Sclerostin Antibody Stimulates Bone Regeneration After Experimental Periodontitis

Andrei D Taut,1 Qiming Jin,2 Jong-Hyuk Chung,1,3 Pablo Galindo-Moreno,1,4 Erica S Yi,1 James V Sugai,1 Hua Z Ke,5 Min Liu,5 and William V Giannobile1,6

1Department of Periodontics and Oral Medicine, School of Dentistry, University of Michigan, Ann Arbor, MI, USA
2Department of Cariology, Restorative Sciences, and Endodontics, School of Dentistry, University of Michigan, Ann Arbor, MI, USA
3Department of Periodontology, School of Dentistry, Kyung Hee University, Seoul, South Korea
4Department of Oral Surgery and Implant Dentistry, University of Granada, Granada, Spain
5Department of Metabolic Disorders, Amgen Inc., Thousand Oaks, CA, USA
6Department of Biomedical Engineering, College of Engineering, University of Michigan, Ann Arbor, MI, USA

ABSTRACT

The reconstruction of large osseous defects due to periodontitis is a challenge in regenerative therapy. Sclerostin, secreted by osteocytes, is a key physiological inhibitor of osteogenesis. Pharmacologic inhibition of sclerostin using sclerostin-neutralizing monoclonal antibody (Scl-Ab) thus increases bone formation, bone mass and bone strength in models of osteopenia and fracture repair. This study assessed the therapeutic potential of Scl-Ab to stimulate alveolar bone regeneration following experimental periodontitis (EP). Ligature-induced EP was induced in rats to generate localized alveolar bone defects. Following 4 weeks of disease induction, Scl-Ab (+/−EP) or vehicle (+/− EP) were systemically delivered, twice weekly for up to 6 wks to determine the ability of Scl-Ab to regenerate bone around tooth-supporting osseous defects. 3 and 6 wks after the initiation of Scl-Ab or vehicle treatment, femur and maxillary jawbones were harvested for histology, histomorphometry, and micro-computed tomography (micro-CT) of linear alveolar bone loss (ABL) and volumetric measures of bone support, including bone volume fraction (BVF) and tissue mineral density (TMD). Serum was analyzed to examine bone turnover markers during disease and regenerative therapy. Vehicle + EP animals exhibited maxillary bone loss (BVF, TMD and ABL) at ligature removal and thereafter. 6 weeks of Scl-Ab significantly improved maxillary bone healing, as measured by BVF, TMD and ABL, when compared to vehicle + EP. After 6 weeks of treatment, BVF and TMD values in the Scl-Ab + EP group were similar to those of healthy controls. Serum analysis demonstrated higher levels of bone formation markers osteocalcin and PINP in Scl-Ab treatment groups. Scl-Ab restored alveolar bone mass following experimental periodontitis. These findings warrant further exploration of Scl-Ab therapy in this and other oral bone defect disease scenarios. © 2013 American Society for Bone and Mineral Research.

KEY WORDS: PERIODONTAL DISEASES; REGENERATIVE MEDICINE; BONE HEALING; BONE ANABOLIC AGENTS; TISSUE ENGINEERING

Introduction

Periodontal disease is a destructive disease that targets tooth-supporting structures of the dentition through complex and multifactorial pathogenic processes. They are initiated by an interaction between bacterial components of tooth-related biofilms and host response mechanisms.1 Although gingivitis represents the reversible inflammatory reaction to biofilms, periodontitis is the nonreversible destructive stage of a persistent bacterial infection.2 Left untreated, periodontitis results in soft tissue destruction and progressive bone destruction, leading to tooth mobility and subsequent tooth loss.3 Various treatment procedures have been widely used for periodontal regeneration therapy. However, reconstruction of large osseous defects resulting from periodontal disease remains a challenge, and procedures such as bone grafts and guided bone regeneration remain unpredictable in their ability to consistently regenerate bone.4,5 Studies on cellular and molecular mechanisms of Wnt and BMP signaling on bone homeostasis have suggested that the use of Wnt signal-enhancing agents may be a promising alternative therapy to prevent bone loss and regenerate periodontal supporting tissues.5,6

Sclerostin, a secreted glycoprotein, is the product of the SOST gene. Sclerostin, produced and secreted primarily by osteocytes, has been shown to be a negative regulator of osteoblast differentiation/function and an inhibitor of bone formation. Although the exact mechanism by which sclerostin inhibits bone formation has not yet been fully identified, studies have...
demonstrated that sclerostin may act through LRP5/6 to inhibit Wnt/beta-catenin signaling, impairing osteoblast differentiation and function.\(^6,7\)\) As such, inhibition of sclerostin could then lead to an increase in Wnt/beta-catenin signaling, thereby increasing bone formation.

