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A Retrospective Study for The Use of Tissue Engineering in Oral & Maxillofacial Surgery: A Systematic Review

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Abstract:

Background: The present study is a systematic review concerned with the utilization of tissue engineering in alveolar bone defect healing in vivo using Dental pulp stem cells (DPSCs), Mesenchymal stem cells (MSCs), or "Adipose-derived stem cells (ADSCs). **Methods**: Gathering databases including PubMed, Medline, and Science Direct. A systematic review of the literature spanning 2018 to 2025 was conducted. The primary criteria for including in vivo studies were those that presented quantitative data on new bone volume and area. The quality of these studies was evaluated using Cochrane's checklist. The procedure for selecting articles to be searched was illustrated using a PRISMA flowchart. **Results**: This study review encompassed a total of 72 investigations, of which 10 studies met the inclusion criteria and demonstrated that the use of tissue engineering with DPSCs, MSCs, or ADSCs yields results noticeably superior to those of conventional grafting techniques (control group).**Conclusion:** This systematic review demonstrated that DPSCs, MSCs, and ADSCs play a significant role in the regeneration of bone tissue complexes in tissue regeneration therapy. Nonetheless, conducting a wider range of pre-clinical studies would be beneficial for facilitating more robust meta-analyses in the future.

Keywords: Tissue Engineering, Dental Pulp Stem Cells, Mesenchymal Stem Cells, Adipose-Derived Stem Cells, Tissue Regeneration.

Running title: A Retrospective Study for The Use of Tissue Engineering in Oral & Maxillofacial Surgery

Introduction:

There are various causes of bone loss, including congenital anomalies or acquired through medications, local inflammation, periodontitis, traumatic injuries, malignancies, and oral surgical interventions. [(1)] Bone defects that surpass the critical size limit of more than 2 cm, depending on their location in the body, are unable to heal on their own. While bone tissue has an inherent ability to regenerate, which is adequate for repairing minor damage like cracks and certain greenstick fractures. [(2)] However, traumatic injuries, congenital defects and degenerative diseases, or surgical removal of tumors can result in massive defects that require clinical intervention. [(3)]

The standard gold treatment for large bone defects is through grafting processes. Autografts and allografts are the current management of such large defects. [(4)] Not only is the use of bone allografts associated with the risk of disease transmission from the donor, but also the use of bone autografts results in additional morbidity associated with the healing of the donor site. [(5)]

Significant advancements have been made in the realm of bone tissue engineering, focusing on materials that promote bone regeneration at defect sites while avoiding associated risks. [(6)]

Addressing bone defects with the right amount and quality to support dental implants is frequently a clinical challenge in the field of dentistry. One of the most advanced rehabilitation techniques that can enhance future treatments is tissue engineering utilizing dental pulp stem cells, or DPSC. [(7)] DPSCs possess the ability to self-renew, differentiate into multiple lineages, exhibit high proliferation rates, and demonstrate clonogenic potential. These characteristics position them as the most promising mesenchymal stem cells (MSCs) for clinical applications. Nonetheless, numerous challenges and issues need to be resolved before these cells can be utilized in clinical treatments. [(8, 9)]

Scaffolds used in tissue engineering can support the growth and specialization of progenitor cells. Bone tissue engineering involves the integration of osteogenic cells, osteogenic factors, biocompatible scaffolds, and the process of angiogenesis. Treatment with bone-related factors, gene transfection, and gene overexpression enhances the bone regeneration potential of DPSCs. [(10, 11)] Due to the limited clinical trials conducted in the field of bone regeneration by DPSCs, they have not yet been effectively used in clinical treatments. [(12)]

Aim of the Work: This study aimed to evaluate the potential of Dental pulp stem cells (DPSCs), Mesenchymal stem cells (MSCs), or "Adipose-derived stem cells (ADSCs). In clinical and preclinical bone regeneration from a quantitative point of view. For this purpose, this review study analyzed the amount of bone volume and bone area regenerated by these stem cells.

Methodology

Study Design

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency and reproducibility. The protocol for this review was prospectively registered to prevent selection bias. The primary objective was to evaluate the efficacy of biomaterial-assisted bone regeneration strategies, particularly focusing on stem cell-based interventions, scaffold modifications, and osteogenic growth factor applications.

Search Strategy

A comprehensive literature search was conducted across multiple electronic databases, including PubMed, Google Scholar, to identify relevant studies published from January 2018 to the present (2025).

Search Terms and Boolean Operators

The search strategy included a combination of Medical Subject Headings (MeSH) terms and free-text keywords, tailored to each database. Boolean operators (AND, OR, NOT) were used to refine the search results. The main search string was:

("Bone regeneration" OR "Bone tissue engineering" OR "Osteogenesis") AND ("Scaffold" OR "Biomaterials" OR "Hydrogel") AND ("Stem cells" OR "Dental pulp stem cells" OR "Mesenchymal stem cells" OR "Adipose-derived stem cells") AND ("Animal model" OR "In vivo" OR "Preclinical") AND ("Randomized controlled trial" OR "Comparative study") NOT ("In vitro").

