



# Stem Cell Therapy in Chronic Periodontitis: Host Limitations and Strategies

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The treatment of chronic periodontitis is undergoing a transition from simple plaque removal and replacement with substitute materials to regenerative therapy, in which stem cells play an important role. Although stem cell-based periodontal reconstruction has been widely explored, few clinical regeneration studies have been reported. The inflammatory lesions under the impact of host factors such as local microbial–host responses, may impede the regenerative properties of stem cells and destroy their living microenvironment. Furthermore, systemic diseases, in particular diabetes mellitus, synergistically shape the disordered host-bacterial responses and exacerbate the dysfunction of resident periodontal ligament stem cells (PDLSCs), which ultimately restrain the capacity of mesenchymal stromal cells (MSCs) to repair the damaged periodontal tissue. Accordingly, precise regulation of an instructive niche has become a promising approach to facilitate stem cell-based therapeutics for ameliorating periodontitis and for periodontal tissue regeneration. This review describes host limitations and coping strategies that influence resident or transplanted stem cell-mediated periodontal regeneration, such as the management of local microbial–host responses and rejuvenation of endogenous PDLSCs. More importantly, we recommend that active treatments for systemic diseases would also assist in recovering the limited stem cell function on the basis of amelioration of the inflammatory periodontal microenvironment.

**Keywords:** chronic periodontitis, systemic diseases, mesenchymal stromal cells, regenerative medicine, bacterial infections

## INTRODUCTION

The oral cavity is a gateway for the external world and is closely linked to systemic immunity and nutrient sensing, and the distinct symbiotic relationship between microbiota and oral tissues renders the bacteria influential to oral health importantly. For example, periodontal homeostasis is susceptible to overwhelmed bacteria–immune responses, which may dramatically call for the construction of chronic infection (1, 2). Until now, chronic periodontitis is the primary cause of tooth loss in adults. Once it occurs, the junctional epithelium migrates apically and transforms into the pocket epithelium, accompanied by a massive inflammatory infiltration culminating with impairment of bone homeostasis (3). Particularly, as oral bacteria and their components can

enter the circulation through the ulcers within the periodontal pockets, periodontitis affects the occurrence and development of many systemic diseases, such as cardiovascular disease and diabetes (4, 5). Reciprocally, the morbidity and progression of periodontal inflammation are also affected by systemic conditions (6, 7). Therefore, chronic periodontitis is an important disease that is related to the whole body rather than localized infection.

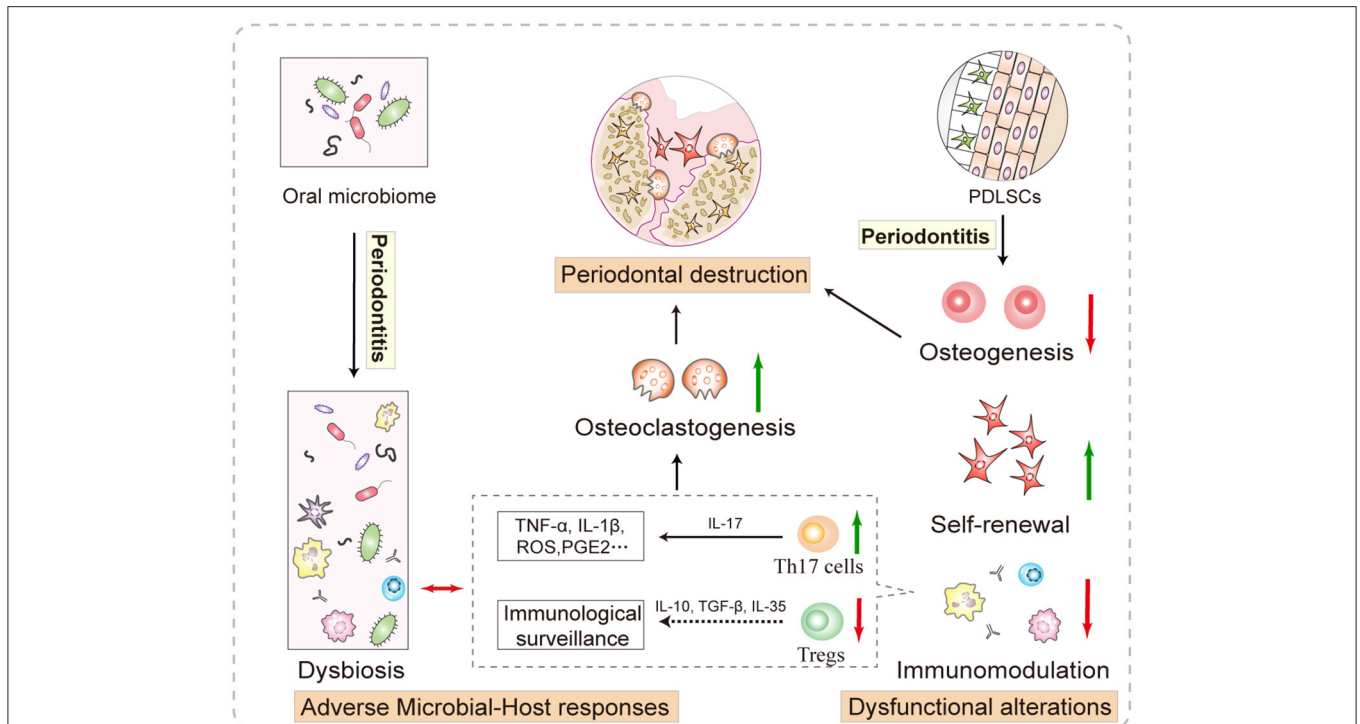
The previous pathogenic understanding of periodontitis has prompted the mechanotherapy based on physically removing the bacterial plaques within periodontal pockets, including scaling and root planning, which can effectively prevent the progression of periodontitis but fail to recover the destroyed periodontal tissue (8). The subsequent introduction of guided tissue regeneration and bone substitute materials ushered in an era of periodontal regenerative therapies because of their positive effects in providing space and fundamental structure for nascent tissue regeneration, and the application of growth factors accelerates this process (9, 10). However, the inherent instability and delivery controllability of growth factors make their effects still controversial. In addition, the present regenerative periodontal therapies are usually conducted with artificial materials in surgery, which remains challenging to reduce surgical trauma and reconstruct the structural support upon massive defects (11). Therefore, current treatments of chronic periodontitis are still challenging in achieving functional recovery of the periodontium.