Human genetics show that patients with loss-of-function mutations in the SOST gene have high bone mass and density, including in their jawbones.\(^8\)\(^\text{–}\)\(^10\) SOST null mice have increased bone formation, bone mass, and bone strength.\(^11\) Sclerostin deficiency in human sclerosteosis, together with the high bone mass phenotype of SOST null mice, suggest that sclerostin inhibition may be a viable approach for developing novel bone anabolic agents for treatment of bone disorders, including alveolar bone loss. A murine anti-sclerostin monoclonal antibody that neutralizes sclerostin (Scl-Ab) has been developed,\(^12\) and has been shown to restore bone formation, bone mass, and bone strength in a rat model of postmenopausal osteoporosis.\(^13\)

Furthermore, rat and nonhuman primate models show that systemic Scl-Ab administration can locally increase bone formation to enhance fracture healing.\(^14\) Clinical studies indicate that Scl-Ab administration to healthy men and postmenopausal women increased bone formation markers and bone mineral density (BMD), suggesting therapeutic potential for Scl-Ab in osteoporosis and other bone disorders that might benefit from increased bone formation.\(^15\)

The current study provides one of the first tests of the hypothesis that Scl-Ab administration can have favorable impacts on disease-affected craniofacial tissues. This study aimed to determine the role of a neutralizing anti-sclerostin antibody (Scl-Ab), as a bone anabolic agent, to regenerate alveolar bone after experimental periodontitis.

Materials and Methods

Scl-Ab administration in healthy (intact) Sprague-Dawley rats

All animal procedures were performed under guidelines approved by the University of Michigan Unit for Laboratory Animal Medicine (ULAM), University Committee on Use and Care of Animals (UCUCA), and compliant with Animals Research: Reporting In Vivo Experiments (ARRIVE) guidelines. To examine the effect of sclerostin blockade in alveolar bone, healthy (intact) rats were treated with either vehicle (\(n = 10\)) or Scl-Ab (ratized monoclonal Scl-Ab III, Amgen Inc., Thousand Oaks, CA, USA; \(n = 8\))\(^1\) via subcutaneous injections into the dorsa of 9-week-old adult male Sprague-Dawley rats (Charles River Laboratories International, Inc., Wilmington, MA, USA), twice weekly for 2 and 4 weeks. Phosphate-buffered saline (1X PBS; Life Technologies, Grand Island, NY, USA) served as the vehicle control in all studies. Maxillary alveolar bone specimens including first, second, and third molars were collected for \(\mu\)CT scanning and volumetric analysis (described below). Femora were harvested to assess the systemic bone anabolic effects of Scl-Ab after 4 weeks. Serum was also collected at 2 and 4 weeks to evaluate systemic biochemical markers of bone formation and resorption.

Experimental periodontal disease (EP) model

After a 1-week acclimation period, adult male Sprague-Dawley rats (approximate weight 250 to 300 g, aged 9 to 10 weeks in regenerative study) were anesthetized using isoflurane inhalation (Charles River Laboratories International, Inc.). Silk sutures (3/0) were placed unilaterally into the gingival sulci of the left maxillary first and second molar teeth to induce experimental periodontitis (EP) through: 1) activation of inflammatory responses against the increased presence of microbial biofilm; and 2) physical irritation and destruction of alveolar bone as a result of subgingival placement of suture material.\(^16\) Sutures were displaced apically into the gingival sulci twice weekly to ensure a subgingival position and were replaced as necessary.

