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Review Article

Advancements and challenges in stem/progenitor cell transplantation for dentin-pulp regeneration: a systematic review of animal studies (part I)

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Abstract: Dentin-pulp regeneration through stem/progenitor cell transplantation represents a promising frontier in regenerative endodontics. This systematic review meticulously evaluates animal studies to investigate the efficacy of stem cell therapy in repairing/regenerating the dentine-pulp complex in mature/immature animal teeth. Employing a comprehensive electronic search of PubMed and Scopus databases up to October 2023, relevant English studies were identified/assessed. Evaluation parameters encompassed radiographic and histological assessments of dentin-pulp complex formation. Outcome measures included pulp-like and dentin-like tissues regeneration, apical healing, dentin thickening, apical closure, and dentinal bridge formation. The risk-of-bias assessment adhered to the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) guidelines. Out of 3250 identified articles, 23 animal experiments were included, categorized into regenerative procedures in mature teeth (n=11), regenerative procedures in immature teeth (n=4), and vital pulp therapy (n=8). Despite the promising potential, the bias in the included studies was high. Notably, Various scaffolds, and growth factors were employed, highlighting the heterogeneity across the studies. Dental pulp stem cells (DPSCs) and bone marrow stem cells, especially specific subfractions, demonstrated notable regenerative potential: hypoxic conditions and extracellular vesicles from preconditioned DPSCs enhanced regeneration, with considerations of cell fate. Donor age impacted regeneration, and challenges persisted in pulpotomy and direct pulp capping. Scaffold and growth factor choices influenced outcomes, underscoring the need for standardized strategies. Despite the promise, clinical viability faces hurdles, necessitating further investigation into adverse effects, optimized scaffolds, and regulatory considerations. This systematic review illuminates the potential of stem cell transplantation for dentin-pulp complex regeneration. The overall evidence quality, influenced by study heterogeneity and biases, underscores the need for cautious interpretation of findings. Future studies should refine methodologies and establish reliable histological parameters for meaningful advancements in dentin-pulp regeneration.

Keywords: Dentin-pulp complex, dental pulp stem cells, pulpotomy, regenerative endodontics, stem cell transplantation, systematic review, vital pulp therapy

Introduction

Regenerative endodontics encompasses biologically based treatments to replace the pulp and restore damaged dentin and root structures, particularly in immature teeth [1]. This innovative approach leverages tissue engineering principles to rejuvenate root canals, fostering the ongoing development of the root and its surrounding tissues. The procedures within regenerative endodontics strive either to induce the formation of a physiologically akin dentine-pulp tissue (regeneration) or to create a new tissue that mimics the native pulp-dentine complex at the histologic level, exhibiting anticipated physiological functions (repair) [2].

The scope of regeneration in endodontics spans vital pulp therapy (VPT), designed to preserve dental pulp vitality and prompt healing/regeneration of the pulp-dentin complex in vital teeth with inflamed pulp [3-5]. Additionally, regenerative endodontic procedures aim to revitalize and regenerate dentin-like and pulp-like tissues in both mature and immature permanent teeth with necrotic pulp [1, 6]. Regenera-

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tive endodontics extends beyond VPT and comprises a spectrum of innovative approaches that restore the vitality and function of diseased or damaged dental pulp. While VPT focuses primarily on preserving pulp vitality and healing inflamed pulp, regenerative endodontic procedures focus on tissue engineering principles to form functional dentin-pulp complexes. These procedures target necrotic mature/immature permanent teeth, where traditional treatment options, i.e., root canal treatment or apical plug, are not considered a biologically based treatment [7]. By leveraging advancements in stem cell biology, tissue engineering, and regenerative medicine, researchers aim to facilitate the growth of new dentin and dental pulp tissues. This broader scope of endodontic regeneration reflects a paradigm shift in treatment philosophy, from mere preservation to active restoration of the natural tooth structure/function [1, 8]. Besides, biologically based treatments refer to therapeutic approaches that use the body's natural healing potential/mechanisms to restore damaged pulp and dentin tissues [9]. These treatments focus on stimulating the regeneration of functional pulp-dentin complexes by using biological agents such as stem cells, growth factors, and scaffolds [8, 10]. With these regenerative strategies, clinicians can explore novel approaches to endodontic therapy that improve outcomes and enhance patient care.

While clinical and animal studies on revitalization procedures for necrotic immature permanent teeth demonstrated increased dentinal wall thickening and root development by attracting blood into root canal spaces, histological observations revealed a lack of dentin-pulp complex formation. Instead, newly grown tissues in the root canal space exhibited characteristics resembling cementum, periodontal ligament (PDL), or bone-like tissue [11-13]. This outcome is postulated to result from the absence of stem cells derived from the remaining vital pulp tissue and apical papilla. Stem cells crucial for the regeneration of tissues might originate from alternative sources such as systemic blood or other local tissues like bone and PDL. The transplantation of stem/progenitor cells for tissue regeneration, with proven success in various medical fields such as cardiovascular diseases [14, 15], skin wound healing [16, 17], and periodontal regeneration [18] has recently gained attention. The stem/progenitor cell transplantation exerts its effects locally at numerous levels, including neovascularization [19], immunomodulation [20], and tissue regeneration [18]. Recently, mesenchymal stem cell transplantation in the root canal has been suggested as essential for the regeneration of the dentin-pulp complex [21].

Although two prior systematic reviews explored the impact of stem cell transplantation on regenerative endodontics outcomes [22, 23], the evidence remained inconclusive. In recent years, the literature has seen a surge in new animal studies featuring mature/immature tooth models. Thus, the present study systematically reviews data from these animal studies, focusing on stem/progenitor cell transplantation for pulp-dentin complex regeneration in both mature and immature teeth.

Methods

Protocol

The present study adheres to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) statement [24]. The PICOS question was formulated as follows: "What are the effects of stem cell transplantation on the regeneration of the dentin-pulp complex in mature/immature teeth in animal models"?

Search strategy

A systematic electronic search was conducted in Pub-Med/Medline, Scopus, and Scholar databases to identify eligible English papers up to October 2023. The search terms included "Dentin OR odontoblast" AND "regeneration" AND "cell". Grey literature was explored through OpenSigle/OpenGrey, and reference lists of reviews and selected studies were also screened for additional papers.

Selection criteria

- Study design: Animal studies assessing the stem/progenitor cell transplantation approach in mature or immature teeth requiring regeneration therapy, including VPT or regenerative procedure.

- Population: Animals such as dogs, rats, mice, and minipigs need regeneration treatment.

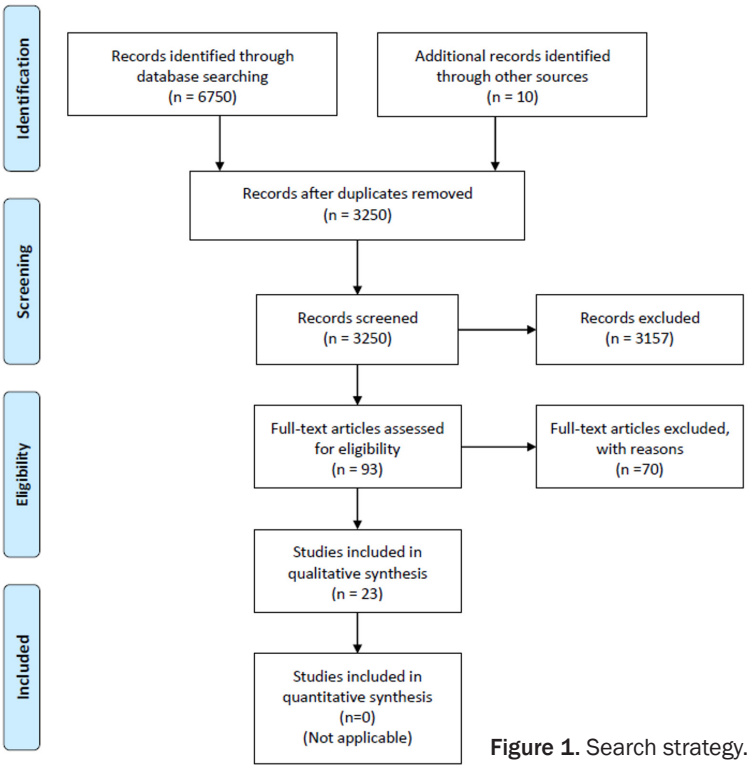

Figure 1. Search strategy.

● Intervention: Stem/progenitor cell transplantation with/without additional treatment involving growth/differentiation factors and/or scaffolds.

● Comparison: No stem/progenitor cell transplantation, scaffold/carrier and/or growth factors, or no treatment.