Over the past decades, mesenchymal stromal cells (MSCs) have journeyed from discovery to mechanistic studies and periodontal regenerative applications. For example, several studies reported the excellent capacity of transplanted periodontal ligament stem cells (PDLSCs) or adipose-derived stem cells (ADSCs) for repairing multiple periodontal lesions in animal models (12–14). Interestingly, PDLSCs appear to be better candidates for regenerative periodontal therapy than other types of MSCs because of their easy accessibility in the oral maxillofacial region (15). Furthermore, PDLSCs exhibited preferable self-renewal capacity than bone marrow MSCs and superior differentiation potential compared with other orofacial MSCs like gingival MSCs under conditioned medium (16, 17). Inspired by the excellent therapeutic potentials of PDLSCs, several preclinical and clinical studies applying PDLSCs for oral regeneration have been performed. However, the results of a recent clinical trial using autologous PDLSCs derived from the impacted tooth are not satisfactory, because there is no significant advantage to restoring the defective periodontium by transplanting PDLSCs, as the control group (cell-free group) had comparable tissue reconstruction (18). These observations appear to support the assumption that the living environment might be the primary reason affecting the regenerative process (19). Specifically, the endogenous stem cells in animal models may shape a healthy stem cell niche and possess a periodontal regenerative property but are impaired in periodontitis patients, which may explain the parallel mandibular defect regeneration in rabbits between the autologous bone graft group and the ADSC-containing group (20). Further, the artificial defects in animal models may not

suffer from some decisive pathogenic factors, such as prolonged inflammatory stimuli and influences from systemic diseases (21). Therefore, mitigating host factors and recreating a beneficial microenvironment could be a promising approach for improving stem cell-based periodontal regeneration. Here, we summarize limitations from the host and coping strategies that influence resident or transplanted stem cell-mediated periodontal regeneration, such as the management of local microbial–host responses and rejuvenation of endogenous PDLSCs. More importantly, we recommend that active treatments for systemic diseases would also assist in recovering the limited stem cell function on the basis of amelioration of the inflammatory periodontal microenvironment.

