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Review

Exosomes derived from odontogenic stem cells: Its role in the dentin-pulp complex

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ABSTRACT

Odontogenic stem cells originate from cranial neural crest cells and offer unique advantages in the regeneration of dentin-pulp complex. There is increasing evidence that stem cells exert their biological functions mainly through exosome-based paracrine effects. Exosomes contain DNA, RNA, proteins, metabolites, etc., which can play a role in intercellular communication and have similar therapeutic potential to stem cells. In addition, compared with stem cells, exosomes also have the advantages of good biocompatibility, high drug carrying capacity, easy to obtain, and few side effects. Odontogenic stem cell-derived exosomes mainly affect the regeneration of the dentin-pulp complex by regulating processes such as dentintogenesis, angiogenesis, neuroprotection and immunomodulation. This review aimed to describe "cell-free therapies" based on odontogenic stem cell-derived exosomes, which aim to regenerate the dentin-pulp complex.

Contents

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1. Introduction

Dentin, a highly mineralized substance, forms the primary hard tissue of the tooth. Dentin composed predominantly of dentinal tubules, odontoblastic cell processes, and an intercellular matrix, which impart strength and hardness of the tooth structure [1]. The pulp tissue, situated within the pulp cavity at the dentin's center, comprises blood vessels, nerves, lymphatic vessels, a variety of cells, and an extracellular matrix, providing dentin-forming, nutritive, sensory, and defensive functions [2]. While dentin lacks blood circulation and nerve innervation, it can respond to external stimuli such as temperature, chemical, and physical changes through interactions with the pulp tissue via dentin tubules. In fact, pulp and dentin are closely related in embryonic development and their responses to external stimuli are interrelated. They form a biological entity, collectively referred to as the dentin-pulp complex. Dental caries, trauma, and retrograde periodontitis can result in pulpitis or pulp necrosis, traditional root canal treatment prevents infection by removing dental pulp. However, the affected tooth loses vitality due to the absence of blood supply and innervation after endodontic-treatment, increasing its brittleness and susceptibility to fractures and loss [3,4]. In recent years, with the ongoing advancements in tissue engineering and regenerative medicine, an increasing amount of research has focused on regenerating the dentin-pulp complex to achieve biomimetic restoration, primarily encompassing vascular and nerve regeneration, inflammation regulation, and promotion of dentin regeneration and mineralization [5].

Mesenchymal stem cells (MSCs) are a unique cell type characterized by self-renewal and multi-lineage differentiation capabilities. Initially isolated from bone marrow by Friedenstein et al. [6]. MSCs hold immense potential in regenerative medicine, playing roles in immune regulation, inflammatory response, and apoptosis [7,8]. Due to their inherent ability to differentiate from primary tissues, tooth-derived stem cells exhibit distinct advantages in dentin-pulp complex regeneration. Researchers have identified various mesenchymal stem cells from dental tissues, with these odontogenic stem cells originating from intracranial neural cells [9]. To date, approximately six primary types, including dental pulp stem cells (DPSCs), stem cells from apical papillae (SCAPs), periodontal ligament stem cells (PDLSCs), dental follicular progenitor cells (DFPCs), and gingival mesenchymal stem cells (GMSCs), have been extensively studied, isolated, and characterized.

Although stem cells have been widely employed in tissue repair and regeneration, their clinical translation faces challenges due to storage, transportation, high cost, and ethical concerns. The potential tumorigenic risk associated with cell therapy poses safety concerns that cannot be overlooked. Furthermore, cell therapy may exhibit poor biocompatibility and provoke immune rejection following transplantation [10]. In recent years, a cell-free therapy centered on exosomes has been proposed [11], which can play a similar therapeutic potential as stem cells. Exosomes play a role in cell-to-cell communication and usually represent the biological characteristics of their parental cells. Exosomes facilitate cell-to-cell communication and typically reflect the biological characteristics of their parent cells. They can be stored at −80 °C, maintaining their biological activity during storage, which renders them more convenient and manageable compared to cells [12]. In fact, exosomes secreted by stem cells from different parts have innate differences, which further lead to their different biological functions, because they reflect the genomic characteristics of their parental cells [13]. Exosomes derived odontogenic stem cells may have unique functions compared with other mesenchymal stem cell-derived exosomes. For example, the function of DPSCs-Exo is more manifested in vascular regeneration, immune regulation, osteogenic differentiation and facilitating tooth differentiation, while the function of PDLSCs-Exo is more manifested in periodontal membrane and bone tissue regeneration [14]. This review aims to describe the cell-free therapy based on exosomes derived from odontogenic stem cells, which aims to regenerate the dentin-pulp complex and provide new research methods and ideas for clinical treatment.

2. Biological characteristics of exosomes

2.1. Classification and biogenesis

Extracellular vesicles (EVs) are nanoscale vesicles featuring a bilayer membrane structure, secreted by cells. In the early stages of

research, they were not well understood and often dismissed as "cellular waste". It wasn't until 1967 that Wolf observed a tiny particulate matter under an electron microscope through high-speed centrifugation and called it "platelet dust" [15]. At present, the subtypes of exosomes are still controversial. EVs can be divided into exosomes (40 nm < diameter <160 nm, average about 100 nm), microvesicles (100–1000 nm) and apoptotic bodies (>1000 nm in diameter) according to their diameter size. The term "exosome" was first formally proposed in 1987 [16].

The biogenesis of exosomes is a dynamic process involving a series of endocytosis and exocytosis. Exosomes are derived from endosomes, whose diameter ranges from 40 to 160 nm, and the cytoplasmic membrane invaginates to form a cup-like structure, which contains cell surface proteins and soluble proteins related to the extracellular environment, forming early endosomes. Early endosomes can merge with other early endosomes, and endosomes mature to form late endosomes, which further form multivesicular bodies (MVBs) containing intralumenal vesicles (ILVs). This is also the second invagination of multivesicular bodies of the cytoplasmic membrane that can be decomposed by lysosomes/autophagosomes or fuse with the plasma membrane to release intralumenal vesicles (ILVs)/exosomes. Surface molecules on exosomes enable them to target receptor cells. They can conduct signals through receptor-ligand interaction, or internalize through endocytosis or phagocytosis, fuse with the membrane of target cells and deliver contents to their cytoplasmic matrix, playing a role in intercellular communication [17] (Fig. 1).

