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Sclerostin antibody enhances implant osseointegration in bone with Col1a1 mutation

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Abstract

We evaluated the potential of sclerostin antibody (SclAb) therapy to enhance osseointegration of dental and orthopaedic implants in a mouse model (Brtl/+) mimicking moderate to severe Osteogenesis Imperfecta (OI). To address the challenges in achieving stable implant integration in compromised bone conditions, our aim was to determine the effectiveness of sclerostin antibody (SclAb) at improving bone-to-implant contact and implant fixation strength. Utilizing a combination of micro-computed tomography, mechanical push-in testing, immunohistochemistry, and Western blot analysis, we observed that SclAb treatment significantly enhances bone volume fraction (BV/TV) and bone-implant contact (BIC) in Brtl/+ mice, suggesting a normalization of bone structure toward WT levels. Despite variations in implant survival rates between the maxilla and tibia, SclAb treatment consistently improved implant stability and resistance to mechanical forces, highlighting its potential to overcome the inherent challenges of OI in dental and orthopaedic implant integration. These results suggest that SclAb could be a valuable therapeutic approach for enhancing implant success in compromised bone conditions.

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Declaration of competing interest

KMK is a consultant for NextCure, Inc. The remaining authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

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Keywords

Osteogenesis imperfecta; Maxilla; Alveolar bone; Tibia; Sclerostin

1. Introduction

In contemporary healthcare, dental implants play a crucial role in restoring oral function and aesthetics for individuals suffering from tooth loss [1]. These implants are typically made from biocompatible materials such as titanium and require surgical placement into the bone. A key factor in their success is osseointegration, the process through which the implant integrates with the surrounding bone tissue [2]. Osseointegration is facilitated by the implant's stability and involves two stages: primary stability, which is mechanical and occurs immediately after placement, and secondary stability, a longer-term biological integration that ensures the implant's lasting stability [3,4].

Excessively brittle and soft bone can compromise the primary and secondary stability of dental implants. Specifically, implants in the maxilla that lack primary stability often exhibit persistent fibrous encapsulation and mobility upon histological examination [5,6]. Osteogenesis imperfecta (OI) results in impaired collagen synthesis due to mutations in collagen (COL1A1 and COL1A2) or collagen-associated genes, leading to reduced bone quality and quantity [7,8]. Successfully achieving osseointegration in patients with OI is particularly challenging due to the complex nature of their bone condition. Although, some studies suggest a high success rate for dental implants in patients with OI, implying that implants could be a viable treatment [9,10]. However, these findings are primarily based on a limited number of cases, predominantly involving mild forms of OI [9,10]. The actual failure rates are not well documented, representing a significant gap in current research. These observations highlight the critical importance of achieving initial implant stability in OI to prevent long-term complications [11,12].

Recent studies have highlighted the potential of Sclerostin antibody (SclAb) therapy in enhancing bone mineral density and improving the structural integrity of OI bones [13–17]. Sclerostin, a protein expressed by osteocytes that regulates bone metabolism, acts by inhibiting Wnt signaling, thus reducing bone formation [18,19]. The inhibition of sclerostin by SclAb has been shown to improve bone quality and promote bone regeneration and osseointegration in preclinical studies involving both maxilla and long bones [20–25]. However, the efficacy of such treatments in OI models, which exhibit unique pathophysiological features affecting bone quality and biomechanics, remains unknown. Gaining insights from these preclinical studies could lead to a better understanding of this treatment and its potential applicability for OI patients.

Many SclAb-fixation studies in rat models are conducted in long bones and show increased osseointegration induced by SclAb [22,23,26,27]. However, these findings are not directly extrapolatable to the clinical conditions of dental implant placement in humans, which typically occur in the jawbones (maxilla and mandible). The jawbones differ significantly in characteristics from long bones; for example, the maxilla presents a moist, microbially rich environment and is more challenging to access compared to the tibia. In contrast, the tibia,

being easily accessible and shielded by muscle and skin, is a convenient model for studying implant osseointegration, yet it may not capture the full complexity of osseointegration in the jawbones [6,28,29].

Our study aims to analyze the effects of SclAb treatment on osseointegration around maxillary implants, using the Brtl/+ mouse model that closely resembles moderate to severe OI in humans [30]. We used the tibia as a reference to explore SclAb effects on osseointegration in long bones, commonly analyzed in fixation osseointegration studies. We propose that the structural and environmental uniqueness of the maxilla may respond differently to SclAb compared to tibia, potentially causing variations in implant osseointegration in an OI mouse model. This study allows us to examine the effects of SclAb on osseointegration in these bones, with the ultimate goal of improving oral rehabilitation for individuals affected by this condition.

2. Material and methods

2.1. Animals

All protocols and procedures involving animals were approved by the University of Michigan's Committee on Use and Care of Animals (UM-IACUC PRO00011108, approved on 11/29/22). Wildtype (WT) and Brtl/+ mice with a mixed background of SV129/CD-1/C57BL/6S were derived from heterozygous Brtl/+ and WT parental strains. The Brtl/+ mouse is a heterozygous knock-in model for OI, featuring a Gly349Cys substitution in a COL1A1 allele, which has also been found in subjects affected by a moderately severe form of OI (type IV) [31]. A total of 48 female mice (3-month-old) underwent bilateral maxillary and tibial implant placement under inhaled isoflurane anesthesia. Buprenorphine was administered pre-operatively and again 12 h later. Carprofen was administered immediately post-operatively and again 24 h later, covering 48 h post-surgery pain management. All surgical animals were housed in specific pathogen-free cages with standard 12-h light/dark cycles and had access to a soft diet following surgery for up to 72 h. Mice ($n = 48$) were randomly assigned to SclAb (25 mg/kg SclAb VI, Amgen, Thousand Oaks, CA) or vehicle injection (PBS) subcutaneously starting on the day of the implant placement, two times per week for 5 weeks following our previously described protocol [15]. No antibiotics were administered to the animals after implantation surgery. These mice were euthanized at 5 weeks (35 days) post-implant placement by CO2 inhalation (Fig. 1).

2.1.1. Maxilla implant bed—Implants were placed transmucosally without incision or flap. An implant bed (0.4 mm width x 1 mm depth) was drilled with a bur at 650 rpm in the alveolar ridge between the upper central incisor and the first molar (Fig. 1).

2.1.2. Tibia implant bed—5 mm skin incisions were performed in the anteromedial of the proximal tibia without reflecting the periosteum. The implant bed (0.4 mm width × 1 mm depth) was drilled with a bur at 650 rpm in the union of the proximal metaphysis with the diaphysis. Incisions were re-approximated with a 6–0 nylon suture (Ethilon Monofilament 6–0, BV100–3, 5 in., Johnson & Johnson Medical, USA).

2.1.3. Implant selection—To simulate the clinical implant placement, we used 'Retopins' made with a titanium-6 aluminum-4 vanadium alloy (NTI Kahla GmbH, Thüringen, Germany). These screws, with a diameter of 0.4 mm are the smallest commercially available that fit the mouse maxilla, as demonstrated in previous studies using CD1 and C57Bl/6 mice [6,32].

2.1.4. Implant placements—Each titanium implant, 1 mm in length, was screwed into the implant bed finger-tight using the integrated handle of the Retopins, which was then manually cut with a cutter after placement. Each mouse received bilateral maxillary and tibial implants (Fig. 1).

