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Systematic Review

Local Immunomodulation of Human Periodontal Ligaments Stem Cells (PDLSC) in Periodontitis: A Systematic Review

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ABSTRACT

Background: *Human periodontal ligament stem cells (PDLSC) are multipotent cells, derived from periodontal ligament (PDL), that can attain the formation of new bone, cementum, and periodontal ligament. However, the quantity of stem cells found in PDL tissues is deficient. Therefore, in periodontitis, the limited PDLSCs make regeneration of the periodontium challenging and unpredictable.* **Objectives**: *To critically appraise the available experimental evidence of the local immunomodulation signaling systems and their surrounding functional molecules for repair and application in periodontal regeneration therapy.* **Methods:** *This systematic review was conducted using PRISMA 2020 guidelines and research papers were selected based on strict exclusion and inclusion criteria. PubMed, Cochrane Library, Google Scholar and Web of Science databases were used to collect all the research papers ranging from January 2012 to December 2022.* **Results:** *Nine out of 586 research papers that focused on PDLSCs during inflammatory conditions were selected based on the Cochrane Risk of Bias Assessment. Seven articles documented that non-coding microRNAs (ncRNAs) played critical roles in the modification of PDLSC multipotency in periodontitis. An article showed that the post-translational modifications are influenced by histone deacetylases (HDAC) indicating the involvement of novel epigenetic signaling systems. A natural product-derived functional molecule resveratrol is described to have anti-inflammatory properties through the nuclear factor kappa B (NF-kB) pathway.* **Conclusion:** *The results of our present systematic review clearly apprise that the ncRNAs signaling system and natural products derived anti-inflammatory functional molecules play a clinically significant therapeutic target in novel periodontitis therapy as preventive, diagnostic, therapeutic, and prognostic markers.*

Keywords: *immunomodulatory factors, PDLSCs, multipotency, periodontal regenerative medicine, miRNA*

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Abbreviations: *CircRNA-Circular RNA, ceRNA-competitive endogenous RNA, HDAC-Histone Deacetylases, lncRNA-long non-coding RNA, MALAT1-metastasis-associated lung adenocarcinoma transcript 1, ncRNAs-non-coding RNA, NF-kB-Nuclear factor-kB, PDLSCs-Periodontal Ligament Stem Cells, TRAF6-TNF Receptor Associated Factor 6*

INTRODUCTION

Periodontal disease is a chronic infectious disease that includes a variety of inflammatory conditions that affect the periodontium which leads to the loss of teeth and contributes to systemic inflammation (Cho et al., 2021). A recent systematic review has shown that periodontitis is highly prevalent, with approximately 10% of the global population affected by advanced periodontitis (Maeda et al., 2013). According to the World Health Organization (2022), advanced periodontal diseases are predicted to involve around 19% of the global adult population, representing more than 1 billion cases worldwide [WHO].

Periodontal regeneration is considered to be a promising strategy for the repair of lost or damaged tissues, and also to improve a patient's quality of life (Cho et al., 2022). For successful regeneration, cells that have the ability to form cementum, periodontal ligament (PDL), and alveolar bone should relocate to the location of the defect and activate the differentiation potential from progenitor cells (Ivanov et al., 2021; Corrado et el., 2013). Periodontal ligament-derived stem cells (PDLSC) are adult multipotent mesenchymal-like stem cells (MSCs) that can induce promising immunomodulation to interact with immune cells for disease treatment (Arora et al., 2022). These PDLSC are multipotent cells that have an important role in regenerating fibers in order to fight against bacterial attack during periodontitis (Iwasaki et al., 2013).

PDLSCs have the ability to develop into PDLs, alveolar bone, cementum, blood vessels, and peripheral nerves (Zhang et al, 2020). They also have a strong proliferation potential and the ability to self-renew (Zhu et al., 2015; Menicanin et al., 2014). Stem cell behavior at sites of inflammation appears to be an important strategy in developing new approaches for in situ tissue regeneration (Nuñez et al., 2019). Exosomes may serve as regulators of cell-to-cell communication and are now recognized as a crucial component of the intercellular microenvironment (Corrado et al., 2013). They may serve as immune modulators by transferring proteins or nucleic acids to recipient cells, which may have immunosuppressive or immunoactivating effects (Iwasaki et al., 2013).

Hence, in this systematic review, we attempt to critically appraise the available experimental evidence of the local immunomodulation signaling systems and their surrounding functional molecules for repair and application in periodontal regeneration therapy.

MATERIALS AND METHODS

The 27 new updated checklists of Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) 2020 are used in this systematic review. (Appendix 2)

Research question

The research question was designed based on PICO (Population, Intervention, Comparison, Outcome) criteria. Hence, two research questions were successfully established;

1. What are the signaling molecules for effective immunomodulatory factors of PDLSCs multipotency in periodontitis patients?

2. How can possible differences between studies be explained?

Eligibility criteria

Two reviewers (NZ) (WN) determined the inclusion and exclusion criteria for the research paper collection under the supervision of two supervisors (KZ) (ER). The reliable papers were collected in accordance with the inclusion and exclusion criteria below:

1. Inclusion criteria
    a) Study characteristics (PICO)
        i. Patient/Population: Patient diagnosed with periodontitis
        ii. Intervention: the signaling molecules for effective immunomodulatory factors of PDLSCs multipotency
        iii. Comparison: different methods used to identify the effective immunomodulatory factors of PDLSCs
        iv. Outcome: Potential molecules for periodontal regenerative medicine
    b) Report characteristics
        i. Publication year: Published within 10 years (between 2012 to June 2022)
        ii. Language: studies reported in the English language
        iii. Study design: Observational and experimental study
2. Exclusion criteria
    a) Study characteristics (PICO)
        i. Patient/Population: Patient without periodontitis
        ii. Intervention: signaling molecule present for other stem cell immunomodulation except for PDLSCs
        iii. Comparison: methods used to identify the effective immunomodulatory factors for another stem cell except for PDLSCs
        iv. Outcome: Potential molecules for other than periodontal regenerative medicine
    b) Report characteristics
        i. Publication year: article published before 2012
        ii. Language: non-English language
        iii. Study design: other than observational and experimental study

Information sources

All reliable research papers and articles were collected from PubMed, Cochrane Library, Google Scholar and Web of Science databases starting from March 2022 until December 2022. All the research papers and articles were gathered in the PICO portal to ease the paper screening process and keep all data on track.

