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REVIEW ARTICLE

MicroRNAs Function in Dental Stem Cells as a Promising Biomarker and Therapeutic Target for Dental Diseases

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Abstract

Undifferentiated, highly proliferative, clonogenic, and self-renewing dental stem cells have paved the way for novel approaches to mending cleft palates, rebuilding lost jawbone and periodontal tissue, and, most significantly, recreating lost teeth. New treatment techniques may be guided by a better understanding of these cells and their potential in terms of the specificity of the regenerative response. MicroRNAs have been recognized as an essential component in stem cell biology due to their role as epigenetic regulators of the processes that determine stem cell destiny. MicroRNAs have been proven to be crucial in a wide variety of molecular and biological processes, including apoptosis, cell proliferation, migration, and necrocytosis. MicroRNAs have been recognized to control protein translation, messenger RNA stability, and transcription and have been reported to play essential roles in dental stem cell biology, including the differentiation of dental stem cells, the immunological response, apoptosis, and the inflammation of the dental pulp. Because microRNAs increase dental stem cell differentiation, they may be used in regenerative medicine to either preserve the stem cell phenotype or to aid in the development of tooth tissue. The development of novel biomarkers and therapies for dental illnesses relies heavily on progress made in our knowledge of the roles played by microRNAs in regulating dental stem cells. In this article, we discuss how dental stem cells and their associated microRNAs may be used to cure dental illness.

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Graphical Abstract

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PEER-REVIEWED FEATURE

MicroRNAs function in dental stem cells as a promising biomarker and therapeutic target for dental diseases

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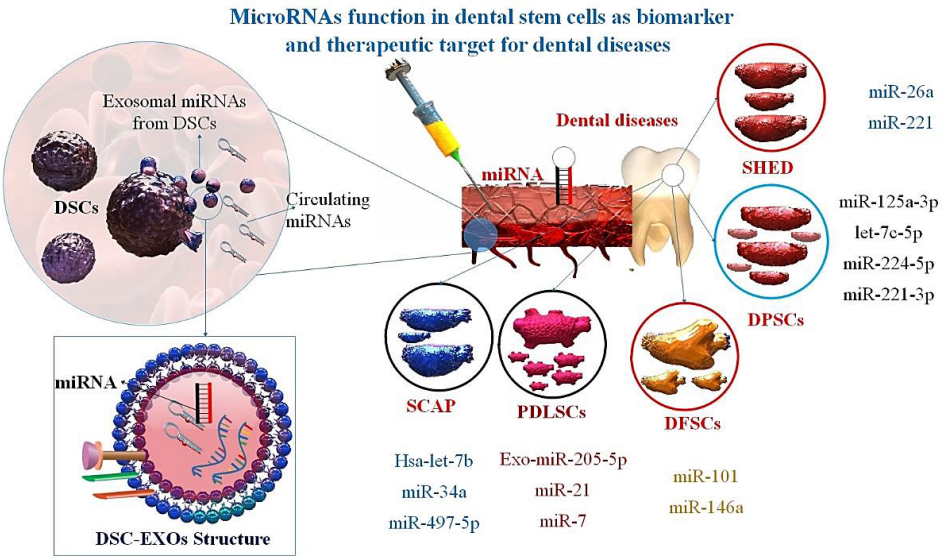


MiRNAs have various functions in the regulation of DSCs and understanding these roles of is crucial for the development of new therapeutics in dentistry. The widespread use of such methods in the future will alter how many dental syndromes and disorders are diagnosed and treated.

[DSCs: dental stem cells]

Key Points

Dental stem cells have various capabilities, including the ability of self-renewal, mesenchymal stem cell characteristics, multi-lineage differentiation, and immunomodulation, making them promising biological tools for the treatment of dental diseases.

Regulating the expression of functional microRNAs in dental stem cells through mimicking microRNAs or suppressing microRNAs can be used as a therapeutic tool.

MicroRNAs have several roles in the regulation of dental stem cells, and the recognition of these functions is essential for the development of novel therapeutics and diagnostic methods in dental diseases.

1 Introduction

In treating a wide range of diseases, the therapeutic use of stem cells (SCs) has shown to be an invaluable tool. Primitive SCs have the potential to self-renew and differentiate in several directions, allowing them to give rise to a wide variety of specialized cell types and tissues [1]. Two primary sources for this method, that have been utilized to treat a wide range of disorders are embryonic stem cells and mesenchymal stem cells (MSCs) [2]. Because of the many patients involved, dental care (including restoration, replacement, and repair) stands out from other clinical procedures. Furthermore, teeth contain stem cell populations that are remarkably proliferative and may be readily extracted from teeth that have been lost or removed surgically. Not only may these SCs be utilized for dental purposes such as repairing, replacing, and regenerating teeth, but they also have tremendous potential for usage in areas such as research into treatments for severe life-threatening disorders that rely on solutions based on SCs [3, 4]. Postnatal root creation, a significant provider of dental stem cells (DSCs), is a developmental process. As a result, the cells engaged in root development are more embryonic like than cells derived from other DSC sources. This is an advantage of teeth as a source of SCs that is frequently neglected, even though it is an essential benefit [5]. Human DSCs may be acquired from removed wisdom teeth, postnatal teeth, or exfoliated deciduous teeth [6]. Because of their availability and convenience, DSCs such as periodontal ligament stem cells (PDLSCs), dental pulp stem cells (DPSCs), SCs from the apical papilla (SCAP), SCs from human exfoliated deciduous teeth (SHED), and dental follicle stem cells (DFSCs) have become promising cell sources for bone and dental regeneration [7, 8] (Fig. 1). As a result of their inherent capacity for tissue-specific differentiation, self-renewal, and multipotency, DSCs have recently emerged as a promising tool for regenerating tooth and periodontal tissues. In light of this, DSC-based regenerative therapy offers a potentially valuable means by which to restore dental tissues that have been destroyed or to create new teeth [9, 10]. Additionally, in comparison to DPSCs, DSCs that have been extracted from exfoliated deciduous teeth have a significant ability for osteogenic regeneration and an elevated proliferation level. Pulp-derived DSCs were tested first and mainly for their capacity to differentiate into osteogenic, odontogenic, and neurogenic cells. The microenvironment of DSCs is crucial in modulating their inherent diversity [11]. After mild-to-severe dental trauma or carious lesions, DSCs in perivascular niches are attracted and encouraged to develop into odontoblast-like cells. During reparative dentinogenesis, a series of molecular processes play a role in mediating this differentiation [12]. The areas of tissue engineering and regenerative medicine and dentistry employ physiologically based treatment procedures for essential tissue regeneration and hence have the capacity to regenerate living tissues. An in-depth knowledge of the molecular signaling systems controlling average tooth growth helps develop methods to generate bioengineered substitute teeth [13] (Table 1).

Extracellular vesicles released by DSCs (DSCs-EVs) have recently been proposed as a cell-free therapeutic option for DSCs. Extracellular vesicles released by DSCs comprise exosomes, microvesicles, and apoptotic bodies. In addition, exosomes (EXOs) play a significant role in the medicinal properties of DPSCs by mediating the paracrine effect of extracellularly secreted components. Moreover, recipient cells take up EXOs by linking to the plasma membrane, attaching to the host receptor, and ultimately endocytosis. Exosomes comprise DNA, proteins, cholesterol, and RNA (circular RNA, miRNA, messenger RNA [mRNA], long non-coding RNA, and transfer RNA) [14, 15]. MicroRNAs are forms of short, single-stranded, endogenous non-coding RNAs that are highly conserved. They are responsible for primary regulatory activities in various cellular and physiological processes, including cellular proliferation, neoplastic cell transformation, differentiation, and cell regeneration. MicroRNA may control a wide range of physiological activities and pathological functions of DPSCs, they have been shown to play critical roles in various processes, including the differentiation of DPSCs into odontogenic cells, the immunological response, and the inflammation of the dental pulp, as validated by several studies [16–18]. The creation of novel therapies in regenerative dental hygiene relies on a thorough knowledge of the roles played by miRNAs in the modulation of distinct DSCs [19]. This article provides

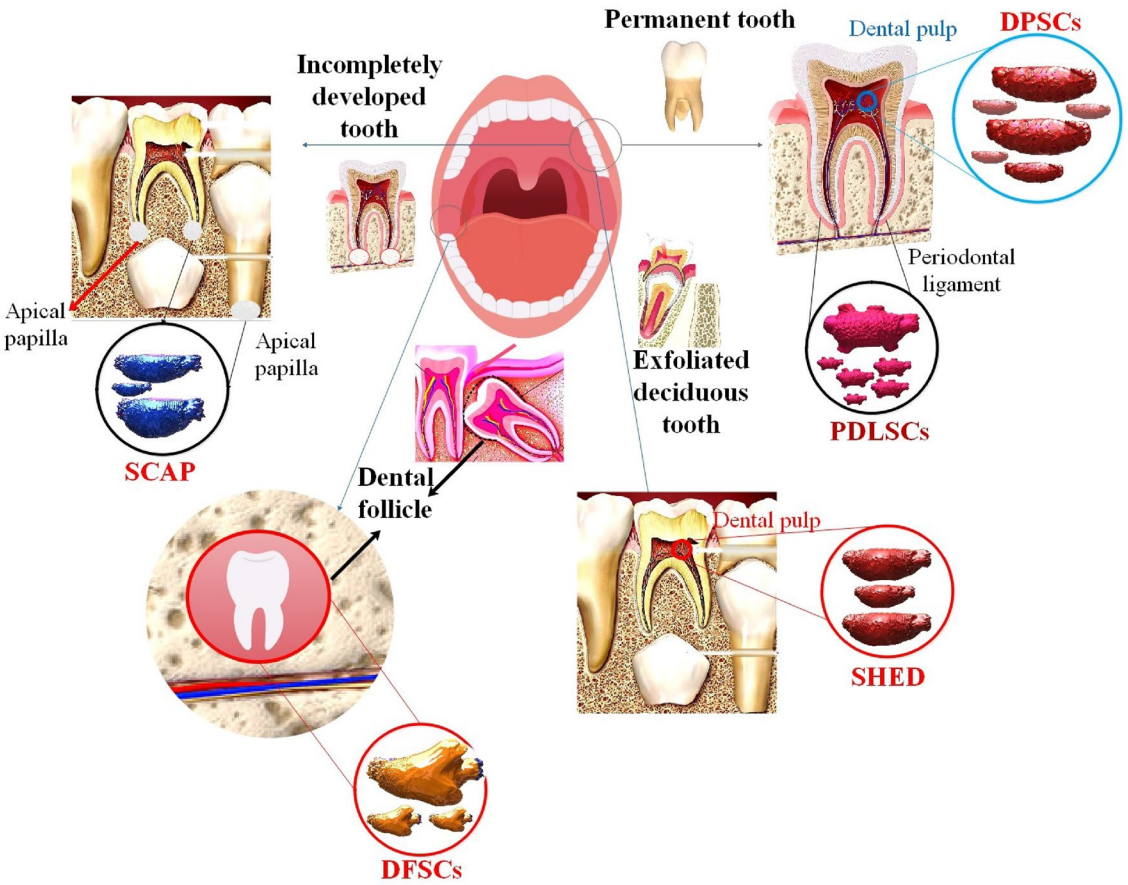


Fig. 1 Dental stem cells from a variety of populations and origins. Dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHEDs) can be derived from the internal dental pulp of permanent and deciduous exfoliated teeth, respectively. Stem cells from the apical papilla (SCAPs) can be isolated from the apical papilla; periodontal ligament stem cells (PDLSCs) can be isolated from the periodontal ligament; and dental follicle stem cells (DFSCs) can be isolated from the dental follicle. Dental follicle stem cells are a type of mesenchymal progenitor cells that surround the tooth germ

up-to-date information on the present state of miRNAs in different DSCs by summarizing the most recent research that supports the biomarker and potential health benefits of miRNAs in PDLSCs, DPSCs, SCAP, SHED, and DFSCs. Additionally, the unique features of these SCs and the current challenges to expanding this approach to dental disease detection and therapy are discussed.

