Review Article

Shenghao Jin, Haitao Jiang, Yue Sun, Fang Li, Jianglan Xia, Yaxin Li, Jiwei Zheng*, Ying Qin

Osteogenic differentiation of periodontal membrane stem cells in inflammatory environments

https://doi.org/10.1515/biol-2022-0474
received March 14, 2022; accepted July 07, 2022

Abstract: Periodontitis is a common disease that is difficult to treat, and if not controlled in time, it causes severe conditions, such as alveolar bone resorption and tooth loosening and loss. Periodontal ligament stem cells constitute a promising cell source for regenerative treatment of periodontitis due to their high osteogenic differentiation capacity. PDLSC osteogenesis plays a central role in periodontal regeneration through successive cytokine-mediated signaling pathways and various biochemical and physicochemical factors. However, this process is inhibited in the inflammatory periodontitis environment due to high concentrations of lipopolysaccharide. Here, we review the mechanisms that influence the osteogenic differentiation of periodontal stem cells in this inflammatory microenvironment.

Keywords: periodontal membrane stem cells, osteogenic differentiation, inflammatory environment, lipopolysaccharide

Periodontitis is an infectious disorder that occurs in the supporting tissues of the teeth and, if left untreated, often results in loosening and loss of teeth and jawbone loss. The commonly used clinical treatments, such as physiotherapy and periodontal surgery, do not yield favorable results. Therefore, tissue regeneration and the use of stem cells to reconstruct periodontal and bone tissues have recently become a major research topic. In recent years, with the development of the field of oral tissue regeneration, the discovery of oral-derived seed cells has enabled the complete regeneration of the periodontal tissue. The cells [1] that have been found to play a regenerative role are partially used in clinical applications include dental pulp stem cells, stem cells from the apical papilla, dental follicle progenitor cells, and periodontal ligament stem cells (PDLSCs). Many studies [2–4] have shown that odontogenic stem cells are superior to non-dental stem cells in regenerative treatment, and periodontal stem cells are recognized as one of the safest and most effective stem cells for this process. PDLSCs are mesenchymal stem cells with self-renewal and multi-directional differentiation potentials (Figure 1), whereby they can differentiate into various types of mesenchymal cells [5], such as osteoblasts, adipocytes, and chondrocytes, in addition to cardiomyocytes, endothelial cells, and ectoderm-derived neural cells, namely neurons, oligodendrocytes, astrocytes, and Schwann cells. Thus, PDLSCs constitute a promising cell source for the repair of various types of tissues in regenerative medicine. Liu et al. [6] and Nagata et al. [7] have investigated the role of PDLSCs in the repair of periodontal bone defects and demonstrated that PDLSC-derived cells are highly effective in osteogenesis and in the formation of a periodontal-like tissue, whereby the regeneration and repair of the periodontal attachment tissue are promoted. These studies have laid the foundation for the development of regenerative treatment approaches in periodontitis.

The bacteria [8] that presumably cause periodontitis include 11 species, such as Actinobacillus, Actinomyces, Porphyromonas gingivalis, and Fosetanella, most of which are G-bacteria. Lipopolysaccharide (LPS) is an important component of the G-bacterial membrane, and the LPS-induced inflammatory microenvironment is an important and typical manifestation of periodontitis. This inflammatory...
microenvironment induces production of numerous inflammatory factors, such as TNF-α, IL-6, and IL-1β, and involves various signaling pathways. According to the recent advances in periodontal disease microbiology, there is conclusive evidence [9] that some of these bacteria, such as *P. gingivalis* and *Bacillus actinomycetemcomitans*, play important roles in the pathogenesis of periodontitis, including the activation of the inflammatory response and promotion of bone loss, both of which are inextricably linked to the production of LPS by bacteria. Although the effects of different bacterial species on periodontal regeneration after bone loss are unclear, the effect of LPS on the osteogenic differentiation of stem cells has commonalities. Croes et al. have found [10] that osteogenesis is active after stimulation with small amounts of inactivated bacteria (low LPS levels). Based on this observation, numerous studies [11–13] have confirmed the protective, proliferative, and osteogenic effects of low concentrations of LPS (generally <5 μg/mL) on periodontal cells. The underlying mechanism may stem from an adaptive mechanism of PDLSCs at low concentrations of LPS. Alternatively, low concentrations of LPS may induce PDLSCs to activate the macrophage NRF2 signaling pathway to regulate the oxidative stress and downregulate macrophage pro-inflammatory factor expression. However, this is obviously not consistent with the microenvironment of severe periodontitis, and thus, high LPS concentrations should be considered in studies. At high LPS concentrations [14], cell proliferation and osteogenic differentiation are significantly reduced, presumably due to the induction of apoptosis through the classical NF-κB pathway and the action of cystein. Studies have generally explored LPS concentration gradients to identify the mechanism(s) underlying these two opposing effects of LPS. However, Albiero et al. [15] have observed that the osteogenic potential of LPS on periodontal stem cells is not affected. This observation has encouraged researchers to seek substances that favor the osteogenic differentiation of stem cells in the periodontitis environment, with the ultimate aim to improve the efficacy of current therapies and to find new therapeutic strategies. The following is a summary of the various mechanisms and factors influencing the osteogenic differentiation of PDLSCs under LPS-induced inflammatory conditions.