## ORAL MICROBIAL INFECTION AND HOST DEFENSES WITHIN PERIODONTAL LESIONS

Whether involving transplanted or resident stem cells, an instructive microenvironment is a prerequisite for their function (22). The term “instructive microenvironment” within defects refers to a precise regulation between multiple cells and extracellular stimuli that provides inductive signaling to tissue regeneration. A typical example is that stem cells from human exfoliated deciduous teeth (SHED) can differentiate into sensory neurons in rat dorsal root ganglia, and SHED-originated aggregates can regenerate the functional pulp in the acellular and protective space of the pulp cavity (23). Specifically, effective root canal disinfection and the relatively closed space can allow pulp regeneration to escape bacterial interference (24), while the periodontal lesions in chronic periodontitis are usually accompanied by an imbalance of the oral microbiome and host immunity, which dramatically affects stem cell function (25, 26). Host responses to the microbiome orchestrate an inflammatory immunological niche, and we have summarized the inflammatory alteration of immune cell subsets and abnormal production of irritating cytokines, which significantly lead to irreversible periodontal inflammation (2). For example, dysbiosis in periodontitis triggers T helper (Th) 17 cells to produce inflammatory cytokines, such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$ , which promote osteoclastogenesis and bone loss, and the Treg-mediated dysregulation of immunological surveillance under dysbiotic background promotes the above inflammatory cascade (**Figure 1**). However, recent evidence on the differentiation of bone-damaging Th17 cells has rehabilitated their role in restricting periodontal infection, although it is manifested as unacceptable tooth loss (27). Of course, during periodontitis treatment, the management of microbial–host responses is very important. For example, the application of resolvin D2 in periodontitis mice inhibits the adaptive immune responses mediated by Th1 cells, promotes M2 polarization of macrophages, and suppresses neutrophil accumulation and release of inflammatory factors, which synergistically reduces periodontal bone loss (28). Therefore, an instructive microenvironment is necessary, and balancing the interaction between the oral microflora and host



**FIGURE 1** | Local factors that promote periodontal destruction. The adverse immunity and continuous stimulation of the dysbiotic microbiome reinforce each other and create a hostile microenvironment for stem cell function. More importantly, the compensatory and decompensatory responses of resident P-PDLSCs are involved in shaping the local microenvironment. Specifically, the increased proliferation of P-PDLSCs cannot offset osteogenesis disorder, which indirectly leads to bone homeostasis imbalance. In addition, abnormal P-PDLSC immunoregulation increases Th17 cell differentiation. These IL-17-releasing cells trigger massive production of inflammatory cytokines, such as TNF- $\alpha$  and IL-1 $\beta$ , which promote osteoclastogenesis and bone loss, and the Treg-mediated dysregulation of immunological surveillance promotes the above inflammatory cascade.

defenses may be a promising approach in stem cell-mediated periodontal regeneration.

## THE DYSFUNCTIONAL ALTERATIONS OF RESIDENT PDLSCS

Except for the inevitable pulp extirpation post-infection, resident stem cells within other oral defects remain, whose functional status is closely related to tissue restoration (29). It has been suggested that transplanted stem cells may rejuvenate resident stem cells by improving the surrounding microenvironment in a paracrine or other manner, rather than facilitating tissue regeneration directly (30). However, overwhelmed inflammation leads to dysfunction in PDLSCs from periodontitis (P-PDLSCs), especially the decreased osteogenesis and immunomodulation capacities, and it is challenging for these defective cells to repair the periodontitis-damaged tissue under dysbiotic background (Figure 1). Specifically, the compensatory alterations in P-PDLSC, observed as high proliferation and migration rates, are considered a resistance response to the inflammation (31). In addition, angiogenin and basic fibroblast growth factor are expressed at higher levels in P-PDLSCs compared to healthy

PDLSCs, suggesting that P-PDLSCs retain stronger angiogenesis-promoting capacity (32). However, the compensatory responses of P-PDLSCs are often insufficient to counterbalance the negative effects under continuous inflammatory stimulation, and subsequently show functional impairments as a decompensation functional phase. On the one hand, a functional study of P-PDLSCs mentioned the correlation between their impaired cell aggregates formation and osteogenesis disorder, which may lead to the inevitable progressive loss of the periodontium (33). Importantly, the lack of natural interaction between the cells may decrease the developmental signal in tissue recovery (23, 34). On the other hand, P-PDLSCs exhibit diminished immunosuppressive capability (31, 35). Typically, the P-PDLSC-induced imbalanced ratio of Th17 and Tregs facilitates the aggravation of periodontitis, the alteration thereof may be associated with the massive proinflammatory cytokine release and affected immunological surveillance (35). These studies suggest that resident P-PDLSC functional impairments may contribute to restrained regeneration in periodontitis, and the recruitment of resident stem cells applying low-intensity pulsed ultrasound and plant extracts such as resveratrol facilitated resident P-PDLSC functional recovery and reduced bone resorption in animal models (33, 36). Previously, we have reviewed affected intracellular

**TABLE 1** | Known molecular mechanisms underlying P-PDLSC dysfunction and targeted therapies to improve the impaired function of P-PDLSCs.