2 Characteristics of Different Dental Stem Cells

2.1 Characteristics of Dental Pulp Stem Cells

The dental pulp is derived from the dental papilla and is a soft tissue with mesenchymal and ectomesenchymal origins. They originate at various points in the tooth's growth process [20]. The first MSCs isolated from human teeth were DPSCs, also known as DPSCs. These cells can develop into osteoblasts, odontoblasts, myocytes, chondrocytes, and adipocytes, as well as neurocytes, in vitro and in vivo [10]. Their natural purpose is to form odontoblasts, which are used to build reparative dentin and assist implementations in dentistry that include the regeneration of dental structures. Because of their high differentiation potential, ease of culture, and simple accessibility from excised teeth that would otherwise be thrown away, research into DPSCs is a promising new area [21–23].

2.2 Characteristics of Stem Cells Exfoliated from Deciduous Teeth

Stem cells exfoliated from deciduous teeth in humans, also known as SHEDs, are a form of MSC and provide an excellent cell source for regenerative therapy. They are highly proliferative, multipotent, and immunosuppressive while carrying a low risk of oncogenesis [24]. Stem cells exfoliated from deciduous teeth in humans are postnatal

Table 1 Various dental disorders and DSC therapeutic properties

DSCs	Characteristics	Therapeutic effects	References
DPSCs	Convenient to culture, with a high differentiation potential, and readily available from abandoned tooth extractions	DPSCs have the potential to transform into odontoblasts, making them useful for both immediate dental treatment and as an in vitro model system for testing and refining novel bioactive materials for use in prospective dental treatment (including bone, pulp, and periodontal regeneration)	[21, 22, 132–134]
PDLSCs	PDLSCs are a form of somatic stem cell that is capable of undergoing vigorous clonal self-renewal and displaying the capacity to develop into a wide variety of cell types	These stem cells are employed in restorative therapy in the periodontium and are a potential technique for periodontal regeneration. Reducing proinflammatory cytokines and increasing anti-inflammatory cytokines lead to a reduction in inflammation and an inhibiting of the immune response	[30, 31, 135]
SHED	These stem cells are highly proliferative, and multipotent and inhibit immune system function with a low risk of oncogenesis	SHED could be utilized to regenerate dental pulp tissue. In vitro experiments showed that SHED was able to adhere to dentin and multiply inside root canals	[24, 136]
SCAP	Dental root-forming stem cells are a distinct population of stem cells with prospective applications in regenerative endodontics and other areas of dentistry	The formation and regeneration of the pulp tissue in open-apex permanent teeth depend on SCAP. SCAP is a potential stem cell source for dental pulp regeneration because it can develop into primary odontoblasts and dental pulp cells, which create root dentine and dental pulp	[137, 138]
DFSCs	DFSCs have been described as a population of heterogeneous cells that might include cellular progenitors for the periodontal development process	During tooth development, periodontal ligament, cementum, and alveolar bone are all produced by DFSCs, a population of mesenchymal progenitor cells that surround the tooth germ	[36, 37, 139]

DFSCs dental follicle cells, *DSCs* dental stem cells, *PDLSCs* periodontal ligament stem cells, *SCAP* stem cells from the apical papilla, *SHED* stem cells from human exfoliated deciduous teeth

stem cells that may differentiate into several other cell types, including bone and tooth-forming osteoblasts, fat-storing adipose cells, and nerve cells [25]. Stem cells exfoliated from deciduous teeth in humans may be a promising cell source for future therapeutic uses because of their increased proliferative capacity, substantial cell source, and the convenience of stem cell harvest with little invasion [26]. In vitro studies demonstrated that SHED was capable of adhering to the dentin walls and proliferating within the root canals [27].

2.3 Characteristics of Stem Cells from the Periodontal Ligament

Stem cells from the periodontal ligament, known as PDLSCs, may be found in the perivascular area of the periodontium. These cells have similar properties to MSCs and show great promise for use in periodontal regeneration. Success in isolating multipotent PDLSCs from human-impacted third molars was reported in 2004 by Seo et al. [28], and these cells showed the ability to develop into periodontal ligaments, alveolar bone, cementum, peripheral nerves, and blood vessels [29]. Consequently, PDLSCs are an essential stem cell type for periodontium regenerative treatment; however, their scarcity hinders the development of scientific and clinical studies [30, 31].

2.4 Characteristics of Apical Papilla Stem Cells

Apical papilla stem cells (SCAPs), a distinct population of DSCs associated with root development, have shown great promise as a regenerative endodontic technique. Investigations have determined the phenotypic properties and other regenerative capabilities of SCAPs. Specific SCAP subpopulations have been highlighted, as well as their neurogenic and angiogenic attributes [32]. As SCAP was shown to be expressed by the stem cell marker *STRO-1*, providing strong evidence for the hypothesis that SCs exist in the apical tissues of teeth [33]. To a large extent, this papilla tissue represents the apical portion of the previously recognized dental papilla, which served as the mesenchymal counterpart in the epithelial-mesenchymal interaction process that resulted in tooth creation [32]. Apical papilla stem cells are a potential stem cell source for dental pulp regeneration because they can develop into primary odontoblasts and dental pulp cells, which create root dentine and dental pulp [32, 34]. As the apical tissues are made to bleed, SCAP transit with the blood and deposit in the blood clot formed within the disinfected

canal, where they aid in the regeneration of the dentin pulpal organ and hasten the tooth's development [35].

2.5 Characteristics of Dental Follicle Cells

Dental follicle cells (DFSCs) are a type of mesenchymal progenitor cells that surround the tooth germ. These cells are crucial for creating cementum, periodontal ligament, and alveolar bone throughout the process of odontogenesis. When guiding tooth eruption and root formation, DFSCs rely on cascades of signaling routes and transcriptional factors. The first DFSCs were isolated from the mouse molar area in 2002 and were coaxed to develop into an osteoblast phenotype in vitro using exogenous bone morphogenetic protein 2. Since then, it has been observed that under certain induction circumstances, DFSCs may develop into various cell types, including cement oblasts, osteoblasts, chondrocytes, adipocytes, and neuron-like cells [36, 37]. Additionally, it is postulated that DFSCs may develop into cementum, intrinsic alveolar bone, and the periodontal ligament [38].

3 The Biogenesis and Function of MicroRNAs

Inhibiting protein translation by binding to the 3′-untranslated region (UTR) of the target mRNA, microRNAs (miRNAs) are short non-coding RNAs that typically range in length from 22 to 25 nucleotides [49, 50]. They play a crucial role in cellular communication and are seen as potentially valuable indicators and treatment tools across various disorders. One way miRNAs are used in medicine currently is to block carcinogenic miRNAs using miRNA antagonists, notably antimiRs and antagomiRs. Another way is to introduce tumor suppressor miRNAs, using synthetic miRNA imitators or vector-based transfection of genomes coding for miRNAs [50, 51]. The expression patterns of human miRNAs have shown that a large number of miRNAs are dysregulated in a variety of disorders and have demonstrated that normal cells and diseased tissues express miRNAs differently. For this reason, miRNA profiling is used to generate disease-specific signatures, with the expectation that this profile will contribute to developing miRNA-based molecular prognosis, diagnosis, and treatment [52–54]. The classic microRNA synthesis pathway is that first, microRNAs are transcribed in the nucleus under the action of RNA polymerase (Pol) into cap-structured primary transcripts, namely pri-miRNAs. When pri-miRNAs are exposed to the endonuclease Drosha and the cofactor double-stranded RNA-binding protein DGCR8, the resulting hairpin precursor microRNAs have roughly 70 nucleotides. Once Exportin5 delivers the pre-miRNA to the cytoplasm, Dicer endoribonuclease performs a clipping reaction to create double-stranded miRNAs of around 22 nucleotides. Double-stranded miRNAs contain unstable 5′ ends and make the miRNA-RISC complex by binding with the miRNA-induced silencing complex. Meanwhile, the other strand of the miRNA is degraded by hydrolases [55] (Fig. 2). Proliferation, migration, cell cycle, apoptosis, and tissue formation are only some of the cell biological processes demonstrated to be regulated by miRNAs. MicroRNAs shed light on molecular methods of post-transcriptional control beyond those of transcription components [56–58]. More recently, miRNAs have been identified as essential translation regulators, which may play a role in determining the behavior and destiny of SCs [59, 60]. MicroRNAs were recently shown to regulate SC transformation by the post-transcriptional targeting substances involved in stem cell upkeep [61].

4 The Role of MicroRNAs in Dental Stem Cells

Several investigations in which miRNA profiling of DSCs from diverse sources of the mature and immature teeth, such as pulp, periodontal ligament, follicle, and apical papilla tissues, have been made to define the function of particular miRNAs in different roles, which may be significant for DSC biology. MicroRNAs substantially regulate several biological procedures, including apoptosis, proliferation, stem cell function, and survival. All these agents make miRNAs perfect for regulating gene regulation DSC differentiation [62]. Aberrant expression of miRNAs has been reported in DPSCs compared with bone marrow MSCs, which are considered a significant cell source for regenerative medicine; additionally, ectopic expression of particular miRNAs may control the DPSC phenotype and DPSC proliferation and differentiation. Although these miRNAs perform substantial roles in DPSC biological progressions, possible mechanisms that influence DPSCs remain under study [63]. For example, miR-142-3p suppressed Serum/Glucocorticoid Regulated Kinase 1 (*SGK1*) expression. *SGK1* overexpression stimulated human PDLSC proliferation and osteogenic differentiation and reversed the inhibitory role of miR-142-3p on human PDLSCs. Thus, miR-142-3p suppresses osteogenic differentiation of human PDLSCs by decreasing *SGK1* expression [64].

MicroRNAs perform as the primary regulator of SCAP differentiation. For example, miR-143-3p preserved the stemness of human SCAPs and controlled their differentiation negatively by targeting nuclear factor I-C. Therefore, suppressing this miRNA represents a possible approach to improve the renewal of damaged tooth roots [65].

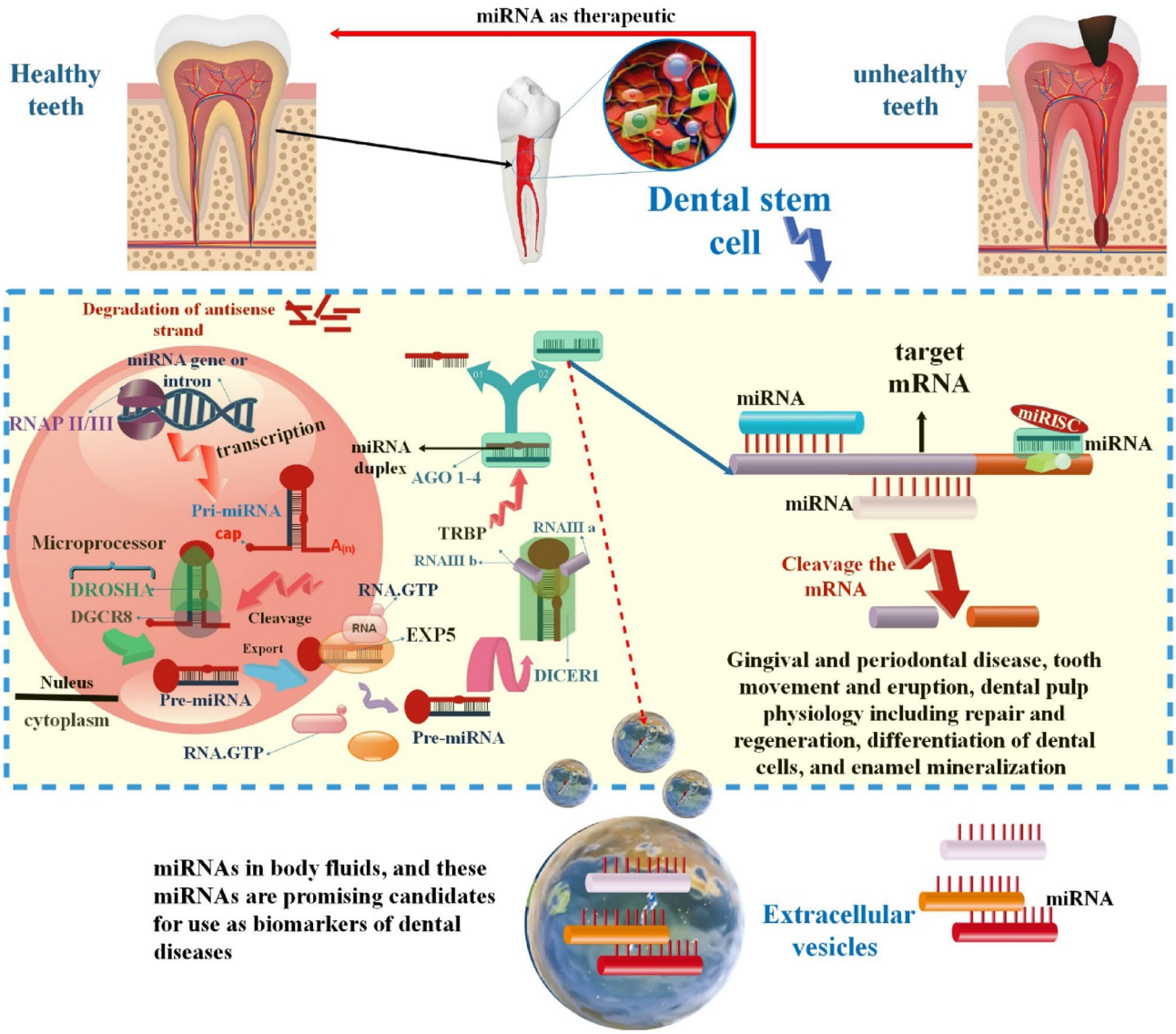


Fig. 2 MicroRNA (miRNA) biogenesis in dental stem cells. A triple mixture structured in the cytoplasm and consisting of Dicer, TRBP, and the tiny miRNA duplexes is responsible for cleaving the pre-miRNA loop. Via extracellular vesicles, miRNAs are transported from donor cells to recipient cells, where they bind to their target messenger RNAs (mRNAs) to regulate gene expression. Moreover, it has been revealed that miRNAs can communicate per se with proteins [127–129]

