EXOSOMES: A BRIDGE OF PERIODONTITIS AND SYSTEMIC DISEASES

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Abstract

Periodontitis, a common oral disease, is featured with complex etiology, progressive and prognosis varies according to the severity of periodontitis. Exosomes belong to a kind of cystic vesicles with biological activity, which widely exist in human body fluids. Exosomes play an irreparable role in signal transmission and material exchange between cells, maintaining cell functions, and regulating body immunity and homeostasis. Exosomes are closely related to periodontitis, and recent study of exosomes has provided new directions and ideas for the diagnosis and treatment of periodontitis. Similarly, as extracellular vesicles, exosomes play a bridging role between periodontitis and some systemic diseases. In this process, exosomes participate in and regulate the process of systemic diseases by carrying nucleic acids, proteins, lipids, etc., and exhibit different bioactive effects according to the different substances carried in exosomes. In this paper, we summarize the latest research progress of exosomes, especially in the periodontitis and some systemic diseases, and review the potential value of exosomes in periodontitis diagnosis and treatments.

Keywords: Periodontitis; Exosomes; Periodontal regeneration; Osteoporosis; Kidney disease

1. Introduction

Periodontitis is an infectious disease that occurs in the supporting tissues of teeth and can result in pathological loss of periodontal ligament and absorption of alveolar bone1,2. The occurrence and development of periodontitis are quite complex, and the pathogenic mechanisms vary from patient to patient. The pathogenesis of periodontitis is now mainly recognized as deregulated inflammatory interactions between periodontal pathogens and host immune system, involving both innate and adaptive responses, which lead to a chronic inflammation in periodontal tissues3,4.

In recent years, the connections between periodontal diseases and systemic conditions have been explored, revealing that periodontal diseases can influence the...
risk of systemic conditions, including osteoporosis\textsuperscript{[5,6]}, kidney diseases\textsuperscript{[7,8]}, Alzheimer’s disease (AD)\textsuperscript{[9,10]}, stroke\textsuperscript{[11]}, and cardiovascular diseases\textsuperscript{[12,13]}. The periodontitis insult and/or the associated pro-inflammatory cascade could contribute to the pathogenesis of these systemic diseases.

Chronic inflammation is driven by various mediators, of which an important part is attributed to the interactions within cytokine networks. Among pro-inflammatory cytokines, interleukin (IL)-1\(\alpha\), IL-1\(\beta\), tumor necrosis factor alpha (TNF-\(\alpha\)), IL-6 and IL-17 accelerate acute and chronic inflammation, and tissue injuries; however, other cytokines have antagonist effects which can restrain inflammation reactions, such as IL-10\textsuperscript{[14]}.

Extracellular vesicles are membranous vesicles that can naturally be released by most cells, which are induced by differentiation, activation, senescence, transformation, etc.\textsuperscript{[15]} Extracellular vesicles can be divided into exosomes and ectosomes categories based on their sizes. The former is 50–150 nm and the latter is 100–500 nm, and they also have different methods of assembly, composition, and release as well as different regulation mechanisms\textsuperscript{[16,17]}. Exosomes act as communication mediators between cells, and according to recent studies, they can even be used as biomarkers for diagnosis and prognosis of diseases\textsuperscript{[18-20]}.

Local injection of gingival mesenchymal stem cells (GMSC)-derived exosomes significantly reduced periodontal bone resorption\textsuperscript{[21]}. Li et al. revealed that exosomal miR-207 alleviated symptoms of depression in stressed mice by targeting Tril to inhibit nuclear factor kappa B (NF-\(\kappa\)B) signaling in astrocytes\textsuperscript{[22]}. Fotuhi et al. compared the levels of long non-coding RNA BACE1-AS levels in plasma and plasma-derived exosomes between AD and healthy people, and found that plasma BACE1-AS level may serve as a potent blood-based biomarker for AD\textsuperscript{[23]}. In this paper, we mainly review the production, composition, and biological function of exosomes, and summarize the research of exosomes in periodontitis and its bridging role between periodontitis and systemic diseases\textsuperscript{[24]}.

2. Pathogenesis of periodontitis

Periodontitis is a kind of chronic inflammation in periodontal tissue, mainly manifested as gingival redness and swelling, periodontal pocket abscess and tooth loosening\textsuperscript{[20]}. The occurrence of periodontitis can be induced by multiple factors, including formation of dental plaque biofilm, defect in neutrophil defense, defect in phagocyte function, immune deficiency, and high level of inflammatory factors\textsuperscript{[26]} (Figure 1).

