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REVIEW

Extracellular Vesicles in Periodontitis: Pathogenic Mechanisms and Therapeutic Potential

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Abstract: Periodontitis is a prevalent yet frequently overlooked oral disease that is linked to a range of systemic conditions. Although basic treatment and periodontal surgery can alleviate the symptoms of periodontitis to a certain extent, the treatment of severe tissue defects or refractory cases is not effective. Extracellular vesicles (EVs) are subcellular lipid bilayer particles that come from a variety of sources and are prevalent in the biological fluids of vertebrates. They play a key role in intercellular communication by transporting multiple signaling molecules. Recent research has indicated that EVs derived from periodontal pathogens can trigger periodontitis, exacerbate the periodontal damage, and potentially disseminate to other parts of the body, leading to systemic conditions. Conversely, extracellular vesicles derived from dental stem cells (DSCs) have demonstrated the ability to regulate the local periodontal immune environment and foster the regeneration and repair of periodontal tissues, positioning them as a promising candidate for cell-free therapeutic approaches to periodontitis. This review aims to summarize the latest research on the involvement of EVs from different sources in the pathogenesis and treatment of periodontitis, especially to systematically elucidate the mechanism of EVs secreted by periodontal pathogens in periodontitis-related systemic diseases for the first time. By uncovering these complex regulatory processes, new and more effective therapeutic approaches can be explored in the battle against periodontitis and its associated systemic diseases.

Keywords: extracellular vesicles, outer membrane vesicles, dental stem cells, periodontitis, pathogenesis, therapy

Introduction

Periodontitis, the primary cause of tooth loss among adults, affects nearly 61.6% of the global population, with severe cases impacting roughly 23.6%.[1] This condition is not only confined to oral health but also has been linked to systemic illnesses such as diabetes mellitus, Alzheimer's disease, cardiovascular disease, and certain types of cancer.[2–4] Nonsurgical periodontal treatment (NSPT) is the basis of periodontitis treatment, mainly including supragingival scaling, subgingival scaling and root planing. Surgical treatment is a supplement to nonsurgical treatment, especially when periodontitis has advanced to a more severe stage. However, these conventional treatments are not effective in treating severe tissue defects or refractory cases. Moreover, given the limitations of the current study design, the potential beneficial effects of nonsurgical periodontal therapy on arterial stiffness in patients with periodontitis still need to be validated in clinical trials.[5] This strongly suggests the need to unlock new therapeutic method.

Extracellular vesicles (EVs) secreted by periodontal pathogens have been proved to be a key link in the occurrence and development of periodontitis and may also be a potential risk factor for related systemic diseases. Higher baseline levels of periodontal pathogens have been found to significantly interfere with the efficacy of one-stage full-mouth subgingival instrumentation for periodontitis.[6] In addition, research has also highlighted the promise of EVs in combating inflammation-related diseases, particularly periodontitis.[7] Compared with a single cell or bacteria, EVs can carry and deliver nucleic acids, proteins, lipids and other bioactive molecules for efficient information exchange between cells, thus

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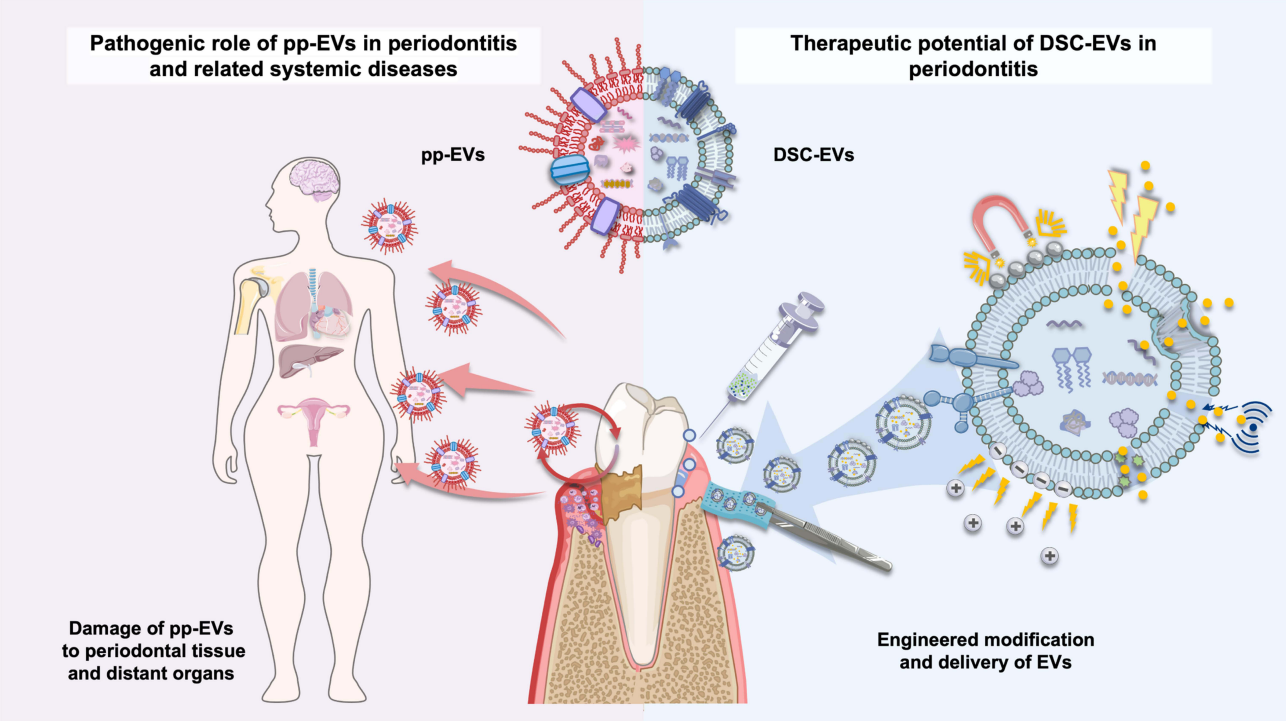
Graphical Abstract



playing a central role in a variety of physiological and pathological processes. In addition, EVs-mediated cell-free therapy effectively avoids the risk of immune rejection and tumor formation that may accompany stem cell transplantation.

