Inhibition of sclerostin activity promotes bone regeneration in experimental periodontal disease: A systematic review and meta-analysis of animal models

MIAOMIAO YANG[1*], YONGQING MA[2*], XIAOFU QU[3], ZHE QU[1] and JIANG SUN[4]

[1]Department of Implantation, Stomatological Hospital of Dalian University (Dalian Stomatological Hospital), Dalian, Liaoning 116021, P.R. China; [2]Department of Oral Surgery, Stomatological Hospital of Dalian University (Dalian Stomatological Hospital), Dalian, Liaoning 116021, P.R China; [3]VIP Clinic, Stomatological Hospital of Dalian University (Dalian Stomatological Hospital), Dalian, Liaoning 116021, P.R. China; [4]Department of Periodontology, Stomatological Hospital of Dalian University (Dalian Stomatological Hospital), Dalian, Liaoning 116021, P.R. China

Received May 12, 2025; Accepted October 31, 2025

DOI: 10.3892/etm.2025.13026

Abstract. Sclerostin, an osteocyte-derived inhibitor of the Wnt/β-catenin pathway, plays a key role in suppressing bone formation. To evaluate the therapeutic efficacy of sclerostin inhibition in mitigating alveolar bone loss and promoting regeneration in animal models of periodontal disease, the present meta-analysis conducted a systematic search of PubMed, Embase, the Cochrane Library and Web of Science for studies published from inception to January 2025, without language restrictions. Eligible studies encompassed *in vivo* experiments investigating pharmacological or genetic strategies targeting sclerostin. A total of eight studies fulfilled the inclusion criteria and pooled analyses demonstrated a notable decrease in the distance between the cementoenamel junction and alveolar bone crest following sclerostin inhibition. Secondary outcomes indicated improvements in bone volume fraction and mineral density and serum osteocalcin levels, with a marked reduction in the number of sclerostin-positive cells. Sclerostin inhibition effectively preserved alveolar bone and enhanced bone quality and metabolic activity in experimental periodontal disease. These findings highlight the potential of sclerostin as an adjunctive strategy for periodontal regeneration.

Correspondence to: Dr Yongqing Ma, Department of Oral Surgery, Stomatological Hospital of Dalian University (Dalian Stomatological Hospital), 935 Changjiang Road, Dalian, Liaoning 116021, P.R. China
E-mail: jasonwillking@163.com

*Contributed equally

Key words: sclerostin antibody, periodontal disease, animal model, bone regeneration, meta-analysis, systematic review

Introduction

Periodontal bone regeneration is a major clinical challenge. Current surgical strategies, such as autologous bone grafting combined with guided tissue regeneration, achieve long-term success rates of ~58% in restoring functional attachment (1-4). These approaches are further constrained by donor site morbidity, unpredictable graft resorption and diminished efficacy in patients with systemic conditions such as diabetes mellitus (5-8). Collectively, these limitations underscore the need for biological therapies that directly modulate bone metabolism.

Sclerostin (Scl; encoded by the SOST gene) is a 22-kDa secreted glycoprotein that has emerged as a key regulator of skeletal homeostasis, particularly in bone remodelling and craniofacial biology. It serves as a key mediator of the interaction between osteoblasts and osteoclasts, maintaining the balance between bone formation and resorption (8). Mechanistically, Scl exerts its effects through a number of interrelated pathways (9-12). By antagonizing the canonical Wnt/β-catenin signaling cascade, Scl binds to low-density lipoprotein receptor-related proteins (LRPs) including LRP5/6 on osteoblast precursors, thereby suppressing osteoblast proliferation, differentiation and matrix mineralization. This inhibition decreases the capacity for new bone formation (13-15). Scl indirectly promotes osteoclastogenesis through bone morphogenetic protein (BMP)/Wnt signaling interactions. It increases the receptor activator of NF-κB ligand (RANKL) to osteoprotegerin (OPG) ratio by stimulating RANKL secretion from osteocytes while decreasing OPG expression, favoring osteoclast differentiation and activity. Thus, Scl not only limits bone formation but also actively enhances bone resorption (16-19).

Beyond local effects in bone, Scl may also act systemically. Evidence suggests (16,17) that it can influence distant organs such as the kidney or vascular system, linking bone metabolism to broader physiological processes. In periodontal disease, these regulatory networks become dysregulated. The alveolar bone undergoes rapid turnover and is subject to continuous mechanical loading from mastication and occlusal

forces (16-18). When combined with chronic biofilm-induced inflammation, these stressors amplify disruption of the Scl-Wnt-RANKL axis, accelerating alveolar bone resorption and undermining periodontal tissue stability (19).

Given its dual role in suppressing osteoblast activity and stimulating osteoclastogenesis, therapeutic inhibition of Scl has attracted extensive research (14-19), particularly following the clinical success of romosozumab in osteoporosis. However, whether these benefits extend to biofilm-driven periodontitis, remains uncertain. The regulatory pathways by which Scl influences alveolar bone remodeling, including inhibition of canonical Wnt/β-catenin signaling, modulation of the RANKL/OPG pathway, interactions with BMP signaling and amplification through inflammatory cytokines are illustrated in Fig. S1.

Research into therapeutic inhibition of Scl has progressed since the approval of romosozumab for osteoporosis and preclinical studies have confirmed its anabolic effects in long-bone defect models (20-22). Whether these effects extend to polymicrobial biofilm-induced periodontitis remains unclear, given the unique challenges of the periodontal environment, including alveolar bone turnover, estimated to be 200-300% faster compared with that of tibial bone (23-27), constant bidirectional loading from mastication (28,29) and chronic biofilm-driven inflammation (30,31).

To the best of our knowledge, the present meta-analysis is the first to synthesize preclinical evidence on Scl inhibition in experimental periodontitis. Existing studies (14-19) demonstrate limited insight into how treatment effects vary by dose, anatomical site or systemic conditions. By integrating available data, the present analysis aimed to outline the dose-response association between SOST suppression and alveolar regeneration, as well as potential differences across defect types such as interproximal and furcation lesions and interactions with systemic bone-modifying factors. Together, these findings may provide a more comprehensive understanding of the therapeutic potential of Scl inhibition in periodontal regeneration.

Materials and methods

Protocol and registration. The present protocol was registered on PROSPERO (https://www.crd.york.ac.uk/PROSPERO; registration ID: CRD42023388345). The present review aimed to evaluate whether inhibition of Scl activity promotes alveolar bone repair and regeneration in animal models of periodontal disease, based on the recommendations from the Cochrane Handbook for Systematic Reviews (32) and PRISMA statement for reporting systematic reviews and meta-analysis (33).

Inclusion criteria. A population, intervention, comparison, outcomes and study design framework was applied to define the criteria. Inclusion criteria were as follows: i) Animal models of experimental periodontal disease; ii) evaluated the inhibition or neutralization of Scl activity; iii) used animal models with periodontal disease but without Scl inhibition as the control group; iv) encompassed primary outcomes associated with decreased alveolar bone loss (ABL) and secondary outcomes including changes in bone volume fraction (BVF), bone mineral density (BMD), bone-specific serum markers and the number of SOST-positive cells; and v) *in vivo* randomized controlled trials.

