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The role of extracellular vesicles in periodontitis: pathogenesis, diagnosis, and therapy

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Periodontitis is a prevalent disease and one of the leading causes of tooth loss. Biofilms are initiating factor of periodontitis, which can destroy periodontal tissue by producing virulence factors. The overactivated host immune response is the primary cause of periodontitis. The clinical examination of periodontal tissues and the patient's medical history are the mainstays of periodontitis diagnosis. However, there is a lack of molecular biomarkers that can be used to identify and predict periodontitis activity precisely. Non-surgical and surgical treatments are currently available for periodontitis, although both have drawbacks. In clinical practice, achieving the ideal therapeutic effect remains a challenge. Studies have revealed that bacteria produce extracellular vesicles (EVs) to export virulence proteins to host cells. Meanwhile, periodontal tissue cells and immune cells produce EVs that have pro- or anti-inflammatory effects. Accordingly, EVs play a critical role in the pathogenesis of periodontitis. Recent studies have also presented that the content and composition of EVs in saliva and gingival crevicular fluid (GCF) can serve as possible periodontitis diagnostic indicators. In addition, studies have indicated that stem cell EVs may encourage periodontal regeneration. In this article, we mainly review the role of EVs in the pathogenesis of periodontitis and discuss their diagnostic and therapeutic potential.

KEYWORDS

periodontitis, extracellular vesicles, stem cell, pathogenesis, diagnosis

1 Introduction

Periodontal disease is the sixth most prevalent disease in the world (1, 2). According to a 2017 report, periodontitis affects 796 million people worldwide (3), places a substantial financial and health burden on those affected, and drastically lowers their quality of life (4, 5).

Abbreviations: MSCs, mesenchymal stem cells; EVs, extracellular vesicles; Th, helper T cells; Treg, regulatory T cell; LPS, lipopolysaccharide; BMSCs, bone mesenchymal stem cells; ADSCs, adipose-derived mesenchymal stem cell; DFSCs, dental follicle stem cells; SHED, stem cells of human exfoliated deciduous teeth; PDLSCs, periodontal ligament stem cells; GMSCs, gingiva mesenchymal stem cells; PRRs, pattern recognition receptors; MMP, matrix metallopeptidase; IL, interleukin; COX-2, cyclooxygenase 2; TNFa, tumor necrosis factor-a.

In recent years, risk factors of periodontitis have been grouped into three main categories: biofilms, host, and environment (6). When local biofilms and the mild host immune are in balance, the immune surveillance and appropriate immune response predominate (7). When exposed to persistent microbial challenges or when the pathogenicity of the local microbiome increases, the balance between biofilms and the host is lost, and the host's immune reactivity is excessive. This results in a highly inflammatory state with immune cell infiltration, pro-inflammatory and inflammatory cytokines up-regulation, excessive osteoclasts activation, ligament fiber degradation, granulation tissue formation, and final periodontal destruction (8–16).

Clinical features of periodontitis include red, swollen, and receding gums, bleeding on periodontal probing, a deeper pocket, the destruction of periodontal tissue, tooth displacement, and eventually tooth loss (17, 18). Unfortunately, due to the low sensitivity and low positive predictive value of these tests, the parameters can only evaluate historical data on periodontal tissue loss and cannot forecast future disease activity (19–21). Furthermore, these parameters vary among dentists, which impacts the accuracy of diagnosing periodontitis (22). To prevent and diagnose periodontitis early and effectively refer patients to specialized therapy, it is crucial to investigate more repeatable, sensitive, and specific methods of periodontal diagnosis that provide current and future disease information (23, 24).

Periodontitis treatment comprises non-surgical treatment, surgical treatment, and adjuvant medicine treatment (25–27). The treatment objectives are to control inflammation, halt disease progression, and help patients reconstruct a healthy and functional dentition (28). Surgical intervention is required when it is necessary to rebuild a bone defect to establish a good bone structure or when regeneration is needed to restore lost periodontal structures (29). Periodontal regeneration is a complex process due to the unique anatomical structure and composition of periodontal tissue, including periodontal ligament, cementum, and alveolar bone (30). Osteogenesis, inflammation control, and angiogenesis play important roles in periodontal regeneration (31, 32). At present, guided tissue regeneration techniques, including the transplantation of soft and hard tissues, the use of growth factors and host regulatory factors, and the use of biomaterials, are the mainstays of periodontal regeneration (33–35). Nevertheless, there are just a few materials with high potential for periodontal regeneration, and present technologies have limitations in attaining periodontal regeneration. Thus, looking for more durable and potent therapies and materials is critical to improving periodontal regeneration (36–38).

EVs are a group of bilayered lipid membrane-structured vesicles secreted by multiple kinds of cells. They carry a variety of substances from the parental cells, like DNA, RNA, lipids, and proteins (23, 39). EVs play a role in various pathological and physiological processes, including immunological regulation, inflammatory response, and tissue healing and regeneration. EVs have been discovered valuable to study physiological processes, pathologies, as well as regeneration (40).

We outlined the function of EVs in the pathogenesis and diagnosis of periodontitis and discussed methods used to isolate and characterize salivary and GCF EVs in this paper. We also reviewed recent research on stem cell-derived EVs in periodontitis therapy and addressed the flaws and future directions.

2 Extracellular vesicles

2.1 Definition of EVs

EVs can be secreted by humans, plants, animals, and microbial origins (40). Only a few nonmammalian sources have been explored in preclinical or clinical settings, and most studies and reviews have concentrated on EVs derived from mammalian cells and body fluids (40). EVs can be classified as endosome-derived exosomes (Exo), plasma membrane-derived microvesicles (MVs), and apoptotic bodies (ApoEVs) based on their secretion processes and characteristics (41, 42). Exosomes (30-100 nm) germinate inward from the endosome membrane, forming multivesicular bodies (MVBs) in the cytoplasm. Some MVBs are degraded by lysosomes, while others fuse with the cell membrane and are discharged into the extracellular environment by exocytosis. MVs (50 nm-2μm) and ApoEVs (50 nm-5 μm) were derived from outward budding. Exosomes and MVs are secreted during normal cellular processes, whereas ApoEVs are only produced during programmed cell death (43–46). In 2018, the International Extracellular Vesicle Society recommended researchers characterize EVs by size: "small EVs" (sEVs < 200 nm) and "medium and/or large EVs" (m/lEVs > 200 nm) unless specific EVs markers are available (47).

EVs can participate in physiological and pathological processes by directly binding to receptors on recipient cells, fusing with the plasma membrane of recipient cells and the membrane of the endosome following endocytosis (48, 49). Various endocytosis-related pathways, including clathrin-dependent endocytosis and clathrin-independent pathways such as caveolin-mediated uptake, macropinocytosis, phagocytosis, and lipid raft-mediated internalization, are thought to be the primary mechanisms of EVs uptake by recipient cells (48, 50).

2.2 Functions of EVs

In physiological and pathological processes, including cancer treatment, early diagnosis, tissue regeneration, and medication, the released EVs can remove metabolic proteins during cell maturation and regulate cell-to-cell communication (39, 51–54).

The RNA composition of EVs varies with pathological situations, and as a result, they have become a source of biomarkers for diagnosing human diseases (55). Proteins, genetic material, and lipids in EVs extracted from oral biofluids (saliva and GCF) have recently emerged as potential sources of biomarkers for periodontal diseases (23).