The implant survival rate is determined by the number of implants that are successfully placed in the implant bed and remain stable for 35 days. Any implant that is lost during this period is classified as a failure.

2.2. Micro computed tomography (μCT)

Prior to scanning, maxilla (n = 24) and tibia (n = 24) samples (Table S1) were embedded in polymethyl methacrylate (PMMA). Samples were scanned at room temperature using an Xradia MicroXCT 520 (Zeiss, Jena, Germany) outfitted with a 0.4× objective lens. The X-ray source was configured to a voltage of 70 kV and a power of 10 W, with no X-ray prefilter utilized. Calibration phantoms were employed, and reference points for each sample were established using both air and implant standards. We developed a segmentation model using the deep learning tool in the Dragonfly 2022 software suite (Object Research Systems, Inc.; QC, Canada). This model was programmed to automatically segment bone, tooth, implant, and air within the sample. Subsequently, the model was employed to automate the segmentation process, enabling quantitative analysis of bone-implant contact and peri-implant bone volume fraction.

To confirm the systemic effect of SclAb treatment observed in our previous studies [13–15], we assessed the femoral trabecular and cortical phenotypes (without implants) of our samples. This evaluation served as an internal control to ascertain that the systemic action of SclAb is consistent with earlier findings, independent of implant-related effects. We used high-resolution μCT (Bruker, Skyscan 1176, Kontich, Belgium), following methodologies previously described [30] to ensure that any changes observed in the surgical site could be attributed to the SclAb effect rather than being merely implant-induced secondary effects.

2.3. Push-in testing

For the mechanical testing of the implants, we used the push-in test because it is reported to be more sensitive than the pull-out test to changes in the mechanical properties at the bone-implant interfaces [33,34]. We utilized an MTS 858 Mini-Bionix servo-hydraulic testing system (MTS Systems Corp., Eden Prairie, MN, USA) to administer a push-in force on the implants. We designed and fabricated a customized probe slightly smaller than the implant to facilitate proper testing of the osseointegration strength at the implant-bone interface. Prior to mechanical testing, each fresh specimen (n = 24) (Table S1) was held and stabilized, but not infiltrated, in light-cured dental acrylic and maintained in a hydrated state

using Phosphate-buffered Saline (PBS). Implants were subjected to a uniaxial compressive load until failure at a consistent displacement rate of 0.1 mm/s. During these tests, a 10-lb load cell (Sensotec, Columbus, OH, USA) continuously recorded the applied force, while an external linear variable differential transducer (LVDT; Lucas Schavitts, Hampton, VA, USA) monitored vertical displacements. Push-in load, defined as the force required to dislodge an implant from the murine maxillary or tibial bone, was precisely measured during these mechanical tests. We employed a customized LABVIEW program (NI, v21.0, Austin, Tx, USA) to perform comprehensive calculations of the mechanical properties under examination.

2.4. Immunohistochemistry (IHC) and Western blot

Maxillae were harvested and fixed in 10 % neutral buffered formalin. Samples ($n = 8$) (Table S1) were decalcified in 20 % EDTA for eight weeks and after complete demineralization, the implants were gently removed from the samples. Specimens were dehydrated through an ascending ethanol series prior to paraffin embedding. 5 μm thick sagittal maxillary sections were cut and collected on Super Frost-plus slides for histology staining including Masson's trichrome. Sample slides were processed for immunohistochemistry (IHC). Before staining, slides were baked at 60° C for at least one hour, then deparaffinized with xylene and rehydrated through graded ethanol baths and MilliQ water. Antigen retrieval was performed in water bath at 65° C for 15 min using R-Universal epitope recovery buffer (EMS 62700–20, PA, USA). Sections were stained using mouse and rabbit specific HRP/DAB (ABC) detection IHC kit (ab64264, Abcam, Cambridge, UK) and processed according to the manufacturer's instructions. Slides were incubated with primary antibody against Sost (ab63097, Abcam, Cambridge, UK) overnight at 4° C and used secondary anti-rabbit (HAF008, R&D systems, Minneapolis, MN, USA) for 1 h at room temperature.

Harvested maxillae ($n = 24$) (Table S1)were processed for western blot as previously described [30]. We used the same primary and secondary antibody for Sost as those used in the IHC) and the housekeeping was Gapdh (CS51724, Cell signaling technology, Boston, MA, USA).

2.5. Statistical analyses

Statistical comparisons among the genotype, control, and treated groups performed using ANOVA, with $p \leq 0.05$ considered significant. We checked the normality assumption using the Shapiro-Wilk test and tested the homoscedasticity assumption using Levene's Test for Homogeneity of Variance. Post hoc analysis was conducted with Welch's two-sample *t*-test to ensure robustness. Pairwise *p*-values were adjusted using the Benjamini-Hochberg (BH) procedure to avoid false positives. We performed linear regression to determine the effect of BIC and BV/TV on push-out force. Additionally, we used Fisher's exact test to assess the relationship between genotype and implant failure. Further analysis also included a comparison of Brtl/+ SclAb to the WT vehicle group, evaluating SclAb's ability to provide restorative anabolic gains as previously described [13–15].

3. Results

3.1. The bilateral implant model shows good postoperative recovery

To assess the healing and response of maxillary and tibial bone in the context of injury, we placed 400 μm diameter implants in the maxilla and tibia of Brtl/+ and WT mice (Fig. 1). Given the limited height of the maxillary alveolar ridge (400 μm), all maxillary implants penetrated into the maxillary sinus, yet without any infections or need for antibiotics.

3.2. Implant survival rates are different in the maxilla compared with tibia

We assessed how these distinct anatomical locations, each with its unique characteristics, influence implant survival. The tibia, shielded by muscle, subcutaneous tissue, and skin, and in direct contact with bone marrow, contrasts with the maxillary environment, which is continuously exposed to moisture and the oral cavity's microbial load. Our findings reveal a significant disparity in immediate post-op implant survival rates, with the tibia demonstrating a superior rate of 97 % compared to 86 % in the maxilla. Notably, in maxilla, failures were predominantly observed in Brtl/+ mice. The estimated odds ratio between genotype and maxilla implants is 0.13 (95%CI: 0.014, 0.68), indicating that there is significant association between the genotype and failed implants ($p = 0.006$). In tibia, Brtl/+ presented higher failure, but there is no significant evidence to show that the genotype and tibial implants have strong association ($p = 0.35$). The estimated odds ratio is 0.296 (95%CI:0.005,3.843). Table 1.