Exclusion criteria. Studies that did not replicate periodontal pathology, *in vitro* experiments and studies lacking a control group or using non-periodontitis animals as controls were excluded.

Search strategy. Studies were identified through a systematic s earch of the following electronic databases: PubMed (https://pubmed.ncbi.nlm.nih.gov/), the Cochrane Library (https://www.cochranelibrary.com/), Embase (https://www.embase.com/) and Web of Science (all databases, https://www.webofscience.com/). Studies published from inception to January 2025 were included, with no language restrictions. The search combined medical subject headings with free-text terms applied to titles, keywords and abstracts. In addition, a manual search was performed by screening the reference lists of articles and related reviews for additional relevant studies. The complete search strategy is provided in Table SI.

Study selection. All retrieved records were imported into EndNote software (version X9; Clarivate Plc) to remove duplicates. A total of two reviewers then independently screened the remaining articles against the inclusion and exclusion criteria. First, titles and abstracts were reviewed to exclude irrelevant studies. Full text of potentially eligible studies was assessed to confirm their relevance. Any discrepancies were resolved by discussion and consensus or consultation with a third reviewer.

Data extraction. Study publication date, author name, sample size, animal species, the sex and diet of the animals, construction of the periodontal disease model, the adopted interventions, route and dose of administration, the duration of administration, outcomes and types of detection (e.g., histological, radiographic or molecular analyses) were collected.

If data were insufficient or presented only graphically, attempts were made to contact the authors and obtain the numerical values. If the information could not be retrieved, WebPlotDigitizer (V5), a digital ruler software, was used to measure graphical data (automeris.io/WebPlotDigitizer/) (34). If studies reported outcomes at multiple time points, only data from the most commonly reported time point across studies (e.g., 3 or 6 weeks) were acquired for meta-analysis. If the experiments included a number of groups, only periodontitis animal models were taken as the experimental and control group for meta-analysis. The data were extracted independently by two authors, with any disagreements being resolved through discussion and consensus.

Quality assessment. Risk of bias was independently evaluated by two reviewers using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool (35), which consists of ten domains adapted from the Cochrane Collaboration tool (36). According to the criteria recommended by Hooijmans *et al* (35), each domain was rated as low risk (score=1) if sufficient methodological details were reported, high risk (score=-1) if clear methodological shortcomings were present or unclear risk (score=0) when information was insufficient to permit judgement. The assessed domains included sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers/experimenters, random outcome assessment,

blinding of outcome assessors, incomplete outcome data, selective outcome reporting and other potential sources of bias. Discrepancies between reviewers were resolved by discussion or consultation with a third reviewer.

Data synthesis and statistical analysis. As only morphometric results, such as the distance between the cementoenamel junction and alveolar bone crest (CEJ-ABC), tissue mineral density (TMD) and BVF, yielded sufficient data, these continuous variables were extracted for meta-analysis. Analysis was conducted using REVMAN (version 5.3; The Cochrane Collaboration) software to determine pooled effects. To identify and measure heterogeneity in the results, χ^2 tests and I^2 statistics were implemented. χ^2 with a significance level of α=0.1 was considered to indicate heterogeneity.

As all outcome measures were continuous variables, the standardized mean difference (SMD) with 95% CI was used to describe the efficacy of the intervention effect. SMD was used instead of weighted mean difference because the studies included reported heterogeneous outcomes (BMD, BVF and ABL) in different units and scales. The use of SMD allowed for standardization and ensured comparability across studies. In accordance with the Cochrane Handbook for Systematic Reviews of Interventions, a random-effects model was applied for all meta-analyses, irrespective of the I^2 value, since heterogeneity is expected across experimental studies.

Subgroup analyses were conducted to explore potential sources of heterogeneity, such as type of intervention (direct vs. indirect inhibition), species, sex, dosage, route of administration, model construction and whether the ligature was removed (most models were ligature-induced). To minimize potential bias from individual studies, sensitivity analysis was conducted by sequentially excluding each trial to evaluate its influence on the pooled results.

Level of evidence. A total of two investigators independently assessed the certainty (level) of evidence for each outcome using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system (GRADEPRO.org), based primarily on five domains, including type of study design, risk of bias, inconsistency, indirectness, imprecision and other comprehensive consideration of sample size and confounding factors. Any disagreements were resolved through discussion or by a third researcher.

Results

Search outcomes. The literature search yielded a total of 99 studies (Fig. S2). After removing the duplicates and screening the remaining publications, 39 articles were eligible for full-text evaluation. Of these, 31 were excluded. A total of eight studies was retrieved for systematic review and assessed by meta-analysis (37-44)

Study characteristics. The present review included articles published from 2013-2023. Although there were no restrictions on the type of animal or publication language, all periodontal disease models identified were rat or mouse studies published in English. The number of animals ranged from 14-40, with a total of 119 males and 64 females [sex not reported in one study (41)]. Models included Sprague-Dawley rats (37,38,42), Wistar albino rats (44), inbred F344 rats (39), OPG-deficient mice (40) and periostin-knockout mice (41). Diet primarily comprised standard chow and tap water, except for the high-sugar water provided to all groups by Chen *et al* (37) and the 10% sucrose given to the experimental group by Yang *et al* (43).

For interventions targeting Scl activity, Scl antibody (Scl-Ab) was used in five studies (37,38,41,42,44), SOST gene knockout was performed in two studies (41,43) and specific pharmacological agents were tested in three studies, namely caffeic acid phenethyl ester (CAPE) (44), low-dose doxycycline (LDD) (44), infliximab (39) and WP9QY (40).

In addition to ligature-induced periodontitis, five studies also applied additional conditions unfavourable for bone regeneration, such as ovariectomy (37,38), periostin knockout (41), OPG deficiency (40) and streptozotocin-induced type I diabetes (37-41,43). A total of one study used silk ligatures saturated with *Porphyromonas gingivalis* (43).

Systemic subcutaneous injection of Scl-Ab was employed in three studies (37,40,42), while intraperitoneal injection was used in two studies (38,41). The dosage and duration included 25 mg/kg twice weekly for 2-8 weeks in four studies (37,41,42,44) and 5 mg/kg weekly for 22 weeks in one study (38). Local Scl-Ab application (125 μg) was tested in one study (42) but proved less effective compared with systemic administration. In addition, Yiğit *et al* (44) applied CAPE and LDD at 10 mg/kg for 14 days, delivered by intraperitoneal injection and oral gavage, respectively. CAPE was more effective compared with LDD at decreasing Scl expression. In the other pharmacological studies, infliximab was administered intraperitoneally at 5 mg/kg once or twice before euthanasia (39) and WP9QY was given subcutaneously at 10 mg/kg three times daily for 5 days (40).

The primary outcome assessment methods included micro-computed tomography (CT) and histological examination to evaluate ABL, BVF and BMD. Immunohistochemistry was used to assess the number of SOST-positive cells and osteoclasts. In addition, serum analysis, ELISA and reverse transcription-quantitative PCR were performed to measure serum biomarkers, such as procollagen type I N-terminal propeptide (P1NP), osteocalcin (OCN), tartrate-resistant acid phosphatase 5b (TRAP5b), RANKL, C-terminal telopeptide of type I collagen (CTx-1) and ALP. The characteristics of the included studies are summarized in Table I.