Intracellular molecular mechanisms		Status in P-PDLSCs	Effects on the function of P-PDLSCs	Identified targets to improve the impaired function of P-PDLSCs	References
NF- $\kappa$ B signaling pathway		Activation	Decreased osteogenic property	I $\kappa$ B $\uparrow$	(39–41)
Wnt signaling pathway	Canonical Wnt pathway	Activation	Increased proliferation and decreased osteogenic property	GSK-3 $\beta$ $\uparrow$	(42, 43)
	Non-canonical Wnt pathway	Inhibition	Decreased osteogenic property	NLK $\uparrow$	(42)
MAPK signaling pathway	ERK pathway	Inhibition	Decreased osteogenic property	MAPK phosphorylation $\uparrow$	(44)
	JNK pathway	Inhibition	Decreased osteogenic property		(44)
	p38 MAPK pathway	Inhibition	Decreased proliferation and osteogenic property		(38, 42, 44)
Epigenetic regulation	Histone acetylation		Decrease	GCN5 $\uparrow$ MORF $\uparrow$	(45, 46)
	Histone deacetylation		Increase	HDAC9 $\downarrow$	(47)
	LncRNA	LncRNA-POIR	Decrease	LncRNA-POIR $\uparrow$	(48)
	miRNA	miR-182	Increase	Decreased osteogenic property	miR-182 $\downarrow$
		miR-17-5p		Increased angiogenesis-promoting property	miR-17-5p $\uparrow$
Autophagy pathway		Activation	Increased osteogenic and angiogenesis-promoting properties, avoidance or acceleration of apoptosis	mTOR $\downarrow$	(32, 50)

$\uparrow$ , up-regulation;  $\downarrow$ , down-regulation.

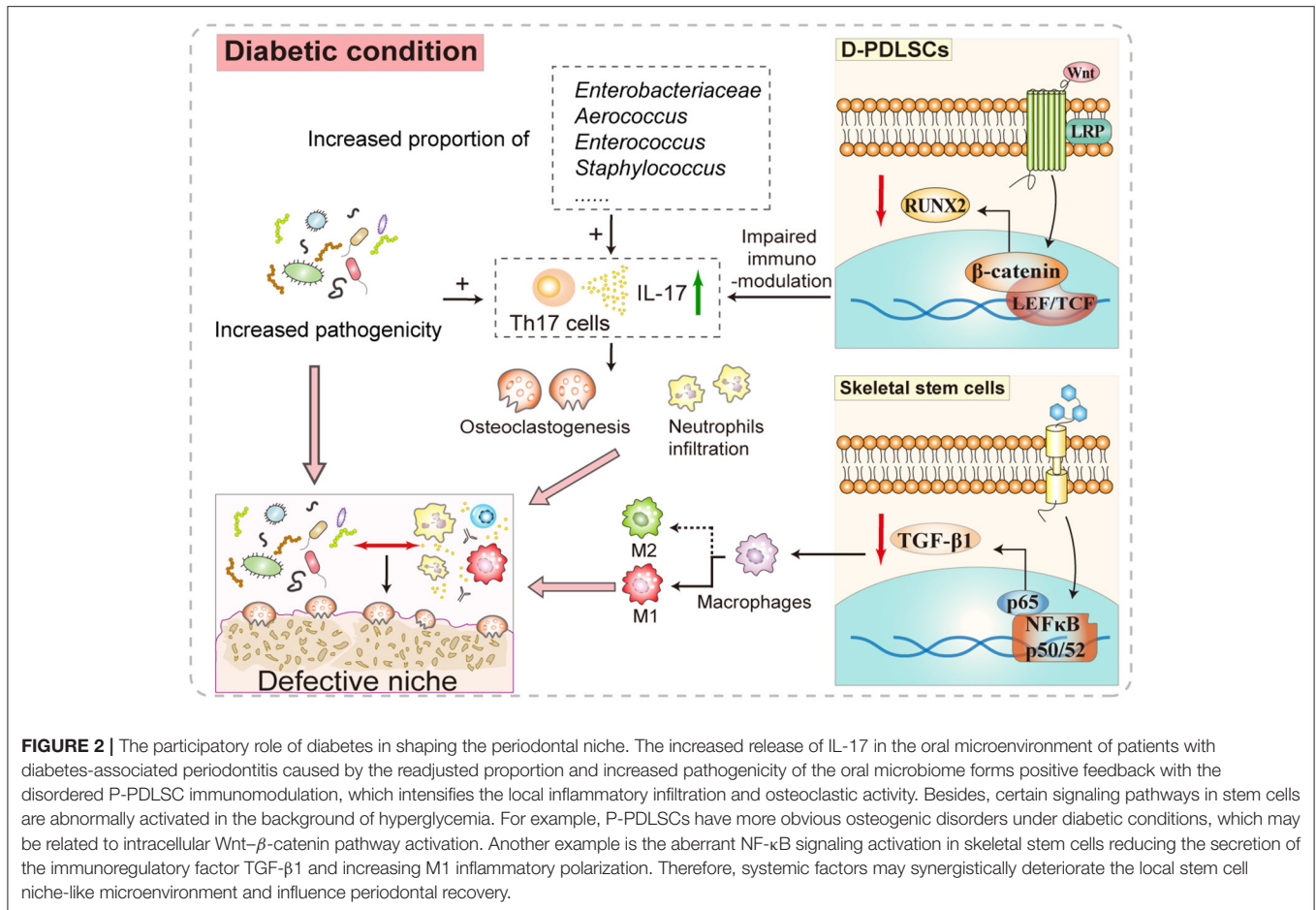
signaling pathways and epigenetic regulation within P-PDLSCs, including but being not limited to nuclear factor-kappa B (NF- $\kappa$ B), Wnt, and mitogen-activated protein kinases (MAPK) pathways, as key mediators of the functional disorders of P-PDLSCs (2). For example, treatment with aspirin induces the expression of general control non-repressed protein 5 (GCN5) in P-PDLSCs, which then upregulates the expression of dickkopf-related protein 1 (DKK1) and inactivates the Wnt- $\beta$ -catenin pathway indirectly, ultimately enhancing the osteogenic differentiation of P-PDLSCs (37). Similarly, the application of erythropoietin could activate the p38 MAPK pathway in P-PDLSCs and promote their bone formation property (38) (Table 1). Taken together, it is still a hotspot and future direction to restore impaired functional phenotypes of P-PDLSCs through molecular targets combined with improving the extracellular microenvironment, which has a promising clinical perspective in realizing endogenous stem cell-mediated periodontal regeneration.