3.3. SclAb increases peri-implant bone volume fraction (BV/TV) across bone types

To assess the peri-implant BV/TV, we delineated a ROI extending 250 μm (μm) radially around the implant surface. Our analysis highlighted notable differences in BV/TV between bone sites (maxilla vs tibia) and genotypes (WT vs Brtl/+). In Brtl/+ mice treated with Phosphate Buffered Saline (PBS-Brtl/+), the BV/TV was significantly lower in the maxilla (22.4 % ± 10.6) compared to the tibia (41.8 % ± 11.0). Similar patterns were observed in PBS-treated WT mice (PBS-WT), which exhibited higher BV/TV in the tibia (60.7 % ± 6.5) compared to the maxilla (46.9 % ± 8.4). When comparing PBS-Brtl/+ with PBS-WT, the BV/TV was significantly higher in PBS-WT for both the maxilla ($p < 0.05$) and tibia ($p < 0.05$). SclAb treatment markedly improved BV/TV in Brtl/+ mice, increasing to 38.3 % ± 5.4 in the maxilla (a 71.3 % increase) and 55.1 % ± 6.4 in the tibia (a 31.9 % increase), suggesting that SclAb treatment effectively mitigates the BV/TV insufficiency in Brtl/+ mice. In WT mice, SclAb administration increased BV/TV to 55.9 % ± 3.3 in the maxilla, which is 19 % higher than PBS-WT mice, but not statistically significant. In the tibia of SclAb-WT, BV/TV increased to 68.5 % ± 7.8, representing a 12.9 % increase compared to the PBS-WT though this increase was not statistically significant. Notably, the maxilla and tibia BV/TV in the SclAb-Brtl/+ mice were comparable to that of PBS-WT mice, highlighting the potential of SclAb treatment to normalize bone structure in Brtl/+ mice. (Fig. 2).

To confirm the effectiveness of SclAb in systemic gains, we analyzed the trabecular and cortical response in femur of our samples without implant placement. SclAb treatment significantly increased distal femoral trabecular BV/TV and mid-diaphyseal cortical

thickness (Ct. Th.) in Brtl/+ mice (8.6 % ± 2.8 and 0.21 mm ± 0.01, respectively) compared to the PBS-Brtl/+ mice (5.1 % ± 2.0 and 0.19 mm ± 0.008, respectively). In wild-type mice, SclAb treatment also significantly improved trabecular BV/TV and Ct.Th (14.0 % ± 3.7 and 0.23 mm ± 0.01, respectively), demonstrating SclAb's positive impact systemically on bone structure and density (Fig. 3).

3.4. SclAb increases bone-implant contact (BIC) in WT and Brtl/+ mice

To assess osseointegration, we evaluated the bone-implant contact (BIC) in both the maxilla and tibia. In the maxilla, BIC for PBS-Brtl/+ mice were significantly lower at 3.4 % ± 2.0 compared to PBS-WT mice, which had a BIC of 18.5 % ± 1.3 ($p < 0.05$), indicating that BIC in WT mice was 444 % higher than in Brtl/+ mice. Following SclAb treatment, BIC in Brtl/+ mice increased to 15.9 % ± 3.0, a significant enhancement of 373 % ($p < 0.05$), compared to PBS-Brtl/+. SclAb-WT mice exhibited a BIC of 25.3 % ± 6.8, slightly, but not significantly, surpassing PBS-WT mice.

In the tibia, PBS-Brtl/+ mice demonstrated a BIC of 58.9 % ± 2.9, substantially lower than PBS-WT mice, which had a BIC of 81.8 % ± 5.1 ($p < 0.05$). Similar to the maxillary findings, SclAb treatment in Brtl/+ mice improved tibial BIC to 77.1 % ± 8.1 ($p < 0.05$), corresponding to a 30.9 % increase. The highest tibial BIC was observed in SclAb-WT mice, reaching 84.6 % ± 5.3, marking a slight but non-significant improvement from PBS-WT mice (81.8 % ± 5.1).

The BIC for both maxillary and tibial bone in SclAb-Brtl/+ mice reached the same levels as those observed in PBS-WT mice (Fig. 4).

3.5. SclAb increases the implant resistance to push-in forces in Brtl/+

In our comparative study of mechanical resistance to push-in forces in implants, we noted distinct responses among our experimental groups. SclAb-Brtl/+ mice demonstrated a push-in force in the tibia that was nearly 150 % higher (17.5 N ± 7.5) compared to the PBS-Brtl/+ (7.1 N ± 4.4, $p < 0.05$). In the maxilla as well, these mice showed a 160 % increased resistance (13.4 N ± 3.2) versus the PBS-Brtl/+ (5.1 N ± 3.4, $p < 0.05$).

Both PBS-WT and SclAb-WT mice demonstrated high push-in resistance, with PBS-WT mice showing values of 14.7 N ± 2.9 in the maxilla and 26.2 N ± 6.1 in the tibia, and SclAb-WT mice showing a slight but non-significant increase in the resistance (17.1 N ± 5.1 and 31.7 N ± 3.4, respectively). The increase in push-in resistance induced by SclAb in WT mice did not reach statistical significance when compared with the PBS-WT, exhibiting only marginal improvements.

Further assessment revealed that the implant push-in resistance of the maxilla and tibia in SclAb-Brtl/+ mice was comparable to the PBS-WT, implying a restoration of mechanical properties toward normal WT levels. (Fig. 5).

3.6. BIC contributes significantly to the fixation push-in resistance

We performed a regression analysis to determine if BIC or BV/TV influences the osseointegration resistance to push-in testing. For the maxilla, the BIC contributes more

to the push-in resistance compared to BV/TV. The estimated BIC coefficient is 0.492 (95 % CI: 0.214, 0.770, $p < 0.002$), while the estimated BV/TV coefficient is 0.015 (95 % CI: −0.156, 0.186, $p < 0.864$). Also for the tibia, the BIC contributes more to the push-in resistance compared to BV/TV. The estimated BIC coefficient is 0.510 (95 % CI: 0.115, 0.905, $p < 0.02$), while the estimated BV/TV coefficient is 0.182 (95 % CI: −0.181, 0.545, $p < 0.33$).

3.7. SclAb induces compensatory production of sclerostin

In a previous study examining the effects of SclAb treatment on alveolar bone regeneration in rats, an increase in sclerostin levels within the alveolar bone was observed [35]. Motivated by these findings, we aimed to determine if a similar response occurred in our implant samples. Enhanced sclerostin immunostaining was observed in the osteocyte-containing lacunae and throughout the network of connecting canaliculi in the alveolar bone (Fig. S1).

Quantification by Western blot, normalized against a housekeeping protein (GapdH), revealed that SclAb treatment significantly increased sclerostin levels in both Brtl/+ and WT samples compared to their respective controls. Specifically, the SclAb-WT group showed a relative sclerostin level increase to 1.72 times that of the control level ($p < 0.05$), while the SclAb-Brtl/+ exhibited a relative increase to 1.82 times the control level ($p < 0.05$).

4. Discussion

Successful osseointegration is critical to long-term health of patients receiving implants in compromised bone conditions, particularly in osteogenesis imperfecta, where impaired bone quality necessitates additional strategies for peri-implant osseointegration. Achieving optimal bone quantity and quality at the implant site is a crucial clinical goal before implant placement. However, understanding the effect of the sclerostin antibody during osseointegration is equally important. Our study investigated whether the sclerostin antibody can rescue osseointegration and implant stability to wild-type (WT) levels in osteogenesis imperfecta (OI) maxilla with poor bone quantity and quality. We found that systemic treatment with SclAb significantly improved implant fixation strength in Brtl/+ mice, as demonstrated by enhanced push-in test results and increased BIC after five weeks therapy. Notably, these improvements were significant in both the maxilla and tibia, indicating SclAb's effectiveness in enhancing osseointegration across different bone sites. In WT mice, however, the increase in BIC from SclAb treatment was modest, suggesting a potential saturation effect in unaffected bone. The increase in SclAb-induced osseointegration observed in this study not only aligns with previous research on osteoporotic rat models [21,36] but also highlights SclAb's potential to improve both the quantity and quality of osseointegration in collagen-compromised bone. Thus, SclAb could enhance the stability and longevity of dental implants, particularly for OI clinical applications.

