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Systematic Review

Application of Periodontal Ligament Stem Cells in Periodontal Regeneration: A Systematic Review

Fatemeh Isamorad [1], Farnaz Kouhestani [2*], Pardis Aghandeh [1], Saeed Reza Motamedian [3]

[1] School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Periodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
[3] Dentofacial Deformities Research Center, Research Institute of Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding Author: Farnaz Kouhestani, Assistant Professor, School of Dentistry, Tehran University of Medical Sciences, North Karegar St, Tehran, Iran. Tel: +98-9128199635, E-mail: dr.f.kouhestani@gmail.com

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Abstract

Introduction: The present study systematically reviews the studies on the application and effect of periodontal ligament stem cells (PDLSCs) in regeneration of periodontal defect in animal and human models.

Materials and Methods: The databases of PubMed, Scopus, Cochrane Library, Embase, and ProQuest, as well as the Web of Science were searched for relevant published articles in English without time limit until August 2022. The eligibility criteria were the studies focusing on the use and effect of PDLSCs on periodontal regeneration. Exclusion criteria included the articles which did not report the extent of periodontal regeneration, and those failing to provide flow cytometry and differentiation tests to prove the stemness of PDLSCs.

Results: In this review, 27 animal and 4 human studies meet the eligibility criteria. Human samples had periodontal disease, and various surgical periodontal defects had been created in the animals (mouse, rat, rabbit, dog, and sheep). Meta-analysis was not possible due to heterogeneity in study designs. Based on the literature review, there was inconsistency in the limited number of conducted human research. However, cementum, PDL, and bone regeneration increased in animal models in the PDLSCs group compared to the control ones. No complication in PDLSCs groups was reported.

Conclusions: The application of PDLSCs as one of tissue engineering components can impose beneficial effects on periodontal tissue regeneration in animal models. However, more human studies with higher quality are required to assess clinical efficacy of this method.

Keywords: Periodontal Diseases, Periodontal Regeneration, Periodontal Ligament, Stem Cells, Systematic Review, Tissue Engineering

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Introduction

Periodontitis is considered as a multifactorial disease, which is caused primarily by dental plaque microorganisms and characterized by periodontal destruction. Ultimately, the inflammation and destruction of periodontal tissues lead to periodontal defect. Additionally, the periodontitis can result in losing tooth in the case of inadequate treatment.[1] The chronic periodontitis has been reported in 48% of adults[2] and advanced periodontitis is more common among older age groups.[2,3] The high prevalence, significant burden, and effect on public health, as well as the quality of life necessitate the more definitive treatments of the disease.[4]

The periodontal therapy ultimately aims at stopping the disease progression and regenerating the lost components of periodontium to their original form and function.[5] The results of many studies have indicated that surgical or non-surgical treatment can stop and cure periodontal disease. This disease is mainly treated through compliance with oral health guidelines and scaling. Further, corrective or reconstructive surgeries may be considered when there are residual pockets after initial non-surgical therapy. These treatments can effectively control inflammation and prevent further disease progression although complete and functional periodontal regeneration is still a clinical challenge.[6,7]

The American academy of periodontology (AAP) defined periodontal regeneration as the formation of a new cementum, alveolar bone, and a functional periodontal ligament (PDL) on the root surface.[8] Accordingly, several methods are applied to achieve periodontal regeneration, some of which are guided tissue regeneration[9] and bone grafting,[10] as well as the use of enamel matrix derivatives[11] and growth factors,[6] or a combination of the above-mentioned techniques. The therapies usually lead to clinical improvement by decreasing probing depth (PD) and clinical attachment level (CAL). However, current therapeutic strategies fail to cause complete and reliable regeneration in all of the tissues and connections injured in severe periodontitis.[5]

The recent advances in tissue engineering and regenerative medicine have enabled the development of new treatments

by using cell-based therapies.[4] Furthermore, stem cells refer to the undifferentiated cells which can proliferate and differentiate into more specialized cells by stimulating with internal and external signals.[5] The utilization of mesenchymal stem cells (MSCs) is among the cell-based approaches for periodontal regeneration. They can be derived from a wide variety of tissues such as bone marrow, adipose, umbilical cord, lungs, or dental tissues.[12] The different types of stem cells like dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), apical papilla stem cells (SCAP) or gingival MSCs (GMSCs) are extracted from teeth. They can be directly injected into periodontal defect as a suspension or by biomaterial scaffolds.[5]

Multipotential stem cells, which were first derived from PDL by Seo et al. in 2004, exhibit the properties similar to bone marrow stem cells, and can differentiate into osteoblast, chondrocyte, and adipose cells under certain conditions.[13] The PDLSCs can generate cementum and PDL structures during transplanting into periodontal defect site in animals, and represent a potential use for periodontal tissue regeneration.[14,15]

Based on the clinical case reports, the MSCs can be successfully used to treat periodontal defects.[16,17] Despite numerous published studies in animal and human models, the effectiveness of MSCs and biomaterials for periodontal regeneration remains controversial.[18]

Thus, the present study aims to provide desired evidence for the evidence-based clinical decision making through the systematic review of the human and animal studies highlighting the application and effect of PDLSCs in periodontal defect treatment and periodontal regeneration.

Materials and Methods

Protocol and Registration

The present systematic review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.[19]

Eligibility Criteria

This review was conducted to answer "Does the periodontal defect treatment using PDLSCs enhance periodontal regeneration in human and animal samples compared to the control groups"?

According to the patient, intervention, comparison, and outcome (PICO) principles,[20] the components are as follows:

P: Animals without systemic disease, adult patients having periodontal disease without systemic disease

I: PDLSC therapy with/without carrier, scaffold, or membrane

C: Treatments other than the utilization of stem cells or those applying the stem cells extracted from other sources

O: Extent of periodontal regeneration (e.g., bone, cementum, or PDL regeneration) based on the clinical, histological or radiological assessment

S: All types of English studies like preclinical animal trials, randomized clinical trials (RCT), cohort, case-control, case report, and case series

Given the exclusion criteria, narrative reviews, commentaries, opinions, conferences, letters, *in vitro* studies, and articles not focusing on the amount of periodontal regeneration were removed from the present review. In addition, the articles applying stem cells from sources other than PDL and those not reporting flow cytometry and differentiation tests to demonstrate the stemness of PDLSCs were not considered.