Regeneration of alveolar bone after experimental periodontitis

Experimental periodontitis was induced for a period of 4 weeks. Intact, healthy controls were not ligated and served as controls. Ligatures were removed at the end of the experimental periodontitis phase before initiating Scl-Ab treatment. To investigate its regenerative potential, Scl-Ab or its vehicle control was administered subcutaneously at a dose of 25 mg/kg, twice weekly for therapeutic periods of 3 and 6 weeks (\(n = 7\) and \(n = 8\), respectively) after a 4-week phase of experimental periodontitis (Fig. 1A), whereas intact, healthy controls were treated with 1X PBS (3 weeks, \(n = 9\); 6 weeks, \(n = 8\)). In other EP animals, Scl-Ab was administered locally, twice weekly for 3 and 6 weeks (\(n = 10\) and \(n = 8\), respectively), into three palatal gingival tissue sites: between the left maxillary first and second molars and near the base of the interproximal gingival papillae (\(5\) \&\& \(10\) mg/mL solution per site, totaling \(15\) mL per animal per treatment session) as previously described, with 1X PBS serving as the control (3 weeks, \(n = 10\); 6 weeks, \(n = 9\)).\(^17\)

Micro-computed tomography scanning and bone analysis

Maxillary bone specimens—including first, second, and third molars—were collected at the designated end points, placed in 10% neutral-buffered formalin for 2 days, and transferred to 70% ethanol (EtOH) for \(\mu\)CT scanning. Fixed, undecalcified maxillae were scanned using a cone beam \(\mu\)CT system (GE Healthcare BioSciences, Model Paxs-928EA, GE Healthcare, London, ON, Canada) at the University of Michigan Orthopaedic Research Imaging Laboratory. The X-ray generator was operated at an accelerated potential of 80 kV with a beam current of 80 mA and the X-ray source 2D detector operated with a shutter speed of 1100 ms to produce images with a voxel size of \(18 \times 18 \times 18\) \(\mu\)m. Scans were reconstructed and three-dimensional (3D) digitized images generated for each specimen. Using GEMS MicroView software (eXplore MicroView v.2.1.2, Analysis Plus, GE Healthcare), each reconstructed image was rotated into a standardized orientation, and a region of interest (ROI) for each specimen was created as previously described with minor modifications.\(^17\) Briefly, for volumetric analysis of the maxillary tooth-supporting alveolar bone, the furcation origin of the first maxillary molar, apices of the mesial root of the first maxillary molar/distal root of
the third maxillary molar, and the mesial roots of the first molar and distal roots of the second molar were used as landmarks for quantifying alveolar bone loss and regeneration within a reproducible region (Fig. 1D–E). Using the average Hounsfield Units (HU) grayscale threshold value for all of the samples, a single, calibrated examiner (AT) volumetrically quantified the alveolar bone surrounding first and second maxillary molars (Fig. 1F), including bone volume fraction (BVF) and tissue mineral density in mg/mm³ (TMD). Additionally, a single, calibrated examiner (EY) linearly measured linear alveolar bone loss (ABL) in millimeters, according to a previously described protocol. ABL is used clinically to quantify the integrity of structural support around a tooth. High ABL values indicate bone loss, whereas low ABL values are correlated with healthy bone levels. Briefly, ABL was measured as the linear distance between the cementoenamel junction and alveolar bone crest at five roots for M1 and four roots for M2 as shown in Fig. 1B, C. ABL data were presented as the overall average of the linear measures for M1 and M2.

**Bone-specific serum biomarkers**

Serum was obtained from blood collected by cardiac puncture at the designated study end points. Serum osteocalcin (OCN) was measured using the rat Osteocalcin Single-plex LINCOplex Kit (RBN-31K-10C, Millipore, St. Charles, MO, USA). Serum procollagen type 1 amino-terminal propeptide (P1NP) and tartrate-resistant acid phosphatase 5b (TRACP 5b) were detected using rat ELISA kits (AC-33F1, SB-TR102, IDS, Fountain Hills, AZ, USA).

**Histology and descriptive bone histomorphometry**

Rats were euthanized in a CO² chamber at the specified time points, and maxillary specimens (containing first/second/third maxillary molars and surrounding alveolar bone) were harvested and fixed in 10% neutral-buffered formalin for 48 hours. After μCT analysis, specimens were decalcified with 10% EDTA disodium salt for 3 weeks, embedded in paraffin, and cut into 5-μm-thick serial sagittal sections of the maxillary first, second, and third molars. Sections were stained with hematoxylin and eosin for descriptive analysis. Remaining sections were deparaffinized and immunostained for detection/localization of SOST and CathK (goat anti-mouse SOST antibody, R&D Systems, catalog no. AF1589; rabbit polyclonal anti-cathepsin K, Amgen, Thousand Oaks, CA, USA). Histological and immunohistochemical images were captured with a Nikon Eclipse 5i microscope (Nikon Inc., Melville, NY, USA) and Nikon Digital Sight DS U1 camera (Nikon Inc.). The fraction of the bone surface covered by osteoclasts on the interproximal bone between M1 and M2 was calculated using NIS Elements Basic Research software (Nikon Inc.).