On the other hand, EVs are used in disease therapy in that the biological properties of EVs can modulate the phenotype and behavior of recipient cells (56, 57). Due to their innate ability to promote tissue regeneration, mesenchymal stem cells (MSCs) have

been employed as a source of regenerative EVs. Besides, MSCs-EVs have the following advantages (1): MSCs-EVs have the innate capacity to cross physiological barriers, such as the blood-brain barrier, due to their nanoscale size (58, 2). The risk of immune rejection and tumorigenicity induced by cell transplantation can be decreased with MSCs-EVs therapy (59, 3). MSCs-EVs are highly stable and biocompatible, and recipient cells may quickly absorb them (60, 4). MSCs-EVs are more convenient to store and transport since they can be kept stable at low temperatures (61, 5). Appropriate modification can enhance the targeting and repair abilities of MSCs-EVs (50, 62, 6). Studies have also demonstrated that MSCs-EVs have no adverse effects in toxicology tests (63, 7). MSCs-EVs are comparable to MSCs in their capacity to repair injured tissues, resist inflammation, inhibit cell apoptosis, and regulate immune responses (64–67). *In vitro* and animal studies have shown the potential of MSCs-derived EVs for treating periodontitis (68, 69). MSCs-EVs have demonstrated potential in the prevention and treatment of periodontal disease as well as periodontal regeneration due to their ability to regulate inflammation and promote osteogenesis (70, 71).

2.3 Extraction and characterization of EVs

Five basic extraction techniques are frequently employed based on the physical (density, size, and solubility) and biological (surface antigen) properties of EVs: precipitation, membrane affinity, size-exclusion chromatography, iodixanol gradient, and phosphatidylserine affinity (72). Additional methods have been developed to improve the specificity of EVs, including tangential flow filtration (73), field-flow fractionation (74), asymmetric flow field-flow fractionation (75), field-free viscoelastic flow (76), alternating current electrophoretic (77), acoustics (78), ion exchange chromatography (75), microfiltration (79), fluorescence-activated sorting (80), etc.

According to the latest MISEV 2018 guidelines for EVs characterization, scientists should include at least three distinct characteristics, such as EVs particle quantity, morphology, and protein markers (47). Dynamic light scattering (81) and nanoparticle tracking analysis (82) are frequently applied to estimate the quantity and size of EVs particles. Transmission electron microscopy (83), scanning electron microscopy (84), and atomic force microscopy (85) can be used to examine the morphology of EVs. Bicinchoninic acid assay (86), fluorimetric assays (87), or the global protein stain on SDS-PAGE (88) were used for EVs protein quantification. Western blot (89), enzyme-linked immunosorbent assay (90), bead-based flow cytometry (91), aptamer- and carbon nanotube-based colorimetric assays (92), and surface plasmon resonance (93) were employed to detect protein markers. Generally, at least one protein from each of the following groups must be assessed (47): (1) Transmembrane or GPI-anchored proteins connected to the plasma membrane or endosomes (Tetraspanins, integrins, etc.). (2) Cytosolic proteins (membrane binding proteins, etc.). (3) Major non-EVs co-isolated structural constituents (lipoproteins, Apolipoproteins, ribosomal proteins, etc.). When claiming specific analysis of sEVs, analysis of transmembrane, lipid-bound and soluble proteins associated with other intracellular compartments (histones, cytochrome C, etc.) is required. Secreted proteins recovered with EVs (cytokines and growth factors, adhesion, extracellular matrix proteins, etc.) are needed to document the functional activities of sEVs. To further indicate particle per volume and particle size distribution, the guidelines also suggested the addition of EVs purity metrics, such as protein/particle ratio, protein/lipid ratio, or RNA/particle ratio.

3 EVs and the pathogenesis of periodontitis

3.1 Direct pathogenic role of outer-membrane vesicles in periodontitis

The Gram-negative bacteria that are closely related to the progression of periodontitis, including Porphyromonas *gingivalis (P. gingivalis), Treponema denticola (T. denticola), Tannerella forsythia (T. forsythia), Actinomyces reticulata (A. reticulata), Fusobacterium nucleatum (F. nucleatum) and Prevotella intermedia (P. intermedia)* have been isolated from the periodontal pocket (94–96). *P. gingivalis* is the primary pathogen responsible for chronic periodontitis (97, 98). It forms the "red complex" along with *T. forsythia* and *T. denticola*, which are accessory pathogens with complementary or supplementary functions (99, 100).

Gram-negative bacteria can selectively export toxins and other virulence factors to host cells through vesicles named OMVs. In light of our current knowledge, OMVs are double-layered spherical membrane-like structures with a diameter ranging from 20 to 250 nm. OMVs contain bacterial parts and products such as fimbriae, lipopolysaccharides (LPS), toxins, outer membrane proteins, peptidoglycans, and bacteria's DNA and RNA (101–107). Yet it is unclear how these elements are packed into OMVs, and how the cargos are selected (101). OMVs can directedly fuse with target cells or be internalized by lipid rafts, micropinocytosis, and clathrin-dependent endocytosis (108–110).

After entering host cells, OMVs can exhibit a variety of virulences (111), and host-derived proteases have little effect on them (112). While requiring much energy, OMVs are crucial for maintaining bacterial virulence, colony formation, material transfer inside bacteria, immune escape, and host cell immune regulation (101, 103, 113–116).

The gingival epithelium is a physical barrier against invasion by biofilms and other nonautologous substances and is the first line of defense in the oral cavity (117, 118). There have been reports of *P. gingivalis* OMVs invading oral epithelial cells (119). By the endocytic pathway, *P. gingivalis* OMVs can efficiently infiltrate human epithelial cells and interfere with their function by destroying signaling molecules necessary for cell migration, such as transferrin receptor, paxillin, and focal adhesion kinase (120, 121). *T. denticola* can disrupt the function of the epithelial barrier and penetrate the epithelial layers by degrading tight junctional proteins like ZO-1 (122). According to Bartruff (123) et al., OMVs derived from *P. gingivalis* significantly inhibited the proliferation of cultured gingival fibroblasts and human umbilical vein endothelial

cells (hUVECs), as well as hUVECs' ability to form capillaries, which restrained periodontal tissue healing.

OMVs could be oral microbial communication between *P. gingivalis* and other oral bacteria (119). Kamaguchi (124) et al. demonstrated that *P. gingivalis* OMVs significantly promoted oral bacteria coaggregation. Grenier (125) noticed that *P. gingivalis* OMVs could mediate the coaggregation between *T. denticola* and *L. saburreum*. *P. gingivalis* and *T. denticola* co-inoculation synergistically triggered host immune responses and alveolar bone loss in a murine experimental periodontitis model (126). According to Inagaki (127) et al., *P. gingivalis* OMVs play an important role in virulence by enhancing *T. forsythia's* adherence and penetration of epithelial cells. *P. gingivalis* OMVs have explicitly been enriched for the heme-binding lipoproteins HmuY and IhtB, which can provide micronutrients to several other subgingival biofilms, resulting in community benefits that encourage biofilm proliferation (128). In addition, *P. gingivalis* OMVs can suppress and disperse rival biofilms in a gingipains-dependent manner to create a favorable environment for *P. gingivalis* (119).

In brief, these findings indicate that OMVs, which can mediate the interaction between biofilms and host cells and hasten the destruction of periodontal tissue, are substantially responsible for the pathogenicity of biofilms (Figure 1). Nevertheless, the precise mechanisms by which OMVs alter the nature of biofilms remain unclear. Further research is required to determine the specific function and associated mechanisms of OMVs in periodontitis.