MicroR-101 was regulated in DFSCs during osteogenic differentiation. In this in vitro study, the expression miR-101 was induced through miR-101-mimic transfection, and the gene expression of osteogenic transcription factors was obtained through real-time, reverse transcription-polymerase chain reactions. In addition, the stimulation of the osteogenic differentiation was assessed through the activity of alkaline phosphatase (ALP). Following miR-101-mimic transfection, the ALP was enhanced, and the gene expression of typical osteogenic transcription factors, including *SP7* (*Osterix*), was upregulated [66]. Furthermore, miR-204 negatively controls the osteogenic differentiation of human DFSCs by targeting the bone-particular transcription agent runt-related transcription factor 2 (*Runx2*), the mineralization maker ALP, and the bone extracellular matrix protein released protein acidic and rich in cysteine (*SPARC*) [67].

5 The Roles of MicroRNAs in Dental Diseases

MicroRNAs control tooth morphogenesis by fine tuning the signaling networks. Particular groups of miRNAs are expressed in the dental epithelium compared with mesenchyme and also in molars compared with incisors. MicroRNAs play significant functions in the growth, maintenance, and repair of human dental tissues, such as but not restricted

to the gingiva, periodontium, dental pulp, tooth erupting, dental cell differentiation, and enamel mineralization [39]. In addition, miRNAs offer a further level of regulation beyond that of transcription agents through regulation of the post-transcriptional regulation of gene expression. For example, periodontal ligament-associated protein-1 (PLAP-1/asporin) is a particular marker in periodontal ligament tissue. It is a significant regulator of osteogenic differentiation of PDLCs. mir-101 and mir-21 target *PLAP-1* to control its expression during the osteogenic differentiation of PDLCs [40]. In addition, increasing data show that miRNAs play crucial functions in pulpal inflammation and pulpal tissue repair. They may be utilized for pulpitis therapy [17, 41].

In a study, researchers showed that miR-22, as a synergetic negative regulator, is implicated in regulating the release of proinflammatory cytokines interceded by the NLRP3/CASP1 inflammasome pathway through targeting *NLRP3* and *HIF-1α* in human dental pulp fibroblasts during the development of pulpitis. The authors have established an in vitro model system. These outcomes offer a new role and mechanism of miR-22-HIF-1α-NLRP3 signaling in regulating proinflammatory cytokine discharge, showing a possible therapeutic approach for prospective endodontic treatment [42]. The miR-200 family was implicated in regulating epithelial SCs, and miR-200c controlled tooth enamel development by inducing signal transduction among ameloblasts [43]. Numerous miRNAs from the miR-181 family (miR-181a*, miR-181b, and miR-181c) are downregulated in unhealthy periapical tissues compared with healthy periapical tissues. These miRNAs are critical in cytokine (interleukin-2 and interleukin-6) and chemokine (CCL-8) regulation. In addition, members of the miR-181 family are considerably downregulated in inflamed compared with healthy pulps [44].

New studies on inflammatory reactions and bone tissue homeostasis have reported a considerable enhancement in salivary expression rates of miR-146a, miR-155, and miR-223, suppressing osteoclast genesis and stimulation of NF-κB pathway activation in patients with periodontitis [45, 46]. Downregulation of miR-26 expression in periodontal disease was returned to levels detected in healthy gingiva after non-surgical periodontal therapy [47, 48]. In vitro miR-26a-5p transfected cells released greater rates of cytokines/chemokines upon induction with peri pathogens and revealed disorder cell migration and cytoskeletal rearrangement. Mechanistically, miR-26a-5p controls inflammation, cell migration, and cytoskeleton arrangements of oral keratinocytes and periodontal ligament fibroblasts through direct interaction with phospholipase C beta 1 [47].

Taken together, these investigations show that miRNAs have an essential function in dental diseases by controlling the development, differentiation, and proliferation of dental cells. Thus, miRNAs are offered to play a joint operation in dental tissue homeostasis and terminal differentiation of cells and exert their role through fine tuning preserved signaling networks [39].

5.1 MicroRNAs in Dental Pulp Stem Cells

Increased expression of *Fyn*, a protein tyrosine kinase, has been linked to several forms of inflammation. The advancement of inflammation was accompanied by a reduction in the levels of expression of miR-125a-3p and an increase in the expression levels of *Fyn*. Neuropilin-1 and Fyn produced a compound that suppressed odontoblastic differentiation and boosted inflammatory reactions in DPSCs using NF-κB signaling pathways. Based on these results, miR-125a-3p targets *Fyn* during DPSC odontoblastic development, suggesting that miR-125a-3p may have therapeutic potential for treating dental caries [68]. Pulpitis healing might include miR-223-3p, which regulates odontoblast maturation. Specifically, miR-223-3p was highly expressed in the inflammatory dental pulp [19, 69, 70]. Dentine sialophosphoprotein (*DSPP*) and dentine matrix protein 1 (*DMP-1*) levels were both dramatically raised after miR-223-3p upregulation in DPSCs ($p < 0.05$). Nevertheless, compared with control DPSCs, the protein level of SMAD family member 3 (*SMAD3*) was considerably decreased ($p < 0.05$). Using a combination of bioinformatics and a reporter test based on luciferase activity, researchers found that miR-223-3p potentially targeted *Smad3* [69].

In contrast to juvenile dental pulp tissue, aged dental pulp tissue increased miR-584 expression and decreased transcriptional co-activator with PDZ-binding motif (*TAZ*) expression. Additionally, it was revealed that miR-584 expression was downregulated, and *TAZ* expression was upregulated in DPSCs that were undergoing proliferation. Mimics of miR-584 inhibited DPSCs proliferation, movement, and *TAZ* synthesis, whereas suppression of miR-584 had the opposite effect. When the *TAZ* was knocked down in DPSCs, it had the same effect as when miR-584 was overexpressed. Additionally, a luciferase reporter test confirmed that miR-584 could attach to the 3-UTR of the *TAZ* mRNA and therefore inhibit the translation of that mRNA. Upregulation of *TAZ* has the potential to restore the suppression of proliferation caused by miR-584 mimics partially. Through the AKT signaling pathway, miR-584 was also able to suppress cell growth and reduce the production of cell-cycle proteins [71].

The amount of let-7c-5p, which inhibits High Mobility Group AT-Hook 2 (*HMGA2*) and stimulates osteogenesis and bone growth, was decreased because of the inflammation caused by pulpitis. When DPSCs were exposed to lipopolysaccharide (LPS) to cause inflammation, let-7c-5p expression was increased. Inflamed DPSCs had their cell growth stifled after exposure to LPS, along with ALP

activity, calcium deposition, and the expression of inflammatory markers such as *OPN*, *OCN*, *MSX2*, *OSX*, and *RUNX2* [72, 73]. Elevated levels of p-PI3K, HMGA2, and p-Akt were linked to the poor osteogenic differentiation of irritated DPSCs. Overexpression of let-7c-5p in inflammatory DPSCs rescued their proliferation and also osteogenic differentiation capability and suppressed PI3K/Akt/HMGA2 signaling. The osteogenic differentiation capability of dental pulp cells was recovered after administration of let-7c-5p agomir in rat models with pulpitis, and PI3K/Akt/HMGA2 signaling was blocked [73].

In this investigation, to determine DPSCs and the effect of miRNAs on DPSC attributes, a miRNA array was performed between dental periodontal ligament cells (DPLCs) and DPSCs. According to Qiao et al., DPLCs had significantly higher levels of miR-224-5p expression than DPSCs. The transfection of DPSCs with a miR-224 suppressor decreased cell viability. Moreover, miR-224 suppression considerably increased cell apoptosis in DPSCs compared with the normal cell group. An in silico study and a dual-luciferase reporter method showed that miR-224 targets the 3′-UTR of the Rac family small GTPase 1 (Rac1) gene. The miR-224 downregulation led to the enhanced expression of Rac1 in DPSCs compared with DPLCs. Moreover, miR-224 suppressor caused increased mitogen-activated protein kinase 8, caspase-3, caspase-9, and Fas ligand expression in DPSC, which may be recovered by Rac1 silencing with transfection with short hairpin RNA-Rac1. In addition, Annexin V-fluorescein isothiocyanate/propidium iodide flow cytometry showed that the silencing of Rac1 restored the pro-apoptotic DPSC cell number with miR-224 transfection. Thus, miR-224 in DPSCs plays an important function in protecting cells from apoptosis by downregulating Rac1 expression and also identifies miR-224 as a new miRNA in regulating DPSC characteristics [74, 75]. The upregulation of miR-21 may simplify the odontoblast differentiation of DPSCs that coordinate with STAT3. An miR-21/STAT3 signal may serve as a regulator within a complex network of agents to control odontoblast differentiation of human DPSCs. These findings may offer a perspective on treating bone or tooth diseases [76]. Sirtuin 7 (*SIRT7*) was recognized as a target of miR-152. *SIRT7* was downregulated in aging human DPSCs, whereas miR-152 suppression upregulated *SIRT7* and inhibited the aging phenotype, and *SIRT7* overexpression rescued miR-152-stimulated senescence. The miR-152/SIRT7 axis plays a primary function in regulating human DPSC senescence and offers a candidate target to enhance the functional and therapeutic capability of human DPSCs [77].

5.2 MicroRNAs in Periodontal Ligament Stem Cells

In PDLSCs undergoing osteogenic differentiation, miR-21 was repressed. MiR-21 overexpression markedly suppressed osteogenesis of human periodontal ligament stem cells, while miR-21 suppression showed the opposite impact. Most importantly, PDLSCs stimulated toward osteogenesis were shown to up-regulate *Smad5*, which was expected to be a downstream target of miR-21 [78].

A recently discovered long noncoding RNA called cytoskeleton regulator RNA (CYTOR) has been implicated in a wide variety of biological mechanisms and has been shown to act as a competing endogenous RNA [79]. There is still debate about its function during PDLSC osteogenic differentiation. Tu et al. first discovered that throughout the osteogenic differentiation of PDLSCs, CYTOR expression rose, and CYTOR was mostly sub-localized in the PDLSC cytoplasm [80]. Importantly, CYTOR directly adheres to miR-6512-3p and miR-6512-3p about *SOX11* levels and osteogenic development of PDLSCs. Furthermore, the expression of *SOX11* also rose dramatically when PDLSCs underwent osteogenic differentiation. Overexpression of CYTOR promoted osteogenic differentiation of PDLSCs, although downregulation of *SOX11* mitigated this impact. There is potential for the miR-6512-3p/CYTOR/SOX11 axis to serve as a new therapeutic target in periodontal regeneration therapy [80].

miR-21 interacts with the 3′-UTR repeat sequence of activin receptor type IIB (*ACVR2B*) mRNA. Mechanical stretch inhibited ACVR2B protein rates in PDLSCs, and this preventative efficacy was regulated when endogenous miR-21 rates were either increased or suppressed. Both stretch and the expression of miR-21 changed endogenous ACVR2B protein amounts and, therefore, the osteogenic differentiation of PDLSCs. Furthermore, the gain and loss of function of ACVR2B interceded the osteogenic differentiation of PDLSCs [81].