SclAb therapy effectively mitigated BV/TV deficits in the maxilla and tibia of Brtl/+ mice, demonstrating its efficacy in addressing bone structural alterations associated with OI. The increases in maxillary and tibial peri-implant BV/TV in Brtl/+ mice align with findings from studies on ovariectomized rats [22,23], showcasing SclAb's broad utility in enhancing bone quantity, even in the presence of genetically altered collagen and under injury stimuli

like implant placement. Moreover, the SclAb-induced increase in BV/TV elevated the phenotypic characteristics of Brtl/+ mice to levels comparable to those in PBS-WT mice across bone types, underscoring SclAb's clinical relevance in restoring bone quantity under pathological conditions.

In WT mice, there is a response to SclAb in the tibia and maxilla, yet the effects are milder compared to the femoral BV/TV. This non-significant enhancement in peri-implant BV/TV for SclAb-WT mice is likely due to the restricted ROI analyzed (250 μm) and the nature of new bone, in comparison to the femoral BV/TV, which ROI represents 10 % of the total femur. The enhancements in BV/TV observed in SclAb-Brtl/+ mice in both the maxilla and tibia are consistent with systemic gains in the femur (without implant placement). This supports the findings from our previous research on the SclAb-induced systemic bone gain [13–15]. Enhancements were noted across all examined bone sites, indicating SclAb's widespread efficacy. However, anatomical and structural differences likely affect the magnitude of SclAb's response, a factor that is also relevant to new bone formation induced by implants.

Our study highlighted the distinct processes of peri-implant osseointegration in the maxilla and tibia, corroborating previous research that demonstrated higher osseointegration in the tibia of healthy mouse models [6,37]. Despite the collagen mutation in our Brtl/+ mice, we observed a pattern of osseointegration akin to that in healthy bone. The differences we observed can be attributed to the unique compositions and remodeling mechanisms of these bones. The maxilla, predominantly composed of trabecular bone, remodels through the periosteum, leading to less dense bone formation [37–39]. In contrast, the tibia, with its dense cortical structure, remodels via both the periosteum and bone marrow, facilitating stronger and more stable bone repair [6,29]. This distinction is crucial for osseointegration, as the tibia's bone marrow and mechanical loading enhance osteoprogenitor cell availability and new bone formation [40]. Additionally, the differences in osseointegration between the tibia and maxilla could be influenced by the oral environment. The maxilla, exposed to a moist environment, faces additional challenges for implant integration, such as a higher risk of inflammation and infection [41,42], compared to the tibia, which is protected by muscle and skin. All these factors may collectively contribute to the differences in osseointegration observed in these bones. In our Brtl/+ mice, SclAb treatment was more effective in enhancing osseointegration in the maxilla than in the tibia. This is likely due to the maxilla's lower baseline bone density, which provides greater potential for growth and improvement.

In this study, we observed an increased presence of sclerostin in the peri-implant alveolar bone of both WT and Brtl/+ mice following SclAb treatment. Western blot analysis confirmed a significant increase in sclerostin levels in both SclAb treated WT and Brtl/+ mice. This elevation was primarily localized within the osteocyte-containing lacunae and the extensive network of canaliculi in the alveolar bone. Previous research suggests that this increase of local sclerostin could be the accumulation of inactive, neutralized sclerostin protein trapped within the bone matrix [35]. Alternatively, the presence of sclerostin could counteract the enhanced bone formation induced by SclAb treatment, potentially through negative feedback regulation, where SclAb binding disrupts normal protein degradation

or clearance, leading to sclerostin accumulation. Furthermore, SclAb treatment may alter cellular signaling, causing increased sclerostin synthesis via activated compensatory pathways. This explanation is supported by previous studies showing that SclAb treatment increases both gene expression and circulating levels of sclerostin [43], possibly due to the combined detection of unbound and antibody-bound sclerostin and the stabilization of the bound form by SclAb.

Our study encountered some limitations, with one notable challenge being the difficulty in standardizing the implant insertion process and achieving precise placement. This issue was exacerbated by the limited mouth opening and the fragility of the surgical site, particularly in Brtl/+ mice, where extreme care is necessary to prevent bone damage. Although the small diameter of the implant and its handle aids in insertion, sectioning of the implant handle after the positioning of the implant may disturb the bone at the implant bed, a concern that is especially relevant in the Brtl/+ mouse model as it could potentially compromise the implant's primary stability. We were not able to measure the implant insertion torque due to the small size of the implants, the restricted sites chosen for placement, and the design of the Retopin. As a result, we used finger-tight torque as an alternative measure. Previous study has shown that the finger-tight torque for female participants averaged 19 Ncm [44]. Additional studies support that lower insertion torques (< 20 Ncm) not only achieve comparable success rates for osseointegration with favorable gains in stability over the osseointegration phase but also reduce damage to the surrounding tissues [45,46]. Based on these studies, the torque range achieved with finger-tight insertion is within the acceptable range for primary stability and osseointegration. All dental implants in our study were placed extending into the maxillary sinus due to the limited height of the alveolar bone. While this raises concerns about potential complications [47], clinical research supports the viability of such placements. Studies have shown that dental implants protruding into the maxillary sinus cavity by 4 mm or less have a survival rate of 99.5 %, suggesting that sinus penetration does not negatively impact implant success [48]. Our study corroborated these findings, with no instances of infection or the need for antibiotics observed.

In the general population, the dental implant survival rate is reported to be around 96 % [49–53]. In contrast, for OI patients, this rate ranges between 80 % to 94 %, with those experiencing more severe OI exhibiting lower survival rates [9,10]. Interestingly, in our study, the maxillary implant survival rates closely match these findings (WT:96 %, Brtl/+: 76 %). This similarity further supports our conclusions that the increase in osseointegration observed with the SclAb treatment in our model may have relevant implications for clinical application in OI patients.

5. Conclusion

Our research demonstrates that systemic administration of SclAb significantly enhances peri-implant osseointegration, highlighting its potential to improve implant success in conditions like OI, both anatomically and functionally. Dental implants in OI patients tend to have higher failure rates, particularly in severe forms of the disease. However, SclAb presents a promising solution for implant-supported dental rehabilitation, potentially reducing the risk of implant failure. This finding not only offers a new avenue of hope for

OI patients but also suggests that targeted therapies can effectively address the challenges of dental implantation in the presence of compromised bone quality, thereby improving treatment outcomes and enhancing patients' quality of life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data availability

Data will be made available on request.