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**Fig. 1.** Study timeline and three-dimensional measures for analysis of tooth-supporting alveolar bone. (A) Regenerative study timeline began with 4 weeks of experimental periodontitis, followed by ligature removal and 3 and 6 weeks of subcutaneous and local Scl-Ab administration. Lingual (B) and buccal (C) 3D views of maxilla at the site of ligature placement. Linear alveolar bone loss (ABL), or the linear distance (orange line) between the cementoenamel junction (CEJ; yellow-dashed line) and alveolar bone crest (green line), was measured along five roots for M1 and four roots for M2. (D) Anatomical landmarks of M1 and M2 were used to create a three-dimensional ROI (yellow box). (E) M1, M2, and M3 were digitally removed and tooth-supporting bone within the disease region was isolated for volumetric quantification (F).
Fluorescent calcein labeling

To assess the mineral deposition rate of alveolar bone during the regenerative phase, rats were given two subcutaneous injections of calcein, 9 and 2 days before euthanasia according to a standard protocol.19 Mandibulæ from each animal (n = 2) were harvested, dehydrated, and embedded undecalciﬁed in methylvemethacrylate for sectioning. Image capture and descriptive histomorphometry were performed on two consecutive nontained 4-μm sections.

Femur bone mineral density

To assess systemic bone-building effects of Scl-Ab, femura from each animal were harvested at euthanization, ﬁxed in 10% phosphate-buffered formalin for 48 hours at 4°C, and stored in 70% EtOH. Areal BMD of left femur was determined ex vivo by dual-energy X-ray absorptiometry (DXA; Hologic QDR 4500a, Bedford, MA, USA). The DXA machine was calibrated according to the manufacturer’s instructions.

Prevention of alveolar bone destruction during experimental periodontitis

We also conducted a study to examine the potential for Scl-Ab to prevent the progression of alveolar bone destruction with EP, by initiating Scl-Ab treatment just before the placement of ligatures. Sprague-Dawley rats with EP were assigned to receive either vehicle or Scl-Ab and intact animals served as the control. Three days before EP induction, a single-dose Scl-Ab (25 mg/kg) was administered via subcutaneous injection. From then onward, Scl-Ab and vehicle injections continued twice weekly for 2 and 4 weeks, concurrently with EP, starting on the day of ligature placement. Maxillary alveolar bone specimens were collected for μCT scanning and volumetric analysis (as described above).

Statistical analysis

Statistical analyses were performed using GraphPad Prism software. Data were pooled by experimental group, and the mean and standard deviation (SD) were calculated. One-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc tests were performed for measuring statistically signiﬁcant differences between groups for serum biomarker levels, volumetric and linear bone levels, and osteoclast number. A p value less than 0.05 was considered to be statistically signiﬁcant. Statistical analysis was carried out in consultation with the Center for Statistical Consultation and Research (CSCAR) at the University of Michigan.

Results

Pharmacological inhibition of sclerostin in healthy alveolar bone

A study was conducted to ﬁrst address whether Scl-Ab had bone anabolic effects on healthy, intact maxillary alveolar bone in rats. The μCT data in Fig. 2A indicate that Scl-Ab signiﬁcantly increased maxillary BVF and TMD in intact healthy rats lacking ligatures compared with vehicle treatment at 2 weeks (BVF, p = 0.001; TMD, p = 0.003) and at 4 weeks (BVF, p = 0.001; TMD, p = 0.010). DXA scanning and analysis of animal long bone was performed to conﬁrm the systemic treatment effects of Scl-Ab in other skeletal sites. As expected, the areal femoral BMD, serum P1NP, and OCN were increased (p < 0.0001, p = 0.0003, and p = 0.058, respectively) in Scl-Ab–treated intact animals compared with the vehicle control group at 4 weeks (Fig. 2B, C), consistent with the systemic bone anabolic effects. This result provided the rationale for examining the therapeutic potential of Scl-Ab in periodontal disease-related bone loss. Such conditions were then mimicked in a separate study by initiating Scl-Ab treatment after ligature-induced inﬂammation and maxillary bone loss had already occurred.

