Highlights

- Craniofacial bones appear to have unique mechanism in bone metabolism
- Whether craniofacial bones respond to TPTD/PTH is largely unknown
- Three-dimensional morphometric analyses of osteocytic lacunae were conducted
- Active changes in osteocytic lacunae size were uniquely observed in jaw bone

Dynamic morphometric changes in the mandibular osteocytic lacunae of ovariectomized rats in response to teriparatide, as revealed by three-dimensional fluorescence analyses: possible involvement of osteocytic perilacunar remodeling

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Abstract

Objectives: Teriparatide [TPTD; human parathyroid hormone (hPTH)] is an anti-osteoporotic drug with bone anabolic effects. Clinical and preclinical studies have indicated that TPTD has value in oral and maxillofacial bone therapies, including jawbone regeneration, periodontal tissue repair, and the treatment of medication-related osteonecrosis of the jaw. However, it is unclear whether craniofacial bones respond to TPTD in a manner similar to the responses by axial and appendicular bones. Recent studies showed that TPTD acts on both osteocytes and osteoblasts. This study was conducted to characterize distinct craniofacial bone sites, with a focus on morphometric changes in osteocytic lacunae in ovariectomized rats receiving TPTD.

Methods: Conventional bone histomorphometric analyses of mandibular and parietal bones sections were conducted. High-resolution confocal imaging-based three-dimensional fluorescence morphometric analyses of osteocytic lacunae in distinct sites of mandibular and parietal bones were further conducted.

Results: We observed dynamic changes in the morphometric characteristics of osteocytic lacunae specifically in alveolar bone and other mandibular bone sites upon administration of TPTD.

Conclusions: These findings suggest that osteocytes in mandibular bone (specifically,

alveolar bone) have unique functional characteristics of osteocytic perilacunar remodeling.

Key words:

Teriparatide, PTH, craniofacial bone, mandibular bone, alveolar bone, osteocytic lacunae, osteocytic perilacunar remodeling, three-dimensional morphometry

1. Introduction

Teriparatide [TPTD; human parathyroid hormone (hPTH)] is considered a biologically active treatment for osteoporosis because of its bone anabolic effects during intermittent administration. The pharmacological activity of TPTD fundamentally increases bone turnover by stimulating bone formation and resorption. Although continuous TPTD administration (similar to the elevated level of serum PTH in hyperparathyroidism [HPT]) reduces bone mass by enhancing bone resorption relative to bone formation, intermittent TPTD administration increases net bone mass by enhancing bone formation relative to bone resorption[1-5]. Three TPTD dosing regimens (once-daily, once-weekly, and twice-

weekly) are clinically available in Japan; they are used for the treatment of osteoporosis according to each patient's requirements and clinical conditions [6-9].

Because of its anabolic effects on bone, TPTD may be beneficial in situations where enhanced bone repair is desired[10,11], including mandibular damage[12]. Experiments with animal models have provided evidence to support the use of TPTD in oral and maxillofacial bone therapies[13-17]. Clinical studies of TPTD have shown promising results in periodontal regeneration and the management of medication-related osteonecrosis of the jaw[18-21].

Studies of human genetic disorders have indicated that PTH signaling has important roles in craniofacial bone. A loss-of-function mutation in exon 3 of the *PTH1R* gene, which encodes PTH/PTH-related protein (PTHrP) Type I receptor (PTH1R), is responsible for Blomstrand chondrodysplasia (OMIM #215045); this fatal condition is characterized by accelerated skeletal maturation, shortened long bones, and increased bone density[22]. Thus, PTH signaling is expected to play an essential role in overall fetal skeletal development. Another loss of function mutation in the *PTH1R* gene causes ankylosed and distorted teeth, along with defects in endochondral bone formation[23]. Mutations in the *PTH1R* gene causing disruption of PTH1R-mediated G-protein signaling are responsible for primary failure of tooth eruption (PFE) (OMIM #125350) [24,25].

Preclinical studies have shown that PTH signaling participates in periodontal ligament maintenance and is required for tooth eruption[26,27]. Because ablation of the mouse *PTH* gene was not associated with any overt changes in teeth or alveolar bone[28], PTHrP presumably is the main functional ligand for PTH1R in jawbones. These findings indicate that loss of PTH signaling function affects physiological development and the maintenance of craniofacial bones, including jawbones.