Quantitative synthesis and data analysis. The outcome variables with sufficient data, including changes in the CEJ-ABC distance, ABL, BMD, BVF, number of Scl-positive cells and bone-specific serum biomarkers such as OCN, P1NP and TRAP5b, were pooled for further analysis (Table SII).

Primary outcomes

ABL. ABL was evaluated by measuring the linear distance or volume between the CEJ and ABC before and after treatment. This parameter directly reflects periodontal tissue changes as assessed by histological or micro-CT examination. All studies (37-44) reported quantitative changes in ABL, therefore this was used as the primary outcome variable of the

Table I. Characteristics of included studies.

Characteristic	First author, year							
	Taut *et al*, 2013	Ren *et al*, 2015	Chen *et al*, 2015	Hadaya *et al*, 2019	Yang *et al*, 2016	Yiğit *et al*, 2022	Kim *et al*, 2016	Ozaki *et al*, 2017
Sample size	16	16	24	40	20	20	16	14
Animal	SD rat	Periostin knockout mouse	SD rat	SD rat	C57BL/6 mouse	Wistar albino rat	Inbred F344 rat	OPG-/- C57BL/6 mouse
Sex	Male	N/A	Female	Female	Male	Male	Male	Male
Diet	Regular rodent chow diet	N/A	Food and high-sugar drinking water	Standard diet NIH-31 modified	Standard solid mouse chow, 10% sucrose for EX group and sterile water for CON group	Standard rat food and tap water *ad libitum*	Standard rat chow and water *ad libitum*	Sterilized water and diet *ad libitum*
Construction of periodontal disease model	Ligature	Periostin knockout	Ligature; OVX	Ligature; OVX	Ligature of silk saturated with P.g	Ligature	Ligature; streptozotocin	OPG deficiency
Intervention, EX/CON	Scl-Ab/PBS	Scl-Ab, SOST and periostin double knockout/ PBS	Scl-Ab/ empty vehicle	Scl-Ab/empty vehicle	SOST gene knockout/none	CAPE; LDD/ PBS	Infliximab/ PBS	WP9QY/PBS
Route of administration	s.c.	i.p.	s.c.	i.p.	N/A	CAPE, i.p.; LDD, oral gavage	i.p.	s.c.
Dose	25 mg/kg	25 mg/kg	25 mg/kg	5 mg/kg Scl-ab	N/A	CAPE, 10 μmol/ kg/day; LDD, 10 mg/kg/day	5 mg/kg	10 mg/kg
Duration	Twice/week for 3 or 6 weeks	Twice/week for 8 weeks	Twice/week for 6 weeks	Weekly for 12 weeks before OVX and 10 weeks after OVX	N/A (constructive knockout); 2 months	Daily for 14 days	Once for the 3 day group (on day 0); twice for the 20 day group (on days 7 and 14)	3 times/day for 5 days

Table I. Continued.

Characteristic	First author, year							
	Taut *et al*, 2013	Ren *et al*, 2015	Chen *et al*, 2015	Hadaya *et al*, 2019	Yang *et al*, 2016	Yiğit *et al*, 2022	Kim *et al*, 2016	Ozaki *et al*, 2017
Secondary outcomes	BVF, TMD. OCN, P1NP, TRAP5b	Bone deposition rate, morphological changes of osteocytes, BV/TV	TRAP5b, CTx-1, BVF, BMD	P1NP, TRAP5b	OPG, RANKL, JNK, p38MAPK, ERK1/2	PNMLS, BMP2 and Scl-positive expression	IL-1β, Scl, cathepsin K-, RANKL- and Scl-positive cells	BV/TV; number of TRAP-5b-, sterix- orScl-positive cells; serum ALP
Detection technique	Bone-specific serum biomarkers; micro-CT; histology immunohistochemistry; fluorescent calcian labeling	Electron microscopy; micro-CT; histology/ immunohistochemistry; FITC staining and Imaris analysis	Bone-specific serum biomarkers; micro-CT; histology	Serum analysis; micro-CT; histology	Immunohistochemistry; micro-CT	Histomorphometry, histopathology and immunohistochemistry	Histology, histomorphology and immunohisto chemistry; RT-PCR	Micro-CT; histomorphology and immunohistochemistry; ELISA (serum marker)
(Refs.)	(42)	(41)	(37)	38)	(43)	(44)	(39)	(40)

Primary outcome was alveolar bone loss in all studies. SD, Sprague-Dawley; OPG, osteoproteprin; BV, bone volume; TV, total volume; TMD, tissue mineral density; PNMLS, polymorphonuclear leukocytes; RANKL, receptor activator of NF-κB ligand; i.p., intraperitoneal; s.c., subcutaneous injections; OVX, ovariectomy; TRAP5b, tartrate-resistant acid phosphatase; OCN, osteocalcin; P1NP, procollagen type I N-terminal propeptides; CAPE, caffeic acid phenethyl ester; LDD, low-dose doxycycline; SOST, sclerostin; Scl-Ab, sclerostin antibody; EX, experimental; CON, control; BMP2, bone morphogenetic protein 2; BVF, bone volume fraction; BMD, bone mineral density; CTx-1, C-terminal telopeptide of type I collagen; ALP, alkaline phosphatase; RT, reverse transcription; FITC, fluorescein isothiocyanate; N/A, not applicable; NIH-31, standard laboratory rodent diet; P.g, *Porphyromonas gingivalis*; CT, computed tomography.

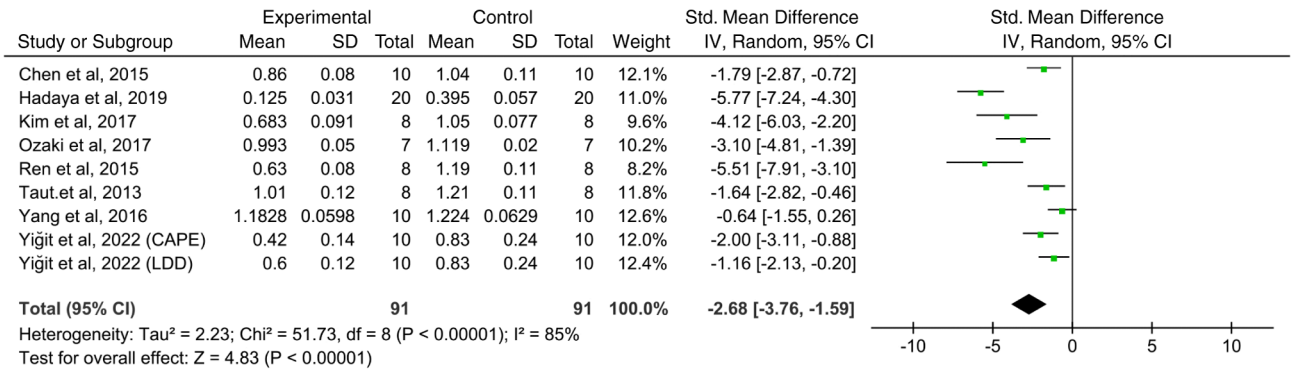

Figure 1. Forest plot of alveolar bone loss. Scl, sclerostin; df, degrees of freedom; LDD, low-dose doxycycline; CAPE, caffeic acid phenethyl ester; IV, inverse variance model.

present meta-analysis. Extracted data were expressed as linear distance, surface area or volume.