● Outcomes: Histological and radiographical parameters were employed to evaluate the following outcomes: 1) regeneration of pulp-like and dentin-like tissue, 2) apical healing, 3) dentin thickening, 4) apical closure, and 5) dentinal bridge formation.

Study selection

Titles/abstracts identified in the electronic search were independently screened by two reviewers (S.S and A.S) based on the selection criteria. Discrepancies among the authors were resolved through discussion. Subsequently, full texts of the selected papers were screened.

Data extraction

Two authors (S.S and S.A) independently extracted the following data from the included papers: First author and year of publication, sample size, cell type and concentrations, growth factor(s) used, scaffold/carrier used, animal species, tooth type, animal model, and outcome (radiographic and histological results).

Risk of bias assessment

For the risk of bias assessment, guidelines outlined by SYRCLE [25] were followed. The evaluation covered the following domains: selection bias (method of sequence generation, baseline characteristics, allocation concealment), performance bias (random housing, blinding of operators and personnel), detection bias (blinded and random outcome assessment), reporting bias (selective reporting, incomplete outcome data), and other sources of bias.

Results

Search results

The search results and the study selection process are illustrated in **Figure 1**. The initial search yielded 3250 records, with 93 papers undergoing full-text evaluation after removing duplicates and assessing titles and abstracts. Ultimately, 23 papers met the criteria for qualitative synthesis, with exclusions based on the use of a subcutaneous model or the unavailability of full text.

Study characteristics

Tables 1-**3** provide an overview of study characteristics for the included articles. The publication timeline spans from 2009 to 2022, with the evaluation of stem/progenitor cell transplantation effects categorized into three groups: i) regenerative endodontic procedure in mature teeth, ii) regenerative endodontic procedure in immature teeth, and iii) VPT. Autologous stem cells were consistently isolated in all studies. Prior to cell transplantation, routine procedures included immunocytochemistry, MTT assay, and flow cytometry analyses to characterize the transplanting stem cells.

Table 1. Animal models used for regenerative endodontic procedure

	Author (year)	Animal	Teeth	Infected/non-infected model	Bleeding induction	Irrigation used	Anti-bacterial agent used	N visit	Coronal seal	Restorations
Mature teeth	Zayed M (2021)	Dog	Incisor	Non-infected	NS	NS	NS	1	NS	NS
	Chen WJ (2021)	Rat	Molar	Non-infected	NS	NS	Calcium hydroxide	2	MTA	Composite
	Iohara K (2016)	Dog	Incisor	Non-infected	NS	NS	-	1	ZPC	Composite
	Xu W (2016)	Chimeric mice	Molar	Non-infected	+	Saline	-	1	MTA	Composite
	Kuang R (2016)	Nude rat	Molar	Non-infected	+	NaOCl	-	1	-	GIC
	Murakami M (2015)	Dog	Canine	Non-infected	NS	NS	-	1	ZPC	Composite
	Iohara K (2014)	Dog	Incisor	Non-infected	NS	NS	NS	1	NS	NS
	Iohara K (2013)	Dog	Incisor	Non-infected	NS	NS	NS	1	NS	NS
	Ishizaka R (2012)	Dog	Incisor	Non-infected	NS	NS	-	1	ZPC	Composite
	Zhu X (2012)	Dog	Premolar	Non-infected	+	NS	-	1	MTA	Composite
	Iohara K (2011)	Dog	Incisor	Non-infected	NS	NS	-	1	ZPC	Composite
Immature teeth	Zhu W (2018)	Dog	Premolar	Infected	+	NaOCl	TAP	2	MTA	Composite
	Wang Y (2013)	Dog	Incisor	Non-infected	NS	NaOCl/saline	-	1	MTA	Composite
	Al Eshiry AE (2018)	Dog	Incisor	Infected	NS	NaOCl	TAP	2	MTA	Composite
	Ling L (2010)	Dog	Premolar	Non-infected	NS	NaOCl/saline	-	1	MTA	Composite

GIC, Glass ionomer cement; MTA, Mineral trioxide aggregates; NaOCl, Sodium hypochlorite; NS, Not stated; TAP, Triple antibiotic paste; ZPC, Zinc phosphate cement.

Table 2. Animal models used for vital pulp therapy

Vital pulp therapy	Author (year)	Animal	Teeth	Hemostasis	Irrigation used	Coronal seal	Restoration
Pulpotomy	Kaneko T (2019)	Rat	Molar	NS	NaOCl/EDTA	MTA	Composite
	Mangione F (2017)	Minipig	Premolar/molar	Cotton-pellet	NS	Biodentine	Composite
	Sueyama Y (2017)	Rat	Molar	NS	NaOCl/EDTA	MTA	Composite
	Jia W (2016)	Dog	Premolar	NS	NS	MTA	GIC/composite
Direct pulp capping	Mohajeri SF (2022)	Dog	Premolar	Cotton-pellet/saline	Saline	-	GIC
	Obeid M (2013)	Dog	Premolar	Cotton-pellet	NaOCl/saline	-	GIC
	Ji YM (2013)	Dog	Premolar	Cotton-pellet	Saline	-	GIC
	Zheng Y (2012)	Minipig	Molar	NS	NS	ZOE	GIC

GIC, glass ionomer cement; EDTA, Ethylenediaminetetraacetic acid; MTA, Mineral trioxide aggregates; NS, Not stated; NaOCl, Sodium hypochlorite; ZOE, Zinc oxide eugenol.

Table 3. Study characteristics of the included studies

	Aim	Author (year)	Animal (Age)	Teeth	Stem cells (Concentrations)	Growth factors	Carrier/ Scaffold	Groups (n)	Outcome assessment (days)
Regenerative procedure in mature teeth	Different cell type	Murakami M (2015)	Dog (9-11 m)	Canine	DPSCs $CD31^-$, ADSCs $CD31^-$, BMSCs $CD31^-$ (5×10^5)	G-CSF	Atecollagen	G1: DPSCs/scaffold/GF (5) G2: ADSCs/scaffold/GF (5) G3: BMSCs/scaffold/GF (5)	Histologic (14 d)
		Ishizaka R (2012)	Dog (9-11 m)	Incisor	DPSCs $CD3^-$, ADSCs $CD31^-$, BMSCs $CD31^-$ (5×10^5)	SDF-1	Collagen	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third) (10) G2: ADSCs/scaffold (apical third), GF/scaffold (middle third) (10) G3: BMSCs/scaffold (apical third), GF/scaffold (middle third) (10)	Histologic (14, 28 d)
	DPSCs/GF and/or collagen	Iohara K (2016)	Dog (8-10 m)	Incisor	mDPSCs (NS)	G-CSF	Atecollagen	G1: Pulpectomy (4) G2: Control (4) G3: DPSCs/scaffold/GF (4) G4: Scaffold (4)	Histologic, radiographic (3, 6 m)
		Iohara K (2013)	Dog (9-11 m)	Incisor	mDPSCs (NS)	G-CSF	Atecollagen	G1: DPSCs/GF/scaffolds (12) G2: Un-fractioned DPSCs/GF/Scaffold (12) G3: DPSCs (12) G4: Un-fractioned DPSCs/scaffold (12) G5: GF (12) G6: Scaffold (12)	Histologic, radiographic (6 m)
	Total pulp stem cell, DPSC or ADSC $CD105^+$	Iohara K (2011)	Dog (NS)	Incisor	Total pulp cells, DPSCs $CD105^+$, ADSCs $CD105^+$ (1×10^6)	SDF-1	Collagen	G1: DPSCs $CD105^+$/GF/scaffold (10) G2: ADSCs $CD105^+$/GF/scaffold (5) G3: DPSCs/GF/scaffold (5) G4: GF/scaffold (5) G5: DPSCs $CD105^+$/scaffold (5) G6: Scaffold (5) G7: Control (7)	Histologic (3 m)
	Hypoxia-treated or mobilized DPSCs	Zayed M (2021)	Dog (12 m)	Incisor	hpDPSCs; mDPSCs (5×10^5)	G-CSF	Atecollagen	G1: hpDPSCs/scaffold/GF (6) G2: mDPSCs/scaffold/GF (6)	Histologic (4 w)
	DPSCs-derived Evs with or without BMSCs	Chen WJ (2021)	Rat (6-8 w)	Molar	sEV or LPS-sEV DPSCs (200 μg/mL), BMSCs (1×10^6)	-	Puratamix	G1: Normal (6) G2: L-DPSCs-sEV/scaffold (5) G3: DPSCs-sEV/scaffold (4) G4: BMSCs/scaffolds (3) G5: BMSCs/L-DPSCs-sEV/scaffold (4) G6: BMSCs/DPSCs-sEV/scaffold (3) G7: Scaffold (2)	Histologic (1 m)
	Systemic BMSCs	Xu W (2016)	Chimeric mice (6 w)	Molar	GFP^+ BMSCs via tail vein (2×10^6)	-	Blood clot	G1: Blood clot (8) G2: Control (8)	Histologic (1, 4, 8 w)
	Normal or hypoxia-treated DPSCs	Kuang R (2016)	Nude rat (6-8 w)	Molar	Human DPSCs (8×10^6)	-	Nanofibrous Microsphere	G1: hDPSCs/microsphere (6) G2: DPSCs/microsphere (6) G3: Control (6)	Histologic (1 m)
	Age of donor	Iohara K (2016)	Dog (5-6 y)	Incisor	DPSCs (NS)	G-CSF	Atecollagen	G1: DPSCs/GF/scaffold (4) G2: Control (4)	Histologic, radiographic (14 d, 4 m)
	DPSCs/PRP	Zhu X (2012)	Dog (12 m)	Premolar	DPSCs (10^6)	-	Blood clot/PRP	G1: Blood clot (8) G2: DPSCs (8) G3: PRP (8) G4: DPSCs/PRP (8)	Histologic (6 m)