References

[1]. Quadri MFA, Alamir AWH, John T, Nayeem M, Jessani A, Tadakamadla SK, Effect of prosthetic rehabilitation on oral health-related quality of life of patients with head and neck cancer: a systematic review, Transl. Cancer Res. 9 (4) (2020) 3107–3118. [PubMed: 35117674]

[2]. Walter N, Stich T, Docheva D, Alt V, Rupp M, Evolution of implants and advancements for osseointegration: a narrative review, Injury 53 (Suppl. 3) (2022) S69–S73. [PubMed: 35948509]

[3]. Javed F, Ahmed HB, Crespi R, Romanos GE, Role of primary stability for successful osseointegration of dental implants: factors of influence and evaluation, Interv. Med. Appl. Sci. 5 (4) (2013) 162–167. [PubMed: 24381734]

[4]. Swami V, Vijayaraghavan V, Swami V, Current trends to measure implant stability, J. Indian Prosthodont. Soc. 16 (2) (2016) 124–130. [PubMed: 27141160]

[5]. Wazen RM, Currey JA, Guo H, Brunski JB, Helms JA, Nanci A, Micromotion-induced strain fields influence early stages of repair at bone-implant interfaces, Acta Biomater. 9 (5) (2013) 6663–6674. [PubMed: 23337705]

[6]. Mouraret S, Hunter DJ, Bardet C, Brunski JB, Bouchard P, Helms JA, A pre-clinical murine model of oral implant osseointegration, Bone 58 (2014) 177–184. [PubMed: 23886841]

[7]. Marini JC, Forlino A, Bachinger HP, Bishop NJ, Byers PH, Paepe A, Fassier F, Fratzl-Zelman N, Kozloff KM, Krakow D, Montpetit K, Semler O, Osteogenesis imperfecta, Nat. Rev. Dis. Primers. 3 (2017) 17052. [PubMed: 28820180]

[8]. Marini JC, Forlino A, Cabral WA, Barnes AM, San Antonio JD, Milgrom S, Hyland JC, Korkko J, Prockop DJ, De Paepe A, Coucke P, Symoens S, Glorieux FH, Roughley PJ, Lund AM, Kuurila-Svahn K, Hartikka H, Cohn DH, Krakow D, Mottes M, Schwarze U, Chen D, Yang K, Kuslich C, Troendle J, Dalgleish R, Byers PH, Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans, Hum. Mutat. 28 (3) (2007) 209–221. [PubMed: 17078022]

[9]. Oelerich O, Kleinheinz J, Bohner L, Wiesmuller V, Hanisch M, Dental implants in people with osteogenesis imperfecta: a systematic review, Int. J. Environ. Res. Public Health 19 (3) (2022).

[10]. Fogh FM, Jensen SS, Kofod T, Lauridsen E, Dental implants in patients with Osteo-genesis imperfecta-clinical and radiographic outcome in six patients, Oral Health Prev. Dent. 21 (1) (2023) 33–40. [PubMed: 36727836]

[11]. Mouraret S, Hunter DJ, Bardet C, Popelut A, Brunski JB, Chaussain C, Bouchard P, Helms JA, Improving oral implant osseointegration in a murine model via Wnt signal amplification, J. Clin. Periodontol. 41 (2) (2014) 172–180. [PubMed: 24164629]

[12]. Lioubavina-Hack N, Lang NP, Karring T, Significance of primary stability for osseointegration of dental implants, Clin. Oral Implants Res. 17 (3) (2006) 244–250. [PubMed: 16672018]

[13]. Sinder BP, Eddy MM, Ominsky MS, Caird MS, Marini JC, Kozloff KM, Sclerostin antibody improves skeletal parameters in a Brtl/+ mouse model of osteogenesis imperfecta, J. Bone Miner. Res. 28 (1) (2013) 73–80. [PubMed: 22836659]

[14]. Sinder BP, Salemi JD, Ominsky MS, Caird MS, Marini JC, Kozloff KM, Rapidly growing Brtl/+ mouse model of osteogenesis imperfecta improves bone mass and strength with sclerostin antibody treatment, Bone 71 (2015) 115–123. [PubMed: 25445450]

[15]. Sinder BP, White LE, Salemi JD, Ominsky MS, Caird MS, Marini JC, Kozloff KM, Adult Brtl/+ mouse model of osteogenesis imperfecta demonstrates anabolic response to sclerostin antibody treatment with increased bone mass and strength, Osteoporos. Int. 25 (8) (2014) 2097–2107. [PubMed: 24803333]

[16]. Glorieux FH, Devogelaer JP, Durigova M, Goemaere S, Hemsley S, Jakob F, Junker U, Ruckle J, Seefried L, Winkle PJ, BPS804 anti-Sclerostin antibody in adults with moderate osteogenesis imperfecta: results of a randomized phase 2a trial, J. Bone Miner. Res. 32 (7) (2017) 1496–1504. [PubMed: 28370407]

[17]. Scheiber AL, Barton DK, Khoury BM, Marini JC, Swiderski DL, Caird MS, Kozloff KM, Sclerostin antibody-induced changes in bone mass are site specific in developing Crania, J. Bone Miner. Res. 34 (12) (2019) 2301–2310. [PubMed: 31441963]

[18]. Diegel CR, Kramer I, Moes C, Foxa GE, McDonald MJ, Madaj ZB, Guth S, Liu J, Harris JL, Kneissel M, Williams BO, Inhibiting WNT secretion reduces high bone mass caused by Sost loss-of-function or gain-of-function mutations in Lrp5, Bone Res. 11 (1) (2023) 47. [PubMed: 37612291]

[19]. Yavropoulou MP, Xygonakis C, Lolou M, Karadimou F, Yovos JG, The sclerostin story: from human genetics to the development of novel anabolic treatment for osteoporosis, Hormones (Athens) 13 (4) (2014) 323–337.

[20]. Korn P, Kramer I, Schlottig F, Todtman N, Eckelt U, Burki A, Ferguson SJ, Kautz A, Schnabelrauch M, Range U, Kneissel M, Stadlinger B, Systemic sclerostin antibody treatment increases osseointegration and biomechanical competence of zoledronic-acid-coated dental implants in a rat osteoporosis model, Eur. Cell. Mater. 37 (2019) 333–346. [PubMed: 31112281]

[21]. Liu S, Virdi AS, Sena K, Sumner DR, Sclerostin antibody prevents particle-induced implant loosening by stimulating bone formation and inhibiting bone resorption in a rat model, Arthritis Rheum. 64 (12) (2012) 4012–4020. [PubMed: 23192793]

[22]. Virdi AS, Irish J, Sena K, Liu M, Ke HZ, McNulty MA, Sumner DR, Sclerostin antibody treatment improves implant fixation in a model of severe osteoporosis, J. Bone Joint Surg. Am. 97 (2) (2015) 133–140. [PubMed: 25609440]

[23]. Virdi AS, Liu M, Sena K, Maletich J, McNulty M, Ke HZ, Sumner DR, Sclerostin antibody increases bone volume and enhances implant fixation in a rat model, J. Bone Joint Surg. Am. 94 (18) (2012) 1670–1680. [PubMed: 22992878]

[24]. Yu SH, Hao J, Fretwurst T, Liu M, Kostenuik P, Giannobile WV, Jin Q, Sclerostin-neutralizing antibody enhances bone regeneration around oral implants, Tissue Eng. Part A 24 (21–22) (2018) 1672–1679. [PubMed: 29921173]

[25]. Yao Y, Kauffmann F, Maekawa S, Sarment LV, Sugai JV, Schmiedeler CA, Doherty EJ, Holdsworth G, Kostenuik PJ, Giannobile WV, Sclerostin antibody stimulates periodontal regeneration in large alveolar bone defects, Sci. Rep. 10 (1) (2020) 16217. [PubMed: 33004873]

[26]. Makhdom AM, Rauch F, Lauzier D, Hamdy RC, The effect of systemic administration of sclerostin antibody in a mouse model of distraction osteogenesis, J. Musculoskelet. Neuronal Interact. 14 (1) (2014) 124–130. [PubMed: 24583547]

