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Sclerostin Antibody (Scl-Ab) Improves Osteomalacia Phenotype in Dentin Matrix Protein 1 (*Dmp1*) Knockout Mice with Little Impact on Serum Levels of Phosphorus and FGF23

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Abstract

Unlike treatments for most rickets, the treatment using 1,25-(OH)₂ vitamin D₃ has little efficacy on patients with hypophosphatemic rickets, a set of rare genetic diseases. Thus, understanding the local cause for osteomalacia in hypophosphatemic rickets and developing an effective treatment to restore mineralization in this rare disease has been a longstanding goal in medicine. Here, we used *Dmp1* knockout (KO) mice (whose mutations led to the same type of autosomal recessive hypophosphatemic rickets in humans) as the model in which the monoclonal antibody of sclerostin (Scl-Ab) was tested in two age groups for 8 weeks: the prevention group (starting at age 4 weeks) and the treatment group (starting at age 12 weeks). Applications of Scl-Ab greatly improved the osteomalacia phenotype (>15%) and the biomechanical properties (3-point bending, ~60%) in the treated long-bone group. Our studies not only showed improvement of the osteomalacia in the alveolar bone, which has the highest bone metabolism rate, as well as the long bone phenotypes in treated mice. All these improvements attributed to the use of Scl-Ab are independent of the change in serum levels of phosphorus and FGF23, since Scl-Ab had little efficacy on those parameters. Finally, we propose a model to explain how Scl-Ab can improve the *Dmp1* KO osteomalacia phenotype, in which the sclerostin level is already low.

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Keywords

DMP1; Hypophosphatemic rickets; PDL; SOST; Sclerostin antibody; Osteocytes

INTRODUCTION

Dmp1 (Dentin matrix protein 1) was initially identified in dentin but later found to be highly expressed in bone, mainly in osteocytes [1–3]. The deletion of murine *Dmp1* causes striking defects in tooth and bone during postnatal development [4, 5]. One of the most common deformities is the presence of large amounts of osteoid in bone (osteomalacia) and short long-bone length, which is closely associated with a sharp reduction in serum phosphorus (without any apparent change in serum calcium) and elevated circulating fibroblast growth factor 23 (FGF23) [6]. Thus we propose that the *Dmp1* knockout (KO) mouse is a hypophosphatemic rickets model. Using an *ex vivo* metatarsal organ culture and an application of neutralizing FGF23 antibodies to treat *Dmp1* KO mice, we proved that: 1) phosphorus plays an important role in growth plate maturation and secondary ossification center formation; 2) osteoblast differentiation is phosphate-dependent; 3) bone extracellular matrix mineralization is partially dependent on the phosphorus level; and 4) neutralizing FGF23 antibodies fully restores *Dmp1* KO bone length but only partially improves the osteomalacia phenotype, indicating that other local factors are partly responsible for abnormalities in bone mineralization [7].

In humans, hypophosphatemic rickets is a group of rickets with an incidence of approximately 4 per 100,000 live births [8]; it is characterized by low serum phosphate levels and is resistant to treatment with ultraviolet radiation or vitamin D ingestion. This disease can cause bone deformity (such as short stature and genu varum) and dentin defects (such as dental abscesses) in children. With continuing osteomalacia and joint defects, pseudofractures, enthesopathy, osteophytes, and osteoarthritis may occur as later complications in many patients [9]. The most common form is X-linked hypophosphatemic (XLH) dominant disorder, which is associated with mutations in the phosphate-regulating endopeptidase homologue X-linked (PHEX) [10]. Another autosomal dominant form of the disease is mutations in FGF23 [11]. Recently we and others have identified mutations in DMP1 [6, 12–18], which are extremely rare due to their autosomal recessive nature. Regardless, clinical, biochemical, and histomorphometric parameters are essentially identical in both the dominant and recessive form of hypophosphatemic rickets.