2.2. Composition and distribution of exosomes

Exosomes are secreted into the extracellular environment by prokaryotic and eukaryotic cells, which contain many components of parental cells, including DNA, RNA, metabolites, and cell surface proteins. These molecules are directly released into target cells by exosomes through different signaling pathways, thus changing the biological characteristics of target cells [18]. The components in exosomes remain relatively stable and do not degrade. Surface labeled proteins mainly include tetraspanins (CD9,CD63,CD81,CD82), integrins, immunomodulatory proteins. Integral proteins include Alix, TSG101, heat shock proteins (Hsp70, Hsp90), etc. Exosomes are widely distributed, almost all mammalian cells are able to secrete exosomes. In addition to exosomes are widely present in all body fluids, including blood, urine, saliva, peritoneal effusion, bile, etc. In the latest progress, exosomes are also extracted from plants and bacteria [19]. However, the purpose of exosome production is not clear, and it is speculated that it may be to remove unnecessary components in cells to maintain cell homeostasis.

2.3. Isolation of exosomes

At present, six technologies for the isolation of exosomes have been established: ultracentrifugation, ultrafiltration, antibody affinity capture, microfluidic chip technology, size exclusion chromatography(SEC) and precipitation based isolation [20–22]. Among them, ultracentrifugation is the most widely used exosome separation technology and is considered to be the "gold standard" for exosome isolation. The ideal method for exosome isolation should be relatively simple, fast, inexpensive and efficient. Nevertheless, different principles of isolation can impose unique limitations on various techniques, and as of yet, there is no universally applicable isolation technique for all types of research. The combination of two or more isolation techniques can optimize the isolation efficiency and quality of exosomes [23]. Ultracentrifugation and precipitation are the most common isolation techniques for exosomes derived from odontogenic stem cells [24].

2.3.1. Ultracentrifugation

Ultracentrifugation was the first method for exosome isolation and remains the most commonly used isolation method. Ultracentrifugation produces centrifugal forces of up to 1,000,000 × *g* to precipitate and purify vesicles of the same size from the sample by high-speed centrifugation. This method is easy to scale up to large-scale exosome preparation, and the technical expertise requirements are low, but the purity of the isolated exosomes is low and time-consuming [25].

2.3.2. Ultrafiltration

Using the interception ability of porous membranes, it is physically intercepted and separates the particles of substances of different sizes in the solution, so as to achieve the purpose of concentration, purification and screening of different components in the solution. However, the adhesion of the filter membrane may lose exosomes, and the deformation of exosomes may be impaired due to the shear forces and pressures generated by filtration [26].

2.3.3. Size exclusion chromatography

Size exclusion chromatography (SEC) is a method for separating particles in solution based on particle size, resulting in exosomes that are uniform in size, structurally intact, and take short time. However, exosomes and microvesicles of the same size cannot be separated, resulting in reduced purity [27,28].

2.3.4. Polymer precipitation method

Polymer precipitation was originally used for the isolation of viruses. Because viruses and exosomes have similar biophysical properties, they are used for the isolation and purification of exosomes [29]. This method is fast, simple, and does not require expensive equipment, and most kits for the rapid isolation of exosomes are based on this method. However, polymer precipitation may also cause protein contamination (including lipoproteins).

2.4. Identification of exosomes

According to the morphology, size and formation process of exosomes, the identification of exosomes mainly depends on the detection of morphological characteristics, particle size and surface markers.

2.4.1. Electron microscopy detection

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and cryo-electron microscopy (cryo EM) are commonly used to characterize the microstructure of exosomes. Electron microscopy can directly observe the structure and morphology and identify exosomes of different sizes. SEM can obtain the microscopic morphology of exosome surface, and TEM has superior resolution to observe the internal structure and morphology. However, during steps such as fixation and staining of exosomes, their size and morphology may be affected, allowing them to dry and form a collapsed cup-like structure [30]. Cryo-electron microscopy, on the other hand, avoids the effects of chemical fixatives and dehydration, and observes circular structures and morphologies [31].

2.4.2. Exosome particle size and concentration detection

Nanoparticle tracing (NTA) and dynamic light scattering (DLS), which detect suspended particle distribution, have been used as one of the methods for exosome characterization. NTA uses the