1 Roles of major signaling pathways

1.1 Wnt/β-catenin signaling pathway

Transcriptional co-activator with PDZ binding motif (TAZ, also known as WWTR1) is a transcriptional co-regulator that promotes the osteogenic differentiation of PDLSCs. Xing et al. [16] observed that LPS-induced osteogenic differentiation of PDLSCs is suppressed upon knocking down TAZ, indicating that TAZ is required for LPS-induced osteogenesis. They also found that the use of the Wnt/β-catenin pathway inhibitor DKK inhibits LPS-induced TAZ activation and osteogenesis, indirectly confirming that TAZ is a downstream component of the Wnt signaling pathway, thus inferring that low concentrations of *Escherichia coli* LPS promote the osteogenic differentiation of human PDLSCs (hPDLSCs) through Wnt/β-catenin-induced TAZ upregulation. This result is encouraging as it explains to some extent the osteogenic effect of LPS at low concentrations. Furthermore, Li et al. [17] found that, in PDLSCs,
removal of the histone acetyltransferase GCN5 downregulates DKK1, whereby the Wnt/β-catenin pathway is activated and osteogenesis is promoted. Thus, it is reasonable to infer that GCN5 regulates the osteogenic differentiation of periodontal stem cells in an inflammatory microenvironment through DKK1 acetylation (Figure 2).

Recently, Notum, a glycoprotein glycan and Wnt/β-catenin-associated ligand, has been discovered. It is a phospholipase shed on the cell surface. Yang et al. [18] examined the expression of Notum in LPS-treated PDLSCs and the osteogenic markers under the influence of Notum. By inhibiting the effect of Notum on the Wnt/β-catenin pathway, they found that this protein may inhibit the osteogenic differentiation of hPDLSCs through this pathway. In addition, lithium chloride [19,20] significantly suppressed the inhibitory effect of Notum on the osteogenic differentiation of hPDLSCs. These observations provide a new thinking for the treatment of periodontitis.

1.2 Toll-like receptor TLR 4/nuclear factor NF-κB signaling pathway

TLR4 is a transmembrane receptor involved in the activation of signaling pathways under inflammatory conditions. Yu et al. [21] applied TLR 4 agonists or antagonists to hPDLSCs extracted from patients with periodontitis alongside those from healthy individuals and analyzed indicators of cell proliferation and differentiation. The authors found that TLR4 activation decreases the ALP activity and expression of osteogenic markers but increases the expression of adipogenesis-related genes poly(AdP ribose) polymerase gamma and lipoprotein lipase, demonstrating that LPS can inhibit the osteogenic differentiation of hPDLSCs and induce their adipogenic differentiation under inflammatory conditions by activating the TLR4 signaling (Figure 3).

The TLR4 receptor-mediated signaling is common, and it is also closely associated with other inflammatory

![Figure 2: Mechanism of Wnt/β-catenin signaling pathway (Description: Wnt/β-catenin pathway is important for osteogenic differentiation of PDLSCs. In the presence of LPS, histone acetyltransferase GCN5 can inhibit this pathway through the acetylation regulation of DDK1. TAZ, which is necessary for LPS-induced osteogenesis, is a downstream component of this signal pathway. Interference with it by ShRNA or under the influence of phospholipase notum can inhibit Wnt/β-catenin pathway-mediated osteogenic differentiation of PDLSCs, and LiCl could reverse the effect of Notum).]
pathways. By using anti-TLR4 antibodies or antagonizers of the TLR4 or NF-κB signaling, Li et al. [14] found that LPS reduces the osteogenic differentiation of hPDLSCs by activating NF-κB through the TLR4 signaling pathway. The LPS-induced reduction in osteogenesis suppressed the alveolar bone loss in LPS-induced periodontitis in rats. Furthermore, Duan et al. [22] observed that salvianolic acid C promoted osteogenesis by attenuating LPS-induced inflammation and apoptosis through the inhibition of the TLR4/NF-κB pathway.