Search strategy

MeSH (Medical Subject Headings) keywords were used to conduct the search, and the search items were merged using the Boolean operators (OR, AND). The keywords such as PDLSCs, multipotency, periodontal regenerative medicine, exosomes, miRNA, and immunomodulatory factors were used to search and filter the relevant articles from the databases (Table 1).

Table 1: Search strategy used in PubMed, Cochrane Library, Web of Science and Google Scholar.

Search terms
("PDLSCs" OR "Periodontal ligament stem cell" OR "stem cell" OR "immunomodulation" OR "immunomodulatory factors" OR "multipotency" OR "multipotent cell" OR "regenerative medicine" OR "miRNA" OR "periodontal regenerative medicine" OR "immunomodulation" OR "local immunomodulation" OR "periodontitis")

Selection process

The titles and abstracts for all 421 papers collected were examined independently by two reviewers. After the abstract screening, 141 full articles were reviewed to have a thorough understanding of each paper to make a decision. Data were excluded if there was a discrepancy unless an acceptable supplementary explanation was given. For the purpose of the final selection of research papers, strict inclusion and exclusion criteria had been used. (Figure 1)

Data collection process

Two reviewers independently obtained data from relevant studies upon reading the full-text article. The crucial data were taken from the included studies such as the publication year and type of study. From this filtering process, 9 papers were considered good quality and relevant for this systematic review topic (Table 2).

Data items

Data which include name of authors, year of publication, study design, methodology of research, study of interest, result of the research and conclusion, outcomes of the studies, and funding for each papers were extracted on a pre-defined spreadsheet (Table 3).

Study risk of bias assessment

Using the Cochrane Risk of Bias tool, two reviewers independently evaluated the included studies' risk of bias. If there were any inconsistencies, discussion will be made for clear comprehension. This was reported in the "risk of bias" table (Table 3).

The assessment tool includes 7 specific domains:

1. Random sequence generation
2. Allocation concealment
3. Blinding of participants and personnel
4. Blinding of outcome assessment
5. Incomplete outcome data
6. Selective reporting
7. Other bias

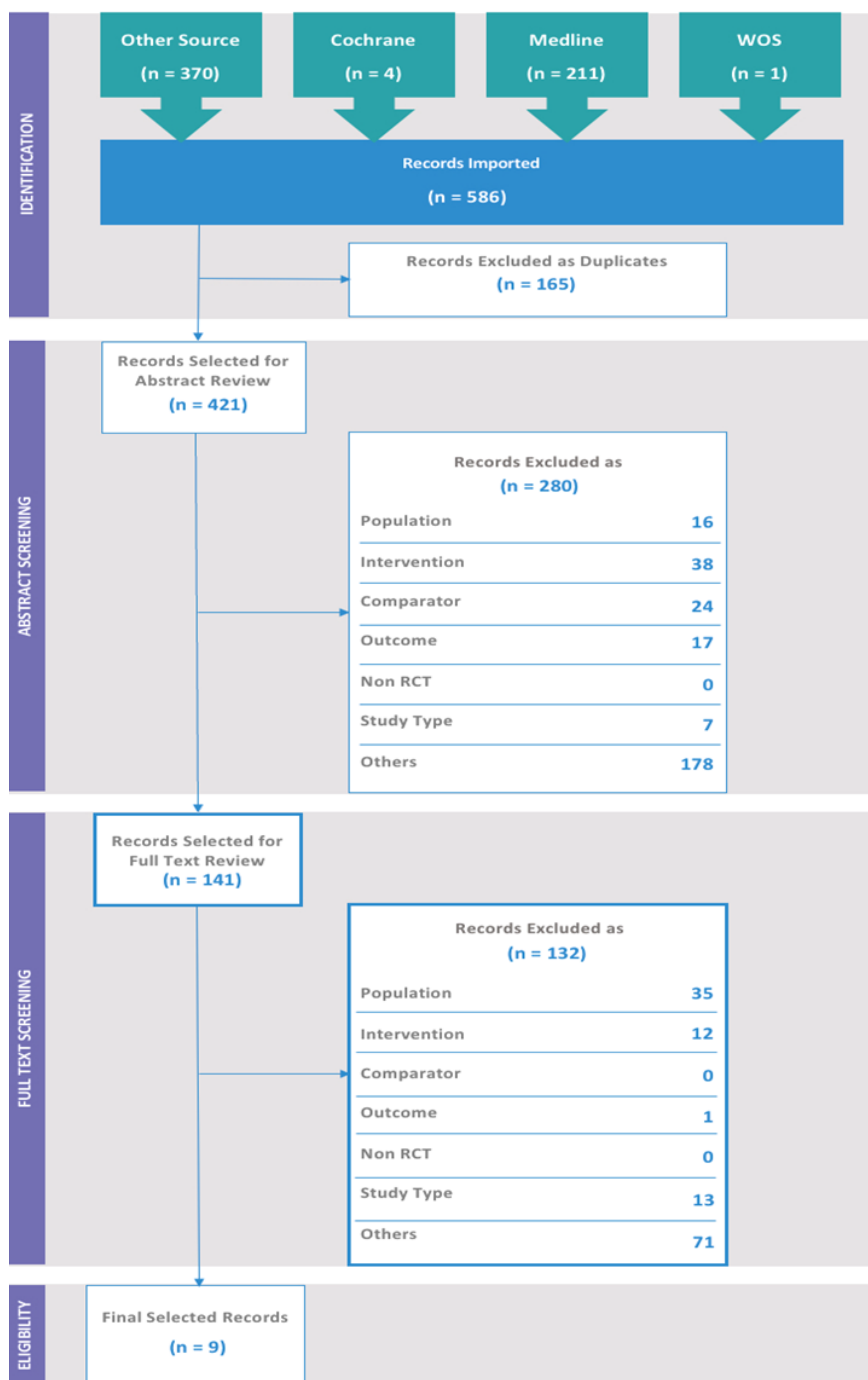

Figure 1: Preferred Reporting Items for Systematic Review and Meta-Analyses flowchart followed in this systematic review

Table 2. The selected papers that were considered as good quality and relevant for this systematic review topic.