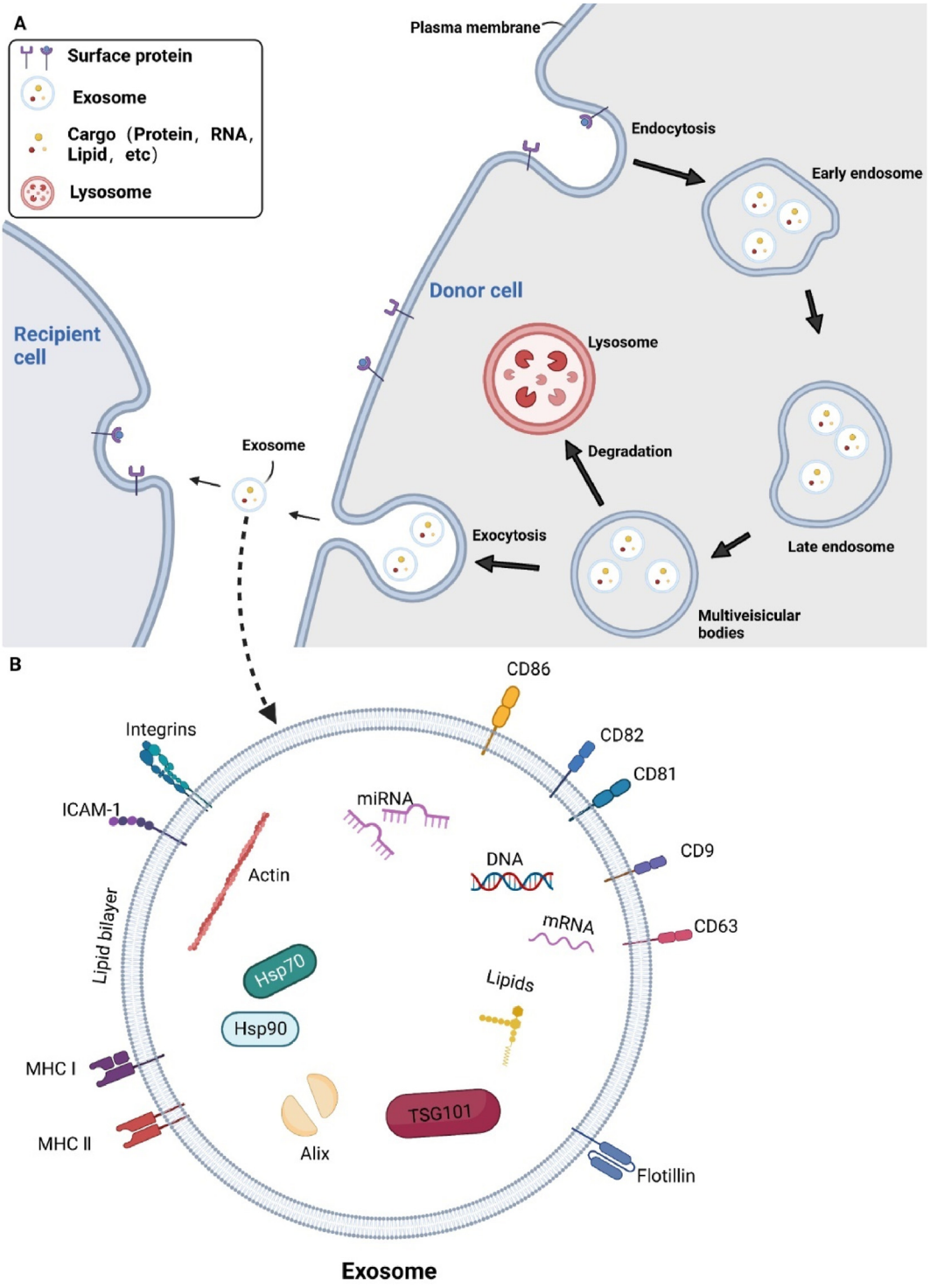

Fig. 1. **A** Biogenesis of exosomes. The biogenesis of exosomes is a dynamic process. First, through endocytosis, the plasma membrane of the cell invaginates to form early endosome (ESE) containing proteins and nucleic acids. Early endosome may merge with other early endosome or form late endosome (LSE). The late endosomal membrane undergoes a second invagination, further forming multiveisicular bodies (MVBs) containing intraluminal vesicles (ILVs). The multiveisicular bodies (MVBs) can be degraded by lysosomes or fused with the plasma membrane of the cell to release the so-called exosomes through exocytosis. **B** Structure of exosomes. Exosomes are nanoscale vesicles wrapped by phospholipid bilayer structure, containing DNA, RNA, lipids, tetraspanins (CD9, CD63, CD81, CD82), heat shock proteins (Hsp70, Hsp90), integrins, metabolites, etc.

characteristics of light scattering and Brownian motion to obtain the particle distribution in the suspension, and its sample processing is simple, the detection speed is fast, and the size distribution and concentration of particles can be directly and real-time. The main difference between DLS and NTA is the concentration range, DLS can only detect samples with higher concentrations.

2.4.3. Identification of surface markers

Exosomes contain nucleic acids, proteins, lipids, etc. Common exosome surface marker proteins include heat shock protein (HSP70, HSP90), protein involved in MVB formation (Alix, TSG101), tetraspanins protein (CD9, CD63, CD81, CD82), integrin, immunomodulatory protein, etc. Flow cytometry and Western blot

techniques are utilized to detect the expression of these unique exosome marker proteins, enabling the identification of exosomes at the protein level.

3. The effect of exosomes derived from odontogenic stem cells on the dentin-pulp complex

3.1. Dentinogenesis

3.1.1. Odontoblastic differentiation

Odontoblastic differentiation is a multi-gene regulated process involving growth factors and dentin matrix proteins, et [32]. During odontogenic differentiation and tertiary dentin formation, Exosomes Derived from Odontogenic Stem Cells induces the differentiation of odontogenic stem cells into odontoblasts. Studies have shown that DPSCs-Exo and exosomes derived from odontoblasts have the effect of inducing the migration of DPSCs and differentiating into odontoblasts, forming tertiary dentin to prevent further destruction of tooth tissue [33]. DPSCs-Exo cultured in odontogenic media are internalized by DPSCs and significantly upregulate dentin sialphosphoprotein (DSPP), alkaline phosphatase (ALP), growth factors (GDF10, BMP9), transcription factors (RUNX2), and type I collagen [34]. Stem cells from apical papilla (SCAPs) are derived from the papilla and differentiate into odontoblastic, which secrete DSPP and DMP-1 to form tubular dentin, and vascular endothelial cells(ECs) [35]. Recent studies have shown that dental pulp cell-derived exosomes loaded with nuclear factor IC(NFIC) can promote SCAPs migration, proliferation and dentin production [36]. NFIC is a key transcription factor for tooth and bone development, which can regulate the expression of DSPP and DMP-1 during the differentiation of odontoblasts [37]. Abundant evidence has proved that exosomes derived from odontogenic stem cells pretreated with LPS can promote the migration, proliferation and differentiation of odontogenic stem cells [38], while exosomes derived from dental pulp cells pretreated with LPS can inhibit the odontoblast differentiation of SCAPs by down-regulating NFIC [36]. The contradictory effect of LPS on exosomes may be attributed to the heterogeneity between odontogenic stem cell-derived exosomes different subtypes. MiRNAs accounts for about 50% of the RNA content in MSCs-Exo and are considered to be a key regulator of the function of exosomes. Exosomes promote odontogenic differentiation of DPSC by regulating miRNA. Exosomes isolated from DPSC cultured under odontogenic conditions cause odontogenic differentiation of DPSC by upregulating DSP, DMP-1, ALP and RUNX2, and the expression of miR-27a-5p is significantly upregulated [39].

In addition, exosomes can also form dentin by regulating Schwann cells (SCs). SCs are precursors of dentin cells [40], the main source of dentin cells, which migrate to the site of injury and differentiate into odontoblasts/pulp cells. DPSC-Exo can promote SCs differentiation into odontoblasts by regulating the migration and proliferation of SCs [41]. On the other hand, DPSC-Exo pretreated with lipopolysaccharide has better regulation of SCs, better odontogenic differentiation and dentin formation.