3.2 Pathogenic role of OMVs in periodontitis by affecting immunity and inflammation

Pattern recognition receptors (PRRs), expressed by host immune cells, are essential molecules that trigger local immune responses. OMVs from periodontal pathogens can induce PRRs reactions (112). OMVs can activate PRRs in gingival epithelial cells, causing the secretion of pro-inflammatory and anti-inflammatory cytokines and activating neutrophils, T and B lymphocytes, and osteoclasts. These reactions promote connective tissue destruction and alveolar bone resorption (129). Choi (130) et al. derived that OMVs secreted by major periodontal pathogens transferred microRNA (miRNA) to immune cells to suppress target genes related to immune response, thereby evading the host adaptive immune responses. The potent but flexible immunostimulatory effects of *P. gingivalis* OMVs may help manipulate and dysregulate host immune responses to initiate disease, and the pro-inflammatory effects of other bacteria may contribute to the disease progression (100). *P. gingivalis* OMVs can selectively promote tumor necrosis factor (TNF) tolerance in a Toll-Like Receptor 4 and mTOR-Dependent manner, leading to local immune evasion (131). *P. gingivalis* OMVs can selectively entrap and activate human neutrophils to initiate degranulation without being destroyed by neutrophils, and they can also breakdown components of secretory granules with antibacterial activity,

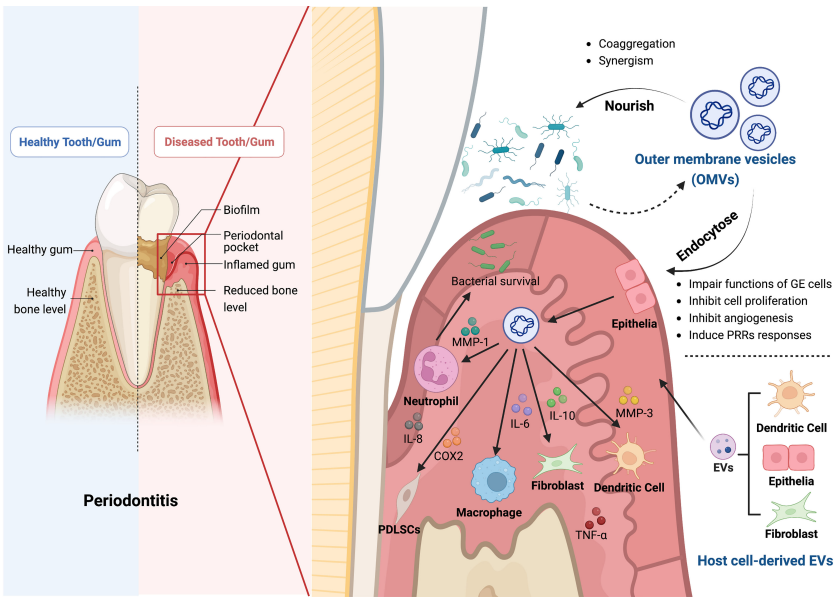

FIGURE 1
The roles of bacterial outer membrane vesicles (OMVs) and host cell-derived EVs in the pathogenesis of periodontitis. OMVs produced by Gram-negative bacteria contain bacterial components and bacterial products, such as outer membrane proteins, peptidoglycan, and lipopolysaccharide, which play a crucial role in the pathogenesis of periodontitis. Firstly, OMVs can enter the gingival epithelial cells through endocytosis and thus impair the function of gingival epithelial cells, inhibiting epithelial cell proliferation, slowing down angiogenesis, and inducing the response to PRRs. Secondly, OMVs can also inhibit bacterial clearance by immune cells by affecting a variety of cellular functions through different inflammatory mediators, including neutrophils, macrophages, fibroblasts, periodontal stem cells, and dendritic cells, which impede the host immune response. OMVs can cause aggregation of bacteria and act synergistically with them to induce the onset and progression of periodontitis. In addition, the EVs secreted by the host cells, such as dendritic cells, fibroblasts, and epithelial cells, can also cause alveolar bone loss, periodontitis and tissue damage. The Graph was created with BioRender.com. PRRs, pattern recognition receptors; MMP, matrix metallopeptidase; IL, interleukin; COX2, cyclooxygenase 2; TNF-α, tumor necrosis factor-α.

especially LL-37 and myeloperoxidase (MPO), to ensure bacterial survival (132).

The levels of inflammatory mediators, particularly interleukin (IL)-1, TNF, prostaglandin E2, and cyclooxygenase-2 (COX-2), are correlated with the severity of inflammatory response and periodontal disease (133, 134). Kou (135) et al. discovered that co-culturing immortalized human gingival epithelial cells with *P. gingivalis* OMVs elevated the production of inflammatory factors as COX-2, IL-6, IL-8, matrix metalloproteinase (MMP)-1, and MMP-3. Another study demonstrated that *P. gingivalis* OMVs enhanced the expression of IL-6 and IL-8 in human gingival epithelial cells *via* activating the signaling pathways Erk1/2, JNK, MAPK, STING, and NF-κB (136). Fleetwood (137) et al. confirmed that *P. gingivalis* OMVs could penetrate the gingival tissue and stimulate macrophages to produce large amounts of TNF-α, IL-12, IL-6, IL-10, IFN-β, and NO, resulting in tissue inflammation and damage. OMVs from *P. gingivalis* and *T. forsythia* induced the expression of pro-inflammatory cytokines like IL-1β, IL-6, IL-23, and IL-12p70 in bone marrow-derived dendritic cells (DCs) (138). Human monocyte cell line U937 and periodontal ligament fibroblasts were activated by *T. forsythia* OMVs in a concentration-dependent manner to produce pro-inflammatory mediators, and the inflammatory response was noticeably greater than that induced by whole *T. forsythia* cells (139).

OMVs should be considered as part of a larger picture because they not only contribute to the local problem of periodontitis (40). As EVs communication is not confined to species, OMVs from periodontal pathogens are also involved in human systemic diseases (104), for instance, Alzheimer's disease (140), neuroinflammation and neurodegeneration (141), cardiovascular disease (142), and diabetes mellitus (98). Investigating interkingdom communication of EVs from different origins may help discover new pathologic mechanisms and innovative therapies.

OMVs derived from probiotic strains contain immunomodulatory molecules that decrease pro-inflammatory cytokines and strengthen epithelial barriers (143, 144). According to reports, OMVs are protease resistant, can withstand long-term storage, and their structural stability makes it easier for them to deliver contents into the host immune system. Moreover, OMVs are attractive vaccines against pathogenic bacteria due to their immunogenicity (145, 146). Specific antibodies against *P. gingivalis* can be produced in mice's blood and saliva by intranasal inoculating OMVs (147, 148). Whereas, because they are still in the very early stages of development, periodontal vaccines face obstacles such as limited yield, unfavorable toxicity, and insufficient immunogenicity (149).

3.3 Host cell-derived EVs in the pathogenesis of periodontitis

Immune senescence plays a pivotal role in the pathophysiology of experimental periodontitis. *P. gingivalis* directly invades DCs to cause premature senescence and dramatically accelerates the senescence of normal bystander DCs by secreting inflammatory exosomes (150). Exosomes of the *P. gingivalis*-invaded DCs transmit senescence to normal bystander DCs and T cells, resulting in the loss of alveolar bone, according to recently published research (151). Another study demonstrated that biofilms could contribute to inflammation and periodontal destruction by promoting gingival fibroblasts to exhibit a tissue-destructive phenotype *via* increased secretion of epithelial EVs (152). Otherwise, LPS-treated periodontal ligament fibroblasts induce inflammation and inhibit the osteogenic activity of osteoblasts by releasing exosomes (153). Presumably, EVs secreted by host cells significantly impact periodontal disease.

In conclusion, OMVs from periodontal pathogens and host cell-derived EVs are critical in the pathogenesis of periodontitis. Nevertheless, there is still much to learn about the precise molecules and mechanisms by which EVs mediate innate and acquired immune response in periodontitis (6).

4 EVs and diagnosis of periodontitis

4.1 Diagnostic role of EVs in saliva and GCF

Saliva, a hypotonic solution composed of GCF, serum, salivary glands secretion, oral mucosal secretion, and microorganisms, is responsible for oral cleaning, antibacterial effect, and host's resistance to oral infections (154, 155). Another important oral biofluid is GCF, a serum exudate of periodontal tissue, presented in the healthy gingival sulcus or the periodontal pocket. Saliva and GCF are rich in biomolecules from the host and microorganisms, such as inflammatory mediators, cytokines, tissue breakdown products, DNA, RNA, EVs, etc. (23, 154, 156–158). Therefore, saliva and GCF can be applied as promising non-invasive indicators for periodontitis (159).