Year	Author name	Title	Database
2015	Zheng *et al*	Periodontitis promotes the proliferation and suppresses the differentiation potential of human periodontal ligament stem cells	PubMed
2019	Chen *et al*	MALAT1 overexpression promotes the proliferation of human periodontal ligament stem cells by upregulating fibroblast growth factor 2	Google Scholar
2013	Chen *et al*	Nuclear factor-kB modulates osteogenesis of periodontal ligament stem cells through competition with β-catenin signaling in inflammatory microenvironments	PubMed
2019	Wang *et al*	Long non-coding RNAs mortal obligate RNA transcript regulates the T proliferation of human periodontal ligament stem cells and affects the recurrence of periodontitis	PubMed
2022	Deng *et al*	Circ_0138959/miR-495-3p/TRAF6 axis regulates proliferation, wound healing and osteoblastic differentiation of periodontal ligament cells in periodontitis	PubMed
2019	Liu *et al*	Down-regulation of long non-coding RNA MEG3 suppresses osteogenic differentiation of periodontal ligament stem cells (PDLSCs) through miR-27a-3p/IGF1 axis in periodontitis	PubMed
2020	Zhang *et al*	PDLSCs Regulate Angiogenesis of Periodontal Ligaments via VEGF Transferred by Exosomes in Periodontitis	PubMed
2018	Li *et al*	Mutual inhibition between HDAC9 and miR-17 regulates osteogenesis of human periodontal ligament stem cells in inflammatory conditions	Google Scholar
2018	Wang *et al*	Resveratrol enhances the functionality and improves the regeneration of mesenchymal stem cell aggregates	Google Scholar

Table 3: Summary of the final included studies.

Author	Year	Study design	Methodology	Study interest	Result	Conclusion	Outcomes	Funding
Zheng *et al*	2015	Case control	1. Patient: Premolars and third molars that were free of disease were taken from ten individuals, whereas diseased teeth were taken from ten patients who had generalised chronic periodontitis. 2. Cell culture: In the middle section of the root, the PDL tissue was gently scraped from the surface. 3. Flow cytometric analysis. 4. Isolation of PDLSCs. 5. Colony-forming unit-fibroblast assay. 6. Osteogenic and adipogenic differentiation. 7. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). 8. Alkaline phosphatase (ALP) activity assay. 9. In vivo differentiation assay. 10. Statistical analysis.	PDLSCs	1.The colony formation of PDLSCs in periodontitis-affected donor is greater than healthy donor. 2. Pluripotency capacity of PDLSCs including osteogenic and adipogenic effect is greater in healthy donors compared to periodontitis-affected PDLSCs. 3.As compared to PDLSCs from healthy donors, those with periodontitis showed lower levels of the osteoblast markers Runx2, collagen 1, and osteocalcin and had lower levels of ALP activity. 4. When compared to a donor with periodontitis, the PDLSC transplants from healthy donors were shown to have a higher tissue-regenerative potential to produce mineralized tissue that resembles cementum.	It was concluded that the PDLSCs from donors with periodontitis may have a decreased ability to differentiate, which may limit their ability to create periodontal tissue. Healthy PDL is a better source if PDLSCs turn out to be a potential cell source for periodontal engineering. PDLSCs may commit to periodontal tissues by modifying cytokines and/or other differentiation methods if they come from damaged teeth.	Immunomodulatory cytokines play role in PDLSCs regeneration	Nature Science Foundation of China
Chen *et al*	2019	Case control	1. Patients: During orthodontic treatment, 12 patients' disease-free third molars and premolars were collected, while 12 patients with generalised chronic periodontitis had their diseased teeth extracted. 2. PDL tissues were taken from the central root of healthy and periodontitis-affected teeth for cell culture and transfection.	PDLSCs	1. In PDLSCs generated from periodontitis-affected teeth, MALAT1 and FGF2 mRNA expression is increased. 2. In PDLSCs generated from healthy and periodontitis-affected individuals, MALAT1 and FGF2 mRNA expression are positively associated. 3. As compared to PDLSCs produced from healthy teeth, those from periodontitis-affected teeth have a greater rate of cell growth.	In conclusion, periodontitis causes an increase in MALAT1 and FGF2 expression. Moreover, through encouraging FGF2 expression and PDLSC proliferation, MALAT1 may contribute to periodontitis.	MALAT1 is regulator of FGF2 which improve the proliferation of PDLSCs	1)Natural Science Foundation of Tibet Autonomous Region 2)The Scientific Research Project of Southern Medical University Stomatological Hospital

			3. RNA isolation and reverse transcription-quantitative PCR (RT-qPCR). 4. Cell proliferation assay. 5. Western blot analysis. 6. Statistical analysis.		4. MALAT1 promotes the growth of PDLSCs generated from healthy and periodontitis-affected teeth. 5. MALAT1 increases the expression of the FGF2 protein in PDLSCs obtained from both healthy people and those with periodontitis.			
Chen *et al*	2013	Case control	1. Human premolars or third molars that were removed for orthodontic treatment from 10 otherwise healthy people served as the study sample and the cell culture. Eight individuals with mild to severe chronic periodontitis had their inflammatory tissues isolated. Using a scalpel, the author removed PDL tissues from either healthy (H-PDLSCs) or patient-derived (P-PDLSCs) root surfaces. Using the limited dilution method, H-PDLSCs and P-PDLSCs were produced. 2. Induction of osteogenic differentiation: H-PDLSCs and P-PDLSCs (P4) were seeded into six-well culture dishes. 3. Total RNA extraction and RT- PCR. 4. LiCl/ LY 294002/ BAY 11-7082/ PDTC treatment. 5. Protein isolation and western blot analysis.	PDLSCs	1. Inhibiting GSK-3b may enable P-PDLSCs to differentiate again into osteogenic cells. 2. GSK-3b modulated WNT and NF-kB signaling in PDLSCs. 3. Activation of GSK-3b could decrease the osteogenic differentiation of PDLSCs. 4. Inhibition of NF-kB inhibited higher NF-kB activation in P-PDLSCs and reduced osteogenesis.	Nuclear factor kB's processes demonstrate how it functions as a regulator, regulating a variety of aspects essential for the osteogenic development of PDLSCs derived from inflammatory microenvironments. The ability of the IkBa phosphorylation inhibitor to partially restore the osteogenic capability of P-PDLSCs raises the possibility that NF-kB is involved in the osteogenic differentiation of P-PDLSCs.	The differentiating potential of PDLSCs in inflammation is restored by NF-B inhibitors.	1)National Major Scientific Research Program of China 2)Nature Science Foundation of China
Wang *et al*	2019	Cohort	1. 48 individuals with periodontitis who had their teeth extracted provided the periodontal ligament tissues for collection. 2. Both healthy and periodontitis-affected third	PDLSCs	1. In comparison to PDLSCs from healthy teeth, the level of MORT expression was noticeably downregulated in PDLSCs isolated from periodontitis-affected teeth (periodontitis) (control). 2.	In conclusion, the multiplication of human PDLSCs and the recurrence of periodontitis are both impacted by lncRNA MORT.	lncRNA MORT plays role in periodontitis recurrence	Health and Family Planning Research Subject of Jilin Province, China