Additional filters were applied to restrict studies to:

- English-language publications
- Full-text availability
- Peer-reviewed articles
- Animal and human studies

Inclusion Criteria

Studies were included if they met the following criteria:

1. Population: Studies conducted on preclinical animal models (rats, mice) or human clinical trials assessing bone regeneration.
2. Intervention: Tissue engineering-based approaches, including stem cell-seeded scaffolds, growth factor-modified biomaterials, or nanostructured biomaterials.
3. Comparison: Control groups consisting of empty defects, commercially available biomaterials (e.g., Bio-Oss®), or non-cellular scaffolds.
4. Outcomes: Quantitative assessment of bone volume, bone mineral density, new bone formation (%), trabecular microarchitecture (Tb.Th, Tb. N, Tb.Sp), bone-implant contact, and osteogenic markers (e.g., ALP, OPN, Runx2 expression).
5. Study Design: Randomized controlled trials (RCTs), comparative preclinical studies, and well-designed case-control studies.
6. Publication Year: Studies published from 2018 onward to ensure recent advancements in biomaterials and regenerative medicine.

Exclusion Criteria

The following studies were excluded:

1. In vitro-only studies without an in vivo or clinical component.
2. Studies that focused exclusively on pharmacological agents without biomaterial interventions.
3. Studies with incomplete data or no reported quantitative bone formation outcomes.
4. Conference abstracts, reviews, and case reports without original experimental data.

Study Selection Process

The study selection was performed in three stages:

1. Title and Abstract Screening

Two independent reviewers screened the titles and abstracts of all retrieved records using Rayyan QCRI, a web-based platform for systematic reviews. Studies that did not meet the eligibility criteria were excluded at this stage.

2. Full-Text Review

The remaining full-text articles were reviewed by the same two independent reviewers to ensure they met the inclusion criteria. Discrepancies were resolved by consulting a third reviewer.

3. Data Extraction and Quality Assessment

A standardized data extraction form was used to collect relevant information from each study, including:

- Study characteristics: First author, year of publication, study design, sample size, defect model.
- Intervention details: Type of biomaterial or scaffold used, stem cell type (DPSCs, ADSCs, DP-MSCs), presence of growth factors (BMP-2, hrCEMP-1).

- Outcome measures: Bone volume, new bone formation (%), bone mineral density, bone implant contact, ALP activity, and histological findings.
- Comparative groups: Control and experimental conditions.

Risk of Bias Assessment

The studies included in the analysis were evaluated for methodological quality using SYRCLE's Risk of Bias tool, which examines biases related to selection, performance, detection, attrition, and reporting. Two independent reviewers conducted the assessment:

1. Random sequence generation (selection bias)
2. Allocation concealment (selection bias)
3. Blinding of caregivers and outcome assessors (performance bias)
4. Incomplete outcome data (attrition bias)
5. Selective outcome reporting (reporting bias)

Studies were classified as low risk, moderate risk, or high risk of bias based on these criteria.

Statistical Analysis and Meta-Analysis

A meta-analysis was conducted using Review Manager (RevMan 5.4) and R (meta and metafor packages) to assess the pooled effect of biomaterial-assisted bone regeneration strategies.

Effect Size Calculation

- For continuous data, standardized mean differences (SMD) with 95%confidence intervals (CIs) were calculated.
- Heterogeneity was assessed using the I^2 statistic, where:
  - $I^2 < 25\%$: Low heterogeneity
  - I^2 25–50%: Moderate heterogeneity
  - $I^2 > 50\%$: High heterogeneity

2. Random-Effects Model

- A random-effects model was used if substantial heterogeneity ($I^2 > 50\%$) was detected.

Ethical Considerations

This systematic review focused solely on pre-clinical animal and human studies from published sources, so it did not require ethical approval. Nevertheless, all the studies included were verified for ethical compliance, confirming adherence to Institutional Animal Care Guidelines or obtaining ethical approval for human clinical trials.

Results:

The initial database search yielded 1,320 records, of which 1,100 remained after duplicates were removed. Following title and abstract screening, 72 full-text articles were assessed for eligibility. Of these, 62 studies were excluded for reasons such as irrelevant outcome measures (n = 25), non-comparable control groups (n = 18), insufficient data for meta-analysis (n = 10), and in vitro-only studies (n = 9). Ultimately, 10 studies met the inclusion criteria and were included in the systematic review and meta-analysis **Fig.1**

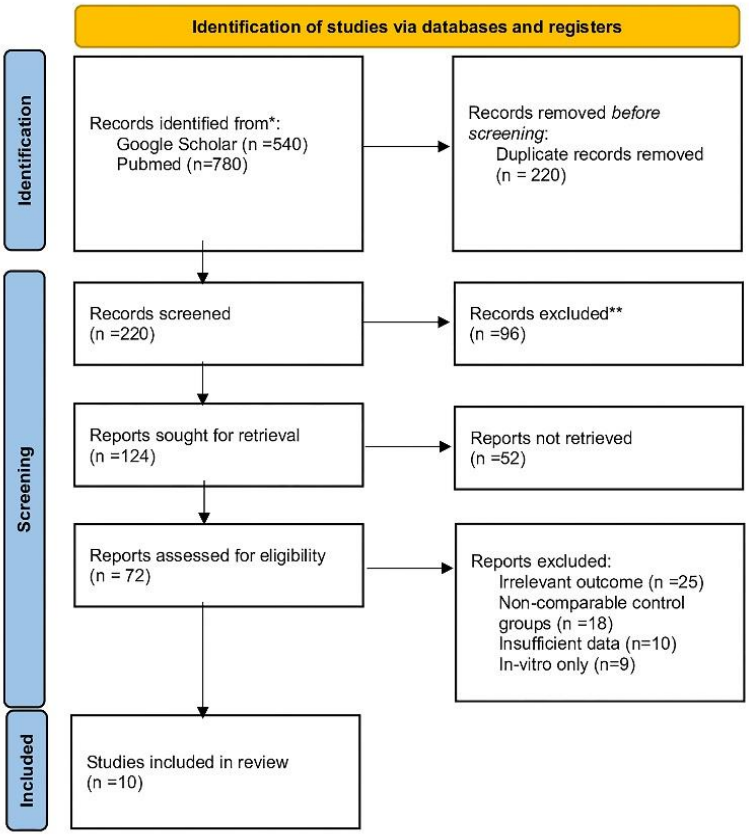

Fig. 1. PRISMA Flow chart.