Many factors affect the TLR4 signaling pathway. Wang et al. [23] found that various concentrations of LPS downregulated the mRNA level of EphrinB2, a cellular marker of osteodifferentiation. In contrast, blocking the TLR4 pathway partially suppressed this effect of LPS. Therefore, it is inferred that LPS partially inhibits the osteogenesis of hPDLSCs by downregulating EphrinB2 through the TLR4 signaling.

In addition to TLR 4 receptor, experiments on other receptors of the same type confirmed that a common signaling feature of all TLRs is the activation of the transcription factor NF-κB, which controls the expression of inflammatory cytokines and factors involved in cell maturation. Through analyses based on polymerase chain reaction, Zhu et al. [24] found that high doses of TLR1, 4, and 6 ligands inhibit the expression of NF-κB. This inhibition occurs through Myd88 or the TRIF-dependent signaling pathway to inhibit Akt activation and hPDLSC osteogenic differentiation in turn. In addition, it has been shown that rutin [25] and extracellular vesicles [26] or astaxanthin [27] can increase the anti-oxidative stress capacity, proliferation, and osteogenic differentiation of PDLSCs by inhibiting the activity of the NFκB signaling, all of which lay the foundation for the development of new treatment strategies in periodontitis.

1.3 ERK1/2 signaling pathways [28]

It has been shown that activation of the ERK1/2 signaling pathways by LPS is at least partially involved in the alteration in the differentiation capacity of PDLSCs as well as in the acquisition of myofibroblast and immunoregulatory properties of these cells. LPS downregulates regulatory
genes related to osteogenesis, such as Runx2, ALP, and Ocn, thereby altering the differentiation commitment of PDLSCs and acting as a suppressor of osteogenesis. In addition, LPS upregulates Sox9 and PPARγ, which promote chondrogenesis and adipogenesis. PDLSC-derived myofibroblasts acquire significant contractile motility upon activation of the ERK1/2 signaling, indicating the effect of this signaling pathway in cell differentiation.

2 Effect of microRNAs (miRs) and IncRNAs

miRs are a class of non-coding single-stranded RNAs that are approximately 22 nucleotides in length. They are encoded by endogenous genes and involved in post-transcriptional regulation in plants and animals. Bao et al. [29] found that miR-148a is upregulated whereas neurofibrillary protein1 (NRP1) is downregulated when LPS inhibits PDLSC proliferation. Conversely, downregulation of miR-148a or upregulation of NRP1 increased the osteogenic capacity of LPS-stimulated PDLSCs. Inhibition of NRP1 could negate the promotion of osteogenesis by miR-148a inhibitors. These two actions are complementary and not independent, confirming the osteogenic potential of PDLSCs in an LPS-induced inflammatory environment. The decrease in the osteogenic potential of PDLSCs in such an environment is associated with the miR-148a/NRP1 functional axis (Table 1).

Yang et al. [30] found that miR-21 can promote the lipogenesis and osteogenesis of PDLSCs by directly inhibiting Spry1, a negative regulator of the ERK and FGF signaling pathways. However, TNF-α may control the expression of inflammatory cytokines, adipogenesis, and osteogenic differentiation of PDLSCs by targeting the miR-21/Spry1 functional axis.

In addition, several studies have identified other miRs that affect the osteogenic differentiation of PDLSCs. For instance, miR-200cN [31] inhibits LPS-induced IL-6, IL-8, and CCL-5 production in hPDLSCs and increases cellular calcium, ALP, and Runx2 levels, consequently promoting osteogenic differentiation. Another miRNA, miR-138, hinders the osteogenic differentiation of PDLSCs by downregulating osteocalcin, Runx2, and type I collagen. Conversely, miR-26a-5p [32] promotes the osteogenic differentiation of PDLSCs both in vitro and in vivo, presumably by targeting Wnt5a and thereby inhibiting the activation of the Wnt/Ca2+ signaling. Xu et al. [33,34] found that the inflammation-mediated changes in P2X7R-overexpressing PDLSCs in an inflammatory osteogenic microenvironment were significantly reduced. Another study revealed that P2X7R overexpression could significantly enhance the osteogenic differentiation of PDLSCs through

