Stem Cell Research & Therapy

REVIEW

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Dental stem cell dynamics in periodontal ligament regeneration: from mechanism to application

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Abstract

Periodontitis, a globally prevalent chronic inflammatory disease is characterized by the progressive degradation of tooth-supporting structures, particularly the periodontal ligament (PDL), which can eventually result in tooth loss. Despite the various clinical interventions available, most focus on symptomatic relief and lack substantial evidence of supporting the functional regeneration of the PDL. Dental stem cells (DSCs), with their homology and mesenchymal stem cell (MSC) properties, have gained significant attention as a potential avenue for PDL regeneration. Consequently, multiple therapeutic strategies have been developed to enhance the efficacy of DSC-based treatments and improve clinical outcomes. This review examines the mechanisms by which DSCs and their derivatives promote PDL regeneration, and explores the diverse applications of exogenous implantation and endogenous regenerative technology (ERT) aimed at amplifying the regenerative capacity of endogenous DSCs. Additionally, the persistent challenges and controversies surrounding DSC therapies are discussed, alongside an evaluation of the limitations in current research on the underlying mechanisms and innovative applications of DSCs in PDL regeneration with the aim of providing new insights for future development. Periodontitis, a chronic inflammatory disease, represents a major global public health concern, affecting a significant proportion of the population and standing as the leading cause tooth loss in adults. The functional periodontal ligament (PDL) plays an indispensable role in maintaining periodontal health, as its structural and biological integrity is crucial for the long-term prognosis of periodontal tissues. It is widely recognized as the cornerstone of periodontal regeneration Despite the availability of various treatments, ranging from nonsurgical interventions to guided tissue regeneration (GTR) techniques, these methods have shown limited success in achieving meaningful PDL regeneration. As a result, the inability to fully restore PDL function underscores the urgent need for innovative therapeutic strategies at reconstructing this essential structure. Stem cell therapy, known for its regenerative and immunomodulatory potential, offers a promising approach for periodontal tissue repair. Their application marks a significant paradigm shift in the treatment of periodontal diseases, opening new avenues for functional PDL regeneration. However, much of the current research has primarily focused on the regeneration of alveolar bone and gingiva, as these

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hard and soft tissues can be more easily evaluated through visual assessment. The complexity of PDL structure, coupled with the intricate interactions among cellular and molecular components, presents significant scientific and clinical hurdles in translating DSC research into practical therapeutic applications. This review provides a thorough exploration of DSC dynamics in periodontal regeneration, detailing their origins, properties, and derived products, while also examining their potential mechanisms and applications in PDL regeneration. It offers an in-depth analysis of the current research, landscape, acknowledging both the progress made and the challenges that remain in bridging the gap between laboratory findings and clinical implementation. Finally, the need for continued investigation into the intricate mechanisms governing DSC behavior and the optimization of their use in regenerative therapies for periodontal diseases is also emphasized.

Keywords Periodontitis, Periodontal ligament regeneration, Dental stem cell, Stem cell therapy

Introduction

Periodontitis, a chronic inflammatory disease of the periodontal tissues driven by biofilm-induced dysbiosis, has merged as a global public health concern and ranks among the most prevalent oral diseases [1]. Currently, approximately 1.1 billion individuals worldwide (13.1% of the global population) are affected by severe periodontitis [2], making it the leading cause of tooth loss in adults. The primary objective of periodontal therapy is to halt the progression of periodontal inflammation while restoring both the structure and function of damaged periodontal tissues. According to the American Academy of Periodontology, periodontal regeneration entails the formation of cementum, alveolar bone, and functional periodontal ligament (PDL) on the root surface within the affected area [3]. The PDL plays a critical role in physical, nutritional, formative, and sensory functions, and its condition greatly influences the prognosis of periodontitis. Research has shown that the deliberate removal of PDL cells from cementum can lead to ankylosis, potentially impairing tooth function and resulting in root resorption of nonresilient-supported tooth. Moreover, regenerated PDL with well-organized fibers inserted into newly formed cementum can prevent apical migration of epithelium and contain precursors necessary for the regeneration of lost bone and cementum, thereby promoting superior clinical outcomes [4]. However, both nonsurgical treatments and regenerative approaches based on guided tissue regeneration (GTR) [5] using various biological products, have demonstrated limited success in achieving true periodontium regeneration [3]. It is widely accepted that the chronic inflammation responsible for periodontal soft and hard tissue defects—particularly PDL—cannot be reliably repaired using current symptomatic treatments, which only reconstruct the junctional epithelium. Thus, there is an urgent need for novel therapies capable of reconstructing functional PDL.

Stem cells have garnered significant attention in the field of tissue engineering due to their robust regenerative capabilities and immunomodulatory properties. Dental stem cells (DSCs) are MSCs with superior multilineage differentiation capacity, which are able to differentiate into odontoblasts, osteoblasts, etc. depending on certain inductive conditions [6]. The utilization of homogeneous MSCs in situ represents a promising strategy for reversing chronic dysbiosis. DSC-based periodontal regenerative cell therapy has emerged as a transformative approach in periodontal regeneration, offering the potential for functional PDL restoration. This review explores the origins, properties, and secretomes of DSCs while examining their underlying mechanisms and applications in PDL regeneration, along with recent research advancements in this field (Fig. 1).

DSCs and its products in PDL regeneration

DSCs

Various types of DSCs are derived from the neural crest, a multipotent and migratory origin [7], including dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), gingival mesenchymal stem cells (GMSCs), stem cells from apical papilla (SCAPs), and dental follicle stem cells (DFSCs). These cells exhibit MSC markers (such as Stro-1, CD146, CD106, CD90, CD73, CD29, and CD13), while consistently testing negative for hematopoietic stem cell markers (such as CD34, CD45, CD14, and CD19) and the MHC class II antigen (HLA-DR) [6]. Under certain culture conditions, DSCs demonstrate the ability to adhere to plates and form colonies. Their robust proliferative capacity and multilineage differentiation potential—spanning osteogenic, chondrogenic, and adipogenic lineages [6] —make them highly suitable candidates for tissue regeneration applications. Additionally, DSCs exhibit immunomodulatory properties, which enable them to regulate and maintain the homeostasis of the periodontal microbiome through immune response [8]. Notably, endogenous DSCs residing in specific niches can be recruited and mobilized by injury signals to damaged areas through a process known as homing. At the injury site, they differentiate into various cell types, facilitating tissue regeneration. Beyond these shared characteristics, each type of DSC possesses unique attributes, as outlined in Table 1.

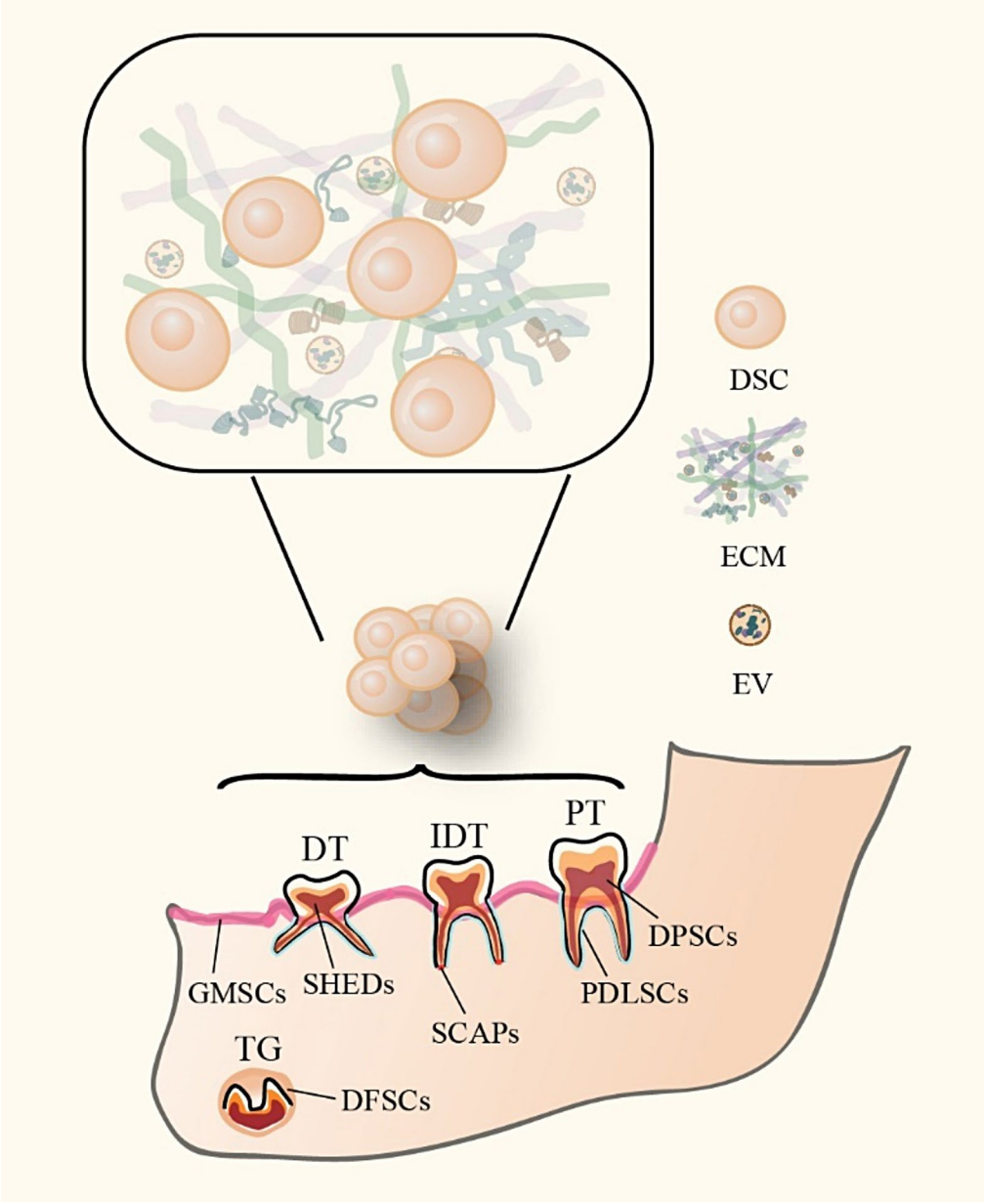

Fig. 1 Dental stem cells and their products in PDL regeneration. DSC: Dental stem cell ECM: Extracellular matrix EV: Extracellular vesicle DFSCs: Dental follicle stem cells GMSCs: Gingival mesenchymal stem cells SHEDs: Stem cells from human exfoliated deciduous teeth SCAPs: Stem cells from apical papilla PDLSCs: Periodontal ligament stem cells DPSCs: Dental pulp stem cells TG: Tooth germ DT: Deciduous tooth IPT: Incompletely developed tooth PT: Permanent tooth

Products of DSCs

Beyond the focus on living cells, increasing attention is being directed toward the physiological regulatory functions of certain secreted products from DSCs, which provide a foundation and theoretical support for cell-free therapies with reduced immunogenicity.

Extracellular vesicles (EVs)

As outlined by the International Society for Extracellular Vesicles (ISEV) in 2018, EVs are a heterogeneous group of membrane-derived lipid bilayer structures, categorized based on their cellular origin, such as DPSC-EVs [15]. EVs play a pivotal role as communication mediators between cells, with those released or deposited in the ECM targeting recipient cells through ligand binding on their surface, thereby triggering proximal or distal signaling cascades. Additionally, EVs facilitate interactions between secretory cells and ECM proteins, while also contributing to immunomodulation and the epigenetic reprogramming of recipient cells [16].