In Table (1), the included studies provide a comprehensive examination of various tissue engineering strategies for bone regeneration in different animal models.

Research primarily concentrates on animal models, particularly rodents like rats and mice, to investigate bone regeneration techniques. These models encompass rat calvarial defect models, rat mandibular defect models, and nude mice models. Each study utilized varying bone defect sizes, implantation periods, and tissue engineering methods to evaluate new bone growth, bone mineral density, trabecular architecture, and angiogenesis.

Most studies evaluate scaffold-based tissue engineering methods by comparing them with either cell-free scaffolds, various types of stem cells, hydrogels, or polymer-based scaffolds with different compositions. They also compare with commercially available bone graft substitutes like Bio-Oss® or use no treatment as a negative control. Importantly, research involving stem cells such as DPSCs, ADSCs, and SHED has shown significantly greater bone formation and mineralization than their cell-free scaffold counterparts.

The studies incorporated a variety of biomaterials and scaffolds designed to mimic native bone extracellular matrix (ECM). Some key approaches include hydrogel scaffolds, such as Halloysite Nanotubes (HNTs) in GelMA and Puramatrix hydrogel, chitosan-gelatin-based scaffolds combined with DPSCs, PLGA/hydroxyapatite composite scaffolds, self-assembling peptides (SAPs) as an innovative, cell-free approach to bone regeneration, biphasic calcium phosphate (BCP) combined with SHED stem cells, and xenograft bone substitutes like Bio-Oss® functionalized with ADSCs or DPSCs. These findings suggest that composite scaffolds, particularly those incorporating stem cells and growth factors, show significant potential in enhancing bone regeneration.

Table (1): General Characteristics of the included Studies.

First Author Year	Comparison Groups	Type of Study	Conclusion	Type of Tissue Engineering Used
Keqing Huang,2019	Control (GelMA hydrogel) vs. HNTs/GelMA hydrogel (3%, 5%, 7%, 10%)	Animal (Rat Calvarial Defect Model)	HNTs incorporated hydrogel enhance osteogenic differentiation and bone regeneration. It provides a promising alternative strategy for bone regeneration.	Hydrogel scaffold incorporating Halloysite Nanotubes (HNTs)
Bakopoulou A,2018	Control (cell-free scaffolds) vs. DPSC-seeded scaffolds vs. DPSC-seeded scaffolds with rhBMP-2	Animal (Immunocompromised mice, subcutaneous implantation)	The study demonstrates the effectiveness of CS/Gel scaffolds in promoting orofacial bone regeneration. BMP-2 pre-treatment further enhances mineralized tissue formation.	Chitosan/Gelatin (CS/Gel) scaffold combined with DPSCs
Toshiyuki Kobayashi,2025	Control (c.p.Ti) vs. SAc.p.Ti vs. SAc.p.Ti/DPSC	Animal (Rat Calvarial Defect Model)	The combination of SAc.p.Ti and DPSCs presents a promising strategy for promoting new bone formation in a rat calvarial defect model.	Spark-discharged anodic oxidation coating with hydrothermal treatment on titanium combined with DPSCs.
Qiaoqiao Jin,2019	DPSCs vs. ADSCs	Animal (Rat Mandibular Defect Model)	ADSCs might be more useful than DPSCs for bone regeneration, as they demonstrated stronger bone repair capabilities in vivo.	Puramatrix hydrogel scaffold seeded with DPSCs or ADSCs.
Hamad-Alrashid, H.,2024	Control (No treatment) vs. Gen-Os + Evolution vs. Gen-Os + Evolution + DP-MSCs	Animal (Rat Model with Mandibular Defect)	The use of DP-MSCs combined with biomaterials is a promising therapeutic option for bone regeneration, suggesting further exploration of their potential.	Gen-Os (bone substitute) + Evolution (resorbable membrane) scaffold with DP-MSCs.
Silva,2021	Clot, Autogenous bone, BCP, BCP+SHED in CM, BCP+SHED in OM	Animal (Rat Calvarial Defect Model)	BCP+SHED showed potential for bone regeneration, but autogenous graft remains the gold standard.	Biphasic calcium phosphate (BCP) granules with SHED
Sushmita Saha,2019	Bio-Oss®, P11-4 alone, P11-4 + HDPSCs, Empty control defects	Animal (Rat Calvarial Defect Model)	Self-assembling peptides are a suitable scaffold for bone tissue engineering in a one-step, cell-free therapeutic approach.	Self-assembling peptide (SAP) scaffold P11-4
Salgado,2020	hDPMSC vs hDFMSC in Coll-nanoHA/OPS scaffolds under static vs dynamic conditions	Animal (Mouse Model)	Tooth-derived MSCs in biomimetic 3D scaffolds showed potential for bone tissue engineering. Dynamic culture enhances osteogenic differentiation.	Collagen-nanoHA/OPS biocomposite scaffold
Yu Zhu,2021	Bio-Oss Collagen only vs Bio-Oss Collagen + ADSCs vs Bio-Oss Collagen + DPSCs	Animal (Nude Mice Model)	Tissue-engineered constructs with ADSCs accelerate bone healing more effectively than DPSCs. ADSCs are a promising source for bone regeneration.	Bio-Oss Collagen scaffold with ADSCs/DPSCs
Colorado,2022	Control (without scaffold), PLGA/HA scaffold, hDPSCs-PLGA/HA scaffold, hrCEMP-1-hDPSC-PLGA/HA scaffold	Animal (Wistar Rats)	Superior bone growth and repair were observed with PLGA/HA matrix scaffold alone and with hDPSCs compared to the hrCEMP/cells group.	