Pulpectomy Immature teeth	DPSC and/or Gelfoam	Wang Y (2013)	Dog (4-5 m)	Incisor	DPSCs (2×10^7)	-	Gelfoam	G1: DPSCs/scaffold (6) G2: Scaffold (6) G3: DPSC (6)	Histologic, radiographic (6 m)
	Stem cells from inflamed/normal pulp	Ling L (2020)	Dog (4-5 m)	Premolar	DPSCs from inflamed/normal pulp (10^6)	-	Puramatrix	G1: DPSCs/scaffold (10) G2: iDPSC/scaffold (10) G3: Control (10)	Histologic Radiographic (3 m)
	GF/scaffold and/or DPSCs	Al Eshiry EA (2018)	Dog (6 m)	Incisor	DPSCs (10^5)	PDGF/NGF/bFGF/BMP-7	Chitosan hydrogel	G1: DPSCs/GF/Scaffold (18) G2: GF/Scaffold (18)	Histologic, radiographic (4 m)
	DPSC and/or PRP	Zhu W (2013)	Dog (6 m)	Premolar	DPSCs (5×10^6)		PRP	G1: Blood clot (10) G2: DPSCs (10) G3: PRP (10) G4: DPSCs/PRP (10) G5: Control (8)	Histologic (6 m), radiographic (3 m)
Pulpotomy	DPSCs and/or scaffold	Mangione F (2017)	Minipig (18-20 m)	Premolar/molar	DPSCs (2×10^7)	-	Puramatrix	G1: DPSCs/scaffold (16) G2: Scaffold (16)	Histologic, radiographic (21 d)
	BMSCs and/or endothelial cells	Sueyama Y (2017)	Rat (7 w)	Molar	BMSCs (2×10^5)/Endothelial cells (10^5)	-	PLLA/matrigel encapsulating cells	G1: BMSCs-endothelial cells/scaffold (8) G2: BMSCs/scaffold (8) G3: Scaffold (8) G4: Pulpotomy (8) G5: Control (8)	Histologic (14 d)
	Matrigel and/or BMSCs vector	Kaneko T (2019)	Rat (6-7 w)	Molar	BMSCs (2×10^5)	-	PLLA/matrigel	G1: BMSC-lacz/scaffold (6) G2: BMSC-vector/scaffold (6) G3: Scaffold (6) G4: Control (6)	Histologic (14 d)
	Simvastatin/DPSCs/gelatin	Jia W (2016)	Dog (5 m)	Premolar	DPSCs (1×10^7)	Simvastatin	Gelatin sponge	G1: MTA (4) G2: Scaffold (4) G3: DPSCs/scaffold (5) G4: Simvastatin/DPSCs/Scaffold (5)	Histologic, radiographic (10 w)
Direct Pulp Capping	DPSCs with/without scaffold	Zheng Y (2012)	Minipig (6-7 m)	Incisor	GFP^+/DPSCs (5×10^5)	-	B-TCP	G1: Calcium hydroxide (16) G2: Scaffold (16) G3: GFP^+/DPSCs/scaffold (16)	Radiographic histologic (1 m)
	BMSCs with/without scaffold	Obeid M (2013)	Dog (NS)	Premolar/canine	BMSCs (2.5×10^6)	-	HA-TCP	G1: MTA (14) G2: BMSCs/scaffold (14) G3: Scaffold (14)	Radiographic histologic (3 m)
		Mohajeri SF (2022)	Dog (18-24 m)	Premolar/canine	BMSCs (NS)	-	Collagen/HA	G1: MTA (13) G2: Scaffold (13) G3: BMSCs/scaffold (13)	Histologic (6 m)
	DPSCs alone or treated with calcium hydroxide	Ji YM (2009)	Dog (12 m)	Premolar	DPSCs (NS)	-	-	G1: GIC (6) G2: DPSCs (6) G3: Calcium hydroxide (6) G4: Calcium hydroxide treated DPSCs (6)	Histologic (4-6 w)

ADSCs, Adipose derived stem cells; bFGF, Basic fibroblast growth factor; BMP-7, Bone morphogenic protein-7; BMSCs, Bone marrow stem cells; DPSCs, Dental pulp stem cells; DPSCs sEV, Extracellular vesicles derived from dental pulp stem cells; hpDPSCs, Hypoxia-treated DPSCs; mDPSCs, mobilized dental pulp stem cells; sEV, Small extracellular vesicles; GF, Growth factors; G-CSF, Granulocyte-colony stimulating factor; GIC, Glass ionomer cement; HA, hydroxyapatite; L-DPSCs sEV, Extracellular vesicles derived from lipopolysaccharide preconditioned dental pulp stem cells; MTA, Mineral trioxide aggregates; NGF, Nerve growth factor; PRP, Platelet rich plasma; PDGF, Platelet-derived growth factor; SDF-1, Stem cell-derived factor-1; HA-TCP, Hydroxyapatite tricalcium phosphate.

Regenerative endodontic procedure in mature teeth

Eleven studies systematically assessed the impact of cell transplantation on dentin-pulp complex regeneration [26-36]. Various animal models were employed, including dogs [26-30, 34-36], rats [31, 33] and mice [32], utilizing non-infected models. Diverse scaffolds were utilized, such as collagen [28, 29], Atecollagen [26, 27, 30, 34, 36], puramatrix [31, 37, 38], microsphere aggregates [33], platelet rich in plasma (PRP) [35] and blood clot [32, 35]. The experiments encompassed the use of different cell types, including DPSCs [33-35], bone marrow stem cells (BMSCs) [32], DPSCs $CD105^+$ SP [28], adipose-derived stem cells (ADSCs) $CD105^+$ SP [28], DPSC $CD31^-$ SP [26, 29, 35], BMSCs $CD31^-$ SP [26, 29], ADSCs $CD31^-$ SP [26, 29], mobilized DPSCs [27, 30, 36], and hypoxia-treated DPSCs [30, 33]. Autologous stem cells were consistently isolated in studies using DPSCs, except in one case where DPSCs were derived from human healthy pulp tissue for use in nude rats [33]. Growth factors, such as granulocyte colony-stimulating factor (G-CSF) [26, 30, 33, 34, 36] or stromal cell-derived factor-1 (SDF-1) [28, 29], were employed. Radiographic techniques, including periapical [34, 36] and magnetic resonance imaging (MRI) [27], were evaluated in three studies [27, 34, 36]. Histological assessments involved hematoxylin and eosin (H&E) staining [26-36], matrix formation via Masson trichrome staining [26, 29, 31], vascularization via BS1-lectin staining [26, 29, 34, 36], neurogenesis via PGP9.5 staining [26, 29], mRNA expression (dentin sialophosphoprotein [DSPP] [26, 33, 36], enamelysin/MMP20 [29, 36], angiogenic/neurotrophic factors [29], periodontal markers [28, 29, 36], markers of bone/adipose tissue [29], pulp markers [28, 29, 36]) and the assessment of the regenerated pulp area to total canal volume [28, 29, 34, 36].

Regenerative endodontic procedure in immature teeth

Four studies investigated the impact of cell transplantation on dentin-pulp complex regeneration [38-41]. Dogs were chosen as the animal models for both infected and non-infected regeneration models. Various scaffolds were employed, including gel foam [39], puramatrix [38], PRP [41], and chitosan hydrogel [40]. The experiments were conducted using DPSCs, and autologous stem cells were isolated in all cases. One study utilized a combination of platelet-derived growth factor (PDGF), nerve growth factor (NGF), basic fibroblast growth factor (bFGF), and bone morphogenic protein-7 (BMP-7) growth factor [40].