## WHAT IS THE ROLE OF SYSTEMIC CONDITIONS WHEN STEM CELLS WORK?

As the important correlation between oral and general health becomes more widely known, the positive role of systemic diseases, such as diabetes, in hindering recovery from oral defects has been recognized (51–53). Here, we summarize the impact of some systemic factors, particularly high blood glucose, on stem cell-mediated periodontal, including aggravating the microbial–host responses and exacerbating the dysfunction of resident stem cells (Figure 2).

## Aggravating the Microbial–Host Responses

The remodeled oral microbiome and aggravated host adverse immunity under systemic diseases have drawn much research attention, which may hinder stem cell-based therapy. For example, the pathogenicity of the oral microbiome under diabetic conditions has been increased, as inoculating similar bacteria into diabetic mice led to greater inflammation than that in the control group (54). Further, elevated blood glucose may alter the resident microbiome composition and pathogenicity. Specifically, the bacteria from diabetic mice induced more IL-17 production, which may be responsible for the increased neutrophil infiltration and osteoclast formation in germ-free mice (55). The theory that glucose influences the oral microbiome composition may only hold under the periodontitis background, as diabetic people and healthy controls have similar salivary microbiomes (56). Generally, systemic diseases that interfere with oral microbiome establishment are common (53), e.g., reduced microbial diversity and a higher proportion of pathogenic bacteria can be detected in patients with periodontitis with systemic lupus erythematosus (57). Further complicating this item, systemic diseases also aggravate the host's inappropriate immunity to oral bacteria (55, 57, 58). Typically, the NF- $\kappa$ B signaling pathway in skeletal stem cells from diabetic patients has activated aberrantly, which reduces the expression of transforming growth factor- $\beta$  (TGF- $\beta$ 1) and inhibits M2 polarization of anti-inflammatory macrophages (59). Additionally, high blood glucose can increase the expression of bone-resorbing cytokines, such as receptor activator for NF- $\kappa$ B ligand (RANKL), in periodontal fibroblasts and promote osteoclastogenesis in alveolar bone (55, 58). In short, the establishment of chronic periodontitis is a dynamic and holistic



process, whereby distinct but disturbed host defenses are engaged at different stages.