5.3 MicroRNAs in Stem Cells from Human Exfoliated Deciduous Teeth

Wu et al. demonstrated that EXOs produced from SHED aggregates (SA-Exo) markedly enhanced angiogenesis and pulp tissue regeneration in vivo. Subsequently, researchers used an in vitro tube formation experiment to show that SA-Exo boosted the angiogenic capacity of human umbilical vein endothelial cells and accelerated SHED endothelial differentiation. Through modulating SMAD2/3/TGF-β signaling, miR-26a, which is upregulated in SA-Exo, promoted angiogenic differentiation of both SHED and human umbilical vein endothelial cells. MiR-26a, transported by SA-Exo, stimulates angiogenesis in a SMAD2/3/TGF-β signaling-dependent manner, which aids in SHED aggregate-based pulp tissue regeneration. This new understanding of SA-Exo may pave the way for innovative pulp regeneration approaches [82].

5.4 MicroRNAs in Stem Cells from the Apical Papilla

An experiment was conducted to look at whether reprogramming affected the expression of miRNAs in SCAPs and DPSCs. By comparing pre-programmed and post-reprogrammed DPSC-/SCAP-induced pluripotent stem cells (iPSCs) using a miRNA microarray, it was found that 134 and 265 distinctively expressed miRNAs were up-regulated. One hundred and seventeen unique miRNAs were found to be upregulated by more than two-fold in both SCAP-iPSCs and DPSC-iPSCs. Three co-regulated miRNAs—miR-92b-3p, miR-19a-3p, and miR-130b-3p—involved in the TGF-β signaling pathway, the cell cycle, and epithelial-mesenchymal transition exhibited the most variation. Expression of miR-92b-3p, miR-19a-3p, and miR-130b-3p in SCAP-iPSCs and DPSCs-iPSCs was significantly upregulated using a quantitative real-time polymerase chain reaction analysis. MicroRNAs were shown to be involved in differentiating DPSC-iPSCs from DPSCs and SCAP-iPSCs from SCAP. The varied features that are induced by the creation of pluripotent stem cells were shown by the varying levels of miRNA expression in reprogrammed dental-derived pluripotent stem cells [83].

The expression of hsa-let-7b did not affect the spread of SCAPs. Alkaline phosphatase activity and SCAP calcification nodule formation were boosted by hsa-let-7b interference. Moreover, the protein levels of ALP, DSPP, OSX, RUNX2, OPN, and OCN all rose significantly, as did the mRNA levels of the osteoblastic markers RUNX2, ALP, OSX, and OPN. In contrast, hsa-let-7b overexpression suppressed SCAPs' odonto/osteogenic differentiation potential. After hsa-let-7b suppression, SCAPs showed enhanced odonto/osteogenic differentiation potential, which was abolished by co-transfection with siMMP1. Odonto/osteogenic differentiation potential of SCAPs may be inhibited through hsa-let-7b targeting of matrix metalloproteinase 1 (*MMP1*) [84]. Apical papilla stem cells play a critical role in the development and repair of root dentin. In SCAPs, NOTCH activation prevented cells from differentiating and increased the expression of miR-34a, while miR-34a blocked Notch signaling by downregulation of N2ICD, NOTCH2, and HES1. This was achieved when miR-34a specifically targeted the *NOTCH2* and *HES1* mRNA 3′UTR. Therefore, there was a rise in the levels of *RUNX2*, *DSPP*, *OSX*, and *OCN* expression. Hence, Notch signaling is essential for the survival of human SCAPs. Through regulating Notch signaling, miR-34a encourages SCAPs to differentiate into odontogenic and osteogenic cells [85]. MiR-124-3p.1 was shown to have a suppressive impact on SCAPs osteo/odontogenic development in a separate investigation. Silence and overexpression of miR-124-3p.1 considerably raised and suppressed MACF1 protein levels in SCAPs, respectively. Regulation of MACF1/smad7 by miR1243p.1 is critical for SCAPs to undergo osteo/odontogenic differentiation along the MACF1/smad7 axis [86]. Throughout the osteo/odontogenic differentiation of SCAP, miR-497-5p was upregulated. MiR-497-5p is a potent inducer of the SCAP osteo/odontogenic differentiation as upregulation of miR-497-5p increased this differentiation, but downregulation of miR-497-5p decreased it. Based on results from a bioinformatics study and a dual luciferase reporter test, we know that miR-497-5p targets SMAD-specific E3 ubiquitin protein ligase 2 (*Smurf2*). *Smurf2* was shown to affect SCAP osteo/odontogenic development adversely, and its silencing was found to counteract the inhibitory impact of miR-497-5p. A pathway analysis revealed that miR-497-5p enhances osteo/odontogenic development by employing the Smad signaling pathway [87]. Apical papilla stem cells undergo osteogenic differentiation when circular SIPA1L1 binds to miR-204-5p and increases *ALPL* expression [88]. Overexpression of miR-141-3p suppressed the proliferative potential of SCAPs. In addition, transfection of miR-141-3p suppressor increased the proliferative capability of SCAPs and delayed their senescence. Yes-associated protein (*YAP*) was detected as the downstream target gene of miR-141-3p. Generally, miR-141-3p prevented proliferative capacity and promoted senescence of SCAPs via post-transcriptionally downregulating *YAP* [89].

5.5 MicroRNA in Dental Follicle Cells

To learn more about miR-146a, its mimics and blockers were transfected into DFSCs, and the expression of genes related to tooth eruption was assessed. Expression of *CSF-1*, *RUNX2*, *EGFR*, and *OPG* was considerably affected when miR-146a was overexpressed or repressed, and miR-146a levels were significantly reduced in RUNX2+/m DFSCs. Mutations in the *RUNX2* gene have a role in the phenotypic shift seen in DFSCs, and cross-talk between the miRNAs and the *RUNX2* gene may constitute a critical regulatory process governing the differentiation of DFSCs [90]. The *RUNX2* mutation causes deferred tooth eruption due to dental follicle dysfunction in individuals with Cleidocranial dysplasia (CCD). Mounting evidence points to *RUNX2*, miR-31, and specific AT-rich binding protein 2 (*SATB2*) as critical players in a regulatory circuit essential for maintaining and regulating mesenchymal stem cell activity and homeostasis. Dental follicle stem cell CCD exhibited decreased *RUNX2* (a transcriptional suppressor of miR-31), increased miR-31, and downregulated *SATB2* in comparison with DFSCs from healthy donors and showed impaired osteoclast-inductive, osteogenic, and matrix-degrading activities [91]. In DFSC CCD, osteoclasts would be inactivated, and bone matrix remodeling would be suppressed because of relatively low

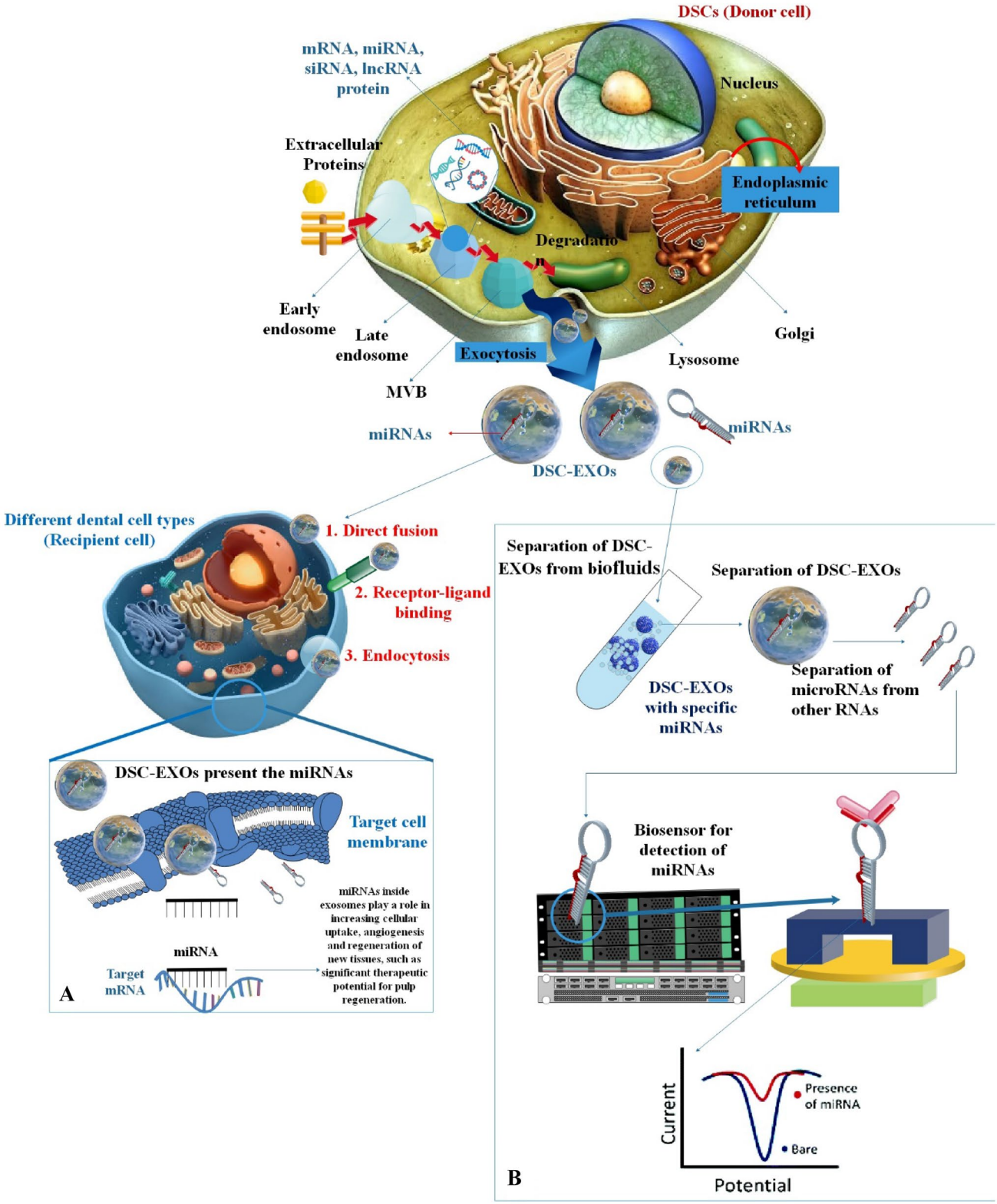
ratios of RANKL/OPG and RANKL/RANK, as well as downregulation of matrix metalloproteinase 2 (*MMP2*) and matrix metalloproteinase 9 (*MMP9*). In addition, endogenous miR-31 knockdown highlighted the functions of the RUNX2-miR-31-SATB2 loop in DFSC CCD, with enhanced *SATB2*, *RUNX2*, osteoclast-inductive, and matrix degradation abilities. When miR-31 was overexpressed ectopically in normal DFSCs, the expression of osteoclast-inductive factors such RUNX2, SATB2 MMP2, and MMP9 decreased. Taken together, our findings raise the possibility that postponed tooth eruption in patients with CCD is caused by a disruption in osteoclast-inductive signaling in DFSCs caused by *RUNX2* mutation/haploinsufficiency [91]. The RUNX2-miR-31-SATB2 loop has been investigated as a therapeutic target for facilitating tooth eruption in patients with CCD [91]. The expressions of miR-29 family members (miR-29a, miR-29b, and miR-29c) were remarkably reduced in human DFSC during osteogenic differentiation. When miR-29s were delivered into human DFSC, collagen type I generation was reduced. Moreover, human DFSC transfected with miR-29 mimics demonstrated delayed mineralization compared with human DFSC transfected with negative control and non-transfection culture. miR-29 negatively controls the osteogenic differentiation/mineralization of human Dental Follicle stem cells by targeting collagen type I [92].

