

Influence of substrate stiffness on the mechanoregulation of dental pulpal stem cells: A systematic review

Background: Dental pulp stem cells (DPSCs) possess significant regenerative potential, and their biological behavior is strongly influenced by the mechanical properties of the extracellular matrix. Among these, substrate stiffness plays a pivotal role in regulating stem cell fate through mechanotransduction pathways. This systematic review evaluated the influence of substrate stiffness on DPSC behavior and its implications for pulpo-dentin regeneration.

Methods: This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines and prospectively registered in PROSPERO (CRD420251129030). Electronic searches of PubMed/MEDLINE, Cochrane Library, and Google Scholar identified studies evaluating DPSC responses to substrates with varying stiffness. Eight eligible studies were included for qualitative synthesis. Methodological quality was assessed using a modified QUIN-2 risk-of-bias tool.

Results: Substrate stiffness exerted a significant influence on DPSC behavior. Compliant substrates preserved stemness, maintained cellular quiescence, and delayed senescence, whereas stiffer substrates promoted odontogenic and osteogenic differentiation. Intermediate stiffness enhanced paracrine activity and angiogenic potential. Three-dimensional and in vivo studies demonstrated that gradient or tri-phasic scaffolds closely mimicking the native mechanical microenvironment facilitated vascularized pulpo-dentin complex regeneration.

Conclusion: Matrix stiffness is a critical regulator of DPSC function and should be considered an essential design parameter in regenerative endodontic scaffolds. Biomimetic scaffolds incorporating spatial mechanical gradients may provide a promising strategy for achieving predictable pulpo-dentin regeneration.

Keywords: Dental pulp stem cells; extracellular matrix; mechanobiology; pulpo-dentin regeneration; scaffold design; substrate stiffness.

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I. Introduction

The untreated caries and the trauma on the teeth usually causes destruction of the pulp, the conventional treatment can treat but the pulp is replaced by inert material which does not provide the biological environment necessary. This critical limitation has driven the field of regenerative endodontics to seek ways to completely reconstitute the functional pulpo-dentin complex.^[1] Achieving true regeneration relies on a triad of dental pulp stem cells (DPSCs), bioactive signaling molecules, and carefully engineered scaffolds.

Dental pulp stem cells (DPSCs) are central to these strategies due to their high proliferative capacity and multilineage differentiation potential. DPSCs can differentiate into odontoblasts, osteoblasts, chondrocytes, endothelial, and neuronal-like cells,^[2,3] making them particularly suitable for pulpo-dentin regeneration.

Beyond biochemical signals, the extracellular matrix (ECM) provides critical biophysical cues that regulate stem cell behavior. Among these, substrate stiffness has emerged as a key determinant of cell fate. The pulp–dentin complex exhibits a pronounced mechanical gradient, ranging from approximately 0.5–5 kPa in lowest stiffness pulp tissue to 150000–20000 kPa in mineralized dentin.^[4] Through mechanotransduction pathways, cells convert these mechanical signals into intracellular responses that influence proliferation, migration, and lineage commitment.^[5, 6]

The 3D technology targeted developments of scaffold that is lowest in stiffness in the core intermediate in the perivascular region and rigid on the surface.^[7] However, many experimental models remain limited to two-dimensional systems and lack vascular or neural integration, while key signaling pathways such as Wnt/ β -catenin and NF- κ B require further clarification.^[7]

The aim of this systematic review is to evaluate the influence of substrate and scaffold stiffness on DPSC behavior and pulpo-dentin regeneration, synthesize available evidence, and identify mechanotransductive pathways relevant to scaffold design and regenerative endodontic applications. Although individual experimental studies have demonstrated the influence of matrix mechanics on DPSC behavior, the available evidence remains fragmented across different scaffold systems, stiffness ranges, and biological outcomes. To date, no

comprehensive synthesis has critically evaluated how substrate stiffness regulates DPSC fate and its translational significance for pulpo-dentin regeneration.