Table 1 Specificity of DSCs from different tissue

Dental stem cells	Advantages	Disadvantages	Isolation time
DPSCs	• easily accessibility and sufficient source • be isolated first, have more studies and clinical applications • potent capacity for forming vessel-rich tissue • higher cementoblasts/osteoblasts differentiation capacity	• Limited source for healthy autologous cells	2000 [9]
SHEDs	• higher proliferative and potential activity than DPSCs • potent capacity for forming neural tissue • remain stemness after cryopreservation	• limited source • lack of studies and clinical applications	2003 [10]
PDLSCs	• higher proliferation potential • higher self-renewal capacity • potent capacity for forming cementum/PDL-like structure with functional attachment • homogeneous for functional regeneration • possess low immunogenicity	• Limited source for healthy autologous cells • lower osteogenic capacity	2004 [11]
DFSCs	• have specific markers • higher proliferation potential • greater plasticity and multipotential capacity (derived from developing tissue) • potent capacity for forming connective tissue and PDL-like tissue • low immunogenicity	• Limited source for autologous cells • low cell abundance • inferior hard tissue-forming potential • lack of studies and clinical applications	2005 [12]
SCAPs	• have specific markers • higher proliferation potential • greater plasticity and multipotential capacity (derived from developing tissue) • grater mineralization ability • low immunogenicity	• Limited source for autologous cells • low cell abundance • lack of studies and clinical applications	2006 [13]
GMSCs	• easily and sustainably accessibility • higher proliferation potential • rapid wound healing property with minimal scar-formation • potent capacity for forming connective and bone-like tissues • immunomodulatory and anti-inflammatory characteristics	• Limited source for healthy autologous cells • low cell abundance	2009 [14]

DPSCs: Dental pulp stem cells SHEDs: Stem cells from human exfoliated deciduous teeth PDLSCs: Periodontal ligament stem cells DFSCs: Dental follicle stem cells SCAPs: Stem cells from apical papilla GMSCs: Gingival mesenchymal stem cells PDL: Periodontal ligament

DSCs, originating from the neural crest, possess exceptional capabilities in immunomodulation, wound healing, odontogenic differentiation, and neuronal differentiation. DSC-EVs have exhibited significant therapeutic potential in addressing neurological disorders, immune diseases, and wound healing [17]. As cell-derived products from dental tissues, DSC-EVs from various sources have been shown to promote the regeneration of dental tissues such as pulp, dentin, and periodontal tissues, by mediating key processes like cell migration, proliferation, and differentiation required for tissue regeneration. Notably, research on DPSC-EVs has provided solid evidence supporting their role in pulp regeneration. DSC-EVs hold considerable promise as tools for PDL regeneration by leveraging their immunomodulatory properties to counteract the excessive immune response typical of the periodontitis environment, promoting differentiation into periodontal lineages essential for PDL regeneration, stimulating vascular regeneration to supply nutrients to newly formed PDL, and ultimately enabling more effective restoration of the PDL [18]. Future research must focus on clarifying the mechanisms, optimal delivery strategies, and ideal DSC-EV subpopulations for use in periodontal regeneration.

Extracellular matrix (ECM)

ECM is the non-cellular component that surrounds cells and tissues, providing essential three-dimensional structural support and containing intrinsic biochemical and biomechanical cues vital for tissue regeneration [19]. Composed primarily of water, proteins, and polysaccharides, the ECM includes components such as glycosaminoglycan (GAG)-based ultrastructures, growth factors (GFs), and adhesion molecules. The ECM plays a critical role in directing cellular behavior and determining cell fate by mediating cell adhesion, migration, and signal transduction, while also regulating cell phenotype and function. As a result, it is indispensable in tissue development, homeostasis, injury response, and regeneration, and is even involved in pathological processes [20].

As a reservoir of growth and regulatory factors, the ECM from allogenic or xenogeneic tissues without nuclei possesses low immunogenicity, offering advantages such as long-term storability and cost-effectiveness [21]. These properties have generated significant interest in the field of tissue regeneration. Studies have shown that ECM derived from MSCs can effectively recreate the stem cell niche, protecting reseeded MSCs from oxidative stress and thereby promoting cell proliferation and maintaining stemness. For instance, research has demonstrated that human urine-derived stem cells enhance the regenerative capacity of PDLSCs [22]. Advanced ECM-based scaffolds, both with and without additional scaffolds, derived from non-dental tissues, have already been applied in periodontal and peri-implant soft tissue regeneration [23, 24]. Numerous studies confirm the efficacy of DPSC-ECM in facilitating pulp tissue regeneration [25]. Moreover, DPSC-ECM has been shown to

possess osteoinductive properties [26], with pretreatment enhancing the odonto/osteogenic differentiation of SCAPs [27], underscoring the superior regenerative potential of DSC-ECM across a wide range of applications. Thus, DSC-ECM from different sources offers a diverse range of periodontal regeneration environments, supplying essential molecules that can recreate the homeostatic microenvironment of the periodontium under inflammatory conditions and mediate periodontal regeneration [26, 28, 29]. It is anticipated that DSC-ECM holds the potential to become a leading therapeutic approach for PDL regeneration in the near future.

Mechanisms

PDL predominantly consists of fibers, cells, and the surrounding ECM, with nourishment supplied by blood vessels and nerve bundles.

A significant portion of the PDL fibers, constituting approximately 50–75% of its volume, is composed largely of collagen fibrils, primarily type I collagen, with smaller contributions from types III, IV, V, VI, and XII collagen [30]. These collagen fibers form fiber bundles, with the principal fibers being the most critical components. These fibers anchor the tooth by inserting into the cementum on one side and the alveolar bone on the other. Additionally, the principal fibers contain elastic fibers (oxytalan) [31], which contribute to the formation of the neural and vascular network within the PDL and regulate vascular flow.

Principal fibers are categorized into five groups based on their spatial arrangement and location, collectively contributing to tooth support and stabilization, particularly during mastication. Among these, the fibers that insert into both the cementum and alveolar bone are referred to as Sharpey's fibers. These fibers primarily insert into the acellular extrinsic fiber cementum (AEFC) and the alveolar bone matrix, where they undergo mineralization, strengthening their anchorage. This mineralized anchorage is essential for tooth fixation and for buffering against external forces, presenting a notable challenge in PDL regeneration.

The PDL's most prevalent and functionally significant cells are fibroblasts, specifically referred to as periodontal ligament cells (PDLCs). These cells are responsible for producing collagen fibers and play a pivotal role in the continuous remodeling and regeneration of the PDL. Together, these components are essential for PDL regeneration.

PDL development is a complex and dynamic process. As the tooth root forms, PDL fiber bundles are initially established, followed by the differentiation of DSCs within the dental follicle adjacent to the root. These DSCs subsequently differentiate into cementum and alveolar bone, where collagen fibers secreted by PDLCs, along with Sharpey's fibers, mineralize to form a mature PDL. This structure provides mechanical support and nutrition and facilitates ongoing remodeling. The following sections will delve into the processes initiated by DSCs, including collagen turnover, microenvironmental changes that contribute to tissue destruction, and other periodontium regeneration, as well as the orderly regeneration of the PDL (Fig. 2). These discussions aim to elucidate the mechanisms by which stem cell therapy influences PDL regeneration (Fig. 3).

Repair of impaired collagen turnover

The structural integrity of PDL tissue is maintained through the rapid turnover of collagen, with collagen fibers having a notably short half-life of only a few days [32]. This process is primarily attributed to the activity of PDLCs, which function as a self-renewing system to sustain tissue homeostasis. PDLCs produce new collagen fibers through proliferation, counterbalancing their degradation at a high rate. In this process, PDLCs effectively remove senescent or damaged collagen fibrils and replace them with newly synthesized ones. However, this regenerative capacity is notably impaired under inflammatory conditions. A series of studies have demonstrated that PDLCs exhibit heightened inflammatory gene expression in cases of periodontitis [33], leading to increased susceptibility to pathogens such as P. gingivalis [34]. Furthermore, PDLCs in periodontitis can be induced into distinct pro-inflammatory phenotypes that perpetuate destructive signaling *in suit*; however, this condition may be reversible.

DSCs not only contribute to the differentiation of fibroblasts and enhance collagen production for PDL repair, but also exert anti-inflammatory effects on fibroblasts by modulating their migratory and secretory behaviors. In murine models, DPSCs and their ECM have been shown to promote wound healing by inhibiting the inflammatory response of fibroblasts and upregulating key proteins involved in the healing process [35], which is primarily mediated through the TGF-β/Smad pathway. Additionally, PDLSCs demonstrate multidirectional differentiation capabilities, spontaneously differentiating into a subpopulation originating from fibroblasts during in vitro expansion [36]. PDLSC-ECM has also been found to significantly enhance the migration of human gingival fibroblasts (HGFs).

In summary, PDLCs, which belongs to fibroblasts, can be quantitatively and functionally repaired through the involvement of DSCs. It is responsible for the repair of impaired collagen turnover caused by periodontitis, and consequently enhance the efficacy of PDL regeneration. However, limited research has been conducted on other types of DSCs. GMSCs, which share similar morphology and phenotypic characteristics with HGFs [37], and

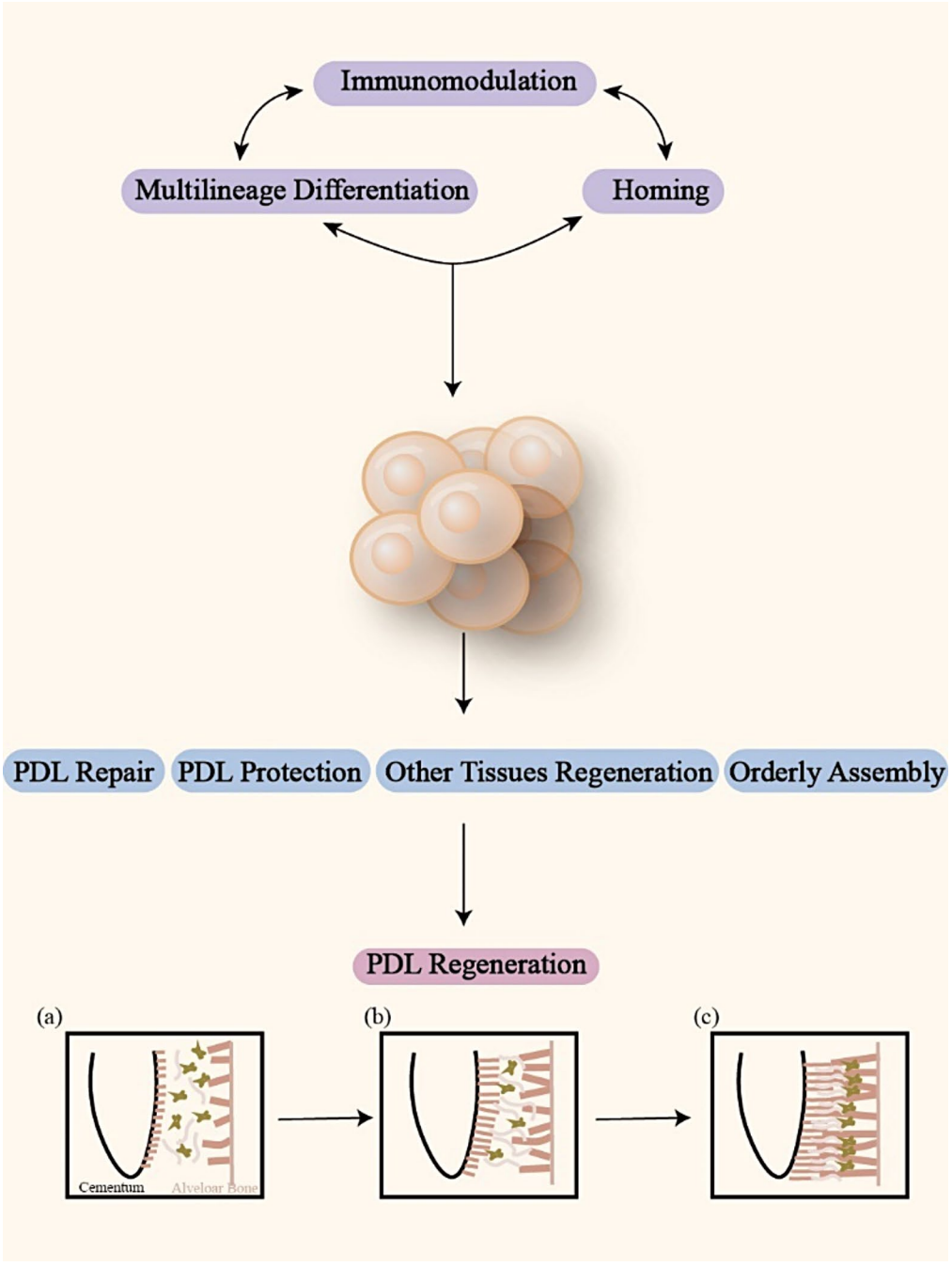

Fig. 2 Overview of mechanism of PDL regeneration of DSCs (**a**) Fibers oriented perpendicular to the longitudinal axis of the root are observed to protrude from the cementum, while the fibers originating from the alveolar bone are thicker and less. (**b**) Fibers from both sides extend into the PDL space without connection, separated by irregular-distributed collagen and fibroblasts. (**c**) A complicated network is observed in PDL space with continuous Sharpey's fibers as well as numerous collagen and fibroblasts. PDL: Periodontal ligament

SHEDs, which share a common origin with DPSCs, are potential candidates for PDLC repair. Despite these findings, current studies have largely focused on wound healing rather than periodontal regeneration. Consequently, further research is required to explore the relationship between DSCs and PDLCs, particularly in the context of PDL impairment.

Tissue destruction protection

The host's excessive immune response to periodontitis results in significant changes in the number and function of immune cells within the PDL, alongside the degradation of the ECM and fibers, further exacerbating the condition.