Therefore, local injection of anti-IL-17 antibody suppressed the pathogenicity of oral bacteria under diabetes, manifested as reduced neutrophil infiltration and bone resorption (55). Further, active treatments for systemic diseases would also assist periodontal regeneration based on the amelioration of the inflammatory niche and maintenance of the instructive environment. For example, metformin hypoglycemic therapy and rheumatoid arthritis treatment restored the dynamic balance of the oral microbiome, thereby relieving periodontitis (56, 60). In addition, bindarit suppressed the persistently elevated chemokine (C-C motif) ligand 2 in diabetes-associated periodontitis, which decreased proinflammatory monocyte recruitment and alleviated periodontitis (61).

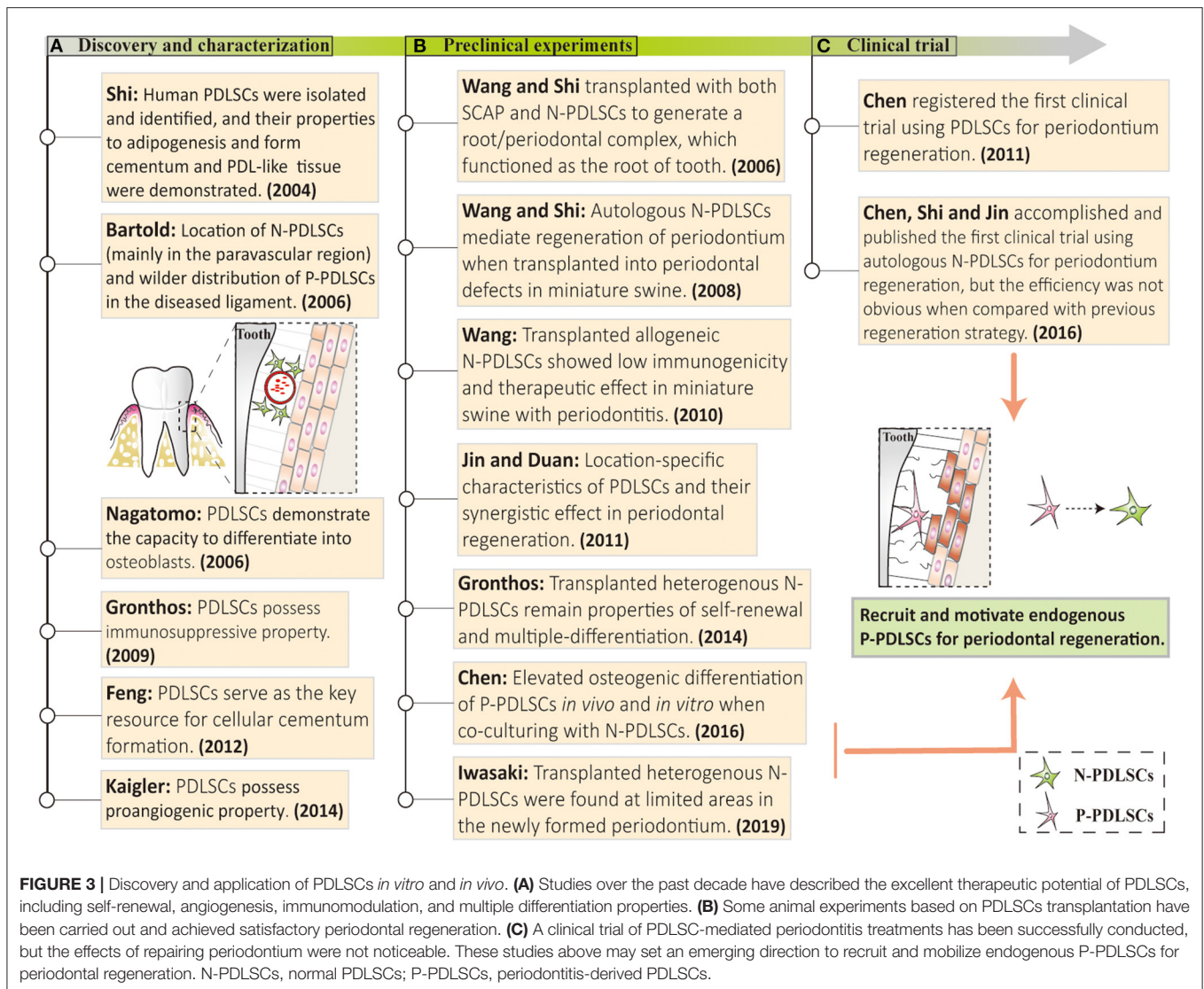
### Exacerbating the Dysfunction of Resident PDLSCs