II. Method

Protocol and Registration

This systematic review followed the PRISMA 2020 guidelines.^[8] The protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO 2025 CRD420251129030). The full protocol, including eligibility criteria, search strategy, and planned analyses, can be accessed at <https://www.crd.york.ac.uk/PROSPERO/view/CRD420251129030>

Eligibility Criteria

The inclusion and exclusion criteria for this systematic review were established using the PICOS framework to capture relevant literature published in English-language peer-reviewed journals between January 2000 and February 2025. Study inclusion and exclusion criteria were established using the PICOS (Population, Intervention, Comparison, Outcome, Study design) framework

- **Population (P):** Dental pulp stem cells (DPSCs) derived from human or animal models, cultured in either two-dimensional (2D) or three-dimensional (3D) systems.
- **Intervention/Exposure (I):** Exposure of DPSCs to culture substrates or engineered scaffolds with defined, variable elasticities.
- **Comparison (C):** Comparisons between different substrate stiffness conditions (e.g., lowest stiffness, intermediate stiffness, and highest stiff matrices).
- **Outcomes (O):** Quantitative or qualitative changes in DPSC biological behavior, specifically including cellular proliferation, migration, odontogenic/osteogenic differentiation, paracrine signaling, or pulpo-dentin tissue regeneration.
- **Study Design (S):** *In vitro* mechanobiology studies, preclinical animal models, and translational *in vivo* studies

Electronic searches were conducted in PubMed/MEDLINE(90),Cochrane Library(48),Google scholar (n = 7,724). Reference lists of included articles and relevant reviews were also hand-searched.

Information Sources and Search Strategy Comprehensive electronic searches were conducted across three primary databases: PubMed/MEDLINE, Cochrane Library, and Google Scholar. The search captured literature published between January 2000 and February 2025. The search strategy utilized a combination of MeSH terms and free-text keywords connected by Boolean operators ("AND", "OR").

The primary search string utilized was: ("*dental pulp stem cells*" OR "*DPSC*" OR "*human dental pulp stem cells*" OR "*HDPSC*") AND ("*stiffness*" OR "*elasticity*" OR "*substrate stiffness*" OR "*matrix stiffness*" OR "*mechanical properties*" OR "*biomechanics*") AND ("*scaffold*" OR "*extracellular matrix*" OR "*tissue engineering*" OR "*regeneration*").

Appropriate syntax modifications were applied for each database, and the final electronic search was completed on 1 February 2025. Reference lists of included articles were also hand-searched, and during manuscript preparation two additional eligible studies published in April 2025 were identified through citation tracking and added before qualitative synthesis, ensuring inclusion of the most recent evidence.

Study Selection The initial database search yielded 7,862 records (PubMed/MEDLINE = 90, Cochrane Library = 48, Google Scholar = 7,724 and hand searching=2). All records were imported into EndNote X9 (Clarivate Analytics) for reference management, where 1,532 duplicate records were removed. The remaining 6,330 studies underwent title and abstract screening by two independent reviewers, resulting in the exclusion of 5,870 studies that did not meet the eligibility criteria.

Full texts were retrieved for 460 potentially eligible studies, of which 452 were excluded after detailed assessment. Main exclusion reasons were lack of quantitative stiffness data (n = 312), non-DPSC or non-human cells (n = 80), insufficient outcome reporting (n = 57), and non-English publication (n = 3). Discrepancies were resolved by discussion or by a third reviewer, and 8 studies met all criteria for qualitative synthesis.

Methodological Quality and Risk of Bias Assessment

The methodological quality and risk of bias of the included *in vitro* studies were assessed using a modified QUIN framework adapted for DPSC mechanobiology and scaffold-based research. The 12 domains were scored on a 3-point scale: 2 = low risk, 1 = unclear risk, and 0 = high risk, yielding a total score of 0–24. Criteria were calibrated to the mechanobiological context, and studies were classified as low risk (≥ 17), moderate risk (11–16), or high risk (≤ 10). The detailed QUIN-2 adaptation and domain-wise modifications are presented in Table 2.

Data extraction

A standardized data extraction form was used, and two reviewers independently collected study data; disagreements were resolved by discussion or by a third reviewer. Extracted items included study characteristics (author, year, country), experimental design (DPSC source, 2D/3D culture, scaffold/substrate type), biomechanical parameters (stiffness values in kPa), and outcomes (mechanotransduction, migration, proliferation, and lineage-specific differentiation). For narrative synthesis, substrates were grouped within each study as compliant, intermediate, or highest stiffness tested; glass controls were treated as rigid, non-physiological comparators and interpreted qualitatively rather than quantitatively.