Information Sources

The search was performed in English in the databases of PubMed, Scopus, Cochrane Library, Embase, and Web of Science for relevant published articles, as well as in ProQuest for theses and Gray Literature without time limit until August 2022.

Table 1. Search Strategy

Pubmed, Scopus, Web of Science, Cochrane library, Proquest	(Periodont* OR "Periodontium"[Mesh] OR "Alveolar Process"[Mesh] OR "Dental Cementum"[Mesh] OR "Gingiva"[Mesh] OR "Periodontal Ligament"[Mesh] OR "Epithelial Attachment"[Mesh]) AND ((("Periodontal Ligament"[Mesh] OR periodont*) AND "Stem Cells"[Mesh]) OR "Periodontal ligament stem cells" OR "Periodontal stem cells" OR "Periodontal ligament-derived stem cells" OR "PDL stem cells" OR "PDLSCs")
Embase	(Periodont*:ti,ab,kw OR 'Periodontium':ti,ab,kw OR 'Alveolar Process':ti,ab,kw OR 'Dental Cementum':ti,ab,kw OR 'Gingiva':ti,ab,kw OR 'Periodontal Ligament':ti,ab,kw OR 'Epithelial Attachment':ti,ab,kw) AND ((('Periodontal Ligament':ti,ab,kw OR periodont*:ti,ab,kw) AND 'Stem Cells':ti,ab,kw) OR 'Periodontal ligament stem cells':ti,ab,kw OR 'Periodontal stem cells':ti,ab,kw OR 'Periodontal ligament-derived stem cells':ti,ab,kw OR 'PDL stem cells':ti,ab,kw OR 'PDLSCs':ti,ab,kw)

Search Strategy

The different combinations of the keywords taken from MeSH and free text (for the cases in which MeSH is not defined) were applied for search according to the guidelines of each database. Table 1 outlines the obtained keywords.

Study Selection

The articles were independently selected by two researchers. After searching the studies, their abstracts were entered into Endnote software, and duplicates were removed, followed by initial screening based on their title and abstract. Then, the full text of appropriate articles was carefully evaluated based on the eligibility and exclusion criteria, and the final ones were obtained. Any disagreement between the two individuals was resolved through discussion with a third one.

Data Collection and Data Items

Considering the objectives, two researchers independently

Table 2. Comparative Table of Human Studies

Study	Study type	Number of cells transplanted	Samples (sex/age/ number)	Defects (type/ location/size)	Treatment groups	Observation period	Parametrs	Outcome	Complications
Chen FM et al 2016[23]	Randomized clinical trial	-	F, M 18 to 65 26.05±4.44-30.04±7.90 years 41	Intrabony defect 3 mm in depth	A) Autologous PDLSCs+ Bio-oss® B) Bio-oss®	3, 6 and 12 months	Clinical: CAL, PD, GR, blood tests Radiological: dental X-ray	Bone fill: in both groups Bone-defect depth: A=B CAL: A=B PD: A=B GR: A=B	Swelling and pain
Shalini HS et al 2018[25]	Randomized controlled trial	-	F, M 30–50 years 28	Intrabony defect	A) Autologous PDLSCs+ Abgel (gelatin sponge) + OFD B) OFD	3, 6, 9, and 12 months	Clinical: CAL, PD, GR, PI, BOP, GBI, GMP, GT Radiological: dental X-ray	GBI: A<B PPD: A<B CAL gain: A>B CEJ-ABC: A>B Bone density:A>B	-
Sánchez N et al 2020[24]	A 12-month quasi-randomized controlled pilot clinical trial	1 x 10^7	F, M XBS + PDL-MSCs: 38-60 years (mean=48.8) XBS + saline: 49-65 years (mean=57.5) 20	Intrabony defect Incisors, canines, premolars and molars	A) Autologous PDLSC+ XBS B) XBS + saline	12 months	Clinical: FMPI, CAL, PPD, GR Radiological: dental X-ray	FMPI: A=B CAL: A=B PPD: A=B REC: A=B	Mild-moderate pain and swelling and mild dentine hypersensitivity
Vanada K et al 2017[26]	case report	-	M 28 years 1	Grade II furcation Distal of mandibular left first molar to distal of mandibular left second molar 9 mm in depth	A) Autologous PDLSC+Abgel® B) OFD	6, 12 months	Clinical: CAL, PD, GR, GMP, GT, relative attachment Radiological: dental X-ray	Furcation involvement: A<B PD: A<B CAL: A<B GMP: A>B GT: A>B Bone regeneration: new in group A	-

BOP, bleeding on probing; CAL, clinical attachment level; CEJ-ABC, cementoenamel junction to alveolar bone crest; FMPI, full-mouth plaque index; GBI, gingival bleeding index; GMP, gingival marginal position; GR, gingival recession; GT, gingival thickness; OFD, open flap debridement; PD, probing depth; PDLSCs, periodontal ligament stem cells; PI, plaque index; PPD, probing pocket depth; REC, Recession of the gingival margin; XBS, xenogeneic bone substitute.