The impact of systemic diseases on resident stem cells has been proposed for years. Recently, a study on P-PDLSCs under diabetes (D-PDLSCs) has provided evidence of their impaired osteogenic differentiation, while the underlying mechanism may differ from that of simple P-PDLSCs (39). It appears that high blood glucose readjusts the intracellular cascade

effects of NF-κB signaling in D-PDLSCs, as inhibiting the NF-κB signaling increases the P-PDLSC osteogenesis, whereas it does not upregulate D-PDLSC osteogenesis (2). Surprisingly, suppressing Wnt-β-catenin signaling reversed the osteogenic potential of D-PDLSCs, which is consistent with that of simple P-PDLSCs (39, 48). However, inhibiting NF-κB signaling in diabetic mice reduced osteoclast numbers and alveolar bone loss (58). Hence, further studies can explore the balance of Wnt-β-catenin and NF-κB signaling in D-PDLSCs to achieve optimal periodontal recovery. Until now, few studies have established the functional status and reparative role of resident PDLSCs under systemic diseases, and more interventions are still required for rejuvenating the resident PDLSCs and achieving periodontal regeneration.

### PERSPECTIVES ON CURRENT STRATEGIES FOR STEM CELL-MEDIATED PERIODONTITIS THERAPY

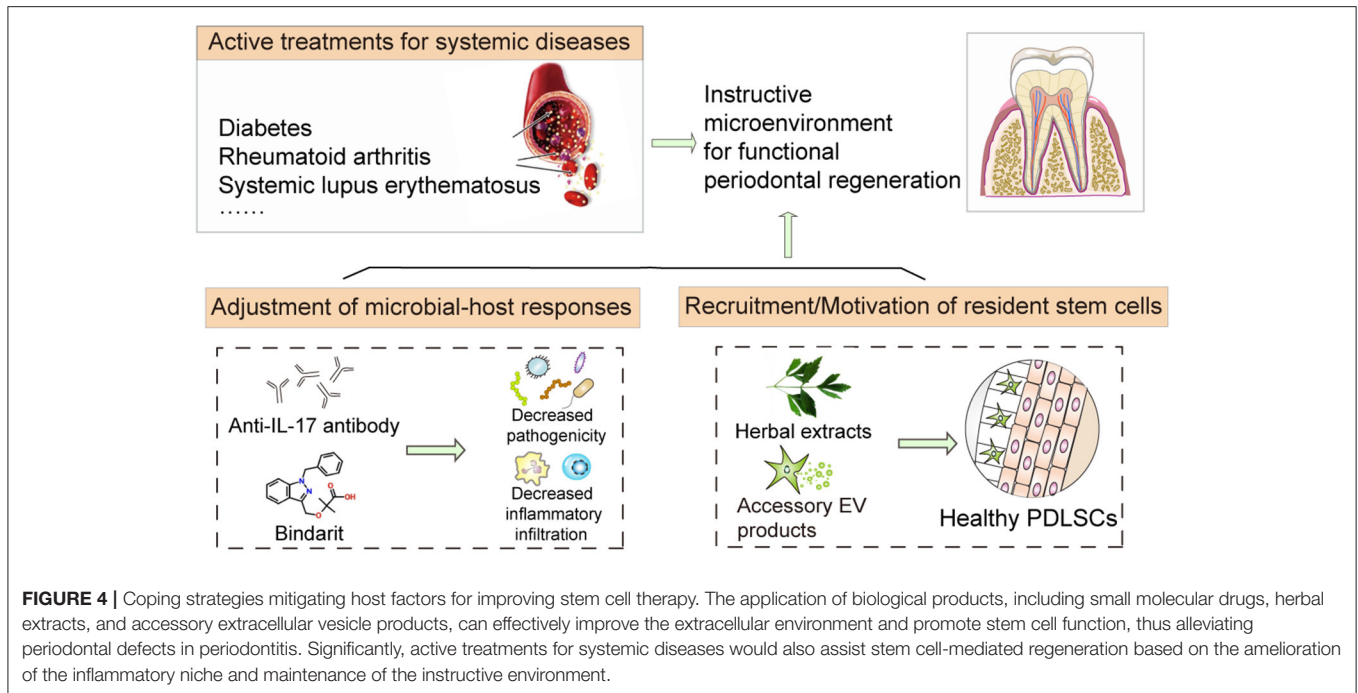
In the past 20 years, there has been considerable progress in stem cell-mediated oral tissue reconstruction, and promoting resident or transplanted stem cells has demonstrated therapeutic prospects (62, 63). However, until now, only the pulp has been