6 MicroRNAs in Dental Stem Cell-Derived Extracellular Vesicles

As critical paracrine effectors, DSC-derived extracellular vesicles (DSC-EVs) are increasingly associated with the positive effects of DSCs through an evolving body of in vivo investigations [93, 94]. By delivering a bioactive cargo and offering curative potential in dental diseases, DSC-EVs have been presented as an encouraging treatment method [93]. Exosomes isolated from DPSCs can be utilized as biomimetic tools to stimulate the odontogenic differentiation of SCs. Exosomes were derived from human DPSCs cultured undergrowth and odontogenic differentiation conditions, named UN-Exo and OD-Exo, respectively [95]. In a study, the miRNA expression profiles of EXOs isolated from DPSCs were recognized [96] (Fig. 3). OD-Exo derived under odontogenic conditions were better stimulators of DPSC differentiation. As is known, exosomal microRNAs can negatively regulate gene expression by binding to their target mRNAs through base pairing to the 3′-UTR, there may exist some other inhibitory molecules between the miR-27a-5p and TGF-β1 signaling. One of the substances known to suppress TGF-β1 signaling is latent TGF-β-binding protein 1 (*LTBP1*). As LTBP1 was shown to covalently attach the TGF-β propeptide (LAP) through disulfide bonds, it was hypothesized that LTBP1's primary function was to preserve TGF-β latency by anchoring TGF-β to the extracellular matrix. Scientists used western blot analysis and a two-fold luciferase experiment to see whether miR-27a-5p might directly suppress *LTBP1* expression. Western blot assays revealed an interesting phenomenon; miR-27a-5p mimics decreased LTBP1 protein quantities. In addition, they reported that miR-27a-5p dramatically stopped luciferase activity in the WT-LTBP1 group while having no impact in the MUT-LTBP1 group. miR-27a-5p directly targeted *LTBP1*, according to a luciferase reporter gene assay. Together, these results suggest that exosomal microRNAs downregulate *LTBP1*, an inhibitory protein, to stimulate odontogenic differentiation through the TGF-β1/smads signaling pathway [96].

MicroRNA expression patterns of DPSC-derived EXO were determined in a study by Hu et al. The analysis revealed that OD-Exo isolated in an odontogenic environment acted as a potent stimulant of DPSC differentiation. In the TGF-β1/smads signaling pathway, exosomal miRNAs repressed an inhibitory factor, *LTBP1*, which stimulated odontogenic differentiation [96]. Exosomes from PDLSCs reduced inflammatory microenvironment via the Th17/Treg/miR-155-5p/SIRT1 regulatory network [97]. Nakao et al. detected the efficacy of tumor necrosis factor-α preconditioned DSC-EVs on mouse periodontal maxillary bone loss. They demonstrated that the upregulated miR-1260b within DSC-EVs accounted for the improved inhibition potential. Mechanistically, miR-1260b suppressed the osteoclastogenic action of periodontal ligament cells by targeting the Wnt5a-interceded *RANKL* pathway [93, 98]. The angiogenesis-linked miRNA miR-378a was detected to be enriched in periodontitis-compromised DPSCs-EVs (P-EVs), and its function in P-EVs improved cell angiogenesis was established, wherein *Sufu* was recognized as a downstream target gene of miR-378a. Functionally, silencing *Sufu*-induced endothelial cell proliferation, migration, and tube development through triggering Hedgehog/Gli1 signaling. Additionally, researchers revealed that incubation with P-EVs enabled the transmission of P-EV-miR-378a to endothelial cells. Therefore, the expressions of *Sufu*, *Gli1*, and vascular endothelial growth factor in endothelial cells were considerably affected through P-EV-interceded miR-378a transmission [99].

The inflammatory microenvironment induced osteogenic and odontogenic differentiation of DPSCs, and inflammatory DPSC-EVs behaved similarly to PDLSCs. MiR-758-5p was upregulated in inflammatory DPSC-EVs and was revealed to play a vital function in the osteogenic and odontogenic commitment of PDLSCs. A dual-luciferase reporter assay established the binding site between miR-758-5p and limb development membrane protein 1 (*LMBR1*). Similarly, inhibiting *LMBR1* increased the generation of osteogenic and odontogenic genes and proteins. In addition, *LMBR1* has been offered to promote bone morphogenetic protein



signaling. Osteoblasts are differentiated from SCs through a complex gene-signaling system involving members of the bone morphogenetic protein family. Commonly, EVs from the inflammatory microenvironment improved the osteogenic and odontogenic differentiation of PDLSCs partially through shuttering *LMBR1*-targeting miR-758-5p via bone morphogenetic protein signaling [100].

◂**Fig. 3** The biogenesis of dental stem cells-exosomes (DSC-EXOs) and their role in pathways to the recipient cell. The mechanism of EXO production during EXO biogenesis comprises the start; the plasma membrane is internalized to create an endocytic vesicle named an early endosome, and after that, early endosome to late endosome alteration. The budding of late endosomal membranes leads to the creation of intraluminal vesicles (ILVs) within large MVBs. Furthermore, MVBs can be transferred to lysosomes for decay or move along microtubules to combine with the cell membrane and release EXOs into the extracellular environment. Exosomes intercede their influences on the recipient cells via three essential methods: (1) receptor-mediated signal amplification; (2) endocytosis; and (3) membrane fusion [15, 130, 131]. **A** Exosomal microRNA (miRNA) from dental stem cells (DSCs) as a therapeutic agent. **B** Exosomal miRNAs from the DSC detection method. Simultaneous and multiplexed diagnosis of miRNAs in a whole DSC-EXOs is developed, which can be used as a polymerase chain reaction-free effective diagnosis technique for dental diseases. Because miRNAs circulate throughout bodily fluids, exosomal miRNAs propose excellent benefits for the assessment of dental diseases. *lncRNA* long noncoding RNA, *mRNA* messenger RNA, *siRNA* small interfering RNA

Compared with healthy EXOs, miR-143-3p was enhanced in inflammatory PDLSC-EXOs, which targeted and suppressed the expression of *PI3Kγ* and enhanced M1 macrophage polarization through inhibiting PI3K/AKT signaling and triggering NF-κB signaling. At the same time, an agonist of the PI3K pathway reversed this efficacy. Furthermore, EXO-shuttled miR-143-3p from PDLSCs drove M1 macrophage polarization and exacerbation of periodontal inflammation in a mouse periodontal model. Inflammatory PDLSCs simplify M1 macrophage polarization via the exosomal miR-143-3p-interceded regulation of PI3K/AKT/NF-κB signaling, providing a potential novel target for periodontitis treatment [101].

In another study, researchers showed that SHED-EXOs (SA-Exos) considerably enhanced pulp tissue renewal and angiogenesis in vivo. In addition, SA-Exos improved SHED endothelial differentiation and increased the angiogenic capability of human umbilical vein endothelial cells. Mechanistically, miR-26a, which is augmented in SA-Exos, enhanced angiogenesis both in SHED and human umbilical vein endothelial cells through controlling TGF-β/SMAD2/3 signaling. SA-Exos shuttled miR-26a enhance angiogenesis through TGF-β/SMAD2/3 signaling contributing to SHED aggregate-based pulp tissue renewal. These new visions for SA-Exos may facilitate the progress of novel approaches for pulp regeneration [82].

7 MicroRNAs as a Biomarker in Dental Diseases

Serum and plasma consist of high levels of specific miRNAs, which can serve as biomarkers for dental diseases. Salivary miRNA biomarkers are emerging as an option for diagnosing dental diseases [102]. In addition, miRNAs facilitate early diagnosis of peri-implant diseases, act as trustworthy indexes of peri-implant health, or help in manage peri-implant diseases [103]. For example, miR-4484 downregulated in the saliva of patients with peri-implantitis. Saliva samples taken 4–6 months following implant implantation had significantly lower rates of miR-4484 than samples collected before implant placement. Salivary miR-4484 has been suggested as a possible peri-implantitis early diagnostic biomarker [104]. MiR-141 was demonstrated to be one of the most frequently expressed miRNAs in human gingival keratinocytes and, therefore, could be used as a potential biomarker for its diagnosis [105]. In another study, researchers evaluated the role of selected miRNAs in different stages of periodontitis and their association with the rates of inflammatory intermediaries in gingival crevicular fluid. The outcomes showed that miRNA-103a-3p, miRNA-23a-3p, miRNA-15a-5p, and miRNA-223-3p were considerably upregulated compared with the healthy control group. Considerable alterations were detected for miRNA-23a-3p, miRNA-103a-3p, and miRNA-423-5p rates according to disease stages. Inflammatory intermediaries assessed in gingival crevicular fluid associate well with the clinical parameters and the severity of the periodontal disease. MicroRNAs can demonstrate biomarkers of disease stage and can be studied as a potential therapeutic agent. Additionally, rates of tumor necrosis factor-α and interleukin-6 may drive the disease development by acting as prognostic markers [106]. miR-28-5p was down-expressed in patients with chronic periodontitis and LPS-stimulated PDLCs. The area under the curve for miR-28-5p was 0.937, with a special diagnostic rate. Furthermore, miR-28-5p was negatively associated with periodontal clinical signs and inflammatory agents. Cell proliferation of PDLCs was suppressed, and inflammation was enhanced under LPS stimulation; however, increased miR-28-5p reduced the influence of LPS. *SPHK1* performances as a miR-28-5p target and promoting *SPHK1* arising from LPS treatment were suppressed through the enhanced miR-28-5p. This miRNA could be used as a possible diagnostic biomarker for chronic periodontitis. Furthermore, miR-28-5p may play a role in chronic periodontitis development by targeting *SPHK1* to control the proliferation and inflammation of PDLCs [107]. Recent studies discovered miRNAs in saliva, which are promising candidates for use as biomarkers of dental diseases [102]. For example, miR-140-5p, miR-146a-5p, and miR-628-5p are overexpressed in small EVs from patients with periodontitis, considering potential biomarkers [108]. In addition, it has been reported that hsa-miR-140-5p and hsa-miR-628-5p in the gingival crevicular fluid are potential diagnostic biomarkers for periodontitis [109]. In addition, using miRNA signatures in humans as a prediction tool will enable us to elucidate the biological processes occurring in DSCs.

Therefore, researchers suggest that the role of hsa-miR-7-5p in gene regulation may suppress cell-cycle progression and proliferation, either for differentiation or to maintain DPSCs in a quiescent state [110] (Table 2).

8 Existing Obstacles and Prospects of This Method

There have been significant advances in DSC research and therapeutic application. However, both areas are still in their infancy. There are still many technical issues to be solved. Nevertheless, its unique advantages in self-renewal, the capacity for heterogeneous differentiation, ease of accessibility, and minimal autologous transplant rejection give dental tissue engineering a bright future. Recent advances in technology and science have made it possible to genetically manipulate and produce DSCs, which might pave the way for novel approaches to tissue regeneration and replacement treatment [111]. The ability of adult dental MSCs bound to collagen to rebuild mandibular bone was recently proven in a human clinical experiment [112–115]. These findings demonstrate the potential of dental tissue engineering to provide a new option for those who have lost teeth owing to pathological conditions, trauma, or congenital absence. Nevertheless, further study is required as implementing these methods may be time consuming, challenging, and costly, making them inaccessible to the general population [112].

Periodontal ligament stem cells and DPSCs are the ideal options for pulp and periodontal regeneration because of their exceptional osteogenic capacity and unique odontogenic potential, respectively. Because of this, research into DPSCs and PDLSCs has focused mostly on their epigenetic processes. Both SCAPs (achieved from dental roots) and SHEDs (gained from exfoliated deciduous teeth) have been the subject of much research owing to their readily available sources and lack of immunogenicity. There are fewer studies on DFPCs because their sources are more restricted [116].

MicroRNAs can control gene roles by stimulating target mRNA degradation or translational suppression. Therefore, the dys-expressed miRNA rate contributes to the abnormality of various biological activities or the onset of various dental diseases [73, 117]. The function of miRNAs in tooth organization has been extensively studied in animal models. Investigation of miRNA expression profiles of the growing mouse molar tooth germ has shown these to be remarkably dynamic [118]. In human dental tissues, miRNAs may show crucial roles associated with periodontal disease, tooth movement and eruption, dental pulp physiology and pathology, differentiation of dental cells, and enamel mineralization [39]. Several studies showed that miRNAs play critical regulatory functions in DSC processes, including development, proliferation, differentiation, growth, neoplastic transformation, and tissue regeneration [62]. Furthermore, miRNAs exhibit minor toxic efficacy and lesser immunogenicity than protein-based medicines and even plasmid-DNA-based gene therapy [50]. Dental stem cell-EXOs have been considered in tooth regeneration and repair. In addition, exosomal miRNAs are used as biomarkers and therapeutic agents in dental diseases. Moreover, these miRNAs function in post-transcriptional gene regulation upon uptake via neighboring or distant dental cells [119–121]. Thus, miRNAs are offered to play a collective function in dental tissue homeostasis and terminal differentiation of DSCs and mainly exert their role through fine tuning maintained signaling networks [39].