Figure 1. Pathogenesis of periodontitis. In the progression of periodontitis, pathogens can directly or indirectly cause periodontal inflammation by producing bacterial enzymes or lipopolysaccharide (LPS), respectively. LPS can stimulate epithelial cells (ECs) to produce exosomes containing prostaglandin E2 (PGE2), resulting in chemotaxis, aggregation and transformation of inflammatory cells, subsequently exacerbating inflammation. Meanwhile, LPS can also act on periodontal cells to produce exosomes containing special signals, which can activate and regulate the signal pathway of immune cells to generate new cytokines and aggravate the development of periodontal inflammation. Abbreviations in the image: ECs: epithelial cells; G: Gram-negative bacteria; LPS: lipopolysaccharide; miR-155-5p: microRNA-155-5p; PDLSCs: periodontal ligament stem cell; PGE2: prostaglandin E2; TNF-\(\alpha\): tumor necrosis factor alpha; TNF-\(\beta\): tumor necrosis factor beta.

2.1. Dental plaque is the initial factor of periodontitis

As the initial factor of periodontitis, plaque biofilm can cause tissue damage and lesions, which is not only related to the virulence and quantity of bacteria, but also the host’s defense ability\textsuperscript{[27]}. The pathogenic microorganisms of chronic periodontitis mainly including Porphyromonas gingivalis, Prevotella nigrescens, Treponema denticola, Prevotella intermedia, Fusobacterium nucleatum, etc.\textsuperscript{[28]} Their pathogenicity is mainly related to lipopolysaccharide (LPS), bacterial enzymes, capsule, cilia and also extracellular vesicles. Recent studies have shown that endotoxin contents are positively correlated to clinical symptoms and alveolar bone absorption\textsuperscript{[29]}. For example, the main pathogenicity of P. gingivalis is related to gingival protease, LPS, indole and organic acids. LPS, a component of cell wall, can directly activate host’s innate immune system and induce a series of inflammatory reactions, which contribute to the damage of periodontal tissue. It has been demonstrated that the periodontal inflammatory response mediated by P. gingivalis is one of the dominant mechanisms of chronic periodontitis\textsuperscript{[30,31]}.

2.2. Different cells involved in the occurrence of periodontitis

The immune reactions and inflammatory responses induced by dental plaque plays an important role during
the occurrence and development of periodontitis. When bacteria invade in periodontal tissue, epithelial cells (ECs) can act as a physical barrier against them, and elicit innate and acquired immune responses. Neutrophils will be recruited and release lysosomal enzymes to kill bacteria, but they also can damage the surrounding normal periodontal tissue when they are hyperactivated. Macrophages can perform chemotaxis in order to phagocytize foreign substances, produce enzymes and many inflammatory factors, such as prostaglandin E2 (PGE2), IL-1β, and TNF-α. These factors play an pivotal role in stimulating the bone resorption activities.

When bacteria and their products invade periodontal tissues as antigens, they will induce a kind of specific immune response. Initially, macrophages in dental pulp matrix and dendritic Langerhans cells within epithelium and odontoblast layer can take up microbial antigenic materials and bring them into the lymphoid tissue to activate lymphocytes. Once lymphocytes reach the damage sites, B cells will transform to antibody-producing plasma cells, which can produce corresponding antibodies to fight against bacteria. The amount and activity of antibodies are considered important factors governing the protection effects against chronic periodontitis. When the antigens enter periodontal tissue again, they can combine with IgG and IgM attached to mast cells; this process can neutralize toxins and assist with the phagocytosis of antigens. Similarly, if the multivalent antigens binds to the prebound IgE, mast cells degranulate and release inflammatory factors, such as histamine, chemotactic factor, PGE2 and leukotriene, and trigger type I hypersensitivity.

In this case, T cells can be activated by antigen-presenting cells, while it can also be activated by inflammatory factors released by mast cells during the degranulation. T cells might contribute to the cell-mediated immune responses by stimulating various T helper (Th) cell responses: Th1, Th2, and Th17, but their specific mechanism and timing of their role are still unclear. Although all these cells have different roles to play in fighting pathogens, it is worth noting that normal immune response can kill bacteria, excessive immune response will aggravate inflammatory reaction and lead to tissue damages.

2.3. inflammatory factors are the mediators between pathogens and periodontitis

As a bridge between immune cells and pathogens, inflammatory factors also play an irreplaceable role in the occurrence of periodontitis. With the onset of inflammation, the necrotic or apoptotic cells can release numerous inflammatory mediators, causing vasodilation, increasing permeability, and promoting neutrophils, macrophages and other inflammatory cells homing into damaged sites. In the current paper, we introduce several common inflammatory factors. TNF-α, produced by macrophages, lymphocytes, and natural killer cells, is a cytokine with extensive biological effects, can initiate the inflammatory reactions. During the pathogenesis of periodontitis, TNF-α not only promote osteoclast maturation and bone resorption, but also reduce the activity of periodontal ligament fibroblasts, thus inhibiting the differentiation of periodontal ligament fibroblasts into osteoblasts and subsequently resulting in osteoclastic absorption. PGE2 also is an inflammatory factor that is closely related to the loss of periodontal attachment. PGE2 has a variety of functions, such as increasing vascular permeability and chemotactic leukocyte, promoting bone resorption, and leading to pain occurrence. Similarly, IL-1β also induces osteoclasts activation, mediates the synthesis of cyclooxygenase, increases the concentration of PGE2 in osteoclasts, and aggravates bone resorption. In addition, IL-1β can also enhance the expression of matrix metalloproteinases (MMPs) and increase the degradation of periodontal extracellular matrix, therefore resulting in the destruction of periodontal tissue. As a polypeptide member of the transforming growth factor-β (TGF-β) superfamily of cytokines, TGF-β1 can mediate chemotaxis of neutrophils and fibroblasts as well as promote the initiation, growth and differentiation of inflammatory cells, which has a high correlation with the occurrence of periodontitis.