3.1.2. Dentin mineralization

After differentiation, odontoblasts begin to secrete extracellular matrix. Most of the organic matrix is type I collagen, and the inorganic component is mainly hydroxyapatite (HA). Mineralization is the process of hydroxyapatite deposition in the extracellular matrix (ECM). Dentin and bone share many common features and are very similar in composition and mineralization process. Bone marrow mesenchymal stem cells (BMMSCs) act as a source of mesenchymal stem cells within the bone marrow and exhibit the potential to differentiate into multiple cell types, including the ability to differentiate into osteoblasts [42]. Research evidence indicates that the biochemical pathways involved in the differentiation of DPSCs into odontoblasts and BMMSCs into osteoblasts share similarities [43]. However, DPSCs exhibit a higher proliferation rate, colony formation rate, and mineralization potential when compared to BMMSCs [44]. Dentin sialoprotein (DSP), dentin phosphoprotein (DPP) and dentin matrix protein-1 (DMP-1) are the SIBLING family proteins that mainly exist in dentin matrix and play important roles in dentinogenesis and mineralization. DPSCs-Exo can promote mineralization and upregulate the expression of specific odontogenic genes (such as DSPP, VEGF and BSP) by activating MAPK signaling pathway, thereby promoting dentin regeneration [33]. SCAPs-Exo can be endocytosed into bone marrow mesenchymal stem cells (BMSCs), promote the formation of mineralized nodules and regulate the formation of dentin by upregulating the expression of DSPP in BMSCs [45]. In addition, exosomes derived from the epithelial root sheath can increase the activity of alkaline phosphatase (ALP), the formation of mineralized nodules, and induce the activation of Wnt-β-catenin signaling in vitro [46].

3.2. Angiogenesis

Angiogenesis is a key factor in the success of pulp regeneration, providing oxygen and nutrients to the tissues to achieve regeneration [47]. Clinical studies have shown that culture in conditioned medium for exosomes deletion impairs the angiogenic response of MSCs, so exosomes are speculated to play an important role in angiogenesis, and exosomes have been proposed as key drugs for regulating angiogenesis [48,49]. The regulatory mechanism of exosomes on angiogenesis is not clear, and it is speculated that it may be related to the pro-angiogenic factors, microRNAs secreted by exosomes. Exosomes can not only directly regulate pro-angiogenic factors, but also affect pro-angiogenic factors by regulating the expression of microRNAs, and ultimately promote angiogenesis. Furthermore, alterations in the microenvironment play a critical role in exosomes regulating the expression of pro-angiogenic factors [50](Fig. 2).

3.2.1. Pro-angiogenic factors

Odontogenic stem cells are not only directly involved in angiogenesis by differentiation into ECs, but also by releasing pro-angiogenic factors through paracrine action. The process of angiogenesis is regulated by a variety of molecules, including vascular endothelial growth factor(VEGF), platelet-derived growth factor (PDGF), placental growth factor (PGF), epidermal growth factor (EGF), angiopoietin (ANG), transforming growth factor β (TGF-β), and tumor necrosis factor α (TNF-α) [51]. These growth factors work synergistically with a large number of MMPs to promote angiogenesis.

MSCs-Exo has special properties that depend on the type of stem cell, so the appropriate stem cell type is important in inducing angiogenesis. For odontogenic stem cells, the most common assessment of regenerative potential and pro-angiogenic capacity are DPSCs, PDLSCs, SHEDs and GMSCs. Stimulated by LPS, the mRNA expression levels of VEGF and KDR in DPSCs-Exo are significantly increased [52]. Similarly, SHED-Exo significantly enhances blood vessel formation in a time- and dose-dependent manner. After co-culture of SHED and SHED aggregate exosomes (SA-Exo), VEGF, angiogenin and PDGF expression levels in SHED treated with SA-Exo were significantly upregulated [53]. PDLSCs-Exo can promote angiogenesis in HUVECS by upregulating the expression of the vascular-specific markers CD31 and VEGF-A[54]. In vitro studies have shown that isolation of GMSCs-exo and binding to hydrogel/chitosan scaffolds results in epithelial growth and angiogenesis at the wound site in rat models [55].

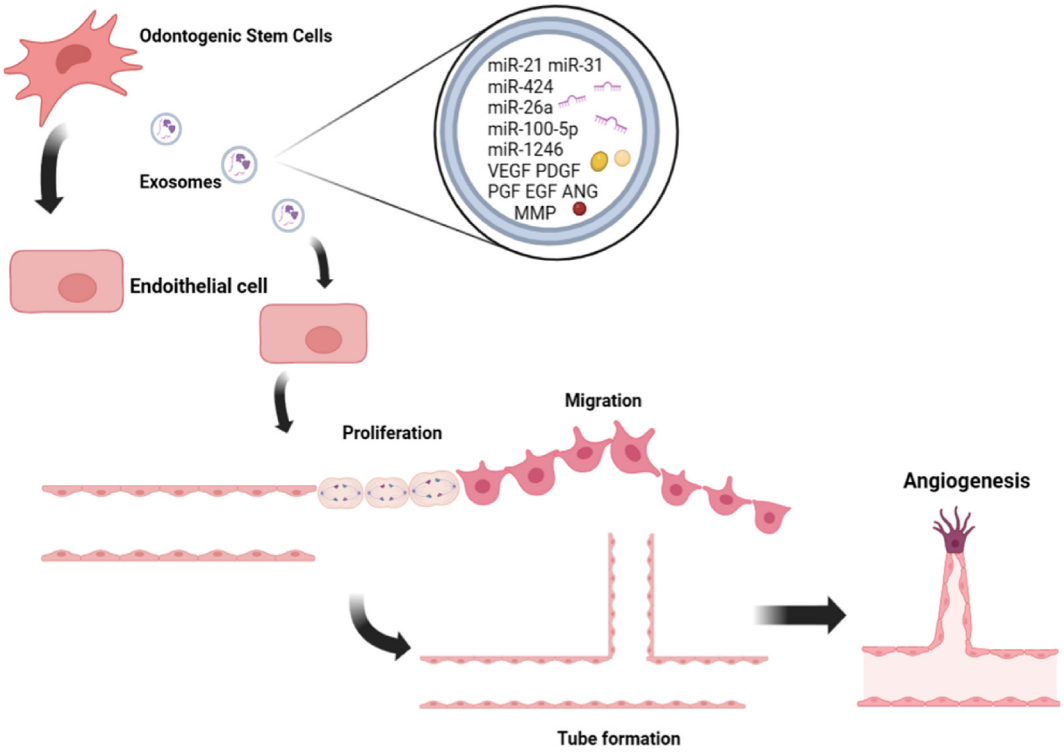

Fig. 2. Angiogenesis of exosomes. In addition to direct differentiation into ECs involved in angiogenesis, odontogenic stem cells can also be represented by paracrine. Odontogenic stem cells indirectly promote angiogenesis by releasing exosomes carrying pro-angiogenic factors and microRNAs, regulating the proliferation and migration of ECs.