Data synthesis

Because the included studies differed substantially in scaffold composition, fabrication methods, stiffness measurement techniques, stiffness ranges, culture systems, mechanotransduction pathways, and outcome measures, quantitative meta-analysis was not appropriate. Findings were therefore synthesized narratively, with studies organized by 2D versus 3D culture and by outcomes such as proliferation, migration, and odontogenic/osteogenic differentiation. Cross-study trends on compliant versus rigid microenvironments were summarized textually and supported by structured tables.

III. RESULTS:

4.1 Study characteristics

A comprehensive search following PRISMA (Figure 1) identified 460 full texts for screening; eight studies met inclusion criteria for qualitative synthesis. Included reports comprised combined *in vitro* and *in vivo* preclinical models and mechanobiology experiments using human dental pulp stem cells (DPSCs) across two-dimensional and three-dimensional culture systems (Table 1). Substrate elasticities were standardized to kilopascals (kPa) for consistency across studies. Where glass was used as a rigid control, its stiffness is reported in kPa for uniformity but interpreted qualitatively because its modulus is orders of magnitude higher than hydrogel-based substrates.

4.2 Risk of bias

Using a modified QUIN-2 rubric as given in table 2, five studies were judged low risk of bias and three studies were judged moderate risk. The moderate ratings primarily reflected incomplete reporting of randomization and blinding. The modified QUIN-2 applied a fixed 12-domain scoring scale (0–24 points) with thresholds for Low (≥ 17), Moderate (11–16), and High (≤ 10) risk; Table 3 indicates the assessment as per the QUIN2 modification.

4.3 Stemness and senescence

Ghaffari & Shrestha demonstrated that culture on the lowest-stiffness substrates (2 kPa in 2D; 0.46–46.9 kPa range in 3D) markedly upregulated stemness-associated genes (NANOG, SOX2) and attenuated cellular senescence markers (reduced SA- β -gal, lower p16, preservation of SIRT1, XRCC5), effects linked to chromatin remodeling and modulation of NF- κ B/Notch signaling^[9]. Gross et al. reported that an intermediate 15 kPa substrate optimally supported secretion of angiogenic cytokines and pulp-like marker expression, indirectly preserving pulp stem-like features at that stiffness^[10]. These findings indicate that the lowest stiffness conditions best preserve DPSC self-renewal and delay senescence, whereas intermediate stiffness supports trophic factor secretion.

4.4 Migration

Ehlinger et al. performed live-cell tracking and durotaxis assays on laminin-coated PDMS and bi-rigidity gels (range 1.5–400 kPa and higher controls) and found that DPSC migration speed did not follow conventional durotaxis.^[11] Migration was weakly influenced by substrate rigidity and depended primarily on Arp2/3 complex dynamics; YAP signaling and focal adhesion accumulation were not the principal drivers of motility in their system.^[11] Datko Williams et al. and other studies reported limited stiffness-dependent changes in migratory behavior, which suggests mechanosensitive migration in DPSCs is atypical and more dependent on actin nucleation machinery than on substrate rigidity.^[12]

4.5 Odontogenic differentiation

Liu et al. demonstrated increased expression of odontoblast/osteoblast markers (DSPP, DMP-1, RUNX2) on higher-stiffness PDMS substrates (1.4, 16, 54, 135 kPa), implicating canonical Wnt/ β -catenin activation in mechanotransduction.^[13] Datko Williams et al. primarily reported increased alkaline phosphatase activity and enhanced mineralization on stiffer substrates (notably ≥ 75 kPa) and showed that inclusion of hydroxyapatite microparticles augmented the mineralized area.^[12] Gross et al. found that 28 kPa favored osteo/odontogenic differentiation with focal adhesion clustering and activation of FAK/ERK pathways.^[10] Collectively, these studies indicate a consistent shift from quiescence toward mineralizing phenotypes as substrate stiffness increases

4.6 Angiogenesis

Li et al. evaluated tri-phasic GelMA hydrogels and reported that medium-stiffness zones (6.37 kPa) optimized angiogenic responses, as evidenced by enhanced endothelial ingrowth and angiogenic marker expression, whereas high-stiffness zones (24.12 kPa) favored odontogenic outcomes.^[15] Gross et al. also identified an intermediate (15 kPa) condition that maximized secretion of angiogenic cytokines relevant to vascularization. These concordant observations support a stiffness-dependent specialization in which intermediate mechanical cues best promote angiogenesis.^[10]