Excess PTH, such as the levels observed in primary HPT, has been associated with bone loss. Padbury et al. evaluated oral health in patients with primary HPT[29]. They did not detect alterations in periodontal parameters such as attachment level, alveolar bone, and probing depth; however, they observed a greater incidence of oral tori (bony growths in the oral cavity) and reduced cortical bone thickness at the mandibular angle. Their findings suggested that increased PTH activity in humans has both catabolic and anabolic effects, but it does not adversely affect the periodontium. This hypothesis was supported by the results of a study involving hemodialysis patients with secondary HPT[30]. The findings thus far indicate that enhanced PTH production has minimal effects on oral health.

The catabolic effects of pharmacological levels of TPTD/PTH on axial (vertebrae) and appendicular (limbs) bone density have been extensively explored. However, it is unclear

whether craniofacial bones exhibit similar responses to TPTD/PTH. Because some characteristics of craniofacial bone development differ from those at other skeletal sites, functional responses to TPTD/PTH may vary according to location. Craniofacial bones are derived from cranial neural crest cells and are mainly formed by intramembranous ossification, whereas axial and appendicular bones differentiate from mesodermal cells through endochondral ossification. Thus, detailed comparative studies concerning the effects of TPTD/PTH on distinct skeletal sites are needed to guide its craniofacial applications. We recently reported that expansions of the osteocytic lacunar–canalicular system resulted from the pharmacological activity of TPTD, suggesting that TPTD contributes to osteocytic bone remodeling[31]. This study was conducted to characterize distinct craniofacial bone sites, with a focus on morphometric changes in osteocytic lacunae, via high-resolution three-dimensional fluorescence morphometry in ovariectomized (OVX) rats receiving TPTD.

2. Materials and Methods

2.1. Animals and bone specimen preparation

Ten-week-old female Sprague–Dawley rats (Charles River, Kanagawa, Japan) were

maintained under a 12-h/12-h light/dark cycle with unrestricted access to tap water and a standard diet containing 1.2% Ca, 0.9% P, 22% protein, and vitamin D3 (6.2 IU/g) (CRF-1; Oriental Yeast, Tokyo, Japan). Rats underwent sham surgery or ovariectomy at 12 weeks of age. Four weeks later, rats who had undergone sham surgery were divided into two groups and subcutaneously injected with 30 μg/kg of TPTD (Asahi Kasei Pharma Corporation, Tokyo, Japan) or saline three times weekly (Sham + TPTD and Sham + Vehicle group, n = 5) for 4 weeks (Figure 1, Table 1). Concurrently, rats who had previously undergone ovariectomy were divided into two groups and subcutaneously injected with 30 μg/kg of TPTD or saline three times weekly (ovariectomized [OVX] + TPTD and OVX + Vehicle group, n = 5) for 4 weeks (Figure 1, Table 1). Four weeks and 2 days after the last subcutaneous injection, rats were euthanized by exsanguination under anesthesia. The mandibular and parietal bones were fixed in 70% ethanol, stained with Villanueva bone stain, dehydrated in a graded ethanol series, cleared in acetone, and embedded in polymethyl methacrylate (Wako Pure Chemical Industries, Osaka, Japan). Thin sections (thickness: 5 μm) were prepared from mandibular and parietal bone for histomorphometry analyses. All experimental protocols were approved by the experimental animal ethics committee at the Asahi Kasei Pharma Corporation and conducted in accordance with the guidelines for management and handling of

experimental animals. Animal care technicians and staff members who administered treatments were not involved in sample measurements. All sample measurements were performed using objective methods.

2.2. Widefield fluorescence and differential interference contrast (DIC) imaging

A widefield fluorescence microscope (ECLIPSE Ni; Nikon, Tokyo, Japan) equipped with differential interference filters, as well as 10x and 20x objectives (Plan Apo λ × 10 [numerical aperture (NA) = 0.45], Plan Apo λ × 20 (NA = 0.75)), was used for fluorescence and DIC observations. The fluorescence filter was Texas Red (excitation wavelength 540–580 nm, dichroic mirror: 595 nm, emission wavelength 600–660 nm); autofluorescence imaging was used to measure mandibular and parietal bone morphologies. Morphometric analyses of mandibular and parietal bones were conducted using autofluorescence.