### Table 1: Effect of miRs and IncRNAs and other genes (description: the table lists some genes that affect the osteogenic differentiation of periodontal stem cells and their mechanisms, especially miRNA and IncRNA)

| Gene             | Mechanism                                                                 | Effect on osteogenesis                                      |
|------------------|----------------------------------------------------------------------------|-------------------------------------------------------------|
| miR-148a/NRP1    | Downregulating miR-148a or upregulating of NRP1                           | Promoting the osteogenic capacity of LPS-stimulated PDLSCs  |
| miR-21 [30]      | Inhibiting a negative regulator (Spry1) of the ERK and FGF signaling pathways | Promoting lipogenesis and osteogenesis of PDLSCs          |
| miR-200cN [31]   | Inhibiting LPS-induced IL-6, IL-8, and CCL-5 production in hPDLSCs and increasing cellular calcium, ALP, and Runx2 levels | Promoting osteogenic differentiation                      |
| miR-138 [31]     | Downregulating osteocalcin, Runx2, and type I collagen                    | Inhibiting the osteogenic differentiation of PDLSCs         |
| miR-26a-5p [32]  | Presumably targeting Wnt5a and thereby inhibiting the activation of the Wnt/Ca2+ signaling | Promoting the osteogenic differentiation of PDLSCs         |
| miR-302b [37]    | Using Chuanxiongzin (TMP) to reduce inflammation and apoptosis          | Promoting osteogenesis of LPS-stimulated human periodontal membrane cells |
| IncRNA [36]      | Presumably regulating the metabolism of various amino acids              | Promoting the osteogenic differentiation of PDLSCs         |
| P2X7R [33,34]    | Affecting the PI3K/Akt/mTOR signaling                                     | Promoting the osteogenic differentiation of PDLSCs         |
| HOXA10 [35]      | Regulating β-linked protein localization and DKK1                        | Inhibiting the osteogenic differentiation of periodontal stem cells |
| PLAP-1 [38]      | Using 1,25(OH)2D3 to inhibit the transcription of PLAP-1                 | Promoting osteogenesis                                     |
the PI3K/Akt/mTOR signaling. These two studies paved the way for the gene modification of stem cells for the treatment of periodontitis. miR species and effects are far from that. Recently, Wang et al. [35] demonstrated experimentally that HOXA10 inhibits the osteogenic differentiation of periodontal stem cells through the regulation of β-linked protein localization and DKK1.

IncRNA is a non-coding RNA of >200 nucleotides. Based on the known IncRNAs, Zhang’s team [36] predicted 63 new IncRNAs that regulate the metabolism of various amino acids. They demonstrated that these metabolic pathways are important for the osteogenic differentiation of PDLSCs, thereby largely unraveling the mechanism underlying IncRNA regulation of osteogenesis. In addition, with the ultimate aim to improve the differentiation potential of periodontitis-derived PDLSCs, the same authors pursued the mechanism, whereby an inflammatory microenvironment hinders osteogenesis and consequently identified 318 differentially expressed IncRNAs between healthy PDLSCs and LPS-mediated PDLSCs. The list of these IncRNAs is expected to be a useful tool to study the osteogenic differentiation of PDLSCs under inflammatory microenvironment. These IncRNAs are expected to be valuable in studying the mechanisms underlying the osteogenic differentiation of PDLSCs in an inflammatory microenvironment. Such studies on the mechanisms whereby IncRNAs regulate the osteogenic differentiation of PDLSCs have recently flourished.

In addition, several scholars have explored chemical approaches to improve osteogenesis. For example, tetramethylpyrazine (TMP) [37] improved the osteogenesis of LPS-stimulated human periodontal membrane cells by reducing inflammation and apoptosis through downregulation of miR-302b. In addition, 1,25(OH)2D3 has been shown to promote osteogenesis by inhibiting the transcription of PLAP-1 [38], but clinical trials have not yielded promising results.

3 Effects of biochemical and other mechanisms

Epigenetic modifications, such as DNA methylation and histone acetylation, manifest at the molecular level. Diomede et al. [39] detected that protein levels are altered in association with DNA methylation and histone acetylation in LPS-treated hPDLSCs and inferred that LPS can affect the osteogenic differentiation of periodontal stem cells through epigenetic modifications.