As with the success with *Dmp1*-KO mice, anti-FGF23 antibody has been effectively applied to treat Hyp mice (a *Phex*-deficient hypophosphatemic mouse model), in which the hypophosphatemia and 1,25(OH)₂D levels, growth retardation, defective mineralization, and malformed cartilage are greatly improved [19]. Importantly, KRN23 (a neutralizing anti-FGF23 monoclonal antibody) was proven to significantly increase the maximum renal tubular threshold for phosphate reabsorption, serum Pi, and 1,25(OH)₂D [20].

Concurrently, studies of rare bone sclerosing dysplasias such as sclerosteosis and van Buchem disease, have led to the discovery of sclerostin (SOST) [21], which is mainly expressed in osteocytes and binds competitively to its receptors with Wnt ligands [22]. The

deletion of *Sost* led to excessive bone formation with better-differentiated osteocytes [23]. Applications of the monoclonal sclerostin antibody (Scl-Ab) in a number of pre-clinical animal models, clinical trials in osteoporosis and bone fracture healing [24–29], and inflammation-caused bone loss models such as colitis [30] and periodontitis [31] [32] were very successful. Importantly, our recent studies in periostin KO mice (a periodontitis animal model) showed that the restoration of bone defects in alveolar bone, a type of bone with the highest bone metabolism in the body, is directly linked to improving osteocyte function and morphology [32].

In the current study, we attempted to test whether Scl-Ab can improve the *Dmp1* KO phenotype. In two different age groups of *Dmp1* KO mice, the application of Scl-Ab successfully rescued the major defects in both long bone and alveolar bone but had little effect on serum levels of FGF-23 and phosphorus. These studies demonstrate that some local factors play an important role and that Scl-Ab can be used to treat patients with hypophosphatemic rickets in the future, as a high-phosphorus diet and FGF23 antibody cannot fully restore local bone defects.

RESULTS

Applications of Scl-Ab greatly improved the *Dmp1* KO cortical bone phenotype

Previously we showed the full restoration of serum levels of phosphorus and short status (cartilage defects) in *Dmp1*-KO mice using injections of FGF-23 antibodies, although the osteomalacia was only partially improved (indicating local pathological factors involved in bone mineralization defects) [7]. To improve the local bone defects, Scl-Ab was injected into the *Dmp1* KO mice at the age of 1 month (early rescue) and 3 months (late rescue) 8 weeks apart. The representative X-ray images showed great improvement of the cortical bone (red arrows) in both treatment groups but no apparent effect on the knee phenotype (increases in width and flaring at the ends of the femur Fig 1a). Similarly, the micro-CT images displayed an increase in the cortical thickness and reduction in porosity (Fig 1b). Quantitative analyses from the micro-CT data revealed a significant increase in the ratio of BV/TV (bone volume/total volume) in the middle of the femur cortical bone in early rescue (Fig 1c, +10.7%) and late rescue situations (Fig 1d, +6.4%). Concurrently, we measured the parameters of serum levels of phosphorus, calcium and FGF23, which showed that injections of Scl-Ab had no apparent effect on these parameters (Supplementary Fig 1). Furthermore, we analyzed the efficacy of Scl-ab on the cortical bone, articular and growth plate using H&E stains, and observed a partial improvement of bone porosity and an increase in the cortical bone thickness, which is statistically different from the non-treated group (Supplementary Fig 2). However, there was no apparent change in the articular cartilage and growth plate (the disorganized cartilage phenotype) in the treatment. Taken together, the above findings support the notion that Scl-Ab significantly improves the bone phenotype in *Dmp1* KO mice, which is independent from the levels of FGF23 (abnormally high) and Pi (very low).