Based on the above findings, this study was conducted by searching PubMed, Web of Science, Cochrane and Embase databases for all publications related to this topic up to October 2024, especially those published in the last 5 years. Non-English, non-availability of full text, duplication of publications, abstracts of meetings and policy papers were excluded. This review synthesizes current understanding of EVs' involvement in periodontitis, emphasizing their bifurcated roles. In particular, it is the first comprehensive summary of how EVs released by periodontal pathogens are involved in the pathogenesis of periodontitis-related systemic diseases. This study was aimed to fully explore the potential of EVs in periodontitis treatment by revealing the nuances of the dual role of EVs and refining preclinical approach to EVs application to compensate for the shortcomings of conventional treatments.

Overview of EVs

EVs, subcellular particles sized 30 nm to 1 μm and enclosed by a lipid bilayer, display a cup-shaped morphology under electron microscopy.[8] The membrane structure of EVs facilitates the carriage and delivery of various cellular components, including proteins, nucleic acids, and lipids. Surface-specific proteins or glycoproteins on EVs act as markers for EV type identification and parent cell tracing. Additionally, receptors or ligands on EVs engage with target cells, mediating binding and fusion.[9] Changes in the sugar or protein composition of EV membrane affect vesicle tropism and physiological properties. For example, exposure to the integrin CD47 on the surface of EVs can protect EVs from phagocytosis, thereby increasing the circulation time of EVs in the bloodstream.[10] In addition, the lipid membrane bilayer of EVs allows a variety of hydrophobic therapeutic ingredients to be incorporated to improve the stability and efficacy of these drugs.[11] Therefore, taking full advantage of membrane modules for modification is an innovative way to design EVs. Moreover, the content of different cargos (such as proteins, transcription factors, nucleic acids, etc) in EVs

will vary depending on the parental cell type, physiological conditions, and biogenesis.[12] As paracrine messengers, EVs transport signaling molecules, thereby regulating a myriad of cellular pathways either in the vicinity or at a distance.

Bacterial-Derived EVs

The majority of EVs released by bacteria, especially Gram-negative bacteria, are classified as bacterial outer membrane vesicles (OMVs).[13] These vesicles serve as a crucial conduit for interactions within microbial communities and between microbes and their hosts. Unattached subgingival plaque is the key initiating factor of periodontitis, which is rich in gram-negative bacteria. Research has demonstrated that OMVs derived from periodontal pathogens (hereinafter referred to as pp-EVs) play a significant role in the establishment of bacterial biofilms.[14] Upon interaction with pathogen-associated molecular patterns (PAMPs) present on pp-EVs, pattern recognition receptors (PRRs) on the host's immune cells can trigger inflammatory responses and initiate EV-mediated adaptive immune responses.[15] Kim et al suggested that pp-EVs accelerate bone loss through inhibiting bone formation and increasing bone resorption.[16] In addition, pp-EVs can restrain the proliferation and growth of fibroblasts, resulting in chronic periodontitis.[17]

Dental Stem Cell-Derived EVs

Beyond the pathological impact of pp-EVs on periodontal tissues, EVs released by dental stem cells (hereinafter referred to as DSC-EVs) are instrumental in periodontitis therapy. They achieve this by modulating the immune microenvironment and fostering the regeneration of periodontal tissues.[18,19] To date, seven distinct types of DSCs have been identified and isolated, encompassing tooth germ stem cells (TGSCs), dental follicle stem cells (DFSCs), stem cells from the apical papilla (SCAPs), dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), stem cells from exfoliated deciduous teeth (SHEDs), and gingival mesenchymal stem cells (GMSCs).[20] Hu et al found that GMSC-derived EVs (hereinafter referred to as GMSC-EVs) effectively cross-regulate NF-κB and Wnt/β-catenin signaling pathways to promote osteogenic differentiation of PDLSCs in inflammatory environment.[21] In a rat model of periodontal defect, EVs derived from DFSCs have been shown significant periodontal regeneration potential.[22] On one side of the spectrum, DSC-EVs have been found to stimulate neovascularization and encourage the osteogenic differentiation of stem cells, which in turn mitigates the loss of alveolar bone.[23,24] Conversely, DSC-EVs also bolster the reparative capacity of compromised periodontal tissues by augmenting the proliferation, migration, and collagen production of periodontal cells (Figure 1).[25]

Hence, EVs originating from various sources may exhibit distinct roles in the pathogenesis and treatment of periodontitis. The revelation of these related regulatory mechanisms may provide new strategies to tackle periodontitis.

Pathogenesis of pp-EVs in Periodontitis and Related Systemic Diseases

pp-EVs instigate inflammation and tissue destruction in periodontitis by breaching the oral epithelial barrier and perturbing the periodontal immune microenvironment. The possible pathogenesis of pp-EVs in periodontitis has been adequately described in Cai and Huang's review and will not be repeated here.[26,27] Emerging data from both epidemiological and experimental studies have demonstrated a connection between periodontitis and multiple systemic diseases, including diabetes mellitus, Alzheimer's disease, cardiovascular disease, and osteoporosis. And the presence of periodontitis is likely to affect the prognosis and outcome of these diseases. The bacterium *Porphyromonas gingivalis (P. gingivalis)*, a key player in periodontitis, has been identified not just in the oral lesion sites but also in distant organs such as the brain, cardiovascular system, liver and fallopian tube-ovaries.[28] Beyond its nanoscale dimensions and robust structural integrity, OMVs also boasts exceptional environmental adaptability and the ability to evade immune detection. These traits facilitate its ability to surpass the localized regions of its parent bacteria, thereby enhancing its invasiveness.[29] Moreover, the relatively high concentration and stable composition of pathogenic agents in OMVs enable them to establish bacteria-host dialogue and instigate disease processes even when live bacteria are not present. Hence, pp-EVs are potentially a significant nexus connecting periodontitis with its associated systemic diseases. Through in-depth understanding of the mechanisms involved, potential therapeutic intervention targets can be found in the treatment of periodontitis-related systemic diseases.