Pooled analysis revealed a significant decrease in the CEJ-ABC distance in the Scl inhibition group compared with the control group (Fig. 1; SMD: -2.68; 95% CI: -3.76 to -1.59; I^2=85%; P<0.0001). In a study by Yiğit *et al* (44), two drugs (LDD and CAPE) with Scl-inhibiting potential were tested and results were analysed separately. While numerous ABL data were derived from micro-CT measurements, two studies employed histological assessment (39,44), resulting in different measurement units (linear distance, area or volume).

Subgroup analysis was performed according to species, sex, dose, route of administration, type of Scl inhibition and periodontitis model. With the exception of dose and route of administration, no significant subgroup differences were observed (Fig. 2). Low-dose (5 mg/kg; Z=7.70) and subcutaneous (Z=5.26) treatments yielded improved outcomes compared with high-dose (25 mg/kg; Z=3.00; P=0.006; Fig. 2B) and intraperitoneal (Z=4.06; P=0.04) treatments (Fig. 2E).

A number of studies used a therapeutic design initiated after ligature removal (37,41-43). Taut *et al* (42) also incorporated a preventive arm, but no quantitative data were reported and this subgroup could not be included in the present meta-analysis. By contrast, Hadaya *et al* (38), in which ligatures were not removed and chronic inflammation persisted during treatment, exhibited a larger effect size compared with ligature-removed models (Fig. S3). These findings suggested that sustained inflammation may modify the response to Scl inhibition and decrease its net regenerative benefit.

Sensitivity analysis was conducted using a leave-one-out approach. After sequentially removing each included study, the pooled effect size and its 95% CI did not notably change (Fig. S4). This indicated that no single study had a decisive influence on the overall results, supporting the validity of the present meta-analysis.

Secondary outcomes

BVF. BVF is a volumetric parameter calculated as the ratio of BV to tissue volume based on micro-CT scanning. A total of three studies (37,40,42) reported the BVF and the newly formed bone area was significantly larger in the experimental groups compared with the control groups (Fig. 3A; SMD: 2.70; 95% CI: 0.69-4.71; I^2=82%; P=0.009). In a study by Taut *et al* (42), both systemic and local administration were tested, but only systemic data were extracted for meta-analysis to ensure consistency.

BMD/TMD. A total of two studies (37,42) reported BMD/TMD and revealed that compared with control treatment, systemic Scl-Ab treatment significantly increased BMD (Fig. 3B; SMD: 2.51; 95% CI: 1.58-3.44; I^2=0%; P<0.00001).

Other bone-specific serum biomarkers. Bone-associated serum markers (P1NP, OCN, OPG, RANKL, TRAP5b, CTx-1 and ALP) were evaluated in four studies (37,38,42,43). Except for OCN in two studies (37,42), no significant pooled results were obtained (Fig. 4A). Hadaya *et al* (38) reported that P1NP levels were notably higher in the Scl-Ab group at 6 and 12 weeks post-ovariectomy but decreased below baseline at the end of treatment (38). Taut *et al* (42) reported that OCN levels were notably elevated in the Scl-Ab group at 3 and 6 weeks. P1NP was also notably higher at 3 but not at 6 weeks (42). Chen *et al* (37) observed marked increases in OCN and OPG in the experimental group after 6 weeks, accompanied by decreases in TRAP5b and CTx-1 (37). Ozaki *et al* (40) reported that WP9QY did not reduce ALP levels in OPG-deficient mice but increased osteoblast differentiation in the M1 interradicular septum.

For serum TRAP5b, no notable differences were observed between the groups. The detection methods varied: Two studies (37,42) assessed TRAP5b expression through Scl-associated assays, while two others (38,40) used histological staining to quantify the number of osteoclasts/unit area (Fig. 4C). Although Taut *et al* (42) concluded that Scl inhibition had no effect on TRAP5b expression, the remaining studies indicated that Scl-Ab therapy decreased TRAP5b expression, which was consistent with increases in the expression of other osteogenic markers.

Number or ratio of SOST-positive cells. A total of three studies (39,40,44) using specific drugs assessed SOST-positive cells, consistently showing that the number of Scl-positive cells increased significantly in untreated periodontitis models but decreased markedly after treatment (Fig. 4B; SMD: -6.06, 95% CI: -10.29 to -1.84; I^2=93%; P=0.005).

Risk of bias. The risk of bias across ten domains was assessed using SYRCLE (Table SIII). Overall, the methodological quality of the included studies was moderate, with a