Injections of Scl-Ab significantly improved *Dmp1* KO long-bone quality

One of the key phenotypes in *Dmp1* KO mice is osteomalacia (defects in mineralization). To address whether Scl-Ab can improve the mineralization in *Dmp1* KO mice, we analyzed the

changes in the mineral contents using the backscatter SEM technique. In the wild type (WT), the Scl-ab treatment group displayed an increase in mineral content (more white) and more osteocyte dendrites. Similarly, there was a great improvement in the KO group treated with Scl-Ab regarding mineralization and osteocyte morphologies (Fig 2a). Furthermore, the Goldner stain images exhibited a reduction in osteoid areas (under-mineralization, red in color) in the KO cortical bone (Fig 2b, *right panel*). This improvement was significantly based on the ratio of mineralized area/total bone area (Fig 2d, 15.4% increase). We also did 3-point bending on the mouse tibia to address whether using Scl-Ab can improve the mechanical properties in bone. The statistical analysis revealed that the Scl-Ab treatment significantly increased bone strength in both WT (150%) and KO (60%) (Fig 2d). Finally, we performed a calcein and alizarin-red double labeling assay, which revealed a diffused smear in the *Dmp1* KO bone surface (a sign of poor mineralization, Fig 2e), which was greatly narrowed down and even a sign of the separation of both labeling lines in the Scl-ab-treated KO mice.

Scl-Ab treatment partially restored *Dmp1* KO alveolar bone morphology and mineralization

Our previous work showed the severe alveolar bone loss of *Dmp1* KO mice [33]. We then tested the efficacy of Scl-Ab treatment on the periodontal phenotype. The X-ray images showed improvement in mineralization in both treatment groups (Fig 3a), which was further confirmed by the micro-CT images (Fig 3b). Similar to responses observed in the long bone, the quantitative data displayed significant improvements in mineralization for both age groups (Fig 3c–d). Furthermore, we showed poor mineralization in the *Dmp1* KO alveolar bones in the ages of 3 and 5 months using the backscattered SEM image technique (Fig 4, the white color indicates better mineralization and the grey or dark color suggests poor mineralization), which was partially rescued by doses of Scl-ab (Fig 4, thick red arrows). In addition, injections of Scl-ab greatly improved the cementum phenotype (yellow arrows), suggesting a mechanism similar to bone mineralization in cementogenesis.

Scl-Ab treatment recovered the loss of collagen fibers in *Dmp1* KO PDL

Although DMP1 is not expressed in the PDL, there is a severe defect in the *Dmp1* KO mice [33]. Using Sirius red stain, we showed irregularly patterned PDL fibers in the KO mice, which were marginally improved by the Scl-ab treatment (Fig 5a). Interestingly, the polarized light images displayed a much clearer view of the improvements in both the amount of collagen fibers and the distribution pattern (Fig 5b). This finding raises two points: 1) The polarized light microscope techniques are more sensitive at reflecting a change in collagen fibers; and 2) The great improvement of the collagen fibers by Scl-Ab treatment makes an important clinical impact, as currently there appears to be no effective way to restore PDL phenotypes either in translational research or clinical treatment for periodontal disease-caused PDL damage.

The combination of a high-phosphate diet and Scl-Ab injection rescued both cartilage and bone defects in *Dmp1* KO mice

Because the Scl-Ab treatments had no apparent effect on the cartilage phenotype and failed to restore the biochemical changes in the *Dmp1* KO serum, we combined the phosphate diet and Scl-Ab treatment together in the 4-wk-old *Dmp1* KO mice for two months. The

representative X-ray images revealed full restoration of the knee phenotype in the phosphorus and combined groups (yellow arrows, Fig 6a). Additions of Scl-Ab further increased the cortical bone thickness (red arrow) in the combined group only. The immunohistochemistry data showed a similar reduction in the expression of Biglycan, a mark to reflect mineralization status (Fig 6b). The FGF23 level in the high-Pi diet group was similar to that in the non-treated KO bone. The level of FGF23 in bones appears only moderately decreased in the combined treatment group, indicating that a local factor might contribute to the changes in FGF23 (Fig 6c).