Pharmacological inhibition of sclerostin in established alveolar bone loss

Four weeks of ligature-induced EP led to signiﬁcant reductions in alveolar bone volume and density around maxillary ﬁrst (M1) and second (M2) molars compared with the intact control group (week 0/BL data, Fig. 3A, B). After ligature removal, natural bone healing led to a rebound in BVF and TMD in vehicle-treated rats lasting 3 weeks. However, after 3 weeks, alveolar bone healing plateaued in rats receiving vehicle treatment (week 3 data, Fig. 3A, B). Three weeks after commencement of systemic Scl-Ab therapy, Scl-Ab–treated animals exhibited a trend toward increased BVF (p < 0.05) and TMD (p < 0.05) compared with the vehicle group. Most notably, systemic Scl-Ab therapy reversed ligature-induced bone loss after the 6-week treatment period, with no differences evident between BVF (p = 0.3378) and TMD (p = 0.4196) of EP animals treated with Scl-Ab and the intact animal group (week 6 data, Fig. 3A, B; Fig. 4B). Furthermore, BVF and TMD of both Scl-Ab and intact animal groups were signiﬁcantly greater compared with the vehicle treatment group after 6 weeks of Scl-Ab treatment (Fig. 4). In contrast, local injection of Scl-Ab after experimental periodontitis exhibited a limited regenerative effect on volumetric alveolar bone healing, as indicated by μCT analysis (Supplemental Fig. S1).

Systemic Scl-Ab administration showed no effects on the EP-related linear alveolar bone loss (ABL) at 3 weeks (p > 0.05), whereas it led to a modest but statistically signiﬁcant improve-ment in linear ABL from EP (p < 0.05) at 6 weeks when compared with vehicle treatment (Fig. 3C). Site-speciﬁc analysis revealed that ABL gain occurred primarily on the buccal regions of M1 and M2 (data not shown).

DXA scanning of femurs was performed to evaluate systemic (post-cranial) skeletal responses to Scl-Ab. Femur BMD was signiﬁcantly increased after 3 weeks (p < 0.001) and 6 weeks (p < 0.001) in Scl-Ab–treated animals compared with both intact and vehicle-treated EP groups (Fig. 5A).

Regeneration mode: bone-formation biomarkers

During early healing, serum OCN concentration was signiﬁcantly higher in animals treated with Scl-Ab compared with intact (p = 0.0019) and vehicle (p = 0.0001) groups after 3 weeks of treatment (Fig. 5B). At 6 weeks post-treatment commencement, serum OCN levels in Scl-Ab–treated animals were still elevated (p = 0.034) compared with the intact control group. Scl-Ab

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increased serum P1NP levels 3 weeks after the start of treatment compared with both intact (p = 0.10) and vehicle-treated EP (p = 0.058) groups (Fig. 5C), whereas there were no statistical differences, in P1NP levels, detected between intact, vehicle-treated EP, and Scl-Ab–treated EP groups at 6 weeks. In addition to the increase in systemic bone-formation biomarkers, Scl-Ab treatment exhibited no change on serum TRAP 5b levels during the 6-week therapeutic phase after ligature-induced EP (Fig. 5D).

Regeneration mode: detection of SOST in alveolar bone tissues

Anabolic effects of Scl-Ab on alveolar bone regeneration were assessed at tissue level by histology. Hematoxylin and eosin (H&E) images of the tooth-root encompassing bone confirmed the sustained bone loss after EP induction. Six weeks of Scl-Ab treatment restored the lost bone microarchitecture, volume, and density to levels comparable to intact control (Fig. 6A–C). Immunostainings for sclerostin and cathepsin K expression were performed to monitor the target protein and active osteoclasts at the site of EP. After 6 weeks of Scl-Ab therapy (Fig. 6D–F), IHC revealed an increased presence of the protein sclerostin within the lacunae, canaliculi, and marrow spaces of the alveolar bone of maxillary M1 and M2 (Fig. 6F). In comparison, sclerostin was minimally detected within the alveolar bone of both the intact and vehicle-treated EP control groups after 6 weeks (Fig. 6D, E). IHC for cathepsin K was applied to detect the presence of active osteoclasts at the site of EP; 6 weeks of Scl-Ab therapy did not lead to a change in osteoclast number and surface coverage (Fig. 6G–I and data not shown) in the eroded interdental regions of alveolar bone, which was consistent with unchanged serum TRAP 5b concentrations with Scl-Ab (Fig. 5C). Descriptive analysis of a small subset of calcein-labeled mandibular samples revealed that Scl-Ab increased bone apposition rate compared with the vehicle-treated EP and intact groups (Fig. 6J–L), as demonstrated by the increased distance between fluorescent bands in the Scl-Ab–treated EP group at 6 weeks.