Vital pulp therapy

Eight studies assessed the impact of cell transplantation on the dentin-pulp complex regeneration through VPTs encompassing pulpotomy [37, 42-44] and direct pulp capping (DPC) [45-48]. The animal models included rats [42, 43], dogs [44] and minipigs [37] for the pulpotomy model and minipigs [45] and dogs [46-48] for the DPC model. Scaffolds and carriers varied with puramatrix [37], gelatin sponge [44], and PLLA/matrigel [42, 43] used in the pulpotomy model and β-TCP [45], hydroxyapatite (HA)-collagen [48], and HA-β-TCP [46] used in the DPC model. The stem cells employed consisted of DPSCs [37, 44], BMSCs [43] and BMSCs/endothelial cells [42] in the pulpotomy model and DPSCs [45], calcium hydroxide treated [47] and BMSCs [46, 48] in the DPC model.

Risk of bias assessment

The risk of bias in all the included animal studies was deemed high, indicating elevated levels of selection, performance and detection bias (**Table 4**).

Results of the included studies

Histologic evaluation

Table 5 outlines the histological findings from the included studies as follows.

Regenerative endodontic procedure in mature teeth

Pulp-like tissue by H&E staining: The generation of pulp-like tissue in the canal space was observed through various approaches: 1) Transplantation of $CD105^+$ pulp cells with growth factors/scaffold demonstrated superior outcomes compared to the transplantation of unfractionated total pulp cells [28]; 2) Hypoxia-treated DPSCs showed better results than normal DPSCs [33] or mobilized DPSCs [30] in pro-

Table 4. Risk of bias assessment

Author (Year)	Random Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding Care-giver	Random outcome assessment	Blinding outcome assessment	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Mohajeri SF (2022)	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Zayed M (2021)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Chen WJ (2021)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Ling L (2020)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Kaneko T (2019)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Al Eshiry E (2018)	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Mangione F (2017)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Sueyama Y (2017)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Iohara K (2016)	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	No	No
Xu W (2016)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Kuang R (2016)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Jia W (2016)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Murakami M (2015)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Iohara K (2014)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	No	No
Iohara K (2013)	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	No	No
Obeid M (2013)	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	No	No
Wang Y (2013)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Zhu W (2013)	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Ishizaka R (2012)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Zheng Y (2012)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Zhu X (2012)	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	No	No	No
Iohara K (2011)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	No	No
Ji YM (2009)	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	No	No

Table 5. Histological findings of the included studies

Treatment approach	Histological evaluation	Author (Year)	Groups	Results
Regenerative procedure in mature teeth	H&E staining	Murakami M (2015)	G1: ADSCs/GF/scaffold; G2: BMSCs/GF/scaffold; G3: DPSCs/GF/scaffold.	Higher pulp-tissue formation in G3 than G2 (1.9-fold) and G1 (1.4-fold).
		Iohara K (2016)	G1: Pulpectomy; G2: Control; G3: DPSCs/scaffold/GF; G4: scaffold.	Higher pulp-like Tissue/blood vessels/secondary dentin formation in the apical part and dentinal wall in G3.
		Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third); G2: ADSCs/scaffold (apical third), GF/scaffold (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	Greater pulp-like tissue with vasculature and innervation (G1, G2>G3). Fibrous matrix/matrix formation in some part of G2.
		Kuang R (2016)	G1: hpDPSCs/microsphere; G2: DPSCs/microsphere; G3: Control.	Higher pulp-like tissues/vascularity in G1>G2, small amount of connective tissue/no pulp-like tissue and blood vessel in G3.
		Iohara K (2014)	G1: DPSCs/GF/scaffold; G2: Control.	Formation of pulp-like loose connective tissue with vasculature/odontoblast-like cells attached to the dentinal wall/osteodentin in the coronal part of G1.
		Xu W (2016)	G1: Blood clot; G2: Control.	Connective tissue/blood vessels in half of the canal/calcified tissue in apical portion/odontoblast-like and bone-like cells inside the root canal in G1; no tissue/some inflammatory cells in G2.
		Chen WJ (2021)	G1: Normal; G2: L-DPSCs sEV/scaffolds; G3: DPSCs-sEV/scaffolds; G4: BMSCs/scaffolds; G5: BMSCs/L-DPSCs-sEV/scaffolds; G6: BMSCs/DPSCs-sEV/scaffolds; G7: Scaffold.	More blood vessels/loose connective tissues in G5 and G2; fewer blood vessels/more mineralization tissues in G4, G6, and G3; no cell or blood vessel with some amorphous matrix in the root canal in G7.
		Zayed M (2021)	G1: hpDPSCs/scaffold/GF; G2: mDPSCs/scaffold/GF.	Well-vascularized, well-innervated loose connective tissue/little infiltration of inflammatory cells/osteoblastic-like and odontoblast-like cells/similar regenerated pulp area and neovascularization in G1-2 (No significant difference between the groups).
		Iohara K (2011)	G1: DPSCs $CD105^+$/GF/scaffold; G2: ADSCs $CD105^+$/GF/scaffold; G3: DPSCs/GF/scaffold; G4: GF/scaffold; G5: DPSCs $CD105^+$/scaffold; G6: scaffold; G7: Control.	Greater pulp-like tissue G1>G4 (4.2-fold)>G5 (3.3-fold)/tubular odontoblast along dentinal wall in G1.
		Zhu X (2012)	G1: Blood clot; G2: DPSCs; G3: PRP; G4: DPSCs/PRP.	New vital tissue (G1: 100%, G2: 57.1%, G3: 75%, G4: 85.7%): bone-like tissue in the coronal part/cementum-like/PDL-like tissue in the middle-apical part. No dentin-like/pulp-like tissue.
		Ishizaka R (2012)	G1: DSCs/GF/scaffold; G2: un-fractioned/GF/Scaffold; G3: DPSCs; G4: un-fractioned/scaffold; G5: GF; G6: scaffold.	Higher pulp-like loose connective tissue/vasculature, similar to the normal pulp/Odontoblast-like cells attached to the dentinal wall in G1.
	Matrix formation by Masson trichome staining	Murakami M (2015)	G1: ADSCs/GF/scaffold; G2: BMSCs/GF/scaffold; G3: DPSCs/GF/scaffold.	Increased in G2 (2.3-fold) and G1 (1.6-fold) than G3.
		Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third); G2: ADSCs/scaffold (apical third), GF/scaffold (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	Positive area was increased in G2 (3-fold) and G3 (2-fold) compared with that in G1.
		Chen WJ (2021)	G1: Normal; G2: L-DPSCs sEV/scaffolds; G3: DPSCs-sEV/scaffolds; G4: BMSCs/scaffolds; G5: BMSCs/L-DPSCs-sEV/scaffolds; G6: BMSCs/DPSCs-sEV/scaffolds; G7: Scaffold.	Collagen fiber density: Increased in all groups than normal group. Collagen fibers arrangement: arranged in G2 and G5 along the dentin wall incorporated with abundant blood vessels. Disorderly arranged collagen deposits diffused in the root canal in G3, G4, and G6.