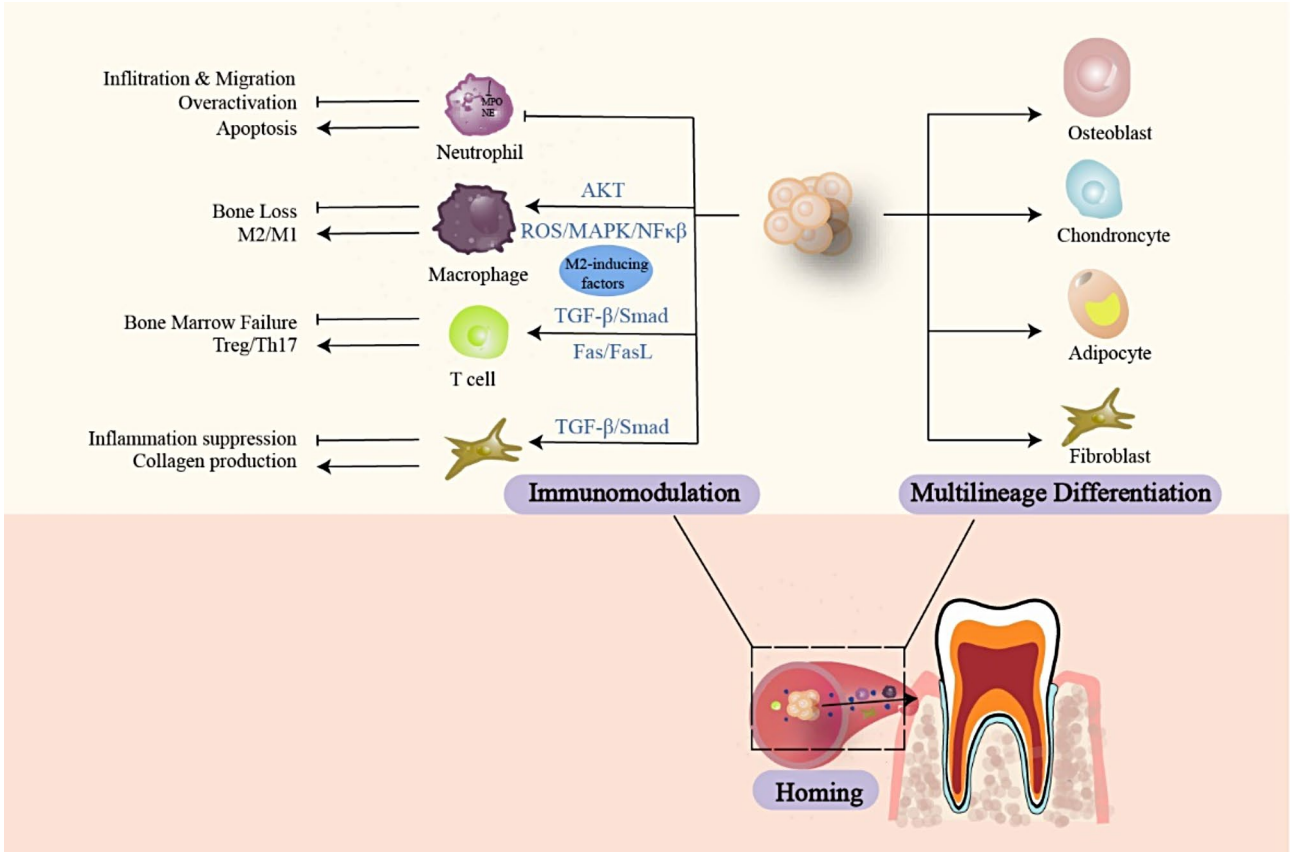

Fig. 3 Mechanisms in PDL regeneration of DSCs and its products. M1: Macrophage differentiation M1 M2: Macrophage differentiation M2 Th: Helper T cell Treg: Regulatory T cell

Matrix metalloproteinases (MMPs), which are secreted by connective tissue cells, pro-inflammatory cells, including fibroblasts and osteoblasts, as well as immune cells, have the capability to degrade various components of the ECM and fibers. In cases of periodontitis, elevated levels of MMPs, particularly MMP-8, MMP-9, and MMP-13, are observed in saliva and gingival crevicular fluid [38], contributing to the extensive degradation of PDL that is rich in collagen fibers. Additionally, in vitro studies have shown that oxytalan fibers can be degraded by MMP-2 [39]. Consequently, maintaining the balance between MMPs and their tissue inhibitors (TIMPs) is essential for the regeneration of PDL. Moreover, neutrophil-derived enzymes such as neutrophil elastase (NE) and myeloperoxidase (MPO) can activate pro-MMPs and inactivate TIMP-1, thereby amplifying the destructive MMP cascades [40, 41]. Certain cytokines, such as TGF-β, have been shown to regulate this process by downregulating MMP synthesis while upregulating TIMP expression [42].

DSCs appear to play a regulatory role in modulating MMP cascades. In vitro studies have demonstrated that DPSCs can reduce MMP-9 expression [43], while in vivo, they have been shown to downregulate MPO expression [44]. Given that DPSCs and other MSCs have the capacity to produce TGF-β [45, 46], they are effective in inhibiting MMP-mediated degradation. Similarly, SHEDs and their derivatives, also originating from dental pulp, are recognized for their potent immunoregulatory effects on MMP-TIMP balance [47, 48]. Research has shown that the PDLSC-ECM can inhibit IL-1β-induced MMP-13 expression [49]. Experiments introducing SCAP-EVs and bioactive scaffolds into murine bone defects in the presence of MMP-9 have demonstrated enhanced periodontium regeneration [50]. Furthermore, GMSCs have been shown to reduce the expression of MMP-9 and NE, in a mouse model of pulmonary fibrosis [51].

Given the well-established role of MMP cascades in tissue destruction, numerous studies have explored the regulatory effects of various DSC sources and their products in this domain. These stem cells primarily modulate the MMP-TIMP balance through immunomodulatory pathways, thereby protecting the PDL tissue including ECM and fibers from destruction. However, the limitation lies in the absence of periodontitis models for DSCs in relation to MMP-TIMP, further research into periodontitis models is essential to deepen our understanding of these mechanisms.

Regeneration of other tissues

In addition to serving as the essential material for collagen fibers, regenerated PDL fibers must be reinserted into the alveolar bone and cementum to form Sharpey's fibers, achieving functional regeneration. Under physiological conditions, PDL remodeling on the alveolar bone side occurs at a faster rate compared to the cementum side, likely due to the higher maturity of collagen on the cementum surface [30]. This disparity may be attributed to the significantly more rapid turnover of alveolar bone. Therefore, facilitating the reconstruction of alveolar bone is critical for the proper formation of Sharpey's fibers.

The regenerative potential of DSCs in promoting alveolar bone repair and mitigating inflammation-induced bone loss has been well-documented in both in vivo and in vitro studies. Substantial evidence supports the osteogenic and osteoinductive properties of DPSCs [52] and their EVs [53] in jawbone regeneration, akin to the effects of PDLSC-EVs [54], which function through the suppression of Wnt signaling [55]. Notably, a study demonstrated that GMSC-EVs pretreated with TNF-α [56] could target Wnt-mediated RANKL [57] leading to the inhibition of osteoclastogenesis. An investigation earlier revealed that GMSCs itself possessed the capacity to alleviate T cell-mediated bone marrow failure by modulating the ratio of helper T cells (Th) to regulatory T cells (Treg) [58], similar to the mechanism observed in SHEDs [59]. Moreover, SHED-ECM [60] and PDLSCs [61] have been shown to reduce bone loss through M2 macrophage polarization, while SHED-EVs enhance osteogenesis, potentially by activating AMPK signaling [62]. In addition to healthy DSCs and their products, some inflamed DSCs may exhibit enhanced regenerative capacity. It has been demonstrated that the osteogenic potential of DPSCs and GMSCs is not only preserved but also augmented in the inflammatory environment due to the upregulation of various stress response proteins, which are dependent on proinflammatory cytokines [63]. This enhanced capacity has been further confirmed, as these cells exhibit superior proliferation and migration abilities in inflammatory conditions [64].

Under physiological conditions, the turnover rate of cementocytes is notably low, which may be attributed to the limited presence of stem cells within the cementum. As a result, the therapeutic use of DSCs to differentiate into dental tissue is essential for cementum redeposition on the tooth root and the formation of Sharpey's fibers. Transplantation of PDLSCs and DFSCs has been shown to regenerate cementum with PDL [65], a process potentially mirrored by co-culturing GMSCs with PDLSCs, likely through M2 macrophage induction [66]. However, research specifically targeting cementogenesis remains scarce, possibly due to the unclear conditions required to induce cementogenesis and the limited recognition of its importance in PDL regeneration. Current research on cementum regeneration is predominantly centered on treating dentin hypersensitivity caused by cervical exposure. The absence of regenerated AEFC complicates the stabilization of mineralized anchorage for newly formed PDL fibers, potentially weakening the reparative PDL's supportive function for the teeth. Nonetheless, experimental evidence suggests that DSCs have considerable potential for inducing cementogenesis under specific conditions, making the identification of these conditions and their underlying mechanisms a critical area of interest for PDL regeneration research.

The field of immunomodulation has garnered increasing attention. In 2023, a debate emerged regarding whether the primary effectors in regeneration were endogenous immune cells rather than DSCs, given that only 40% of xenogenous DSCs survived by the seventh day [67]. A 2024 study provided further evidence that DFSC implantation stimulated the host's innate immune response by inducing neutrophil activity [68], an observation that aligns with earlier findings related to PDLSCs in 2019 [69]. Recent investigations have highlighted DSC-induced modifications in immune cells, such as macrophages [70], T cells [71–73], and B cells [74]. These studies suggest that the immune cascade initiated by DSCs may play a predominant role in the regenerative effects observed, emphasizing the need to explore the mechanisms of DSC-mediated immunomodulation, which may occur through DSCs' products *via* paracrine pathways.

In conclusion, DSCs possess significant multipotent differentiation and immunomodulatory capabilities, enabling them to mitigate inflammation-induced damage and promote the regeneration of alveolar bone and cementum that are essential for PDL function [75]. By effectively controlling periodontitis and promoting the regeneration of other periodontal tissues either directly or indirectly, DSCs present a promising resource for functionally PDL regeneration. While there have been notable advancements to date, certain limitations persist. Contemporary research has predominantly concentrated on alveolar bone rather than cementum, thereby highlighting the necessity for scholars to undertake comprehensive investigations into the latter in future studies. What's more, research suggests that in situ DSCs achieve the best outcomes for PDL regeneration. PDLSCs and DFSCs demonstrate superior potential in forming an organized PDL structure compared to DPSCs alone, which require supplementary bioactive factors [52, 65]. This discrepancy may be due to the distinct differentiation patterns inherent to various stem cell types. Understanding these differentiation preferences and mechanisms will assist in the clinical selection of suitable DSCs and may even allow for the use of induced pluripotent stem cells (iPSCs) to

customize tissue regeneration strategies based on specific clinical needs in the future.

Promotion of orderly PDL regeneration

The PDL is not merely a "sandwich" structure composed of PDL fibers, alveolar bone, and cementum, but rather a highly organized construction shaped by its complex developmental process. As a result, strategies aimed solely at reproducing PDL fibers fall short of achieving true PDL regeneration and should instead be viewed as forms of "repair". Under physiological conditions, the remodeling of PDL is driven by the involvement of PDLSCs and bone marrow stem cells (BMSCs) that enter the PDL *via* blood vessels. Stem cells are notable not only for their multipotent differentiation capabilities but also for their critical spatiotemporal regulatory functions. The precise coordination of signaling pathways in time and space is essential to ensure that regenerated tissues closely mimic the form and function of their original counterparts, thereby accomplishing the goal of true tissue "regeneration".

It is well-established that the gene expression of DSCs is tightly controlled to ensure that appropriate proteins are expressed at the correct time and location, facilitating the orderly regeneration of PDL [76]. The balance between Wnt and BMP signaling pathways is particularly important in determining the fate of DFSCs, which subsequently influences PDL formation by regulating osteogenesis, as previously mentioned. Additionally, the autocrine signaling pathway involving parathyroid hormone-related peptide (PTHrP) and its receptor (PTH1R) plays a pivotal role in promoting the development of aligned PDL fibers. PTHrP(+) DSCs differentiate into PDLCs and acellular cementoblasts, primarily influencing cementogenesis [77].

Furthermore, the spatiotemporal regulatory capacity of stem cells extends beyond tissue regeneration and includes endogenous cell homing and mobilization of host-derived stem cells. This refers to the natural process by which cells are recruited to specific tissues or organs, and the activation of host stem cells to support tissue repair and regeneration [78].

DPSCs can be recruited to sites of pulpitis inflammation through chemokine interactions such as TGF-β1 [79], as well as through EVs interacting with neighboring cells and the ECM [80]. Current clinical interventions also utilize this characteristic. During regenerative endodontic procedures, the process of inducing bleeding leads to the migration of undifferentiated stem cells into the canal space, which can contribute to pulp tissue regeneration [81]. This homing process is largely mediated by chemotactic factors from stem cells, including stromal-derived factor 1 (SDF-1) [82], stem cell factor (SCF), and bone morphogenetic protein-2/7 (BMP2/7) [83]. Notably, SDF-1, whose expression increases following injury [84], binds to C-X-C chemokine receptor type 4 (CXCR4), facilitating stem cell homing to the wound site and subsequent differentiation through the SDF-1/CXCR4 axis [82, 85]. This axis also plays a key role in promoting PDLSC proliferation, migration, and osteogenesis [86].