[27]. Agholme F, Li X, Isaksson H, Ke HZ, Aspenberg P, Sclerostin antibody treatment enhances metaphyseal bone healing in rats, J. Bone Miner. Res. 25 (11) (2010) 2412–2418. [PubMed: 20499342]

[28]. Son C, Choi MS, Park JC, Different responsiveness of alveolar bone and long bone to epithelial-mesenchymal interaction-related factor, JBMR Plus 4 (8) (2020) e10382. [PubMed: 32803111]

[29]. Mavropoulos A, Rizzoli R, Ammann P, Different responsiveness of alveolar and tibial bone to bone loss stimuli, J. Bone Miner. Res. 22 (3) (2007) 403–410. [PubMed: 17181394]

[30]. Sung HHH, Spresser WJ, Hoffmann JP, Dai Z, Van der Kraan PM, Caird MS, Blaney Davidson E, Kozloff KM, Collagen mutation and age contribute to differential craniofacial phenotypes in mouse models of osteogenesis imperfecta, JBMR plus 8 (1) (2024).

[31]. Forlino A, Porter FD, Lee EJ, Westphal H, Marini JC, Use of the Cre/lox recombination system to develop a non-lethal knock-in murine model for osteogenesis imperfecta with an alpha1(I) G349C substitution. Variability in phenotype in BrtlIV mice, J. Biol. Chem. 274 (53) (1999) 37923–37931. [PubMed: 10608859]

[32]. Biguetti CC, Cavalla F, Silveira EM, Fonseca AC, Vieira AE, Tabanez AP, Rodrigues DC, Trombone APF, Garlet GP, Oral implant osseointegration model in C57Bl/6 mice: microtomographic, histological, histomorphometric and molecular characterization, J. Appl. Oral Sci. 26 (2018) e20170601. [PubMed: 29898187]

[33]. Seong WJ, Grami S, Jeong SC, Conrad HJ, Hodges JS, Comparison of push-in versus pull-out tests on bone-implant interfaces of rabbit tibia dental implant healing model, Clin. Implant Dent. Relat. Res. 15 (3) (2013) 460–469. [PubMed: 22172015]

[34]. Chang PC, Seol YJ, Goldstein SA, Giannobile WV, Determination of the dynamics of healing at the tissue-implant interface by means of microcomputed tomography and functional apparent moduli, Int. J. Oral Maxillofac. Implants 28 (1) (2013) 68–76. [PubMed: 23377049]

[35]. Taut AD, Jin Q, Chung JH, Galindo-Moreno P, Yi ES, Sugai JV, Ke HZ, Liu M, Giannobile WV, Sclerostin antibody stimulates bone regeneration after experimental periodontitis, J. Bone Miner. Res. 28 (11) (2013) 2347–2356. [PubMed: 23712325]

[36]. Liu M, Kurimoto P, Zhang J, Niu QT, Stolina M, Dechow PC, Feng JQ, Hesterman J, Silva MD, Ominsky MS, Richards WG, Ke H, Kostenuik PJ, Sclerostin and DKK1 inhibition preserves and augments alveolar bone volume and architecture in rats with alveolar bone loss, J. Dent. Res. 97 (9) (2018) 1031–1038. [PubMed: 29617179]

[37]. Covani U, Giammarinaro E, Panetta D, Salvadori PA, Cosola S, Marconcini S, Alveolar bone remodeling with or without collagen filling of the extraction socket: a high-resolution X-ray tomography animal study, J. Clin. Med. 11 (9) (2022).

[38]. Lin Z, Fateh A, Salem DM, Intini G, Periosteum: biology and applications in craniofacial bone regeneration, J. Dent. Res. 93 (2) (2014) 109–116. [PubMed: 24088412]

[39]. Jonasson G, Skoglund I, Rythen M, The rise and fall of the alveolar process: dependency of teeth and metabolic aspects, Arch. Oral Biol. 96 (2018) 195–200. [PubMed: 30292055]

[40]. Ma Q, Miri Z, Haugen HJ, Moghanian A, Loca D, Significance of mechanical loading in bone fracture healing, bone regeneration, and vascularization, J. Tissue Eng. 14 (2023), 20417314 231172573.

[41]. Kunrath MF, Dahlin C, The impact of early saliva interaction on dental implants and biomaterials for oral regeneration: an overview, Int. J. Mol. Sci. 23 (4) (2022).

[42]. Liao M, Shi Y, Chen E, Shou Y, Dai D, Xian W, Ren B, Xiao S, Cheng L, The bio-aging of biofilms on behalf of various oral status on different titanium implant materials, Int. J. Mol. Sci. 24 (1) (2022).

[43]. Carpenter KA, Davison R, Shakthivel S, Anderson KD, Ko FC, Ross RD, Sclerostin antibody improves phosphate metabolism hormones, bone formation rates, and bone mass in adult Hyp mice, Bone 154 (2022) 116201. [PubMed: 34537437]

[44]. Kanawati A, Richards MW, Becker JJ, Monaco NE, Measurement of clinicians' ability to hand torque dental implant components, J. Oral Implantol. 35 (4) (2009) 185–188. [PubMed: 19813423]

[45]. Norton MR, The influence of low insertion torque on primary stability, implant survival, and maintenance of marginal bone levels: a closed-cohort prospective study, Int. J. Oral Maxillofac. Implants 32 (4) (2017) 849–857. [PubMed: 28708918]

[46]. Rea M, Botticelli D, Ricci S, Soldini C, Gonzalez GG, Lang NP, Influence of immediate loading on healing of implants installed with different insertion torques—an experimental study in dogs, Clin. Oral Implants Res. 26(1) (2015) 90–5. [PubMed: 24313303]

[47]. Kim SW, Lee IH, Kim SW, Kim DH, Points to consider before the insertion of maxillary implants: the otolaryngologist's perspective, J. Periodontal. Implant Sci. 49 (6) (2019) 346–354. [PubMed: 31886027]

[48]. Ragucci GM, Elnayef B, Suarez-Lopez Del Amo F, Wang HL, Hernandez-Alfaro F, Gargallo-Albiol J, Influence of exposing dental implants into the sinus cavity on survival and complications rate: a systematic review, Int. J. Implant Dent. 5 (1) (2019) 6. [PubMed: 30719578]

[49]. Clark R, Burren CP, John R, Challenges of delivery of dental care and dental pathologies in children and young people with osteogenesis imperfecta, Eur. Arch. Paediatr. Dent. 20 (5) (2019) 473–480. [PubMed: 30868445]

[50]. Howe MS, Keys W, Richards D, Long-term (10-year) dental implant survival: a systematic review and sensitivity meta-analysis, J. Dent. 84 (2019) 9–21. [PubMed: 30904559]

[51]. Ivanova V, Chenchev I, Zlatev S, Mijiritsky E, Correlation between primary, secondary stability, bone density, percentage of vital bone formation and implant size, Int. J. Environ. Res. Public Health 18 (13) (2021).