BS1-lectin IHC staining	Murakami M (2015)	G1: ADSCs/GF/scaffold; G2: BMSCs/GF/scaffold; G3: DPSCs/GF/scaffold.	Higher in G3 than G2 and G1.
	Iohara K (2014)	G1: DPSCs/GF/scaffold; G2: Control.	Newly formed vessels similar to the normal pulp.
	Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third); G2: ADSCs/scaffold (apical third), GF/scaffold (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	No significant differences in neovascularization in the regenerated tissues of all groups.
	Iohara K (2013)	G1: DPSCs/GF/scaffold; G2: un-fractioned DPSCs/GF/Scaffold; G3: DPSCs; G4: un-fractioned DPSCs/scaffolds; G5: GF; G6: scaffold.	There was little difference in BS-1 lectin staining after all transplantations except for transplantation of collagen only.
PGP9.5 IHC staining	Murakami M (2015)	G1: ADSCs/GF/scaffold; G2: BMSCs/GF/scaffold; G3: DPSCs/GF/scaffold.	Higher in G3 than G2 and G1.
	Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third); G2: ADSCs/scaffold (apical third), GF/collagen (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	Positive staining, with no significant differences in neovascularization in the regenerated tissues of all groups.
TRH-DE staining	Murakami M (2015)	G1: ADSCs/GF/scaffold; G2: BMSCs/GF/scaffold; G3: DPSCs/GF/scaffold.	Similar expression in G1-3.
Double staining DSPP	Murakami M (2015)	G1: ADSCs/GF/scaffold; G2: BMSCs/GF/scaffold; G3: DPSCs/GF/scaffold.	Positive in cell lining of dentinal wall in G1, G2 and some area of pulp-tissue.
	Iohara K (2013)	G1: DPSCs/GF/scaffold; G2: un-fractioned DPSCs/GF/Scaffold; G3: DPSCs; G4: un-fractioned DPSCs/scaffold; G5: GF; G6: scaffold.	Positive in G1.
	Kuang R (2016)	G1: hpDPSC/microsphere; G2: DPSCs/microsphere.	Strongly positive in G1, a weaker staining in G2.
mRNA expression (Enamelysin/MMP20)	Iohara K (2013)	G1: DPSCs/GF/scaffold; G2: un-fractioned DPSCs/GF/scaffold; G3: DPSCs; G4: un-fractioned DPSCs/scaffold; G5: GF; G6: scaffold.	Positive in G1.
	Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third); G2: ADSCs/scaffold (apical third), GF/scaffold (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	Higher expression in odontoblastic layer along dentinal wall and some part of the regenerated tissue in G2.
	Iohara K (2011)	G1: DSCs $CD105^+$/GF/scaffold; G2: ADSCs $CD105^+$/GF/scaffold; G3: DSCs/GF/scaffold; G4: GF/scaffold; G5: DSCs $CD105^+$/scaffold; G6: Scaffold; G7: Control.	Positive in G1.
mRNA expression (Angiogenic/neurotrophic factors)	Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third); G2: ADSCs/scaffold (apical third), GF/scaffold (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	High expression of VEGF, MMP3, GM-CSF, Neuropeptide and BDNF in G1.
mRNA expression of (Periodontal marker)	Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third); G2: ADSCs/scaffold (apical third), GF/scaffold (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	Lower expression of periostin mRNA in all groups than that in the normal periodontal ligament.
	Iohara K (2011)	G1: DPSCs $CD105^+$/GF/scaffold; G2: ADSCs $CD105^+$/GF/scaffold; G3: DPSCs/GF/scaffolds; G4: GF/scaffold; G5: DPSCs $CD105^+$/scaffold; G6: scaffold; G7: Control.	G1: Higher expression of axin2, periostin, and asporin/PLAP-1 mRNA (25,531-fold, 179-fold, and 11-fold) in normal PDL than G1, and lower in normal pulp than G1 (0.4-fold, 0.4-fold, and 2.4-fold, respectively).
	Iohara K (2013)	G1: DSCs/GF/scaffold; G2: un-fractioned/GF/Scaffold; G3: DPSCs; G4: Un-fractioned/scaffold; G5: GF; G6: Scaffold.	Higher expression levels of periostin and *PLAP-1* were (47.5-fold and 8.9-fold, respectively) in the normal PDL than G1.
mRNA expression (Bone marker)	Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/scaffold (middle third); G2: ADSCs/scaffold (apical third), GF/scaffold (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	Adipose tissue and bone markers were not expressed.

	mRNA expression (Pulp marker)	Ishizaka R (2012)	G1: DPSCs/scaffold (apical third), GF/collagen (middle third); G2: ADSCs/scaffold (apical third), GF/scaffold (middle third); G3: BMSCs/scaffold (apical third), GF/scaffold (middle third).	Syndecan3 and TRH-DE were similarly expressed in all groups.
		Iohara K (2011)	G1: DPSCs CD105$^+$/GF/scaffold; G2: ADSCs CD105$^+$/GF/scaffold; G3: DSCs/GF/scaffold; G4: GF/scaffolds; G5: DPSCs CD105$^+$/scaffold; G6: scaffold; G7: Control.	More expression of Syndecan3 (14.3 times) and Tenascin C (50.0 times) in G1 than periodontal ligament, although those expressions in G1 were similar to those in normal pulp.
		Iohara K (2013)	G1: DSCs/GF/scaffold; G2: Un-fractioned/GF/Scaffold; G3: DPSCs; G4: Un-fractioned/scaffold; G5: GF; G6: Scaffold.	Higher expression of tenascin C (62.5 times), syndecan 3 (7.7 times), and TRH-DE (5.0 times) in G1 than periodontal ligament. Similar expression levels to those in normal pulp.
	Regenerated area	Iohara K (2013)	G1: DSCs/GF/scaffold; G2: Un-fractioned/GF/Scaffold; G3: DPSCs; G4: Un-fractioned/scaffold; G5: GF; G6: Scaffold.	Larger area in G1 than G6 (4.6-fold), G3 (3.1-fold), and G5 (3.3-fold). G2 resulted in less regenerated pulp tissue (0.6-fold) than G1.
		Ishizaka R (2012)	G1: DPSCs/collagen (apical third), GF/collagen (middle third); G2: ADSCs/collagen (apical third), GF/collagen (middle third); G3: BMSCs/collagen (apical third), GF/collagen (middle third).	G1>G2>G3.
		Iohara K (2014)	G1: DPSCs/GF/scaffold; G2: Control.	Cover ~60% of the root canal total area, which was reduced on 120 d than the previous result in young Teeth (90% on 60 d).
		Iohara K (2011)	G1: DSCs CD105$^+$/GF/scaffold; G2: ADSCs CD105$^+$/GF/scaffold; G3: DSCs/GF/scaffold; G4: GF/scaffold; G5: DSCs CD105$^+$/scaffold; G6: scaffold; G7: Control.	G1>G3>G5>G4>G6>G2.
Regenerative procedure in immature teeth	H&E staining	Al Eshiry EA (2018)	G1: DPSCs/GF/scaffold; G2: GF/scaffold.	Greater pulp-like tissue/fibrous tissue with detached pulp stone and blood vessels/non-organized odontoblast-like cells in G1; no soft tissues in G2/and non-organized odontoblast-like cells in G2.
		Wang Y (2013)	G1: DPSCs/scaffold; G2: Scaffold; G3: DPSCs.	Higher pulp-like tissue/odontoblast-like cells along the radicular inner wall in G1, some fiber-like tissue in G2, no tissue in G3.
		Ling L (2020)	G1: DPSCs/scaffold; G2: iDPSCs/scaffold; G3: Control.	Pulp-like tissue up to coronal (40% G1, 30% G2), Middle (20% in G1-2), apical third (40% G1, 50% G2). Osteoblast-like/odontoblast-like cells in G1-2.
		Zhu W (2013)	G1: Blood clot; G2: DPSCs; G3: PRP; G4: DPSCs/PRP; G5: Control.	Vital tissues (new hard tissues/blood vessel in a matrix of fibrous connective tissue). % vital tissue area to the total canal area (G1: 34; G2: 48; G3: 75; and G4: 70); Cementum-like tissue % (G1: 50; G2: 100; G3: 60; G4: 100); Bone-like tissue % (G1: 50; G2: 60; G3: 60; G4: 70).
Pulpotomy	H&E staining	Sueyama Y (2017)	G1: BMSCs-endothelial cells/scaffold; G2: BMSCs/scaffold; G3: scaffold; G4: Pulpotomy; G5: Control.	Pulp healing/complete dentin bridge formation in G1, Incomplete, thinner dentin bridges in G2. Hard tissue formation at the border between the implanted area and the remaining pulp in G3. No pulp tissue in G4.
		Kaneko T (2019)	G1: BMSC-lacz/scaffold; G2: BMSCs vector/scaffold; G3: scaffold; G4: Control.	Pulp-like tissue/almost complete dentin bridge formation in G1-2. Incomplete mineralized tissue formation at the border between the implanted area and the remaining pulp in G3. Mineralized tissue formation at the border between the MTA-filled area and the remaining pulp in G4.
		Jia W (2016)	G1: MTA; G2: scaffold; G3: DPSCs/scaffold; G4: Simvastatin/DPSCs/Scaffold.	Dental bridge formed/pulp-like tissue/odontoblast-like cells in G1; a small amount of pulp-like tissue/calcification in G2; pulp-like tissue in G3; pulp-like tissue/odontoblast-like cells in G4; Pulp regenerated area: G2 (47.3%), G3 (76.8%), G4 (85.8%). Apical closure in all groups.
	Matrix formation (Masson trichome staining)	Mangione F (2017)	G1: DPSCs/scaffold; G2: Scaffold.	G1-2: The reparative bridge closed the root canal entrance, no difference.
	DSP expression	Mangione F (2017)	G1: DPSCs/scaffold; G2: Scaffold.	Cell with a palisade organization, DSP expression in G1.