			molars were used to create periodontal ligament (PDL) tissues. 3. Patients with periodontitis were monitored for 2 years after therapy to check for periodontitis recurrence. 4. The total RNA were isolated from PDLSCs using RNA-spin™ Total RNA Extraction Kit (iNtRON Biotechnology DR). Reverse transcriptions were performed using Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit.		Four instances of periodontitis-affected teeth had considerably more PDLSC proliferation than the group of healthy teeth (4 cases). 3. When MORT was overexpressed, PDLSC proliferation from periodontitis-affected teeth was dramatically suppressed. 4. Patients with poor MORT expression had a considerably greater incidence of periodontitis recurrence.			
Deng *et al*	2022	Case control	1. At the Gaoxin branch of Jinan Stomatological Hospital, 35 patients with chronic periodontitis (CP) had their premolars extracted when periodontal ligament tissues were obtained from the surface of the gingival sulcus. 30 healthy people (male:female ratio: 19:11; age range: 55) provided normal tissues. 2. PDLCs were isolated from the periodontal ligaments of 26 teeth removed from 12 healthy people in order to undergo orthodontic treatment. The middle third of the roots' middle third was used to delicately scrape out the periodontal ligaments, which were then chopped into 1 mm3 pieces and plated into 35 mm cell culture dishes. 3. PDLCs were given varied doses of 1 mg/mL	PDLSCs	1. PDLCs treated with LPS and periodontitis samples both showed significant levels of Circ 0138959 expression. 2. In PDLCs exposed with LPS, the absence of circ 0138959 promoted proliferation, wound healing, and osteogenic differentiation but suppressed apoptosis and inflammation. 3. Circ 0138959 made miR-495-3p act like a sponge. 4. In PDLCs, the Circ 0138959/miR-495-3p axis controlled the LPS-induced cell damage. 5. Circ 0138959 controlled TRAF6 expression by acting as a miR-495-3p sponge.	As a result of controlling the miR-495-3p/TRAF6 axis, this study showed that circ 0138959 accelerated the pathological development of periodontitis in vitro. Most importantly, by lowering the expression of circ 0138959, periodontitis therapy may be enhanced.	circ_0138959 plays a part in periodontitis progression	Shandong Provincial Nature Science Foundation

			LPS (Sigma) to cause periodontitis-like damage (12 h, 24 h, 48 h).					
Liu *et al*	2019	Case control	1. From the Gene Expression Omnibus database, microarray data of the expression profiles of three periodontal tissues with periodontitis and three healthy tissues were retrieved. 2. Periodontal ligament tissues from 20 patients with periodontitis (ten males and ten females, ages 24-38), who had premolar extraction at the stomatological hospital of Shandong University, were used to create human periodontitis PDLSCs (pPDLSCs). 20 healthy patients provided periodontal ligament tissues from their removed premolars in order to obtain human healthy PDLSCs (hPDLSCs) (10 males, 10 females, age 18-25). 3. Premolars were first cleaned with PBS and then disinfected with 75% ethanol. The centre third of dental roots were then divided into normal and periodontitis periodontal ligament tissues. Collagenase type I was used to shred and digest separated tissues.	PDLSCs	Comparing periodontal tissues from people with periodontitis to healthy periodontal tissues, it was found that IGF1 was down-regulated and the PI3K/Akt signalling pathway was dysregulated. In periodontitis periodontal tissues relative to healthy periodontal tissues, lncRNA MEG3 was down-regulated and associated with IGF1 via miR-27a-3p. A co-expression network was established, PDLSCs were identified, and the lncRNA MEG3 was positively correlated with PDLSC osteogenic development. Via miR-27a-3p, LncRNA MEG3 controlled PDLSC osteogenic differentiation. IGF1 is a target of MiR-27a-3p, which controls PDLSC osteogenic development. The PI3K/Akt signalling pathway was controlled by the LncRNA MEG3/miR-27a-3p/IGF1 axis to influence PDLSC osteogenic differentiation.	To summarise, lncRNAMEG3 and IGF1 expression levels were both down-regulated, but miR-27a-3p expression levels were increased in periodontitis periodontal tissues compared to healthy periodontal tissues. Additionally, the lncRNA/miRNA/mRNA regulatory network has been widely established, and it influences miRNAs at posttranscriptional processing steps by acting as a ceRNA, which inhibits the expression of miRNA target genes. In summary, the lncRNA MEG3 promoted PDLSC osteogenic development in periodontitis via sponging miR-27a-3p to up-regulate IGF1 and activate the PI3k/Akt signalling pathway.	lncRNA MEG3 improves PDLSC multipotency and differentiation	1)National Natural Science Foundation of China 2)Natural Science Foundation of Shandong Province 3)China Postdoctoral Science Foundation 4) Special funds for Postdoctoral Innovation Projects of Shandong Province 5) the Fundamental Research Funds of Shandong University 6) Students Research Fund of Shandong University 7) the Construction Engineering Special Fund of "Taishan Scholars" 8)Key research and development program of Shandong Province
Zhang *et al*	2020	Case control	The centre of the root surfaces of donors with normal and periodontitis-related periodontal ligaments were cut into tiny pieces (approximately	PDLSCs	markers particular to the veins In the periodontal ligaments of people with periodontitis, CD31 and VEGFA were shown to be strongly expressed. When co-	The findings showed that the exosome-mediated transfer of VEGFA, which miR-17- targeted, was regulated by the inflammatory	miR-17-5p/VEGFA signaling pathway is the regulator in periodontits	1) Key R & D Plan Projects of Shaanxi Province 2)Natural Science Foundation of Jiangsu Province, China 3)Natural Science