In addition to those mentioned above, there are also many other inflammatory factors work for the development of periodontitis. It is worth to note that these different inflammatory factors are encapsulated by different extracellular vesicles to secrete and play various physiological roles.

3. Formation and physiological functions of exosomes

3.1. Biogenesis of exosomes

Extracellular vesicles mainly include ectosomes and exosomes, but they are produced via different mechanisms. Ectosomes are generated by cells and released through plasma membrane budding followed by pinching off, and then released into the extracellular space. However, exosomes are generated de novo by cells through the endocytosis process and plasma membrane invagination. The invagination of plasma membrane is a cup-shaped structure that includes cell-surface proteins and soluble proteins, lipids, and nucleic acids, which is driven by endosomal sorting complex required for transport. This leads to the formation of an early-sorting endosome (ESE),
which can directly merge with a preexisting ESE. The trans-Golgi network (TGN) and endoplasmic reticulum (ER) also can contribute to the formation and contents of ESE. ESEs can mature into late-sorting endosomes (LSEs), they can become multivesicular bodies (MVBs) due to the LSEs accumulation. This process results in MVBs containing several exosomes. MVBs can fuse with autophagosomes, and the contents are degraded in the lysosomes ultimately, MVBs can also directly fuse with lysosomes for degradation. The degradation products could be recycled by the cells. Besides, MVBs can be transported to plasma membrane and docked on the luminal side of plasma membrane by the help of MVB-docking proteins to release these exosomes. Through the above process, cells can package cargoes consisting of selective lipids, proteins, and nucleic acids in exosomes, and such cargoes can be transported to recipient cells, contributing to expressional and functional changes (Figure 2).

3.2. Uptake of exosomes

As a kind of extracellular vesicles, exosomes can be released from a variety of cells including fibroblasts, intestinal ECs, neurons, and adipocytes. Exosomes tend to move through the intercellular junctions, leave their initial fluid and move to adjacent areas of tissues, and communicate with neighboring cells through cell-cell connections, which is the primary function of exosomes. Meanwhile, exosomes also enter serum, lymph and cerebrospinal fluid, breast milk, urine, saliva, and other body fluids to play different biological roles.

After released by parent cells, exosomes can navigate through extracellular fluid for varying times and distances. When exosomes reach the location of particular tissue, they are internalized by recipient cells through receptor-mediated endocytosis, pinocytosis, and phagocytosis, or through the fusion with cell membrane, which results in direct release of contents into cytoplasm. The receptor-mediated endocytosis plays a critical role during the entry of exosomes into target cells. Exosomes can interact with recognized specific target cells and then establish interactions with the surface of recipient cells by binding to their receptors or other appropriate sites, which is followed by exosomes fusion with plasma membrane. After this process, exosomes can discharge the luminal cargoes into the recipient cells, resulting in the physiological change of recipient cells. After that, exosomes components can be reassembled in new vesicles that are recycled by other cells to activate effector networks. Exosomes can also release contents into recipient cells by fusing with cells. Subsequently, exosomes membranes and cargoes can redistribute in the recipient cell, which can then be recycled for MVBs or plasma membrane assembly, respectively. Recycled exosomes are released to the extracellular fluid.
by the previous target cell, which becomes the parent cell eventually\(^{61,64}\). Another process that does not occur frequently is that exosomes directly recognize the cell surface receptors, which is not followed by exosome fusion but results in the activation of intracellular signaling\(^{65}\).

### 3.3. Composition and main contents in exosomes

The contents of exosomes mirror the composition of donor cells; therefore, they contain various kinds of constituents due to diverse cell origin, including lipids, cytosolic and cell-surface proteins, metabolites, DNA, RNA, and so on\(^{57}\). Lipids in exosomes are not only involved in the formation of exosome, but also play an irreplaceable influence in the physiological and pathological processes of cells. As mentioned above, lipids and lipid-metabolizing enzymes are involved in the formation and release of exosomes\(^{66}\).