Prevention mode

To examine whether Scl-Ab could prevent alveolar bone loss in maxillary bone affected by periodontal disease, Scl-Ab was injected concurrently with placement and during the continuous
different (compared with vehicle treatment for 6 weeks led to a significant recovery of disease-induced ABL compared with vehicle-treated EP group (\( p < 0.05 \) versus Scl-Ab; \( p < 0.05 \) versus intact; \( p < 0.05 \) versus vehicle).

Our study provides early preclinical evidence that sclerostin has an ongoing role in minimizing alveolar bone mass postdevelopmentally in young growing rats. Sclerosteosis patients display increased jawbone growth because of the genetic deficiency of sclerostin during early growth and development. We now document that blockade of sclerostin via neutralizing Scl-Ab displays an anabolic effect on alveolar bone, both under physiologic conditions and therapeutically after EP-induced bone loss. Within 2 weeks of subcutaneous Scl-Ab administration, intact tooth-supporting alveolar bone exhibited significantly greater bone volume fraction and mineral density relative to vehicle control, and this effect was maintained through week 4. Under active inflammatory conditions such as the prevention study shown here, Scl-Ab administration in EP groups had limited effects on focal BVF and TMD when compared with corresponding vehicle controls. Although Scl-Ab has been shown to halt systemic bone loss under inflammatory conditions in a mouse model of chronic colitis, specific interactions between Scl-Ab and inflammatory pathways in alveolar bone merit further investigation. Furthermore, additional studies with longer treatment duration will be needed to determine the preventative potential of Scl-Ab under active periodontal inflammatory conditions.

In healthy intact animals, the increases by Scl-Ab in alveolar bone volume and density corresponded with increased long bone mass and density at 4 weeks. Furthermore, increases in bone volume and density were consistent with a trend toward increased serum OCN at 2 and 4 weeks and a significant increase in the bone formation marker P1NP at 4 weeks. Femur bone density and serum bone formation markers in an aged, gonad-intact male rat model with the same treatment regimen showed similar treatment response. In general, Scl-Ab has been shown to either decrease or have no effect on serum TRAP 5b levels across a variety of preclinical animal models. In addition to the bone anabolic response, Scl-Ab also showed beneficial anti-resorptive effect as evidenced by the decreased level of CTXI in a recent clinical trial.

Our findings also suggest that under the current experimental conditions, Scl-Ab administration reverses alveolar bone loss in a preclinical animal model of periodontitis. After EP induction—which resulted in decreases of 30% and 32% in BVF and TMD, respectively—animal groups receiving Scl-Ab exhibited higher alveolar bone volume and density compared with vehicle controls after just 3 weeks of therapy. Additionally, although presence of ligatures. Systemic Scl-Ab administration in the EP group resulted in only nonsignificant, modest increases in both BVF and TMD at 2- and 4-week time points when compared with corresponding vehicle controls (Supplemental Fig. S2A, B). This result indicates that Scl-Ab might be well suited as an adjunctive therapy, in conjunction with aggressive management of inflammation.

**Discussion**

Outcomes of sclerostin inhibition using a neutralizing monoclonal antibody have been studied in clinical trials for postmenopausal osteoporosis, and in preclinical models of osteoporosis, osteogenesis imperfecta, rheumatoid arthritis, bone repair, and fracture healing. In both the preclinical and clinical settings, administration of Scl-Ab is consistently associated with increased bone formation, bone mass, and bone density at several sites throughout the body, but effects within the craniofacial complex have never been investigated.