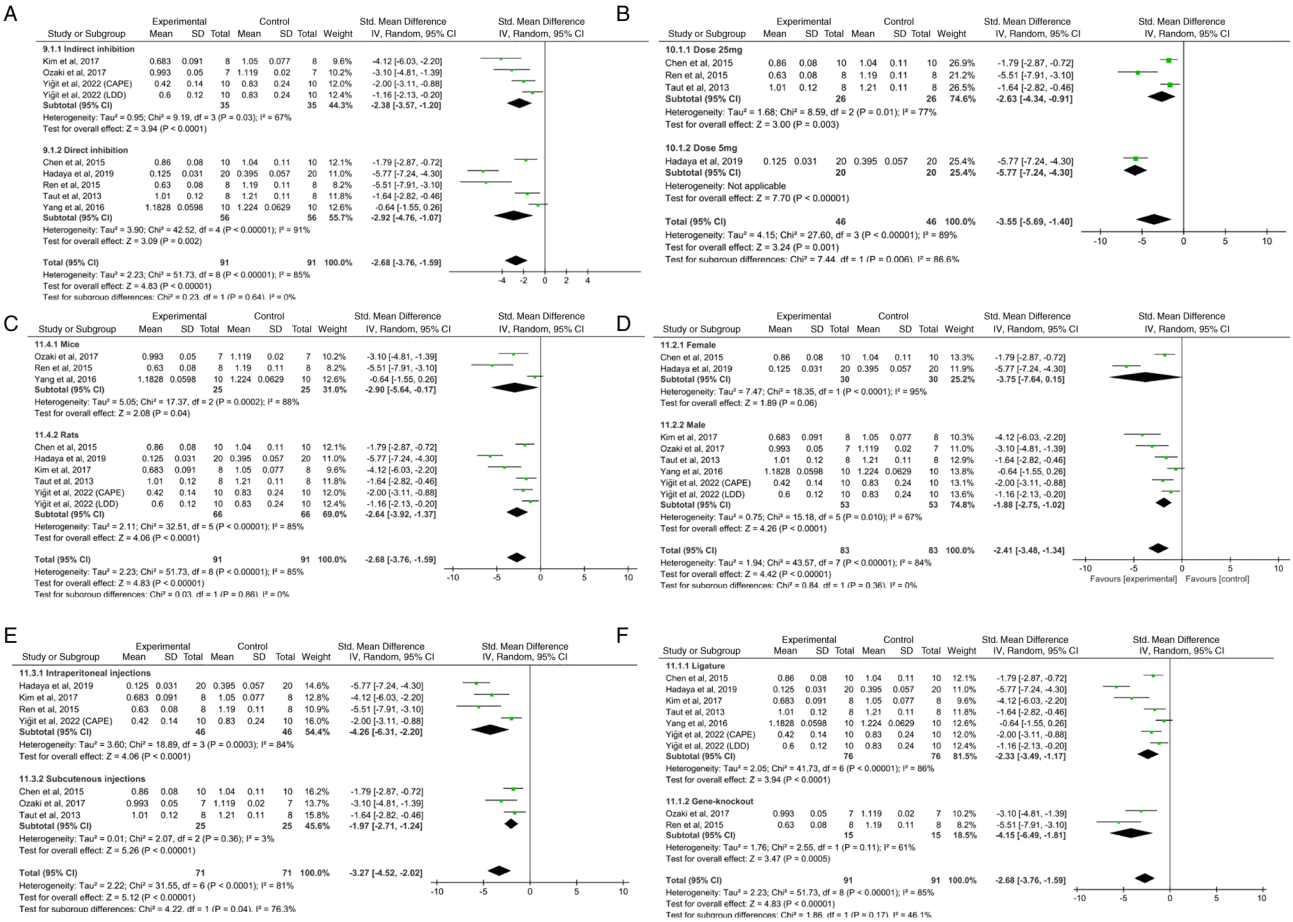

Figure 2. Subgroup analysis of alveolar bone loss. (A) Direct versus indirect inhibition. (B) Dose. (C) Species. (D) Sex. (E) Route of administration. (F) Induction method (ligature and gene-knockout). ABL, alveolar bone loss; Scl, sclerostin; df, degrees of freedom; LDD, low-dose doxycycline; CAPE, caffeic acid phenethyl ester; SMD, standardized mean difference; IV, inverse variance model.

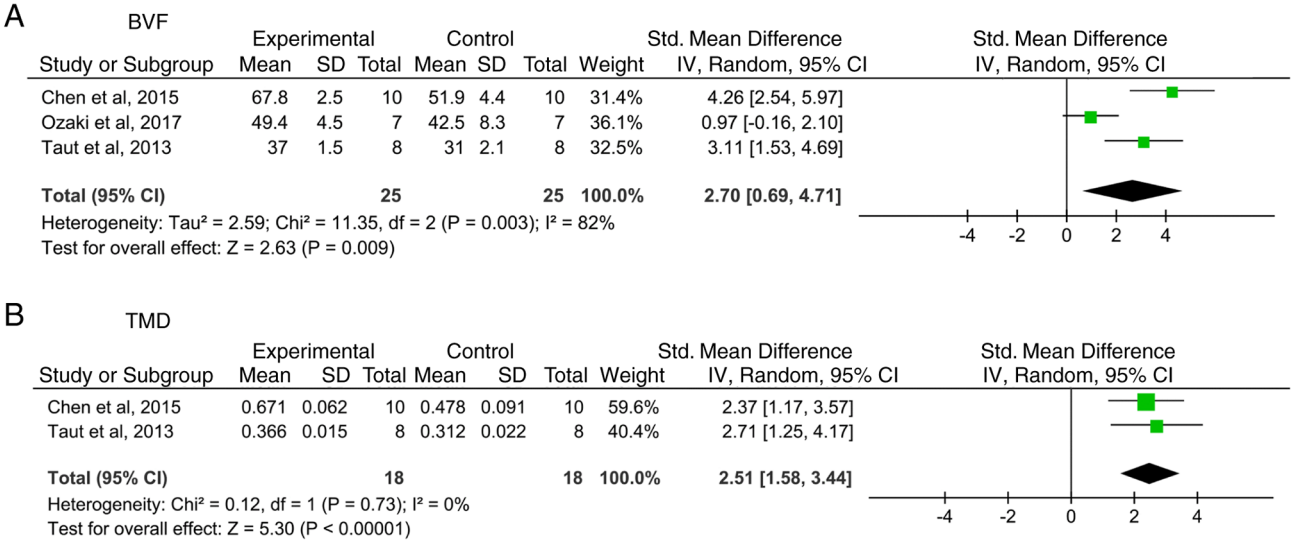

Figure 3. Forest plots of BVF and TMD. (A) Bone volume fraction. (B) Tissue mineral density. Scl, sclerostin; df, degrees of freedom; SMD, standardized mean difference; IV, inverse variance model; BVF, bone volume fraction; TMD, tissue mineral density.

number of domains judged as unclear or high risk. Adequate sequence generation was reported in 1/8 studies, while baseline characteristics were comparable in 7/8 (37-41,43,44). Allocation concealment was not described in any report and therefore was rated as high risk across all the studies. Random housing and outcome assessments were not reported in any study, resulting in universally unclear ratings. A total of three studies (37,38,44) described the blinding of experimenters