In summary, DSCs promote the orderly regeneration of PDL by activating the "wound healing cascade" through autocrine and chemotactic signaling pathways, allowing it to self-regenerate spatiotemporally. The regenerative potential is further enhanced by recruiting endogenous stem cells for precise tissue regeneration, a process known as endogenous regenerative technology (ERT). By leveraging the ability of MSCs to mobilize and direct themselves toward injury sites under inflammatory conditions, this approach broadens the applications of DSCs in tissue regeneration. The recruitment of MSCs to injury sites enables the use of cell-free homing therapy, which directs nearby DSCs to periodontal defect sites, ultimately achieving orderly PDL regeneration [83, 86].

Application

As previously mentioned, DSCs and their derivatives hold the potential to functionally repair PDL damage, improve the prognosis for patients with periodontitis, and offer a novel strategy for periodontal regenerative therapy. Numerous studies, both in vitro and in vivo, have investigated stem cell-based approaches to promote PDL regeneration, either by introducing exogenous stem cells or regulating endogenous stem cells (Fig. 4).

A major point of contention in stem cell therapy, across various fields, lies in the appropriate selection of cell types. The comparative osteogenic potential of different DSCs has not been conclusively determined, largely due to the diverse states of DSCs and the lack of standardized experimental conditions [87–89]. This variability makes it more challenging to establish the optimal type of DSC for PDL regeneration, as no universally accepted in vitro culture methods or in vivo research models currently exist. However, increasing attention is being paid to this issue. For instance, a 2020 study demonstrated that PDLSC sheets were superior for PDL regeneration [90], as they were able to generate directionally organized fibers, a finding that aligns with previous research [91]. Additionally, in 2023, GMSCs and SHEDs were identified as promising candidates for PDL regeneration [92]. Despite these advancements, further comprehensive research is needed to reach definitive conclusions regarding the most suitable DSC types and conditions for optimal PDL regeneration.

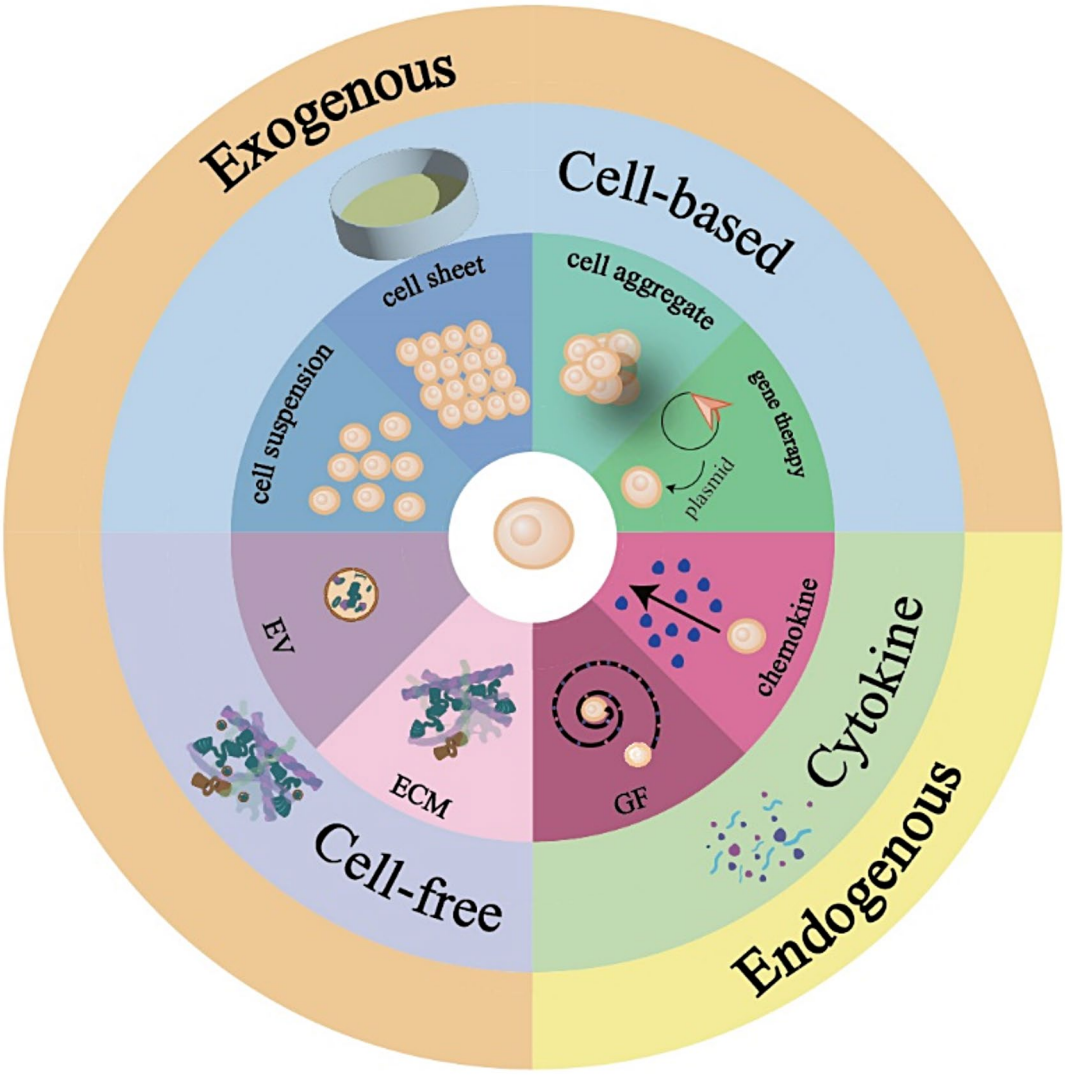

Fig. 4 Application of DSCs and its products. EV: Extracellular vesicle ECM: Extracellular matrix GF: Growth factor

Exogenous DSCs

In regard to the source of DSCs, autologous, allogenous, and xenogenous grafts have been explored as potential sources for tissue regeneration in preclinical studies [93]. However, most advanced clinical trials have focused primarily on autografts. In periodontal tissue regeneration, PDLSCs are considered the optimal choice for seed cells due to their homology with the target tissue. Stem cells can be delivered either systemically, through injection into the circulation, or locally, by direct transplantation into the defect site. The systemic approach relies on the regulation and homing of exogenous stem cells to target lesions, making it more suitable for treating systemic disorders [59]. Conversely, direct transplantation offers higher tissue specificity and reduced stem cell loss, making it more applicable for periodontal regeneration, where defects are relatively superficial and accessible. Currently, the advanced stem cell therapies mentioned below are replacing the less efficient traditional methods of transplantation, and stem cell products with superior properties have emerged as the primary areas of investigation in PDL regenerative cell therapy.

Cell suspension

The traditional cell therapy is the single-cell suspension injection into the body, frequently encountered with challenges such as low cell survival rates and insufficient structural support [94]. To address these limitations, researchers are now investigating the use of scaffold materials to improve the efficacy of stem cell transplantation and enhance regenerative outcomes.

Scaffold-assisted cell suspensions have demonstrated improved outcomes for PDL regeneration. These scaffolds, such as calcium phosphate combined with

injectable hydrogels, support the regeneration of periodontal tissues, including the PDL [95, 96]. The addition of bioactive agents, such as connective tissue growth factor (CTGF), to these cell suspension-scaffold complexes stimulates the differentiation of stem cells into fibroblasts, thereby promoting PDL regeneration [97]. Additionally, studies have shown that co-culturing DPSCs and PDLSCs on material scaffolds can upregulate mRNA expression related to osteogenesis and odontogenesis [98]. Since cell suspensions do not require specialized culture techniques, they allow for the prompt clinical application of autologous DSCs in patients. Notably, the seeding of DPSCs [99, 100], PDLSCs [99], and GMSCs with associated gingival fibroblasts [101] into bioscaffolds has demonstrated significant clinical attachment level (CAL) gains in periodontal defects, further elucidating the regenerative capacity of PDL.

In summary, DSC suspensions offer the benefit of simple culture procedures, which are appropriate for clinical application and commonly applied with scaffolds. Despite the promising results of scaffold-based approaches, there are still notable limitations to using cell suspensions. These include the vulnerability of suspended cells to external factors such as mechanical stress, temperature fluctuations, and ex vivo conditions, as well as difficulties in organizing them into ordered cell clusters. These challenges hinder the ability to fully address the regenerative requirements of the PDL due to its complex microstructure. As a result, alternative approaches, such as cell sheet and cell aggregate technologies, are gaining increasing attention for their potential to overcome these limitations and improve PDL regeneration outcomes.

Cell sheets

Cell sheets are defined as layers of cells cultivated to a hyper-confluent state, allowing for extensive cell-cell interactions with deposited ECM, and the prevention of anoikis [102]. Initially, cell sheets were created using temperature-responsive culture dishes, but more recent systems, such as ion-induced cell detachment surfaces and electro-responsive surfaces, have been developed to avoid the adverse effects of low temperatures on cell functions [103]. Current research is increasingly focused on improving the functionality of cell sheets. For instance, the vitamin C-induced method has been shown to enhance the proliferation capacity and osteogenic differentiation efficiency of PDLSCs [104].

The cell sheet technique has been widely employed in PDL regeneration studies and has demonstrated superiority over cell suspension injections [52]. PDL regeneration was observed in both autologous and allogeneic PDLSC transplantation in vivo [105], as well as in human DPSCs [52], SHEDs [106], and DFSCs [107, 108] especially when assembled into multilayer structures. The regenerative capabilities of DSCs are undisputed, making strategies to enhance their effectiveness a prominent research topic. For example, reimplantation of avulsed teeth using PDLSC sheets combined with platelet-rich fibrin (PRF) granules has led to improved periodontal healing, evidenced by the regeneration of PDL-like tissues and a reduction in ankylosis and inflammation [109]. Composite sheets co-culturing PDLSCs with jaw-derived BMSCs [110], DFSCs [111], and human umbilical vein endothelial cells (HUVECs) [112] have successfully induced organized PDL with Sharpey-like fibers in nude mice. PDLSC sheets pretreated with recombinant human BMP-2 (rhBMP-2) [113] have shown increased proliferation, differentiation, and ECM protein production. Additionally, scaffolds like natural human tooth root fragments [112] and artificial polycaprolactone membranes [114] can be employed to provide structural support and stabilization for these cell sheets.

In the past, DSC implantations were primarily cultured in basic media, but researchers have since shifted to using DPSC sheets cultured in osteoinductive media [115]. Since 2019, this modification has demonstrated superior bone regeneration effects compared to traditional methods, favoring the mineralized anchorage of Sharpe's fibers. This progression highlights the potential for further improvement in DSC implantation outcomes through the modification of cell sheet materials and the application of PDL induction methods.

Overall, DSC sheets have demonstrated better regenerative outcome of PDL than DSC suspension. And it is encouraging to observe a growing array of advanced techniques aimed at enhancing the regeneration efficiency of DSC sheets, which include specific culture methods, the incorporation of bioactive factors, and the utilization of scaffolds. However, a 2016 randomized controlled trial (NCT01357785) involving 30 patients found no significant differences between groups treated with and without PDLSC sheets, a result that contradicts preclinical studies [116, 117]. The discrepancy may be due to several factors, including the small sample size, limited number of experimental centers, impractical cell dosage in the delivery system, and potential functional impairment of the cells during processing.

To address these challenges, future investigations should include larger patient cohorts, human-derived bio-delivery systems, and more refined pretreatment techniques to better assess the efficacy of DSC-based therapies for PDL regeneration.

Cell aggregates

Cell aggregates are generated through micro-mass particle culture of clustered cells under specific environmental conditions, creating 3D structures with a higher concentration of ECM, which can enhance their biological

and inductive properties. These aggregates can take on various forms, such as microparticles, microtissues/spheroids, or even organoids, with microparticles and spheroids being most commonly applied in PDL regeneration [118].

PDLSC aggregates alone have demonstrated superior ECM secretion and anti-inflammatory properties, leading to functional recovery of the PDL in rat models of inflammatory periodontal defects [119]. Similar efficacy has been observed in the application of DPSC [120], and DFSCs aggregates [121] within "sandwich-like" structures. One study highlighted that PDLSC pellets, composed of PDLSC aggregates and apical tooth germ cell-conditioned medium (APTG-CM), exhibited a connective tissue-like morphology in vitro and induced a cementum/PDL-like complex in vivo [122]. Beyond ECM production, DSCs can also be co-cultured with MSCs from other sources. For instance, co-culturing PDLSCs with jaw-derived BMSCs in aggregates has shown better outcomes than any mono-aggregate [123]. These findings suggest that 3D-structured implantations can be tailored in various morphologies that are favorable for periodontal regeneration, where cell adhesion is preserved, cell-cell interactions remain intact, and an optimal environment for ECM secretion is provided. This structure enhances the quality of cell engraftment, resulting in improved PDL regeneration outcomes.

In a significant advancement, researchers successfully created an in vitro PDL model in 2024 using PDLCs with tensile loading, marking a notable step forward in PDL regeneration through organoids [124]. Although the model cannot yet be directly compared to in vivo PDL due to its limited size and the forces applied, it represents a milestone in PDL regeneration. Organoids rely on self-organization, where cells sort and commit to specific lineages in a spatiotemporally restricted manner, forming organ-like structures [125]. While this approach shows promise for larger organs, its randomness and scale limit its immediate application to PDL regeneration [126]. Further research into microscopic applications is necessary to explore the full potential of organoids in PDL regeneration.