Table 3. Comparative Table of Pre-clinical Animal Studies

Study	Number of cells transplanted	Samples (sex/age/number)	Defects (type/location/size)	Treatment groups	Observation period	Parameters	Outcome	Complications
Duan XJ et al 2018[27]	-	Nude mice M 58 weeks 25-30 g 25	Fenestration defect Bilaterally at the lower border of the mandible first molar 4x3x2	A) Allogeneic PDLSCs + PRF membrane B) Allogeneic PDLSCs + collagen membrane C) PRF membrane D) Collagen membrane E) Blank	Day 12 and 24	Histological: HES, Azan staining Radiological: micro-CT	New bone area: A>B>C>D Cementum regeneration: a thin layer in group A and little new cementum in group B PDL regeneration: disordered and not perpendicular to the root surface in group A and new PDL fibres were sparse and loose without periodontal fibre bundle formation	-
Wen Y et al 2012[43]	5 x 10^5	SD rats 6	Fenestration defect Mandibular first molar 1x3	A) hPDLSCs+ eGFP+ collagen B) hPDLSCs+ collagen C) None cells	6 weeks	Histological: HES Histomorphometric IHC	Bone,cementum, PDL regeneration: new in all groups but less in group C	-
Han J et al	1 x 10^6	SD rat	Fenestration defect		1, 2, and 4	Histological: HES,	New bone area: A>B=C	No

2014[29]		F Adult 220-250 g 36	Buccal and distal roots of the first molar and the buccal root of the second molar 2x3	A) Allogeneic PDLSCs+ Gelfoam B) Gelfoam alone C) Untreated	weeks	modified tetrachrome Histomorphometric IHC:BrdU	New bone length: A>B=C New cementum length: A>C	
Nagata M et al 2017[31]	1 x 10^5	SD rat M 8 weeks 50	Intrabony defect Mandibular first molar roots to second molar mesial root 3x2	A) Allogeneic PDLSCs-CM-high+ collagen+ fibrin glue B) Allogeneic PDLSCs-CM-moderate+ collagen+ fibrin glue C) Allogeneic PDLSCs-CM-low+ collagen+ fibrin glue D) Fibroblast-CM+ collagen+ fibrin glue E) Control-CM	4 weeks	Histological: HES and azan staining Radiological: micro-CT	Alveolar bone level:higher in groups A and B than groups C and D Exposed root area: A>B>C=E tissue regeneration: A>B>C=D=E Bone regeneration: in groups A,B Fibrous connective tissues: in groups C,D and E Collagen bundles: new in all groups	No
Sun J et al 2017[49]	1 x 10^6	SD rat M 180 ± 15 g 15	Intrabony defect Roots of the mandibular molars 3x2	A) Healthy-hPDLSCs+DMSO B) Periodontitis-hPDLSCs+ DMSO C) Periodontitis-hPDLSCs+ Osthole D) No defects and defects without anything	4 weeks	Radiological: micro-CT	Bone regeneration: A=C>B	-
Wang YJ et al 2018[50]	1 x 10^6	SD rats F 8 weeks 200-220 g 8	intrabony defect Mandibular first and second molars 1mm apical of the alveolar bone crest 2x1 2.5x1.5	A) Normal-hPDLSCs+ CBB B) Peridontitis-hPDLSCs+ CBB C) RSV-treated hPDLSCs+ CBB D) CBB transplantation alone	4 weeks	Histological: HES, Masson's trichrome Radiological: micro-CT IHC, (qRT-PCR), Western blot	Defect area: A<C<B=D BV/TV:A>C>B=D Bone area: A>C>B=D Collagen matrix area: A>C>B=D	-
Iwasaki K et al 2019[51]	5 x 10^5	Athymic nude rats M 7-8 weeks 22	Intrabony defect Mesial root of the mandibular first molar to the mesial root of the second molar 3x2	A) hPDLSCs+ amnion B) Amnion	4 weeks	Histological: HES, Azan staining Radiological: micro-CT	Bone regeneration: A>B Exposed root area: A>B Cementum-like tissue regeneration: new in group A Sharpey's fibers: new in group A	-
Liu JY et al 2019[52]	5 x 10^6	SD rats 7 weeks 42	Intrabony defect Roots of first and second molars 5x1.5x0.8	A) Allogeneic PDLSCs+ collagen membrane B) Collagen membrane only C) Blank (defects only)	3 weeks	Histological: HES, Masson's trichrome Radiological: micro-CT IHC	Bone regeneration: A>B>C BV/TV>: A>B=C TbTh: A>B=C TbN:A=B=C Bone density: A=B=C Cementum-like regeneration: more in group A Collagen fiber regeneration: more in group A	-
Qiu J et al 2020[28]	-	Wistar rats M 6-7 weeks 200-230 g 90	Intrabony defect Mesial root of the first mandibular molar to the mesial root of the second mandibular molar 3x2x1	A) hPDLSCs-CM group+ collagen membrane B)GMSCs-CM group+ collagen membrane C) GF-CM group+ collagen membrane D) Control group	1, 2, and 4 weeks	Histological: HES, Masson's trichrome IHC	Collagen membrane: clearly visible New bone formation: A=B>C=D New cementum formation: A=B>C>D Connective tissue: more organized in groups A and B, less orderly in other groups Height of new bone:A=B>C>D New bone area:A=B>C>D	No
Liu YS et al	1 x 10^6	SD rats	Intrabony defect	A) hPDLSCs	12 weeks	Clinical: GM-CEJ, PD,	GM-CEJ: A=B	-