Genetic analysis of saliva sEVs showed that innate immune response proteins were considerably enriched in patients with severe periodontitis, particularly the complement component 6 (C6) (160). One study proposed that the DNA methylation pattern of 5-methylcytosine (5mC) in saliva EVs was comparable in the groups with healthy gums, gingivitis, and periodontitis (161). According to another study, patients with periodontitis had considerably fewer CD9+ and CD81+ saliva exosomes than healthy controls (162). CD63+ exosomes in GCF were increased in patients with periodontitis compared with those without periodontitis (157). In addition to EVs surface markers, miRNAs were abundant in EVs, and the level of hsa-miR-199a-3p decreased with the development and progression of periodontitis (163). There was a decrease in miR-223-3p concentration in periodontitis (164). In contrast, periodontitis dramatically enhanced the expression of hsa-miR-140-5p, hsa-miR-146a-5p, hsa-miR-628-5p, and PD-L1 mRNA (165, 166). A *P. gingivalis* saliva diagnostic kit for the detection of *P. gingivalis* and *P. gingivalis* OMVs has been developed by researchers using monoclonal antibodies that identify the conserved *P. gingivalis* virulence factor RgpA-Kgp complex (167).

These findings demonstrate that EVs released by saliva and GCF can reveal alterations in the local microenvironment and may indicate periodontitis (157, 168, 169). Owing to that, saliva and

GCF EVs may one day become simple and fast chair-side methods for diagnosing and evaluating periodontitis activity.

4.2 Collection of saliva and GCF and isolation of EVs

Patients should refrain from drinking and eating for at least one hour before sampling to avoid contamination of saliva and GCF with food and drink (23, 161, 163). Saliva can be collected either stimulated or unstimulated. Stimulated saliva is collected by chewing or gustatory stimulation (such as chewing paraffin or placing citric acid). In contrast, unstimulated saliva is collected by spitting or drooling without chewing or gustatory stimulation (170). Techniques used to collect saliva can affect its composition and the ultimate determination of particular biomarkers (171). As a result, saliva collection should closely resemble actual clinical conditions, and sample collection and processing should be consistent throughout.

Subgingival biofilms should be removed, and teeth should be blown dry to exclude saliva interference before GCF collection. After gently sampling with filter paper strips to prevent contamination from bleeding, GCF is eluted with PBS (172). Researchers should make it clear whether the GCF is from the healthy or the diseased site and correctly record the clinical parameters of each site, which is of great value in parsing the biochemical information (159, 168).

Although there is no optimal method for isolating saliva and GCF EVs, several researchers have compared different isolation protocols. In a comparison of saliva sEVs obtained by ultracentrifugation (UC) and ExoQuick-TC (TM) (EQ) precipitation, Zlotogorski - Hurvitz (173) et al. found that EQ generated a larger shape/aggregation pattern and a higher CD63/CD9/CD81+ sEVs subset than UC. Other investigators compared the particle production and particle/protein ratio of UC-sEVs and SEC-sEVs in saliva, showing that SEC-sEVs were superior in both categories (23).

In summary, mounting evidence points to the possibility that EVs generated from GCF and saliva may serve as vital diagnostic biomarkers for periodontitis owing to their cargo of proteins, RNA, and DNA (Table 1). But there is still a long way to go before EVs can be used for clinical diagnosis because the techniques for collecting EVs are currently only in-vitro or pre-clinical. The primary challenge is standardized techniques for isolating and characterizing EVs. More specific, sensitive, and practical biomarkers should be developed (174). An analysis with a large

TABLE 1 Application of exosomes in the diagnosis of periodontitis.

Author (Year)	Research type	Origin of EVs	Contents/ markers	Groups	Sample size	Conclusions	Reference
Chaparro Padilla A, et al. (157)	cross-sectional case-control study	saliva, GCF	CD9, TSG101, Alix	Periodontitis(stages II, III, and IV)	41	hypersecretion, pro-inflammatory	(141)
				Gingival health or Gingivitis	45		
Huang X, et al. (160)	cross-sectional case-control study	saliva	CD9, CD81	Severe Periodontitis (SP)	11	protein expression difference, pro-inflammatory	(144)
				Periodontal health	11		
Han P, et al. (161)	cross-sectional case-control study	saliva	CD9, TSG101	Periodontitis	8	hypersecretion, pro-inflammatory	(145)
				Gingivitis	7		
				Periodontal health	7		
Tobon-Arroyave SI, et al. (162)	cross-sectional case-control study	saliva	CD9, CD81	Periodontitis	104	hyposecretion	(146)
				Periodontal health	45		
Nik Mohamed Kamal NNS, et al. (163)	cross-sectional case-control study	saliva, plasma	unspecified	Chronic Periodontitis(CP)	8	miRNA expression difference, expression downregulated	(147)
				Periodontal health	8		
Xia Y, et al. (164)	cross-sectional case-control study	saliva	miR-223-3p	Periodontitis(stages III and IV)	none	expression downregulated, anti-inflammatory	(148)
				Periodontal health	none		
Yu J, et al. (165)	prospective observational investigation	saliva	PD-L1	Periodontitis	61	expression upregulated, pro-inflammatory	(149)
				Periodontal health	30		
Han P, et al. (166)	cross-sectional case-control study	saliva	unspecified	Periodontitis(stages III and IV)	10	expression upregulated	(150)
				Gingivitis	9		
				Periodontal health	10		

sample size is required to establish proper EVs-periodontitis diagnostic criteria matched with different ages, genders, etc.

5 MSCs-derived EVs and therapy of periodontitis

5.1 Role of MSCs-EVs in periodontitis treatment *via* anti-inflammatory and immune regulation

As an essential component of the innate immune system, macrophages mediate the onset and progression of periodontitis (175). Macrophages can differentiate into either a pro-inflammatory (M1) or an anti-inflammatory (M2) phenotype in reaction to local microenvironments, with each playing a unique role in a variety of physiological or pathological conditions (176, 177). Cytokines like TNF- and IL-6, which are produced by M1 macrophages, increase inflammation, activate osteoclasts, and result in the resorption of alveolar bone. In contrast, factors such as IL-10 and transforming growth factor (TGF) -β, produced by M2 macrophages, have anti-inflammatory and angiogenic effects and can activate osteoblasts (175, 176, 178, 179). Consequently, modulating the macrophage M1/M2 polarization ratio is an effective strategy for intervening in diseases.

In a rat calcaneus defect model, TNF-α-pretreated MSCs-EVs possess stronger immunomodulatory properties that can suppress M1 macrophages markers like IL-1β and iNOS and increase M2 macrophages markers like Arg1 and CD206, thereby promoting bone formation (180). MSCs-derived exosomes can improve the treatment of periodontitis by reestablishing the equilibrium of T helper 17/regulatory T cells (Th17/Treg) in inflamed periodontal tissues (181).

Bone mesenchymal stromal cells (BMSCs)-derived EVs regulate the inflammatory immune response and promote periodontal regeneration by inhibiting osteoclast activity, influencing macrophage polarization to M2, and regulating the production of TGF-β1 (68). Xu (182) et al. found that upon LPS stimulation, BMSCs-EVs converted macrophages from M1 to M2 phenotype *in vitro*, which decreased inflammation. In addition, ApoEVs derived from BMSCs can inhibit the polarization of macrophages into the M1 phenotype, reduce the COX-2 expression, down-regulate the TNF-α secretion, and inhibit adjacent osteoclasts, which serve as the foundation for the treatment of periodontitis (183). BMSCs-EVs have become promising therapeutic strategies for managing periodontitis (184).