Direct Pulp Capping	H&E staining	Obeid M (2013)	G1: MTA; G2: BMSCs/HA-TCP; G3: HA-TCP.	G1: Calcific bridge with some irregularities/no soft-tissue inclusions in G1; stem cells residing beneath the exposure site/osteodentin in G2; superficial necrosis/chronic inflammatory cell infiltration in G3.
		Ji YM (2009)	G1: GIC; G2: DPSCs/GIC; G3: calcium hydroxide/GIC; G4: Calcium hydroxide treated DPSCs/GIC.	Necrotic tissue debris inflammatory cell mixture above the dentin layer in G3; dentin-like tissue in G4.
		Mohajeri SF (2022)	G1: MTA; G2: Scaffold; G3: BMSCs/scaffold.	Continuous and complete dentinal bridge/odontoblast-like cell/osteodentin in some cases/mild inflammation/no pulp necrosis in G1; No dentinal bridge/mild osteodentin/no odontoblast-like cell/no pulp necrosis/moderate-severe inflammation in some cases. The mean thickness of dentinal bridge (G1: 0.12 μm; G2: 0.00 μm; G3: 0.06 μm).
		Zheng Y (2012)	G1: Calcium hydroxide; G2: Scaffold; G3: GFP^+ DPSCs/scaffold.	Dentin-like tissue which didn't cover the root canal orifice completely with uneven thickness/inflammatory cells and blood vessel dilation in pulp tissue in G1; dentin-like tissue only on the root canal orifice/no osteodentin formation/pulp tissue with some blood vessel dilation in G2; uniform dentin-like tissue with higher thickness/no inflammation and blood vessel dilation/some osteodentin formation in G3; % regenerated dentin area: G1 (13.9), G2 (34.6), G3 (81.4).

ADSCs, Adipose derived stem cells; BMSCs, Bone marrow stem cells; DPSCs, Dental pulp stem cells; DPSCs sEV, Extracellular vesicles derived from dental pulp stem cells; hpDPSCs, hypoxia-treated dental pulp stem cells; H&E, Hematoxylin and eosin; MTA, Mineral trioxide aggregates; GIC, Glass ionomer cement; GF, Growth factors; HA, hydroxyapatite; L-DPSCs sEV, Extracellular vesicles derived from lipopolysaccharide preconditioned dental pulp stem cells; PRP, Platelet rich plasma; DSP, Dentin sialophosphate; VEGF; Vascular endothelial growth factor.

moting the formation of pulp-like tissue; 3) Implantation of CD31$^-$ DPSCs/growth factor/scaffold showed more effective pulp-like tissue regeneration compared to CD31$^-$ ADSCs/growth factors/scaffold and CD31$^-$ BMSCs/growth factors/scaffold [27, 29]; 4) Extracellular vesicles derived from lipopolysaccharide (LPS)-preconditioned DPSCs/BMSCs/scaffold resulted in greater regeneration of pulp-like tissue compared to the use of extracellular vesicles derived from DPSCs or BMSCs alone [31]; 5) Transplantation of the DPSCs/growth factors/scaffold demonstrated superior results compared to the use of growth factors alone [28], DPSCs alone [28] or scaffold alone [27, 28]. However, applying DPSCs, PRP, or the combination of DPSCs/PRP led to the formation of PDL-like tissue in the canal space [35].

Dentin-like tissue by H&E staining: The presence of odontoblast-like cells was identified through the following approaches: 1) Transplantation of DPSCs in combination with growth factors/scaffold when compared to the control, demonstrated the detection of odontoblast-like cells. Notably, in one study, transplantation of DPSCs from an aged donor led to the formation of odontoblast-like cells in the radicular dentin and osteodentin in the coronal part [34]; 2) Transplantation of CD105$^+$ DPSCs with growth factors/scaffold yielded superior results compared to various conditions, including transplantation of DPSCs/growth factors/scaffold, CD105$^+$ DPSCs/scaffold, CD105$^+$ ADSCs/growth factors/scaffold or scaffold alone [28].

Systemic administration of BMSCs [32] and transplantation of hypoxia-treated DPSCs [30, 33] resulted in the formation of both osteoblast-like and odontoblast-like cells within the canal space. Implantation of DPSCs, PRP or the combination of DPSCs/PRP led to the development of bone-like and cementum-like tissues within the canal space [35].

Matrix formation: Matrix formation assessed through trichrome staining was investigated in three studies. The following approaches demonstrated enhanced matrix formation: 1) Transplantation of CD31$^-$ DPSCs with growth factors/scaffold exhibited higher matrix formation compared to CD31$^-$ ADSCs with growth factors/scaffold and CD31$^-$ BMSCs with growth factors/scaffold [26, 29]. 2) Extracellular vesicles derived from LPS-preconditioned DPSCs/BMSCs with scaffold resulted in greater matrix formation when compared to the matrix formation observed with extracellular vesicles released by DPSCs or BMSCs alone [31].

Vascularization: The assessment of vascularization through BS1-lectin staining was conducted in four studies. In comparing CD31$^-$ DPSCs with growth factors/scaffold to CD31$^-$ ADSCs with growth factors/scaffold and CD31$^-$ BMSC with growth factors/scaffold, DPSCs demonstrated superior results in one study [26]. However, another study indicated similar outcomes among these stem cells [28]. Moreover, implantation of DPSCs from an aged donor in combination with growth factors/scaffold exhibited positive staining [34]. In addition, transplantation of DPSCs with growth factors/scaffold, DPSCs alone, or growth factors resulted in more pronounced staining compared to the transplantation of scaffold alone [36].

Neurogenesis: Neurogenesis was evaluated in two papers through PGP9.5 staining. In one study, transplantation of 31$^-$ DPSCs with growth factors/scaffold resulted in greater neural staining compared to CD31$^-$ ADSCs with growth factors/scaffold and CD31$^-$ BMSCs with growth factors/scaffold [26]. However, another study indicated similar outcomes among three types of stem cells [28].

DSPP staining: Positive DSPP staining was observed in the following approaches: 1) Transplantation of CD31$^-$ ADSCs with growth factors/scaffold and CD31$^-$ BMSCs with growth factors/scaffold compared to CD31$^-$ DPSCs with growth factors/scaffold [26]. 2) Transplantation of hypoxia-treated DPSCs, when compared to normal DPSCs [33]. 3) Implantation of DPSCs with growth factors/scaffold demonstrated more positive staining than implantation of DPSCs alone, growth factor alone or scaffold alone [36].

mRNA expression: Enamelysin/MMP20 exhibited greater expression in the following scenarios: 1) Transplantation of CD31$^-$ ADSCs with growth factors/scaffold and CD31$^-$ BMSCs with growth factors/scaffold compared to transplantation of CD31$^-$ DPSCs growth factors/scaffold [26]. 2) Implantation of DPSCs with growth factors/scaffold compared to implantation of growth factors or scaffold alone [36]. 3) Transplantation of CD105$^+$ DPSCs with growth

factors/scaffold compared to DPSCs with growth factors/scaffold, DPSCs alone, growth factors alone or scaffold alone [28].

Angiogenic/neurotrophic factors expression: Higher expression of angiogenic/neurotrophic factors was observed after the implantation of $CD31^-$ ADSCs with growth factors/scaffold compared to the transplantation of $CD31^-$ BMSCs with growth factors/scaffold and $CD31^-$ DPSCs with growth factors/scaffold [29].

Regenerated pulp area to total canal volume: Greater regenerated pulp area to total canal volume in the included studies was reported in the following situations: 1) Transplantation of $CD31^-$ DPSCs with growth factors/scaffold compared to the implantation of $CD31^-$ ADSCs with growth factors/scaffold and $CD31^-$ BMSCs with growth factors/scaffold [26]. 2) Transplantation of DPSCs with growth factors/scaffold in an aged donor compared to a young donor [34]. 3) Transplantation of DPSC with growth factors/scaffold compared to DPSCs alone, growth factor alone or scaffold alone [36]. 4) Transplantation of $CD105^+$ DPSCs with growth factors/scaffold compared to DPSCs with growth factors/scaffold, DPSCs alone, growth factors alone or scaffold alone [28].

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Pulp-like tissue by H&E staining: The formation of pulp-like tissue with blood vessels was observed through the following approaches: 1) Transplantation of DPSCs with growth factors/scaffold, compared to transplantation of growth factors/scaffold alone [40]. 2) Transplantation of DPSCs with scaffold, when compared to transplantation of scaffold alone; implantation of only scaffold resulted in the formation of fibrous tissue [39].

In addition, implantation of DPSCs from inflamed/non-inflamed pulp with scaffold resulted in the formation of pulp-like tissue in the canal space [38].

Dentin-like tissue by H&E staining: Observation of odontoblast-like cells occurred with the transplantation of DPSCs with scaffold, compared to transplantation of scaffold alone, or DPSCs alone [39]. Non-tubular odontoblast cells were formed when either DPSCs with a combination of growth factor/scaffold or growth factors/scaffold were implanted [40]. The formation of odontoblast-like and osteoblast-like cells was observed when DPSCs (from normal or inflamed pulp) with scaffold were transplanted [38]. In another study, transplantation of DPSCs, PRP or DPSCs/PRP resulted in the formation of bone-like or cementum-like tissues [41].