3.2.2. MicroRNA

miRNAs are a group of small non-coding RNAs that do not encode proteins themselves, but they can bind to the 3′ untranslated region of the target mRNA to induce mRNA cleavage or inhibit its transcription, thereby acting as a negative regulator of the gene of interest. miRNAs are important regulators of function of ECs and angiogenesis. In fact, during angiogenesis, MSCs-Exo can release several miRNAs with pro-angiogenic abilities to promote angiogenesis in tissue repair [56,57], such as miRNA-31, miRNA-21, etc. In vitro studies have shown that miRNA-424 may play a negative regulatory role in ECs differentiation and regeneration of human dental pulp cells. Overexpression of miR-424 inhibits ECs differentiation, while inhibition of miR-424 expression promotes angiogenesis [58]. MSCs-Exo has been shown to promote vascularization via the p38 MAPK signaling pathway [34]. MiR-26a transferred from SHED aggregate exosomes (SA-Exo) promotes angiogenesis via the TGF-β/SMAD2/3 signaling pathway, thereby promoting pulp regeneration [53]. It is worth mentioning that miR-100-5p and miR-1246 enriched in SHED-Exo and transferred to endothelial cells can also inhibit angiogenesis by downregulating the expression of VEGFA, while the opposite mechanism of action exhibited is still unclear [59]. As a result, miRNAs are able to regulate VEGF in multiple ways and thus participate in VEGF-mediated angiogenesis [60].

3.3. Neuroprotection and neuroregeneration

A series of biological changes occur after nerve damage, including clearance of damaged axons and myelin sheaths and proliferation of SCs. Nerve regeneration after injury refers to the growth of neurites, especially neuronal axons after damage, to re-establish synaptic structures with target tissues and restore physiological functions. Exosomes are involved in a range of mechanisms in post-injury neuroprotection and neuroregeneration, including activation of SCs, axon regeneration, angiogenesis and inflammation regulation (Fig. 3).

3.3.1. Activation of SCs

SCs are important glial cells of the peripheral nervous system, and their migration and proliferation play an important role in neural repair [61]. When peripheral nerves are damaged, SCs undergo dedifferentiation, synaptic transmission, axon regeneration and other reactions. At the same time, SCs also secrete various cytokines, growth factors, neurotrophic factors and extracellular matrix molecules, thereby promoting the survival of neurons [62]. Exosomes have been shown to promote peripheral nerve regeneration by regulating the biological function of SCs. Studies have shown that GMSCs-Exo, after being engulfed by SCs, can significantly promote SCs proliferation, DRG axon growth, and promote myelination to repair peripheral nerve damage in rats [63]. MiRNAs contained in exosomes may be the main effector molecules, influencing gene expression of SCs through post-transcriptional mechanisms in response to nerve damage. Qin et al. confirmed that in vitro GMSCs-Exo promotes the expression of SCs dedifferentiation/repair phenotype-related genes, restoring function through activation of SCs during regeneration [64]. Thus, exosomes can exert their neuroprotective functions through activation of SCs [65].

3.3.2. Axon regeneration

Axon regeneration is a fundamental process of nerve repair. During peripheral nerve repair, axons express various adhesion molecules that promote the migration of SCs. In addition, SCs dedifferentiation relies on axon contact, and in the absence of axon contact, the regenerative capacity of SCs decreases and the number of cells decreases, so accelerating axon regeneration is critical for neural repair [66]. MSC-Exo promotes axon regeneration by

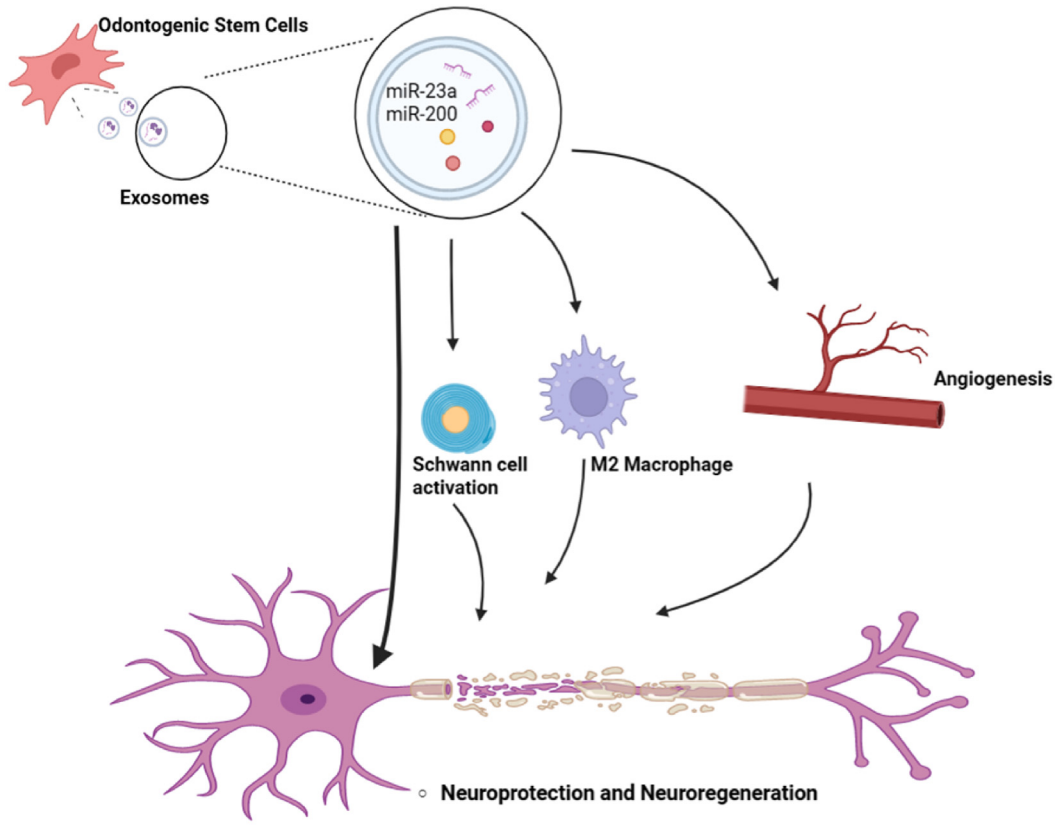

Fig. 3. Neuroprotection and neuroregeneration. Odontogenic stem cells are derived from the neural crest, so they have good neurogenetic potential. Exosomes derived from odontogenic stem cells can play neuroprotective and neuroregenerative roles similar to stem cells through a series of interactions, such as axon regeneration, activation of SCs, angiogenesis and inflammatory regulation.

carrying miRNAs and growth factors. Exosome-specific expression of miRNAs, such as miRNA-23a, miRNA-200, miRNA-133b overexpression is involved in phenotypic transformation, axon growth and macrophage migration of Schwann cells.