4.7 In vivo regeneration

Woloszyk et al. showed that stiffer PEG-fibrinogen hydrogels (1.05 kPa vs 0.18 kPa) promoted odontogenic gene expression and mineralization while softer gels supported proliferation and pulp-like tissue formation in preclinical models.^[14] Qu et al. demonstrated that nanofibrous gelatin scaffolds with graded stiffness (0.89, 6.10, 18.23 kPa) enabled regeneration of a vascularized pulp-dentin complex in vivo, with higher-stiffness zones driving odontogenesis and graded scaffolds recapitulating functional tissue architecture.^[16] Li et al. reported that tri-phasic GelMA scaffolds (0.79, 6.37, 24.12 kPa) achieved complete pulp-dentin regeneration in vivo by combining angiogenic (medium) and odontogenic (high) zones.^[15] These 3D in vitro + in vivo studies together indicate that recreating native mechanical gradients, rather than uniform stiffness, is important for full pulp-dentin regeneration.

IV. Discussion

This systematic review shows that substrate stiffness is a major regulator of dental pulp stem cell (DPSC) behavior, but its effects depend on the biological outcome being assessed. Across the included studies, softer substrates generally supported stemness maintenance and delayed senescence, whereas stiffer matrices promoted odontogenic differentiation and mineralization. Intermediate stiffness often favored angiogenic signaling, suggesting that DPSCs respond to a mechanical spectrum rather than a single optimal stiffness value.

Soft substrates were particularly important for preserving the undifferentiated state of DPSCs. Ghaffari et al. reported that low-stiffness conditions upregulated stemness-associated markers and reduced senescence-related features, indicating that a compliant microenvironment helps maintain cellular quiescence and self-renewal capacity.^[9] Gross et al. also suggested that intermediate stiffness can support secretion of trophic and pro-angiogenic factors, but the strongest stemness-preserving effect was seen at the lowest stiffness conditions. Together, these findings suggest that a softer matrix is more suitable for maintaining the regenerative reserve of DPSCs before differentiation is required.^[10]

In contrast, increased stiffness consistently favored odontogenic and mineralizing phenotypes. Liu et al. demonstrated that stiffer PDMS substrates enhanced odontogenic marker expression, including DSPP, DMP-1, and RUNX2, indicating activation of a dentin-forming program.^[13] Datko Williams et al. similarly showed that alkaline phosphatase activity and mineral deposition increased with substrate stiffness, reinforcing the association between rigidity and matrix mineralization.^[12] These findings indicate that stiffer environments shift DPSCs from a maintenance state toward a differentiated osteo/odontogenic lineage.

Angiogenesis appeared to be optimized at intermediate stiffness rather than at the softest or stiffest extremes. Gross et al. found that mid-range stiffness supported secretion of pro-angiogenic cytokines and pulp-like marker expression, suggesting that vascular support requires a balanced mechanical niche.^[10] This is important because pulp regeneration depends not only on odontoblastic differentiation but also on timely revascularization. Therefore, intermediate stiffness may serve as a transitional mechanical environment that promotes tissue support functions before full mineralizing differentiation occurs.

Migration represented a distinct mechanobiological outcome and did not follow classical stiffness-dependent durotaxis. Ehlinger et al. showed that DPSC motility was only weakly influenced by substrate rigidity and was instead closely linked to Arp2/3 complex dynamics.^[11] This suggests that DPSC migration is regulated more by intracellular actin remodeling than by external stiffness gradients alone. As a result, migration should be considered a separate mechanistic endpoint rather than simply another stiffness-driven response.

The in vivo and three-dimensional studies further strengthen the argument that DPSC responses are spatially and temporally regulated by substrate mechanics. Woloszyk et al. showed that stiffer hydrogels promoted odontogenic gene expression and mineralization, whereas softer gels supported proliferation and pulp-like tissue formation.^[14] Qu et al. demonstrated that graded scaffolds could regenerate a vascularized pulp-dentin complex in vivo, supporting the value of mechanical heterogeneity rather than uniform substrate stiffness.^[16] Li et al. extended these findings by showing that tri-phasic scaffolds could promote angiogenesis, odontogenesis, and complete pulp-dentin regeneration in vivo, indicating that distinct stiffness zones may be needed for different stages of tissue repair.^[15]

Mechanistically, these outcomes are consistent with the idea that extracellular stiffness is translated into intracellular signaling through mechanotransduction pathways such as YAP/TAZ, Wnt/ β -catenin, FAK/ERK, and

NF- κ B. Softer environments appear to preserve stemness and suppress senescence, whereas stiffer environments promote cytoskeletal tension and lineage commitment. DPSCs respond to stiffness in a cspecific manner influenced by scaffold composition, culture, and the cellular endpoint being assessed. Clinically, this suggest a single uniform scaffold stiffness is unlikely to meet the dynamic needs of the pulp-dentin complex.

