peri-Implant Bone Loss: A Systematic Review". International Journal of Molecular Sciences 24.4 (2023).
12. Fujimori Kohei., et al. "Detection of Salivary miRNAs Reflecting Chronic Periodontitis: A Pilot Study". Molecules 24.6 (2019).
13. Hadjidakis Dimitrios J and Ioannis I Androulakis. "Bone Remodeling". Annals of the New York Academy of Sciences 1092 (2006): 385-396.
14. Hammerschmidt Nikolaus., et al. "Continuous Polyethylene Glycol Precipitation of Recombinant Antibodies: Sequential Precipitation and Resolubilization". Process Biochemistry 51.2 (2016): 325-332.
15. Han Pingping., et al. "Detection of Salivary Small Extracellular Vesicles Associated Inflammatory Cytokines Gene Methylation in Gingivitis". International Journal of Molecular Sciences 21.15 (2020): 1-17.
16. Han Pingping., et al. "Salivary Outer Membrane Vesicles and DNA Methylation of Small Extracellular Vesicles as Biomarkers for Periodontal Status: A Pilot Study". International Journal of Molecular Sciences 22.5 (2021): 1-15.
17. Han Pingping., et al. "Salivary Small Extracellular Vesicles Associated miRNAs in Periodontal Status—A Pilot Study". International Journal of Molecular Sciences 21.8 (2020).
18. Han Pingping, Peter Mark Bartold and Sašo Ivanovski. "The Emerging Role of Small Extracellular Vesicles in Saliva and Gingival Crevicular Fluid as Diagnostics for Periodontitis". Journal of Periodontal Research 57.1 (2022): 219-231.
19. Holliday L Shannon., et al. "RANKL and RANK in Extracellular Vesicles: Surprising New Players in Bone Remodeling". Extracellular Vesicles and Circulating Nucleic Acids 2.1 (2021): 18-28.
20. Huang Xiaoyi., et al. "Analysis of Salivary Exosomal Proteins in Young Adults with Severe Periodontitis". Oral Diseases 26.1 (2020): 173-181.
21. Jansson Leif., et al. "Intra-Individual Cytokine Profile in Peri-Implantitis and Periodontitis: A Cross-Sectional Study". Clinical Oral Implants Research 32.5 (2021): 559-568.
22. Karttunen Jenni., et al. "Precipitation-Based Extracellular Vesicle Isolation from Rat Plasma Co-Precipitate Vesicle-Free MicroRNAs". Journal of Extracellular Vesicles 8.1 (2019).
23. Kassebaum NJ., et al. "Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors". Journal of Dental Research 96.4 (2017): 380-387.
24. Kinane Denis F and P Mark Bartold. "Clinical Relevance of the Host Responses of Periodontitis". Periodontology 2000 43.1 (2007): 178-193.
25. Kosaka Nobuyoshi., et al. "Secretory Mechanisms and Intercellular Transfer of MicroRNAs in Living Cells". Journal of Biological Chemistry 285.23 (2010): 17442-17452.
26. Liu Chun., et al. "Immunoaffinity-Enriched Salivary Small Extracellular Vesicles in Periodontitis". Extracellular Vesicles and Circulating Nucleic Acids 4.4 (2023): 698-712.
27. Menini Maria., et al. "Prediction of Titanium Implant Success by Analysis of MicroRNA Expression in Peri-Implant Tissue: A 5-Year Follow-Up Study". Journal of Clinical Medicine 8.6 (2019).
28. Monje Alberto., et al. "Insights into the Clinical Diagnosis of Peri-Implantitis: To Probe or Not to Probe". Current Oral Health Reports 7.3 (2020): 304-312.
29. Monteiro Lara J.., et al. "Oral Extracellular Vesicles in Early Pregnancy Can Identify Patients at Risk of Developing Gestational Diabetes Mellitus". PLoS ONE 14.6 (2018).