clinically regenerated, which may be due to the applied young SHED aggregates and the natural shape of the pulp cavity, while the results of a recent clinical trial using autologous healthy PDLSCs derived from the impacted tooth are not satisfactory (18, 23). Of note, pulpitis is a localized disease that is rarely affected by persistent host factors, and the pulp cavity confers physical protection for pulp regeneration (64). Other than pulpitis, however, periodontal lesions lack protective space for stem cell function and are closely linked to systemic inflammation, which may partially explain the difficulty in their regeneration. Accordingly, a reasonable breakthrough for ensuring periodontal regeneration could be the improvement of adverse effects from local or systemic factors. For example, decreasing the pathogenicity of oral bacteria and suppressing the recruitment of proinflammatory monocyte by using small molecule drugs can alleviate diabetes-associated periodontitis (55, 61). Furthermore, active treatments for systemic diseases, such as diabetes and rheumatoid arthritis, would aid recovery

based on ameliorating the bacterial inflammatory responses and stem cell status (56, 60). However, most strategies for intervening microbial-host responses are based on mechanistic *in vitro* or preclinical studies, which require further clinical verification, presenting more challenges for future treatment.

The unsatisfactory outcomes in autologous healthy PDLSCs-mediated periodontal regeneration may also attribute to the functional condition of endogenous PDLSCs, as the endogenous PDLSCs in animal models may possess the periodontal regenerative property but are impaired in periodontitis patients. Previous studies on PDLSCs from discovery to mechanistic studies and regenerative applications may set an emerging direction to recruit and mobilize endogenous P-PDLSCs for periodontal regeneration (Figure 3). As current studies have revealed the molecular mechanisms involved in the inflammatory responses of P-PDLSCs, restoration and/or mobilization of P-PDLSCs can be targetedly achieved by using small molecule drugs, herbal extracts, and accessory extracellular vesicle (EV)



products (2). However, it should be noted that precise delivery of drugs to modulate PDLSCs *in vivo* remain as a major challenge and a potential direction in this field. In this regard, as programmed cell death protein 1 (PD1) represents a functional surface marker of orofacial MSCs, targeted techniques such as aptamers may serve as effective tools for precise modulation (65). More importantly, the self-assemble SHED aggregates in pulp regeneration may initiate the redevelopment process, implicating the significance of natural signals inductive of tissue regeneration (23, 66). Notably, induction by stem cell subsets may evolve a natural condition for periodontal regeneration, as shown by the improved expression of extracellular matrix and bone-related genes and the regeneration of complex periodontium-like structures *in vivo* when using composite cell sheets composed of PDLSCs and jaw bone marrow MSCs (67). Therefore, the activation of resident P-PDLSCs and prudent administration of cellular interaction are necessary, but require multiple considerations to ensure the natural process of stem cell-mediated periodontal recovery, which is a lesson we should learn from the clinical success of pulp regeneration.

## CONCLUSIONS

Chronic periodontitis exerts a great influence on the patient's life quality and interpersonal communication. The current treatment for periodontitis is moving toward the goal of functional periodontal regeneration, in which MSCs play an integral role in future therapy. Importantly, MSCs may shape a distinct niche with surrounding extracellular matrix and cytokines, which is related to stem cell fate. Therefore, the prerequisite for stem cell therapy in chronic periodontitis is the adjustment of local stem cell niche, which refers to the improvement

of the systemic condition and the abnormal bacterial-host responses (Figure 4). Beyond that, continuous inflammation in the periodontal tissue induces functional impairments of endogenous P-PDLSCs, which contribute to the disturbed stem cell niche and restrained regeneration in periodontitis. Therefore, the functional condition of endogenous PDLSCs may be a key approach in periodontal regeneration (Figure 4). Although prompting endogenous stem cells to treat periodontitis has been validated in animal models, it is difficult to precisely improve the function of therapeutic stem cells and the mutual induction of cell subsets, and more in-depth research is still needed for functional periodontal regeneration.

## AUTHOR CONTRIBUTIONS

ZZ contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. MD and MH contributed to conception, design, and interpretation and critically revised the manuscript. JT contributed to conception, design, and data interpretation and critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

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