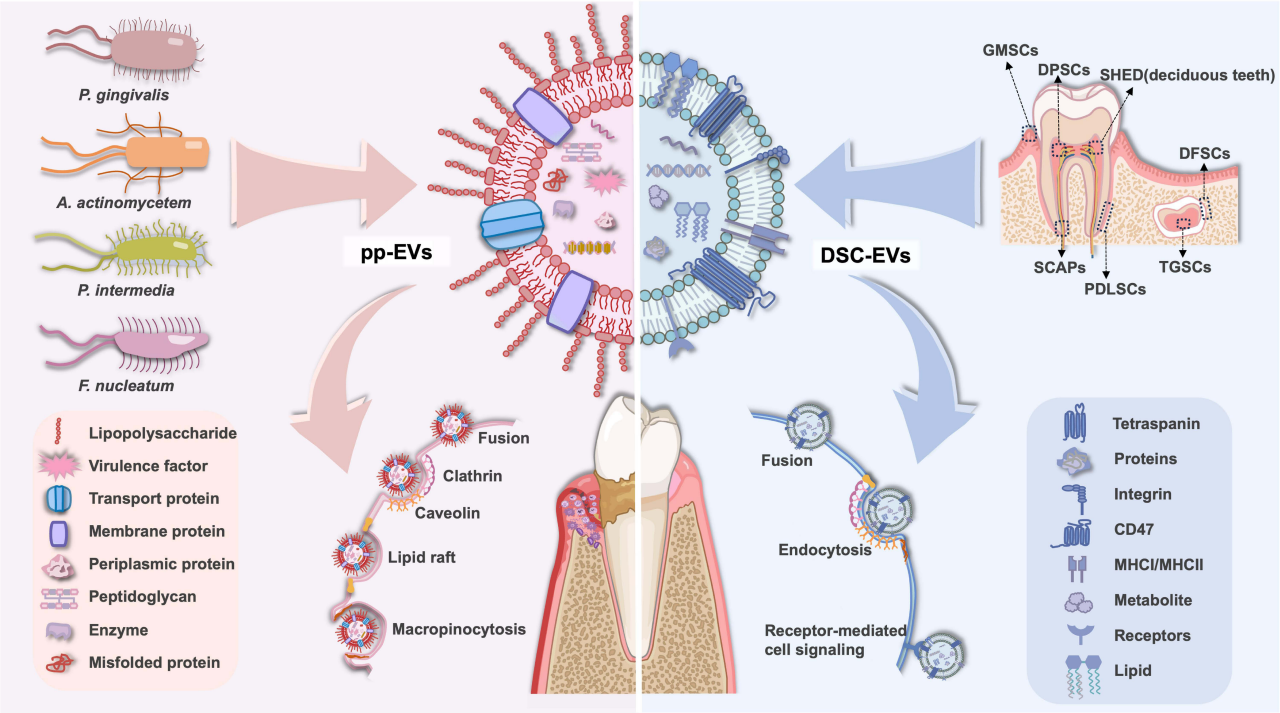


Figure 1 Duality of EVs in periodontitis. The pink area shows common periodontal pathogens and the pathways by which EVs secreted by them enter non-phagocytic host cells. In contrast, the blue region highlights common dental stem cells, illustrating how the EVs they produce are taken up by recipient cells.
Abbreviations: *P. gingivalis, Porphyromonas gingivalis; A. actinomycetemcomitans, Actinobacillus actinomycetemcomitans; P. intermedia, Prevotella intermedia; F. nucleatum, Fusobacterium nucleatum.*

pp-EVs and Diabetes Mellitus

Diabetes mellitus is caused by defects in insulin secretion or activity. Periodontitis and diabetes mellitus have a bidirectional association, which can mutually promote their prevalence and severity.[30] The mechanisms by which diabetes mellitus facilitates periodontitis have been comprehensively described by Zhao et al, including microbiome factors, host immune factors, and oxidative stress.[31] Meanwhile, several studies have shown that *P. gingivalis* may play a major role in the development of type 2 diabetes mellitus by inducing insulin resistance. For example, *P. gingivalis*-induced endotoxemia can exacerbate nonalcoholic fatty liver disease by increasing insulin resistance and inhibiting glucose metabolism.[32] Seyama et al reported that gingipains, an important virulence factor secreted by *P. gingivalis*-derived OMVs (hereinafter referred to as *Pg*-EVs), can inhibit glycogen synthesis in liver cells by targeting the Akt/GSK-3β signaling pathway and thus maintain a high level of blood sugar in experimental mice.[33] Moreover, in an experiment by Huang, *Pg*-EVs were shown to release arginine gingival proteinase (Rgp) into the aqueous humor when injected into diabetic mice via venules. This process increased the level of oxidative stress in retinal microvascular endothelial cells and lead to mitochondrial dysfunction, thereby aggravating retinopathy in diabetic mice.[34]

pp-EVs and Alzheimer's Disease

Alzheimer's disease is a prevalent neurodegenerative condition in the elderly. Evidence suggests that periodontal pathogens and their virulence factors can cross the blood-brain barrier (BBB), facilitate the penetration of related pathogenic factors and cause pathological changes similar to those observed in Alzheimer's disease.[35] Nonaka found that *Pg*-EVs delivered gingipain to cerebral microvascular endothelial cells through the blood circulation and degrades tight junction proteins including Zonula occludens-1 (ZO-1) and occludin. Such pathological changes destroyed the BBB and promoted *P. gingivalis* and its virulence factors to infiltrate into the brain parenchyma.[36] Consistent with these findings, Elashiry et al demonstrated for the first time in a mouse model that *Pg*-EVs can metastasize and cross the BBB to participate in the pathogenesis of AD.[37] *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*) is an

equally essential periodontal pathogen, and *A. actinomycetemcomitans*-derived OMVs (*Aa*-EVs) can also penetrate the BBB and invade the brain.[38] Choi and his collaborators indicated that *Aa*-EVs provoke neuroinflammation by delivering extracellular RNA (exRNA) to activate the proinflammatory factors IL-6 and NF-κB in brain monocyte/microglia.[39]