On the one hand, exosomes are formed by lipid bilayer membrane, and released by cells after fusion of MVBs with the plasma membrane. Although the molecular mechanism of this process is still unclear, a study has already shown that glycosphingolipids can be involved in the release of exosomes\(^{67}\). On the other hand, exosomes contain lots of other lipids, including cholesterol, sphingolipid, sphingomyelin, phosphatidylserine, arachidonic acid, and other fatty acids; prostaglandins and leukotrienes in these fatty acids can be utilized as inflammation biomarkers\(^{18}\).

Exosomes also include some specific proteins and non-specific proteins. Specific proteins include integrins, tetraspanins, major histocompatibility complex (MHC) Class I, II, and so on. Among these proteins, tetraspanins can assemble into functionally active membrane structures together with other transmembrane proteins\(^{68}\). Moreover, CD63 and CD81 are recognized as specific markers of exosomes\(^{69}\). A range of fusion and transferring proteins such as Rab2, Rab7, flotillin, and annexin, heat shock proteins (HSP) such as HSP70 and HSP90, cytoskeleton proteins such as actin, myosin and tubulin, and proteins that mediate MVBs formation such as Alix belong to non-specific protein types in exosomes\(^{70}\). The nucleic acids contained in exosomes include mRNA, miRNA as well as long non-coding RNA, cyclic DNA, and so on\(^{71}\). Some studies have proven that nucleic acids in exosomes are involved in many diseases, such as tumors, cardiovascular diseases, and autoimmune diseases\(^{69,72}\). MiRNAs perform negative regulation and confer characteristic changes in the expression levels of target genes\(^{73}\). Interestingly, some miRNAs were found to have novel functions when they are in exosomes. Exosomal miR-21 and miR-29a, in addition to the classic role of targeting mRNA, were first discovered to have the capacity to act as ligands that bind to toll-like receptors (TLRs) and activate immune cells\(^{74}\). Furthermore, miRNAs in the upstream of mRNA can also affect cell survival, especially changing the levels of components essential for the control of cell migration, development and metastasis\(^{75}\).

### 3.4. Physiological and pathophysiological functions of exosomes

The function of exosomes depends on the status of original cells or tissues at the stage of exosome generation\(^{61}\). Exosomes can regulate many pathophysiological processes including immune responses, inflammation, tumor growth, and infections\(^{71}\). As mediators of cell-cell communication, exosomes play a crucial role in the maintenance of cells, homeostasis and regulation of cellular functions. Exosomes are involved in the recycling of cell surface proteins and signaling molecules to affect cell proliferation, differentiation, and apoptosis through paracrine pathways. Meanwhile, exosomes as cellular garbage bags can expel excess and nonfunctional cellular components to maintain normal cellular functions\(^{61,64}\).

In the inflammatory responses, exosomal cargos, such as interferon alpha, can suppress the infection effects by limiting viral replication or enhancing antiviral immunity\(^{18}\). Similarly, exosomes can regulate the function of immune cells which mediate immune responses. Some studies have shown that exosomes derived from antigen presenting cells, such as dendritic cells, can express MHC Classes I and II molecules and costimulatory signals on the cells surface to present the peptide antigen to specific T cells directly to induce their activation which contributes to the induction of specific immune responses\(^{76,77}\).

### 3.5. Different isolation methods of exosomes

There are different methods for separating exosomes based on their size, shape, flotation density, and labeled protein. Exosomes can be isolated by using ultracentrifugation, density gradient centrifugation, ultrafiltration, precipitation, and immunoaffinity capture from conditioned media or body fluids\(^{78}\). However, each method has its advantages and disadvantages as shown in Table 1.

### 4. The role of exosomes in periodontitis

Exosomes are associated with a variety of inflammatory diseases, and they can be treated as vehicles to transfer inclusions from donor cells to target cells and influence their metabolism\(^{18}\). Exosomes secreted by human periodontal ligament stem cells (PDLCs) are a kind vesicle containing lipids, proteins, mRNAs, and non-coding RNAs, all of them are important during the intercellular communications and periodontitis occurrence\(^{69}\).

While invading periodontal tissues, pathogenic factors stimulate the production of exosomes in periodontal
cells, which contain a variety of inflammatory factors. When exosomes reach their destination, their contents are released into the receptor cells and then activate TLRs and subordinate signaling pathway, which activates the expression of cytokines and regulatory factors in inflammatory reactions\textsuperscript{88}. In addition, the interaction of inflammatory cells and tissue cells can produce a series of immune responses, which subsequently lead to immune system dysregulation and occurrence of periodontitis.

4.1. The role of nucleic acids in exosomes in periodontitis

At present, studies on nucleic acids related to periodontitis in exosomes mainly focus on miRNAs. Exosomes containing miRNAs regulate cells through surface proteins in the form of ligands and receptors, and they have different effects on periodontitis according to the different type of miRNAs\textsuperscript{87}.