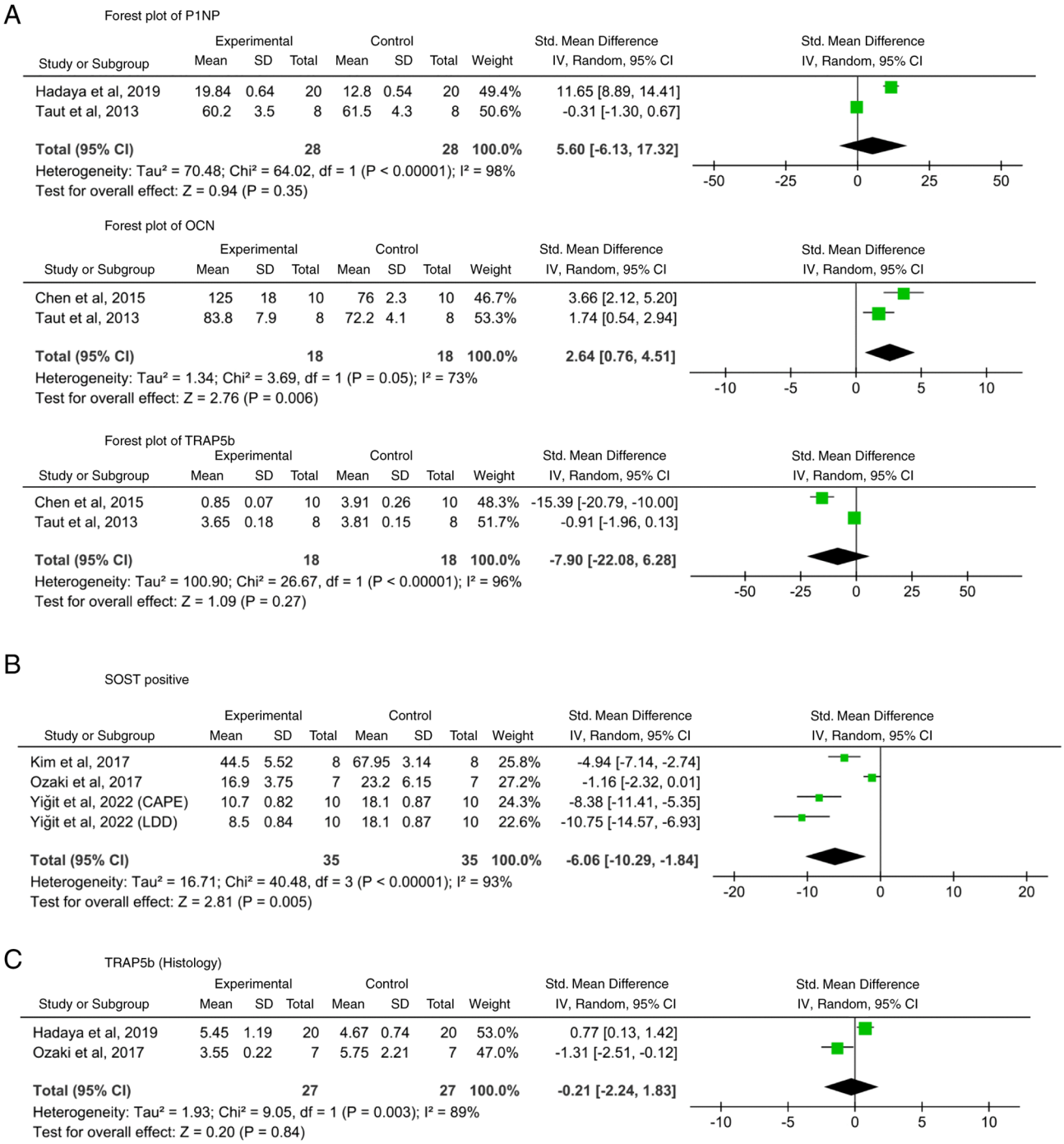

Figure 4. Forest plots of bone-specific biomarkers and SOST-positive cells. (A) Bone-specific serum biomarkers (P1NP, OCN and TRAP5b). (B) Histological test for TRAP5b. (C) SOST-positive cells. Scl, sclerostin; df, degrees of freedom; LDD, low-dose doxycycline; CAPE, caffeic acid phenethyl ester; SMD, standardized mean difference; TRAP5b, tartrate-resistant acid phosphatase 5b; SOST, sclerostin; IV, Inverse Variance Model; Pp1NP, procollagen type I N-terminal propeptide; OCN, osteocalcin; TRAP5b, tartrate-resistant acid phosphatase 5b.
Supplementary figure legends

and the blinding of outcome assessors. By contrast, incomplete outcome data were adequately addressed in all studies and selective outcome reporting was judged as low risk in 7/8 (37-41,43,44). Other sources of bias were consistently rated as low risk. Collectively, these findings indicated that although the internal validity of the included studies was limited by insufficient reporting of randomization, housing and blinding, the risk of attrition and reporting bias was generally low.

Level of evidence. Level of evidence for SOST-positive cells, ABL and related subgroups was determined to be moderate. However, the levels of evidence for TMD, BVF, OCN, P1NP and TRAP5b were lower (Table SIV). The decrease in the level of evidence was primarily due to inconsistency and imprecision or less overlap in CI, small P-values for the heterogeneity test, large I^2 value and failure to perform sensitivity analyses. None of the included studies reported osteonecrosis or other adverse events associated with sclerostin inhibition

Discussion

The present analysis highlighted the potential of Scl inhibition in mitigating ABL and promoting osteogenesis in experimental periodontitis models (37,38,41-43). The mechanisms underlying these effects are complex but may involve key pathways including Wnt/β-catenin signaling, which regulates osteoblast differentiation and bone formation (13-15,41,43). In addition, Scl inhibition may act via the RANKL/OPG pathway, thereby balancing osteoclast activity and limiting excessive bone resorption (16,37,40,42). The decrease in the number of SOST-positive cells further suggested that Scl inhibition effectively targets its intended biological pathway at the histological level (39,41,44).

Scl inhibition not only attenuated alveolar bone loss (ABL) but also promoted regeneration through three primary mechanisms. First, activation of the canonical Wnt pathway restores β-catenin translocation to the nucleus, rescuing osteogenic differentiation in periodontal ligament stem cells (13-15,41,43,45). Second, modulation of the RANKL/OPG pathway limits osteoclastogenesis by suppressing macrophage colony-stimulating factor secretion from platelet-derived growth factor receptor-β (PDGFRβ)-positive osteoprogenitors (16,37,40,42,46,47). Third, disruption of the TNF-α/NF-κB/SOST feedback loop reduces inflammation-induced osteocyte apoptosis and RANKL production, thereby supporting bone preservation and regeneration (39,48,49). Together, these multi-pathway interactions highlight the key role of Scl in periodontitis, namely bone microenvironment crosstalk.

Low-dose regimens (5 mg/kg) (38) showed greater efficacy compared with higher doses (25 mg/kg), particularly in maintaining bone formation and decreasing ABL (37,41,42,44). This paradox suggests that treatment duration and timing may be more influential compared with dose alone. In periodontal models, longer treatment (22 vs. 4-6 weeks) promoted cumulative Wnt activation, underscoring the importance of optimizing both dose and duration for clinical translation (38). Administration route also influenced outcomes, with subcutaneous delivery (37,40,42) outperforming intraperitoneal injection, potentially due to decreased hepatic first-pass metabolism (38,41,45). However, the current evidence is limited and the superiority of lower-dose regimens should be interpreted cautiously. More data are needed to confirm the optimal dose-time association and future studies should systematically examine dosing schedules and treatment durations to validate these observations.

The present study primarily reflects therapeutic models, as preventive evidence was scarce. A number of studies applied therapeutic interventions following ligature removal (37,41-43). Taut *et al* (42) tested both preventive and therapeutic arms, but only the therapeutic arm reported quantitative data, hindering pooled analysis of preventive effects. Hadaya *et al* (38) retained ligatures during treatment to model persistent inflammation. Compared with inflammation-resolved therapeutic models, this design produced a larger effect size for ABL but less pronounced regenerative benefits. These findings suggested that persistent inflammation limits the anabolic effects of Scl inhibition, even when short-term improvements in bone turnover markers are observed. The coexistence of preventive and therapeutic designs may therefore obscure biological interpretation and should be considered when extrapolating to clinical contexts.

To assess the robustness of the present results, a leave-one-out sensitivity analysis was performed, sequentially excluding each study and recalculating the pooled effect size. Results remained consistent across all iterations, indicating that no single study disproportionately influenced the overall findings. This supported the validity of the present conclusions despite heterogeneity and small sample sizes.

Notably, transient increases in bone formation markers such as P1NP have been reported, consistent with findings from osteoporosis models (38,42,46). None of the included studies reported osteonecrosis or similar adverse events (38). This suggested a favorable safety profile compared with antiresorptive therapies such as bisphosphonates, which are associated with medication-related osteonecrosis of the jaw. The absence of major adverse outcomes supports the translational potential of Scl inhibition.