Future biomaterials should therefore incorporate mechanical gradients or time-dependent stiffening so that early soft conditions preserve viability and stemness, while later stiffer regions promote odontogenic differentiation and mineralization. This might allow the natural progression of pulp healing and tissue regeneration.

The included studies were strengthened by advanced 3D and in vivo models, but most still used static substrates that do not mimic the viscoelastic, remodeling nature of the pulp microenvironment. This limits direct clinical translation, since real tissue healing occurs in a changing mechanical landscape rather than under fixed laboratory conditions.

The evidence base is also small and heterogeneous in scaffold composition, fabrication methods, stiffness measurement, and outcome assessment, which prevented meta-analysis. Future work should focus on temporally responsive, multi-phasic scaffolds and mechanogenomic profiling to define how cytoskeletal tension, chromatin remodeling, and lineage programs interact during DPSC differentiation.

In addition, scaffold systems combining soft, intermediate, and stiff regions may be the best strategy for simultaneously supporting stemness, angiogenesis, and odontogenic repair. Such designs may ultimately improve the clinical relevance of regenerative endodontic therapies.

V. Conclusion

This review suggests that substrate stiffness is an important regulator of DPSC behavior. Current evidence indicates that compliant matrices preserve stemness whereas relatively stiffer substrates promote osteogenic and odontogenic differentiation. Although these findings support biomaterial optimization for regenerative endodontics, most evidence originates from in vitro and preclinical studies. Clinical validation is required before these observations can be translated into routine therapeutic applications.

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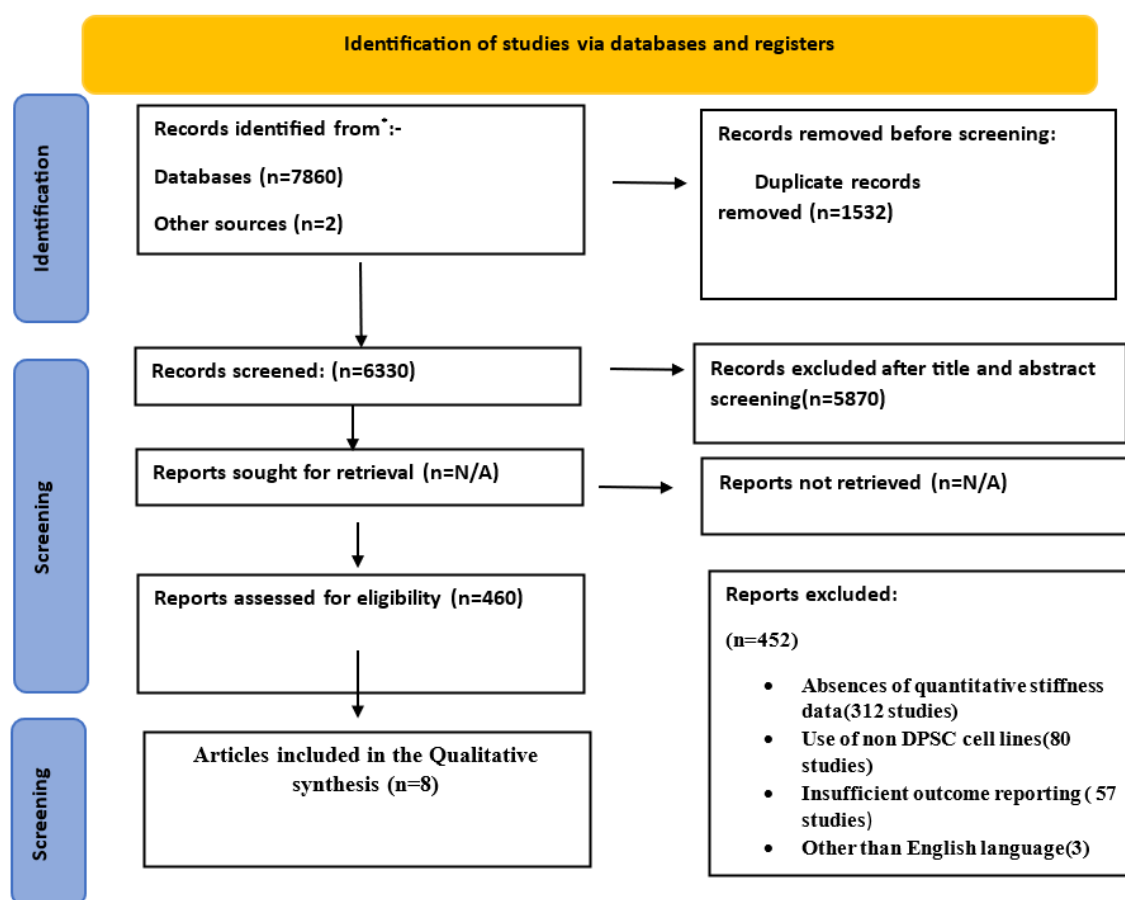