There is an imbalance of Th17/Treg in the peripheral blood of periodontitis patients, characterized by upregulated Th17 or downregulated Treg\textsuperscript{88}. Zheng et al. found exosomes from PDLSCs in periodontal inflammatory environment induced with LPS have a regulatory effect on the inflammatory microenvironment by regulating Th17/Treg differentiation and homeostasis. They used inhibitors to knock down miR-155-5p in normal PDLSCs and found that the Th17/Treg ratio increased and then lead to an inflammatory status\textsuperscript{89}.

Among the main bacteria implicated in the pathology of periodontal disease, \textit{Aggregatibacter actinomycetemcomitans} is well known for causing loss of periodontal attachment and systemic diseases\textsuperscript{89}. Han et al. analyzed the small RNA expression profiles in activated human macrophage-like cells infected with exosomes from \textit{A. actinomycetemcomitans} and the result provided evidence that these cells can harbor small RNAs of bacterial origin, which contribute to the production of TNF-\textalpha through TLR-8 and NF-kB signaling pathways\textsuperscript{91}.

This indicates that exosomes play an irreplaceable role in the occurrence of periodontitis caused by periodontal pathogens. In addition, Yu et al. showed that the detected expression levels of salivary exosomal PD-L1 mRNA were significantly higher in periodontitis patients when compared with non-periodontitis patients, and the mRNA level also has significant differences at all stages of periodontitis\textsuperscript{92}.

4.2. The role of proteins in exosomes in periodontitis

Proteins in exosomes include transmembrane proteins and embedded proteins which play an important role in the occurrence of periodontitis\textsuperscript{93}. ‘Tetrapeptide superfamily’ is a specific protein of transmembrane proteins with various biological activities, such as cell adhesion, motion, migration, growth, signal transduction, differentiation, and sperm-egg fusion\textsuperscript{94}. Zhao et al. isolated and identified exosomes derived from PDLSCs, and found PDLSC-derived exosomes could express the common surface adhesion molecules CD9, CD63, CD81 and TSG101\textsuperscript{95}. This suggests that exosomes secreted by periodontal cells have the potential to act as vesicles for inflammatory factors.

Huang et al. extracted salivary exosomes from 11 young patients with severe periodontitis and identified 26 proteins that were relatively specific to normal people, and found obvious immune correlations among these proteins, suggesting that exosomes may be involved in the immune response during the development of periodontitis\textsuperscript{93}. Zhao et al. established an inflammatory model in vitro and isolated exosomes from primary human periodontal ligament fibroblasts (hPDLFs) which were treated with LPS from \textit{P. gingivalis}. The results showed that the levels of total protein and exosome-enriched protein were higher in LPS-treated group than in controls, indicating that exosome secretion was enhanced by LPS. Moreover, they found that the expression of IL-6 and TNF-\textalpha was upregulated at both gene and protein levels after treated with LPS, which also has an influence on bone remodeling\textsuperscript{96}. These suggest that

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Exosomes connect periodontitis and systemic diseases

5. Bridging role of exosomes in systemic diseases accompanied by periodontitis

Periodontal pathogenic bacteria invade periodontal tissue and cause local inflammation, and the dynamic imbalance between pathogen and host immune response contributes to the process of periodontitis\cite{34,97}. In the acute stage of chronic periodontitis, inflammatory periodontal tissues produce many inflammatory mediators, such as IL-6, TNF-α, and PGE2, and release them into exosomes. Subsequently, these exosomes can be transported within body fluids to various organs and tissues, thereby giving rise to the corresponding response and increasing the systemic inflammatory load\cite{98}. A growing line of evidence demonstrated that exosomes play an indispensable role in the pathological development of various systemic diseases, such as osteoporosis, kidney disease, AD, stroke, and cardiovascular disease (Figure 3).

5.1. Bone resorption is the main consequence of both osteoporosis and periodontitis

Osteoporosis is a systemic disease that weakens bones due to decreased bone density and bone mass, which mostly affects cancellous bone\cite{99}. Periodontitis involves local inflammatory alveolar ridge loss, following an infectious breach of the alveolar cortical bone, and then may result in tooth loss\cite{100}. Obviously, osteoporosis and periodontitis are both diseases characterized with bone resorption, and many studies also support the hypothesis that systemic osteoporosis relates to local osteoporotic changes in the loss of tooth-supporting tissues, such as alveolar bone\cite{96}.

Increasing evidence indicates that local periodontal inflammation can affect the bone remodeling process by releasing exosomes. Zhao et al. evaluated the role of exosomes derived from hPDLFs in the progression of periodontitis, and the results showed that inflammatory hPDLFs which were pretreated with LPS can inhibit the osteogenic activity of osteoblasts by secreting exosomes\cite{96}. Sun et al. found that the expression of miR-214 from osteoclast-derived exosomes in the serum of osteoporosis patients and mice was significantly higher than that in normal people and mice, the results proved that miR-214 could affect osteoblasts and inhibit their osteogenic activity through exosomes\cite{101}. Proteins in exosomes may also play an important role during the occurrence of osteoporosis. Huo et al. extracted exosomes from the serum of osteoporosis patients and found that the expression of 17 proteins, including integrin β3, integrin α2β1, talin 1, and gelsolin, was significantly changed in osteoporosis group and osteopenia group\cite{102}. They are likely to function by working together, suggesting that these proteins in exosomes affect the process of systemic bone changes.