pp-EVs and Cardiovascular Disease

Disruption of intercellular junctions between endothelial cells impairs the normal function and integrity of the endothelial barrier, which further exacerbates vascular failure and cardiovascular disease.[40] To determine whether pp-EVs can mediate endothelial dysfunction, Farrugia et al extracted *Pg*-EVs and infected human umbilical vein endothelial cells (HUVECs). Interestingly, the gingipains in pp-EVs suppress the expression of platelet-endothelial cell adhesion molecule 1 (PECAM-1) on the cell surface and loosen intercellular contact, thereby increasing the permeability of endothelial cells.[41] Moreover, Yue and cooperator reported that Rho kinase (ROCK) mediates pp-EV-induced endothelial nitric oxide synthase (eNOS) suppression through ERK1/2 and p38 MAPK, thereby destroying vascular integrity and homeostasis.[42] In addition, pp-EVs can also elicit cardiovascular disease by facilitating the formation of atherosclerosis. *Pg*-EVs upregulate the expression of Runt-related transcription factor-2 (RUNX2) by rapidly activating ERK1/2 in vascular smooth muscle cells (VSMCs), which is conducive to cell calcification and atherosclerosis.[43]

pp-EVs and Other Diseases

Currently, the significance of pp-EVs in other systemic diseases has been gradually emphasized. For instance, He et al proposed that *Pg*-EVs destroy the function of the lung epithelial barrier and inflict inflammatory lung diseases.[44] EVs derived from *P. gingivalis* or *Filifactor alocis (F. alocis)* are concentrated in long bones after intraperitoneal administration and subsequently activate osteoclast precursors to yield transcription factors, ultimately leading to osteoporosis.[45,46] It can be seen that pp-EVs may travel throughout the body by blood circulation and trigger or aggravate pathological changes in the distal organs by carrying multiple bioactive molecules. This realization underscores that a single treatment may not be sufficient to address the complexity of systemic diseases associated with periodontitis. When devising treatment strategies for patients grappling with periodontitis alongside associated systemic conditions, it is essential to integrate a multifaceted approach that encompasses periodontal therapy. The pathogenesis of pp-EVs in periodontitis related systemic diseases are summarized in Figure 2 and Table 1.

Restoration Mechanisms of DSC-EVs in Periodontitis Therapy

Traditional stem cell therapy excels at facilitating tissue repair, regeneration, and addressing stubborn medical conditions. Nonetheless, it carries inherent risks, including unpredictable cell differentiation, procedural complexity, and safety concerns. In this context, EV-mediated cell-free therapy stands out for its ability to mimic parental cell functions more effectively. It is characterized by heightened safety, enhanced tissue penetration, accurate targeting, and a multiplicity of functions. These attributes render EVs a compelling option for therapeutic interventions in periodontitis.[52,53] Figure 3 shows the restoration mechanisms of DSC-EVs in periodontitis therapy.

The Role of microRNAs in DSC-EVs

MicroRNAs (miRNAs) in EVs are essential for cell communication and signal transduction, affecting physiological and pathological processes. In cell-free therapies, DSC-derived EVs are key vectors for miRNA delivery and precise cell function regulation. Liu et al first identified miRNAs in PDLSC-derived EVs that promote osteogenesis in recipient cells, shedding light on miRNA regulatory mechanisms in DSC-EVs during this process.[54] Similarly, Shen et al suggested that miR-1246 enrichment in DPSC-EVs protects experimental mice from alveolar bone loss, while antiagomiR-1246 reverses these effects.[55] To dissect the regulatory effects of miRNAs in EVs on angiogenesis, Pizzicannella analyzed differentially expressed miRNAs in GMSC-EVs. These authors suggested that GMSC-EVs enhance the expression of VEGF, OPN, and RUNX2 in a rat skull defect model by delivering miR-2861 and miR-210.[56] Furthermore, periodontal cell senescence and impaired multifunctional differentiation caused by oxidative stress are not conducive to the repair and regeneration of periodontal defects. Accordingly, delaying cell senescence may be an emerging alternative for

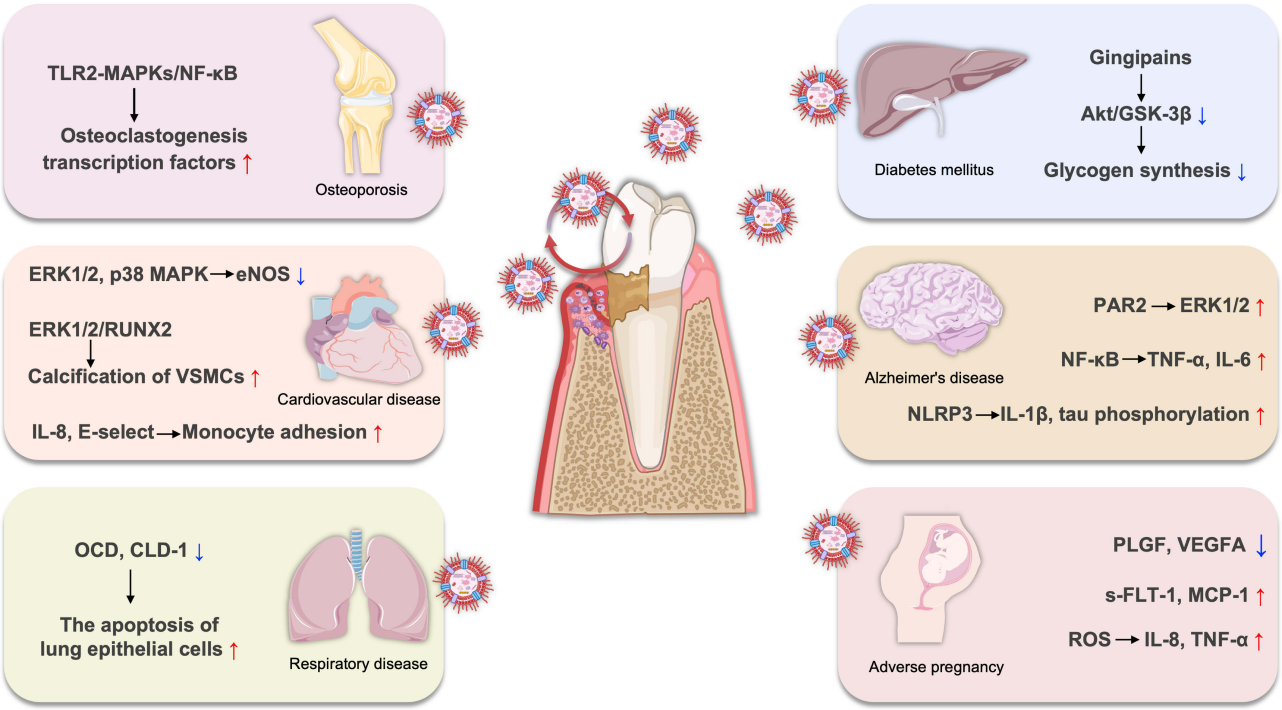


Figure 2 Promotional roles of pp-EVs in periodontitis related systemic diseases. pp-EVs not only affect local periodontitis lesions but also delivery to distal organs via the bloodstream.