Fig. 3. Micro-CT assessment of alveolar bone BVF, TMD, and ABL during Scl-Ab therapy after induction of experimental periodontitis. Change in BVF (A) and TMD (B) throughout the regenerative study with subcutaneous dosing. Three weeks after ligature removal, BVF and TMD of vehicle-treated EP and intact groups were still statistically different (\( p < 0.05 \) versus intact). After 6 weeks of Scl-Ab treatment, BVF and TMD showed no statistical differences compared with the intact group (\( p < 0.05 \) versus Scl-Ab). (C) Mean linear ABL around first and second maxillary molars measured using micro-CT software. Scl-Ab treatment for 6 weeks led to a significant recovery of disease-induced ABL compared with vehicle-treated EP group (\( p < 0.05 \) versus Scl-Ab; \( p < 0.05 \) versus intact; \( p < 0.05 \) versus vehicle).
**Fig. 4.** Sclerostin antibody facilitates alveolar bone regeneration after experimental periodontitis. Representative micro-CT images of maxillary alveolar bone surrounding the first (M1) and second (M2) molars at (A) baseline and (B) 6-week time points for intact and EP treatment groups. Representative sagittal and transverse slices (2D) as well as 3D images of maxillary samples help showcase the visual differences between the amount of bone regenerated around M1 and M2 in Scl-Ab–treated animals versus vehicle-treated animals after 6 weeks.

**Fig. 5.** Scl-Ab increases serum concentration of bone formation markers osteocalcin and P1NP in regeneration study. Femur BMD (A) and serum concentrations of OCN (B), P1NP (C), and TRAP 5b (D) at baseline, 3-week, and 6-week time points.
natural healing of bone plateaued approximately 3 weeks after ligature removal, BVF and TMD continued to increase with Scl-Ab treatment for up to 6 weeks. After 6 weeks of systemic Scl-Ab therapy, significant increases in alveolar bone volume and density were observed compared with the vehicle-treated EP group. Furthermore, no statistical difference was found between alveolar bone BVF and TMD of EP compared with intact, healthy animals.

The lack of effect of locally administered Scl-Ab on alveolar bone regeneration may be owing to the absence of special carriers for maintaining high local drug exposure, and/or the insufficient amount of total Scl-Ab administered via this route (approximately 25-fold lower than the systemic dose). More sophisticated local delivery approaches with sustained controlled release profiles may have therapeutic potential and should be explored. The local anabolic bone response detected in animals treated with systemic Scl-Ab may have been owing in part to the activation of downstream systemic modulators that influence bone formation and remodeling. Recent studies suggest that sclerostin affects bone metabolism by decreasing concentrations of both vitamin D and PTH. Therefore, a systemic sclerostin blockade may prove more effective in activating bone anabolic signaling pathways in addition to Wnt signaling to a greater extent than what may be achieved with local delivery.

Collectively, the current data suggest that systemic Scl-Ab administration results in a global skeletal anabolic effect and active mineralization on the surface of both cortical and trabecular bone in the jaws. Despite the positive outcomes of systemic Scl-Ab delivery on volumetric measures of alveolar bone, the effects on linear bone height under the current conditions were limited. The kinetics of linear ABL changes associated with systemic Scl-Ab administration suggest that greater improvements might be found with longer-term treatment, a hypothesis that warrants further study. Nonetheless, the overall alveolar bone responses to systemic Scl-Ab.

Fig. 6. Histological evaluation and IHC detection of bone markers in tooth-supporting alveolar bone after 6 weeks of Scl-Ab administration in regeneration study. ROI for H&E (A–C) and SOST IHC (D–F) marked as black dotted rectangular outline on sagittal section (2× magnification) of M1/M2 and supporting alveolar bone. Red, dotted rectangular outline marks ROI for CathK IHC (G–I). H&E sections (A–C) of alveolar bone surrounding the roots of M2 (20× magnification). (D–F) IHC staining for SOST (40× magnification) indicated an increased presence of sclerostin protein in osteocytes and marrow spaces of tooth-supporting alveolar bone after Scl-Ab treatment. Red triangles indicate SOST-positive osteocytes and white triangles indicate SOST-negative osteocytes. (G–I) CathK IHC staining (10× magnification) for osteoclast cells present on the surface (indicated by yellow triangles) of the interproximal bone of M1 and M2. CathK-positive cells (marked by asterisk) also present within the marrow spaces. (J–L) Calcein double-labeling images of interradicular alveolar bone from intact, vehicle-treated EP, and Scl-Ab-treated EP groups. Scl-Ab treatment increases the mineral apposition rate in tooth-supporting alveolar bone as detected by fluorescent calcein labeling (yellow-outlined orange triangles).
administration improved alveolar bone stock, which might result in higher-quality tooth-supporting alveolar bone. Collectively, the observed increases in alveolar BVF and TMD and the decrease in ABL with systemic Scl-Ab might serve to better preserve at-risk natural teeth in the periodontitis setting, and/or to better prepare affected regions of bone for prosthetic implants.