Endoplasmic reticulum stress (ERS) is a pathophysiological process [40,41] in which an imbalance in the action of the endoplasmic reticulum in response to a noxious stimulus leads to a decrease in the ability of the endoplasmic reticulum to fold proteins and an increase in the amount of unfolded proteins. Xue et al. [42] applied endoplasmic reticulum activators to LPS-treated PDLSCs and detected significant downregulation of the osteogenic markers RUNX2 and ALP. Zhang et al. [43] found that inflammatory periodontal stem cells mediated IL-1β secretion by macrophages through regulation of macrophage endoplasmic reticulum stress, which is suggestive of the involvement of endoplasmic reticulum in osteogenesis. Zhai et al. [44] found that upregulation of mitochondrial fusion protein 2 (mitofusin 2, Mfn2), a factor mediating endoplasmic reticulum-mitochondria coupling, suppresses osteogenesis. Recently, Feng et al. have found that 4-phenylbutyric acid [45] can inhibit inflammation and improve osteogenesis through ERS-related mechanisms and the NF-κB pathway.

From a systemic perspective, Plemmenos and Piperi [46] found that AGEs, glycosylation end products in diabetic metabolism, enhance LPS-induced expression of inflammatory factors in hPDLSCs and reduce osteogenic differentiation. It is suggested that diabetes may exacerbate the inflammation in patients with periodontitis and thus must be considered in periodontal treatment.

4 Conclusion

Periodontitis is a common disease of the periodontal supporting tissues, and most patients present to the clinic with severe symptoms, such as significant alveolar bone resorption and tooth loosening or even loss. However, there is currently no effective therapeutic strategy in periodontitis. In recent years, the development of tissue stem cell engineering has led researchers to seek breakthroughs in periodontitis treatment from a new perspective, such as regenerative approaches using periodontal stem cells. However, in the oral cavity of patients with severe periodontitis, the inflammatory microenvironment [47] caused by the high concentrations of LPS hampers the osteogenic differentiation of periodontal stem cells and, thus, impedes the success of the regenerative therapy. Therefore, the mechanisms underlying this phenomenon should be elucidated to find effective solutions that can improve the success rate.

PDLSC osteogenesis is differentially affected by LPS through different signaling pathways. The osteogenic
differentiation of hPDLSCs is promoted by E. coli LPS at low concentrations through the Wnt/β-catenin–induced upregulation of TAZ. However, both the histone acetyltransferase GCN5 and phospholipase Notum can suppress this pathway, thereby inhibiting the osteogenic differentiation. LPS can also inhibit osteogenesis and instead induce adipogenesis of hPDLSCs under inflammatory conditions by activating the TLR4 signaling and presumably through the resulting EphrinB2 downregulation, and by activating the NF-κB pathway. In addition, high doses of TLR1, 4, and 6 ligands inhibit Akt activation through the Myd88- or TRIF-dependent signaling pathways, which also inhibit hPDLSC osteogenesis. Activation of the ERK1/2 signaling can also affect cell differentiation. Studies at the gene level have revealed that the miR-148a/NRPI functional axis and miR-138 have inhibitory effects on osteogenesis, whereas miR-200c/N, miR-26a-5p, and P2X7R have stimulatory effects. Studies on micro-RNAs and lncRNAs have expanded the spectrum of mechanistic studies related to osteogenesis. In addition, epigenetic modifications, ERS, endoplasmic reticulum mitochondrial coupling, and diabetic metabolites have inhibitory effects on the osteogenic differentiation of periodontal stem cells.

Immense progress [48] has been made on the mechanisms affecting the osteogenic differentiation of PDLSCs in an LPS-induced inflammatory environment. These stem cells constitute a physiologically suitable cell population that can be used for the regenerative treatment of periodontitis [49]. Furthermore, they can be easily obtained and cultured and are tolerant to the LPS-induced inflammatory microenvironment of periodontitis. Of course, there are still many mechanisms yet to be clarified. First, the connections between the signaling pathways should be studied to further investigate the mechanisms affecting the osteogenic differentiation of PDLSCs in an LPS-induced inflammatory environment. Second, PDLSCs should be compared with other stem cells in osteogenic capacity in such an environment. Finally, although many factors affecting osteogenesis have been clinically explored, only a few have been applied to clinical experiments, and thus, basic research findings should be evaluated through additional clinical studies for bench-to-bedside translation.

Funding information: The research has been financially supported by: (1) Xuzhou science and technology program (kc15sh015); (2) Jiangsu Province’s “six talent peaks” funded project (2014-wsw-068); (3) General project of natural science research plan of colleges and universities in Jiangsu Province (12kjb320015); (4) Key project of Jiangsu college students’ innovation and entrepreneurship training program (202010310888); and (5) Open project of Jiangsu Key Laboratory of oral diseases (jsklod-kf-1905)

Author contributions: Jin conceived the idea and completed the writing and subsequent revision of the article. Jiang suggested ideas and participated in revisions. Sun, Fang Li, Xia, and Yaxin Li are members of the subject group and provided help with the conception and writing of the article. Zheng and Qin are supervisors and guided us through the article. Zheng is the corresponding author. The authors applied the SDC approach for the sequence of authors. Authors are sorted in order of upload (under the heading). Each author has consensus.

Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Sybil D, Jain V, Mohanty S, Husain SA. Oral stem cells in intraroral bone formation. J Oral Biol. 2020;62(1):36–43. doi: 10.1016/j.job.2019.12.001.
[2] Ye L, Chen L, Feng F, Cui J, Li K, Li Z, et al. Bone marrow-derived stromal cells are more beneficial cell sources for tooth regeneration compared with adipose-derived stromal cells. Cell Biol Int. 2015;39(10):1151–61. doi: 10.1002/cbin.10488.
[3] Cui D, Li H, Wan M, Peng Y, Xu X, Zhou X, et al. The origin and identification of mesenchymal stem cells in teeth: From odontogenic to non-odontogenic. Curr Stem Cell Res Ther. 2018;13(1):39–45. doi: 10.2174/1574888X12666170913150403.
[4] Lambrits I, Driesen RB, Dillen Y, Gervois P, Ratajczak J, Vangansweinkel T, et al. Dental pulp stem cells: Their potential in reinnervation and angiogenesis by using scaffolds. J Endod. 2017;43(9, Supplement):S12–S6. doi: 10.1016/j.joen.2017.06.001.
[5] Chew JR, Chuah SJ, Teo KYW, 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. doi: 10.1016/j.actbio.2019.03.021.
[6] 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(1):320. doi: 10.1186/s13287-019-1409-4.
[7] Nagata M, Iwasaki K, Akazawa K, Komaki M, Yokoyama N, Iizumi Y, et al. Conditioned medium from periodontal ligament stem cells enhances periodontal regeneration. Tissue Eng Part A. 2016;23(9–10):367–77. doi: 10.1089/ten.tea.2016.0274.
[8] Abusleme L, Hoare A, Hong B-Y, Diaz PI. Microbial signatures of health, gingivitis, and periodontitis. Periodontol 2000. 2021;86(1):57–78. doi: 10.1111/prd.12362.
Bartold PM, Van Dyke TE. An appraisal of the role of specific bacteria in the initial pathogenesis of periodontitis. J Clin Periodontol. 2019;46(1):6–11. doi: 10.1111/jcpe.13046.

Croms M, Kruyt MC, Boot W, Pouran B, Brahram MV, Pakpahan SA, et al. The role of bacterial stimuli in inflammation-driven bone formation. Eur Cell Mater. 2019;37:402–19. doi: 10.22203/ecm.v037a24.

Keong JY, Low LW, Chong JM, Ong YY, Pulikkotil SJ, Singh G, et al. Effect of lipopolysaccharide on cell proliferation and vascular endothelial growth factor secretion of periodontal ligament stem cells. Saudi Dent J. 2020;32(3):148–54. doi: 10.1016/j.sdent.2019.08.001.

Wang YZ, Fei DD, Zhang Y, Zhang XG, Wang Y, Wang QT. Role and mechanism of low-dose lipopolysaccharide-treated human periodontal ligament stem cells on the expression of macrophage pro-inflammatory factors. Zhonghua Kou Qiang Yi Xue Za Zhi. 2021;56(7):672–8. doi: 10.3760/cma.j.cn12144-20210329-00046.

Tomokiyo A, Wada N, Maeda H. Periodontal ligament stem cells: Regenerative potency in periodontium. Stem Cell Dev. 2019;28(15):974–85. doi: 10.1089/scd.2019.0031.

Li C, Li B, Dong Z, Gao L, He X, Liao L, et al. Lipopolysaccharide differentially affects the osteogenic differentiation of periodontal ligament stem cells and bone marrow mesenchymal stem cells through Toll-like receptor 4 mediated nuclear factor κB pathway. Stem Cell Res Ther. 2014;5(3):67. doi: 10.1186/srt134.

Albiero ML, Amorim BR, Casati MZ, Sallum EA, NOCITI FH, Silverio KGBOR. Osteogenic potential of periodontal ligament stem cells are una.. published online 2019;34(1):1–13. doi: 10.1111/omi.12249.

Li B, Sun J, Dong Z, Xue P, He X, Liao L, et al. GCN5 modulates differentiation of periodontal ligament stem cells via Toll-like receptors 4 (TLR4)/nuclear factor kappa B (NF-κB) pathway. Med Sci Monit. 2019;25:949–508. doi: 10.12659/MSM.918940.