In short, DSC aggregates have facilitated superior PDL regeneration in its 3D structure. Apart from the latest breakthrough in PDL organoids, efforts are ongoing to enhance the efficiency of cell aggregates. Beyond the incorporation of bioactive agents, pharmacological interventions, such as resveratrol, have been shown to boost the anti-oxidative, anti-inflammatory, and osteogenic potential of endogenous MSCs *via* specific signaling pathways [127]. Additionally, the application of personalized scaffolds to construct DSC aggregates has significantly improved their regenerative effect on PDL [128]. This suggests that further investigation into methods for enhancing the regenerative potential of cell aggregates, including improving immunomodulation and ECM synthesis, could be a promising area for future research.

Cell products

The secreted products of exogenous stem cells, known as cell-free therapy, offer remarkable potential for tissue regeneration. These products are non-immunogenic, easy to mass-produce, convenient to store, and long-term stable. Additionally, they circumvent challenges associated with donor aging and cell source availability, making them an optimal strategy for PDL regeneration.

EVs, one of the most important paracrine mediators, have demonstrated significant advantages in preclinical trials. For example, DPSC-EVs [129], and SHED-EVs [62] have shown therapeutic effects in mouse experimental periodontitis by accelerating the healing of periodontal tissues. Similarly, EVs from healthy PDLSCs [54], DFSCs (with or without LPS pretreatment) [18], and GMSC pretreated with TNF-α [56] have been shown to rescue the osteogenic capacity of endogenous cells within the periodontal environment under inflammatory conditions, thereby promoting PDL regeneration. Furthermore, treatment with MSC-derived EVs resulted in notable improvements in PDL length and reductions in bone loss, suggesting the formation of new PDL [130]. Most recently in 2024, SCAP-EVs demonstrated significant potential in regenerating soft tissue such as gingiva, which shares similar histological characteristics with PDL [131]. Hence, DSC-EVs are rich in bioactive agents capable of enhancing cell function, promoting neurovascular regeneration, and facilitating PDL regeneration. Despite their promise, current research on EVs has primarily focused on alveolar bone regeneration rather than PDL, likely due to the difficulty in quantifying and comparing PDL as a microstructure in animal models.

The ECM, a complex mixture of proteins, cytokines, GFs, enzymes, and even EVs, also exhibits significant regenerative potential. Compared to DSC-EVs, DSC-ECM has been more extensively studied for PDL regeneration, as ECM is rich in bioactive molecules and more easily accessible. ECM derived from PDLSCs and GMSCs has demonstrated outstanding regenerative outcomes, including the formation of new PDL [28, 29]. As previously mentioned, APTG-CM co-cultured with PDLSCs has been shown to enhance PDL regeneration. DPSC-ECM has also exhibited significant osteo/odonto-inductive capacity [26, 27]. Additionally, a cytokine cocktail mimicking MSC-ECM has facilitated PDL regeneration by promoting the migration of BMSCs and PDLCs [132]. These findings highlight the accessibility and promise of DSC-ECM in PDL regeneration, establishing a solid foundation for future research in this field.

In brief, EVs and ECM from DSCs have shown the ability to enhance PDL regeneration, because they are rich in bioactive agents essential for PDL regeneration.

Others

The recognized regenerative potential of exogenous DSCs has led to an intensified focus on enhancing their efficacy in tissue regeneration. As previously noted, the inclusion of specific bioactive elements can significantly boost regenerative outcomes. However, GFs applied locally are prone to rapid dispersion from the implantation site due to physical degradation, resulting in a limited duration of effect. To address this, gene therapy, characterized by the prolonged sustainability of specific proteins, offers a compelling alternative. Featured as a greater sustainability of specific proteins, gene therapy is defined as the process of treating disorders by transferring genetic materials to introduce, suppress, or modulate the expression of target genes within cells, thereby enabling the production of therapeutic proteins [133].

One example is the introduction of hepatocyte growth factor (HGF) into DPSCs, which has been demonstrated to induce angiogenesis in vivo as an endothelial growth factor [134]. When HGF-DPSCs were transplanted into swine periodontal defects, newly formed Sharpey's fibers were observed, embedded within a larger volume of regenerated mineralized tissue [135], paralleling the effects seen with secreted frizzled-related protein 2 (SFRP2)-modified SCAPs, where Wnt signaling was suppressed [136, 137]. The capacity to maintain the stemness and increase the multi-differentiation in SCAPs has been also reported before [138, 139].

Similar approaches have been applied to other cell types, with significant PDL regeneration observed following the use of BMP2-modified BMSCs [140], and BMP7/platelet-derived growth factor (PDGF)-transfected fibroblasts [141] *in vivo.*

In summary, the potential of DSCs in gene therapy for PDL regeneration is considerable. Future research is expected to explore different DSC subtypes, gene variants, and optimized delivery mechanisms tailored to various periodontal defect conditions [142].

Endogenous DSCs

As discussed above, cell therapy based on exogenous DSCs demonstrates exceptional regenerative potential, yet most strategies have not reached clinical application due to high costs and limited accessibility. Therefore, altering impaired tissue to enhance self-regeneration ability presents an appealing alternative to conventional therapeutic interventions, which is referred to as ERT. Recent research emphasizes the critical role of stem cell niches in determining stem cell fate, which brings about a bright approach in periodontal tissue engineering by utilizing biochemical factors and suitable materials to establish an artificial ECM framework or material niche, facilitating cell homing and tissue regeneration.

Regenerative ability

While DSCs present within periodontal defects possess the ability to self-renew and differentiate into multiple lineages, poor clinical outcomes are frequently observed. This is largely due to the dysregulation of bioactive factors and immune imbalances in pathological conditions, which diminish the regenerative potential of DSCs. Therefore, a key challenge in ERT is the reactivation of the innate regenerative potential of DSCs.

GFs are inherent biological agents that regulate critical cellular processes related to tissue regeneration through interactions with specific cell receptors. Typically produced by healthy cells, these factors are often depleted in chronic inflammatory environments, necessitating exogenous administration to stimulate the targeted cellular activities required for tissue repair. Key GFs involved in DSC therapies include PDGF, TGF-β, fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF) [78, 143], all of which play pivotal roles in the proliferation, differentiation, maintenance of stemness, and paracrine signaling of DSCs [144–146].

In addition, since periodontitis is featured as the dysbiotic microbial environment exacerbated by an overactive host immune response, host immunomodulation therapies have become increasingly recognized as a key factor in the management of tissue regeneration. Proinflammatory cytokines, such as TNF-α and IFN-γ, have been demonstrated to impair the regenerative capacity of MSCs [147].

Delivering the bioactive cues in need along with their stimulators or inhibitors directly to the defect sites offers another promising strategy. Conventional tissue-engineering approaches, such as transplantation with or without scaffolds, are commonly used. More cutting-edge techniques like scaffold-releasing agents with sustainability, are under investigation [148]. However, limitations such as reduced bioactivity over time, challenges in dosage control, and non-targeted delivery hinder their widespread adoption in patient treatment [149]. Gene therapy, which enables the delivery of biologically active proteins *via* gene transfer, holds potential to overcome these challenges.

In clinical practice, regenerative therapies such as platelet-rich plasma (PRP) are commonly employed, which contains a variety of GFs essential for wound healing, including PDGF, TGF-β, and VEGF [78]. Systemic drug therapy also offers a more convenient option compared to local administration. It was demonstrated that aspirin treatment *p.o.* was able to lower the expression levels of

TNF-α and IFN-γ, protecting PDLSCs from osteogenic impairment and mitigating inflammatory bone loss [150].

Overall, GFs and anti-inflammatory agents enhance the regenerative ability of DSCs by modulating the inflammatory microenvironment. To optimize the efficacy of xenogeneic transplantation, advancements in delivery systems are essential, including factor-releasing scaffold and gene therapy. The latest research has introduced a hydrogel capable of slowly releasing ions intraorally, which potentially augmented the therapeutic effects of bioactive factors through synergy [151]. Ongoing efforts continue to explore more efficient and accessible methods to improve clinical outcomes in PDL regeneration.

Homing ability

As previously mentioned, the modulation of DSC activation and suppression is influenced by specific signals originating from injured tissues, which play a critical role in recruiting and mobilizing resident cells. Given that DSCs migrate from the neural crest during embryonic development, MSCs from other regions of the body can be home to the dental tissues when necessary [152]. Therefore, leveraging certain chemokines to attract an optimal number of responsive stem cells to defect sites has emerged as a key strategy in ERT for facilitating PDL regeneration.

The SDF-1/CXCR4 axis, a critical regulator of stem cell recruitment, migration, and differentiation [82], has been frequently applied in ERT. One study demonstrated that the implantation of SDF-1-loaded membranes into periodontal defects in rats enhanced bone formation, hypothesizing through the increased migrative and regenerative ability of PDLSCs [86]. Similarly, another animal model using SDF-1 showed significantly increased PDL length and decreased inflammatory response [153].

The role of FGF signaling in the migration of neural crest cells is currently well-established in the scientific literature [154]. FGF-2, in particular, has been shown to recruit DSCs and activate their mitogenic and angiogenic properties [155], leading to the formation of PDL-like structures in furcation class II animal models [156]. Recently, advanced delivery techniques have been in preclinical applications, for example, a shell fibrous superassembled framework with controlled release of FGF-2 and BMP-2 have demonstrated greater fibers alignment mimicking the mature PDL in vivo, through the recruitment of PDLSCs in vitro [157]. However, some clinical trials have not shown significant differences in CAL outcomes [158], likely due to inappropriate doses, application methods, and observation periods.

Combining chemokines with GFs offers a promising approach to maximizing regenerative outcomes. It has been reported that the SDF-1/BMP complex can significantly enhance PDL regeneration [83], particularly loaded with a a scaffold designed for precise, sustained release [159]. Additionally, as a natural reservoir of various GFs, PRP has demonstrated the ability to regenerate tooth roots, including PDL, by promoting the homing of PDLSCs and BMSCs [160].

In conclusion, chemokine therapies for DSC homing hold the potential to diminish the immunogenicity associated with allogeneic transplantation, while simultaneously enhancing the activation and targeted migration of endogenous cells. Furthermore, these therapies can be effectively combined with other treatments including gene therapy and pharmacological interventions, to augment the overall efficacy. Future research should focus on expanding the repertoire of available cytokines, developing cutting-edge delivery systems, and establishing standardized protocols for ERT.

Conclusions

Given the widespread prevalence of periodontitis and its threat to maintaining complete dentition, the focus has shifted towards microscopic PDL reconstruction to enhance the prognosis for the growing population of patients with severe periodontitis. This review focuses on DSCs that are identified as remarkable multi-lineage differentiation and non-immunogenic properties, providing a physiological overview as well as elucidating the types and formats employed in regenerative therapies. The main mechanisms underlying PDL regeneration involve repairing impaired collagen turnover, inhibiting tissue destruction, promoting the regeneration of other affected tissues, and inducing orderly PDL regeneration.

What's more, this review first provides a comprehensive summary of the application formats and current development of DSCs in PDL regeneration, which highlights the significance of functional PDL in patients with periodontitis. It outlines future research directions in periodontal regeneration and establishes a foundational framework for preliminary studies in PDL regeneration. While there has been notable progress to date, certain limitations persist. Several bottlenecks require attention and resolution. First, a comprehensive understanding of the mechanisms governing DSCs and their associated regeneration pathways is still in its infancy. Second, the optimal source of DSCs for PDL regeneration continues to be debated, necessitating further investigation into various DSC types, donor selection criteria, and standardized protocols for cell acquisition and preservation. Third, discrepancies between preclinical and clinical regenerative outcomes highlight the need for a thorough exploration of the underlying mechanisms, optimization of DSC protocols, and the incorporation of cutting-edge technologies, such as bioactive factor integration, sophisticated delivery systems, and targeted functional modifications. Finally, it is crucial to explore application

methods that are tailored to the different stages of periodontitis, aligning more closely with clinical realities.

In conclusion, DSCs hold significant potential for PDL regeneration due to their high efficiency, regeneration capacity, and immunomodulatory property. This review highlights the significance of functional PDL for patients with periodontitis, identifies future research priorities in PDL regeneration and provides a framework for the foundational studies in PDL regeneration. It aims to offer a concise and insightful overview, serving as a valuable resource for further research in related fields.