A number of limitations should be acknowledged. First, heterogeneity across studies was moderate-high, stemming from differences in animal species, periodontitis induction method, intervention protocol (dose, duration, route of administration, preventive vs. therapeutic design and presence or absence of residual inflammation). These variations complicate direct comparisons and may explain the variability in effect sizes. However, sensitivity analysis demonstrated that the overall conclusions remained robust despite this heterogeneity. Second, the overall risk of bias was high or unclear across a number of domains. A number of studies did not adequately describe randomization procedures and allocation concealment was rarely reported, raising concerns regarding potential selection bias. While a number of studies reported blinding of investigators or outcome assessors, these practices were inconsistent, increasing the risk of performance and detection bias. In addition, small sample size and incomplete methodological reporting further decreased the certainty of evidence and may limit reproducibility. These shortcomings highlight the need for stricter adherence to established guidelines such as the ARRIVE guidelines (50) or SYRCLE (35), which emphasize transparent reporting of randomization, allocation concealment and blinding. Strengthening these aspects in future animal studies may minimize bias and improve reliability. Third, the use of different outcome assessment methods created additional inconsistency. Techniques such as micro-CT, histology, immunohistochemistry and serum biomarkers vary in sensitivity, quantification accuracy and the biological processes they capture. For example, micro-CT provides precise three-dimensional measurements of alveolar bone, whereas histology and immunohistochemistry yield localized and often semi-quantitative information. Serum biomarkers, by contrast, reflect systemic rather than site-specific changes in bone turnover. Furthermore, different biomarkers (such as OCN, P1NP and TRAP5b) and detection approaches (immunohistochemistry vs. serum assays) were used across studies. These discrepancies complicate comparisons, contribute to variability in effect sizes and may explain why some results had low statistical significance. Such inconsistencies may also affect the reliability of the

results. Consequently, the overall certainty of evidence was downgraded to moderate to low according to GRADE. Future studies should use standardized and validated detection protocols or coordinate primary outcome measures and biomarker panels, to enhance comparability, reproducibility and reliability. Finally, the predominance of ligature-induced models (7/8 studies) limits the generalizability of findings to bacterially driven periodontitis (37-44). These limitations underscore the need for more rigorous and standardized protocols in future. Clear reporting of randomization, allocation concealment and blinding, along with coordination of biomarker detection methods and outcome assessments, are key in reducing heterogeneity and improving evidence quality. In parallel, systematic investigation of preventive vs. therapeutic settings, optimized dose-time regimens, alternative delivery routes and diverse disease models are necessary to delineate the translational potential of Scl inhibition in periodontal regeneration (37-44).

In conclusion, the present analysis suggested Scl inhibition notably decreased ABL and enhanced the expression of bone formation markers in preclinical periodontitis models. The improved efficacy of low-dose subcutaneous regimens (5 mg/kg) suggested that sustained Wnt activation rather than transient peak-dose stimulation may underlie the superior therapeutic response. These findings warrant clinical trials evaluating Scl-Ab as an adjunct to conventional periodontal therapy (for example, scaling and root planing), prioritizing dose optimization and standardized imaging protocols.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MY contributed to data analysis using RevMan and EndNote software, constructed figures, analyzed data, and wrote and revised the manuscript. YM conceived the study, constructed figures, analyzed data, and wrote and revised the manuscript. XQ designed the study methodology, contributed to project administration and resources, and provided supervision. ZQ contributed to the study conception, critical revision of the manuscript for important intellectual content, and overall supervision of the project. JS contributed to study validation, data interpretation and resource coordination. MY and YM confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Abdulkareem AA, Al-Taweel FB, Al-Sharqi AJB, Gul SS, Sha A and Chapple ILC: Current concepts in the pathogenesis of periodontitis: From symbiosis to dysbiosis. J Oral Microbiol 15: 2197779, 2023.
2. Meyle J and Chapple I: Molecular aspects of the pathogenesis of periodontitis. Periodontol 2000 69: 7-17, 2015.
3. Hajishengallis G and Chavakis T: Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat Rev Immunol 21: 426-440, 2021.
4. Tonetti MS, Greenwell H and Kornman KS: Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Clin Periodontol 45 Suppl 20: S149-S161, 2018.
5. Kwon T, Lamster IB and Levin L: Current concepts in the management of periodontitis. Int Dent J 71: 462-476, 2021.
6. Graziani F, Karapetsa D, Alonso B and Herrera D: Nonsurgical and surgical treatment of periodontitis: How many options for one disease? Periodontol 2000 75: 152-188, 2017.
7. Slots J: Periodontitis: Facts, fallacies and the future. Periodontol 2000 75: 7-23, 2017.
8. Haque MM, Yerex K, Kelekis-Cholakis A and Duan K: Advances in novel therapeutic approaches for periodontal diseases. BMC Oral Health 22: 492, 2022.
9. Preshaw PM and Bissett SM: Periodontitis and diabetes. Br Dent J 227: 577-584, 2019.
10. Zhou N, Zou F, Cheng X, Huang Y, Zou H, Niu Q, Qiu Y, Shan F, Luo A, Teng W and Sun J: Porphyromonas gingivalis induces periodontitis, causes immune imbalance, and promotes rheumatoid arthritis. J Leukoc Biol 110: 461-473, 2021.
11. Cardoso EM, Reis C and Manzanares-Céspedes MC: Chronic periodontitis, inflammatory cytokines, and interrelationship with other chronic diseases. Postgrad Med 130: 98-104, 2018.
12. Persson GR: Periodontal complications with age. Periodontol 2000 78: 185-194, 2018.
13. Napimoga MH, Nametala C, da Silva FL, Miranda TS, Bossonaro JP, Demasi AP and Duarte PM: Involvement of the Wnt-β-catenin signalling antagonists, sclerostin and dickkopf-related protein 1, in chronic periodontitis. J Clin Periodontol 41: 550-557, 2014.
14. Mihara A, Yukata K, Seki T, Iwanaga R, Nishida N, Fujii K, Nagao Y and Sakai T: Effects of sclerostin antibody on bone healing. World J Orthop 12: 651-659, 2021.
15. Jacobsen CM: Application of anti-Sclerostin therapy in non-osteoporosis disease models. Bone 96: 18-23, 2017.
16. Kitaura H, Marahleh A, Ohori F, Noguchi T, Shen WR, Qi J, Nara Y, Pramusita A, Kinjo R and Mizoguchi I: Osteocyte-related cytokines regulate osteoclast formation and bone resorption. Int J Mol Sci 21: 5169, 2020.
17. Liao C, Liang S, Wang Y, Zhong T and Liu X: Sclerostin is a promising therapeutic target for oral inflammation and regenerative dentistry. J Transl Med 20: 221, 2022.
18. Chatzopoulos GS, Costalonga M, Mansky KC and Wolff LF: WNT-5a and SOST levels in gingival crevicular fluid depend on the inflammatory and osteoclastogenic activities of periodontal tissues. Medicina (Kaunas) 57: 788, 2021.
19. Yakar N, Guncu GN, Akman AC, Pınar A, Karabulut E and Nohutcu RM: Evaluation of gingival crevicular fluid and peri-implant crevicular fluid levels of sclerostin, TWEAK, RANKL and OPG. Cytokine 113: 433-439, 2019.
20. Chatzopoulos GS, Koidou VP and Wolff LF: Expression of Wnt signaling agonists and antagonists in periodontitis and healthy subjects, before and after non-surgical periodontal treatment: A systematic review. J Periodontal Res 57: 698-710, 2022.
21. Chatzopoulos GS, Mansky KC, Lunos S, Costalonga M and Wolff LF: Sclerostin and WNT-5a gingival protein levels in chronic periodontitis and health. J Periodontal Res 54: 555-565, 2019.