The TNF-α-pretreated exosomes derived from gingiva mesenchymal stem cells (GMSCs) are notable for their ability to induce M2 polarization and prevent osteoclast formation (185). Wang (186) et al. observed that macrophages co-cultured with GMSCs exosomes in the inflammatory microenvironment showed significantly lower levels of M1 markers but somewhat raised levels of M2 features. In other words, GMSCs exosomes could trigger the transformation of M1 macrophages into M2 macrophages and lessen the pro-inflammatory substances that M1 macrophages release (186). Another study showed that GMSCs-sEVs significantly improved periodontal regeneration by inhibiting the release of pro-inflammatory cytokines from T cells and monocytes/macrophages, blocking T cells activation, and inducing the creation of Tregs (187).

Zheng (188) et al. discovered that periodontal ligament stem cells (PDLSCs)-derived exosomes alleviated the inflammatory microenvironment in chronic periodontitis *via* the Th17/Treg/miR-155-5p/SIRT1 regulatory network. EVs derived from LPS-pretreated PDLSCs induced M1 polarization of macrophages, whereas DNase I-treated EVs abolished M1 polarization. EVs derived from PDLSCs may be a potential therapeutic target for periodontal inflammation (189).

LPS-pretreated dental follicle stem cells (DFSCs)-sEVs polarized macrophages to an M2 phenotype through the ROS/ERK signaling pathway, inhibiting the alveolar bone loss and promoting periodontal regeneration in dogs with experimental periodontitis (69).

DCs-derived exosomes are relevant to immune therapy of periodontitis (190, 191). It has been demonstrated that engineered EVs derived from DCs can modulate the immune response in periodontitis and prevent inflammatory bone loss (192).

Consequently, studies have presented that MSCs-EVs from various sources can promote M2 macrophage polarization, restrict osteoclast activity, and reduce alveolar bone resorption, which paves the way for the development of periodontitis therapy (Figure 2; Table 2).

5.2 Role of MSCs-EVs in periodontal regeneration

To achieve functional periodontal regeneration, periodontal ligament fibers need to be inserted between the newly produced cementum and alveolar bone (193). Recent studies are concerned mainly with the osteogenic and angiogenic properties of MSCs-EVs, which are critical elements of periodontal regeneration.

Zhu B (194) et al. co-cultured MSCs-Exo with PDLSCs and noticed increased proliferation and osteogenic differentiation of PDLSCs. Furthermore, *in vitro* experiments demonstrated that MSCs-Exo promoted the PDLSCs proliferation and migration by activating AKT and ERK signaling pathways (70). Hypoxic preconditioning of MSCs-sEVs significantly enhanced the proliferation, migration, and angiogenesis of human umbilical vein endothelial cells (UVECs) and promoted the formation of vascularized bone (32). In a rat model of the alveolar bone defect, Chew (70) et al. transplanted a collagen sponge loaded with MSCs-Exo and observed the regeneration of alveolar bone and functional periodontal ligament fibers.

Wei (195) et al. proposed that BMSCs-Exo derived from different stages of osteogenic induction could exert a sustained anti-inflammatory effect during osteogenesis, up-regulate genes associated with osteogenesis at the early stage, and promote MSCs migration at the later stage. EVs derived from neural

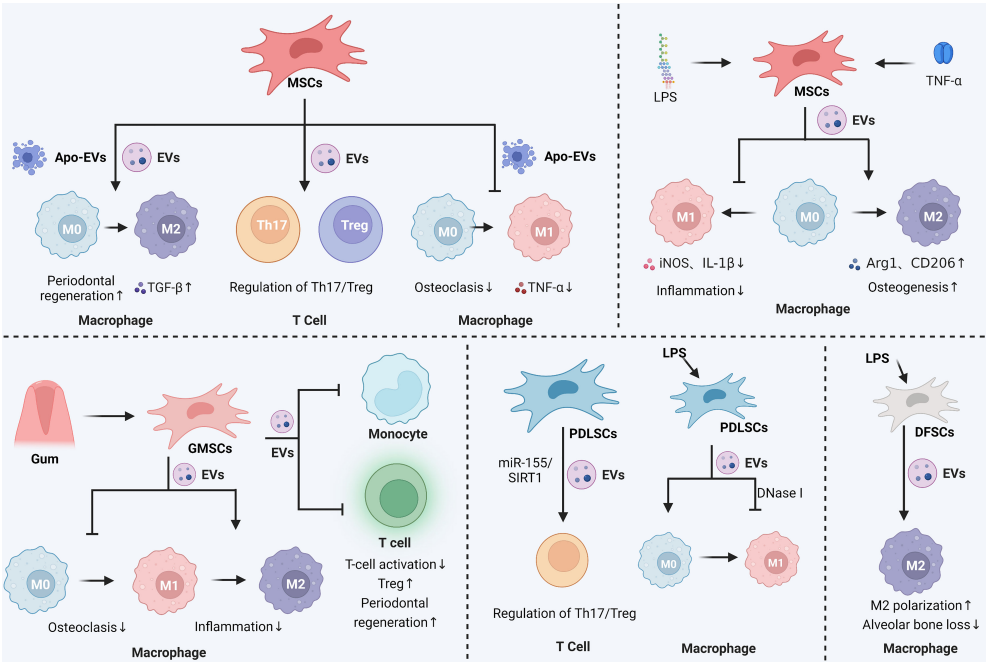

FIGURE 2
Immunomodulatory and anti-inflammatory effects of stem cell-derived EVs in periodontitis. In periodontitis, MSCs secreted EVs were able to promote the M2 polarization of macrophages and maintain the balance of Th17 and Treg cell ratio. MSCs secreted apoptotic vesicles were also able to inhibit the M1 polarization of macrophages, reduce the level of inflammatory mediators such as TNF-α, and promote the M2 polarization of macrophages. Pretreatment of MSCs with LPS or TNF-α enhanced the effect of MSCs-EVs, mainly by affecting macrophage polarization to control the inflammatory response and promote osteogenesis. Gingival mesenchymal stem cells (GMSCs) secrete EVs that affect macrophage polarization and inhibit osteoclastic and inflammatory reactions in periodontitis. GMSCs-EVs also inhibit monocyte and T-cell activation and promote periodontal tissue regeneration. Periodontal membrane stem cells (PDLSCs)-derived EVs could regulate the Th17/Treg ratio *via* the miR-155/SIRT1 axis and inhibit macrophage M1 polarization. LPS-stimulated dental follicular stem cells (DFSCs)-derived EVs also promoted macrophage M2 polarization and reduced alveolar bone loss. The Graph was created with BioRender.com. MSCs: mesenchymal stem cells; EVs, extracellular vesicles; Th, helper T cells; Treg, regulatory T cell; LPS, lipopolysaccharide.

TABLE 2 Anti-inflammatory and immunomodulatory effects of stem cell exosomes in periodontitis.