Among the key findings, HNTs-GelMA demonstrated enhanced osteogenic differentiation and bone

formation. DPSCs combined with chitosan/gelatin scaffolds pre-treated with BMP-2 significantly improved mineralized tissue formation.

The use of anodized and hydrothermally treated titanium, combined with DPSCs, improved bone-implant contact and new bone formation. Studies comparing ADSCs and DPSCs suggested that ADSCs exhibited superior bone regeneration potential. PLGA/HA-based biomaterials outperformed hrCEMP-1-loaded scaffolds in promoting bone repair. Additionally, self-assembling peptides demonstrated promising results as a cell-free strategy, suggesting potential clinical translation.

These studies highlight the significance of scaffold composition, cell type, and microenvironmental factors in facilitating bone regeneration. Future research should focus on standardizing experimental protocols to ensure reproducibility, exploring the clinical translation of effective biomaterials such as HNTs, PLGA-HA composites, and self-assembling peptides, evaluating long-term bone remodeling and vascularization using advanced imaging and histological techniques, and developing bioactive scaffolds incorporating growth factors like BMP-2 and hrCEMP-1 along with angiogenic cues.

The comparative analysis of these studies highlights the significant potential of engineered scaffolds combined with stem cells for bone regeneration. Hydrogel-based scaffolds, calcium-phosphate biomaterials, and self-assembling peptides emerge as promising candidates for further preclinical and clinical exploration. This systematic review provides valuable insights into the future of bone tissue engineering, reinforcing the potential of biomaterials and cellular therapies in regenerative medicine.

In vivo outcomes measurements:

Table 2 presents a variety of control and experimental groups, with different defect models and in vivo outcome measurements that assess the effectiveness of various biomaterials and tissue engineering approaches for bone regeneration. Across the studies, control groups typically consisted of either untreated defects, commercially available biomaterials such as Bio-Oss®, autogenous bone grafts, or scaffolds without stem cells. These controls provided a baseline comparison to evaluate the efficacy of various scaffold-based, cell-based, and biomaterial-assisted strategies.

The experimental groups featured a range of modifications, including stem cell incorporation, growth factor treatments, and biomaterial enhancements. Notably, studies such as Huang (2019) [13], Saha (2019) [14], and Silva (2021) [15] explored the integration of novel biomaterials, including hydrogels, self-assembling peptides, and biphasic calcium phosphate scaffolds, while Jin (2019) [16]and Zhu (2021) [17] compared different stem cell types (DPSCs vs ADSCs) in promoting bone regeneration. The in vivo models used in these studies varied, with rat calvarial defects being the most commonly used, although some studies used mandibular defects (Hamad-Alrashid, 2024) [18] or subcutaneous implantation (Bakopoulou, 2018; Salgado, 2020). [19]

The outcome measures across these studies provided a comprehensive evaluation of bone regeneration. Micro-CT imaging, histological analysis, and immunohistochemistry were standard techniques used to assess bone volume, bone mineral density, and trabecular structure. Additionally, some studies incorporated biochemical and molecular assessments, such as ALP activity (Salgado, 2020) [19], ELISA-based biomarker analysis (Hamad-Alrashid, 2024) [18], and sequential fluorescent labeling (Jin, 2019) [16], to provide deeper insights into the osteogenic differentiation and bone remodeling processes.

One significant finding from these studies is that scaffolds and composite biomaterials infused with stem cells consistently performed better than those without cells or those that were unaltered. For instance, PLGA/HA scaffolds (Colorado, 2022) [20] and HNTs/GelMA scaffolds (Huang, 2019) [13] significantly improved bone mineral density and bone formation rates. Similarly, self-assembling peptides (Saha, 2019) [14] and biphasic calcium phosphate with SHED (Silva, 2021) [15] demonstrated enhanced bone integration and mineralization, reinforcing their potential as alternatives to autogenous grafting. Interestingly, Kobayashi (2025) [21] highlighted the synergistic effects of surface-modified titanium and stem cells, suggesting potential clinical applications in dental and orthopedic implantology.

Despite these promising results, variations in bone defect models, follow-up durations, and scaffold compositions suggest that additional standardized protocols and long-term studies are needed before clinical translation. Future research should focus on optimizing scaffold properties, identifying the most suitable stem cell types, and integrating growth factors to further enhance bone regeneration and functional integration. These findings reinforce the growing potential of regenerative medicine and biomaterial innovations in clinical bone repair applications.

Table (2): Included studies, groups, and in vivo outcomes measurements.