			1 mm3) and digested with collagenase type I. PDLSCs used for exosome separation were grown in vesicle-free media and serum. Centrifuging was used to separate the remaining cells from the obtained conditioned media for 10 min. at 500 g.		cultured with inflamed PDLSCs, HUVEC tube formation improved while the expression of VEGFA was up-regulated in inflamed PDLSCs compared to control. Inflammation increased PDSLC exosome production, which in turn boosted HUVEC angiogenesis. In contrast, inhibiting exosome secretion caused HUVEC angiogenesis to decline.	microenvironment of periodontitis, which improved the vascularization of the periodontal ligaments and enabled pro-angiogenesis of PDLSCs.		Foundation of China
Li *et al*	2018	Case control	1. Sample collection: Twenty teeth from patients who had chronic periodontitis and had to have their teeth pulled were taken. To create the experimental periodontitis model, SD rats were employed. 2. Human PDLSC isolation and culture. 3. 3-2, tests for the dye reduction of 5-Diphenyltetrazolium bromide (MTT). Inhibition of RNA. 5. Western blotting evaluation. 6. ALP test and Alizarin red staining. 7. Analysis using real-time quantitative PCR. Chromatin immunoprecipitation, number eight (ChIP). 9. Immunohistochemistry with paraffin embedding (IHC-P).	PDLSCs	1. Inflammation reduced PDLSCs' ability to promote osteogenesis. 2. HDAC9 is implicated in the epigenetic alterations brought on by inflammation in hPDLSCs. 3. Restoring osteogenic differentiation in P-PDLSCs with HDAC9 inhibition. 4. In vitro osteogenic development of PDLSCs is regulated by the interaction of mir-17 and HDAC9. 5. In vivo, NaB restores osteo-differentiation.	In conclusion, si-miR-17 and NaB both inhibit HDAC and restore osteogenesis in human inflammatory PDLSCs. We present novel evidence for the role of miRNAs in histone modification-related disorders as well as how NaB may serve as potential therapeutic targets for periodontitis and inflammatory diseases.	HDAC inhibitors or si-HDAC9 rescued the osteogenic differentiation capacity of inflammatory PDLSC	1) the National Key Research and Development Program of China 2) Nature Science Foundation of China 3) National Natural Science Foundation (NNSF) of China
Wang *et al*	2018	Case control	1. Human PDLSC isolation, culture, and verification: Normal periodontal conditions and periodontitis donors are used as donors. By scraping the middle third of the root surface, PDL samples were obtained	PDLSCs	1. Cell aggregate osteogenesis and cell aggregate formation are reduced in PDLSCs from patients with periodontitis. 2. RSV protects N-PDLSC aggregates' capacity to form aggregates and osteogenesis when exposed to the pro-	In conclusion, using RSV-based cell aggregate engineering is the best way to enhance the ability of MSCs produced from both healthy and inflammatory microenvironments to regenerate tissue.	RSV can be a therapeutic agent for periodontal regeneration	State Scholarship Fund of China

			and placed in culture medium. Based on flow cytometry, surface marker profiling of human PDLSCs was carried out. 2. Formation of PDLSC aggregates: PDLSCs were further grown in medium that promotes cell aggregation. 3. Osteogenic differentiation: PDLSC aggregates were subsequently cultivated in osteogenic-inducing medium containing 50 g/ml ascorbic acid in order to produce osteogenic differentiation. 4. Ectopic tissue regeneration of PDLSC aggregates in naked mice: The dorsal area of naked animals received transplants of N-PDLSC aggregates, P-PDLSC aggregates, and P-PDLSC aggregates that had been exposed to RSV.		inflammatory cytokine TNF-. 3. RSV therapy enhances N-PDLS C and P-PDLSC aggregate formation and osteogenic potential. 4. RSV therapy improves in situ PDLSC aggregates' osteogenesis and periodontal regeneration.			

RESULTS

Study selection

A total of 586 studies were identified from Cochrane, Medline, Web of Science and other databases which resulted in 421 studies left for abstract screening after removing duplicates. During the abstract screening process, 280 studies were disqualified based on PICO exclusion criteria, leaving 141 studies for full-text screening. Only 11 studies are eligible for final selected records that meet all PICO inclusion and exclusion criteria. (Figure 1) (Table 2)

However, during the risk of bias assessment, 2 reviewers managed to identify 2 studies that were not suitable for this systematic review even though both of the studies met all the inclusion criteria. These 2 studies were excluded because they were not experimental or Randomized Control Trial studies. Thus, another criterion was added to the exclusion and inclusion criteria leaving only 9 studies for the final selection of papers. List of excluded studies in the systematic review, along with the reason for exclusion summarized in Appendix 3

Study characteristics

The general characteristics of the included studies such as title, name of the author, year of publication, and database of the studies - are provided in Table 2. Of the nine studies, four studies were from the PubMed database on "Periodontitis promotes the proliferation and suppresses the differentiation potential of human periodontal ligament stem cells", "Nuclear factor-kB modulates osteogenesis of periodontal ligament stem cells through competition with β-catenin signaling in inflammatory microenvironments", "Long non-coding RNAs mortal obligate RNA transcript regulates the T proliferation of human periodontal ligament stem cells and affects the recurrence of periodontitis", "Circ_0138959/miR-495-3p/TRAF6 axis regulates proliferation, wound healing and osteoblastic differentiation of periodontal ligament cells in periodontitis", "Down-regulation of long non-coding RNA MEG3 suppresses osteogenic differentiation of periodontal ligament stem cells (PDLSCs) through miR-27a-3p/IGF1 axis in periodontitis" and "PDLSCs Regulate Angiogenesis of Periodontal Ligaments via VEGF Transferred by Exosomes in Periodontitis". 3 studies were from Google Scholar database on "MALAT1 overexpression promotes the proliferation of human periodontal ligament stem cells by upregulating fibroblast growth factor 2", "Mutual inhibition between HDAC9 and miR-17 regulates osteogenesis of human periodontal ligament stem cells in inflammatory conditions", and "Resveratrol enhances the functionality and improves the regeneration of mesenchymal stem cell aggregates". All these studies involve periodontitis patients as a sample and control with the sample from a healthy group. Study design for all the studies included in this systematic review were RCTs and each of the studies used a small number of participants.

Risk of Bias in Studies

Quality assessment was independently performed by two calibrated authors (NZ and WN) using the Cochrane Risk of Bias tool for RCTs studies. Any doubt was resolved by discussion with the supervisor. There were three studies presented with a high risk of bias while only two studies presented with a low risk of bias and the rest of the studies presented with a moderate risk of bias. The risk of bias in this review is summarized in Table 4.

Table 4: Risk of bias assessment for studies included in this systematic review.