Abbreviations: eNOS, endothelial nitric oxide synthase; VSMCs, vascular smooth muscle cells; PAR2, protease-activated receptor 2; NLRP3, NOD-like receptor thermal protein domain associated protein 3; OCD, occludin; CLD-1, claudins-1; VEGFA, vascular endothelial growth factor A; PLGF, placental growth factor; sFLT-1, soluble fms-like tyrosine kinase-1; MCP-1, monocyte chemoattractant protein-1; ↑, upregulation; ↓, downregulation.

elucidating the intrinsic repair mechanism of periodontal tissue.[57] Mas-Bargues found that high oxygen tension causes DPSC senescence, but EV-derived miR-302b can delay this and restore DPSC pluripotency.[58]

In fact, in addition to miRNAs, there are other nucleic acid components in EVs, such as mRNAs, circRNAs, and lncRNAs. Xie et al demonstrated that elevated circLPAR1 in DPSC-EVs, by competitive binding to hsa-miR-31, enhances SATB2 expression and osteogenic differentiation in recipient cell.[59] Furthermore, SHED-derived EVs (SHED-

Table 1 The Pathogenic Role of pp-EVs in Systemic Diseases

Disease Type	EVs Source	Recipient Cells	Cargo	Primary Effect and Related Mechanism	Ref
Diabetes mellitus	*Pg*	HepG2 cells	Gingipains	Promote diabetes mellitus via inhibiting the Akt/GSK-3β pathway in hepatocytes	[33]
	Pg	HRMECs	–	Aggravate diabetic retinopathy by activating PAR-2	[34]
Alzheimer's Disease	*Pg*	HMC3 cells	Gingipains	Induce neuroinflammation via increasing proinflammatory cytokines	[47]
	Pg	BV2 microglia cells	exRNA	Induce neuroinflammation through activating NF-κB pathway	[39]
	Pg	hCMEC/D3	Gingipains	Disrupt the BBB via degradation ZO-1 and occludin	[36]
	Pg	HBMEC	Gingipains, LPS	Disrupt the BBB via degradation ZO-1	[37]
	Aa	U937 cells	exRNA	Induce neuroinflammation through TLR-8 and NF-κB pathway	[48]

(*Continued*)

Table 1 (Continued).

Disease Type	EVs Source	Recipient Cells	Cargo	Primary Effect and Related Mechanism	Ref
Cardiovascular disease	*Pg*	HUVECs	–	Suppress eNOS production and endothelial cell function via ERK1/2 and p38 MAPK pathways	[42]
	Pg	HMEC-1	Gingipains	Increase endothelial cell permeability by degradating recombinant PECAM-1	[41]
	Pg	VSMC	–	Induce RUNX2 expression and VSMC calcification through ERK pathway	[43]
	Pg	HUVEC	–	Promote monocyte adhesion by elevating IL-8 and E-selectin expression	[49]
	Pg	J774.A1 cell line	LPS	Promote the formation of foam cells by inducing the binding of LDL to macrophages	[50]
Respiratory disease	*Pg*	A549 cells	–	Destruct the barrier system via activating caspase-3 and degradating PARP	[44]
Osteoporosis	*Fa*	BMMs	–	Induce osteoclast differentiation and bone resorption via activating the TLR2-MAPKs/NF-κB pathway	[45]
Adverse pregnancy	*Pg*	HTR-8 cell line	–	Disrupt placental homeostasis by disrupting trophoblast for vascular transformation and immune homeostasis maintenance	[51]

Notes: –, unknown.
Abbreviations: *Pg, P. gingivalis; Aa, A. actinomycetemcomitans; Fa, F. alocis*.

EVs) enhanced glutamate metabolism and oxidative phosphorylation activity in DPSCs through delivery of the mRNA encoding mitochondrial transcription Factor A (TFAM), which was conducive to bone regeneration in DPSCs.[60]

The Role of Proteins in DSC-EVs

Besides miRNAs, proteins are also essential constituents of EVs, facilitating intercellular communication and the transduction of signals through these vesicular shuttles. Furthermore, cargo proteins can be used as biomarkers for disease diagnosis and prognosis assessment. Transcriptome sequencing has revealed that GMSCs participate in regulating the processes of bone homeostasis and angiogenesis by secreting TGF-β, BMPs, VEGF and so on.[61] Luo et al found that

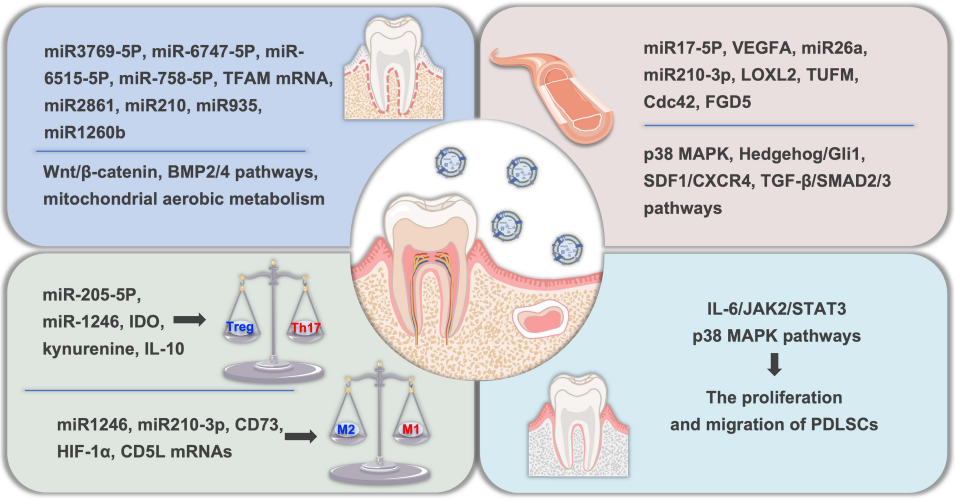


Figure 3 Inhibitory roles of DSC-EVs in periodontitis. DSC-EVs inhibit the development of periodontitis and promote periodontal tissue regeneration by promoting osteogenesis (upper left area), promoting angiogenesis (upper right area), regulating the immune microenvironment (lower left area), and improving soft tissue repair (lower right area).
Abbreviations: LOXL2, lysyl oxidase like 2; Cdc42, cell division cycle 42; IDO, indoleamine 2,3-dioxygenase.