Duan Y, An W, Wu H, Wu Y. Salvinorin C attenuates LPS-induced inflammation and apoptosis in human periodontal ligament stem cells via Toll-like receptors 4 (TLR4)/nuclear factor kappa B (NF-κB) pathway. Med Sci Monit. 2020;26:3274–83. doi: 10.12659/MSM.918940.

Cheng Y, Yuan C, Meng T, Liu Y, Zhu S, Zhang C, et al. Lipopolysaccharide inhibits osteogenic differentiation of periodontal ligament stem cells partially through toll-like receptor 4-mediated ephrinB2 downregulation. Clin Oral Investigations. 2020;24(10):3407–16. doi: 10.1007/s00784-020-03211-w.

Zhu Y, Li Q, Zhou Y, Li W. TLR activation inhibits the osteogenic potential of human periodontal ligament stem cells through Akt signaling in a MyD88- or TRIF-dependent manner. J Periodontol. 2019;90(4):400–15. doi: 10.1002/jper.2019.0026.

Zhao B, Zhang W, Xiong Y, Zhang Y, Jia L, Xu X. Rutin protects human periodontal ligament stem cells from TNF-α induced damage to osteogenic differentiation through suppressing mTOR signaling pathway in inflammatory environment. Arch Oral Biol. 2020;109:104584. doi: 10.1016/j.archoralbio.2019.104584.

Čebaturānienu A, Kriauciūnaitė K, Prunskaitė J, Tunačaitis V, Pivoriūnas A. Extracellular vesicles suppress basal and lipopolysaccharide-induced NFκB activity in human periodontal ligament stem cells. Stem Cell Dev. 2019;28(15):1037–49. doi: 10.1089/scd.2019.0021.

Xie C, Lin M, Tian H, Zhang L, Ren A. Astaxanthin inhibits inflammation of human periodontal ligament stem cells induced by lipopolysaccharide. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2021;46(3):227–33. doi: 10.1007/s11741-2020-00146.

Kukolj T, Trivanović D, Djordjević IO, Mojsilović S, Krstić J, Obradović H, et al. Lipopolysaccharide can modify differentiation and immunomodulatory potential of periodontal ligament stem cells via ERK1,2 signaling. J Cell Physiol. 2018;233(1):447–62. doi: 10.1002/jcp.25904.

Bao L, Zhang X, Xu Y, Wang M, Song Y, Gu Y, et al. Dysfunction of mIR-148a-NRP1 functional axis suppresses osteogenic differentiation of periodontal ligament stem cells under inflammatory microenvironment. Cell Reprogram. 2019;21(6):314–22. doi: 10.1089/cell.2019.0026.

Yang N, Li Y, Wang G, Ding Y, Jin Y, Xu Y. Tumor necrosis factor-α suppresses adipogenic and osteogenic differentiation of human periodontal ligament stem cell by inhibiting miR-21-Spry1 functional axis. Differ Res Biol Diversity. 2017;97:33–43. doi: 10.1007/j.diff.2017.08.004.

Hong L, Sharp T, Khorsand B, Fischer C, Eliason S, Salem A, et al. MicroRNA-200c represses IL-6, IL-8, and CCL-5 expression and enhances osteogenic differentiation. PLoS One. 2016;11(8):e0160915. doi: 10.1371/journal.pone.0160915.

Zhang KK, Geng YD, Wang SB, Hua L. MicroRNA-26a-5p targets Wnt5a to regulate osteogenic differentiation of human periodontal ligament stem cell from inflammatory microenvironment. Zhonghua Kou Qiang Yi Xue Za Zhi. 2019;54(10):662–9. doi: 10.3760/cma.j.issn.1002-0098.2019.10.003.

Xu HY, He XT, Wang J, Li X, Xia Y, Tan YZ, et al. Role of the P2X7 receptor in inflammation-mediated changes in the...
osteogenesis of periodontal ligament stem cells. Cell Death Dis. 2019;10(1):20. doi: 10.1038/s41419-018-1253-y.

[34] Xu XY, Tian BM, Xia Y, Xia YL, Li X, Zhou H, et al. Exosomes derived from P2X7 receptor gene-modified cells rescue inflammation-compromised periodontal ligament stem cells from dysfunction. Stem Cell Transl Med. 2020;9(11):1414–30. doi: 10.1002/sctm.19-0418.