3.3.3. *Inflammatory regulation*

The inflammatory response is an essential process for nerve repair [67]. After peripheral nerve injury, SCs are activated to release chemokines and pro-inflammatory cytokines through a pro-neuroinflammatory response. Subsequent aggregation of macrophages and some peripheral immune cells to the site of injury accelerates further clearance of myelin sheath and axon debris [68]. MSCs-Exo has similar anti-inflammatory effects to parent cells, which plays an important role in nerve repair and regeneration. MSCs-Exo contains transforming growth factor-β, interferon γ, prostaglandin E2 and other inflammatory regulators, which are involved in the process of mediating the immune inflammatory response. Exosomes also alter the polarization of macrophage M1 to M2 phenotypes, which helps to inhibit excessive inflammatory responses. There is evidence that GMSCs-Exo regulates the inflammatory response by secreting biologically active factors with immunosuppressive and anti-inflammatory functions, exerting powerful therapeutic potential in peripheral nerve injury [69]; DPSCs-Exo inhibits inflammation by reducing protein expression of IL-6, IL-1β, and TNF-α to slow brain edema and nerve damage in mice [70]. In addition, there is a close relationship between angiogenesis and nerve repair [71] More and more evidence shows that exosomes can participate in angiogenesis of damaged peripheral nerves, which is conducive to tissue regeneration and repair of the nervous system [65]. For example, LPS-treated DPSC-EXO has been shown to promote the proliferation, migration, and tube-forming capacity of HUVECs in vitro by altering miRNA expression profiles and levels of VEGF, thereby promoting angiogenesis [52]; PDLSC-Exo and GMSC-Exo also play an important role in angiogenesis [14,54].

Odontogenic stem cells are derived from neural crest, so they seem to have more prominent neurogenesis potential than stem cells from other sources [9]. Similarly, exosomes derived from odontogenic stem cells can play a tissue repair and regeneration function similar to stem cells, which can deliver a variety of neurotrophic factors, proteins and miRNAs to axons, restore microenvironment homeostasis, and show stronger nerve regeneration and neuroprotection [72]. In summary, exosomes, as key molecules for intercellular communication, are involved in a series of interacting mechanisms in the process of neural repair, including axon regeneration, activation of SCs, angiogenesis and inflammation regulation, which is conducive to the establishment of the microenvironment required for peripheral nerve repair. The vascularization and neuralization of the pulp allow the tooth to produce a protective response to external stimuli such as machinery, temperature or chemistry, and supports the formation of dentin. Although exosomes have been poorly studied in promoting pulp nerve regeneration, existing studies have shown that odontogenic exosomes have strong therapeutic potential in pulp nerve protection and regeneration.

3.4. *Immune regulation and anti-inflammation*

Inflammation has been underestimated in pulp healing and regeneration, and in the past was only considered an undesirable effect. There is now evidence that pulp inflammation is a prerequisite for the regeneration of the dentin-pulp complex [73]. Early

studies attributed the anti-inflammatory and immunosuppressive effects of MSCs to direct contact with immune cells [74]. However, MSCs survive less than a week after systemic administration, but their therapeutic effect persists, suggesting that the immune function of MSCs is at least partly due to its paracrine effects, and exosomes may be the main active substance [75,76]. Similarly, exosomes derived from odontogenic stem cells have also shown properties such as anti-inflammatory and immunomodulatory properties. DPSCs-Exo have been shown to have stronger immunomodulatory abilities than BMMSCs-Exo, which may be beneficial in the treatment and prevention of pulp inflammation [77]. Exosomes derived from odontogenic stem cells exerts anti-inflammatory immunomodulatory effects mainly through four aspects: 1. Reduce inflammatory response; 2. Regulate macrophage phenotype and function; 3. Regulate T cells and B cells; 4. Regulate DC phenotype and NK proliferation (Fig. 4).

3.4.1. Macrophages

When the dentin-pulp complex is stimulated externally, the immune response is activated, and the relative immune cells are concentrated at the site of inflammation by the chemotaxis of chemokines secreted by macrophages. Macrophages play an important role in the inflammatory environment and tissue regeneration. The transformation of macrophages from pro-inflammatory M1 to anti-inflammatory M2 is essential for tissue regeneration. M1 macrophages produce pro-inflammatory factors such as TNF-α, IL-6 and IL-1β to promote inflammation, and M2 macrophages produce IL-10, TGF-β and other anti-inflammatory factors to relieve inflammation. Exosomes derived from odontogenic stem cells exert its anti-inflammatory and immunomodulatory effects by modulating the transformation of macrophages from pro-inflammatory M1 or anti-inflammatory M2 phenotypes. Recent studies have shown that miRNAs contained in exosomes play an important role in regulating the phenotype of macrophages. DPSCs-Exo can promote the transformation of macrophage M1 to M2 type, which is achieved by DPSCs-Exo by inhibiting the TLR and NFκB pathways. The high expression of miR-125a-3p in exosomes inhibits of NFκB and TLR pathways by targeting IKKBB, while DPSCs-Exo and the miR-125a-3p contain promote macrophages to release BMP-2, which in turn activates the odontogenic differentiation of DPSCs through the BMP-2 pathway [78]. The transformation of macrophages from M1 phenotype to M2 phenotype in pulp tissue creates a suitable microenvironment for repair and regeneration [79]. Since exosomes contain a large number of bioactive molecules, such as proteins, mRNA, miRNAs, and lipids, MSCs may produce more immunoreactive exosomes under inflammatory stimulation. There is substantial evidence that the microenvironment modulates the effects of exosomes on macrophages. For example, under inflammatory conditions, GMSCs-Exo can promote the transformation of macrophages from M1 phenotype to M2 phenotype and reduce the pro-inflammatory factors produced by M1 macrophages [80]; GMSCs-Exo inhibits the inflammatory response of M1 macrophages and reduces lipid accumulation in M1 macrophages in a high-lipid microenvironment [81].