Vital pulp therapy (pulpotomy)

Pulp-like tissue formation was achieved through the following approaches: 1) Transplantation of simvastatin-treated DPSCs with a scaffold and DPSCs with a scaffold compared to BMSCs/scaffold or scaffold alone [44]. 2) Transplantation of BMSCs with a scaffold compared to scaffold transplantation alone [43]. However, transplantation of BMSCs with endothelial cells in combination with a scaffold resulted in pulp healing when compared to transplantation of BMSCs with a scaffold or scaffold alone [42]. Transplantation of DPSCs with a scaffold resulted in a greater volume of dentin formation compared to the scaffold-only group, found only at the canal orifice [37].

Vital pulp therapy (DPC)

Complete dentinal bridge formation was observed through the following approaches: 1) Implantation of BMSCs with scaffold resulted in the formation of osteodentin, compared to pulp capping with mineral trioxide aggregate (MTA), which led to the formation of odontoblast-like cells [46, 48]. 2) Transplantation of DPSCs with scaffold showed similar results to the scaffold implantation, indicating reparative bridge formation [45]. 3) Transplantation of calcium-hydroxide treated cells resulted in the formation of odontoblast-like cells compared to DPSCs alone [47].

Radiographic evaluation

Table 6 presents the radiographic findings of the included studies as follows.

Regenerative endodontic procedure in mature teeth

1) Transplantation of DPSCs from an aged donor in combination with growth factor/scaffold resulted in the obliteration of the enlarged

Table 6. Radiographic findings of the included studies

Treatment approach	Author (Year)	Technique used	Groups	Findings
Regenerative procedure in mature teeth	Iohara K (2014)	Periapical	G1: DPSCs/GF scaffold; G2: scaffold	Obliteration of the enlarged apical portion following pulpectomy.
	Iohara K (2016)	MRI	G1: pulpectomy; G2: Control; G3: DPSCs/scaffold/GF; G4: Scaffold	Higher SI in G3-4 than others at 1 d; Lower SI in the apical part and higher SI in the coronal part in G4 at 90 d. Similar SI to the normal pulp in G3 at 180 d.
Regenerative procedure in mature teeth	Al Eshiry EA (2018)	Periapical	G1: DPSCs/GF/Scaffold; G2: GF/Scaffold	Healed periapical lucency: 83.33% in both groups. Radicular thickening: G1 (91.67%); G2 (25%). Radicular lengthening: G1 (75%); G2 (16.67%). Apical closure: G1 (75%); G2 (16.67%).
	Wang Y (2013)	Periapical	G1: DPSC/scaffold; G2: scaffold; G3: DPSCs	Complete root development (continuity of root length and closure of the apical foramen) was detected; G1 (all), G2 (4), G3 (0). Radicular thickening and foramen closure was observed in G1.
	Ling L (2020)	Periapical	G1: DPSCs/scaffold; G2: iDPSCs/scaffold; G3: Control	No periradicular lesion/root development in all groups.
	Zhu W (2013)	Periapical	G1: Blood clot; G2: DPSCs; G3: PRP; G4: DPSCs/PRP; G5: Control	Periapical healing: G1 (90%); G2 (80%); G3 (100%); G4 (90%); Root thickening: G1 (60%); G2 (100%); G3 (30%); G4 (90%); no increased root thickness in the control.
Pulpotomy	Mangione F (2017)	Micro CT	G1: DPSCs/scaffold; G2: Scaffold	Reparative mineralized bridge formation at each root canal entrance in G1-2; lower mineralized volume/less dense/higher porosity percentages/more connected non-mineralized areas/affected the microarchitecture of the dentin bridge in G1.
Direct pulp capping	Obeid M (2013)	CBCT	G1: MTA; G2: BMSCs/Scaffold; G3: Scaffold	Formation of a calcific, thick, and mostly continuous barrier in G1-2, faint radiopaque patches were observed within the radiolucent pulp space (intrapulpal calcification) in G3.
	Zheng Y (2012)	CT	G1: Calcium hydroxide; G2: Scaffold; G3: GFP^+/DPSCs/scaffold	G3: Almost complete dentin regeneration, even on the roof of the pulp chamber in G3; less regeneration in G2; very little alteration in G1.
	Jia W (2016)	Periapical	G1: MTA; G2: scaffold; G3: DPSCs/scaffold; G4: Simvastatin/DPSCs/scaffold	Increased root length/apical closure in all groups.

ADSCs, Adipose derived stem cells; BMSCs, Bone marrow stem cells; CT, Computed tomography; CBCT, Cone beam computed tomography; DPSCs, Dental pulp stem cells; GF, Growth factors; MTA, Mineral trioxide aggregates; HA, Hydroxy appetite; iDPSCs, Dental pulp stem cells from inflamed pulp; MRI, Magnetic resonance imaging; PRP, Platelet rich plasma; SI, Signal intensity.

apical portion [34]. 2) Transplantation of DPSCs with growth factors/scaffold when compared to transplantation of scaffold alone or the control, showed signal intensity similar to normal pulp [27].

Regenerative endodontic procedure in immature teeth

1) Transplantation of either DPSCs with growth factors/scaffold or growth factors/scaffold led to the healing of periradicular lesion. However, radicular thickening and apical closure were more pronounced when DPSCs were used [40]. 2) When DPSCs were implanted with scaffold, greater radicular thickening and apical closure were observed compared to the implantation of DPSCs or scaffold alone [39]. 3) Transplantation of DPSCs from inflamed or non-inflamed pulp, in combination with scaffold, resulted in complete root development [38].

Vital pulp therapy (pulpotomy)

Transplantation of DPSCs with scaffold or scaffold alone resulted in the formation of a reparative mineralized bridge at the root canal entrance. However, when DPSCs were implanted, a dentinal bridge with lower mineralized volume/density and a higher porosity percentage was observed [37].

Vital pulp therapy (DPC)

In one study, transplantation of BMSCs with a scaffold or MTA led to the formation of a calcific continuous thick barrier. However, using the scaffold alone resulted in the faint radiopaque patches within the radiolucent pulp space [46]. Additionally, almost complete dentin regeneration was observed when DPSCs with a scaffold were implanted [45].

Discussion

The regeneration of the dentin-pulp complex through stem/progenitor cell transplantation represents a challenging yet critical goal in regenerative endodontics [8]. This review systematically assesses the growing body of literature, aiming to synthesize comprehensive findings on different outcomes associated with the transplantation of stem cells for dentin-pulp regeneration in both mature and immature teeth. The study incorporates twenty-three animal experiments, characterized by a predominantly high risk of bias, to evaluate the impact of stem cell transplantation across three treatment approaches: pulpectomy in mature/immature teeth, pulpotomy, and DPC.

The reviewed studies exhibit various methodological limitations. Firstly, diverse models were employed, encompassing different animals, carriers/scaffolds, cellular sources, and experimental pulpal defects. Secondly, the studies demonstrated a high risk of selection, performance, detection, and reporting bias.

Recent studies have explored various stem cell types from different sources for regenerating the dentin-pulp complex, with DPSCs being the preferred choice due to their regenerative capacity [49]. Our review emphasizes that application some subfractions of DPSCs, such as $CD31^-$ or $CD105^+$, combined with growth factor and scaffold, had greater efficacy in regenerating pulp-like tissue. In addition, transplantation of these DPSCs subfractions with growth factors resulted in a much higher volume of regenerated pulp tissue compared with transplantation of DPSCs subfractions only; this could be due to the effect of SDF-1-CXCR4 axis on homing of CXCR4+ stem cells during pulp regeneration [50].

It has been shown that MSCs cultured under hypoxic conditions could improve their regenerative potential in various tissues; several studies found that the hypoxic condition of MSCs can improve vascular tube formation [51] and neurogenesis [52]. Researchers demonstrated that 3D culturing of conditions human DPSCs with nanofibrous microspheres under hypoxic conditions resulted in the activation of the HIF-1α in DPSCs, and thus, up-regulating vascular endothelial growth factor (VEGF) expression [33]. In the current review, we found appropriate pulp-tissue formation after applying hypoxia-treated DPSCs; however, this treatment approach led to the formation of osteoblast-like and odontoblast-like cells in the canal space [30, 33].

The presence of osteoblast-like and odontoblast-like cells in the root canal space is paramount in the context of dentin-pulp regeneration, contributing to the formation of bone-like and dentin-like tissues, respectively. The functional impact of these cells on the overall archi-

tecture and composition of the regenerated tissue within the root canal is clear. This holds significant clinical relevance, underscoring the imperative for precise control over differentiation pathways during regenerative procedures. The intricate involvement of osteoblast-like and odontoblast-like cells emphasizes the necessity for targeted modulation to enhance the success of dentin-pulp regeneration in clinical settings.