2.3. onfocal fluorescence imaging

Confocal imaging of Villanueva-stained bone sections was performed using a confocal laser microscopy system (A1-ECLIPSE Ti2; Nikon), with an HP Apo TIRF 100 × oil DIC N2 objective (NA = 1.49). Two laser lines at 488 nm and 561 nm were used for excitation;

two filter cubes at 480 nm/560 nm and 560 nm/640 nm were used for detection. Images were acquired with a density of 0.10 μm/pixel using the 100 × objective, along with 12-bit color depth. For three-dimensional fluorescence morphometry, confocal images were acquired with a 0.3-μm step size for voxel sampling using the 100 × objective.

2.3. Region of interest (ROI) establishment and histomorphometry

For morphometric analyses of mandibular bone, the following parameters were measured on transverse sections of first molars according to the method of Inoue et al.: a, distance from the lowermost mandibular margin to the alveolar apex; b, vertical line from the buccal apex of the mandible to a; c, vertical line from the lingual apex of the mandible to a; and d, angle formed by the lowest mandibular margin, alveolar apex, and buccal apex of the mandible (Figure 2). For measurements of alveolar bone volume (BV)/tissue volume (TV), a 0.5 mm × 0.5 mm square was established, extending from the furcation area to the root apex (Figure 3). For morphometric analyses of parietal bone, three ROIs (1000 μm wide × 1400 μm long) were established on transverse sections of parietal bone. Based on histological observations of DIC images, the parietal bone was divided into three layers: outer plate (a, the external side), middle plate (b, containing marrow), and inner plate (c, the internal side). The thickness of each layer was measured

as the length of both ends of a fixed rectangle. The total thickness (d) of the three layers was measured (Figure 4b). For BV/TV measurements, three ROIs (1000 μm long × 1400 μm wide) were established in each section; each ROI was divided into three layers, as in the morphometric analyses above. No ROIs were established for morphometric analyses of middle plate bone (Figure 4d). Morphometric parameters of the middle plate in parietal bone (e.g., TV, BV, osteoid volume [OV], osteoid length, osteoid width, bone marrow area, bone surface, OV/TV, OV/BV, and osteoid length/bone surface) were established as depicted in Figure 4d and Supplementary Figure 1.

2.4. Three-dimensional reconstruction and morphometric analysis of the osteocytic lacunae

Three-dimensional fluorescence images acquired with the HP Apo TIRF 100 × oil DIC N2 objective (NA = 1.49) were constructed from z-series images using IMARIS software (Bitplane, Zurich, Switzerland), as previously described.

2.5. Statistical analysis

All data are presented as means and standard deviations (SDs); they were analyzed by one-way analysis of variance (ANOVA). Sidak's multiple comparisons test was used for

post hoc analyses. GraphPad Prism software (version 9.5.0) was used to create graphs and perform all statistical analyses. The threshold for statistical significance was defined as $p < 0.05$.

3. Results

3.1. Regimen settings of TPTD administration and experimental schedule

To analyze the effects of TPTD administration on ectoderm-derived bones (e.g., mandibular and parietal bones) in OVX rats, we established four experimental groups: sham-operated rats receiving vehicle (Sham + Vehicle), sham-operated rats receiving TPTD (30 μg/kg) (Sham + TPTD), OVX rats receiving vehicle (OVX + Vehicle), and OVX rats receiving TPTD (30 μg/kg) (OVX + TPTD) (Figure 1, Table 1). In each group, injections of vehicle and TPTD were initially given at 4 weeks after surgery, and were repeated three times weekly for 4 weeks.

3.2. Morphometric analyses of mandibular and parietal bone sections

We first conducted morphometric analyses of frontal sections of mandibular bone, as

depicted in Supplementary Figure 1a. The mandibular bone height (denoted by a), buccal extrusion (denoted by b), lingual extrusion (denoted by c), and masseteric ridge angle (denoted by d) parameters did not significantly differ among groups (Supplementary Figure 1b). Accordingly, the BV/TV in alveolar bone at furcation and apical areas of alveolar bone did not significantly differ among groups (Supplementary Figure 2). These observations suggested that OVX and subsequent TPTD administration did not affect the overall morphology of mandibular bone, and that higher-resolution analysis was needed to identify the effects of OVX and subsequent TPTD administration on mandibular bone.