Fig no.1 Prisma framework

Table 1: Summary of findings of the included studies

Author(s) , Year, Country	Study Design	DPSC Source	Cultu re Type	Scaffold / Material	Stiffness (kPa)	Mechanotransduc tion Pathways Studied	Migration / Proliferatio n Tests	Key Outcomes
Woloszyk et al., 2014[14], Switzerland	In-vitro + in-vivo preclinical	Human DPSCs (P3–P4)	3D	PEG-fibrinogen hydrogels (soft vs. stiff)	0.18 kPa vs. 1.05 kPa	Not mechanistically probed	Proliferation: PicoGreen DNA assay	Stiffer hydrogels promoted odontogenic genes and mineralization; softer gels supported proliferation and pulp-like tissue
Qu et al., 2015[16], USA/China	In-vitro + in-vivo regeneration	Human DPSCs (P3–P5)	3D	Nanofibrous gelatin scaffolds	0.89, 6.10, 18.23 kPa	Not mechanistic (matrix-focused)	Proliferation: EdU, DNA quantification	High stiffness promoted mineralization; graded scaffolds regenerated vascularized pulpodentin complex in vivo
Liu et al., 2017, [13]China	In-vitro mechanotransduction	Human DPSCs (<25 y)	2D	PDMS substrates	1.4, 16, 54, 135 kPa	Canonical Wnt/ β -catenin	Proliferation: CCK-8 assay	Stiffer substrates promoted proliferation and odontogenic gene

								expression via Wnt activation
Datko Williams et al., 2018[12], USA	In-vitro mechanobiology	Human DPSCs (STRO-1 ⁺ , third molars)	2D	Fibronectin-coated hydrogels ± HA microparticles	3, 10, 45, 75 kPa; glass 50,000,000–60,000,000 kPa	None directly interrogated	Proliferation: BCA assay; mineralization: ALP, Alizarin Red	Substrates at 75 kPa or greater enhanced ALP and mineralization; HA microparticles increased mineralized area
Ehlinger et al., 2021[11], France	In-vitro mechanobiology + inhibitor assays	Human DPSCs (immature third molars)	2D	Laminin-coated PDMS + bi-rigidity gels	1.5–400 kPa; 2,500 kPa; 69,000,000 kPa	YAP, myosin II, Arp2/3, ROCK	Migration: live-cell tracking; durotaxis assay	Migration was stiffness-independent, but Arp2/3-dependent and YAP-independent; no durotaxis detected
Gross et al., 2023[10], Germany	In-vitro mechanobiology	Human DPSCs (STRO-1 ⁺)	2D	Fibronectin-coated PDMS	1.5, 15, 28 kPa	FAK, ERK1/2, focal adhesion proteins	Proliferation: microscopy confluence; pulp marker gene expression	15 kPa optimized cytokine secretion; 28 kPa promoted osteo/odontogenic differentiation and focal adhesion clustering
Ghaffari & Shrestha, 2025,[9] Canada	In-vitro mechanobiology (2D & 3D)	Human DPSCs (Lonza)	2D & 3D	PDMS + porous scaffolds	2, 50 kPa; 3D 0.46–46.9 kPa	NF-κB, Notch, chromatin remodeling	Senescence: SA-β-gal, p16, SIRT1, XRCC5	Lowest stiffness substrates preserved stemness and reduced senescence; 3D scaffolds provided partial protection
Li et al., 2025[15], China	In-vitro + in-vivo mechanobiology	Human DPSCs (P3)	3D	GelMA hydrogels (5–15%)	0.79, 6.37, 24.12 kPa	Wnt/β-catenin, PI3K-Akt, TGF-β	Proliferation: CCK-8, live/dead; migration: scratch assay	Medium stiffness optimized angiogenesis; high stiffness enhanced odontogenesis; tri-phasic scaffolds achieved complete pulpodentin regeneration in vivo