[35] Wang C, Li Y, Yu K, Jiang Z, Wang Y, Yang G. HOXA10 inhibit the osteogenic differentiation of periodontal ligament stem cells by regulating β-catenin localization and DKK1 expression. Connect Tissue Res. 2021;62(4):393–401. doi: 10.1080/03008207.2020.1756271.

[36] Huang Y, Zhang Y, Li X, Liu H, Yang Q, Jia L, et al. The long non-coding RNA landscape of periodontal ligament stem cells subjected to compressive force. Eur J Orthod. 2019;41(4):333–42. doi: 10.1093/ejo/cjy057.

[37] Duan Y, An W, Wu Y, Wang J. Tetramethylpyrazine reduces inflammation levels and the apoptosis of LPS-stimulated human periodontal ligament cells via the downregulation of miR-302b. Int J Mol Med. 2020;45(6):1918–26. doi: 10.3892/ijmm.2020.4554.

[38] Zhang P, Zhang Y, Liu Q, Zhang Y, Ji Y, Xu X. 1,25(OH)2D3 supports the osteogenic differentiation of hPDLSCs under inflammatory conditions through inhibiting PLAP-1 expression transcriptionally. Int Immunopharmacol. 2020;78:105998. doi: 10.1016/j.intimp.2019.105998.

[39] Diomedes F, Thangavelu SR, Merciaro I, D’Orazio M, Bramanti P, Mazzon E, et al. Porphyromonas gingivalis lipopolysaccharide stimulation in human periodontal ligament stem cells: Role of epigenetic modifications to the inflammation. Eur J Histochim. 2017;61(3):2826. doi: 10.4081/ehj.2017.2826.

[40] Yamada H, Nakajima T, Domon H, Honda T, Yamazaki K. Endoplasmic reticulum stress response and bone loss in experimental periodontitis in mice. J Periodont Res. 2015;50(4):500–8. doi: 10.1111/jpr.12232.

[41] Bezamat M, Deoley K, Khaliq S, Letra A, Scariot R, Silva RM, et al. Are mTOR and endoplasmic reticulum stress pathway genes associated with oral and bone diseases? Caries Res. 2019;53(3):235–41. doi: 10.1159/000492675.

[42] Xue P, Li B, Tan J, An Y, Jin Y, Wang Q. Effect of endoplasmic reticulum stress on the expression and osteogenic differentiation of periodontal ligament stem cells. Zhonghua Kou Qiang Yi Xue Za Zhi. 2015;50(9):548–53.

[43] Zhang Y, Wang YZ, Fei DD, Zhang XG, Liao ZX, Liu LX, et al. Inflammatory periodontal stem cells mediate interleukin-1β secretion of macrophage by regulating macrophage endoplasmic reticulum stress. Zhonghua Kou Qiang Yi Xue Za Zhi. 2021;56(4):329–34. doi: 10.3760/cma.j.cn112144-20201105-00553.

[44] Zhai QM, Li B, WangZW, Liu L, Jin Y, Jin F. Endoplasmic reticulum-mitochondrial contact regulates osteogenic differentiation of periodontal ligament stem cells via mitofusion 2 in inflammatory microenvironment. Zhonghua Kou Qiang Yi Xue Za Zhi. 2018;53(7):453–8. doi: 10.3760/cma.j.issn.1002-0098.2018.07.005.

[45] Feng Y, Zhang R, Wang YR, Chen F, Luo Q, Cai C, et al. Inhibition of endoplasmic reticulum stress by 4-phenyl butyric acid presents therapeutic effects on periodontitis: Experimental studies in vitro and in rats. Stem Cell Int. 2021;2021:6618943. doi: 10.1155/2021/6618943.

[46] Plemmenos G, Piperi C. Pathogenic molecular mechanisms in periodontitis and peri-implantitis: Role of advanced glycation end products. Life. 2022;12(2):218. doi: 10.3390/life12020218.

[47] Chen M, Lin X, Zhang L, Hu X. Effects of nuclear factor-κB signaling pathway on periodontal ligament stem cells under lipopolysaccharide-induced inflammation. Bioengineered. 2022;13(3):7951–61. doi: 10.1080/21655979.2022.2051690.

[48] Queiroz A, Albuquerque Souza E, Gasparoni LM, de França BN, Pelissari C, Triveireller M, et al. Therapeutic potential of periodontal ligament stem cells. World J Stem Cell. 2021;13(6):605–18. doi: 10.4252/wjsc.v13.i6.605.

[49] Nuñez J, Vignoletti F, Caffesse RG, Sanz M. Cellular therapy in periodontal regeneration. Periodontol 2000. 2019;79(1):107–16. doi: 10.1111/prd.12250.