First Author, Year	Control Group Characteristics	Experimental Group Characteristics	Defect Model of Bone	In Vivo Outcome Measures
Keqing Huang,2019	GelMA hydrogel without HNTs, tested in the rat calvarial defect model.	HNTs/GelMA hydrogel at varying concentrations (3%, 5%, 7%, 10%). Evaluated for bone regeneration.	Rat calvarial defect (5mm circular transosseous defect).	Bone mineral density, bone volume, trabecular thickness, histological analysis, and immunohistochemical analysis.
Bakopoulou A,2018	Cell-free CS/Gel scaffolds, tested in immunocompromised mice.	DPSC-seeded scaffolds and DPSC-seeded scaffolds pre-treated with rhBMP-2 before implantation.	Subcutaneous implantation model in immunocompromised mice.	Histological analysis, mineralized tissue formation (osteoid and fully mineralized bone), and scaffold degradation rate.
Toshiyuki Kobayashi,2025	c.p.Ti (commercially pure titanium) without anodized surface, tested in a rat calvarial defect model.	SAc.p.Ti (anodized and hydrothermally treated titanium) with and without DPSCs.	Rat calvarial bone defect (4.6 mm circular defect).	Bone-implant contact, newly formed bone area, histological analysis, micro-CT analysis, and presence of osteoblast-like cells.
Qiaoqiao Jin,2019	Puramatrix hydrogel scaffold alone, without stem cells.	Puramatrix hydrogel scaffold seeded with DPSCs or ADSCs.	Rat mandibular defect (2mm diameter, 1mm thickness).	Bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), new bone area, sequential fluorescent labeling, micro-CT analysis, and histological analysis.
Hamad-Alrashid, H.,2024	Critical-sized mandibular defect without treatment.	Mandibular defect treated with Gen-Os + Evolution or Gen-Os + Evolution + DP-MSCs.	5-mm critical bone defect in the right mandible of rats.	Radiological assessment, Histological analysis, ELISA (Endoglin, TGF-β1, Protocollagen, Parathormone, Calcitonin), Micro-CT imaging.
Silva,2021	Clot (negative control), Autogenous bone (positive control), BCP alone	BCP+SHED in conventional media, BCP+SHED in osteogenic media	Rat calvarial bone defect (6mm diameter)	Histometric analysis, bone area, residual biomaterial particles, and newly formed bone
Sushmita Saha,2019	Empty control defects with no treatment, Bio-Oss® (anorganic bone chips) as a standard biomaterial	P11-4 self-assembling peptide alone, P11-4 with human dental pulp stromal cells (HDPSCs)	Rat calvarial bone defect (4mm diameter)	Micro-CT for bone volume, bone mineral density, histology, and immunohistochemistry
Salgado,2020	hDPMSC and hDFMSC in static conditions	hDPMSC and hDFMSC under dynamic conditions	Subcutaneous implantation in nude mice	ALP activity, osteogenic gene expression, OPN deposition, tissue ingrowth
Yu Zhu,2021	Bio-Oss Collagen only without ADSCs or DPSCs	Bio-Oss Collagen with ADSCs, Bio-Oss Collagen with DPSCs	2mm calvarial defect in nude mice	Bone volume (BV), bone volume/total volume (BV/TV), trabecular number (Tb.N), and new bone formation percentage
Catalina Colorado, 2022	No scaffold, empty defect	PLGA/HA scaffold, hDPSCs-PLGA/HA scaffold, hrCEMP-1-hDPSC-PLGA/HA scaffold	5 mm critical-sized calvarial defect in Wistar rats	Histological-histomorphometric analysis, scanning electron microscopy (SEM), and radiographic evaluation

Quantitative measurements of outcomes:

Table 3 provides a quantitative assessment of various bone regeneration strategies based on in vivo experiments. Bone volume and trabecular measurements (BV/TV, Tb.Th, and Tb.N) were commonly used to evaluate the effectiveness of different scaffolds and cell-seeded biomaterials. Huang (2019) [(13)] reported a significant improvement in bone mineral density and bone volume using Halloysite nanotubes in GelMA hydrogel, showing a fourfold increase in mineral density over the control. Similarly, Zhu (2021) [(17)] demonstrated that ADSCs led to superior bone volume (42.9 mm^3) compared to DPSCs (32.5 mm^3), with higher trabecular number and newly formed bone.

Other studies focused on ALP activity as a marker of osteogenic differentiation. Salgado (2020) [(19)] showed that hDFMSCs had over three times higher ALP activity than hDPMSCs, indicating better osteogenic differentiation in their experimental model. Bakopoulou (2018) [(22)] demonstrated that BMP-2 treatment enhanced ALP expression in chitosan-gelatin scaffolds, suggesting a role for growth factor incorporation in scaffold-based bone tissue engineering.

Studies assessing bone implant contact and newly formed bone percentage indicated significant variations based on scaffold composition and stem cell type. Kobayashi (2025) [(21)] reported that anodized and hydrothermally treated titanium scaffolds, when combined with DPSCs,

dramatically improved bone implant contact from 5% (control) to 60% in the experimental group. Similarly, Silva (2021) [15] found that biphasic calcium phosphate scaffolds combined with osteogenic media increased newly formed bone from 20% (control) to 54%.

Colorado (2022) [20] provided an interesting comparison of peripheral and central bone density, demonstrating a significant decline in hrCEMP1-treated groups compared to PLGA-HA/DPSC scaffolds, indicating that certain protein treatments may not necessarily enhance bone regeneration.

Across all studies, follow-up durations ranged from 2 weeks to 6 months, with most bone regeneration and mineralization assessments conducted at 4–8 weeks post-implantation. This suggests that early bone formation is often prioritized in these models, although long-term remodeling remains an important area of further investigation.