Next, we conducted morphometric analyses of frontal sections of parietal bone, as depicted in Supplementary Figure 3a. Although total thickness was significantly greater in the OVX + TPTD group than in the OVX + Vehicle group, TPTD administration in sham-operated rats (Sham + Vehicle vs. Sham + TPTD) and OVX rats (Sham + Vehicle vs. OVX + Vehicle) did not significantly affect this parameter (Supplementary Figure 3b). BV/TV values in the inner, middle, and outer plates did not significantly differ among groups (Supplementary Figure 3c). Because OVX tended to reduce BV/TV in the middle plate, whereas TPTD tended to reverse this change in a non-significant manner (Supplementary Figure 3c), we postulated that TPTD administration affected bone formation on the surface of bone marrow cavities specifically located in the middle plate.

We then analyzed parameters related to osteoid in the middle plate (Supplementary Figure 3d and 4). The osteoid length/bone surface was significantly higher in the OVX + TPTD group than in the OVX + Vehicle group (the right graph in Supplementary Figure 3d). Other parameters (e.g., OV/TV and OV/BV) showed similar tendencies, although the differences were not statistically significant. These observations suggested that, particularly in OVX rats, TPTD administration augmented BV by acting on outer and inner bone surfaces (periosteum).

3.3. Three-dimensional fluorescence morphometry analyses of osteocytic lacunae in distinct bone sites of mandibular and parietal bones

Because osteocytic lacunae exhibit distinct morphology according to bone site[31,32], we compared osteocytic lacunae in distinct mandibular and parietal bone sites among experimental groups via three-dimensional fluorescence morphometry analyses of high-resolution confocal images (Supplementary Figure 5 and Figure 2). In our three-dimensional morphometry, in addition to regular parameters of volume and surface, we scored ellipticity of osteocytic lacunae through introducing prolate/oblate parameters to compare spatial shape (Supplementary Figure 5). Volume, surface area, ellipticity (oblate and prolate), and number of vertices of osteocytic lacunae were significantly greater in

alveolar bone than in other bone sites on the buccal and lingual sides of the mandible (Figure 2), indicating that osteocytic lacunae in alveolar bone exhibited atypical morphology comprising large, oblate, and polygonal shapes. Other distinct features of osteocytic lacunae in alveolar bone included an obviously thin canalicular network compared with other bone sites (Figure 2b).

The same analyses of osteocytic lacunae in parietal bone showed that the volume of osteocytic lacunae was significantly greater in the middle plate than in outer and inner plates (Figure 3). Ellipticity (oblate) was significantly greater in the middle plate than in the outer plate. Surface area, ellipticity (prolate), and number of vertices of osteocytic lacunae did not significantly differ among these bone sites. Our analyses indicated that osteocytic lacunae in parietal bone exhibit a typical oblong shape, and that osteocytic lacunae were larger in the middle plate than in other bone sites.

3.4. The effect of TPTD on morphometrical changes in osteocytic lacunae in mandibular and parietal bones

We previously reported that frequent TPTD administration expanded the size of osteocytic lacunae in a bone site-dependent manner [31]. To assess the effect of TPTD on osteocytic lacunae in mandibular bone, we utilized three-dimensional fluorescence

morphometry as described above (Figures 4 and 5). At alveolar bone sites, the volume and surface area of osteocytic lacunae were significantly greater in the Sham + TPTD group than in the Sham + Vehicle group (Figures 4 and 5a). These parameters were also significantly greater in the OVX + Vehicle group than in the Sham + Vehicle group, while they were significantly reduced in the OVX + TPTD group. The number of vertices showed a pattern similar to these parameters. Overall, the findings indicated that administration of TPTD to sham-operated rats increased the size of osteocytic lacunae in alveolar bone, whereas the administration of TPTD to OVX rats decreased the size of osteocytic lacunae that had been expanded by OVX.