2021[53]	cells/ml	4 weeks 200-300 g 56	maxillary periodontal tissue	B) experimental periodontitis model C) blank		SBI, GI Histological: HES Radiological: micro-CT	PD: A=B SBI: A=B GI: A=B vertical bone regeneration: A>B	
Lei FZ et al 2022[54]	1 x 10^6 cells/ml	SD rats M 103-120 g 36	Intrabony defect buccal alveolar bone of the right mandibular first molar 3x1.5x2	A) hPDLSCs+ β-TCP B) hPDLSCs+ matigel C) β-TCP D) Matigel E) Untreated	8 weeks	Histological: HES, Masson staining Radiological: micro-CT	Bone formation: A>B>C>D>E BV/TV: A>B>C>D=E	-
Iwasaki K et al 2014[30]	-	Nude rat M 7 weeks 6	Bilateral class II furcation defect Palatal aspect of the maxillary first molars 1.5wx2d	A) hPDLSCs+ amnion B) Amnion	4 weeks	Histological: HES and azan staining Radiological: micro-CT	Bone filling: A>B Cementum thickness: A>B Sharpey's fibers regeneration: new in group A	No
Sano K et al 2020[55]	4 x 10^5 2 x 10^3	SD rats 7 weeks 40	Furcation defect Bilateral maxillary first molars mesial furcation 1wx1.3d	A) hPDLSCs spheroids+ Matrigel B) Co-cultured spheroids with hPDLSCs: HUVECs at a ratio of 1:1+matrigel C) Co-cultured spheroids with hPDLMSCs: HUVECs at a ratio of 1:2+ matrigel D) Co-cultured spheroids with hPDLMSCs: HUVECs at a ratio of 2:1+ matrigel E) Untreated	4, 8 weeks	Histological: HES, Azan staining Radiological: micro-CT, X-Ray	Bone fill:A=B=C=D>E BV/TV: A=B=C=D>E New bone area: A=B=C=D>E New cementum: A=C>B>D Sharpey's fiber: new in groups A to D	-
Su F et al 2015[32]	5 x 10^6	New Zealand white rabbits M 2.0-2.5 kg 20	Alveolar bone defect Left alveolar bone of incisors 5x10x4	A) hOPG- Autologous PDLSCs+ β-TCP+ gelatin membrane B) Autologous PDLSCs+ β-TCP+ gelatin membrane C) β-TCP+ gelatin membrane D) No treatment	12 weeks	Histological: Toluidine blue staining Laser confocal microscope, Histomorphometric	New bone area: B>A>C>D	No
Liu Y et al 2008[14]	2 x 10^7	Miniature pig 12 months 30-40 kg 14	Alveolar bone defect Mesial region of the maxilla and mandibular first molars 3x7x5	A) Autologous PDLSCs+ HA/TCP+ gelatin membrane B) HA/TCP+ gelatin membrane C) No treatment	4,12 weeks	Clinical: CAL, PI, PD, GR, BOP Histological: HES, GFP Radiological: CT	PD: A<B<C GR: A<B<C AL: A<B<C Length of bone regeneration: A>B>C Cementum and PDL regeneration: new in group A	-
Ding G et al 2010[56]	2 x 10^6	Mini pig F 6-8 months 30-40 kg 15	Alveolar bone defect Mesial region of the maxilla and mandibular first molars 3x7x5	A) Autologous PDLSCs + HA/TCP+ gelatin membrane B) Allogeneic Guizhou minipig PDLSCs+ HA/TCP+ gelatin membrane C) Autologous heterogenic minipig PDLC + HA/TCP + gelatin membrane D) HA/TCP + gelatin membrane E) Initial periodontal therapy only	12 weeks	Clinical: CAL, PD, GR, blood and biochemical tests Histological: HES Radiological: CT Histomorphometric	PD: A<B<C<E<D GR: A=B<C<E<D AL: A=B<C<E<D Bone, cementum, PDL regeneration: new in groups A and B %of bone in periodontium: A=B>C>D=E	-
Liu OS et al	4 x 10^6	Miniature pig	Alveolar bone defect	A) Autologous pig PDLSCs+	12 weeks	Clinical: CAL, PD, GR,	PD: A=B<C<D	No

2013[57]		F 6-8 months 10-19 kg 12	Mesial region of the maxilla and mandibular first molars 3x7x5	HA/TCP scaffolds B) Allogeneic pig PDLSCs+ HA/TCP scaffolds C) HA/TCP D) Control group		blood and biochemical tests Histological: HES Radiological: Intraoral photographs, CT, X-Ray	GR:A=B<C<D AL: A=B<C<D Periodontal regeneration: partially in groups C and D, significantly more in groups A and B	
Zhu B et al 2017[34]	1x 10^5	Miniature pig 2 years 5	Intrabony defect Maxillary and mandibular jaw bones;where the canine tooth had been extracted three months previously 5.2x5	A) Autologous PDLSCs+ jBMMSC+ TDM B) Autologous PDLSCs+ iBMMSC+ autologous PDLSC+ TDM	12 weeks	Histological: HES, Masson's trichrome IHC: Col 1	Bone-like regeneration: A>B PDL-like regeneration: A>B	-
Fu X et al 2014[33]	1 x 10^6	Miniature pig F 9-12 months 40-45 kg 6	Intrabony defect Mandibular first molars (mesial proximal side, buccal side and furcation erea) 5x7x7	A) Allogeneic PDLSCs+ HA/TCP B) Allogeneic SHED+ HA/TCP C) HA/TCP	12 weeks	Clinical: CAL, PD, GR Histological: HES Radiological: CT	PD: A=B<C GR:A=B<C AL: A=B<C Periodontal tissue regeneration: A=B>C Bone regeneration: A=B>C Furcation regeneration: A=B>C Cementum and PDL regeneration: new in groups A and B HAB-CEJ: A=B>C	-
Basan T et al 2017[58]	10^6	Guttinger minipigs 22 ± 3 months 35 ± 11 kg 15	Class II furcation defect 3rd premolar and 1st molar in both quadrants of the mandible	A) Autologous PDLSCs+ collagen powder+ semipermeable membrane B) Collagen powder+ semipermeable membrane C)Blank	Day 14, 28, 84 and 120	Histological: toluidine blue	New attachment: A=B>C	-
Park J et al 2011[36]	6 x 10^6	Beagle dogs 10 months 10 kg 8	Circumferential defect 2nd and 4th mandibular premolars 3 mm wide	A) Autologous PDLSCs graft B) Autologous DPSCs graft C) Autologous PAFSCs group D) Periodontal defect but no stem cell graft	8 weeks	Clinical: AL, PD, GR Histological: HES, Masson's trichrome Radiological: micro-CT	PD:E<A<C<B<D GR:E<A=B=C<D AL:E<A<C<B<D BV:A>C>B>D TV: A<B=C<D BV/TV: A>C>B>D Bone, cementum, Sharpey's fibers regeneration: new in group A and C Heigh of new bone: A>C>B>D	No
Wang L et al 2011[59]	2 x 10^3	Beagle dog 20 months 6	Alveolar bone defect Mesial and buccal region of maxillary canine 9x13	A) Autologous alveolar bone surface-PDLSCs+ fibrin gel B) Autologous root surface-PDLSCs+ fibrin gel C) Fibrin gel alone	10 weeks	Radiological: CT	Bone regeneration:A>B	-
Nunez J et al 2012[37]	7.5x 10^5	Beagle dog M 1year 10 kg 4	Bilateral 3-wall intrabony defect Mesial aspect of the second premolar to the first molar	A) Autologous PDLSCs+ collagen B) CDCs+ collagen C) Collagen	12 weeks	Histological: Toluidine blue staining Histomorphometric	GM-JE: no significant differences between the groups CEJ-JE:A>B=C JE-CC: no significant differences between the groups	No