	Random sequence generation	Allocation concealment	Blinding of participants & personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Zheng *et al,* 2015	Red	Red	Red	Red	Green	Green	Red
Chen *et al,* 2019	Green	Red	Red	Red	Green	Green	Red
Chen *et al,* 2013	Green	Green	Green	Green	Green	Green	Green
Wang *et al,* 2019	Green	Green	Green	Green	Red	Red	Green
Deng *et al,* 2022	Green	Red	Red	Red	Green	Green	Red
Liu *et al,* 2019	Red	Red	Red	Red	Green	Green	Red
Zhang *et al,* 2020	Red	Red	Red	Red	Green	Green	Red
Li *et al,* 2018	Red	Red	Green	Green	Green	Green	Red
Wang *et al,* 2018	Red	Red	Red	Green	Green	Green	Red

Red: high risk , green: low risk

Results of individual studies

The cellular signaling system within the PDLSCs niche and corresponding functional signaling molecules in healthy and inflamed gingival tissue were analyzed as the population and the function of PDLSC in periodontitis and healthy gingival tissue. Furthermore, the differentiation potency of PDLSC in inflamed conditions was determined.

Out of nine articles, seven documented that non-coding microRNAs (ncRNAs) and long non-coding RNA (lncRNA) played crucial roles in the modification of PDLSC multipotency through lncRNA/miRNA/mRNA/ceRNAs in periodontitis. One article showed in an inflammatory environment histone deacetylases (HDAC) influence the post-translational modifications of histone. Mutual interaction between epi-miRNA, the miR-17 which develops reciprocal signaling with histone regulator HDAC9 demonstrated a novel mechanism to interact with the epi-genome under inflammatory microenvironment. Natural phytoalexin, resveratrol, is outlined to have anti-inflammatory properties and inhibitory effects on the nuclear factor kappa B (NF-kB) pathway, a key inflammatory signaling pathway, and act as a reliable and widespread rejuvenate molecule. Summary of the result of individual studies is summarized in Table 5.

Table 5: Result of individual studies.

Authors/year	Methods	Comment	Predictive preventive/ regenerative model
Zheng *et al*, 2015	hPDLSCs derived from periodontitis-affected and healthy teeth/ cell culture, flow cytometry and RT-qPCR	In periodontitis, the number of PDLSC increases. However, due to certain immunomodulatory components impaired the pluripotential pathway of PDLSCs.	1) This study clearly indicates that certain immunomodulatory cytokines causing the loss of multipotency and regenerative capacity of PDLSCs will be potential interventive molecules for future intervention for periodontitis. 2) PDLSCs for transplant therapeutics regenerative tissue engineering in periodontitis, healthy PDLSCs may retain the regenerative multipotency of periodontium.
Chen *et al*, 2019	hPDLSCs derived from periodontitis-affected and healthy teeth / Cell Counting kit-8 assay	In PDLSCs generated from periodontitis, MALAT1 overexpression greatly increased FGF2 expression.	In periodontitis, MALAT1 promotes the production of FGF2 and the growth of PDLSCs, both of which might be helpful in regenerative treatment.
Chen *et al*, 2013	hPDLSCs. Healthy and inflammatory (moderate or severe chronic periodontitis) / cell culture	Inflammation-related PDLSC differentiation is saved by NF-B inhibitor, which prevents p65 phosphorylation.	In contrast to how NF-B signalling inhibitors can help hPDLSCs recover, inflammation can affect the multipotency and self-renewal of hPDLSCs.
Wang *et al*, 2019	hPDLSCs. Healthy and inflammatory (moderate or severe chronic periodontitis) / cell culture and qRT-PCR	High levels of MORT were strongly associated with a decreased risk of periodontitis recurrence, whereas low levels of MORT expression showed a high rate of periodontitis recurrence. Overexpressed MORT, on the other hand, prevented PDLSCs from proliferating. MORT, a lncRNA, may be a promising therapeutic target.	1) According to this study, lncRNA MORT functions as a predictive biomarker in periodontitis and a therapeutic target to stop the spread of PDLSs in individuals with the condition.
Deng *et al*, 2022	hPDLSCs derived from chronic periodontitis-affected and healthy donor/ qRT-PCR / stability assay / wound healing assay / flow cytometry / ELISA	Circ 0138959 downregulation lessened the effects of LPS-induced osteogenic differentiation and proliferation suppression. PDLSCs are thought to have a pathogenic marker called Circ 0138959.	research found that through controlling the miR-495-3p/TRAF6 axis, circ 0138959 enhanced the pathological development of periodontitis in vitro. Most importantly, decreasing the expression of circ 0138959 may enhance the therapy for periodontitis.

Liu *et al*, 2019	hPDLSCs sample obtained from healthy and periodntitis patient/ bioinformatic differential analysis, cell culture, flow cytometry, Western blot, luciferase reporter gene assay, ALP, ARS	In periodontitis samples, miR27a3p was upregulated whereas lncRNA MEG3 and IGF1 were both downregulated. By increasing IGF1 expression while decreasing miRNA27a3p expression, overexpression of MEG3 enhanced osteogenic differentiation of PDLSC. A biomarker of periodontitis inhibitors was lncRNA MEG3.	1) This work demonstrated that the lncRNA MEG3 enhances the multipotency and differentiation of PDLSCs by sponging miR-27a-3p, which upregulates IGF1.
Zhang *et al*, 2020	hPDLSCs sample obtained from healthy and periodontitis donors. / flow cytometry / exosome isolation / western blot analysis / statistical analysis	Periodontitis' inflammatory microenvironment promoted pro-angiogenesis in PDLSCs through controlling the miR-17-5p-targeted exosome-mediated transfer of VEGFA.	According to this study, the exosome-secreted VEGF is regulated by the miR-17-5p/VEGFA signalling pathway, which is also the regulator of periodontitis. The pro-angiogenesis was suppressed by miR-17-5p overexpression. Overexpression of miR-17-5p inhibited the pro-angiogenic potential of inflamed PDLSCs by reducing the exosome-mediated transfer of VEGFA 3'-UTR. The therapeutic potential of exosomes is important.
Li *et al*, 2018	hPDLSCs from healthy or periodontitis human tissue was compared / cell culture, MTT dye reduction assays, Western blot analysis	In inflammatory circumstances, HDAC9 reduced the ability of PDLSCs to differentiate into osteoblasts. The ability of inflammatory PDLSC to differentiate into osteoblasts was restored to a level comparable to that of healthy PDLSC when HDAC9 was downregulated by HDAC inhibitors or si-HDAC9.	Histone modification is a post-translational process that involves histone deacetylases (HDAC). NaB (Sodium butyrate), an HDAC9 inhibitor, decreased the expression of miR17-92a and restored the differential potential of PDLSCs. As inflammatory-regulated epigenetic mediators, miRNA play important functions.
Wang *et al*, 2018	hPDLSCs from normal control subjects (N-PDLSCs) and periodontitis patients (P-PDLSCs) were investigated/ cell culture,ALP, Micro-computed tomography analysis, qRT-PCR, Immunohistochemistry analysis, Western blot analysis	A natural phytoalexin called resveratrol (RSV) has been shown to enhance cell survival, osteogenesis, and the paracrine action of MSCs through controlling the Sirt1 and AMPK pathways and NF-kB signalling.	RSV can be one of the pharmacological agents that can be naturally obtained from fruits for the future regenerative medicine. Phytochemicals can be the potential regulator to repair impaired regenerative potential of PDLSCs particularly in inflammatory micro-environment and PDLSCs cell aggregate engineering/ Cell sheet technology.