3.4.2. T cells and B cells

T lymphocytes are the main effector cells of acquired immunity and are involved in multiple immune response processes. Differentiated and mature in the thymus, the largest number is in

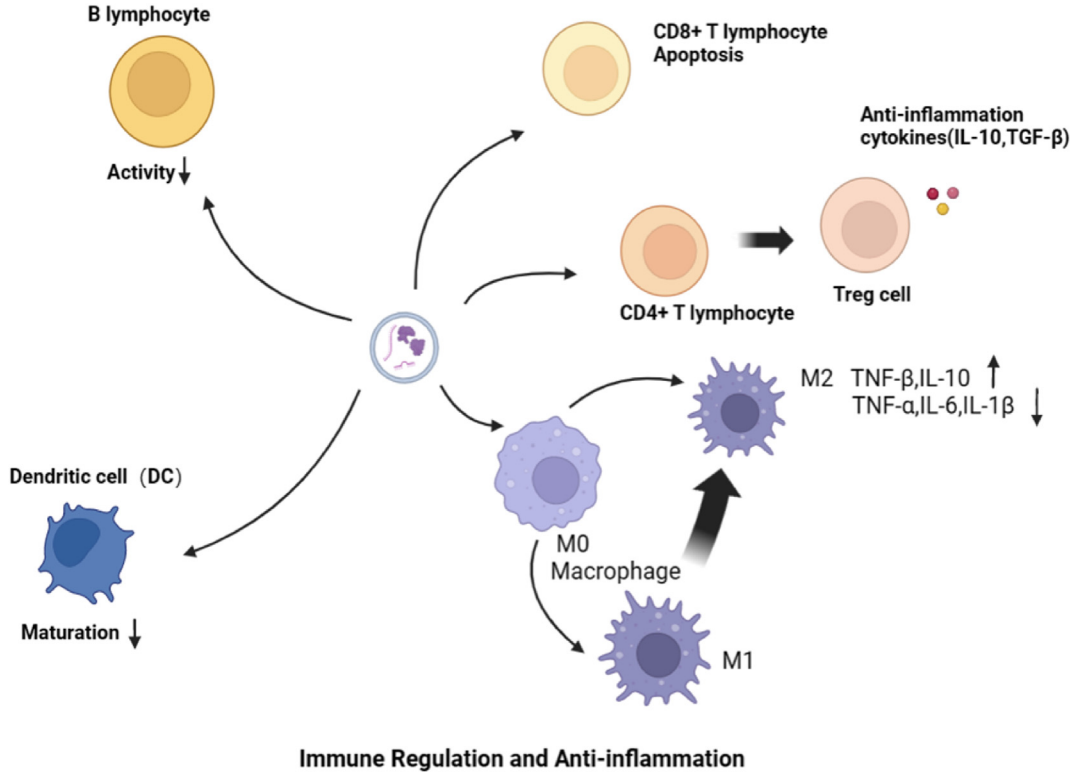

Fig. 4. Immune regulation and anti-inflammation. Exosomes derived from Odontogenic stem cells also have immunomodulatory and anti-inflammatory effects. Odontogenic stem cell-derived exosomes promote the transformation of macrophages to the M2 type, which produces anti-inflammatory factors to relieve inflammation. What's more, when stimulated by inflammation, stem cells may produce more immunoreactive exosomes. Exosomes can inhibit B lymphocyte differentiation, activation and proliferation, induce apoptosis of $CD8^+$ T cells, and promote the transformation of $CD4^+$ T lymphocytes to Tregs. In addition, exosomes can also inhibit lymphocyte activity, promote the secretion of anti-inflammatory factors, and reduce the production of promoting factors by inhibiting dendritic cell maturation.

lymphocytes. According to the different expression of CD4 and CD8, T cells can be divided into $CD4^+$ T lymphocytes and $CD8^+$ lymphocytes. Exosomes can play similar biological roles to parental cells, inhibiting the differentiation, activation and proliferation of T cells, inducing T cell apoptosis, and promoting the production of Treg [82]. Studies have shown that SCAPs-Exo can promote the transformation of $CD4^+$ T cells to Tregs through exosomes in the paracrine pathway, promote the secretion of the anti-inflammatory cytokine IL-10, regulate the immune microenvironment, and effectively reduce pulp inflammation [83,84]. Similarly, DPSCs, as an important stem cell involved in the regeneration of the dentin-pulp complex, DPSCs-Exo reduces the secretion of pro-inflammatory factors IL-17 and TNF-α by inhibiting the differentiation of $CD4^+$ T cells into T helper 17 cells (Th17), while promoting the polarization of $CD4^+$ T cells into Treg, and increasing the release of anti-inflammatory factors IL-10 and TGF-β [74]. In addition, PDLSCs-Exo exert anti-inflammatory effects by regulating microRNA-155-5p to mediate Th17/Treg balance [85]. However, B lymphocytes are not detected in normal pulp, and the number of T and B lymphocytes increases in inflammatory pulp, so B lymphocyte-mediated humoral immunity does not participate in the early immune response of the local pulp. B lymphocytes can activate complement to function in the immune response by secreting antibodies. There is evidence that odontogenic stem cells, such as GMSCs, inhibit B cell proliferation, differentiation, and activation in vivo and in vitro [86]. BMMSCs-Exo regulates cell function by influencing mRNA expression in B lymphocytes [87]. However, studies have also shown that the regulation of B cells by mesenchymal stem cells is independent of extracellular vesicles [88]. Although the ability of odontogenic stem cell-derived exosomes to inhibit B-lymphocyte-mediated immune responses has not been well understood, the potential of their immunomodulation remains promising.

3.4.3. Dendritic cells

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs), which can effectively stimulate the activation of T lymphocytes and B lymphocytes. In the dentin-pulp complex, DCs are often arranged along blood vessels, concentrating around the blood vessels of the internal pulp and next to the dentin cell layer on the periphery of the pulp. Some cytoplasmic protrusions protrude into the dentin tubules and perform immune surveillance functions [89,90]. Several reports have shown that MSCs-Exo are able to inhibit dendritic cell maturation, inhibit lymphocyte activity, promote the secretion of IL-10 and TGF-β, and reduce the secretion of IL-6 [91,92]. This means that exosomes have the fate of regulating DC, exerting immunomodulatory and anti-inflammatory effects.