Although exploiting miRNAs as biomarkers for dental diseases is promising, some constraints should be considered. Differentially expressed candidate miRNAs identified through pilot studies need to be validated with further studies using larger sample sizes with appropriate control groups. Practical focused treatments are necessary for at-risk patients to capitalize on an early diagnosis, and without such treatments, a clear cost-benefit advantage cannot be realized [102]. In addition, EXOs hold high promise as bio-inspired delivery systems for miRNAs in dental diseases [15, 122]. MicroRNAs enriched in dental stem cell EXOs enhance the therapeutic effects of DSCs in dental diseases [122].

The most promising option for successfully regenerating damaged or diseased dental tissues, or even the complete tooth, after tooth loss is stem cell-based therapy. Therefore, it would be interesting to learn more about how innervation functions in these processes. Dentists are only now beginning to use stem cell-based techniques, but there are still many obstacles to be solved, including the need to regenerate complete teeth and enamel. Only a few clinical experiments using SCs have been filed thus far, and the outcomes are still a mystery. Although collected SCs have not yet been used in dental treatments, this is undoubtedly a young field of study that could have significant future implications [6, 123]. Dental stem cells could be a turning point in individualized regenerative therapy. Improvements in DSC population isolation, comprehension, and differentiation capacities may lead to new areas of study and dental disease treatments [124]. Understanding the varied roles that miRNAs play in the regulation of DSCs is essential for the creation of novel dental treatments. Furthermore, miRNAs supported DSC differentiation and may thus represent a viable target in regenerative medicine to either assist bone/mineralized tissue production or retain the stem cell phenotype. Utilizing cutting-edge technologies, the treatment of oral illness might be revolutionized in the future [62, 125, 126].

Table 2 miRNAs in DSCs for dental diseases

DSCs	miRNAs	Study type	Explain	References
DPSCs	miR-125a-3p	In vitro	By targeting Fyn, miR-125a-3p plays a significant part in the odontoblastic differentiation of DPSCs, indicating that it may have therapeutic promise in the treatment of dental caries. Moreover, this miRNA was figured to be the effector because it suppressed TLR and NF-κB, two important mediators of the inflammatory reaction	[68, 140]
DPSCs	let-7c-5p	In vitro and in vivo	Suppression of PI3K/HMGA2/Akt signaling was shown to be critical for the anti-inflammatory and pro-osteogenesis impact of let-7c-5p during the acute phase of pulpitis	[73]
DPSCs	miR-224-5p	In vitro	Both DPSC proliferation and migration were boosted by miR-224-5p suppression	[141]
DPSCs	miR-221-3p	In vitro	Reduced cell viability and subsequent apoptosis were seen in miR-221-3p-depleted DPSCs due to Rac1-mediated cell death induction	[74, 75]
PDLSCs	Exo-miR-205-5p	In vitro and in vivo	PDLSC-derived exo-miR-205-5p targets *XBP1* to reduce inflammation and restore a healthy balance between Th17 and Treg cells in chronic periodontitis	[142]
PDLSCs	miR-21	In vitro and in vivo	In PDLSCs undergoing osteogenic differentiation, miR-21 was knocked down. By focusing on Smad5, miR-21 may regulate the osteogenic development of human PDLSCs	[78]
PDLSCs	miR-7	In vitro	PDLSCs can be controlled in their osteogenic stemness and differentiation by miR-7	[116, 143]
SHED	miR-26a	In vitro and in vivo	Regenerating pulp tissue from SHED aggregates, miR-26a is shuttled there by SA-Exo and stimulates angiogenesis via TGF/SMAD2/3 signaling. These new understandings of SA-Exo may pave the way for innovative pulp regeneration techniques	[82]
SCAP	Hsa-let-7b	In vitro	The odonto/osteogenic differentiation potential of SCAPs may be restricted by Hsa-let-7b via inhibiting *MMP1*	[84]
SCAP	miR-34a	In vitro	miR-34a stimulates SCAP odontogenic and osteogenic development by interacting with notch signaling	[85]
SCAP	miR-497-5p	In vitro	The expression of miR-497-5p rose throughout osteo/odontogenic development in SCAP. Pathway analysis revealed that miR-497-5p stimulates osteo/odontogenic development by activating the Smad signaling pathway	[87]
DFSCs	miR-101	In vitro	Elevated levels of ALP and the expression of common osteogenic transcription factors, including *SP7* (osterix), were seen after transfection with a miRNA101-mimic. DFSCs continue to differentiate into osteoblasts because of the miR-101 effect	[66]
DFSCs	miR-146a	Patient with cleidocranial dysplasia	Overexpression or inhibition of miR-146a dramatically affected the expression of *CSF-1*, *RUNX2*, *EGFR*, and *OPG*. Additionally, miR-146a considerably reduced in RUNX2+/m DFCs	[90]

ALP alkaline phosphatase, *DFSCs* dental follicle stem cells, *DSCs* dental stem cells, *miRNAs* microRNAs, *PDLSCs* periodontal ligament stem cells, *SCAP* stem cells from the apical papilla, *SHED* stem cells from human exfoliated deciduous teeth

9 Conclusions

Current research has linked miRNAs to various biological functions, including cell division, differentiation, apoptosis, and cancer development. Numerous studies have shown the importance of miRNAs in tooth formation. It has been well acknowledged that miRNAs play a pivotal role in stem cell biology by regulating epigenetically the pathways determining stem cell destiny. Because of their plentiful availability, excellent efficiency, and biocompatibility, DSCs hold great promise for use in tissue regeneration. Several mechanisms control how dental-derived SCs perform their biological roles. Recent studies have shown that miRNAs also regulate dental-derived SCs. Because miRNAs increase DSC differentiation, they may be used in regenerative medicine to either preserve the stem cell phenotype or to aid in the development of tooth tissue. Therapeutically, this approach is superior to the norm. Dental tissue engineering has been the subject of several in vitro and in vivo experiments, all showing positive and promising findings for their possible future applications. However, additional study is required to comprehend the role of miRNAs in DSCs and to address the current challenges. The widespread use of such methods in the future will alter how many dental syndromes and disorders are now diagnosed and treated.

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Declarations

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References

1. Yasamineh S, Kalajahi HG, Yasamineh P, Gholizadeh O, Youshanlouei HR, Matloub SK, et al. Spotlight on therapeutic efficiency of mesenchymal stem cells in viral infections with a focus on COVID-19. Stem Cell Res Ther. 2022;13(1):257.
2. Tayanloo-Beik A, Nikkhah A, Roudsari PP, Aghayan H, Rezaei-Tavirani M, Nasli-Esfahani E, et al. Application of biocompatible scaffolds in stem-cell-based dental tissue engineering. Cell Biology and Translational Medicine, Volume 18: Tissue Differentiation, Repair in Health and Disease. 2022:83–110.
3. Chalisserry EP, Nam SY, Park SH, Anil S. Therapeutic potential of dental stem cells. J Tissue Eng. 2017;8: 2041731417702531.
4. Soudi A, Yazdanian M, Ranjbar R, Tebyanian H, Yazdanian A, Tahmasebi E, et al. Role and application of stem cells in dental regeneration: a comprehensive overview. EXCLI J. 2021;20:454.
5. Volponi AA, Pang Y, Sharpe PT. Stem cell-based biological tooth repair and regeneration. Trends Cell Biol. 2010;20(12):715–22.
6. Morsczeck C, Reichert TE. Dental stem cells in tooth regeneration and repair in the future. Expert Opin Biol Ther. 2018;18(2):187–96.
7. Morsczeck C. Dental stem cells for tooth regeneration: how far have we come and where next? Expert Opin BiolTher. 2023;23:1–11.
8. Li P, Ou Q, Shi S, Shao C. Immunomodulatory properties of mesenchymal stem cells/dental stem cells and their therapeutic applications. Cell Mol Immunol. 2023;20(6):558–69.
9. Rajabzadeh N, Fathi E, Farahzadi R. Stem cell-based regenerative medicine. Stem Cell Invest. 2019;6:19.
10. Hu L, Liu Y, Wang S. Stem cell-based tooth and periodontal regeneration. Oral Dis. 2018;24(5):696–705.
11. Granz CL, Gorji A. Dental stem cells: the role of biomaterials and scaffolds in developing novel therapeutic strategies. World J Stem Cells. 2020;12(9):897.
12. Sanz JL, Rodriguez-Lozano FJ, Lopez-Gines C, Monleon D, Llena C, Forner L. Dental stem cell signaling pathway activation in response to hydraulic calcium silicate-based endodontic cements: a systematic review of in vitro studies. Dent Mater. 2021;37(4):e256–68.
13. Zhang W, Yelick PC. Tooth repair and regeneration: potential of dental stem cells. Trends Mol Med. 2021;27(5):501–11.
14. Mai Z, Chen H, Ye Y, Hu Z, Sun W, Cui L, et al. Translational and clinical applications of dental stem cell-derived exosomes. Front Genet. 2021;12: 750990.
15. Oveili E, Vafaei S, Bazavar H, Eslami Y, Mamaghanizadeh E, Yasamineh S, et al. The potential use of mesenchymal stem cells-derived exosomes as microRNAs delivery systems in different diseases. Cell Commun Signal. 2023;21(1):1–26.
16. Gong Q, Wang R, Jiang H, Lin Z, Ling J. Alteration of microRNA expression of human dental pulp cells during odontogenic differentiation. J Endod. 2012;38(10):1348–54.
17. Zhong S, Zhang S, Bair E, Nares S, Khan AA. Differential expression of microRNAs in normal and inflamed human pulps. J Endod. 2012;38(6):746–52.
18. Liu W, Gong Q, Ling J, Zhang W, Liu Z, Quan J. Role of miR-424 on angiogenic potential in human dental pulp cells. J Endod. 2014;40(1):76–82.
19. Kulthanaamondhita P, Kornsuthisopon C, Photichailert S, Manokawinchoke J, Limraksasin P, Osathanon T. Specific microRNAs regulate dental pulp stem cell behavior. J Endod. 2022;48:688–98.
20. Chalisserry EP, Nam SY, Park SH, Anil S. Therapeutic potential of dental stem cells. J Tissue Engineer. 2017;8:2041731417702531.
21. Tsutsui TW. Dental pulp stem cells: advances to applications. Stem Cells Clon Adv Appl. 2020;13:33–42.
22. Kunimatsu R, Nakajima K, Awada T, Tsuka Y, Abe T, Ando K, et al. Comparative characterization of stem cells from human exfoliated deciduous teeth, dental pulp, and bone marrow–derived mesenchymal stem cells. Biochem Biophys Res Commun. 2018;501(1):193–8.
23. Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. J Tissue Engineer Regen Med. 2015;9(11):1205–16.
24. Taguchi T, Yanagi Y, Yoshimaru K, Zhang X-Y, Matsuura T, Nakayama K, et al. Regenerative medicine using stem cells from human exfoliated deciduous teeth (SHED): a promising new treatment in pediatric surgery. Surg Today. 2019;49:316–22.
25. Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, et al. Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther. 2010;1(1):1–11.
26. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod. 2009;35(11):1536–42.
27. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci. 2003;100(10):5807–12.
28. Seo B-M, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004;364(9429):149–55.
29. Gay IC, Chen S, MacDougall M. Isolation and characterization of multipotent human periodontal ligament stem cells. Orthodont Craniofacial Res. 2007;10(3):149–60.
30. Trubiani O, Pizzicannella J, Caputi S, Marchisio M, Mazzon E, Paganelli R, et al. Periodontal ligament stem cells:

current knowledge and future perspectives. Stem Cells Dev. 2019;28(15):995–1003.
31. Tomokiyo A, Wada N, Maeda H. Periodontal ligament stem cells: regenerative potency in periodontium. Stem Cells Dev. 2019;28(15):974–85.
32. Nada OA, El Backly RM. Stem cells from the apical papilla (SCAP) as a tool for endogenous tissue regeneration. Front Bioeng Biotechnol. 2018;6:103.
33. Huang GTJ, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod. 2008;34(6):645–51.
34. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, et al. Comparative analysis of in vitro osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP). Arch Oral Biol. 2011;56(7):709–21.
35. Lovelace TW, Henry MA, Hargreaves KM, Diogenes A. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. J Endod. 2011;37(2):133–8.
36. Zhao M, Xiao G, Berry JE, Franceschi RT, Reddi A, Somerman MJ. Bone morphogenetic protein 2 induces dental follicle cells to differentiate toward a cementoblast/osteoblast phenotype. J Bone Miner Res. 2002;17(8):1441–51.
37. Yao S, Pan F, Prpic V, Wise G. Differentiation of stem cells in the dental follicle. J Dent Res. 2008;87(8):767–71.
38. Guo W, Chen L, Gong K, Ding B, Duan Y, Jin Y. Heterogeneous dental follicle cells and the regeneration of complex periodontal tissues. Tissue Engineer Part A. 2012;18(5–6):459–70.
39. Sehic A, Tulek A, Khuu C, Nirvani M, Sand LP, Utheim TP. Regulatory roles of microRNAs in human dental tissues. Gene. 2017;596:9–18.
40. Li C, Li C, Yue J, Huang X, Chen M, Gao J, et al. miR-21 and miR-101 regulate PLAP-1 expression in periodontal ligament cells. Mol Med Rep. 2012;5(5):1340–6.
41. Jin Y, Wang C, Cheng S, Zhao Z, Li J. MicroRNA control of tooth formation and eruption. Arch Oral Biol. 2017;73:302–10.
42. Jiang W, Sun S, Wang D, Qiu J, Song Y, Zhang Q, et al. MicroRNA-22 suppresses NLRP3/CASP1 inflammasome pathway-mediated proinflammatory cytokine production by targeting the HIF-1α and NLRP3 in human dental pulp fibroblasts. Int Endod J. 2022;55(11):1225–40.
43. Gu M, Yu X, Fan L, Zhu G, Yang F, Lou S, et al. Genetic variants in miRNAs are associated with risk of non-syndromic tooth agenesis. Front Physiol. 2020;11:1052.
44. Al Gashaamy ZJ, Alomar T, Al-Sinjary L, Wazzan M, Saeed MH, Al-Rawi NH. MicroRNA expression in apical periodontitis and pulpal inflammation: a systematic review. PeerJ. 2023;11: e14949.
45. Wu P, Feng J, Wang W. Expression of miR-155 and miR-146a in the saliva of patients with periodontitis and its clinical value. Am J Transl Res. 2021;13(6):6670.
46. Sipert CR, Morandini AC, Dionísio TJ, Trachtenberg AJ, Kuo WP, Santos CF. MicroRNA-146a and microRNA-155 show tissue-dependent expression in dental pulp, gingival and periodontal ligament fibroblasts in vitro. J Oral Sci. 2014;56(2):157–64.
47. Uttamani JR, Naqvi AR, Estepa AMV, Kulkarni V, Brambila MF, Martínez G, et al. Downregulation of miRNA-26 in chronic periodontitis interferes with innate immune responses and cell migration by targeting phospholipase C beta 1. J Clin Periodontol. 2023;50:102–13.
48. Gao J, Li L, Wu M, Liu M, Xie X, Guo J, et al. MiR-26a inhibits proliferation and migration of breast cancer through repression of MCL-1. PLoS ONE. 2013;8(6): e65138.
49. Ortiz GGR, Mohammadi Y, Nazari A, Ataeinaeini M, Kazemi P, Yasamineh S, et al. MicroRNAs: influencers and targets in hematopoietic stem cell aging. 2023. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10123996/.
50. Hu J, Stojanović J, Yasamineh S, Yasamineh P, Karuppannan SK, Hussain Dowlath MJ, et al. The potential use of microRNAs as a therapeutic strategy for SARS-CoV-2 infection. Arch Virol. 2021;166:2649–72.
51. Norouzi M, Yasamineh S, Montazeri M, Dadashpour M, Sheervalilou R, Abasi M, et al. Recent advances on nanomaterials-based fluorimetric approaches for microRNAs detection. Mater Sci Eng C Mater Biol Appl. 2019;104: 110007.
52. Osaki M, Takeshita F, Ochiya T. MicroRNAs as biomarkers and therapeutic drugs in human cancer. Biomarkers. 2008;13(7–8):658–70.
53. Manikkath J, Jishnu PV, Wich PR, Manikkath A, Radhakrishnan R. Nanoparticulate strategies for the delivery of miRNA mimics and inhibitors in anticancer therapy and its potential utility in oral submucous fibrosis. Nanomedicine. 2022;17(3):181–95.
54. Santonocito S, Polizzi A, Palazzo G, Isola G. The emerging role of microRNA in periodontitis: pathophysiology, clinical potential and future molecular perspectives. Int J Mol Sci. 2021;22(11):5456.
55. Han X, Fan Z. MicroRNAs regulation in osteogenic differentiation of mesenchymal stem cells. Front Dent Med. 2021;72.
56. Han T-S, Voon DC-C, Oshima H, Nakayama M, Echizen K, Sakai E, et al. Interleukin 1 up-regulates microRNA 135b to promote inflammation-associated gastric carcinogenesis in mice. Gastroenterology. 2019;156(4):1140-55.e4.
57. Tomé M, López-Romero P, Albo C, Sepúlveda JC, Fernández-Gutiérrez B, Dopazo A, et al. miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells. Cell Death Differ. 2011;18(6):985–95.
58. Fan J, An X, Yang Y, Xu H, Fan L, Deng L, et al. MiR-1292 targets FZD4 to regulate senescence and osteogenic differentiation of stem cells in TE/SJ/mesenchymal tissue system via the Wnt/β-catenin pathway. Aging Dis. 2018;9(6):1103.
59. Gangaraju VK, Lin H. MicroRNAs: key regulators of stem cells. Nat Rev Mol Cell Biol. 2009;10(2):116–25.
60. Li N, Long B, Han W, Yuan S, Wang K. microRNAs: important regulators of stem cells. Stem Cell Res Ther. 2017;8:1–7.
61. Yi R, Poy MN, Stoffel M, Fuchs E. A skin microRNA promotes differentiation by repressing 'stemness.' Nature. 2008;452(7184):225–9.
62. Iranmanesh P, Vedaei A, Salehi-Mazandarani S, Nikpour P, Khazaei S, Khademi A, et al. MicroRNAs-mediated regulation of the differentiation of dental pulp-derived mesenchymal stem cells: a systematic review and bioinformatic analysis. Stem Cell Res Ther. 2023;14(1):1–20.
63. Sun DG, Xin BC, Wu D, Zhou L, Wu HB, Gong W, et al. miR-140-5p-mediated regulation of the proliferation and differentiation of human dental pulp stem cells occurs through the lipopolysaccharide/toll-like receptor 4 signaling pathway. Eur J Oral Sci. 2017;125(6):419–25.
64. Sun Y, Shi J, Luo X, Xu X. microRNA-142–3p regulates osteogenic differentiation of human periodontal ligament stem cells via mediating SGK1. J Stomatol Oral Maxillofac Surg. 2023;124(1): 101369.
65. Gao S, Ge LH, Zhao YM, Li P, Li YY, Zhao W. Hsa-miRNA-143-3p regulates the odontogenic differentiation of human stem cells from the apical papilla by targeting NFIC. Int Endod J. 2022;55(3):263–74.
66. Klingelhöffer C, Codrin C, Ettl T, Reichert T, Morsczeck C. miRNA-101 supports the osteogenic differentiation in human dental follicle cells. Arch Oral Biol. 2016;72:47–50.

67. Ito K, Tomoki R, Ogura N, Takahashi K, Eda T, Yamazaki F, et al. MicroRNA-204 regulates osteogenic induction in dental follicle cells. J Dent Sci. 2020;15(4):457–65.
68. Wang J, Zheng Y, Bai B, Song Y, Zheng K, Xiao J, et al. MicroRNA-125a-3p participates in odontoblastic differentiation of dental pulp stem cells by targeting Fyn. Cytotechnology. 2020;72:69–79.
69. Huang X, Liu F, Hou J, Chen K. Inflammation-induced overexpression of microRNA-223-3p regulates odontoblastic differentiation of human dental pulp stem cells by targeting SMAD3. Int Endod J. 2019;52(4):491–503.
70. Matsui S, Ogata Y. Effects of miR-223 on expression of IL-1β and IL-6 in human gingival fibroblasts. J Oral Sci. 2016;58(1):101–8.
71. Tian S, Liu Y, Dong F, Dou Y, Li W, Wang J. Knockdown of microRNA-584 promotes dental pulp stem cells proliferation by targeting TAZ. Cell Cycle. 2020;19(9):1048–58.
72. Wang Y, Zheng Y, Wang Z, Li J, Wang Z, Zhang G, et al. 10(-7) m 17β-oestradiol enhances odonto/osteogenic potency of human dental pulp stem cells by activation of the NF-κB pathway. Cell Prolif. 2013;46(6):677–84.
73. Yuan H, Zhao H, Wang J, Zhang H, Hong L, Li H, et al. MicroRNA let-7c-5p promotes osteogenic differentiation of dental pulp stem cells by inhibiting lipopolysaccharide-induced inflammation via HMGA2/PI3K/Akt signal blockade. Clin Exp Pharmacol Physiol. 2019;46(4):389–97.
74. Baru O, Raduly L, Bica C, Chiroi P, Budisan L, Mehterov N, et al. Identification of a miRNA panel with a potential determinant role in patients suffering from periodontitis. Curr Issues Mol Biol. 2023;45(3):2248–65.
75. Qiao W, Li D, Shi Q, Wang H, Wang H, Guo J. miR-224-5p protects dental pulp stem cells from apoptosis by targeting Rac1. Exp Ther Med. 2020;19(1):9–18.
76. Xu K, Xiao J, Zheng K, Feng X, Zhang J, Song D, et al. MiR-21/STAT3 signal is involved in odontoblast differentiation of human dental pulp stem cells mediated by TNF-α. Cell Reprogramming. 2018;20(2):107–16.
77. Gu S, Ran S, Liu B, Liang J. miR-152 induces human dental pulp stem cell senescence by inhibiting SIRT 7 expression. FEBS Lett. 2016;590(8):1123–31.
78. Wei F, Yang S, Guo Q, Zhang X, Ren D, Lv T, et al. MicroRNA-21 regulates osteogenic differentiation of periodontal ligament stem cells by targeting Smad5. Sci Rep. 2017;7(1):1–12.
79. Ou C, He X, Liu Y, Zhang X. lncRNA cytoskeleton regulator RNA (CYTOR): diverse functions in metabolism, inflammation and tumorigenesis, and potential applications in precision oncology. Genes Dis. 2021;10(2):415–29.
80. Tu S, Chen Y, Feng Y, Kuang Z, Wang Y, Chen L, et al. lncRNA CYTOR facilitates osteogenic differentiation of human periodontal ligament stem cells by modulating SOX11 via sponging miR-6512-3p. Stem Cells Int. 2023;2023.
81. Wei F, Liu D, Feng C, Zhang F, Yang S, Hu Y, et al. microRNA-21 mediates stretch-induced osteogenic differentiation in human periodontal ligament stem cells. Stem Cells Dev. 2015;24(3):312–9.
82. Wu M, Liu X, Li Z, Huang X, Guo H, Guo X, et al. SHED aggregate exosomes shuttled miR-26a promote angiogenesis in pulp regeneration via TGF-β/SMAD2/3 signalling. Cell Prolif. 2021;54(7): e13074.
83. Tan X, Dai Q. Characterization of microRNAs expression profiles in human dental-derived pluripotent stem cells. PLoS ONE. 2017;12(5): e0177832.
84. Wang Y, Pang X, Wu J, Jin L, Yu Y, Gobin R, et al. MicroRNA hsa-let-7b suppresses the odonto/osteogenic differentiation capacity of stem cells from apical papilla by targeting MMP1. J Cell Biochem. 2018;119(8):6545–54.
85. Sun F, Wan M, Xu X, Gao B, Zhou Y, Sun J, et al. Crosstalk between miR-34a and notch signaling promotes differentiation in apical papilla stem cells (SCAPs). J Dent Res. 2014;93(6):589–95.
86. Li N, Li Z, Yan M, Wang Y, Gu Y, Wu J, et al. miRNA-124-3p. 1 inhibits the osteogenic and odontogenic differentiation of SCAPs via MACF1/smad7 Axis. 2021.
87. Liu J, Wang X, Song M, Du J, Yu J, Zheng W, et al. MiR-497-5p regulates osteo/odontogenic differentiation of stem cells from apical papilla via the Smad signaling pathway by targeting Smurf2. Front Genet. 2020;11: 582366.
88. Li Y, Bian M, Zhou Z, Wu X, Ge X, Xiao T, et al. Circular RNA SIPA1L1 regulates osteoblastic differentiation of stem cells from apical papilla via miR-204-5p/ALPL pathway. Stem Cell Res Ther. 2020;11(1):1–15.
89. Li Z, Ge X, Lu J, Bian M, Li N, Wu X, et al. MiR-141-3p regulates proliferation and senescence of stem cells from apical papilla by targeting YAP. Exp Cell Res. 2019;383(2): 111562.
90. Chen P, Wei D, Xie B, Ni J, Xuan D, Zhang J. Effect and possible mechanism of network between Micro RNA s and RUNX 2 gene on human dental follicle cells. J Cell Biochem. 2014;115(2):340–8.
91. Ge J, Guo S, Fu Y, Zhou P, Zhang P, Du Y, et al. Dental follicle cells participate in tooth eruption via the RUNX2-MiR-31-SATB2 loop. J Dent Res. 2015;94(7):936–44.
92. Tomoki R, Ogura N, Takahashi K, Ito K, Kondoh T. MicroRNA-29 family suppresses mineralization in dental follicle cells. J Hard Tissue Biol. 2015;24(1):23–8.
93. Li Y, Duan X, Chen Y, Liu B, Chen G. Dental stem cell-derived extracellular vesicles as promising therapeutic agents in the treatment of diseases. Int J Oral Sci. 2022;14(1):2.
94. Kim D, Lee AE, Xu Q, Zhang Q, Le AD. Gingiva-derived mesenchymal stem cells: potential application in tissue engineering and regenerative medicine-a comprehensive review. Front Immunol. 2021;12: 667221.
95. Li Y, Liu C, Han G. Research progress of odontogenic extracellular vesicles in regeneration of dental pulp. Oral Dis. 2022.
96. Hu X, Zhong Y, Kong Y, Chen Y, Feng J, Zheng J. Lineage-specific exosomes promote the odontogenic differentiation of human dental pulp stem cells (DPSCs) through TGFβ1/smads signaling pathway via transfer of microRNAs. Stem Cell Res Ther. 2019;10:1–14.
97. Zheng Y, Dong C, Yang J, Jin Y, Zheng W, Zhou Q, et al. Exosomal microRNA-155-5p from PDLSCs regulated Th17/Treg balance by targeting sirtuin-1 in chronic periodontitis. J Cell Physiol. 2019;234(11):20662–74.
98. Nakao Y, Fukuda T, Zhang Q, Sanui T, Shinjo T, Kou X, et al. Exosomes from TNF-α-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss. Acta Biomater. 2021;122:306–24.
99. Zhou H, Li X, Wu RX, He XT, An Y, Xu XY, et al. Periodontitis-compromised dental pulp stem cells secrete extracellular vesicles carrying miRNA-378a promote local angiogenesis by targeting Sufu to activate the Hedgehog/Gli1 signalling. Cell Prolif. 2021;54(5): e13026.
100. Yan C, Li N, Xiao T, Ye X, Fu L, Ye Y, et al. Extracellular vesicles from the inflammatory microenvironment regulate the osteogenic and odontogenic differentiation of periodontal ligament stem cells by miR-758-5p/LMBR1/BMP2/4 axis. J Transl Med. 2022;20(1):208.
101. Wang Y, Zhang X, Wang J, Zhang Y, Ye Q, Wang Y, et al. Inflammatory periodontal ligament stem cells drive M1 macrophage polarization via exosomal miR-143-3p-mediated regulation of PI3K/AKT/NF-κB signaling. Stem Cells. 2023;41(2):184–99.
102. Kim S-H, Lee S-Y, Lee Y-M, Lee Y-K. MicroRNAs as biomarkers for dental diseases. Singapore Dent J. 2015;36:18–22.