Abbreviations

PDL	Functional periodontal ligament
GTR	Guided tissue regeneration
MSCs	Mesenchymal stem cells
DPSCs	Dental pulp stem cells
SHEDs	Stem cells from human exfoliated deciduous teeth
PDLSCs	Periodontal ligament stem cells
GMSCs	Gingival mesenchymal stem cells
SCAPs	Stem cells from apical papilla
DFSCs	Dental follicle stem cells
DSC	Dental stem cell
EV	Extracellular vesicle
ISEV	International Society for Extracellular Vesicles
ECM	Extracellular matrix
GAG	Glycosaminoglycan
AEFC	Acellular extrinsic fiber cementum
PDLCs	Periodontal ligament cells
HGFs	Human gingival fibroblasts
MMPs	Matrix metalloproteinases
TIMPs	Tissue inhibitors of metalloproteinase
NE	Neutrophil elastase
MPO	Myeloperoxidase
Th	Helper T cell
Treg	Regulatory T cell
iPSCs	Induced pluripotent stem cells
BMSCs	Bone marrow stem cells
PTHrP/PTH1R	Parathyroid hormone-related peptide/Parathyroid hormone receptor-1
ERT	Endogenous regenerative technology
SDF-1	Stromal-derived factor 1
SCF	Stem cell factor
BMP	Bone morphogenetic protein
CXCR4	C-X-C chemokine receptor type 4
CTGF	Connective tissue growth factor
PRF	Platelet-rich fibrin
HUVECs	Human umbilical vein endothelial cells
APTG-CM	Apical tooth germ cell–conditioned medium
HGF	Hepatocyte growth factor
SFRP2	Secreted frizzled-related protein
PDGF	Platelet-derived growth factor
GFs	Growth factors
FGF	Fibroblast growth factor
VEGF	Vascular endothelial growth factor
PRP	Platelet-rich plasma

Acknowledgements
This study was supported by Science and Technology Projects in Guangzhou (2024A04J6615) and grants from the Natural Science Foundation of Guangdong Province (2021B1515140008). We express gratitude to the funding sponsors.

Author contributions
Mingdeng Rong and Jincheng Zeng conceived the idea for the review, designed the study framework, and supervised the overall project. Shuyi Wen conducted the literature search, selected relevant studies, extracted the data, wrote the first draft of the manuscript, drew the pictures and table involved. Xiao Zheng and Wuwei Yin played a significant role in writing the initial draft of the manuscript as well as refining the outline. Yushan Liu, Ruijie Wang, Yaqi Zhao and Ziyi Liu played a supportive role in the literature search and participated in revising the manuscript. Cong Li contributed to the manuscript feedback. All authors contributed significantly to the revision and approval of the submitted version. All the authors have read and approved the final manuscript.

Funding
This study was supported by Science and Technology Projects in Guangzhou (2024A04J6615) and grants from the Natural Science Foundation of Guangdong Province (2021B1515140008).

Data availability
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 21 August 2024 / Accepted: 17 October 2024
Published online: 31 October 2024

References

1. Eke PI, Borgnakke WS, Genco RJ. Recent epidemiologic trends in periodontitis in the USA. Periodontol. 2000. 2020;82:257 – 67.
2. Chen MX, Zhong YJ, Dong QQ, Wong HM, Wen YF. Global, regional, and national burden of severe periodontitis, 1990–2019: an analysis of the global burden of Disease Study 2019. J Clin Periodontol. 2021;48:1165–88.
3. Fakheran O, Birang R, Schmidlin PR, Razavi SM, Behfarnia P. Retro MTA and tricalcium phosphate/retro MTA for guided tissue regeneration of periodontal dehiscence defects in a dog model: a pilot study. Biomaterials Res. 2019;23:14.
4. Paknejad M, Eslaminejad MB, Ghaedi B, Rokn A, Khorsand A, Etemad-Moghadam S, et al. Isolation and Assessment of mesenchymal stem cells derived from bone marrow: histologic and histomorphometric study in a Canine Periodontal defect. J Oral Implantol. 2015;41:284–91.
5. Pellegrini G, Pagni G, Rasperini G. Surgical approaches based on Biological objectives: GTR versus GBR techniques. Int J Dent. 2013;2013:521547.
6. Aydin S, Şahin F. Stem cells derived from Dental tissues. Adv Exp Med Biol. 2019;1144:123–32.
7. Sui B, Zheng C, Zhao W, Xuan K, Li B, Jin Y. Mesenchymal condensation in tooth development and regeneration: a focus on translational aspects of organogenesis. Physiol Rev. 2023;103:1899–964.
8. Andrukhov O, Behm C, Blufstein A, Rausch-Fan X. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells: implication in disease and tissue regeneration. World J Stem Cells. 2019;11:604–17.

9. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. P Natl Acad Sci Usa. 2000;97:13625–30.
10. 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 U S A. 2003;100:5807–12.
11. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004;364:149–55.
12. Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, et al. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol. 2005;24:155–65.
13. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS ONE. 2006;1:e79.
14. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol. 2009;183:7787–98.
15. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7:1535750.
16. Janockova J, Slovinska L, Harvanova D, Spakova T, Rosocha J. New therapeutic approaches of mesenchymal stem cells-derived exosomes. J Biomed Sci. 2021;28:39.
17. 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;14:2.
18. Ma L, Rao N, Jiang H, Dai Y, Yang S, Yang H, et al. Small extracellular vesicles from dental follicle stem cells provide biochemical cues for periodontal tissue regeneration. Stem Cell Res Ther. 2022;13:92.
19. Frantz C, Stewart KM, Weaver VM. The extracellular matrix at a glance. J Cell Sci. 2010;123:4195–200.
20. Kapp TG, Rechenmacher F, Neubauer S, Maltsev OV, Cavalcanti-Adam EA, Zarka R, et al. A comprehensive evaluation of the Activity and Selectivity Profile of ligands for RGD-binding integrins. Sci Rep-Uk. 2017;7:39805.
21. Lin H, Chen H, Zhao X, Chen Z, Zhang P, Tian Y, et al. Advances in mesenchymal stem cell conditioned medium-mediated periodontal tissue regeneration. J Transl Med. 2021;19:456.
22. Xiong X, Yang X, Dai H, Feng G, Zhang Y, Zhou J, et al. Extracellular matrix derived from human urine-derived stem cells enhances the expansion, adhesion, spreading, and differentiation of human periodontal ligament stem cells. Stem Cell Res Ther. 2019;10:396.
23. Tavelli L, McGuire MK, Zucchelli G, Rasperini G, Feinberg SE, Wang H, et al. Extracellular matrix-based scaffolding technologies for periodontal and peri-implant soft tissue regeneration. J Periodontol. 2020;91:17–25.
24. Takewaki M, Kajiya M, Takeda K, Sasaki S, Motoike S, Komatsu N, et al. MSC/ECM Cellular complexes Induce Periodontal tissue regeneration. J Dent Res. 2017;96:984–91.
25. Huang CC, Narayanan R, Warshawsky N, Ravindran S. Dual ECM biomimetic scaffolds for Dental Pulp Regenerative Applications. Front Physiol. 2018;9:495.
26. Nowwarote N, Petit S, Ferre FC, Dingli F, Laigle V, Loew D, et al. Extracellular Matrix Derived from Dental Pulp Stem cells promotes mineralization. Front Bioeng Biotechnol. 2021;9:740712.
27. Phothichailert S, Nowwarote N, Fournier BPJ, Trachoo V, Roytrakul S, Namangkalakul W, et al. Effects of decellularized extracellular matrix derived from Jagged1-treated human dental pulp stem cells on biological responses of stem cells isolated from apical papilla. Front Cell Dev Biology. 2022;10:948812.
28. Nagata M, Iwasaki K, Akazawa K, Komaki M, Yokoyama N, Izumi Y, et al. Conditioned medium from Periodontal ligament stem cells enhances Periodontal Regeneration. Tissue Eng Part A. 2017;23:367–77.
29. Qiu J, Wang X, Zhou H, Zhang C, Wang Y, Huang J, et al. Enhancement of periodontal tissue regeneration by conditioned media from gingiva-derived or periodontal ligament-derived mesenchymal stem cells: a comparative study in rats. Stem Cell Res Ther. 2020;11:42.
30. de Jong T, Bakker AD, Everts V, Smit TH. The intricate anatomy of the periodontal ligament and its development: lessons for periodontal regeneration. J Periodontal Res. 2017;52:965–74.
31. Strydom H, Maltha JC, Kuijpers-Jagtman AM, Von den Hoff JW. The oxytalan fibre network in the periodontium and its possible mechanical function. Arch Oral Biol. 2012;57:1003–11.
32. Sodek J. A comparison of the rates of synthesis and turnover of collagen and non-collagen proteins in adult rat periodontal tissues and skin using a microassay. Arch Oral Biol. 1977;22:655–65.
33. Zhang T, Yu J, Wang G, Zhang R. Amyloid precursor protein binds with TNFRSF21 to induce neural inflammation in Alzheimer's Disease. Eur J Pharm Sci. 2021;157:105598.
34. Scheres N, Laine ML, Sipos PM, Bosch-Tijhof CJ, Crielaard W, de Vries TJ, et al. Periodontal ligament and gingival fibroblasts from periodontitis patients are more active in interaction with Porphyromonas gingivalis. J Periodontal Res. 2011;46:407–16.
35. Chin YT, Liu CM, Chen TY, Chung YY, Lin CY, Hsiung CN, et al. 2,3,5,4'-tetrahydroxystilbene-2-o-beta-d-glucoside-stimulated dental pulp stem cells-derived conditioned medium enhances cell activity and anti-inflammation. J Dent Sci. 2021;16:586–98.
36. Iwasaki K, Komaki M, Akazawa K, Nagata M, Yokoyama N, Watabe T, et al. Spontaneous differentiation of periodontal ligament stem cells into myofibroblast during ex vivo expansion. J Cell Physiol. 2019;234:20377–91.
37. Zhang W, Zhou L, Dang J, Zhang X, Wang J, Chen Y, et al. Human gingiva-derived mesenchymal stem cells ameliorate streptozoticin-induced T1DM in mice via suppression of T effector cells and Up-regulating Treg subsets. Sci Rep. 2017;7:15249.
38. Luchian I, Goriuc A, Sandu D, Covasa M. The role of Matrix metalloproteinases (MMP-8, MMP-9, MMP-13) in Periodontal and Peri-implant pathological processes. Int J Mol Sci. 2022;23.
39. Kawagoe M, Tsuruga E, Oka K, Sawa Y, Ishikawa H. Matrix metalloproteinase-2 degrades fibrillin-1 and fibrillin-2 of oxytalan fibers in the human eye and periodontal ligaments in vitro. Acta Histochem Cytochem. 2013;46:153–9.
40. Zeng W, Song Y, Wang R, He R, Wang T. Neutrophil elastase: from mechanisms to therapeutic potential. J Pharm Anal. 2023;13:355–66.
41. Hernandez M, Gamonal J, Tervahartiala T, Mantyla P, Rivera O, Dezerega A, et al. Associations between matrix metalloproteinase-8 and – 14 and myeloperoxidase in gingival crevicular fluid from subjects with progressive chronic periodontitis: a longitudinal study. J Periodontol. 2010;81:1644–52.
42. Edwards DR, Murphy G, Reynolds JJ, Whitham SE, Docherty AJ, Angel P, et al. Transforming growth factor beta modulates the expression of collagenase and metalloproteinase inhibitor. Embo J. 1987;6:1899–904.
43. Kanji S, Sarkar R, Pramanik A, Kshirsagar S, Greene CJ, Das H. Dental pulp-derived stem cells inhibit osteoclast differentiation by secreting osteoprotegerin and deactivating AKT signalling in myeloid cells. J Cell Mol Med. 2021;25:2390–403.
44. Li N, Zhang Y, Nepal N, Li G, Yang N, Chen H, et al. Dental pulp stem cells overexpressing hepatocyte growth factor facilitate the repair of DSS-induced ulcerative colitis. Stem Cell Res Ther. 2021;12:30.
45. Kwack KH, Lee JM, Park SH, Lee HW. Human Dental Pulp Stem cells suppress Alloantigen-induced immunity by Stimulating T Cells to release transforming growth factor Beta. J Endod. 2017;43:100–8.
46. Tomic S, Djokic J, Vasilijic S, Vucevic D, Todorovic V, Supic G, et al. Immunomodulatory properties of mesenchymal stem cells derived from dental pulp and dental follicle are susceptible to activation by toll-like receptor agonists. Stem Cells Dev. 2011;20:695–708.
47. Liu P, Zhang Q, Mi J, Wang S, Xu Q, Zhuang D, et al. Exosomes derived from stem cells of human deciduous exfoliated teeth inhibit angiogenesis in vivo and in vitro via the transfer of mir-100-5p and miR-1246. Stem Cell Res Ther. 2022;13:89.
48. Chen YR, Lai PL, Chien Y, Lee PH, Lai YH, Ma HI et al. Improvement of Impaired Motor Functions by Human Dental Exfoliated Deciduous Teeth Stem Cell-Derived Factors in a Rat Model of Parkinson's Disease. Int J Mol Sci. 2020;21.
49. Huang CY, Vesvoranan O, Yin X, Montoya A, Londono V, Sawatari Y, et al. Anti-inflammatory effects of Conditioned Medium of Periodontal ligament-derived stem cells on chondrocytes, synoviocytes, and Meniscus cells. Stem Cells Dev. 2021;30:537–47.
50. Jing X, Wang S, Tang H, Li D, Zhou F, Xin L, et al. Dynamically Bioresponsive DNA Hydrogel Incorporated with Dual-Functional Stem cells from apical papilla-derived Exosomes promotes Diabetic Bone Regeneration. Acs Appl Mater Interfaces. 2022;14:16082–99.
51. Wang X, Zhao S, Lai J, Guan W, Gao Y. Anti-inflammatory, antioxidant, and Antifibrotic effects of Gingival-Derived MSCs on Bleomycin-Induced Pulmonary Fibrosis in mice. Int J Mol Sci. 2021;23.