The presence of the sclerostin protein was dramatically increased in the alveolar bone of animals treated with Scl-Ab compared with vehicle-treated controls, which might reflect an accumulation of the inactive, neutralized sclerostin protein trapped within the tissues, and/or perhaps an increase in intracellular sclerostin within osteocytes, suggesting a compensatory upregulation of sclerostin upon inhibition by Scl-Ab. As anticipated, sclerostin was localized to the osteocyte-containing lacunae, as well as throughout the connecting network of canaliculi of the alveolar bone. Furthermore, sclerostin was also detected on the bone surface of marrow spaces within alveolar bone, suggesting that Scl-Ab may facilitate bone formation by enhancing differentiation of osteoblast precursors within the bone marrow. (29)

Within a small subset of samples, fluorescent calcein labeling revealed an anabolic effect at both 3 and 6 weeks in groups receiving Scl-Ab therapy. Furthermore, the anabolic effects of sclerostin neutralization on bone appear to be enhanced by normal mechanical loading. (30) Increased regional alveolar bone formation was observed on the mesial surfaces and bone marrow spaces of the mandibular alveolar bone. Sclerostin has been shown to be important in mechano-sensation and the preservation of alveolar bone mechanical properties in response to the masticatory forces acting on the surface of bone. (29)

However, further investigation will be needed to determine the relationship between natural forces and Scl-Ab-mediated bone apposition in alveolar bone.

The prolonged absence of sclerostin, during development and throughout life, in patients suffering from sclerosteosis, leads to several characteristic clinical features evident in their craniofacial tissues. Patients display irregular dentition with dental malocclusion, increased spacing between teeth, and excess cellular cementum, which are believed to be secondary defects resulting from loss-of-function of sclerostin. (31) Additionally, in rare cases, partial anodontia and delayed eruption have also been recorded. (32) A deeper understanding of the mechanisms behind the clinical manifestations in patients with sclerosteosis and SOST knockout animal models could provide insight into the development of models for further studying the effects of sclerostin deficiency on craniofacial tissues.

Several study limitations are worth mentioning. When the regeneration study was terminated, improvements in ABL with Scl-Ab were only beginning to manifest. Because ABL can be an important surrogate for clinical measures of PD, it will be important to examine the reproducibility of this positive effect and to test whether ABL further improves with longer durations of Scl-Ab administration. The modest effects of Scl-Ab on alveolar bone in the prevention protocol, wherein active inflammation and ligatures were present throughout, might suggest that Scl-Ab therapy would be more effective as an adjuvant to standards of care that reduce active inflammation. It also remains unclear as to whether Scl-Ab might impact the periodontal ligament and the strength of tooth attachment. Although sclerostin expression has been localized in tooth-associated cementocytes in rodents/humans, the effects of sclerostin loss-of-function on cementum tissue remain unclear, (33,34) and the role of sclerostin in cementum homeostasis and formation, as well as the effects of sclerostin neutralization on cementum regeneration, warrant further investigation in the future. However, in this study, we noted that along with alveolar bone regeneration, there was a corresponding cementum that formed without significant alterations to the thickness or general morphology, similar to intact cemental surfaces in health animals. Further studies would be required to more carefully determine sclerostin’s role on cementogenesis, especially in situations of complete cemental removal after root-planing procedures, not performed in this study. Future studies will be required to monitor alveolar bone levels after the cessation of Scl-Ab therapy. Additionally, it will be important to monitor the anabolic effects of systemic Scl-Ab delivery at different sites throughout the body to avoid potentially detrimental effects of excess bone formation.

In summary, Scl-Ab treatment induces an anabolic response in alveolar bone under physiologic conditions, as well as during alveolar bone healing in experimental periodontitis by increasing bone formation. Our results support the rationale for the clinical investigation of Scl-Ab in augmenting alveolar bone healing and facilitating bone regeneration in a dental setting for patients with periodontal disease.

Disclosures

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