Author (Year)	MSCs source	Pretreatment of MSCs or EVs	Recipient of EVs	Experimental model	EVs administration	Functional outcome	Reference
Chew JRJ, et al., (70)	hMSC	/	rPDLSCs	①cells co-culture; ②Experimental periodontal defect rat;	①cells co-culture; ②Experimental periodontal defect rat: transplant/ implant with exosome-loaded collagen sponge (CS/Exosome) or control collagen sponge (CS/ Control);	repair periodontal defects, increase PDLSCs migration and proliferation	(67)
Cebatariuniene A, et al., (71)	hPDLSC	/	hPDLSCs	cells co-culture	cells co-culture	suppress basal and LPS-induced activity of NFκB	(68)
Liu L, et al., (68)	rBMSC	/	hPDLSCs/ RAW264.7 cells	①cells co-culture; ②Experimental Porphyromonas-induced periodontitis rats;	①cells co-culture; ②Experimental Porphyromonas-induced periodontitis rats: inject in periodontal pocket;	promote PDLSCs migration, proliferation and osteogenic differentiation	(65)
Huang Y, et al., (69)	hDFMSC	LPS pretreatment	hPDLSCs	①cells co-culture; ②Experimental Porphyromonas-induced periodontitis dogs;	①cells co-culture; ②Experimental Porphyromonas-induced periodontitis dogs: inject into the periodontal pocket;	promote PDLSCs proliferation and migration and macrophage proliferation	(66)

(Continued)

TABLE 2 Continued

Author (Year)	MSCs source	Pretreatment of MSCs or EVs	Recipient of EVs	Experimental model	EVs administration	Functional outcome	Reference
Kang M, et al., (180)	hBMSC	TNF-α pretreatment	mBMMs	①cells co-culture; ②Experimental calvaria defect rat;	①cells co-culture; ②Experimental calvaria defect rat: place on the wound by a clinical grade collagen scaffold (OraPLUG, Salvin);	immunoregulation, anti-inflammatory	(164)
Zhang Y, et al., (181)	hDPSC	3D culture	mouse naive CD4+ T cells	①cells co-culture; ②Experimental Ligature-induced periodontitis mice;	①cells co-culture; ②Experimental Ligature-induced periodontitis mice: inject into the palatal gingiva;	miR-1246 expression upregulated, reactive Th17 cell/ Treg balance, anti-inflammatory	(165)
Xu R, et al., (182)	rBMSC	LPS pretreatment	Raw264.7 cells	①cells co-culture; ②Experimental myocardial infarction mice;	①cells co-culture; ②Experimental myocardial infarction mice: intramyocardial injection at four sites around the infarct border zone;	promote M2 macrophage polarization, attenuat the post-infarction inflammation and cardiomyocyte apoptosis	(166)
Ye Q, et al., (183)	mBMSC	/	mBMDMs	Porphyromonas gingivalis derived LPS (Pg-LPS) induced inflammation of mouse bone marrow-derived macrophages (mBMDMs)	cells co-culture	inhibit M1 macrophage polarization and TNF-α secretion	(167)
Yue C, et al., (184)	hBMSC	/	RAW264.7 cells	cells co-culture	cells co-culture	regulate macrophage metabolism, differentiation, and inflammation resolution	(168)
Nakao Y, et al., (185)	hGMSC	TNF-α pretreatment	hPBMCs	①cells co-culture; ②Experimental wound healing mice; ③Experimental Ligature-induced periodontitis mice;	①cells co-culture; ②Experimental wound healing mice: subcutaneously inject into the cutaneous wounds; ③Experimental Ligature-induced periodontitis mice: inject into the palatal gingiva of the ligated second maxillary molar;	promote M2 macrophage polarization, immunoregulation	(169)
Wang R, et al., (186)	hGMSC	/	THP-1 cells	cells co-culture	cells co-culture	promote M2 macrophage polarization, anti-inflammatory	(170)
Zarubova J, et al., (187)	hGMSC	/	macrophages	cells co-culture	cells co-culture	reactive Th17 cell/ Treg balance, anti-inflammatory	(171)
Zheng Y, et al., (188)	hPDLSC	LPS pretreatment	CD4+ T cells	cells co-culture	cells co-culture	reactive Th17 cell/ Treg balance, anti-inflammatory	(172)
Kang H, et al., (189)	hPDLSC	LPS pretreatment	THP-1 cells	cells co-culture	cells co-culture	inhibit M1 macrophage polarization and TNF-α secretion	(173)

EGFL-like 1 modified BMSCs were more capable of stimulating BMSCs osteogenesis due to the downregulation of miR-25-5p (196). Huang (197) et al. demonstrated that EVs derived from BMP2-overexpressing BMSCs preserved the essential physical and biochemical characteristics of BMSCs-EVs but showed greater bone regeneration capability in a rat calvarial defect model. The ApoEVs from dying BMSCs can effectively promote the viability of endogenous BMSCs and repair bone defects (198). In critical-size calvarial bone defects, BMSCs-EVs positively regulate osteogenic genes and osteoblast differentiation *in vitro* (199). It has also been reported that BMSCs-derived exosomes overexpressing hypoxia-inducible factor (HIF)-1α can increase the packaging of Jagged1 and angiogenesis of endothelial cells (ECs) via the Notch signaling pathway (200). BMSCs-derived Nidogen1-enriched EVs enhanced the migration and angiogenic capacity of rat arterial endothelial cells (AECs) and promoted bone regeneration in rat femoral defect models (201). Hui (202) et al. coated BMSCs-EVs on a demineralized bone matrix to create a functional scaffold with enhanced pro-angiogenic and pro-bone regeneration activities.

Through a rat periodontitis model, Mohammed (203) et al. found that the injection of adipose-derived mesenchymal stem cells (ADSCs) exosomes suspension can be used as an auxiliary tool to promote periodontal regeneration, specifically periodontal fibers, blood vessels, and alveolar bone. The polydopamine-coated poly (lactic-co-glycolic acid) (PLGA/pDA) scaffold combined with ADSCs-EVs significantly induced the alveolar bone defect repair in the rat model (204). The ADSCs exosomes immobilized on the PLGA/pDA scaffolds promote the repair of critical-size skull defects in rats by stimulating osteogenesis and promoting BMSCs migration and homing (205).

In the inflammatory microenvironment, dental pulp stem cells (DPSCs)-EVs may shutter LMBR1-targeting miR-758-5p via the BMP signaling pathway to promote osteogenic and odontogenic differentiation of PDLSCs and provide a potential strategy for bone regeneration (206). DPSCs exosomes can effectively reduce periodontal bone loss by stimulating the migration of human DPSCs and mouse osteoblasts (207). It is addressed that DPSCs-EVs can induce the regeneration of experimental bone defects by enhancing the phosphorylation of ERK 1/2 and JNK and promoting the osteogenic differentiation of ADSCs (208). Xian (209) et al. found that DPSCs exosomes could stimulate endothelial cell proliferation and pro-angiogenic factors production, such as FGF-2, VEGF-A, KDR, and MMP-9. It has been demonstrated that DPSCs-EVs isolated from periodontally healthy and unhealthy teeth can enhance endothelial cell angiogenesis activity, accelerate wound healing, and encourage angiogenesis in mouse skin lesions (210). The co-injection of DPSCs-EVs with collagen, β-tricalcium phosphate, or hydroxyapatite can stimulate new bone formation in rat skull defects (211).

Invitro studies performed by Wang (212) et al. demonstrated that stem cells of human exfoliated deciduous teeth (SHED)-Exo under osteogenic induction conditions could up-regulate the expressions of osteogenic markers like osteopontin (OPN), osteocalcin (OCN), and Runx2 during the osteogenic differentiation of PDLSCs and enhance the osteogenic differentiation of PDLSCs *via* Wnt and BMP signaling pathways. Wei (213) et al. observed that exosomes repaired the defect to the same extent as original stem cells, increased the osteogenic impact of BMSCs, and inhibited adipogenesis after injecting SHED-derived exosomes into the bone defect area of a mouse periodontitis model.

Purified PDLSCs-EVs were discovered to reduce LPS-induced NF-B activity in PDLSCs and enhance osteogenic mineralization in PDLSCs, which may be helpful for the targeted treatment of chronic inflammation in periodontitis (71). Engineered EVs from PDLSCs promoted bone regeneration and angiogenesis of skull defects in rats (214). Collagen membrane enriched with PDLSCs-EVs or polyethylenimine (PEI)-engineered EVs (PEI-EVs) can activate osteogenesis and promote bone regeneration (215). PDLSCs-EVs immobilized in matrigel accelerated bone tissue repair by inducing BMSCs proliferation and migration through increasing the phosphorylation of AKT and ERK1/2 (216).