The dentin-pulp complex has its unique immune microenvironment and immune defense system, containing complex cell composition. Exosomes derived from odontogenic stem cells has immunomodulatory properties and low immunogenicity [93]. Relying on the immunomodulatory and anti-inflammatory effects of exosomes derived from odontogenic stem cells, an appropriate immune microenvironment can be created in the root canal to promote the regeneration of the dentin-pulp complex.

4. Application of exosomes in regeneration of dentin-pulp complex

Exosomes can be combined with biomaterials for application and retention in damaged tissues, especially in the field of tissue engineering, such as collagen, matrix, chitosan, hydrogel, etc. Exosomes bind to matrix proteins and thus to biological materials. Some biomaterials change the release rate of exosomes by adjusting molecular weight, crystallinity or mixing ratio to maintain stability, which helps exosomes to function. Choosing the right scaffold to deliver exosomes to the site of action is important. The geometry and mechanical properties of the stent can influence behavior and adhesion of cell to the scaffold surface [94].

Experiments in animal models have shown that combining exosomes to collagen membranes is used in root canal therapy. DPSCs-Exo and DPSCs-OD-Exo can induce pulp-like tissue regeneration in tooth root section models [34]. Exosomes derived from epithelial root sheaths are loaded in the collagen gel, which can play a role in increasing the survival of papilla cells. In root sections, epithelial root sheath cell-derived exosomes attach to collagen gels and are slowly endocytosis and release by papillary cells, thereby promoting the regeneration of restorative dentin, vessel and nerve [46]. In addition, exosomes bind to type I collagen and fibronectin in a dose-dependent and saturated manner, and this connection can be degraded by RGD peptide integrin [34]. Swanson et al. designed an amphiphilic polymer carrier synthesized from a triblock copolymer and placed in a model of pulp incision in rat molars. Encapsulate exosomes by polymer self-assembly with slow release at the site of action for 8–12 weeks. The results show that after six weeks, the controlled release of exosomes derived from odontogenic stem cells leads to the formation of restorative dentin, and it is more effective than the use of glass ionomer cement in vivo [33].

In addition to the above methods, exosomes can be directly dissolved in PBS/normal saline and injected into the site of action, or directly injected intravenously after dissolution to enter the blood circulation and act on the site of injury [95]. Exosomes are small in size and can avoid phagocytosis by monocytic macrophages. Compared with liposomes, exosomes have better biocompatibility and lower immunogenicity, are well tolerated, and can be repeatedly injected without significant side effects.

5. Discussion and future outlook

Functional pulp is indispensable for the long-term generation of teeth, and ideal pulp-dentin complex regeneration should achieve vigorous regeneration of the whole tooth and restore the normal function of the tooth [96]. Regeneration strategies for the dentin-pulp complex can be achieved with cell therapy or cell-free therapy. However, the clinical translation of MSCs is limited by storage, transportation, and ethical issues. As a current hot spot, exosomes have received extensive attention from researchers at home and abroad. Current evidence suggests that exosome-based cell-free therapies are superior to cell therapies. Compared with odontogenic stem cells, odontogenic stem cell-derived exosomes have the advantages of strong drug carrying ability, high specificity, low immunogenicity, good biocompatibility, easy to obtain, small side effects, and nanoscale size [97]. Odontogenic stem cell-derived exosomes play an important role in enhancing receptor cell function, such as proliferation, dentinogenesis/osteogenic differentiation, anti-inflammatory, etc. Exosomes are expected to replace stem cells in clinical practice in the future. Notably, there is evidence that donor age, health, sex, number of parental cell passages, cell culture conditions, etc. are critical to function [98,99].

In addition to tissue repair and regeneration, exosomes can also be used as drug carriers to achieve targeted drug delivery. Exosomes have the ability to load “cargo” and deliver them to target cells for cell-to-cell communication. The phospholipid bilayer of exosomes protects the contents from rapid decomposition and crosses the blood-brain barrier [100]. Exosomes carrying drugs can be used in the treatment of cardiovascular and cerebrovascular diseases, autoimmune diseases, neurodegenerative diseases. In recent years, artificial exosomes with higher drug loads have been

prepared to overcome the shortcomings of natural exosomes in clinical applications. Artificial exosomes have high drug-carrying capacity, high targeting, similar physicochemical and biological characteristics to natural exosomes, and have higher yields than natural exosomes [101].

However, there is no standardized isolation method for exosomes, and there are disadvantages such as insufficient number of exosomes. The two major technical obstacles restricting the basic research and application of exosomes include: (1) how to simplify the process of exosome extraction and improve the extraction efficiency; (2) How to effectively distinguish exosomes from other extracellular vesicles. In recent years, a combination of several separation methods has been used to improve efficiency. In addition, the mechanisms of exosome secretion and fusion have not been clarified [25]. Regarding the use of exosomes, dose control is critical. There are two routes of administration of exosomes, one is to load exosomes into biomaterials, such as collagen membranes, hydrogels, gelatin sponges, PLA scaffolds, etc., so as to slowly release them to the site. Studies have shown that after dissolution of exosomes directly subcutaneously or intravenously, exosomes can be quickly removed from the blood, accumulate in the stomach, liver, intestine, and finally be absorbed and excreted by macrophages [102]. For topical application of exosomes, it may be removed by external factors more quickly, and sufficient time of action cannot be guaranteed.

Currently, there is still insufficient evidence to evaluate and compare the molecular differences, biological functions, and clinical applications between odontogenic exosomes of different origins, and between odontogenic stem cells-derived exosomes and exosomes derived from other types of mesenchymal stem cells. There is no standardized unification for the isolation, storage, transportation and large-scale production of exosomes derived from odontogenic stem cells, and unnecessary tissues such as the third molar cannot be effectively and stably stored for a long time. Therefore, the clinical application of exosomes derived from odontogenic stem cells cannot be promoted on a large scale. In order for the exosomes to play an ideal role, the dose of exosomes, the treatment method of the parental cells of exosomes and the efficient purification need to be further studied.

6. Conclusion

In conclusion, exosomes derived from odontogenic stem cells have many advantages in biocompatibility, drug loading ability and other aspects. Exosomes derived from odontogenic stem cells can regulate many important biological processes, including dentinogenesis, angiogenesis, neuroprotection and immune regulation, and can be used to regenerate the dentin-pulp complex. Although exosomes have become the focus of research in the past decade, their role in the regeneration of dentin-pulp complex is still in its infancy. There are many challenges to be overcome before exosomes derived from odontogenic stem cells can be widely used in the clinic.

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Declaration of competing interest

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