			3-4 x 3-4 x 3-4				Length of new bone:A>B=C Length of new cementum:A>B>C	
Tsumanuma Y et al 2016[35]	4 x 10^4	Beagle dogs M 1-2 year 10 kg 8	Bilateral critical-size supra-alveolar defects Mandibular third and fourth premolars 5x5	A) Autologous PDLSCs+ PGA+ β-TCP+ collagen+ absorbable GTR membrane B) Allogenic PDLSCs + PGA+ β-TCP + collagen+ absorbable GTR membrane C) β-TCP+collagen+ absorbable GTR membrane	8 weeks	Histological: azan staining Radiological: micro-CT Histomorphometric, Enzyme-linked, immunosorbent assay: CRP, IL-10, IFN-c, CD30	Bone regeneration: A=B=C Cementum regeneration:A=B>C PDL score and ankylosis: A=B=C Collagen fibers: dense and perpendicular in group B, oblique or parallel in groups A and C	No
Shi H et al 2018[60]	2 x 10^7	Beagle dogs M 2 years 16.7 ± 2.1 kg 6	Dehiscences defect Buccal aspect of bilateral lower second premolars 3x5	A) hPDLSCs+ BCP+ Biog-Gide B) OFD	4, 8 and 12 weeks	Histological: Van-Geision staining, Masson staining Radiological: micro-CT Fluorescence labeling	Bone, cementum, PDL regeneration: new in group A epithelium and connective tissue: new in group B BV,TV, BV/TV, TbN, TbTh: enhanced in group A	-
Mrozik K et al 2013[38]	1 x 10^7	Merino ewes 3-5 years 63.5-72.0 kg 13	Rectangular zero-wall dehiscence defect Buccal plate exposing both the mesial and distal roots of the second premolar 10mm in depth	A) Allogeneic PDLSCs + Gelfoam+resorbable barrier membrane B) Gelfoam alone + resorbable barrier membrane C) Unfilled	4 weeks	Histological: HES, modified tetrachrome Histomorphometric	Mean new bone area: A=B>C Mean new bone maximum height:A=B>C Mean new cementum length:A>B>C Mean cementum regrowth: A>B>C Mean new Sharpey's fiber thickness: A=B>C Mean length of new Sharpey's fiber attachment:A>B>C	No
Menicanin D et al 2014[39]	2 x 10^6	Sheep F 7	Intrabony defect bilateral mandibular first premolar 5 mm in depth	A) Autologous PDLSCs+ Gelfoam scaffold+ Gore-Tex membrane B)Gelfoam scaffold alone+ Gore-Tex membrane	8 weeks	Histological: HES, modified tetrachrome histomorphometri, IHC: OPN, BSP, COL-I, αSMA, BrdU	Bone,cementum, PDL regeneration: new in group A	-

ABC, alveolar bone crest; Al, attachment loss; αSMA, alpha smooth muscle actin; BOP, bleeding on probing; BrdU, bromo-deoxyuridine; BSP, bone sialoprotein; β-TCP, β-tricalcium phosphate; BV, bone volume; CAL, clinical attachment level; CBB, calcined bovine bone; CC, coronal cementum; CD, cluster of differentiation; CDC, cementum derived cells; CEJ, cementoenamel junction; CM, conditioned medium; Col, collagen; CRP, serum c-reactive protein; CT, computed tomography; DMSO, dimethyl sulfoxide; DPSC, dental pulp stem cells; eGFP; enhanced green fluorescent protein GF,gingival fibroblasts; GM, gingival margin; GMSC, gingival margin stem cell; GR, gingival recession; GT, gingival thickness; GTR, guided tissue regeneration; HA/TCP, hydroxyapatite/tricalcium phosphate; HAB, height of the alveolar bone; HES, hematoxylin eosin stain; hOPG, human osteoprotegerin; hPDLSC, human periodontal ligament stem cells; HUVEC, human umbilical vein endothelial cell; IHC, Immunohistochemical; iJBMMSC iliac-derived bone marrow mesenchymal stem cell; IL-10, interleukin-10; INF-c, interferon-c; JBMMSC, jaw-derived bone marrow mesenchymal stem cell; JE, junctional epithelium; OFD, open flap debridement; OPN, osteopontin; PD, probing depth; PDL, periodontal ligament; PDLSCs, PDL stem cells; PAFSC, periapical follicular stem cell; PI, plaque index; PRF, platelet-rich fibrin; RSV, Resveratrol; SBI, Sulcus bleeding index; SD, Sprague–Dawley; SHED, stem cells from human exfoliated deciduous teeth; Tb.N, trabecular number; Tb.Th, trabecular thickness; TDM, treated dentine matrix; TV, total volume.

extracted the desired information from the included studies and entered it into the tables, any disagreement between whom was overcome by discussing with a third individual.

Regarding each study, the demographic characteristics of samples (type, number, sex, and mean age), number and status of cell differentiation, type, location, and size of periodontal defect, and treatment of intervention and control groups, as well as author information, study type, follow-up period, employed tests, outcomes in periodontal regeneration, and complications were examined (Table 2 and 3).