Figure 3. The bridging role of exosomes between periodontitis and systemic diseases. The occurrence of periodontitis renders the body in an inflammatory state, which can promote the secretion of exosomes by a variety of cells. Nucleic acid, protein, and lipid can be transmitted between various cells through the exosomes, which can be used as messengers to mediate multiple cells signaling pathways, and then participate in the pathogenesis of osteoporosis, renal fibrosis, AD, stroke, oral cancer, and cardiovascular disease.
5.2. Periodontitis increases the incidence of chronic kidney disease (CKD)

As a progressive pathological condition, renal fibrosis is the final outcome of a variety of CKDs\textsuperscript{103,104}. Renal fibrosis is characterized by excessive accumulation of fibroblasts and cell matrix, which eventually leads to end-stage renal disease\textsuperscript{105,106}. On the one hand, various pathogenic factors can stimulate the intrinsic cells of kidney and promote the proliferation of fibroblasts leading to fibrosis\textsuperscript{107}, and on the other hand, accumulated protein can increase glomerular protein filtration, which contributes to collagen deposition and accumulation, resulting in the gradual hardening of renal parenchyma, the formation of scar, and finally the complete loss of organ functions\textsuperscript{107}. The exact pathogenesis of CKD influenced by periodontitis is currently unclear, but it has been reported that the prevalence of chronic periodontitis would lead to an increase incidence of CKD. TGF-β is not only an important cytokine that promotes the development of periodontitis\textsuperscript{108}, but also a primary factor that drives fibrosis in most forms of CKD\textsuperscript{109,110}. Chen \textit{et al.} found that periodontitis caused the downregulation of matrix MMP2 and upregulation of tissue inhibitors of metalloproteinases-1 and TGF-β1 at transcriptional and translational levels by comparing with normal mice; this is a possible mechanism by which periodontitis aggravates kidney damage\textsuperscript{111}. Borges \textit{et al.} used kidney injury as a model system and demonstrated that injured ECs produce an increased number of exosomes to activate fibroblasts, which depends on the exosomes delivering TGF-β1 mRNA\textsuperscript{112}. In addition, Sonoda \textit{et al.} used the rat kidney ischemia-reperfusion injury as an acute kidney injury model, and found that miR-16, miR-24, and miR-200c in urine exosomes were increased at the injury stage, and miR-9a, miR-141, miR-200a, miR-200c, and miR-429 were upregulated in the early recovery stage, indicating that these miRNAs are related to renal fibrosis\textsuperscript{113}.

5.3. Periodontal pathogens lead to the loss of neurons and induce AD

AD is a neurodegenerative disorder characterized by gradual cognitive decline and memory loss, even intellectual loss, or death in some severe cases\textsuperscript{114}. AD is characterized by the presence of insoluble plaques and tangles composed of Aβ and hyper-phosphorylated tau (p-tau), respectively\textsuperscript{115}. At present, more and more evidence has shown that inflammation plays a key role in the pathophysiological process of AD, and the relationship between periodontitis and AD has also been confirmed by relevant studies\textsuperscript{116}. Periodontal pathogens can induce the mutual activation of microglia and inflammatory factors directly or indirectly, as well as the accumulation of amyloid Aβ in brain, and eventually lead to a vicious cycle and the loss of neurons\textsuperscript{9,117}. Ide \textit{et al.} assessed the cognition of 60 people with mild-to-moderate AD and took blood samples for screening markers of inflammation and found that the presence of periodontitis was associated with a 6-fold increase in the rate of cognitive decline as assessed. This indicates that periodontitis is associated with worsened cognitive decline in AD, which may be mediated through effects on systemic inflammation\textsuperscript{118}.

Exosomes are one of the multiple cellular mechanisms that may link amyloid and tau secretion to both toxicity and neurofibrillary lesion spreading in AD\textsuperscript{119,120}. In the process of the onset of AD, neurons and neuroglial cells can release exosomes into the extracellular space or transport them to the neighboring cells through blood, and then exosomes can fuse with the membrane and release contents, especially miRNAs, into the intracellular plasma to activate TLRs\textsuperscript{121}. TLR7-9 activates the myeloid differentiation factors (MyD88) and then activates nuclear factors and transcription factors activator protein-1, leading to neuroinflammation and neuronal death\textsuperscript{121}. Zheng \textit{et al.} isolated exosomes from peripheral plasma and injected them into the hippocampus of an AD mouse model, and found that exosomes can diffuse throughout the brain and clustered around the Aβ plaques, which indicates that exosomes may contribute to the spread of Aβ\textsuperscript{122}.