GMSCs-exosomes were shown to contain a variety of growth factors, such as transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF), which were shown to promote pre-osteoblast migration and osteogenic differentiation (217). 3D-engineered scaffolds complexed with GMSCs-EVs exhibited strong osteogenic induction ability (218).

According to Ma L (219) et al., DFSCs-sEVs boosted PDLSC migration, proliferation, and osteogenic differentiation, which offers a novel approach to periodontal regeneration in the future.

In conjunction with the current investigations, it is proposed that MSCs-EVs possess the potential to treat periodontitis and promote periodontal regeneration (Figure 3; Table 3). While the effectiveness of MSCs-EVs on periodontal ligament fibers and cementum regeneration needs to be further studied. Moreover, effectively alleviating the homeostasis imbalance is the key to periodontal regeneration. The application of MSCs-EVs in dentistry is restricted to fundamental research, and its clinical use requires more rigorous evidence. There is still a long way to go before MSCs-EVs can be used as an effective and safe dental clinical treatment method (220).

6 Summary and prospect

Several findings imply that OMVs can interfere with host gingival epithelium functions, affect angiogenesis, and induce PRRs reactions. OMVs also play a role in the pathogenicity of biofilms, such as promoting bacteria coaggregation, providing micronutrients to subgingival biofilms, and dispersing rival biofilms. These indicate that OMVs can promote connective tissue destruction and alveolar bone resorption. More research is required on the precise function and related mechanisms of OMVs in periodontitis. For example, the molecules and pathways by which OMVs affect innate and acquired immune defense (6).

Growing evidence suggests that saliva and GCF-derived EVs may serve as periodontitis biomarkers. Although the potential of saliva and GCF-derived EVs is promising, many obstacles must be solved before EVs can be translated into chair-side or off-the-shelf diagnostic tools. The major challenges are: (1) The need for standardized

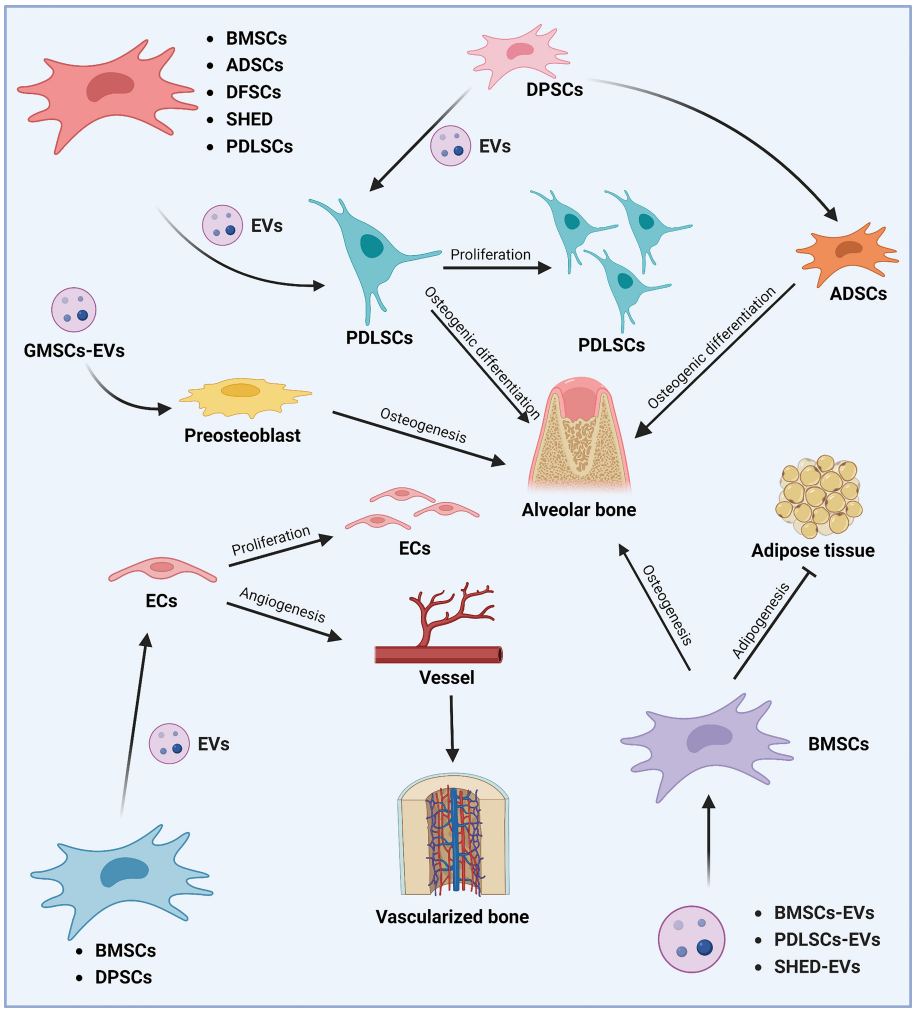

FIGURE 3
Role of stem cell-derived EVs in periodontal tissue regeneration. Different sources of MSCs can promote periodontal tissue regeneration and thus play a role in the treatment of periodontitis. Various sources of MSCs-EVs (including BMSCs, ADSCs, DFSCs, SHED, DPSCs, and PDLSCs) can promote the proliferation of periodontal membrane stem cells and alveolar bone osteogenesis by regulating osteogenic differentiation. DPSCs-derived EVs can promote alveolar bone regeneration by affecting the osteogenic differentiation of ADSCs. In addition, GMSCs-derived EVs can promote alveolar bone regeneration by regulating osteoblast precursor cells. In addition, BMSCs, PDLSCs, and SHED-derived EVs can promote osteogenic differentiation and inhibit lipogenic differentiation of BMSCs, thus promoting alveolar bone regeneration. The Graph was created with BioRender.com. BMSCs, bone marrow-derived mesenchymal stem cells; ADSCs, adipose tissue-derived mesenchymal stem cells; DFSCs, dental follicle stem cells; SHED, stem cells of human exfoliated deciduous teeth; PDLSCs, periodontal ligament stem cells; EVs, extracellular vesicles; GMSCs, gingiva-derived mesenchymal stem cells.

TABLE 3 Tissue regeneration of stem cell exosomes in periodontitis.

Author (Year)	MSCs source	Pretreatment of MSCs or EVs	Recipient of EVs	Experimental model	EVs administration	Functional outcome	Reference
Zhu B, et al. (194)	hPDLSC, hIBMMSC, JBMMSC	/	hPDLSCs	①cells co-culture; ②nude mice;	①cells co-culture; ②nude mice: transplant/implant into;	pro-osteogenic, osteoimmunomodulatory	(178)
Zhuang Y, et al. (32)	hox-rBMSC	hypoxia pretreatment	HUVECs	①cells co-culture; ②Experimental calvarial defect rat;	①cells co-culture; ②Experimental calvarial defect rat: transplant/ implant into;	promote HUVECs proliferation, migration and angiogenesis, pro-osteogenic	(179)
Wei F, et al. (195)	hBMSC	/	hBMDMs and RAW264.7	cells co-culture;	cells co-culture;	pro-osteogenic, osteoimmunomodulatory	(180)

(Continued)