DISCUSSION

Periodontal ligament stem cells (PDLSCs) demonstrate the capacity of self-renewal and multipotency and contribute to the repair of periodontium. However, in an inflammatory microenvironment the differentiation and regenerative potential of human PDLSCs is impaired. Followings are the analysis of nine article extracted from our present systematic review study:

Impairment of multipotency of the PLDSCs:

According to Zheng 2015, In Periodontitis the pluripotential capacity of the PDLSCs is reduced and the impaired ability to regenerate tissues whereas, the proliferation of PDLSCs is increased. This impairment of multipotency of the PLDSCs phenomenon indicated that in an inflammatory micro-environment, certain components not only enhanced the PDLSCs proliferation but also acted to decrease stem cell differentiation ability.

miRNA-mRNA:

Overexpression of miR-17-5p in inflamed PDLSCs reduced the exosome-mediated transfer of VEGFA 3'-UTR thus blocked the pro-angiogenic ability of PDLSCs (Zhang, 2020):

long non-coding RNA (lncRNA MALAT1) and ceRNA:

By the sponging of certain miRNAs, lncRNA MALAT1 may encourage aberrant overexpression of fibroblast growth factor 2 (Chen, 2019).

long non-coding RNA (lncRNA MEG3):

Since lncRNA MEG3 functioned as a ceRNA, or miRNA sponge, it served as an inhibitor of the periodontitis biomarker. The miR-27a-3p as a ceRNA was suppressed by the lncRNA MEG3, which increased IGF1 and activated the PI3k/Akt signaling pathway, amplifying PDLSC osteogenic differentiation in the periodontitis microenvironment (Liu, 2019).

lncRNA MORT-miRNA:

lncRNA MORT and higher cell proliferation rate of PDLSCs in inflammatory micro-environment: High levels of lncRNA MORT rescued abnormal PDLSCs proliferation and low level of lncRNA MORT expression lower recurrence rate of periodontitis after treatment. A recent study demonstrated that lncRNA MORT interacts with miRNA-16 and promotes apoptosis in cancer cells (Wang et al., 2019).

Circular RNAs (circncRNAs miR-495-3p/TRAF6 axis-ceRNAs:

The inflammatory response in periodontitis is mediated by TNF Receptor Associated Factor 6 (TRAF6), and miR-495-3p inhibits this functionality, hence halting the development of periodontitis. The Circ 0138959 overexpressed in the periodontal ligament stem cells during periodontitis and accelerated the pathological development of the disease. It also serves as a miR-495-3p sponge. TRAF6 level was increased by the circ 0138959 and miR-495-3p interaction. Circ_0138959 modulated disease progression by targeting miRNA/mRNA axis. (Deng, 2022).

Epigenetic modulation by periodontitis micro-environment:

Histone deacetylases (HDAC) modify histone core as well as non-histone targets post-translationally. HDAC9 inhibitor NaB (sodium butyrate) downregulated the miR17-92a and preserved the multipotency of

PDLSCs through promoting osteogenesis. As the mediators of inflammatory-regulated epigenetic changes, miRNA plays important functions (Liu et al., 2018).

Nuclear factor-kB (NF-kB) in inflammatory microenvironment:

An inhibitor of GSK-3b that regulates β-catenin and NF-kB signaling and PI3K inhibitor inhibit p65 phosphorylation and interferes with p65 nuclear translocation and restores defective osteogenic differentiation from periodontitis (Chen et al., 2013).

Phytochemicals and Regulation of NF-kB:

Resveratrol, a natural phytoalexin, has been shown to have significant rejuvenative effects, particularly in stem cells, and is gaining therapeutic interest. Also, a strong inhibitor of NF-kB, the master regulator of the inflammatory signaling pathway. Cell aggregate engineering (Cell sheet technology) is a promising autologous MSCs strategy that exhibits facile harvesting and lacks immune rejection while improving functioning and facilitating MSC regeneration. The capability of PDLSCs in forming cell aggregates is not promising in an inflammatory micro-environment, therefore, demonstrating weaker osteogenic and regenerative abilities. Phytochemicals may be the promising approach to repair the diminish regenerative potential of PDLSCs, particularly in inflammatory micro-environment (e.g., cell aggregate engineering) (Wang, 2018).

Around 70% of RNA transcription in the human genome is not involved in protein translation; yet, it circulates throughout the body and plays critical roles in physiological and pathological processes by negatively influencing protein expression. These are non-coding RNAs length is between 200 bp to 10 kb, binds to complementary sequences in the coding or 3' untranslated region of target messenger RNAs, thereby involved in the regulation of a variety of gene expression, growth, differentiation, and development, carcinogenesis, rheumatoid arthritis (RA), and inflammation. Non-coding RNAs (ncRNAs) control the expression of their target genes by either blocking translation or degrading transcribed mRNAs. RNA that contains specific miRNA response elements, can be targeted and regulated by a single RNAs. These RNAs can be controlled by various mRNA.

A competitive endogenous RNA (ceRNA) construction can be established by a complex exit mechanism that allows miRNAs to compete with each other for shared miRNA-response elements in the target messenger RNAs. This competitive interaction between ncRNAs-miRNA establishes a cross-talk with target mRNA for the functional balance of gene networks; destabilization of these regulatory interactions may lead to pathological changes.

CeRNA regulate gene expression at the post-transcriptional level by sponging ncRNAs that inhibit the target messenger RNA (mRNA) from being translated. CeRNA is regarded as diagnostic and prognosis markers or therapeutic targets (Tang et al., 2022; Mehta et al., 2021).