[52]. Wang Y, Baumer D, Ozga AK, Korner G, Baumer A, Patient satisfaction and oral health-related quality of life 10 years after implant placement, BMC Oral Health 21 (1) (2021) 30. [PubMed: 33446161]

[53]. Yang Y, Hu H, Zeng M, Chu H, Gan Z, Duan J, Rong M, The survival rates and risk factors of implants in the early stage: a retrospective study, BMC Oral Health 21 (1) (2021) 293. [PubMed: 34107931]

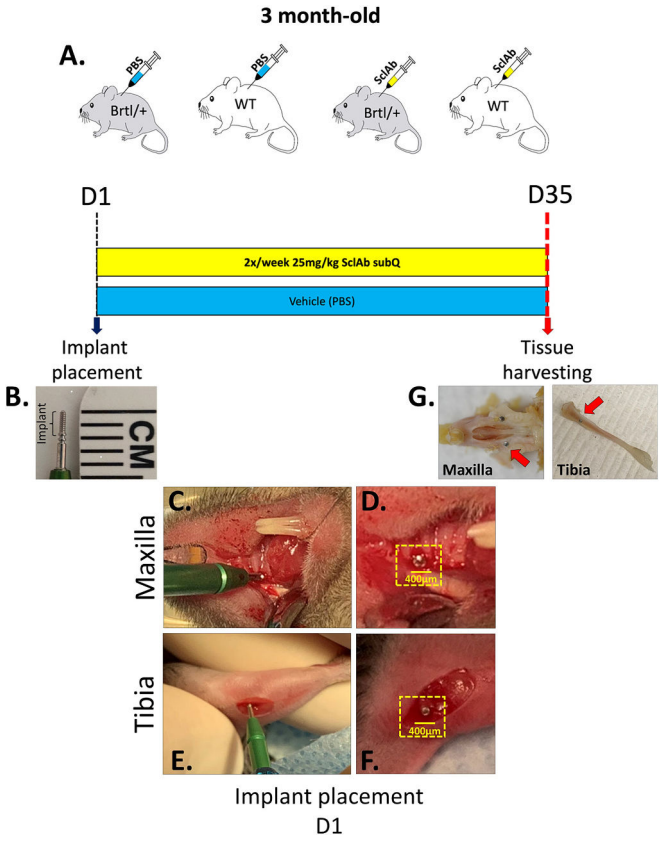

Fig. 1.

Experimental design. A. 3-month-old WT and Brtl/+ mice underwent bilateral maxillary and tibial implant with concomitant SclAb/PBS injections. B. 400 μm diameter titanium implants. C and D. Maxillary implant placement and localization. E and F. Tibial implant placement and localization. G. Dissected specimens with the implants.

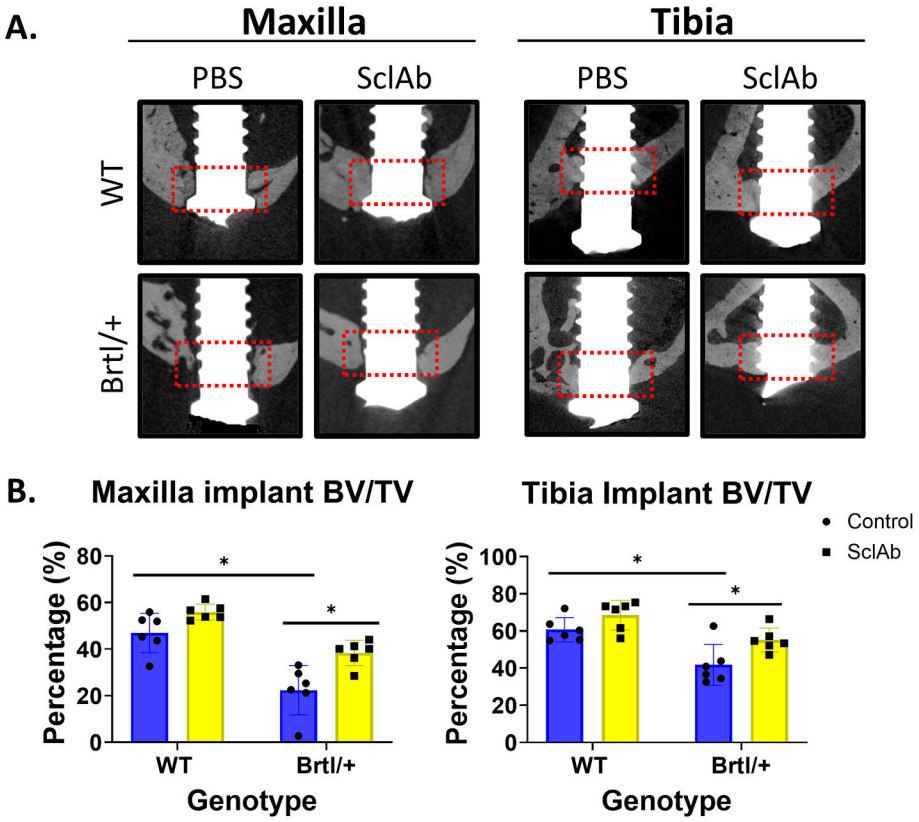

Fig. 2.

Bone volume fraction (BV/TV) of the peri-implant bone (within 250um of the implant). A. Figure shows the peri-implant bone in the PBS and SclAb treated groups in the maxilla and tibia of both genotypes. Red represents the region of interest (ROI). B. Graph represents the BV/TV of the red area in the maxilla and tibia. Asterisk represents $P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