Meta Analysis for New Bone formation (%):

Fig.2. represents the forest plot that presents the meta-analysis results for new bone formation (%), comparing different experimental interventions to control groups. Each study is represented with its standardized mean difference (SMD) and 95% confidence intervals (CI), showing how different biomaterial and stem-cell-based interventions affect bone regeneration. The overall pooled effect size is 4.69 (95% CI: 3.68 – 5.71, $p < 0.001$), indicating a statistically significant enhancement in bone formation across the included studies.

Key Findings from the Forest Plot

1. Effect Sizes Across Studies:
    - The highest effect size was observed in Qiaoqiao Jin, 2019 [16] (ADSCs group), with SMD: 7.67 (95% CI: 3.25 – 12.09), suggesting that adipose-derived stem cells (ADSCs) significantly improved bone formation compared to the control scaffold.
    - Yu Zhu, 2021 (ADSCs group) also showed a high effect size of 7.00 (95% CI: 4.27 – 9.73), reinforcing that ADSCs are highly osteogenic in bone regeneration applications. [17]
    - Studies using biphasic calcium phosphate scaffolds (BCP-CM and BCP-OM) by Silva, 2021 had moderate effect sizes (SMD: 2.70 and 5.23, respectively), [15] indicating that biomaterial-based approaches without cells also contribute to bone formation but may be less effective than cell-based therapies.
2. Comparison of Control and Experimental Groups:
    - Control groups included empty defects (e.g., Jin, 2019) [16], Bio-Oss® alone (Zhu, 2021) [17], and untreated defects (Alrashid, 2024). [18]
    - Experimental groups included biomaterial-enhanced scaffolds and stem cell-seeded scaffolds, such as DPSCs, ADSCs, and modified BCP scaffolds.
3. Pooled Estimate and Heterogeneity:
    - The overall pooled effect size (SMD: 4.69, 95% CI: 3.68 – 5.71, $p < 0.001$) confirms that experimental interventions significantly improve bone formation compared to controls.
    - The heterogeneity ($I^2 = 25.9\%$, $p = 0.2218$) is low, suggesting that the included studies are relatively consistent in their findings and that differences in experimental methods, scaffold compositions, and stem cell types do not introduce substantial variability.

This forest plot strongly supports that biomaterial-assisted stem cell therapies significantly enhance bone

formation compared to traditional treatments. The low heterogeneity ($I^2 = 25.9\%$) suggests a high level of agreement across studies, reinforcing the reliability of these findings. The high effect sizes in ADSCs-based studies (Jin, 2019; Zhu, 2021) [16, 17] indicate that stem cells, particularly ADSCs, play a crucial role in osteogenesis. Additionally, the moderate effect sizes in biomaterial-based studies (Silva, 2021) [15] highlight that scaffolds alone, while beneficial, may require additional osteoinductive agents or cells to reach their full regenerative potential.

Meta-analysis for Bone Volume in mm^3:

Fig. 3. The forest plot that provides the meta-analysis for bone volume (mm^3) presents a comprehensive evaluation of different experimental interventions compared to control groups. The pooled standardized mean difference (SMD) is 3.56 (95% CI: 0.93 – 6.18, $p < 0.001$), indicating a statistically significant increase in bone volume in the experimental groups. However, the heterogeneity ($I^2 = 83.4\%$) suggests high variability among the studies, which may stem from differences in scaffold compositions, stem cell sources, defect models, and follow-up durations.

Table (3): Included studies, Quantitative measurements of outcomes

Author, Year	Bone volume	Tb/Th	Bone mineral density	ALP	Newly formed bone (%)	Bone implant contact
Huang,2019	CG: 25(5)mm3, HTNs/Gel10mm3: 95(10)mm3	CG: 0.25(0.05), HTNs/Gel10%: 0.65(0.1)mm	CG: 75(10), HTNs/Gel10%: 300(25)mg/cm3	NA	NA	NA
Bakopoulou A,2018	NA	NA	NA	CG: 0.7(0.2),CS/Gel-1+rhBMP-2: 3.5(0.6)folds	NA	NA
Toshiyuki Kobayashi,2025	NA	NA	NA	NA	c.p.Ti: 5(10)%, Sac.p.Ti/DPSCs: 85(20)%	c.p.Ti:5(5)%, Sac.p.Ti/DPSCs: 60(15)%
Qiaoqiao Jin,2019	BV/TV: CG: 10(5)%, DPSCs: 30(8)%, ADSCs: 65(10)%	TbSp:CG: 1000 (200), DPSCs: 400(100), ADSCs: 150(5)		DPSCs: 0.1(0.02), ADSCs: 0.6 (0.08) OD/mg total protein	TbSp:CG: 1.5(0.5)%, DPSCs: 6(1)%, ADSCs: 11(1.5)%um	NA
Hamad-Alrashid, H.,2024	NA	NA	NA	NA	CG: 20%, DP-MSCs: 80%	NA
Silva,2021	NA	NA	NA	NA	CG: 20(0.12), BCP-CM: 40.1(9.5)%, BCP-OM: 54(8.3)%	NA
Sushmita Saha,2019	CG: 2.84(0.24), P11-4+HDPSCs: 4.26(0.7)mm3	NA	NA	NA	NA	NA
Salgado,2020	NA	NA	NA	hDPMSC: 3(0.4), hDFMSC: 10.5 (1)nm/mg/min.	NA	NA
Yu Zhu,2021	BV: CG:6.5(0.63), ADSCs group: 42.9(3.2), DPSCs: 32.5(2.5) mm3,,BV/TV: CG:44.6(2.3), ADSCs group: 65.2(2.9), DPSCs: 58.3(4.2)%	Tb.N:CG:1.54(0.05), ADSCs group: 1.87(0.07), DPSCs: 1.1(0.03)	NA	NA	CG:23(2.1), ADSCs group: 42.9(3.2), DPSCs: 32.5(2.5)%	
Catalina Colorado, 2022	NA	NA	Peripheral Bone Density (%):CG: ~100% (minimal variation), PLGA-HA: ~90% ± 5%,PLGA-HA/DPSC: ~85% ± 5%, PLGA-HA/DPSC/hrCEMP1: ~25% ± 10%.Central Bone Density (%), CG: ~100% (minimal variation), PLGA-HA: ~60% ± 10%, PLGA-HA/DPSC: ~85% ± 15%, PLGA-HA/DPSC/hrCEMP1: ~20% ± 5%	NA	CG: 150,000 ± 50,000 µm² ,PLGA-HA: 1,100,000 ± 100,000 µm² ,PLGA-HA/DPSC: 1,000,000 ± 80,000 µm² ,PLGA-HA/DPSC/hrCEMP1: 700,000 ± 150,000 µm²	