Our present systematic review clearly demonstrated that (i) interaction between non-coding RNAs and their target messenger RNAs (mRNAs) played critical roles in regulating PDLSCs in the inflammatory micro-environment. (ii) competitive endogenous RNA (ceRNA) which means miRNA sponges or antagomirs also involved in the above regulatory ncRNAs/miRNAs/mRNAs system, mapping ceRNAs and constructing ceRNAs for impaired PDLSCs may be the promising approach (Gao et al., 2021; Zhou et al., 2019; Santonocito et al., 2021) (iii) Inflammatory immunomodulators (miRNAs) that involved in the regulation of key epigenetic signaling system (histone modifications or DNA methylation) could be the potential target for PDLSCs repair and regenerative pathways (Zhang et al., 2022) (iii) Phytochemicals and exosomes may be the promising approach to repair impaired regenerative potential of PDLSCs, particularly in inflammatory micro-environment (e.g., cell aggregate engineering) (Laurindo et al., 2023; Zhang et al., 2022).

Strength and limitations

Our study has several strengths. We used 4 databases (PubMed, Web of Science, Google Scholar and Cochrane) to identify as many relevant publications as we can according to the keywords used. All the searching, quality assessment and data extraction were performed by two independent assessors. In order to eliminate the bias in the study, the selected study that met all the inclusion criteria was appraised using the Cochrane Risk of Bias Assessment. The aim of this study was to know the local immunomodulation in periodontitis patients, hence only studies published using samples from periodontitis patients were used.

We came across certain limitations in this study. We found quite a number of good and high-quality papers during the abstract screening, however, we needed to subscribe and pay a certain amount of money in order to have full access to the paper. The next limitation is the language barrier, in which all the studies chosen for this systematic review are restricted only to the English language.

Another limitation that is important to take note of is selection bias because of the small number of participants involved in the included study.

Implications

Public awareness of periodontitis is increasing day by day due to its prominent signs and symptoms such as recession and tooth mobility. Although painless, periodontitis is a serious condition to take note of as it may lead to tooth loss if left untreated. With this systematic review, our results can also serve as research guidelines for immunomodulation molecule identification in periodontitis patients. Most importantly, this study can contribute knowledge about functional molecules of local immunomodulation signaling systems for future study on periodontal engineering.

CONCLUSION

The vulnerability of PDLSCs is triggered by the genetic and epigenetic factors (diabetes, smoking) notably, an increase in changing expression profiles of ncRNAs strongly indicating the competitive role among circRNAs/lncRNAs significantly critical PDLSCs pathogenesis. Thus ceRNA Networks study is a hotspot research area in periodontitis (Ye et al., 2021). The results of our present systematic review demonstrate that non-coding microRNAs (ncRNAs) signaling systems specifically ceRNAs, natural products derived anti-inflammatory functional molecules and exosome (Zhang et al., 2022) as well as epigenetic regulatory molecules such as epi-miRNAs could be the potential clinical importance in novel periodontitis treatments as well as preventive, diagnostic, therapeutic and prognostic markers.

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COMPETING INTEREST

The authors declared no conflicts of interest with respect to the research, authorship of this article.

AVAILABILITY OF DATA, CODE AND OTHER MATERIALS

Appendix 1. PRISMA 2020 Abstract Checklist; Appendix 2. PRISMA 2020 Checklist; Appendix 3. List of excluded studies in the systematic review along with the reason for exclusion.

Appendix 1: PRISMA abstract checklist

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	YES
		BACKGROUND	
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	YES
		METHODS	
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	YES
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	YES
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	YES
Synthesis of results	6	Specify the methods used to present and synthesise results.	
		RESULTS	
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	YES
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	YES
		DISCUSSION	
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	NA
Interpretation	10	Provide a general interpretation of the results and important implications.	NA
		OTHER	
Funding	11	Specify the primary source of funding for the review.	NA
Registration	12	Provide the register name and registration number.	NA

Appendix 2: PRISMA checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	YES
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	YES
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	YES
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	YES
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	YES
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	YES
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	YES
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	YES
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	YES
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	YES
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	YES
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	YES
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	NA
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention	NA

Section and Topic	Item #	Checklist item	Location where item is reported
		characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	NA
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	NA
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	NA
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	NA
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	NA
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	NA
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	NA
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	YES
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	YES
Study characteristics	17	Cite each included study and present its characteristics.	YES
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	YES
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	YES
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	NA
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	NA
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	NA

Section and Topic	Item #	Checklist item	Location where item is reported
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	NA
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	YES
	23b	Discuss any limitations of the evidence included in the review.	YES
	23c	Discuss any limitations of the review processes used.	YES
	23d	Discuss implications of the results for practice, policy, and future research.	YES
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	NA
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	NA
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NA
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	YES
Competing interests	26	Declare any competing interests of review authors.	YES
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	NA

Appendix 3: List of excluded studies in the systematic review, along with the reason for exclusion.

Num.	Title	Authors/Year	Summary comment for exclusion
1	The effect of aging on the pluripotential capacity and regenerative potential of human periodontal ligament stem cells	Zhang, 2012	Subscription with payment
2	High-power, red-light-emitting diode irradiation enhances proliferation, osteogenic differentiation, and mineralization of human periodontal ligament stem cells via ERK signaling pathway	Yamauchi, 2018	Donors are not from periodontitis case
3	Decellularized matrix could affect the proliferation and differentiation of periodontal ligament stem cells in vitro	Huang, 2021	Donors are not from periodontitis case
4	The roles of calcium-sensing receptor and calcium channel in osteogenic differentiation of undifferentiated periodontal ligament cells	Koori, 2014	Subscription with payment

5	Effects of lysophosphatidic acid on human periodontal ligament stem cells from teeth extracted from dental patients	Chul, 2019	Donors are not from periodontitis case
6	IL-33 guides osteogenesis and increases proliferation and pluripotency marker expression in dental stem cells .	Kukolj, 2018	Donors are not from periodontitis case
7	Lactate inhibits osteogenic differentiation of human periodontal ligament stem cells via autophagy through the MCT1-mTOR signaling pathway.	Luo, 2022	Subscription with payment
8	Zanthoxylum schinifolium enhances the osteogenic potential of periodontal ligament stem cells	Kim, 2014	Subscription with payment
9	Priming integrin alpha 5 promotes the osteogenic differentiation of human periodontal ligament stem cells due to cytoskeleton and cell cycle changes	Wang, 2018	Subscription with payment
10	Regeneration of periodontal tissues using allogeneic periodontal ligament stem cells in an ovine model	Mrozik, 2013	Subscription with payment

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