DISCUSSION

Hypophosphatemic rickets, a set of rare genetic diseases, is different from most cases of rickets, as the administration of calcitriol (1,25-(OH)₂ vitamin D₃) is relatively ineffective. In the last two decades, great progress has been made in identifying genetic causes of this disease, including discoveries of different mutations in *Phex* [10], FGF23 [11] and *DMP1* [6]. Importantly, successful applications of neutralized FGF23 antibodies in Hyp mice [19] and *Dmp1* KO mice [7] led to clinical trials of the application of this antibody in XLH patients who displayed rickets, osteomalacia, and changes in serum phosphorus and FGF23, similar to these two animal models. The results showed a significantly increased maximum renal tubular threshold for phosphate reabsorption, serum Pi, and 1,25(OH)₂D [20]. However, the local osteomalacic bone phenotype remains in the treated KO mice [7]. In the current study, we followed a different strategy and tested the efficacy of Scl-Ab in *Dmp1* KO mice at two age groups (i.e., early prevention and late rescue), as this neutralizing antibody has been shown to have great efficacy in the treatment of a number of pre-clinical animal models and clinical trials of osteoporosis and bone fracture healing [24–28]. Furthermore, Scl-Ab has been successfully used to treat inflammation-caused bone loss such as the colitis animal model [30] and periodontitis model [31, 32]. The data obtained from both treated groups are exciting, as not only the osteomalacic phenotype is greatly improved in the treatment groups, but the PDL and cementum phenotypes are partially recovered as well in the treated mice.

It is well documented that the pathological change in osteocytes is the primary cause of hypophosphatemic rickets in both *Dmp1* and *Phex* KO mice [6, 34–37]. Recently our group used a novel technique combining FITC confocal 3D imaging and Imaris software to quantify the osteocyte-canalicular system changes between *Dmp1* KO mice and littermate controls. We concluded that there is a dramatic reduction of osteocyte surface area and dendritic processes in the *Dmp1* KO mice [38]. We also showed that a full restoration of serum phosphorus levels using either a high-Pi diet or the FGF23 antibody can completely rescue the rickets phenotype as the cartilage defect is the secondary consequence of these genetic defects [7]. Because of the importance of the phosphorus impact on the maturation of osteoblasts into osteocytes, these treatments greatly improve the osteomalacic phenotype, including osteocyte morphology and function [7]. Interestingly, the SOST levels in the *Dmp1* KO bone matrices are low due to the defect in osteocyte maturation [7]. The logical treatment for *Dmp1* KO mice should increase, instead of decrease, the level of SOST. However, the osteocyte morphology in both long bone and alveolar bone phenotypes is indeed improved by Scl-Ab treatment in both age groups. Although we do not know exactly

why and how this astonishing effect occurs in this animal model, we speculate that a large dosage of Scl-Ab “interferes with”, instead of specifically neutralizes, other unknown molecules that are critical for osteocyte or osteoblast function. This guess is supported in part by three lines of evidence: 1) none of serum levels of biochemical parameters are changed in the Scl-Ab treated mice, supporting the local role of Scl-Ab; 2) there is no apparent change in osteoclasts by TRAP stain (Supplement Fig 3), as a reduction in osteoclast function is partially responsible for the malformed bone in the *Dmp1* KO mice [7]; and 3) there are moderate improvements in some molecules that are critical for the *Dmp1* KO phenotype, including osterix, E11 and FGF23 (Supplement Fig 4). Yet, further detailed research is required to solve this unexpected benefit in controlling osteomalacia.

Periodontitis, the most common human disorder, results in the impairment of the PDL and alveolar bone and is a major cause of tooth loss in adults, occurring in 10–15% of adults in the population [39–41]. Our very recent studies demonstrated unique characteristics of alveolar bone as follows: 1) a high bone formation rate; 2) the progenitor cells in the periodontal ligament (PDL) region play a key role in the alveolar bone formation; 3) a severe alveolar bone loss occurs in the periostin KO mice, whereas there is only a slight change in the KO long bone; 4) applications of Scl-ab fully restore bone loss in the periostin KO alveolar bone, which is directly linked to the restoration of osteocyte morphologies [32]. Similarly in this study, we showed a great improvement in the PDL, cementum and alveolar bone in the treated group, although either DMP1 or SOST is expressed in the PDL. We speculate that the direct improvement of alveolar bone phenotypes by Scl-Ab treatment could somehow affect the Sharpey’s fibers, the connective tissue that links alveolar bone and PDL. This finding is important, as there is currently no surgical or drug method that can be used to restore the damaged PDL structure.