103. Sinha A, Bhattacharjee R, Bhattacharya B, Nandi A, Shekhar R, Jana A, et al. The paradigm of miRNA and siRNA influence in Oral-biome. Biomed Pharmacother. 2023;159: 114269.
104. Urvasizoglu G, Kilic A, Barlak N, Gundogdu M, Karatas OF. MiR-4484 acts as a potential saliva biomarker for early detection of peri-implantitis. Int J Oral Maxillofac Implants. 2021;36(1):115–21.
105. Chen SC, Constantinides C, Kebschull M, Papapanou PN. MicroRNAs regulate cytokine responses in gingival epithelial cells. Infect Immun. 2016;84(12):3282–9.
106. Costantini E, Sinjari B, Di Giovanni P, Aielli L, Caputi S, Muraro R, et al. TNFα, IL-6, miR-103a-3p, miR-423-5p, miR-23a-3p, miR-15a-5p and miR-223-3p in the crevicular fluid of periodontopathic patients correlate with each other and at different stages of the disease. Sci Rep. 2023;13(1):126.
107. Huang P, Jia L. MicroRNA-28-5p as a potential diagnostic biomarker for chronic periodontitis and its role in cell proliferation and inflammatory response. J Dent Sci. 2022;17(4):1501–9.
108. Han P, Bartold PM, Salomon C, Ivanovski S. Salivary small extracellular vesicles associated miRNAs in periodontal status: a pilot study. Int J Mol Sci. 2020;21(8):2809.
109. Kaczor-Urbanowicz KE, Trivedi HM, Lima PO, Camargo PM, Giannobile WV, Grogan TR, et al. Salivary exRNA biomarkers to detect gingivitis and monitor disease regression. J Clin Periodontol. 2018;45(7):806–17.
110. Vasanthan P, Govindasamy V, Gnanasegaran N, Kunasekaran W, Musa S, Abu Kasim NH. Differential expression of basal micro RNA s' patterns in human dental pulp stem cells. J Cell Mol Med. 2015;19(3):566–80.
111. Zhai Q, Dong Z, Wang W, Li B, Jin Y. Dental stem cell and dental tissue regeneration. Front Med. 2019;13:152–9.
112. Lymperi S, Ligoudistianou C, Taraslia V, Kontakiotis E, Anastasiadou E. Dental stem cells and their applications in dental tissue engineering. Open Dentistry J. 2013;7:76.
113. Alarcón-Apablaza J, Prieto R, Rojas M, Fuentes R. Potential of oral cavity stem cells for bone regeneration: a scoping review. Cells. 2023;12(10):1392.
114. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, et al. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater. 2009;18(7):75–83.
115. Sheth N, Parmar B, Patel H, Makwana K. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells: a pilot study. Int J Oral Maxillofacial Surg. 2017;46:215.
116. Chen Y, Wang X, Wu Z, Jia S, Wan M. Epigenetic regulation of dental-derived stem cells and their application in pulp and periodontal regeneration. PeerJ. 2023;11: e14550.
117. Ortiz GGR, Mohammadi Y, Nazari A, Ataeinaeini M, Kazemi P, Yasamineh S, et al. A state-of-the-art review on the MicroRNAs roles in hematopoietic stem cell aging and longevity. Cell Commun Signal. 2023;21(1):1–16.
118. Jevnaker AM, Osmundsen H. MicroRNA expression profiling of the developing murine molar tooth germ and the developing murine submandibular salivary gland. Arch Oral Biol. 2008;53(7):629–45.
119. Xing X, Han S, Li Z, Li Z. Emerging role of exosomes in craniofacial and dental applications. Theranostics. 2020;10(19):8648.
120. Byun JS, Hong SH, Choi JK, Jung JK, Lee HJ. Diagnostic profiling of salivary exosomal micro RNA s in oral lichen planus patients. Oral Dis. 2015;21(8):987–93.
121. Luo H, Chen D, Li R, Li R, Teng Y, Cao Y, et al. Genetically engineered CXCR4-modified exosomes for delivery of miR-126 mimics to macrophages alleviate periodontitis. J Nanobiotechnol. 2023;21(1):1–15.
122. Huang C-C, Narayanan R, Alapati S, Ravindran S. Exosomes as biomimetic tools for stem cell differentiation: applications in dental pulp tissue regeneration. Biomaterials. 2016;111:103–15.
123. Miran S, Mitsiadis TA, Pagella P. Innovative dental stem cell-based research approaches: the future of dentistry. Stem Cells Int. 2016;2016.
124. El Moshy S, Radwan IA, Rady D, Abbass MM, El-Rashidy AA, Sadek KM, et al. Dental stem cell-derived secretome/conditioned medium: the future for regenerative therapeutic applications. Stem Cells Int. 2020;2020.
125. Kulthanaamondhita P, Kornsuthisopon C, Photichailert S, Manokawinchoke J, Limraksasin P, Osathanon T. Specific microRNAs regulate dental pulp stem cell behavior. J Endod. 2022;48(6):688–98.
126. Hussain A, Tebyaniyan H, Khayatan D. The role of epigenetic in dental and oral regenerative medicine by different types of dental stem cells: a comprehensive overview. Stem Cells Int. 2022;2022.
127. Kogure T, Lin WL, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology. 2011;54(4):1237–48.
128. Eiring AM, Harb JG, Neviani P, Garton C, Oaks JJ, Spizzo R, et al. miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. Cell. 2010;140(5):652–65.
129. Chen H, Xue R, Huang P, Wu Y, Fan W, He X, et al. Modified exosomes: A good transporter for miRNAs within stem cells to treat ischemic heart disease. Journal of Cardiovascular Translational Research. 2022:1-10.
130. Ma S, Jiang Y, Qian Y, Du J, Yu X, Luo S, et al. The emerging biological functions of exosomes from dental tissue-derived mesenchymal stem cells. Cell Reprogram. 2023;25(2):53–64.
131. Shi X, Yang G, Liu M-y, Yuan M-t, Wang D, Wang X-f. Exosomes derived from human dental pulp stem cells increase flap survival with ischemia-reperfusion injuries. Regenerative Medicine. 2023(0).
132. Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. J Tissue Eng Regen Med. 2015;9(11):1205–16.
133. Potdar PD, Jethmalani YD. Human dental pulp stem cells: applications in future regenerative medicine. World J Stem Cells. 2015;7(5):839.
134. Shen Z, Kuang S, Zhang Y, Yang M, Qin W, Shi X, et al. Chitosan hydrogel incorporated with dental pulp stem cell-derived exosomes alleviates periodontitis in mice via a macrophage-dependent mechanism. Bioactive Mater. 2020;5(4):1113–26.
135. Rajan TS, Giacoppo S, Diomede F, Ballerini P, Paolantonio M, Marchisio M, et al. The secretome of periodontal ligament stem cells from MS patients protects against EAE. Sci Rep. 2016;6(1):38743.
136. Rosa V, Dubey N, Islam I, Min K-S, Nör JE. Pluripotency of stem cells from human exfoliated deciduous teeth for tissue engineering. Stem Cells International. 2016;2016.
137. Zhuang X, Ji L, Jiang H, Liu Y, Liu X, Bi J, et al. Exosomes derived from stem cells from the apical papilla promote dentine-pulp complex regeneration by inducing specific dentinogenesis. Stem cells international. 2020;2020.
138. Wei J, Song Y, Du Z, Yu F, Zhang Y, Jiang N, et al. Exosomes derived from human exfoliated deciduous teeth ameliorate adult bone loss in mice through promoting osteogenesis. J Mol Histol. 2020;51:455–66.
139. Zhou T, Pan J, Wu P, Huang R, Du W, Zhou Y, et al. Dental follicle cells: roles in development and beyond. Stem Cells International. 2019;2019.

140. Zheng J, Kong Y, Hu X, Li Z, Li Y, Zhong Y, et al. MicroRNA-enriched small extracellular vesicles possess odonto-immunomodulatory properties for modulating the immune response of macrophages and promoting odontogenesis. Stem Cell Res Ther. 2020;11(1):1–14.
141. Ke Z, Qiu Z, Xiao T, Zeng J, Zou L, Lin X, et al. Downregulation of miR-224-5p promotes migration and proliferation in human dental pulp stem cells. BioMed Research International. 2019;2019.
142. Kang L, Miao Y, Jin Y, Shen S, Lin X. Exosomal miR-205-5p derived from periodontal ligament stem cells attenuates the inflammation of chronic periodontitis via targeting XBP1. Immunity Inflamm Dis. 2023;11(1): e743.
143. Gu X, Li X, Jin Y, Zhang Z, Li M, Liu D, et al. CDR1as regulated by hnRNPM maintains stemness of periodontal ligament stem cells via miR-7/KLF4. J Cell Mol Med. 2021;25(9):4501–15.