52. Hu J, Cao Y, Xie Y, Wang H, Fan Z, Wang J, et al. Periodontal regeneration in swine after cell injection and cell sheet transplantation of human dental pulp stem cells following good manufacturing practice. Stem Cell Res Ther. 2016;7:130.
53. Lee AE, Choi JG, Shi SH, He P, Zhang QZ, Le AD. DPSC-Derived Extracellular vesicles promote rat Jawbone Regeneration. J Dent Res. 2023;102:313–21.
54. Lei F, Li M, Lin T, Zhou H, Wang F, Su X. Treatment of inflammatory bone loss in periodontitis by stem cell-derived exosomes. Acta Biomater. 2022;141:333–43.
55. Maeda K, Takahashi N, Kobayashi Y. Roles of wnt signals in bone resorption during physiological and pathological states. J Mol Med (Berl). 2013;91:15–23.
56. Nakao Y, Fukuda T, Zhang Q, Sanui T, Shinjo T, Kou X, et al. Exosomes from TNF-alpha-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss. Acta Biomater. 2021;122:306–24.
57. Leibbrandt A, Penninger JM. RANK/RANKL: regulators of immune responses and bone physiology. Ann N Y Acad Sci. 2008;1143:123–50.
58. Tsukasaki M, Komatsu N, Nagashima K, Nitta T, Pluemsakunthai W, Shukunami C, et al. Host defense against oral microbiota by bone-damaging T cells. Nat Commun. 2018;9:701.
59. Liu Y, Wang L, Liu S, Liu D, Chen C, Xu X, et al. Transplantation of SHED prevents bone loss in the early phase of ovariectomy-induced osteoporosis. J Dent Res. 2014;93:1124–32.
60. Maeda A, Kikuiri T, Yoshimura Y, Yawaka Y, Shirakawa T. Bone resorption improvement by conditioned medium of stem cells from human exfoliated deciduous teeth in ovariectomized mice. Exp Ther Med. 2022;23:299.
61. Liu J, Chen B, Bao J, Zhang Y, Lei L, Yan F. Macrophage polarization in periodontal ligament stem cells enhanced periodontal regeneration. Stem Cell Res Ther. 2019;10:320.
62. Wu J, Chen L, Wang R, Song Z, Shen Z, Zhao Y, et al. Exosomes secreted by stem cells from human exfoliated deciduous Teeth promote alveolar bone defect repair through the regulation of Angiogenesis and Osteogenesis. Acs Biomater Sci Eng. 2019;5:3561–71.
63. Tomasello L, Mauceri R, Coppola A, Pitrone M, Pizzo G, Campisi G, et al. Mesenchymal stem cells derived from inflamed dental pulpal and gingival tissue: a potential application for bone formation. Stem Cell Res Ther. 2017;8:179.
64. Al Bahrawy M, Ghaffar K, Gamal A, El-Sayed K, Iacono V. Effect of inflammation on Gingival Mesenchymal Stem/Progenitor cells' Proliferation and Migration through Microperforated membranes: an in Vitro Study. Stem Cells Int. 2020;2020:5373418.
65. Park JY, Jeon SH, Choung PH. Efficacy of periodontal stem cell transplantation in the treatment of advanced periodontitis. Cell Transpl. 2011;20:271–85.
66. Li X, He XT, Kong DQ, Xu XY, Wu RX, Sun LJ, et al. M2 macrophages enhance the Cementoblastic differentiation of Periodontal ligament stem cells via the akt and JNK pathways. Stem Cells. 2019;37:1567–80.
67. Wei X, Guo S, Liu Q, Liu L, Huo F, Wu Y et al. Dental follicle stem cells promote Periodontal regeneration through periostin-mediated macrophage infiltration and reprogramming in an inflammatory microenvironment. Int J Mol Sci. 2023;24.
68. Liu L, Wen Y, Chen L, Li M, Yu J, Tian W, et al. Xenogenous implanted dental follicle stem cells promote periodontal regeneration through inducing the N2 phenotype of neutrophils. Stem Cell Res Ther. 2024;15:270.
69. Misawa M, Silverio RK, Nociti FJ, Albiero ML, Saito MT, Nobrega SR, et al. Periodontal ligament-derived mesenchymal stem cells modulate neutrophil responses via paracrine mechanisms. J Periodontol. 2019;90:747–55.
70. Yang Z, Ma L, Du C, Wang J, Zhang C, Hu L, et al. Dental pulp stem cells accelerate wound healing through CCL2-induced M2 macrophages polarization. Iscience. 2023;26:108043.
71. Matsumura-Kawashima M, Ogata K, Moriyama M, Murakami Y, Kawado T, Nakamura S. Secreted factors from dental pulp stem cells improve Sjogren's syndrome via regulatory T cell-mediated immunosuppression. Stem Cell Res Ther. 2021;12:182.
72. Yang N, Liu X, Chen X, Yu S, Yang W, Liu Y. Stem cells from exfoliated deciduous teeth transplantation ameliorates Sjogren's syndrome by secreting soluble PD-L1. J Leukoc Biol. 2022;111:1043–55.
73. Yu S, Chen X, Liu Y, Zhuang XY, Wang AC, Liu XM, et al. Exosomes derived from stem cells from the apical papilla alleviate inflammation in rat pulpitis by upregulating regulatory T cells. Int Endod J. 2022;55:517–30.
74. Liu O, Xu J, Ding G, Liu D, Fan Z, Zhang C, et al. Periodontal ligament stem cells regulate B lymphocyte function via programmed cell death protein 1. Stem Cells. 2013;31:1371–82.
75. Song N, Scholtemeijer M, Shah K. Mesenchymal stem cell immunomodulation: mechanisms and therapeutic potential. Trends Pharmacol Sci. 2020;41:653–64.
76. Rao P, Jing J, Fan Y, Zhou C. Spatiotemporal cellular dynamics and molecular regulation of tooth root ontogeny. Int J Oral Sci. 2023;15:50.
77. Sui BD, Zheng CX, Zhao WM, Xuan K, Li B, Jin Y. Mesenchymal condensation in tooth development and regeneration: a focus on translational aspects of organogenesis. Physiol Rev. 2023;103:1899–964.
78. Chen F, Zhang J, Zhang M, An Y, Chen F, Wu Z. A review on endogenous regenerative technology in periodontal regenerative medicine. Biomaterials. 2010;31:7892–927.
79. Howard C, Murray PE, Namerow KN. Dental pulp stem cell migration. J Endodont. 2010;36:1963–6.
80. Ganesh V, Seol D, Gomez-Contreras PC, Keen HL, Shin K, Martin JA. Exosome-based cell homing and angiogenic differentiation for Dental Pulp Regeneration. Int J Mol Sci. 2022;24.
81. 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;37:133–8.
82. Hocking AM. The role of chemokines in Mesenchymal Stem Cell Homing to wounds. Adv Wound Care (New Rochelle). 2015;4:623–30.
83. Kim K, Lee CH, Kim BK, Mao JJ. Anatomically shaped tooth and periodontal regeneration by cell homing. J Dent Res. 2010;89:842–7.
84. Toksoy A, Muller V, Gillitzer R, Goebeler M. Biphasic expression of stromal cell-derived factor-1 during human wound healing. Br J Dermatol. 2007;157:1148–54.
85. Wu N, Song J, Liu X, Ma X, Guo X, Liu T, et al. Effect of an low-energy nd: YAG laser on periodontal ligament stem cell homing through the SDF-1/CXCR4 signaling pathway. BMC Oral Health. 2023;23:501.
86. Liang Q, Du L, Zhang R, Kang W, Ge S. Stromal cell-derived factor-1/Exendin-4 cotherapy facilitates the proliferation, migration and osteogenic differentiation of human periodontal ligament stem cells in vitro and promotes periodontal bone regeneration in vivo. Cell Prolif. 2021;54:e12997.
87. Gao Y, Zhao G, Li D, Chen X, Pang J, Ke J. Isolation and multiple differentiation potential assessment of human gingival mesenchymal stem cells. Int J Mol Sci. 2014;15:20982–96.
88. Kotova AV, Lobov AA, Dombrovskaya JA, Sannikova VY, Ryumina NA, Klausen P et al. Comparative Analysis of Dental Pulp and Periodontal Stem cells: differences in morphology, functionality, osteogenic differentiation and proteome. Biomedicines. 2021;9.
89. Miteva M, Mihaylova Z, Mitev V, Aleksiev E, Stanimirov P, Praskova M et al. A review of Stem Cell attributes derived from the oral cavity. Int Dent J. 2024.
90. Hu L, Zhao B, Gao Z, Xu J, Fan Z, Zhang C, et al. Regeneration characteristics of different dental derived stem cell sheets. J Oral Rehabil. 2020;47(Suppl 1):66–72.
91. Cha Y, Jeon M, Lee HS, Kim S, Kim SO, Lee JH, et al. Effects of in Vitro Osteogenic induction on in vivo tissue regeneration by Dental Pulp and Periodontal Ligament Stem cells. J Endod. 2015;41:1462–8.
92. Zhao J, Zhou YH, Zhao YQ, Gao ZR, Ouyang ZY, Ye Q, et al. Oral cavity-derived stem cells and preclinical models of jaw-bone defects for bone tissue engineering. Stem Cell Res Ther. 2023;14:39.
93. Mendoza AH, Balzarini D, Alves T, Holzhausen M, Rovai ES. Potential of mesenchymal stem cell sheets on Periodontal Regeneration: a systematic review of Pre-clinical studies. Curr Stem Cell Res Ther. 2023;18:958–78.
94. Zhang J, Chan HF, Wang H, Shao D, Tao Y, Li M. Stem cell therapy and tissue engineering strategies using cell aggregates and decellularized scaffolds for the rescue of liver failure. J Tissue Eng. 2021;12:1542251177.
95. Ma Y, Ji Y, Zhong T, Wan W, Yang Q, Li A, et al. Bioprinting-based PDLSC-ECM screening for in vivo repair of alveolar bone defect using Cell-Laden, Injectable and Photocrosslinkable Hydrogels. Acs Biomater Sci Eng. 2017;3:3534–45.
96. Wang W, Wang A, Hu G, Bian M, Chen L, Zhao Q, et al. Potential of an aligned porous hydrogel Scaffold Combined with Periodontal Ligament Stem cells or Gingival Mesenchymal Stem cells to promote tissue regeneration in Rat Periodontal defects. Acs Biomater Sci Eng. 2023;9:1961–75.
97. Lee CH, Shah B, Moioli EK, Mao JJ. CTGF directs fibroblast differentiation from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model. J Clin Invest. 2010;120:3340–9.
98. Suh J, Lim KT, Jin H, Kim J, Choung P, Chung JH. Effects of co-culture of dental pulp stem cells and periodontal ligament stem cells on assembled dual disc scaffolds. Tissue Eng Regen Med. 2014;11:47–58.