Extracellular vesicles released by LPS-preconditioned DPSCs, in combination with BMSCs, demonstrated increased regeneration of pulp-like tissue in the root canal space potentially through the regulation of inflammatory mediators and complementary proteins. This approach may create a mild inflammatory microenvironment, enhancing immunomodulation and anti-inflammatory functions during healing. In addition, this approach might mediate the proliferation, migration, and differentiation of BMSCs [31].

Donor age emerged as a factor influencing the regeneration capacity of stem cells [34]. It has been reported that there was little difference in the regenerative potential of stem cells derived from old or young donors. Since using autologous stem cells is a priority to regulatory policies, there are limitations to harvesting autologous stem cells in elderly patients [53]. In the current review, we found that DPSCs derived from aged donors led to the formation of pulp-like tissue; however, the volume of the regenerated area might be lesser than those obtained from the young donor [34].

Clinically, various vital pulp therapies can be applied to treat pulpitis, and calcium-enriched mixture (CEM) cement or MTA is widely used for DPC and pulpotomy because of their known properties to induce hard tissue repair [3, 54-59]. The cell transplantation approach was examined regarding pulp-tissue regeneration in pulpotomy treatment; Simvastatin-treated DPSCs, when introduced into the pulp chamber, showed promise in coronal pulp and dentin regeneration [44].

Co-transplantation of BMSCs and endothelial cells into pulp chambers and filling with MTA facilitated coronal pulp regeneration and induced the formation of a complete dentin bridge, outperforming the results obtained with BMSCs alone [42]. The choice of scaffold and/or growth factors in these transplantation approaches significantly influenced outcomes. When BMSCs were used combined with PLLA scaffold/matrigel in another study [43], pulp-like tissue and an almost complete dentin bridge were formed. It seems that the use of appropriate scaffold and/or growth factors should also be considered in the cell transplantation approach.

It has been reported that DPC with MTA resulted in forming a greater calcified bridge than that of BMSCs and the hybrid scaffold [48], highlighting the influence of MTA's physical and bioactive properties [60]. However, BMSCs transplantation exhibited hard tissue formation in several samples, suggesting the need for further research on additional factors, such as growth factors, different scaffolds, and cell implantation conditions.

Culturing stem cells traditionally involves fetal bovine serum, raising immunologic response risks. The application of human platelet lysate as an autologous medium offers a potential alternative [61]. The importance of cell concentration in stem cell transplantation for dentin-pulp regeneration is underscored, emphasizing the challenges of estimating precise doses and the direct relationship between cell concentrations and scaffold design.

The carriers/scaffolds selected for the delivery of stem/progenitor cells are believed to influence the regeneration outcomes. In the regeneration process, suitable scaffolds should be designed properly to mimic the native environment [62, 63]. Given the complexity of the pulpal root canal anatomy, injectable carriers/scaffolds have been recommended for the regeneration of the dentin-pulp complex. In regenerative endodontics, numerous scaffolds were examined in in-vitro or ex-vivo studies. However, the included investigations used ß-tricalcium phosphate, collagen, chitosan hydrogel, gelatin sponge, PRP or scaffold-free cell pellets; the impact of the characteristics of scaffolds/carrier on the transplanted stem/progenitor regenerative outcomes remains unclear.

PRP was suggested as a potential scaffold, but it did not enhance the regeneration of pulp-like tissues in some studies, leading to the forma-

tion of cementum-like, PDL-like, and bone-like tissues. The type and concentration of growth factors combined with PRP/DPSCs may influence results [35]. In contrast, the collagen scaffold to carry DPSCs into the canals may provide superior results when compared to that of PRP/DPSCs [28]. PRP contains several growth factors (i.e., transforming growth factor beta 1, PDGF, FGF, VEGF, and epidermal growth factor) [64]. The application of specific growth factors with a defined concentration may influence the results.

Growth factors, including G-CSF, SDF-1 and a combination of PDGF/NGF/bFGF/BMP-7, enhanced regeneration outcomes in reviewed studies. G-CSF is one of the most used growth factors, and it has been approved by the Pharmaceuticals and Medical Devices Agency, Japan, and the U.S. Food and Drug Administration for decreasing the incidence of infection. It has been shown to be safe with only a few well-described side effects. G-CSF has therapeutic potential in neurogenesis and angiogenesis in experimental stroke models [65], retinal ganglion cell axotomy models [66], and spinal cord injury models [67].

Despite these advancements, the clinical viability of pulp regeneration therapy using cell transplantation faces challenges, including difficulties with regulatory approval, high cost associated with storage (cell cryopreservation/banking system) and packaging, and risks of immune rejection, pathogen transmission, and tumorigenesis during engraftment.

Histological examination was employed to investigate the efficacy of dentin-pulp regeneration, encompassing various sub-outcomes such as dentine, pulpal connective tissue, vascular, and neural regeneration. The majority of results were assessed qualitatively, with only a limited number of studies utilizing quantitative evaluations. The challenge of quantitatively assessing successful pulp-dentin complex regeneration persists. Furthermore, the choice of animal models lacked standardization, and there is a dearth of compelling evidence indicating which models more accurately replicate clinical conditions.

This review, based on 23 animal studies featuring diverse designs (including cell types, carriers/scaffolds, growth factors, animal models, tested outcomes, and regenerative treatment approaches), coupled with a high risk of bias, results in overall low-quality evidence.

Potential clinical implications

The systematic review presents a comprehensive assessment of the current literature on dentin-pulp regeneration, offering valuable insights into potential clinical applications of stem cell-based therapies. Advancements in stem cell therapies, particularly employing DPSCs and various growth factors, hold promise for innovative strategies in challenging cases of pulpitis and dentin-pulp injuries. Utilizing the regenerative capacity of stem cells, combined with tailored scaffolds and growth factors, may enhance the success rates of regenerative endodontic procedures, marking a paradigm shift towards biologically driven regenerative solutions. Examples, such as the application of hypoxia-treated DPSCs and LPS-preconditioned DPSCs, underscore the importance of optimizing the microenvironment for enhanced pulp-tissue regeneration. These findings highlight the role of inflammatory modulation and suggest tailored preconditioning methods, providing clinicians with avenues to enhance the therapeutic potential of transplanted stem cells. Additionally, insights into the impact of donor age emphasize the need for careful consideration in stem cell source selection. While autologous stem cells are favored, age-related differences in regenerative potential suggest exploring alternative approaches or combination therapies, especially for aged donor-derived stem cells.

Limitations of the study

While aiming for a comprehensive analysis of dentin-pulp regeneration through stem/progenitor cell transplantation, inherent heterogeneity across studies poses challenges in drawing definitive conclusions. Varied experimental designs, animal models, stem cell types, scaffolds, and growth factors, coupled with the identified high risk of bias, hinder result uniformity. Future research adopting rigorous methodologies is crucial for reliability in regenerative endodontics. Lack of standardization in animal models, outcomes, diverse histological parameters, and short-term focus warrants cautious extrapolation to clinical scenarios. Inadequate temporal assessment of regeneration necessi-

tates addressing sustained efficacy and durability, which is crucial for clinical translation.

Prospects of the study

Promising prospects in advancing dentin-pulp regeneration through stem/progenitor cell transplantation involve identifying specific subfractions of dental pulp stem cells (e.g., $CD31^-$ or $CD105^+$), enhancing efficacy with growth factors and scaffolds for targeted interventions. Exploration of hypoxia-treated dental pulp stem cells suggests a potential avenue for improving regenerative potential. Activation of HIF-1α and upregulation of VEGF expression under hypoxic conditions present valuable mechanisms for more effective dentin-pulp regeneration. The use of extracellular vesicles from LPS-preconditioned dental pulp stem cells, especially with bone marrow-derived mesenchymal stem cells, showcases intriguing approaches. Modulating inflammatory mediators and complementary proteins contributes to a favorable microenvironment, emphasizing immunomodulation in enhancing the regenerative process. We also underscore the necessity for future research to discern the specific characteristics of various scaffolds contributing to optimal regenerative outcomes. Additionally, stem cell transplantation in aged donors raises critical considerations, urging understanding of outcomes in aged populations for clinical translation.

Conclusion

The present systematic review highlights the potential of stem/progenitor transplantation as a promising therapeutic avenue for achieving functional dentin-pulp regeneration. Nevertheless, future investigations are imperative to address critical aspects: *i*) the development of serum- and animal product-free culturing media for cell culture; *ii*) comprehensive evaluation of possible adverse/side effects associated with the stem/progenitor transplantation approach; *iii*) identification of appropriate carriers/scaffolds with optimal characteristics, either independently or in conjunction with growth factors/signaling molecules; and *iv*) utilization of defined and measurable histological parameters to ensure the generation of comparable and reliable results. These considerations underscore the need for future research to refine and advance the application of stem/progenitor cell-mediated approaches in regenerative endodontics.

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Disclosure of conflict of interest

None.

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