Risk of Bias Assessment

The risk of bias in the eligible human articles was determined by using Cochrane Collaboration's tool for appraising risk of bias in randomized trials,[21] and judged as low, high, and unclear risk. In this regard, a study was rated as low risk of bias when all of the key domains were low risk, while an unclear risk of bias study referred to the study having low or unclear risk for all key domains. Further, one or most of key domains were high risk in a study with high risk of bias. Furthermore, the modified systematic review center for laboratory animal experimentation (SYRCLE) tool[22] was used for the risk of bias in animal studies. The terms "yes", "no", and "unclear" were utilized for low risk, high risk, and insufficient data for judgment, respectively. An overall score was calculated based on the number of yes to the total number of items, the greater value of which illustrates the better quality of study. Each study was independently assessed, followed by the evaluation between various studies.

Synthesis of Results

Given the impossibility of meta-analysis due to the diversity in the method and outcomes of studies, their classification and systematic comparison were carried out qualitatively.

Results

Study Selection

A total of 9895 articles were obtained through electronic search in databases, of which 31 (27 animal and 4 human) were included in the study by considering the eligibility and exclusion criteria. Figure 1 shows the flowchart of the search results according to the PRISMA guideline.

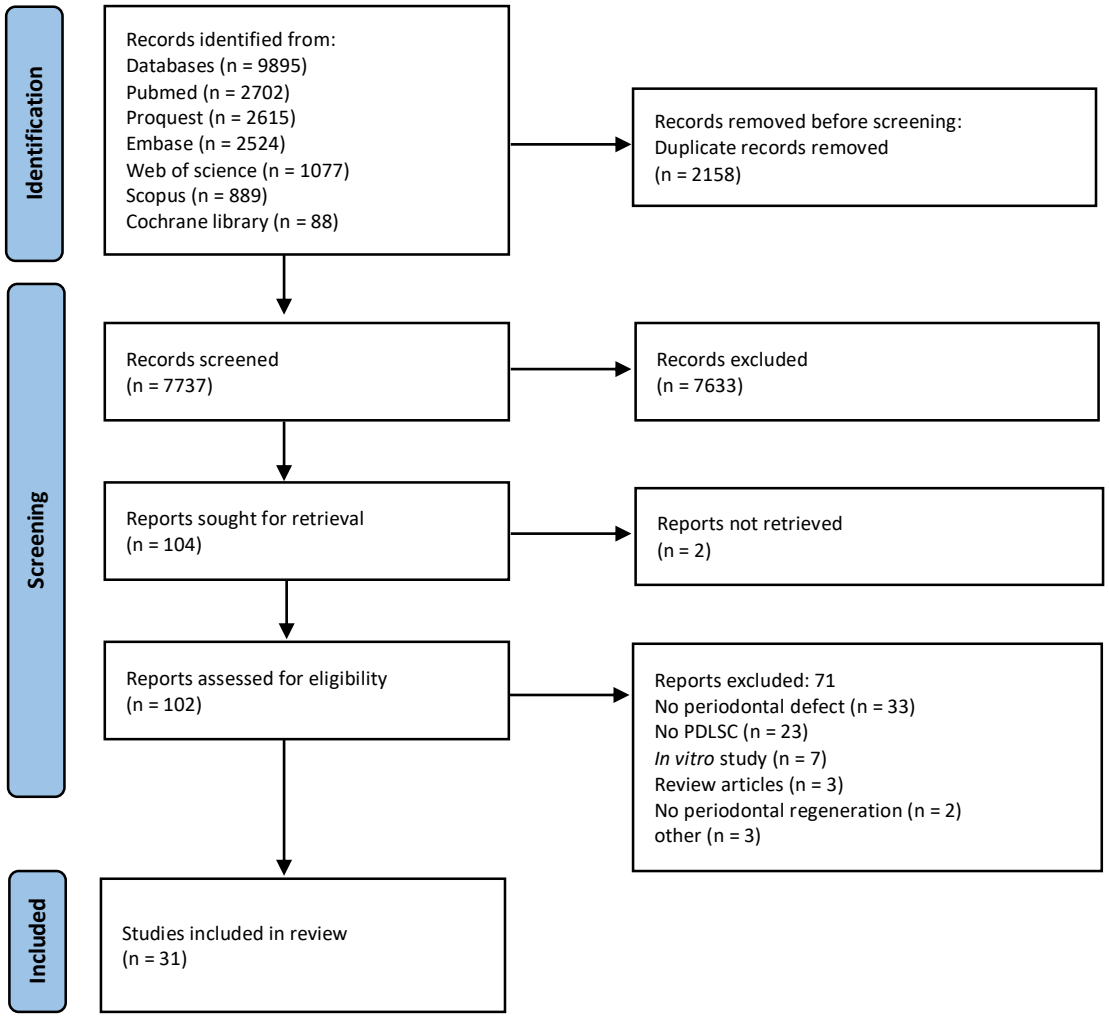

Figure 1. Flow Diagram of Studies Selection According PRISMA Guidelines.

Study Characteristics

Table 2 summarizes the eligible human articles. Among 4 human studies, 3 ones were RCT,[23-25] which examined 89 samples (55 females and 34 males) with intrabony defect. In two studies, the treatment groups received scaffold and autologous PDLSC+scaffold,[23,24] while Shalini et al., performed only open flap debridement (OFD) for the control group.[25] In addition, Abgel and Bio-Oss scaffolds were applied in the studies. The samples were checked up to 12 months based on the clinical and radiological results. The fourth human article, a case report, focused on a 28-year-old male having a grade II furcation defect. In the study, the treatment groups included PDLSC+OFD autologous and OFD, which were clinically and radiologically assessed after 6 and 12 months.[26]

Table 3 indicates a summary of the eligible animal articles. most studies used rats (12studies), followed by miniature pigs (6 studies), beagle dogs (5 studies), and sheep (2 studies). Finally, mice and rabbits were used in only one study each. In total,579 animals were described in this review: 407 rats, 67 miniature pigs, 40 dogs, 25 mice, 20 sheep, and 20 rabbits. Further, the various types of periodontal defects such as intrabony (17 studies), furcation (3 studies), fenestration (3 studies), dehiscence (2 studies), circumferential (1 study), and supra-alveolar (1 study) defects were created through surgery. The PDLSCs originated from human cell (14 studies), autologous (9 studies), allogenic (5 studies), and autologous + allogeneic (3 studies) sources. Furthermore, different scaffolds were utilized, most of which was collagen.