22. Ozden FO, Demir E, Lutfioglu M, Acarel EE, Bilgici B and Atmaca A: Effects of periodontal and bisphosphonate treatment on the gingival crevicular levels of sclerostin and dickkopf-1 in postmenopausal osteoporosis with and without periodontitis. J Periodontal Res 57: 849-858, 2022.
23. Delgado-Calle J, Sato AY and Bellido T: Role and mechanism of action of sclerostin in bone. Bone 96: 29-37, 2017.
24. Iwamoto R, Koide M, Udagawa N and Kobayashi Y: Positive and negative regulators of sclerostin expression. Int J Mol Sci 23: 4895, 2022.
25. Tanaka S and Matsumoto T: Sclerostin: From bench to bedside. J Bone Miner Metab 39: 332-340, 2021.
26. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, Hofbauer LC, Lau E, Lewiecki EM, Miyauchi A, *et al*: Romosozumab treatment in postmenopausal women with osteoporosis. N Engl J Med 375: 1532-1543, 2016.
27. Martiniakova M, Babikova M and Omelka R: Pharmacological agents and natural compounds: Available treatments for osteoporosis. J Physiol Pharmacol 71: 2020.
28. Tinsley BA, Dukas A, Pensak MJ, Adams DJ, Tang AH, Ominsky MS, Ke HZ and Lieberman JR: Systemic administration of sclerostin antibody enhances bone morphogenetic protein-induced femoral defect repair in a rat model. J Bone Joint Surg Am 97: 1852-1859, 2015.
29. Korn P, Kramer I, Schlottig F, Tödtman N, Eckelt U, Bürki A, Ferguson SJ, Kautz A, Schnabelrauch M, Range U, *et al*: Systemic sclerostin antibody treatment increases osseointegration and biomechanical competence of zoledronic-acid-coated dental implants in a rat osteoporosis model. Eur Cell Mater 37: 333-346, 2019.
30. Virdi AS, Irish J, Sena K, Liu M, Ke HZ, McNulty MA and Sumner DR: Sclerostin antibody treatment improves implant fixation in a model of severe osteoporosis. J Bone Joint Surg Am 97: 133-140, 2015.
31. Li X, Ominsky MS, Villasenor KS, Niu QT, Asuncion FJ, Xia X, Grisanti M, Wronski TJ, Simonet WS and Ke HZ: Sclerostin antibody reverses bone loss by increasing bone formation and decreasing bone resorption in a rat model of male osteoporosis. Endocrinology 159: 260-271, 2018.
32. Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP and Thomas J: Updated guidance for trusted systematic reviews: A new edition of the cochrane handbook for systematic reviews of interventions. Cochrane Database Syst Rev 10: ED000142, 2019.
33. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, *et al*: The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372: n71, 2021.
34. Drevon D, Fursa SR and Malcolm AL: Intercoder reliability and validity of WebPlotDigitizer in extracting graphed data. Behav Modif 41: 323-339, 2017.
35. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M and Langendam MW: SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 14: 43, 2014.
36. Higgins JPT and Green S: Cochrane handbook for systematic reviews of interventions. Version 5.1.0. The Cochrane Collaboration, London, 2011. www.handbook.cochrane.org.
37. Chen H, Xu X, Liu M, Zhang W, Ke HZ, Qin A, Tang T and Lu E: Sclerostin antibody treatment causes greater alveolar crest height and bone mass in an ovariectomized rat model of localized periodontitis. Bone 76: 141-148, 2015.
38. Hadaya D, Gkouveris I, Soundia A, Bezouglaia O, Boyce RW, Stolina M, Dwyer D, Dry SM, Pirih FQ, Aghaloo TL and Tetradis S: Clinically relevant doses of sclerostin antibody do not induce osteonecrosis of the jaw (ONJ) in rats with experimental periodontitis. J Bone Miner Res 34: 171-181, 2019.
39. Kim JH, Kim AR, Choi YH, Jang S, Woo GH, Cha JH, Bak EJ and Yoo YJ: Tumor necrosis factor-α antagonist diminishes osteocytic RANKL and sclerostin expression in diabetes rats with periodontitis. PLoS One 12: e0189702, 2017.
40. Ozaki Y, Koide M, Furuya Y, Ninomiya T, Yasuda H, Nakamura M, Kobayashi Y, Takahashi N, Yoshinari N and Udagawa N: Treatment of OPG-deficient mice with WP9QY, a RANKL-binding peptide, recovers alveolar bone loss by suppressing osteoclastogenesis and enhancing osteoblastogenesis. PLoS One 12: e0184904, 2017.
41. Ren Y, Han X, Ho SP, Harris SE, Cao Z, Economides AN, Qin C, Ke H, Liu M and Feng JQ: Removal of SOST or blocking its product sclerostin rescues defects in the periodontitis mouse model. FASEB J 29: 2702-2711, 2015.
42. Taut AD, Jin Q, Chung JH, Galindo-Moreno P, Yi ES, Sugai JV, Ke HZ, Liu M and Giannobile WV: Sclerostin antibody stimulates bone regeneration after experimental periodontitis. J Bone Miner Res 28: 2347-2356, 2013.
43. Yang X, Han X, Shu R, Jiang F, Xu L, Xue C, Chen T and Bai D: Effect of sclerostin removal in vivo on experimental periodontitis in mice. J Oral Sci 58: 271-276, 2016.
44. Yiğit U, Kırzıoğlu FY and Özmen Ö: Effects of low dose doxycycline and caffeic acid phenethyl ester on sclerostin and bone morphogenic protein-2 expressions in experimental periodontitis. Biotech Histochem 97: 567-575, 2022.
45. Ominsky MS, Boyce RW, Li X and Ke HZ: Effects of sclerostin antibodies in animal models of osteoporosis. Bone 96: 63-75, 2017.
46. Ashifa N, Viswanathan K, Sundaram R and Srinivasan S: Sclerostin and its role as a bone modifying agent in periodontal disease. J Oral Biosci 63: 104-110, 2021.
47. de Vries TJ and Huesa C: The osteocyte as a novel key player in understanding periodontitis through its expression of RANKL and sclerostin: A review. Curr Osteoporos Rep 17: 116-121, 2019.
48. Sapir-Koren R and Livshits G: Osteocyte control of bone remodeling: Is sclerostin a key molecular coordinator of the balanced bone resorption-formation cycles? Osteoporos Int 25: 2685-2700, 2014.
49. Li X, Niu QT, Warmington KS, Asuncion FJ, Dwyer D, Grisanti M, Han CY, Stolina M, Eschenberg MJ, Kostenuik PJ, *et al*: Progressive increases in bone mass and bone strength in an ovariectomized rat model of osteoporosis after 26 weeks of treatment with a sclerostin antibody. Endocrinology 155: 4785-4797, 2014.
50. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, *et al*: The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18: e3000410, 2020.