99. Sanchez N, Fierravanti L, Nunez J, Vignoletti F, Gonzalez-Zamora M, Santamaria S, et al. Periodontal regeneration using a xenogeneic bone substitute seeded with autologous periodontal ligament-derived mesenchymal stem cells: a 12-month quasi-randomized controlled pilot clinical trial. J Clin Periodontol. 2020;47:1391–402.
100. Ferrarotti F, Romano F, Gamba MN, Quirico A, Giraudi M, Audagna M, et al. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: a randomized controlled clinical trial. J Clin Periodontol. 2018;45:841–50.
101. Abdal-Wahab M, Abdel GK, Ezzatt OM, Hassan A, El AM, Gamal AY. Regenerative potential of cultured gingival fibroblasts in treatment of periodontal intrabony defects (randomized clinical and biochemical trial). J Periodontal Res. 2020;55:441–52.
102. Owaki T, Shimizu T, Yamato M, Okano T. Cell sheet engineering for regenerative medicine: current challenges and strategies. Biotechnol J. 2014;9:904–14.
103. Thummarati P, Laiwattanapaisal W, Nitta R, Fukuda M, Hassametto A, Kino-Oka M. Recent advances in cell sheet Engineering: from fabrication to clinical translation. Bioeng (Basel). 2023;10.
104. Wei F, Qu C, Song T, Ding G, Fan Z, Liu D, et al. Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity. J Cell Physiol. 2012;227:3216–24.
105. Ding G, Liu Y, Wang W, Wei F, Liu D, Fan Z, et al. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. Stem Cells. 2010;28:1829–38.
106. Yang X, Ma Y, Guo W, Yang B, Tian W. Stem cells from human exfoliated deciduous teeth as an alternative cell source in bio-root regeneration. Theranostics. 2019;9:2694–711.
107. Bai Y, Bai Y, Matsuzaka K, Hashimoto S, Fukuyama T, Wu L, et al. Cementum- and periodontal ligament-like tissue formation by dental follicle cell sheets co-cultured with Hertwig's epithelial root sheath cells. Bone. 2011;48:1417–26.
108. Yang H, Li J, Hu Y, Sun J, Guo W, Li H, et al. Treated dentin matrix particles combined with dental follicle cell sheet stimulate periodontal regeneration. Dent Mater. 2019;35:1238–53.
109. Zhao YH, Zhang M, Liu NX, Lv X, Zhang J, Chen FM, et al. The combined use of cell sheet fragments of periodontal ligament stem cells and platelet-rich fibrin granules for avulsed tooth reimplantation. Biomaterials. 2013;34:5506–20.
110. Zhang H, Liu S, Zhu B, Xu Q, Ding Y, Jin Y. Composite cell sheet for periodontal regeneration: crosstalk between different types of MSCs in cell sheet facilitates complex periodontal-like tissue regeneration. Stem Cell Res Ther. 2016;7:168.
111. Liu J, Wang L, Liu W, Li Q, Jin Z, Jin Y. Dental follicle cells rescue the regenerative capacity of periodontal ligament stem cells in an inflammatory microenvironment. PLoS ONE. 2014;9:e108752.
112. Panduwawala CP, Zhan X, Dissanayaka WL, Samaranayake LP, Jin L, Zhang C. In vivo periodontal tissue regeneration by periodontal ligament stem cells and endothelial cells in three-dimensional cell sheet constructs. J Periodontal Res. 2017;52:408–18.
113. Park JY, Park CH, Yi T, Kim SN, Iwata T, Yun JH. rhBMP-2 pre-treated Human Periodontal ligament stem cell sheets regenerate a mineralized layer mimicking Dental Cementum. Int J Mol Sci. 2020;21.
114. Zhao B, Chen J, Zhao L, Deng J, Li Q. A simvastatin-releasing scaffold with periodontal ligament stem cell sheets for periodontal regeneration. J Appl Biomater Funct Mater. 2020;18:587032254.
115. Takizawa S, Yamamoto T, Honjo KI, Sato Y, Nakamura K, Yamamoto K, et al. Transplantation of dental pulp-derived cell sheets cultured on human amniotic membrane induced to differentiate into bone. Oral Dis. 2019;25:1352–62.
116. Chen FM, Gao LN, Tian BM, Zhang XY, Zhang YJ, Dong GY, et al. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: a randomized clinical trial. Stem Cell Res Ther. 2016;7:33.
117. Liu J, Ruan J, Weir MD, Ren K, Schneider A, Wang P et al. Periodontal Bone-Ligament-Cementum Regeneration via scaffolds and Stem cells. Cells-Basel. 2019;8.
118. Ahrens CC, Dong Z, Li W. Engineering cell aggregates through incorporated polymeric microparticles. Acta Biomater. 2017;62:64–81.
119. Guo W, He Y, Tang X, Chen G, Shi H, Gong K, et al. Scaffold-free cell pellet transplantations can be applied to periodontal regeneration. Cell Transpl. 2014;23:181–94.
120. Meng H, Hu L, Zhou Y, Ge Z, Wang H, Wu CT, et al. A sandwich structure of Human Dental Pulp Stem cell sheet, treated dentin matrix, and Matrigel for tooth Root Regeneration. Stem Cells Dev. 2020;29:521–32.
121. Feng G, Wu Y, Yu Y, Huang L, An S, Hu B, et al. Periodontal ligament-like tissue regeneration with drilled porous decalcified dentin matrix sheet composite. Oral Dis. 2018;24:429–41.
122. Yang Z, Jin F, Zhang X, Ma D, Han C, Huo N, et al. Tissue engineering of cementum/periodontal-ligament complex using a novel three-dimensional pellet cultivation system for human periodontal ligament stem cells. Tissue Eng Part C Methods. 2009;15:571–81.
123. Zhu B, Liu W, Zhang H, Zhao X, Duan Y, Li D, et al. Tissue-specific composite cell aggregates drive periodontium tissue regeneration by reconstructing a regenerative microenvironment. J Tissue Eng Regen Med. 2017;11:1792–805.
124. Mulimani P, Mazzawi NA, Goldstein AJ, Obenaus AM, Baggett SM, Truong D, et al. Engineered 3D Periodontal Ligament Model with magnetic Tensile Loading. J Dent Res. 2024;103:1008–16.
125. Zheng CX, Sui BD, Hu CH, Qiu XY, Zhao P, Jin Y. Reconstruction of structure and function in tissue engineering of solid organs: toward simulation of natural development based on decellularization. J Tissue Eng Regen Med. 2018;12:1432–47.
126. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. Science. 2014;345:1247125.
127. Tamaki N, Cristina OR, Inagaki Y, Fukui M, Nagata T, Ito HO. Resveratrol improves oxidative stress and prevents the progression of periodontitis via the activation of the Sirt1/AMPK and the Nrf2/antioxidant defense pathways in a rat periodontitis model. Free Radic Biol Med. 2014;75:222–9.
128. Daghrery A, Ferreira JA, de Souza AI, Clarkson BH, Eckert GJ, Bhaduri SB, et al. A highly ordered, Nanostructured Fluorinated CaP-Coated Melt Electrowritten Scaffold for Periodontal tissue regeneration. Adv Healthc Mater. 2021;10:e2101152.
129. Shimizu Y, Takeda-Kawaguchi T, Kuroda I, Hotta Y, Kawasaki H, Hariyama T, et al. Exosomes from dental pulp cells attenuate bone loss in mouse experimental periodontitis. J Periodontal Res. 2022;57:162–72.
130. Chew J, Chuah SJ, Teo K, Zhang S, Lai RC, Fu JH, et al. Mesenchymal stem cell exosomes enhance periodontal ligament cell functions and promote periodontal regeneration. Acta Biomater. 2019;89:252–64.
131. Liu Y, Zhuang X, Yu S, Yang N, Zeng J, Liu X, et al. Exosomes derived from stem cells from apical papilla promote craniofacial soft tissue regeneration by enhancing Cdc42-mediated vascularization. Stem Cell Res Ther. 2021;12:76.
132. Sakaguchi K, Katagiri W, Osugi M, Kawai T, Sugimura-Wakayama Y, Hibi H. Periodontal tissue regeneration using the cytokine cocktail mimicking secretomes in the conditioned media from human mesenchymal stem cells. Biochem Biophys Res Commun. 2017;484:100–6.
133. Mitsiadis TA, Smith MM. How do genes make teeth to order through development? J Exp Zool B Mol Dev Evol. 2006;306:177–82.
134. Matsumoto K, Nakamura T. Hepatocyte growth factor (HGF) as a tissue organizer for organogenesis and regeneration. Biochem Biophys Res Commun. 1997;239:639–44.
135. Cao Y, Liu Z, Xie Y, Hu J, Wang H, Fan Z, et al. Adenovirus-mediated transfer of hepatocyte growth factor gene to human dental pulp stem cells under good manufacturing practice improves their potential for periodontal regeneration in swine. Stem Cell Res Ther. 2015;6:249.
136. Li G, Han N, Yang H, Zhang X, Cao Y, Cao Y, et al. SFRP2 promotes stem cells from apical papilla-mediated periodontal tissue regeneration in miniature pig. J Oral Rehabil. 2020;47(Suppl 1):12–8.
137. Jin L, Cao Y, Yu G, Wang J, Lin X, Ge L, et al. SFRP2 enhances the osteogenic differentiation of apical papilla stem cells by antagonizing the canonical WNT pathway. Cell Mol Biol Lett. 2017;22:14.
138. Lin X, Dong R, Diao S, Yu G, Wang L, Li J, et al. SFRP2 enhanced the adipogenic and neuronal differentiation potentials of stem cells from apical papilla. Cell Biol Int. 2017;41:534–43.
139. Yu G, Wang J, Lin X, Diao S, Cao Y, Dong R, et al. Demethylation of SFRP2 by histone demethylase KDM2A regulated osteo-/dentinogenic differentiation of stem cells of the apical papilla. Cell Prolif. 2016;49:330–40.
140. Chen YL, Chen PK, Jeng LB, Huang CS, Yang LC, Chung HY, et al. Periodontal regeneration using ex vivo autologous stem cells engineered to express the BMP-2 gene: an alternative to alveolaplasty. Gene Ther. 2008;15:1469–77.
141. Jin QM, Anusaksathien O, Webb SA, Rutherford RB, Giannobile WV. Gene therapy of bone morphogenetic protein for periodontal tissue engineering. J Periodontol. 2003;74:202–13.
142. Elangovan S, Karimbux N. Review paper: DNA delivery strategies to promote periodontal regeneration. J Biomater Appl. 2010;25:3–18.
143. Kaigler D, Cirelli JA, Giannobile WV. Growth factor delivery for oral and periodontal tissue engineering. Expert Opin Drug Deliv. 2006;3:647–62.

144. Zhang C, Guo H, Yang C, Chen Q, Huang J, Liu L, et al. The biological behavior optimization of human periodontal ligament stem cells via preconditioning by the combined application of fibroblast growth factor-2 and A83-01 in in vitro culture expansion. J Transl Med. 2019;17:66.
145. Yu Y, Mu J, Fan Z, Lei G, Yan M, Wang S, et al. Insulin-like growth factor 1 enhances the proliferation and osteogenic differentiation of human periodontal ligament stem cells via ERK and JNK MAPK pathways. Histochem Cell Biol. 2012;137:513–25.
146. Jaukovic A, Kukolj T, Trivanovic D, Okic-Dordevic I, Obradovic H, Miletic M, et al. Modulating stemness of mesenchymal stem cells from exfoliated deciduous and permanent teeth by IL-17 and bFGF. J Cell Physiol. 2021;236:7322–41.
147. Wang L, Zhao Y, Liu Y, Akiyama K, Chen C, Qu C, et al. IFN-gamma and TNF-alpha synergistically induce mesenchymal stem cell impairment and tumorigenesis via NFkappaB signaling. Stem Cells. 2013;31:1383–95.
148. Ogawa K, Miyaji H, Kato A, Kosen Y, Momose T, Yoshida T, et al. Periodontal tissue engineering by nano beta-tricalcium phosphate scaffold and fibroblast growth factor-2 in one-wall infrabony defects of dogs. J Periodontal Res. 2016;51:758–67.
149. Chen FM, Jin Y. Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. Tissue Eng Part B Rev. 2010;16:219–55.
150. Li B, Sun J, Dong Z, Xue P, He X, Liao L, et al. GCN5 modulates osteogenic differentiation of periodontal ligament stem cells through DKK1 acetylation in inflammatory microenvironment. Sci Rep. 2016;6:26542.
151. Zhang G, Zhang M, Feng Q, Wang R, Mei H, Xing K, et al. Supramolecular Composite Hydrogel loaded with CaF(2) nanoparticles promotes the recovery of Periodontitis Bone Resorption. Acs Appl Mater Interfaces. 2024;16:45929–47.
152. Mayo V, Sawatari Y, Huang CY, Garcia-Godoy F. Neural crest-derived dental stem cells–where we are and where we are going. J Dent. 2014;42:1043–51.
153. Cai X, Yang F, Walboomers XF, Wang Y, van den Jansen JA, et al. Periodontal regeneration via chemoattractive constructs. J Clin Periodontol. 2018;45:851–60.
154. Yardley N, Garcia-Castro MI. FGF signaling transforms non-neural ectoderm into neural crest. Dev Biol. 2012;372:166–77.
155. Fayazi S, Takimoto K, Diogenes A. Comparative Evaluation of Chemotactic Factor Effect on Migration and differentiation of stem cells of the apical papilla. J Endod. 2017;43:1288–93.
156. Murakami S, Takayama S, Kitamura M, Shimabukuro Y, Yanagi K, Ikezawa K, et al. Recombinant human basic fibroblast growth factor (bFGF) stimulates periodontal regeneration in class II furcation defects created in beagle dogs. J Periodontal Res. 2003;38:97–103.
157. Ding T, Li J, Zhang X, Du L, Li Y, Li D, et al. Super-assembled core/shell fibrous frameworks with dual growth factors for in situ cementum-ligament-bone complex regeneration. Biomater Sci. 2020;8:2459–71.
158. Li F, Yu F, Xu X, Li C, Huang D, Zhou X, et al. Evaluation of recombinant human FGF-2 and PDGF-BB in Periodontal Regeneration: a systematic review and Meta-analysis. Sci Rep. 2017;7:65.
159. Tan J, Zhang M, Hai Z, Wu C, Lin J, Kuang W, et al. Sustained release of two bioactive factors from Supramolecular Hydrogel promotes Periodontal Bone Regeneration. ACS Nano. 2019;13:5616–22.
160. Ji B, Sheng L, Chen G, Guo S, Xie L, Yang B, et al. The combination use of platelet-rich fibrin and treated dentin matrix for tooth root regeneration by cell homing. Tissue Eng Part A. 2015;21:26–34.

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