Risk of Bias in Studies

Figure 2a and table 4 displays a summary of appraising the risk of bias in human studies. As depicted, the majority of the articles exhibit unclear risk in random sequence generation, as well as low risk in allocation concealment and incomplete outcome data, as well as the blinding of participants and personnel. Additionally, all studies are rated as low risk in terms of selective reporting, as well as the blinding of outcome assessment. Thus, all articles have unclear risk of bias based on the Cochrane tool.

The appraisal of the risk of bias in animal studies is presented in Figure 2b and table 5. According to the SYRCLE tool, the largest percentage of studies with unclear risk was related to the domains of allocation concealment, random outcome assessment, and blinding against detection bias. Further, the number of high-risk articles was maximized in the random sequence generation, incomplete outcome data, and allocation concealment, respectively. The greatest percentage of studies with low risk was observed in selective outcome reporting (all), followed by baseline characteristics.

Figure 2. Risk of Bias Assessment of Included Studies. **A.** Risk of bias graph for animal studies, using the SYRCLE's tool, averaged per item.; **B.** Risk of bias graph for randomized human trials, using the Cochrane Collaboration's tool, averaged per item. The green, yellow and red colors depict the percentages of studies with low, unclear or high risk of bias of the total number of assessed studies.

Table 4. Summary of Risk Bias in Individual Human Studies

Study	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Anything else, ideally prespecified
Chen et al, 2016[23]	Unclear	Low	Unclear	Low	Low	Low	Low
Shalini et al, 2018[25]	Low	Unclear	Low	Low	Unclear	Low	Low
S6nchez et al, 2020[24]	Unclear	Low	Low	Low	Low	Low	Low

Table 5. Summary of Risk Bias in Individual Pre-clinical Animal Studies

Study	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome Assessment	Blinding	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Liu et al, 2008[14]	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes
Ding et al, 2010[56]	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes
Park et al, 2011[36]	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes
Wang et al, 2011[59]	No	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Nunez et al, 2012[37]	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Unclear	Yes	Yes
Wen et al 2012[43]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Lio et al, 2013[57]	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes
Mrozik et al, 2013[38]	No	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Yes
Fu et al, 2013[33]	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Han et al, 2014[29]	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Yes
Iwasaki et al, 2014[30]	No	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Menicanin et al, 2014[39]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Su et al, 2015[32]	Unclear	Yes w	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes
Zhu et al, 2015[34]	No	Yes a	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Tsumanuma et al, 2016[35]	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes
Basan et al, 2017[58]	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Yes	No	Yes	Yes
Nagata et al, 2017[31]	Unclear	Yes	Yes	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes
Sun et al, 2017[49]	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Unclear	Unclear	Yes	Yes
Duan et al, 2018[27]	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Shi et al, 2018[60]	No	Yes	No	Unclear	Unclear	Unclear	Unclear	No	Yes	Yes
Wang et al, 2018[50]	Unclear	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Iwasaki et al, 2019[51]	Unclear	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes
Liu et al, 2019[52]	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Unclear	Unclear	Yes	Yes
Qiu et al, 2020[28]	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Sano et al, 2020[55]	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Liu et al, 2021[53]	Unclear	Yes	Unclear	Yes	Unclear	Yes	Unclear	No	Yes	Yes
Lei et al, 2022[54]	Unclear	Yes	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes

Results of Individual Sources of Evidence

The clinical and radiological results of two human studies represented insignificant difference in intrabony defect regeneration, as well as an improvement in CAL, PD, and GR in PDLSCs+Bio-Oss® and PDLSCs+XBS groups compared to the XBS+saline and Bio-Oss® control groups.[23,24] However, the PDLSC Ni+Abgel+OFD and PDLSCs+Abgel® groups exhibited bone regeneration, and higher CAL, PD, and GR compared to the OFD group in the two other articles.[25] Furthermore, Vanada et al.,[26] reported improvement in furcation involvement. Chen et al.,[23] reported the complications of swelling and pain, and Sánchez et al.,[24] referred to mild-moderate pain, swelling, and mild dentin hypersensitivity. However, no complication was mentioned in the two other studies.

According to Duan et al.,[27] the only study involving mice, PDLSCs increase cementum, bone, and PDL regeneration.

The results of all studies on rats indicated more bone regeneration after treating with PDLSCs. In addition, this treatment led to improved cementum regeneration, as well the regeneration of collagen, Sharpey's fiber, connective tissue, and PDL in 7, 4, 3, 2, and 1 articles, respectively. The results of a study demonstrated no significant difference between PDLSCs and GMSCs with respect to cementum, connective tissue, and bone regeneration.[28] Further, no post-treatment complication was reported in four articles.[28-31]

Only one study used a rabbit model, in which bone regeneration was higher in the PDLSC group than in the control group, and no complications occurred.[32]

Regarding pigs, four studies revealed greater periodontal regeneration, and lower PD, GR, and Al in the PDLSC group compared to the controls. Fu et al.,[33] mentioned that more furcation, cementum, PDL, and bone are regenerated, and there was no significant difference between the PDLSC and stem cells from human exfoliated deciduous teeth (SHED) groups in any of the parameters, while the results of Zhu et al.,[34] represented an increase in PDL and bone regeneration. Furthermore, no complication was observed in only one article.[32]