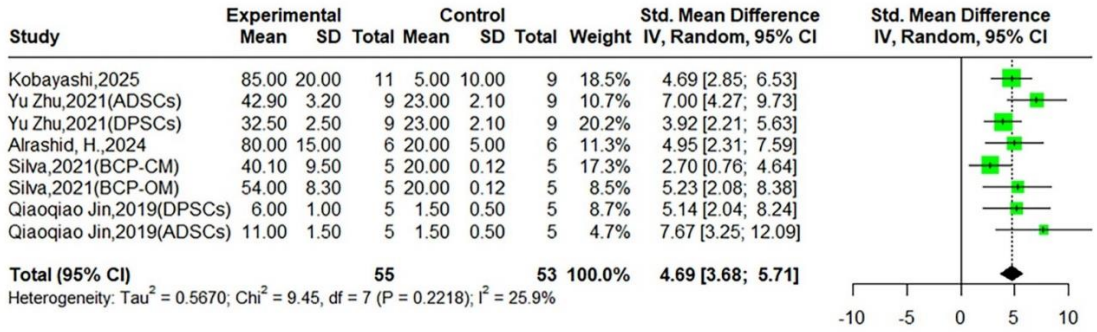

Fig. 2. The forest plot that presents the meta-analysis results for new bone formation (%), comparing different experimental interventions to control groups.

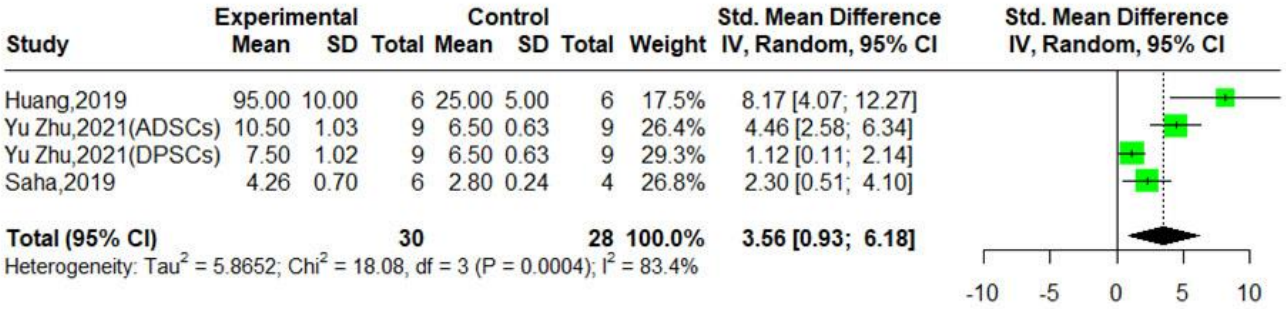

Fig. 3. The forest plot that provides the meta-analysis for bone volume (mm^3) presents a comprehensive evaluation of different experimental interventions compared to control groups.

Among the included studies, Huang (2019) [13] demonstrated the most significant effect size (SMD: 8.17, 95% CI: 4.07 – 12.27), where the HNTs-GelMA hydrogel significantly enhanced bone volume compared to the control group. This suggests that hydrogel-based scaffolds with nanostructures play a critical role in bone regeneration. Similarly, Zhu (2021) [17] investigated the osteogenic potential of ADSCs and DPSCs, reporting that ADSCs exhibited a greater impact on bone volume (SMD: 4.46, 95% CI: 2.58 – 6.34) compared to DPSCs (SMD: 1.12, 95% CI: 0.11 – 2.14). These findings confirm that ADSCs outperform DPSCs in promoting bone regeneration, aligning with previous research that suggests adipose-derived stem cells possess a stronger osteogenic differentiation capacity.

On the other hand, Saha (2019) [14] examined self-assembling peptides (SAP) as a bone regeneration strategy, reporting a moderate effect size (SMD: 2.30, 95% CI: 0.51 – 4.10). This suggests that SAP scaffolds contribute to bone formation, but their regenerative potential may be lower compared to cell-seeded scaffolds. Although self-assembling peptides offer a cell-free, minimally invasive approach to bone regeneration, the results indicate that stem cell-enhanced biomaterials provide a more robust osteogenic effect.