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SHEDs-EVs can mobilize naïve bone marrow mesenchymal stromal cells (BMSCs) through secreting multiple growth factors, thereby promoting bone and periodontal tissue regeneration.[62] Zhang et al reported a significant increase in VEGFA expression in inflamed PDLSCs, resulting in enhanced angiogenesis in HUVECs when co-cultured with inflamed PDLSCs.[63] Given the above findings, EVs play a remarkable role in the treatment of periodontitis by delivering proteins to recipient cells.

Key Pathways Mediated by DSC-EVs

Although the types of molecules transported by EVs are distinctive in various cell types or in diverse growth microenvironments, key pathways in recipient cells are always mobilized by this delivery. Over the years, the pathways affected by DSC-EVs in the treatment of periodontitis have been extensively and intensively studied.

Mitogen-Activated Protein Kinase (MAPK) Pathway

MAPK pathway is a critical bridge connecting extracellular signals with intracellular responses. Co-culturing PDLSCs with DFSCs enhances PDLSC proliferation and osteogenic differentiation via the p38 MAPK pathway, and this effect is confirmed in periodontal tissue regeneration in SD rats.[64] Jin et al confirmed that DPSC-EVs promoted the migration and mineralization of adipose derived stem cells (ADSCs) by activating the MAPK pathway, and significantly improved the regeneration of mandibular defects in rats.[65] Similarly, Zhao et al extracted EVs from PDLSCs to incubate BMSCs and discovered that these cells could expedite cell proliferation and migration by increasing the phosphorylation of AKT and ERK1/2, subsequently accelerating bone tissue repair.[66] Furthermore, DPSC-EVs were shown to promotes migration, proliferation and capillary formation of HUVECs by upregulating Cdc42/p38 MAPK signaling pathway.[67]

Wnt Pathway

The Wnt signaling pathway is recognized for its multifaceted role in periodontal biology. It not only stimulates the proliferation and differentiation of periodontal cells but also governs the cementum formation and development. Furthermore, it exerts a regulatory influence on the dynamics of periodontal tissue destruction and regeneration. It has been reported that GMSC-EVs improve the regenerative potential of PDLSCs in the inflammatory microenvironment by inhibiting NF-κB signaling and Wnt5a expression.[68] Qian et al revealed that EVs extracted from curcumin-treated PDLSCs had an enhanced pro-osteogenic ability, which was due to upregulated p-GSK3β and β-catenin protein levels in the recipient cells.[69] Consistently, after TNF-α pretreatment, miR-1260b in GMSC-EVs downregulated osteoclastogenic activity of periodontal ligament cells by inhibiting the Wnt5a/RANKL pathway.[70] These results provide valuable new ideas for the treatment of periodontitis.

Janus Kinase 2/Signal Transducer and Activator of Transcription 3 (JAK2/STAT3) Pathway

JAK2/STAT3 pathway is involved in the initiation and progression of inflammatory responses and immune responses in diverse pathological processes. For instance, DPSC-EVs effectively downregulated the expression of the IL-6, p-JAK2, and p-STAT3 proteins in LPS-treated PDLSCs. These findings suggested that the JAK2/STAT3 pathway may be involved in regulating the anti-inflammatory and osteogenic effects of DPSC-EVs on PDLSCs.[71] Moreover, recent studies have focused on the role of STAT3 in the regulation of autophagy. Xie et al processed macrophages with SHED-EVs and discovered that STAT3 pathway-related proteins were activated, subsequently promoting the expression of the autophagy-related protein LC3.[72]

Other Signaling Pathway

M1 and M2 macrophage polarization imbalance is key in periodontitis progression. LPS-stimulated PDLSCs secrete EVs that enhance M1 polarization via TLR2/TLR4/NF-κB,[73] while GMSC-EVs promote M2 polarization through HIF-1α/mTOR after TNF-α and IFN-α stimulation.[74] Hypoxic preconditioning has been reported to markedly increase EV release in DPSCs and promote the polarization of M2 macrophages, thus ameliorating LPS-induced inflammatory osteolysis.[75] Table 2 summarizes the restoration mechanism of DSC-EVs in periodontitis therapy and tissue regeneration.

Table 2 Restoration Mechanisms of DSC-EVs in Periodontitis Therapy and Tissue Regeneration