Based on the outcomes related to dogs, the PDLSC group allowed more bone regeneration than the controls in all studies. However, Tsumanuma et al.,[35] referred to insignificant difference in the PDL and bone regeneration compared to the scaffold group. The results of three articles indicated cementum regeneration. According to Park et al.,[36] the comparison between PDLSCs, DPSCs, and periapical follicular stem cells (PAFSCs) reflects new Sharpey's fiber and cementum regeneration in the PDLSC and PAFSC groups. The level of PD and AL was less in the PDLSC group, and the highest extent of bone regeneration was detected in the PDLSCs, PAFSCs, and DPSCs groups, respectively. Researchers declared no complication after treatment in three studies.[35-37]

Mrozik et al.,[38] found that higher cementum regeneration and fiber attachment occurred in the sheep treated with PDLSCs. However, bone regeneration and Sharpey's fiber did not significantly differ from the scaffold groups, and no complication was observed. Finally, Menicanian et al.,[39] reported cementum, PDL, and bone regeneration.

Discussion

Summary of Evidence

Periodontitis is a common disease, the effect of which on oral and public health necessitates the need to regenerate the injured or lost periodontal tissue such as bone, cementum, and PDL. Given the recent advances and research in the tissue engineering, the application of PDLSCs for periodontal regeneration is considered as one of the new therapies. Seemingly, the results of the previous studies have provided promising evidence for the desired effectiveness of PDLSCs on periodontal regeneration in human and animal models. In this regard, the present systematic review highlighted the scientific studies concerning the efficacy of PDLSCs on periodontal regeneration both in human and animal models.

After a careful analysis, our results revealed that it was not possible to perform direct head-to-head comparisons of these studies as a result of variations between studies in terms of animal model used, the defect type and size, the number of cells per defect, the biomaterials applied, healing time after cell transplantation, Methods of evaluating the results and how to report the outcomes. Accordingly, no meta-analysis of the data could be carried out. However, specific markers can be evaluated for periodontal regeneration (new bone, new cementum, and new PDL). Additionally, PDLSCs exhibit the potential to improve periodontal defects so that the amount of bone, cementum, and PDL regeneration is more compared to the control groups in most studies.

The results of the previous systemic reviews in the field of tissue engineering have indicated the ability of MSCs to regenerate periodontal tissues, which is consistent with those of the present study. For example, Bright et al.,[4] reviewed 17 articles and found that PDLSC implantation leads to a positive outcome in terms of an increase in periodontal regeneration in the animal models. Based on the results of reviewing 15 human studies, low-quality evidence suggested that MSC-based treatment may have little positive effect on periodontal regeneration.[5] According to Portron et al.,[18] the results of 50 human and animal articles represented that MSC may beneficially affect periodontal regeneration. Further, most studies have reported the positive and promising effect on the potential to regenerate PDL-derived cells.

Despite the evidence reflecting the possibility of periodontal regeneration in animals, predictable regeneration in human remains an elusive clinical objective.[40] In periodontal regeneration, the potential of stem/progenitor

cells should be examined to explain the key points of periodontal growth. The cell therapy approach to periodontal regeneration is in its infancy although the future of this type of regenerative treatment looks very encouraging.[41,42] The preclinical animal studies are considered as the first step in this process. The results of the present systematic review demonstrated the useful effect of the treatment with PDLSCs on periodontal regeneration in the animal models. Given that limited studies have been carried out in the human models, the results cannot be definitive.

PDLSCs are a candidate cell source for periodontal regeneration, as well as ideal cells for gene therapy in periodontal tissue engineering.[43] PDL cells are expanded *in vitro* in sufficient quantity, which represent a potential to regenerate alveolar bone and cementum. They can be used with appropriate biomaterials to engineer living tissue *in vitro* for subsequent transplantation into defect sites.[44] A large body of research has assessed the regenerative capacity of PDLSCs *in vivo* and in vitro, the results of which have revealed that PDLSCs differentiate in many pathways under defined culture conditions.[45] Furthermore, freshly-isolated and frozen human PDL contains stem cells which can differentiate into cementoblast cells *in vitro* and form cementum/PDL tissues *in vivo*.[13,46] PDLSCs with self-renewability and multilineage differentiability express MSC surface markers such as CD44, CD73, CD 90, CD105, CD106 (VCAM-1), CD146 (MUC-18), and Stro-1, and lead to the non-expression of hematopoietic markers like CD31, CD34, and CD45.[47,48] They exhibit a higher proliferation rate than the bone marrow skeletal stem cells. In addition, PDLSCs express scleraxis, a tendon/ligament-specific transcription factor, at a higher level compared to the stem cells extracted from bone marrow or dental pulp. PDLSCs can form PDL attachment *in vivo* through generating Sharpey's fiber-like collagen bundles connected to cementum-like structures.[13]

Given the current scientific advances, as well as a large number of animal studies, there is good evidence to support the use of PDLSCs for periodontal regeneration. Seemingly, it is the right time to move from preclinical animal research to human one. However, some issues should be highlighted to check treatment stability, as well as the lack of complication, one of which is understanding the safety and immunoregulatory properties of the cell, scaffold, and carrier applied for cell implantation. The others include the examination of the most suitable tissue as donor cells and its isolation method, and accurate quantitative assessment of the extent of periodontal tissue regeneration, as well as comparison with other approaches and long-term evaluation.

Limitation

The inclusion of only English articles increases the possibility of publication bias, which can be addressed as one of the limitations of the present review. To minimize this error, a comprehensive search was conducted in most databases, as well as the gray literature consisting of unpublished studies, theses, lectures, and posters.

Conclusion

The results of this review suggest the useful effects of using PDLSCs as one of tissue engineering components on enhancing periodontal tissue regeneration. Animal studies have frequently revealed the positive effect of this treatment although the outcomes of limited human research are inconsistent. Thus, further human studies with better design and more samples should be performed to examine clinical efficacy. The results provide important information for implementing the stem cell-based approaches in clinical practice as a routine treatment in the future.

Authors' Contributions

All authors contributed equally to the current study.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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