Interestingly, we showed the changes in ECM components such as biglycan in *Dmp1* KO mice, although we do not know how this change affect bone quality. One of our future goals is to study the impact of DMP1 on autophagy, a homeostatic mechanism whereby either a cell-survival or cell-death pathway is regulated [42]. The rationale behind this goal is that some ECM components such as decorin (a small leucine-rich proteoglycan), perlecan (the basement membrane heparan sulfate proteoglycan), and endorepellin (the C-terminal fragment of perlecan) have been demonstrated to control different pathways of autophagy [43].

It is noteworthy that the bone matrix and osteocyte lacunae organization of the long bones and alveolar bones are not fully rescued in the *Dmp1* KO-treated group compared to the WT- or WT- group treated with Scl-Ab, suggesting that the improvement in “bone quality” is more likely due to an increase in bone volume, rather than full restoration of the bone matrices. In other words, an increase in the amount of bone in the treated group is responsible for improvement in mechanical properties.

In summary, these studies showed the unexpected efficacy of Scl-abs in partial restoration of the osteomalacia phenotype in alveolar and long bones with few changes in FGF23 and phosphate levels in KO mice. Furthermore, a combination of a high-phosphorus diet and Scl-ab treatment greatly improved a local bone defect and serum phosphorus levels. We

speculate that this positive impact is independent from the change in Wnt- β -catenin signaling, as SOST levels in *Dmp1* KO are already low. We believe that these findings hold promise as a possible therapy for treating patients with hypophosphatemic rickets.

MATERIALS AND METHODS

Mice, Scl-Ab treatment, High Phosphate Diet and Serum Collection

A total of 96 *Dmp1* KO mice and age-matched littermate control mice with the C56B6 background were divided into two age groups [one and three months ($n = 12$)]. The mice were intraperitoneally (i.p.) injected with either 25 mg/kg Scl-Ab (twice a week) or vehicle for 8 weeks, they were then euthanized at the ages of 3 months and 5 months, respectively. For the Hi-Phosphate diet experiment, 16 wild type and *Dmp1* KO littermates were randomly divided into two groups for each genotype, the control group was fed with standard rodent chow (0.67% P, Ralston Purina, 5010) and the other group with high-phosphate rodent chow (2% P, Harlan Teklad, cat. TD87133). All animal protocols were approved by the Animal Care and Use Committee at Texas A&M University Health Science Center Baylor College of Dentistry.

After anesthetizing the mice, we used the common cardiac puncture method to collect blood from the 3-month-old mice. The total blood was centrifuged at 3000rpm for 20mins, and the serum was then collected and frozen to -80 before analysis. The serum phosphorus was measured using the phosphomolybdate-ascorbic acid method, and the serum FGF23 levels were determined using full-length FGF23 ELISA kits (Kainos Laboratories, Tokyo, Japan), as described previously[6].

Sample preparation and biohistochemistry

After the mice were euthanized, the right lower jaws and right hind-limbs were fixed in 70% ethanol and used for radiographs, μ CT, and SEM examination. The lower left jaws and left hind-limbs were fixed in freshly prepared 4% paraformaldehyde in phosphate-buffered saline (pH 7.4), decalcified in EDTA, and embedded in paraffin using standard histological procedures as previously described [3]. The tissue blocks were cut into 5- μ m-thick serial sections and mounted on glass slides. The sections were used for histological stains like H&E, TRAP (tartrate-resistant acid phosphatase), Sirius red and immunohistochemistry (FGF23: 1:1000 donated by Dr. Chunlin Qin from Baylor College of Dentistry; Biglycan: 1:1000 generously provided by Dr. Larry Fisher from NIH, USA; E11: 1:400, generously provided by Dr. Bonewald from UMKC; and OSX: 1:400, Abcam, Cambridge, MA, USA;).