TABLE 3 Continued

Author (Year)	MSCs source	Pretreatment of MSCs or EVs	Recipient of EVs	Experimental model	EVs administration	Functional outcome	Reference
			cells (macrophages)				
Lan Y, et al. (196)	rBMSC	neural EGFL-like 1 (Nell1) pretreatment	rBMSCs	①cells co-culture; ②Experimental calvarial defect rat;	①cells co-culture; ②Experimental calvarial defect rat: transplant/ implant into;	pro-osteogenic	(181)
Huang CC, et al. (197)	hBMSC	genetically modified by constitutively expressing BMP2 (bone morphogenetic protein 2)	hBMSCs	①cells co-culture; ②Experimental calvarial defect rat;	①cells co-culture; ②Experimental calvarial defect rat: place on the wound;	pro-osteogenic	(182)
Li M, et al. (198)	mBMSC	/	rBMSCs	①cells co-culture; ②Experimental calvarial defect rat;	①cells co-culture; ②Experimental calvarial defect rat: transplant/ implant into;	promote rBMSCs proliferation, migration, and osteogenic differentiation	(183)
Qin Y, et al. (199)	hBMSC	/	human osteoblasts (hFOBs)	①cells co-culture; ②Experimental calvarial defect rat;	①cells co-culture; ②Experimental calvarial defect rat: transplant/ implant into;	promote hFOBs proliferation, migration, and osteogenic differentiation	(184)
Gonzalez-King H, et al. (200)	hDPSC	hypoxia pretreatment	HUVECs	①cells co-culture; ②nude mice;	①cells co-culture; ②nude mice: inject subcutaneously into the flanks;	pro-angiogenic	(185)
Cheng P, et al. (201)	rBMSC	/	RAECs	①cells co-culture; ②nude mice;	①cells co-culture; ②nude mice: inject subcutaneously into the bac;	enhance RAECs migration, pro-angiogenic	(186)
Xie H, et al. (202)	rBMSC	/	HUVECs	①cells co-culture; ②nude mice;	①cells co-culture; ②nude mice: nude mice: implant into subcutaneously;	promote grafts vascularization, pro-angiogenic, pro-osteogenic	(187)
Mohammed E, et al. (203)	hADSC	/	/	Experimental periodontal defect rat;	Experimental periodontal defect rat: inject in periodontal pocket;	immunomodulatory, anti-inflammatory, pro-osteogenic	(188)
Yang Y, et al. (204)	hADSC	/	hPDLSCs	①cells co-culture; ②Experimental alveolar Bone Defects rat;	①cells co-culture; ②Experimental alveolar Bone Defects rat: transplant/implant into;	pro-angiogenic	(189)
Li W, et al. (205)	hADSC	/	hBMSCs	①cells co-culture; ②Experimental calvarial defect mice;	①cells co-culture; ②Experimental calvarial defect mice: transplant/ implant into;	promote hBMSCs osteogenic, proliferation and migration, pro-angiogenic	(190)
Yan C, et al. (206)	ihDPSC	/	hPDLSCs	cells co-culture;	cells co-culture;	promote hPDLSCs osteogenic and osteogenic differentiation	(191)
Shimizu Y, et al. (207)	hDPSC	/	mouse osteoblastic MC3T3- E1 cells	①cells co-culture; ②Experimental periodontal defect mice;	①cells co-culture; ②Experimental periodontal defect mice: directly appliy onto the silk ligature;	promote MC3T3- E1 cells migration	(192)
Jin Q, et al. (208)	hDPSC	/	hADSCs	①cells co-culture; ②Experimental mandible defect rat;	①cells co-culture; ②Experimental mandible defect rat: injecte into the hydrogel scaffold material and sutur;	promoted hADSCs migration, mineralization and osteogenic differentiation	(193)
Xian X, et al. (209)	hDPSC	/	HUVECs	cells co-culture;	cells co-culture;	promote HUVECs proliferation and tube	(194)

(Continued)

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TABLE 3 Continued

Author (Year)	MSCs source	Pretreatment of MSCs or EVs	Recipient of EVs	Experimental model	EVs administration	Functional outcome	Reference
						formation, pro-angiogenic	
Zhou H, et al. (210)	hDPSC	/	ECs	①cells co-culture; ②Experimental skin wound healing mouse;	①cells co-culture; ②Experimental skin wound healing mouse: subcutaneously inject into the full-thickness excisional skin wound;	pro-angiogenic	(195)
Imanishi Y, et al. (211)	rDPSC	/	/	Experimental calvarial defect rat;	Experimental calvarial defect rat: transplant/ implant into;	pro-angiogenic, pro-osteogenic	(196)
Wang M, et al. (212)	hSHED	/	hPDLSCs	cells co-culture;	cells co-culture;	promote hPDLSCs osteogenic differentiation	(197)
Wei J, et al. (213)	hSHED	/	mBMSCs	①cells co-culture; ②Experimental periodontal defect rat;	①cells co-culture; ②Experimental periodontal defect rat: inject into the buccal and lingual sides of the first molar;	promote mBMSCs osteogenesis, differentiation, and bone formation	(198)
Pizzicannella J, et al. (214)	hPDLSC	/	hPDLSCs	①cells co-culture; ②Experimental calvarial defect rat;	①cells co-culture; ②Experimental calvarial defect rat: transplant/ implant into;	pro-angiogenic, pro-osteogenic	(199)
Diomede F, et al. (215)	hPDLSC	/	hPDLSCs	①cells co-culture; ②Experimental calvaria defect rat;	①cells co-culture; ②Experimental calvaria defect rat: transplant/ implant into;	pro-angiogenic, pro-osteogenic	(200)
Zhao B, et al. (216)	hPDLSC	/	hBMSCs	①cells co-culture; ②Experimental calvaria defect rat;	①cells co-culture: none; ②Experimental calvaria defect rat: transplant/ implant into;	promote hBMSCs proliferation, migration, and osteogenic differentiation	(201)
Jiang S, et al. (217)	hPDLSC	/	mouse osteoblastic MC3T3- E1 cells	cells co-culture;	cells co-culture;	promote pre-osteoblasts migration and MC3T3-E1 cells osteogenic differentiation	(202)
Diomede F, et al. (218)	hGMSC	/	hGMSCs	①cells co-culture; ②Experimental calvaria defect rat;	①cells co-culture; ②Experimental calvaria defect rat: transplant/ implant into;	pro-osteogenic	(203)
Ma L, et al. (219)	hDFSC	/	hPDLSCs	①cells co-culture; ②Experimental periodontal defect rats;	①cells co-culture; ②Experimental periodontal defect rats: transplant/ implant into;	promote hPDLSCs proliferation, migration, osteogenic differentiation	(204)

methods for EVs isolation and characterization. (2) More useful, sensitive, and specific biomarkers must be developed. (3) A large sample size analysis is required to establish the diagnostic standards for EVs-periodontitis that are appropriate for individuals of different ages and genders (23, 174). Despite the current challenges, the diagnostic potential of saliva and GCF-derived EVs is compelling, and future clinical applications can be expected.

MSCs-derived EVs have massive advantages, particularly convenient access, a wide range of sources, immunomodulatory ability, and tissue repair and regeneration capacities. EVs derived from MSCs have emerged over the past decades as an alternative therapy for stem cells in the field of regenerative medicine (221), and they are expected to be a novel therapeutic tool for periodontal regeneration (222). However, the oral cavity is a highly complex environment constantly changing, and it is unclear how several elements, including temperature, pH, oxygen, inflammation, and microbiota species, affect EVs. The majority of current MSCs-EVs studies use animal models. Yet the clinical application of MSCs-EVs in periodontal regeneration has not been reported. There are no standardized methods for the clinically graded manufacture and quality control of EVs medicines, which are crucial in following EVs clinical trials (223). In addition, there are still no low-cost technologies to swiftly produce an abundance of highly homogenous MSCs-EVs (224).

It is anticipated that more comprehensive research on EVs affecting periodontitis will be produced, demonstrating tremendous promise for clinical treatment, and opening up new doors for the advancement of stomatology.

Author contributions

CM and JP were responsible for constructing the concept of the paper. JP and WZ contributed to the literature search and analysis. CM, RC, and LW were responsible for writing the manuscript draft. BL and YW participated in editing and finalizing the manuscript. All authors have agreed with the final version of the manuscript before submission. All authors read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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