Table No. 2: Modification of the QUIN-2 tool for DPSC mechanobiology studies

Domain	QUIN-2 basis	Modification for this review	Rationale for modification
C1 Study objective clarity	Explicit statement of study aim and hypothesis	Retained without modification	Clear articulation of the experimental objective is essential for interpreting mechanobiological outcomes
C2 Sample size justification	A priori sample size calculation or justification	Retained, with inclusion of replicate reporting where formal power analysis was absent	Many in vitro biomaterial studies report replicates rather than formal power calculations
C3 Experimental methodology	Overall methodological rigor and design transparency	Expanded to include scaffold fabrication, stiffness characterization, and assay workflow	These elements are critical determinants of reproducibility in DPSC mechanobiology
C4 Biological sample characterization	Adequacy of sample description	Expanded to include DPSC source, donor attributes, passage number, and tissue origin	Stem cell phenotype and mechanosensitivity vary with biological source and culture history

C5 Sequence generation / random allocation	Randomization of group assignment	Retained as applicable	Randomization reduces selection bias where experimental allocation is feasible
C6 Allocation concealment	Protection of group assignment prior to intervention	Retained as applicable	Concealment minimizes allocation-related bias in studies with assignable experimental groups
C7 Operator blinding	Blinding of the operator or experimentalist	Retained with laboratory-specific interpretation	Operator blinding reduces handling and execution bias during scaffold preparation and assay performance
C8 Outcome assessor blinding	Blinding of outcome assessment	Retained with emphasis on imaging and quantitative analysis	Many DPSC endpoints rely on observer-dependent measurements that benefit from blinded assessment
C9 Procedural standardization	Consistency of experimental conditions	Expanded to include substrate fabrication, modulus testing, culture conditions, and assay timing	Standardization is essential because mechanobiological outcomes are sensitive to technical variability
C10 Statistical analysis	Appropriateness and transparency of statistical methods	Retained, with emphasis on stiffness-group comparisons and replicate-level analysis	Appropriate statistics are necessary to support stiffness-dependent conclusions
C11 Reporting completeness	Completeness of outcome and method reporting	Retained, with focus on all mechanobiology endpoints	Selective reporting can distort interpretation when multiple biological outcomes are assessed
C12 Competing interests and funding disclosure	Disclosure of COI and funding sources	Retained without major modification	Transparency in funding and conflicts is necessary for assessing potential bias

Table no. 3: Risk of bias assessment using QUIN2 tool

Study (Year)	C1 Aim	C2 Sample Size Calc	C3 Methodology	C4 Sample Description	C5 Randomization	C6 Allocation Concealment	C7 Operator Blinding	C8 Outcome Assessor Blinding	C9 Standardization	C10 Statistics	C11 Complete Reporting	C12 COI/Funding	Total Score (0 – 24)	Overall RoB
Qu et al., 2015 [16]	2	1	2	2	0	0	1	1	2	2	2	1	18	Low
Woloszyk et al., 2014 [14]	2	0	2	1	0	0	0	0	2	1	2	1	11	Moderate
Liu et al., 2018 [13]	2	1	2	2	1	1	1	1	2	2	2	1	18	Low
Datko Williams et al., 2018 [12]	2	0	2	1	0	0	0	0	2	1	2	1	11	Moderate
Ehlinger et al., 2021 [11]	2	1	2	2	1	1	1	1	2	2	2	1	18	Low
Gross et al., 2023 [10]	2	0	2	2	0	0	0	0	2	2	2	1	13	Moderate
Ghafari &	2	1	2	2	1	1	1	1	2	2	2	1	18	Low

Shrestha, 2025 [9]														
Li et al., 2025 [15]	2	1	2	2	1	1	1	1	2	2	2	1	18	Low