3-point bending experiment

One femur from each mouse at 12 weeks of age was used for mechanical testing ($n=6$ for all four groups). After sacrificing the animal, the femora were removed, stripped of muscle, wrapped in saline gauze, and stored at -80°C until testing. At the time of testing, the femora were thawed in room temperature saline and three-point bending tests were conducted at room temperature. Each femur was placed on the lower support points of a three-point bending fixture with the anterior side up (i.e., bending approximately the medial-lateral plane). With the span between the lower supports set at 5.2 mm, the fresh bones were loaded

to failure at a rate of 2.0 mm/min using a material-testing system (Bionix Test System 858, MTS, Eden Prairie, MN, USA) as previously described [44].

Goldner Staining and Double Labeling

The left long bone specimens were dehydrated through a graded series of ethanol (70–100%) and embedded in methyl-methacrylate (MMA, Buehler, Lake Bluff, IL, USA) without decalcification. Twenty- μ m sections were cut and stained for Masson Goldner trichrome staining as previously described[6]; bioquant (Nashville, TN) was used to quantify the osteoid/mineralized bone areas and ratios.

For the double-labeling experiment, all animals were injected with calcein green (fluka 190167, 5mg/kg) intraperitoneally 7 days before sacrifice, followed by alizarin red (Sigma A3882, 20mg/kg) injection 2 days before sacrifice. The double-labeling distances indicated the bone formation rate during the 5-day interval, and the samples were MMA-embedded and sectioned to be visualized under a fluorescent microscope.

Backscattered scanning electron microscopy (SEM)

For the mandible and long-bone specimens, the MMA-embedded blocks were sectioned through the center of the first mandibular molar and medial-laterally for the long bone using a water-cooled diamond-impregnated circular saw (Isomet Buehler, Lake Bluff, IL USA). The surfaces of the sample blocks were polished using 1, 0.3 and 0.05 μ m alumina alpha micropolish II solutions (Buehler) with a soft cloth rotating wheel [45]. Each sample was then cleaned in an ultrasonic bath followed by air drying for sputter-coating with carbon and scanning using a backscattered electron detector in a JEOL JSM-6300 scanning electron microscope (JEOL, Japan). The parameters were kept constant while the backscattered SEM images were taken.

X-ray Radiography and Micro-CT Quantification

The mandibles and long bones from the four different groups in both age sets (3-month and 5-month) were dissected and analyzed by X-ray radiography (piXarray 100, Micro Photonics, Allentown, PA, USA) and by a μ -CT35 imaging system (Scanco Medical, Basserdorf, Switzerland). For the μ -CT analyses, we initially used a medium-resolution scan (7.0 μ m slice increment) of the whole mandible to obtain an overall assessment of the tooth shape and structure. We then took a high-resolution scan (3.5 μ m slice increment) of the mandible with 400 slices selected for analyses of the alveolar bone around the 1st molar. Of note, the tooth and PDL were excluded when contouring. Long-bone cortical shell data were obtained at the midshaft of the bone by means of serial tomographic imaging at an energy level of 55 kV and an intensity of 145 μ A. One hundred lateral sections of slices above the midshaft of the femur were analyzed at a threshold of 283. The bone volume to total volume ratio (BV/TV) and porosity were analyzed using the Scanco software. Bone thickness quantification was based on micro-CT cross-sectional images at the femur midshaft region, and the distances were measured by Image-J on the anterior-posterior cortical shell in five animals for all four different groups. All measurement data were reported as mean $\pm$ S.E.

Statistical analyses

Statistical significance was determined using a one-way ANOVA followed by Bonferroni post hoc comparisons between two groups using SPSS 13.0. A P value of < 0.05 was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- DMP1 (*italic*) KO mice develop rickets and periodontitis due to matrix mineralization defects
- Scl-Ab improved both bone quality and PDL integrity in DMP1 (*italic*) KO mice
- Combined therapy using Pi diet and Scl-Ab further restored bone/cartilage defects

Figure 1. Restoration of the cortical bone loss in two Scl-Ab-treated age groups

(a), Representative radiographs revealed thin bone thickness in the KO using small arrows and recovery of bone thickness in both treated groups using large arrows; (b), The micro-CT results showed thin and porotic cortical bones in the KO groups using small arrows, and partial restoration of the bone phenotype with large arrows; (c), The quantitative data exhibited a significant increase in BV/TV (bone volume/total volume) in both the WT and KO groups between the groups compared (**, $p < 0.01$; *, $p < 0.05$; $n = 5$).