5.4. Periodontitis promotes platelet aggregation and cerebral arteriosclerosis which lead to stroke

Stroke is a rapidly progressive ischemic or hemorrhagic encephalopathy, which can be divided into ischemic stroke (IS) and hemorrhagic stroke. Hemorrhagic stroke refers to non-traumatic cerebral parenchymal hemorrhage, also known as cerebral hemorrhage, mainly occurs in patients with hypertension and cerebral arteriosclerosis\textsuperscript{124}. IS is mainly caused by thromboembolism of the great arteries supplying blood to brain, which leads to necrosis of brain tissue\textsuperscript{125}. Periodontitis is associated with stroke. Sen \textit{et al.} found that the risk of thrombotic stroke in patients with periodontitis is twice the risk in healthy individuals\textsuperscript{126}. Periodontitis is associated with elevated levels of C-reactive protein, fibrinogen, and cytokines, and these inflammatory factors can invade vascular endothelial cells, thereby promoting platelet aggregation and atherosclerotic diseases as well as inducing formation of foam cells\textsuperscript{127,128}. This suggests that periodontitis may cause cerebral arteriosclerosis and endothelial cell damage, which eventually lead to stroke.

A growing line of evidence demonstrated that exosomes participate in the development of stroke. Some researchers extracted circulating exosomes from the blood of patients...
who have acute stroke within 72 h, and found that the expression of miR-223 was higher than that in control group\(^{[129]}\). Li et al. divided 55 patients with IS into acute phase group and subacute stage group, and the results showed that plasma exosomal mir-422a and mir-125b-2-3p levels in subacute phase group were significantly lower than those in acute phase group, and the expression level of mir-422a in acute phase group was higher than that in control group\(^{[130]}\). Ji et al. detected the alternation in serum exosome concentrations and the levels of serum exosomal miR-9 and miR-124 in 65 patients with acute IS and 66 non-stroke patients, and the results showed that the concentration of serum exosomes was elevated, and the median levels of serum exosomal miR-9 and miR-124 in acute IS patients were significantly higher than those in control group\(^{[131]}\).

### 5.5. Periodontal pathogens increase morbidity and risk of atherosclerosis, which leads to cardiovascular disease

Cardiovascular disease is a common disease that seriously threatens the health of human beings, especially the elder individuals\(^{[132]}\). Cardiovascular disease is a multifactorial disease with complex etiology, and periodontitis may be one of the pathogenic factors. Systemic inflammatory response and vascular endothelial damage caused by periodontitis are related to the development of cardiovascular disease\(^{[133]}\). Eight types of bacteria were identical in both subgingival plaque and atherosclerotic plaque. Among these bacteria, \(P.\) gingivalis and \(Tannerella forsythia\) significantly increase morbidity and risk of atherosclerosis. The results strongly correlate periodontal bacterial co-occurrence and periodontal bacterial adhesion factor to atherosclerosis\(^{[134]}\). In addition, there is a certain relationship between periodontal disease and C-reactive protein level, and C-reactive protein may also be involved in the development of cardiovascular disease\(^{[135]}\).

The exosomes and their miRNAs may be involved in the pathophysiological process of atherosclerosis as a medium of cell communication\(^{[136]}\). Wang et al. have demonstrated that macrophage-derived exosomes can transfer miR-155 to cardiac fibroblasts, thereby inhibiting fibroblast proliferation and enhancing inflammatory response\(^{[137]}\). Widera et al. detected the expression of miRNA in plasma exosomes from a large number of patients with acute coronary syndrome, and the results showed that the expression of miR-1, miR-133a, miR-133b, and miR-208b was upregulated\(^{[138]}\).

### 6. Application prospect of exosomes in periodontitis and other systemic diseases

Specific contents are the attributes of exosomes that allow them to send signals to specific recipient cells or tissues. Therefore, exosomes can be used as promising diagnostic biomarker (Table 2) and therapeutic tool for the treatment of periodontitis and other diseases. These roles are attributed to their abilities to transfer RNA, proteins, enzymes, and lipids, thereby affecting physiological and pathological processes in various diseases\(^{[61]}\).

Recently, many investigations have shed light on periodontitis treatments using exosomes. Modified exosomes can inhibit inflammation and promote periodontal regeneration by affecting the function of periodontal cells or immune cells. Chew et al. observed the effects of mesenchymal stem cells (MSCs) exosomes-loaded collagen sponges on the healing of periodontal defects in immunocompetent rat models, and found that MSC-derived exosomes can increase PDL cells migration and proliferation through CD73-mediated activation of pro-survival AKT and ERK signals to promote periodontal regeneration\(^{[140]}\). Wang et al. used osteoblasts stimulate stem cells from human exfoliated deciduous teeth (SHED) to acquired special exosomes, and demonstrated that they have a facilitating effect on the osteogenic differentiation of PDLSCs \textit{in vitro}. Both Wnt/β-catenin and BMP/Smad signaling pathways are involved in this physiological process\(^{[98]}\), which indicates that exosomes isolated from SHED may have a great therapeutic potential in the loss of periodontal or alveolar bone. Macrophages are involved in the development of inflammation, and different types of macrophages play various roles in different stages of inflammation. In the early stage of inflammation, M1 cells