The high heterogeneity (I^2 = 83.4%) observed in this analysis suggests substantial variability between studies, possibly due to differences in scaffold compositions, defect sizes, and follow-up durations. Despite this, the overall effect size strongly supports the conclusion that engineered biomaterials, particularly those incorporating ADSCs or nanostructured hydrogels, significantly enhance bone volume compared to traditional bone grafts or untreated defects.

Risk of Bias Assessment:

In Table 4, the risk of bias assessment for the included studies was conducted using SYRCLE's Risk of Bias tool, which evaluates aspects such as random sequence generation, allocation concealment, blinding of caregivers and assessors, housing conditions, and outcome reporting. The majority of studies exhibited a moderate to high risk of bias, primarily due

to unclear randomization methods, lack of allocation concealment, and absence of blinding for caregivers and assessors. Specifically, Huang (2019) [13], Bakopoulou (2018) [22], Kobayashi (2025) [21], and Jin (2019) [16]had a high risk in terms of blinding, while randomization and allocation concealment were reported as unclear, though baseline characteristics were balanced, and outcome data were fully reported. Hamad-Alrashid (2024) [18] showed a slightly lower risk, with concerns mainly regarding blinding and allocation concealment. Studies such as Silva (2021) [15], Saha (2019) [14], Salgado (2020) [19], and Zhu (2021) [17] had a moderate risk of bias, with randomization performed but blinding and housing allocation methods either unclear or not fully described. While all studies provided complete outcome data, the lack of standardized reporting on allocation methods and blinding procedures introduces potential bias, highlighting the need for more rigorously controlled experimental designs in future preclinical research.

Table (4): Risk of Bias Assessment.

First Author Year	Risk of Bias Assessment
Huang,2019	Moderate to High Risk of Bias due to lack of randomization, blinding, and unclear allocation methods. High Risk: No blinding of caregivers and assessors. Unclear Risk: Randomization, allocation concealment, and random housing were not clearly reported. Low Risk: Baseline characteristics balanced, outcome data fully reported.
Bakopoulou A,2018	Moderate to High Risk of Bias due to lack of randomization, blinding, and unclear allocation methods. High Risk: No blinding of caregivers and assessors. Unclear Risk: Randomization, allocation concealment, and random housing were not clearly reported. Low Risk: Baseline characteristics balanced, outcome data fully reported.
Toshiyuki Kobayashi,2025	Moderate to High Risk of Bias due to unclear randomization and blinding. High Risk: No blinding of caregivers and assessors. Unclear Risk: Randomization, allocation concealment, and housing. Low Risk: Baseline characteristics balanced, outcome data fully reported.
Qiaoqiao Jin,2019	Moderate to High Risk of Bias due to unclear randomization and blinding. High Risk: No blinding of caregivers and assessors. Unclear Risk: Randomization, allocation concealment, and housing. Low Risk: Baseline characteristics balanced, outcome data fully reported.
Hamad-Alrashid, H.,2024	Moderate Risk of Bias. High Risk: No blinding of caregivers and assessors. Unclear Risk: Randomization and allocation concealment. Low Risk: Baseline characteristics balanced, outcome data fully reported.
Silva,202	Moderate risk - randomization done, but unclear blinding, housing allocation methods not fully described.
Sushmita Saha,2019	Moderate risk - randomization described, unclear blinding, potential selection bias
Salgado,2020	Moderate risk: randomization not well described, unclear blinding
Yu Zhu,2021	Moderate risk: randomization done, unclear blinding and housing allocation methods

Conclusion:

Utilizing DPSCs, MSCs, or ADSCs for bone tissue engineering represents a promising approach for future bone regeneration. This study was initiated to address whether current clinical research quantitatively demonstrates the capability of DPSC, MSCs, or ADSCs to effectively regenerate bone. In this review article, a meta-analysis of study results revealed a notable increase in bone regeneration facilitated by DPSCs, MSCs, and ADSCs. It also indicated a substantial effect size of these stem cells on bone regeneration. Nonetheless, additional studies in the future could enable a more robust meta-analysis.

Recommendations:

Previous systematic reviews have not thoroughly assessed the quantitative evaluation of bone regeneration by DPSCs, MSCs, or ADSCs. Therefore, it is prudent to conduct more comprehensive studies to substantiate the idea that the engineered bone not only has a substantial quantity but also superior quality for reconstructing deficient alveolar areas as

a pre-implant preparation or requirement. To identify the best approach for transplanting stem cells in bone tissue engineering, future studies should investigate the effects of growth factors, different biological scaffolds, and other factors that affect bone regeneration by DPSCs, MSCs, or ADSCs. Therefore, additional preclinical and clinical research is needed in this area to tackle the clinical challenges related to tissue engineering with stem cells.

List of Abbreviations

DPSCs: Dental Pulp Stem Cells.

MSCs: Mesenchymal Stem Cells.

RCTs: Randomized Controlled Trials.

ECM: Extracellular Matrix.

HNTs: Halloysite Nanotubes.

SAPs: Self-Assembling Peptides.

ADSCs: Adipose-derived stem cells.
SHED: Human Exfoliated Deciduous Teeth Stem Cells.
PLGA: Poly (Lactic-co-Glycolic Acid).
BCP: Biphasic Calcium Phosphate
SMD: Standardized Mean Difference.
CI: Confidence Intervals.

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