Similar changes in the size of osteocytic lacunae were observed in buccal sites of mandibular bone (Figures 4 and 5b). TPTD had similar effects on osteocytic lacunae in lingual bone sites, although OVX did not significantly expand the size of lacunae in these bone sites (Figures 4 and 5c).

We assessed the effects of TPTD on osteocytic lacunae in parietal bone sites (Figures 6 and 7). In the outer plate, the volume, surface area, and number of vertices tended to be greater in the OVX + Vehicle group than in the Sham + Vehicle group, suggesting that OVX expanded the size of lacunae in these bone sites; however, the administration of TPTD to sham-operated and OVX rats did not lead to significant changes (Figures 6 and

7a). In other parietal bone sites (i.e., middle and inner plates), morphometric analyses of osteocytic lacunae did not show significant changes in a manner consistent with mandibular bone (Figures 6 and 7b, c). These analytical observations in parietal bone suggested that changes in the size of osteocytic lacunae were less sensitive in parietal bone than in mandibular bone.

4. Discussion

4.1 Animal models of induced osteoporosis

Animal model studies in rats and rabbits have evaluated the effects of PTH administration on osteoporosis-affected craniofacial bones[13,16,33,34]. In those models, osteoporosis was induced by ovariectomy alone, or in combination with glucocorticoid treatment or the extraction of opposing teeth. Model animals intermittently received hPTH (1–34) or hPTH (1–84), then underwent evaluations of bone quality and quantity. The results of these studies suggested that alveolar bone is susceptible to osteoporosis and more responsive to PTH treatment, as indicated by greater increases in bone density in the molar region compared with other jawbone sites. In the present

study, we did not observe significant anabolic effects of TPTD administration on alveolar bone in the mandibular molar region, whereas parietal bone displayed a significant response to TPTD in terms of increased thickness. These results contradict a previous report that, at 1 year after surgery, TPTD administration to OVX rats for 10 weeks (5 days per week) stimulated bone formation in periosteal and cancellous bone surfaces, as well as the mandibular alveolar crest[33]. Compared with the previous work, our low dosing frequency and short treatment period might have been insufficient to exert an obvious anabolic effect on mandibular bone; however, the regimen utilized in the present study is identical to the approach in our previous reports, which demonstrated that TPTD had anabolic effects on trunk bones (e.g., tibial and vertebral bones)[5,35-37]. Nevertheless, our morphometric analyses of osteocytic lacunae demonstrated that lacunar size and shape varied in a more dynamic manner among OVX rats than among sham-operated control rats. These results are consistent with previous reports that osteoporotic alveolar bone is more sensitive to PTH, as mentioned above[33]. We also found that osteocytic lacunae in alveolar bone exhibited more dynamic morphometric changes in response to ovariectomy and TPTD administration, compared with other sites in mandibular and parietal bone. Considering previous reports, it can be speculated that these site-specific differences are related to a distinct rate of remodeling in alveolar bone.

4.2. Periodontitis and TPTD

Preclinical and clinical studies have demonstrated the positive effects of PTH administration on periodontitis-induced alveolar bone loss. The administration of PTH (40 μg/kg), 3 times per week for 30 days, effectively prevented bone loss in OVX rats with ligature-induced periodontitis[38], suggesting that systemic administration of PTH has regenerative effects on both inflammation-mediated bone loss and metabolic bone loss; this hypothesis was supported by the results of clinical studies[21]. It will be insightful to investigate the pathophysiological roles of alveolar osteocytes in these models.

4.3. Osteocytic perilcunar bone remodeling via regulated demineralization and mineralization on the surfaces of osteocytic lacunae

Osteocytic osteolysis is suspected to constitute a phenomenon in which osteocytes participate in bone and mineral metabolism by removing perilacunar matrix[39,40]. Osteocytic osteolysis is primarily characterized by osteocytic lacunae enlargement in the contexts of lactation, hyperparathyroidism, and tumor-associated osteolysis, whereby activated osteocytes exhibit osteoclast activity to remove mineralized bone matrix. Our

previous study of rabbit tibiae supported the conclusion that such demineralization on the surfaces of osteocytic lacunae is involved in the pharmacological activity of TPTD[31]. Frequent administration of high doses of TPTD resulted in expansion of the osteocytic lacunar-canalicular system. This pharmacological phenomenon was highly bone site-specific and observed mainly in the Haversian layer, rather than the outer and inner lamellar layers.