Cargo Type	EVs Source	Recipient Cells	Cargo/Pathway	Primary Effect	Ref
miRNA	PDLSCs	PDLSCs	miR-141-3p	Alleviate PDLSCs senescence induced by hyperglucose	[76]
	SHEDs	PDLSCs	miR-92a-3p	Promote proliferation and osteogenic differentiation, but inhibit apoptosis and inflammation	[77]
	DPSCs	BMDM	miR-1246	Promote M2 macrophage polarization	[55]
	DPSCs	DPSCs	miR-302b	Delay the aging of DPSCs and restore cellular pluripotency	[58]
	DPSCs	Naïve CD4 T cells	miR-1246	Restore Th17/Treg balance	[78]
	DPSCs	PDLSCs	miR-758-5p	Promote osteogenic and odontogenic differentiation	[79]
	GMSCs	GMSCs	miR-2861, miR-210	Promote osteoangiogenesis	[56]
	PDLSCs	HUVECs	VEGFA	Promote angiogenesis	[63]
	PDLSCs	PDLSCs	BMP2/4, VEGFA, VEGFR2	Promote osteogenesis and angiogenesis	[80,81]
	SHEDs	BMSCs	TGF-β 1, PDGF, IGF-1, FGF-2	Promote Naïve BMSCs migration	[62]
	SCAPs	HUVECs	Cdc42	Promote angiogenesis	[82]
Pathway	PDLSCs	BMSCs	AKT and ERK1/2 pathway	Enhance BMSC migration	[66]
	PDLSCs	$CD4^+$ T Cells	ERK pathway	Inhibit T cell proliferation but promote Treg cell differentiation	[83]
	SHEDs	THP-1 cells	AKT, ERK1/2 and STAT3 pathway	Induce autophagy in macrophages	[72]
	SHEDs	HUVECs	TGF-β/SMAD2/3 pathway	Promote angiogenesis	[84]
	DPSCs	ADSCs	MAPK pathway	Promote osteogenic differentiation	[65]
	DPSCs	PDLSCs	IL-6/JAK2/STAT3 pathway	Inhibit inflammation	[71]
	DPSCs	RAW264.7 macrophages	NF-κB1 pathway	Induce M2 macrophage polarization but inhibit osteoclastogenesis	[75]
	GMSCs	PDLSCs	NF-κB and Wnt5a pathway	Suppress inflammation	[68]
	GMSCs	PDLCs	Wnt5a/RANKL pathway	Inhibit osteoclastogenesis	[70]
	DFSCs	PDLSCs	p38 MAPK pathway	Promote PDLSCs proliferation	[64]
	DFSCs	PDLSCs, RAW264.7 macrophages	ROS/MAPK pathway	Enhance antioxidant effects and M2 polarization	[85]
circRNA	DPSCs	DPSCs	circLPAR1	Promote osteogenic differentiation	[59]

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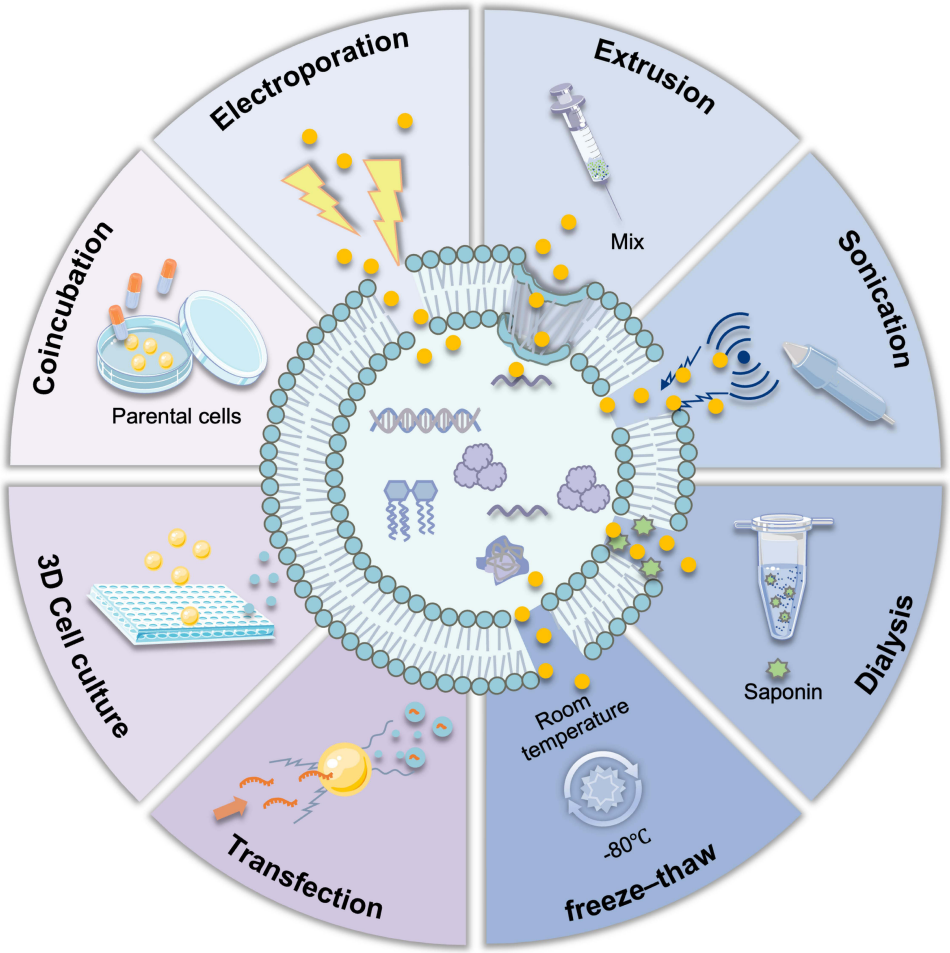

Figure 4 Cargo loading methods for EVs. Based on coincubation, physicochemical direct loading methods such as surfactant treatment, sonication, electroporation, extrusion, freeze–thaw cycling and dialysis have been developed to improve cargo loading efficiency.

Application Strategy of EVs in the Prevention and Treatment of Periodontitis

Taking Full Advantage of pp-EVs for Vaccine Development

OMVs mimic parental bacteria's immunogenicity, elicit host adaptive responses, and are highly stable for <1 year at low temperatures.[86] In 2016, the Food and Drug Administration (FDA) successfully approved an OMV-based vaccine, namely, the meningitis serotype B vaccine BEXSERO (GlaxoSmithKline, London, UK), demonstrating the viability of OMVs as a vaccine or drug delivery platform.[87] Local combination of *Pg*-EVs and TLR3 agonist poly(I:C) on the nasal mucosa has been reported to effectively trigger a specific antibody response significantly reducing *P. gingivalis* levels in mice, with safety confirmed in a trial by Nakao.[88] These findings confirm pp-EVs' feasibility as a safe, efficient intranasal vaccine for periodontitis. While all Gram-negative bacteria produce OMVs with varying yields and cargos, even under different environmental conditions, thorough evaluation is essential before pp-EVs can be developed into vaccines. Maximizing the immunogenicity of pp-EVs is crucial while ensuring safety and tolerability.