Figure 2. Restoration of cortical bone mineralization in two Scl-Ab treated age groups
(a), Backscatter SEM on *Dmp1* long bones showed defects in mineralization and more cuboid and round-shaped osteocyte lacuna. Scl-Ab treatment has both improved bone mineralization as well as osteocyte morphologies in WT and *Dmp1* null mice. **(b)**, Trichrome goldner staining exhibited more red-labelled osteoid area in *Dmp1* bone, which is rescued by Scl-Ab. **(c)**, Quantification data showed significantly improved mineralized bone area ratio according to bioquant (*, $p < 0.05$; $n = 4$). **(d)**, 3-point bending exhibited better mechanical properties in both WT and *Dmp1* null mice after antibody treatment (**, $p < 0.01$; *, $p < 0.05$; $n = 6$). **(e)** The double labeling assay showed no apparent difference in the bone formation rate between the Scl-ab treated and non-treated specimens in the WT group,

although the Scl-Ab treated KO group showed a sharp reduction in dye diffusion areas compared to the non-treated KO mice.

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Figure 3. Rescue of alveolar bone loss by Scl-Ab in both age groups

(a), X-ray images showed alveolar bone loss (thin red arrows) in *Dmp1* null mice in both age groups, which was partially restored with Scl-Ab treatment (wide red arrows). (b), uCT data revealed much thicker cortical walls and less porous matrices (wide red arrows) after Scl-Ab injection compared with untreated mutant littermates (thin red arrows). (c) and (d), μ CT statistical analysis showed a significant rescue of alveolar bone formation in both age groups (c: 3-mon; d: 5-mon; **, $p < 0.01$; $n = 6$). (e) and (f), Porosity quantification indicated rescue of alveolar bone porosity in both age groups (e: 3-mon; f: 5-mon; **, $p < 0.01$; $n = 6$).

Figure 4. Scl-Ab rescued bone and cementum mineralization in *Dmp1*-null mice mandible (a), Backscatter SEM showed increased alveolar bone formation in both WT and *Dmp1* null mice (wide red arrows) after Scl-Ab injection compared with control (thin red arrow). The yellow arrow indicated the rescue of cementum mineralization in both age groups. (b), Enlarged SEM pictures of the 1st molar root revealed rescue of cementum.

Figure 5. Restoration of collagen fibers in the PDL (periodontal ligament) in Dmp1 null mice after Scl-Ab treatment
 (a), Sirius red staining showed loosely bundled collagen fibers in the PDL region in Dmp1 null mice, which is partially restored in the Scl-Ab treated group. (b), polarized picture of the corresponding area revealed the rescue of PDL fiber by Scl-Ab, showing orange- colored rich collagen PDL fibers.

Figure 6. Full restoration of Dmp1 null knee and cortical bone by combined treatment of Scl-Ab and high phosphate diet

(a), X-ray images showed deformity of knee joint (widest yellow arrow) and thin cortical shell in Dmp1 null mice (thinnest red arrow), high-phosphate diet alone partially rescued knee and bone phenotype, but combined high phosphate and Scl-Ab treatment further restored knee joint morphologies (thinnest yellow arrow) and cortical bone thickness (widest red arrow). (b), Biglycan IHC revealed more mature and better mineralized bone matrix due to either the high-phosphate diet or the combined treatment. (c), Dmp1 null mice showed the highest Fgf-23 level in the bone (wide red arrow), whereas a high-phosphate diet alone did not seem to reduce the Fgf-23 levels, and Scl-Ab, high-phosphate diet combined treatment had a significantly decreased Fgf-23 level (white arrow).