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are activated by LPS and secrete pro-inflammatory factors. However, M2 cells are activated by IL-4 produced by Th2 cells, and then release some cytokines to inhibit inflammatory response and promote tissue repair\(^ {165} \). Wang et al. co-cultured macrophages and exosomes that were isolated from GMSC and stimulated by LPS, and found that the levels of M1 markers, including TNF-\( \alpha \), IL-12, CD86, and IL-1\( \beta \), were significantly decreased but the level of M2 marker such as IL-10 increased moderately. GMSC-derived exosomes may promote M1 macrophage transformation into M2 macrophages, reducing the pro-inflammatory factors which are produced by M1 macrophages\(^ {166} \). All of these provide us a new direction for the treatment of periodontitis. As a non-surgical periodontal treatment tool, exosomes showed superior effects on cells, with the additional advantages of safety and easy preparation. They can be applied as a suspension injected locally to the periodontal defects, without additional cost in the preparation of scaffolds\(^ {167} \). Mohammed et al. compared the effects of exosomes and adipose-derived stem cells on the periodontitis mice, and found that the exosomes group showed the most evident proliferation in periodontal tissue with increased cellularity and significant organization, and the alveolar bone was more organized with an osteoid tissue layer\(^ {99,168} \).

In addition to periodontitis, the potential of using exosomes to treat other diseases has also been discovered by more and more researchers. Wei et al. found that exosomes from SHED can directly promote the osteogenesis, differentiation and bone formation of bone marrow MSCs. They applied SHED-exosomes to the mouse model with a bone loss, and then found that Runx2 and Smaad5, the key transcription factors for osteogenic differentiation, were upregulated and bone loss was restored after injecting of SHED-exosomes\(^ {169} \). Xie et al. reported that MSC-derived exosomes could promote osteoblast proliferation through inhibiting cell apoptosis, and significantly increase the expression level of GLUT3, which eventually stimulates the osteogenesis differentiation of osteoblasts; this provides a theoretical basis for the clinical treatment of osteoporosis\(^ {170} \). Recent studies have shown that MSC-derived exosomes therapy improves renal outcomes in several animal models of AKI and CKD, such as ureteral obstruction, ischemia-reperfusion injury, drug/toxin induced nephropathy, and renal vascular disease\(^ {171} \). The exosomes derived from MSCs can regulate the expression of intercellular adhesion molecule-1 and then inhibit the infiltration of dendritic cells into the kidney. In addition, these exosomes can reduce the production of pro-inflammatory cytokines and inhibit renal fibrosis\(^ {172} \). Sato et al. found that exosomes containing \( \beta \)-secretase 1 (BACE1) siRNA or curcumin reached the brain after peripheral injection and ameliorated AD-like pathology in mice\(^ {173} \). In addition, exosomes have great potential in the treatment of stroke. Li et al. found that injection of enriched plasma exosomes from mice treated with remote ischemic preconditioning could significantly reduce the infarct size in a murine model of cerebral ischemia and improve the neurological function\(^ {174} \). Inoue et al. found that dental pulp stem cells-derived exosomes can induce angiogenesis and neurogenesis, and then reduce the infarct volume\(^ {175} \). The important value of exosomes in the diagnosis and treatment of cardiovascular diseases cannot be neglected. Preclinical studies have shown that the exosomes play a protective role in ischemic heart disease by reducing myocardial ischemia-reperfusion injury and promoting vascular regeneration\(^ {176} \). Luo et al. used myocardial infarction rats as animal model and injected exosomes enriched with miR-126, and the results showed that the release of inflammatory factors decreased and the infarct size decreased significantly\(^ {177} \).

7. Conclusion

Exosomes, as functional carriers of nucleic acids, lipids, proteins, and other substances, play an important role in the occurrence and progression of periodontitis. Exosomes carrying some specific substances can promote the occurrence of periodontitis, while exosomes carrying other specific substances can play an anti-periodontitis role. This shows that the exosomes from periodontal tissue or cells are pivotal in the occurrence and recovery of periodontitis. In addition, exosomes with different contents playing a bridge role in patients with periodontitis and other systemic diseases. Exosomes can cause periodontal inflammatory mediators to spread to the whole body, thus promoting or antagonizing the occurrence of specific diseases. The current study of exosomes is helpful to explain the pathogenesis of periodontitis and how to regulate the progression of other systemic diseases in patients with periodontitis.

At present, the understanding of exosomes is still limited. Nevertheless, exosomes could serve as an important biomarker of clinical diagnosis, treatment of periodontitis, and intervention of systemic diseases in future.

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Conflict of interest
None.

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