Functional Modification Strategies for EVs Before Clinical Application

Preclinical studies indicate MSC-EVs' potential in treating inflammation and promoting tissue regeneration. However, the successful clinical application of EVs still faces certain limitations, such as low yield, rapid clearance, unsatisfactory targeting ability, and uncertain loading efficiency.[89] In this regard, Yang et al[8] summarized the emerging technologies for EVs isolation, engineering, and delivery systems (eg, heterogeneous hydrogels, microneedle patches and micro-

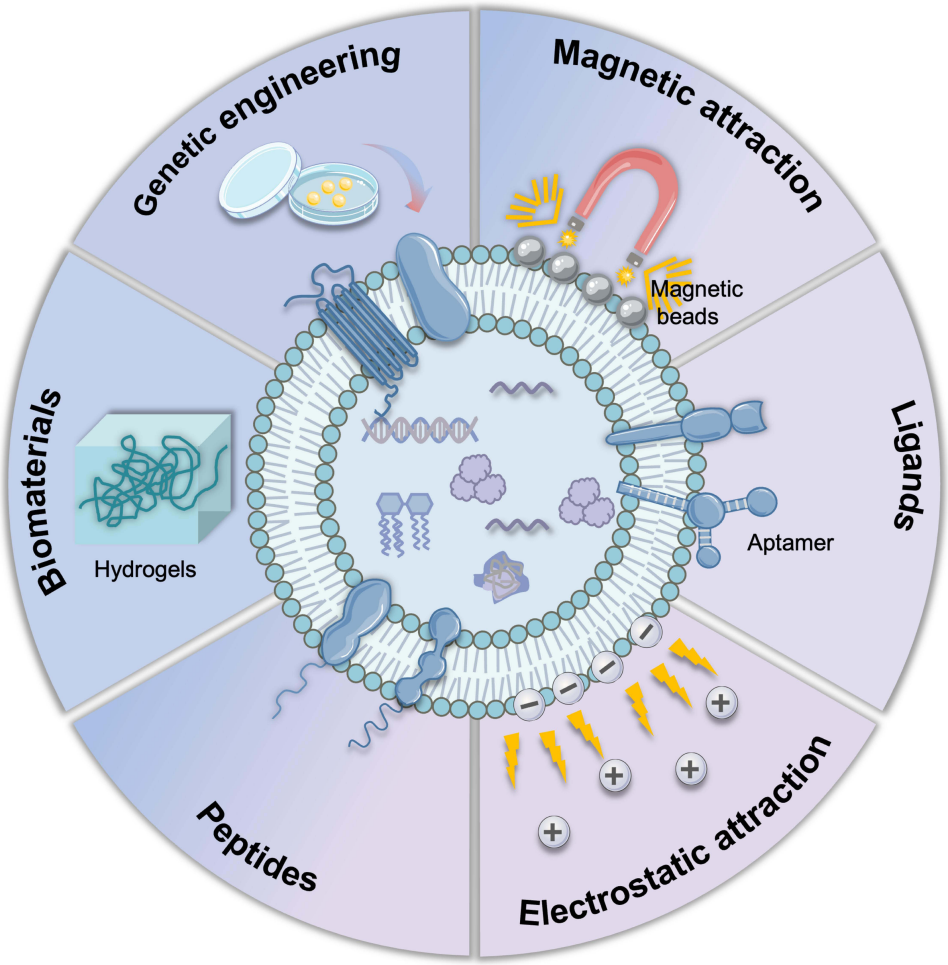

Figure 5 Strategies for targeting EVs. Methods to increase the affinity of EVs for specific sites include genetic engineering, integration of ligands or peptides on the EVs' surface or resorting to biomaterials and electrostatic and magnetic attraction.

nanoparticles). Notably, the autonomous mobility of emerging nanomotor technology is expected to be used in the future to enhance EVs delivery efficiency and targeting.

The most common cargo loading method involves preprocessing parental cells by optimizing culture, co-incubating with therapeutics, and transfecting with RNA, peptides, and proteins. For instance, hypoxia preconditioning stimulated SHED-EVs' potential in angiogenesis and osteogenesis by boosting EVs secretion, VEGF signaling and thyroid hormone synthesis.[90] Xu et al demonstrated that PDLSCs engineered with the P2X7R gene release EVs that enhance osteogenic differentiation in inflammation via differential miRNA expression.[91] Although the abovementioned three loading methods are relatively simple, the loading efficiency and drug toxicity on EV secretion cannot be controlled. Researchers suggest either direct co-incubation of therapeutic agents with EVs or temporary membrane disruption using physicochemical methods for drug loading (Figure 4). After loading BMP2 into EVs via electroporation or sonication, Yerneni et al reported that the loading efficiency of sonication increased by more than 3-fold.[92] Physicochemical methods can enhance cargo loading efficiency in EVs, but they must be carefully optimized to avoid damaging or contaminating the EVs.

Additionally, EVs can be equipped with targeting ligands like antibodies, peptides, or proteins to enhance their ability to recognize and bind specific targets. For instance, CXCR4-overexpressing EVs boost macrophage targeting and M2 macrophage polarization by delivering miR-126.[93] EV targeting can also be enhanced by their surface negative potential for electrostatic attraction or by conjugating them with iron oxide nanoparticles for magnetic attraction.[94,95] Additionally, combining EVs with biomaterials improves targeted drug delivery, reduces EV clearance by the immune system, and enables local sustained release.[96] (Figure 5).

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Conclusions and Future Perspective

Rising as vital mediators of intercellular communication, EVs have been identified to influence the progression of periodontitis from multiple perspectives and seemingly playing a Janus-faced role in regulating the fate of periodontitis. Therefore, giving full play to the protective effect of EVs on periodontal tissue, such as the development of pp-EV vaccine and engineering EVs, may play an unexpected role in periodontitis prevention and periodontal regeneration treatment. Reasonably combined with conventional periodontal therapy, EVs may effectively improve quality of life in patients with periodontitis. Although EVs have shown great potential in disease diagnosis and treatment, the negative outcomes that may accompany their preclinical application have been less reported. In view of the current difficulties in the preparation of therapeutic EVs, it is necessary to further explore the safety risks caused by methodological bottlenecks. In addition, with the further study of EV-mediated systemic interactions and the continuous overcoming of methodological bottleneck, the clinical application prospect of treating periodontitis and related systemic diseases by EVs will be brighter.

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Disclosure

The authors report no conflicts of interest in this work.

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