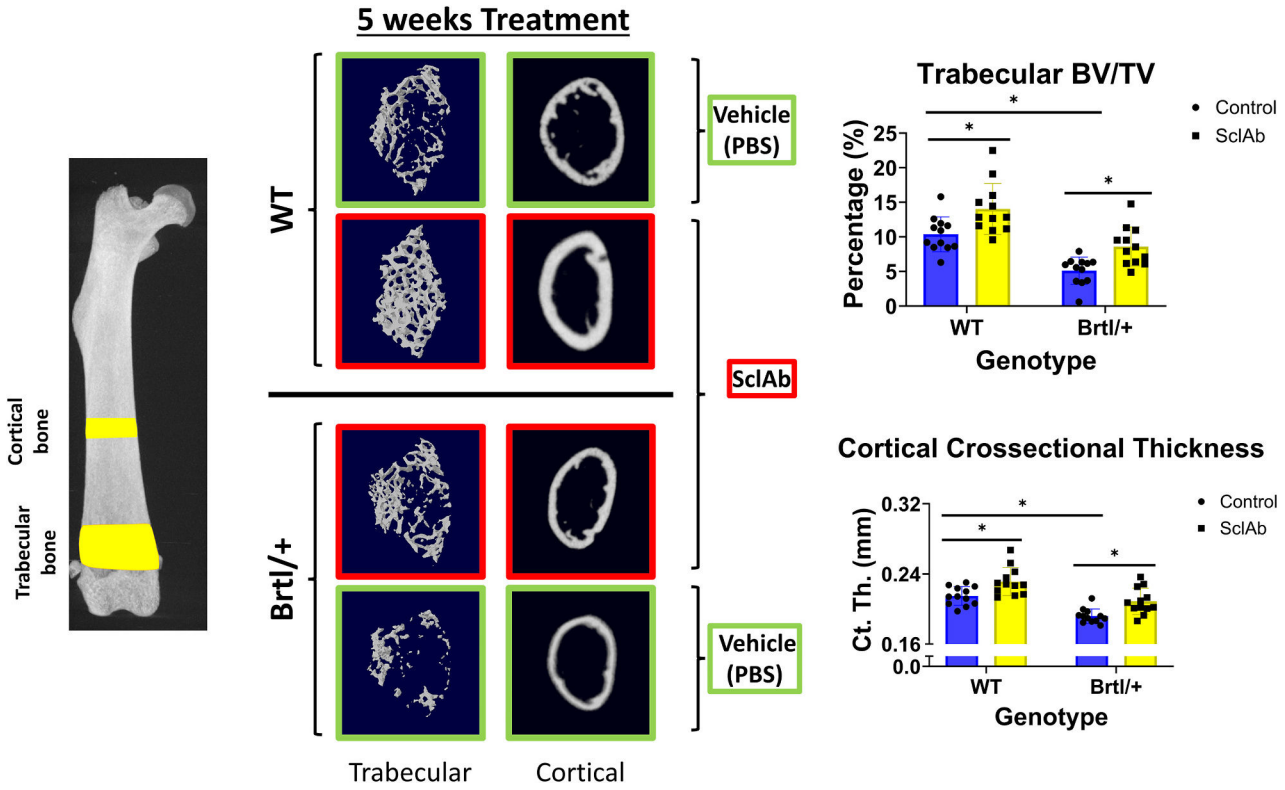

Fig. 3.

Micro computed tomography of the distal femur metaphysis. A. The cortical ROI defined as 15 % of the total femur length situated equidistantly between the distal femur and the third trochanter, the trabecular ROI was defined as 10 % of the total femur, located immediately proximal to the distal femoral end. Representative median BV/TV images reveal reduction in Brtl/+ bone mass and the anabolic effect of SclAb therapy. B. Graphs showing the increase in BV/TV and cortical thickness (ct.th) induced by SclAb for trabecular bone.

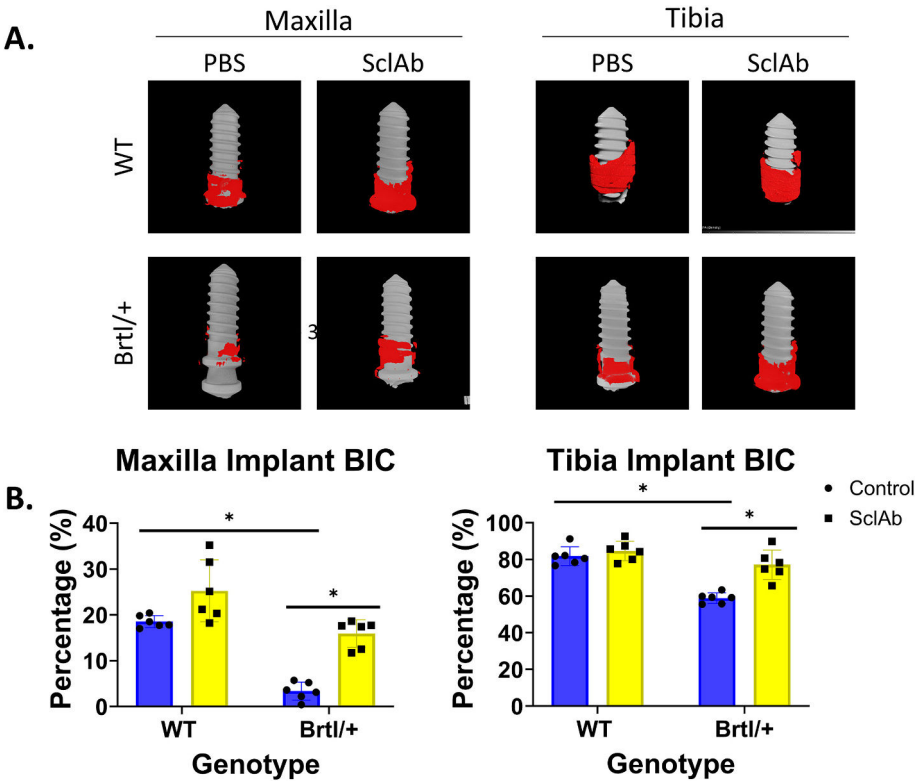

Fig. 4.

Bone-implant contact (BIC). A. Representative illustration of the BIC in the maxilla and tibia. B. Graph showing the percentage of bone -implant contact in the maxilla and tibia for both PBS and SclAb treated WT and Brtl/+ groups. Asterisk represents $P < 0.05$.

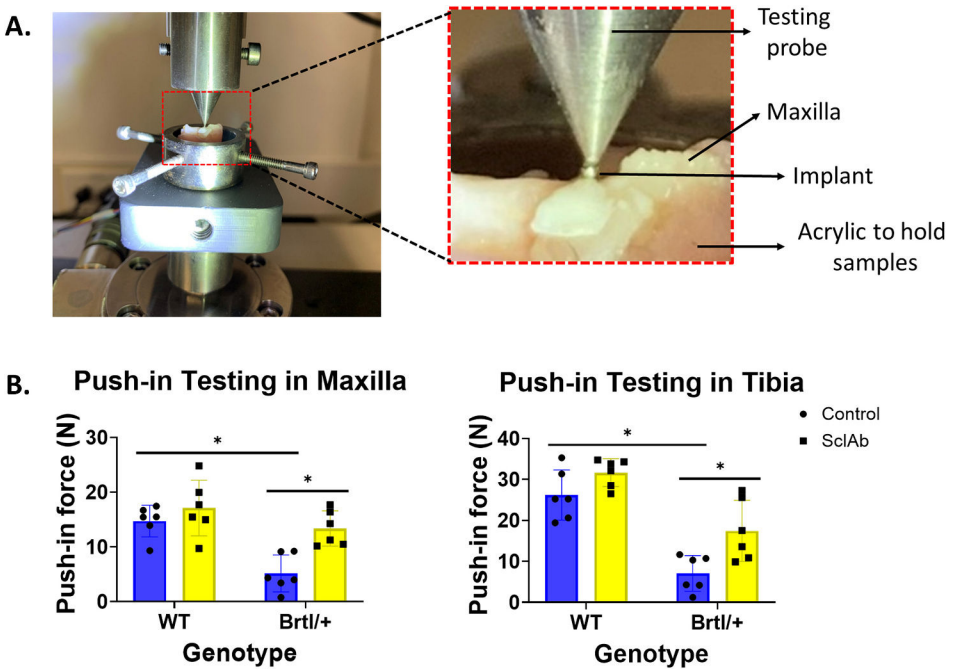

Fig. 5.

Push-in mechanical testing. A. Customized fixture aligned with the maxillary and tibial implants with the samples stabilized in light-cured acrylic to allow implant positioning directly beneath the probe. B. Graph showing maximum load measured in maxilla and tibia. Asterisk represents $P < 0.05$.

Table 1

Numbers of implants placed by genotypes.

Location	Genotype	Total implants By genotype Number (%)	Failed Implants Number (%)	Survived Implants	
				by genotype Number (%)	by bone location Number (%)
Maxilla	WT	50 (100 %)	2 (4 %)	48 (96 %)	83 (86 %)
	Brtl/+	46 (100 %)	11 (24 %) **	35 (76 %)	
Tibia	WT	50 (100 %)	1 (2 %)	49 (98 %)	92 (97 %)
	Brtl/+	46 (100 %)	3 (7 %)	43 (93 %)	

** *p value* ≤ *0.05.*