30. Nik Mohamed Kamal., et al. "Plasma- and Saliva Exosome Profile Reveals a Distinct MicroRNA Signature in Chronic Periodontitis". Frontiers in Physiology 11 (2020).
31. Offenbacher Steven., et al. "Changes in Gingival Crevicular Fluid Inflammatory Mediator Levels during the Induction and Resolution of Experimental Gingivitis in Humans". Journal of Clinical Periodontology 37.4 (2010): 324-333.

32. Papapanou Panos N., et al. "Periodontitis: Consensus Report of Workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions". Journal of Periodontology 89 (2018): S173-S182.
33. Raposo Graça and Philip D Stahl. "Extracellular Vesicles: A New Communication Paradigm?" Nature Reviews Molecular Cell Biology 20.9 (2019): 509-510.
34. Saito Akira., et al. "MicroRNA Profiling in Gingival Crevicular Fluid of Periodontitis—A Pilot Study". FEBS Open Bio 7.7 (2017): 981-994.
35. Salvi Giovanni E., et al. "Prevalence of Periimplant Diseases". Implant Dentistry 28.2 (2019): 100-102.
36. Schwarz Frank., et al. "Peri-Implantitis". Journal of Clinical Periodontology 45 (2018): S246-S266.
37. Théry, Clotilde, Matias Ostrowski., et al. "Membrane Vesicles as Conveyors of Immune Responses". Nature Reviews Immunology 9.8 (2009): 581-593.
38. Théry Clotilde., et al. "Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018): A Position Statement of the International Society for Extracellular Vesicles and Update of the MISEV2014 Guidelines". Journal of Extracellular Vesicles 7.1 (2018).
39. Tobón-Arroyave., et al. "Decreased Salivary Concentration of CD9 and CD81 Exosome-Related Tetraspanins May Be Associated with the Periodontal Clinical Status". Journal of Clinical Periodontology 46.4 (2019): 470-480.
40. Tonetti Maurizio S., et al. "Staging and Grading of Periodontitis: Framework and Proposal of a New Classification and Case Definition". Journal of Clinical Periodontology 45 (2018): S159-S172.
41. Turchinovich Andrey., et al. "Characterization of Extracellular Circulating MicroRNA". Nucleic Acids Research 39.16 (2011): 7223-7233.
42. Van Niel Guillaume., et al. "Shedding Light on the Cell Biology of Extracellular Vesicles". Nature Reviews Molecular Cell Biology 19.4 (2018): 213-228.
43. Wang Tuo., et al. "Blocking Hsa_circ_0006168 Suppresses Cell Proliferation and Motility of Human Glioblastoma Cells by Regulating Hsa_circ_0006168/MiR-628-5p/IGF1R CeRNA Axis". Cell Cycle 20.12 (2021): 1181-1194.
44. Welsh Joshua A.., et al. "Minimal Information for Studies of Extracellular Vesicles (MISEV2023): From Basic to Advanced Approaches". Journal of Extracellular Vesicles 13.2 (2024).
45. Wu Xiaolin., et al. "MiR-27a Targets DKK2 and SFRP1 to Promote Reosseointegration in the Regenerative Treatment of Peri-Implantitis". Journal of Bone and Mineral Research 34.1 (2019): 123-134.
46. Yamaguchi Arisa., et al. "Changes in the Components of Salivary Exosomes Due to Initial Periodontal Therapy". Journal of Periodontal and Implant Science 53.5 (2023): 347-361.
47. Yu Jialiang., et al. "Detection of Exosomal PD-L1 RNA in Saliva of Patients with Periodontitis". Frontiers in Genetics 10 (2019): 202.
48. Yuana Yuana., et al. "Extracellular Vesicles in Physiological and Pathological Conditions". Blood Reviews 27.1 (2013): 31-39.
49. Zitzmann Nicola U and Tord Berglundh. "Definition and Prevalence of Peri-Implant Diseases". Journal of Clinical Periodontology 35 Suppl.8 (2008): 286-291.

Volume 9 Issue 3 September 2025