An intrinsic feedback mechanism to reverse osteocytic osteolysis is presumably activated in the post-lactation period[40,41]. During this reverse phase, osteocytes can replace the depleted matrix as a complement to restored osteoblast function. Therefore, DMP1, PHEX, Phospho1, and other biomineralization-related proteins essential for skeletal development may play important roles in regulating the mineralization of adult osteocytic lacunae, thus contributing to postnatal bone homeostasis and mineral metabolism[40]. The present study revealed dynamic contraction of osteocytic lacunae in alveolar bone and other mandibular bone sites among OVX rats receiving TPTD; lacunae in parietal bone were less responsive to TPTD, although calvariae and mandibular bone have similar developmental origins and ossification mechanisms. Therefore, our findings suggest that osteocytes in mandibular bone (specifically, alveolar bone) have unique functional characteristics that involve dynamic regulation of lacunar

resorptive and reversal phases, thus osteocytic perilacunar remodeling; such characteristics are less obvious in other bones. Moreover, our findings suggest that the pharmacological activity of TPTD, and possibly the physiological role of PTH, includes roles in regulating the demineralization and mineralization of osteocytic lacunae. The unique morphometric characteristics of osteocytic lacunae in alveolar bone, which exhibit polygonal lacunar shapes with small numbers of canaliculi, may be associated with their specific regulatory functions; notably, most lacunae display round, oblong, or spindle shapes with large numbers of canaliculi that constitute a dense cellular network[32]. In support of this hypothesis, alveolar osteocytes specifically respond to distinct mechanical loads induced by orthodontic tooth movement, as indicated by site-specific changes in sclerostin expression in a rat model[42].

5. Conclusions

In this study, we identified dynamic changes in the morphometric characteristics of osteocytic lacunae in alveolar bone and other mandibular bone sites upon administration of TPTD. These findings suggest that osteocytes in mandibular bone (specifically, alveolar bone) have unique functional characteristics of dynamic regulation of osteocytic perilacunar remodeling, whose characteristics are less obvious in other bones.

Considering that osteocytes exhibit functional heterogeneity in the expression of molecules involved in phosphate metabolism, bone formation, and mineralization, as indicated by distinct expression patterns of fibroblast growth factor (FGF)23, sclerostin, and DMP1[43], it is important to conduct comparative studies focused on the molecular characteristics of osteocytes in jawbones (e.g., alveolar bone). Additional comparative studies involving other bones will help to guide clinical applications of TPTD in craniofacial bone.

Ethical Approval

All animal experimental protocols were approved by the Experimental Animal Ethics Committee at Asahi Kasei Pharma Corp (Tokyo, Japan) and conducted in accordance with established guidelines concerning the management and handling of experimental animals.

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Author contributions

A. Nakanishi-Kimura conducted most fluorescence imaging data analyses, including the establishment of AI-driven morphometric analyses, and wrote the manuscript. A. Takakura and R. Takao-Kawabata designed most animal experiments, conducted bone analyses, wrote the manuscript, and supervised the project. M. Hoshi-Numahata, H. Watanabe, and M. Nishiura contributed to statistical analysis and data interpretation. T. Iimura supervised the project, contributed to fluorescence imaging analyses, and wrote the manuscript. All authors approved the final manuscript.

Competing interests

A. Takakura and R. Takao-Kawabata are employees of Asahi Kasei Pharma Corporation. The remaining authors declare no conflicts of interest.

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Figure Legends

Figure 1.

Flowchart of teriparatide (TPTD) administration. (a) Twelve-week-old female rats were divided into four groups: (1) Sham + Vehicle (administration of saline three times weekly), (2) Sham + TPTD (administration of 30 μg/kg TPTD three times weekly), (3) OVX + Vehicle (administration of saline three times weekly), and (4) OVX + TPTD (administration of 30 μg/kg TPTD three times weekly).

Figure 2.

Quantitative three-dimensional analysis of osteocytes in mandibular bone. (a) Schematic

diagram demonstrating locations measured in mandibular bone. (b) Representative images show osteocytes obtained from three different locations in mandibular bone of the Sham + Vehicle group. Scale bars indicate 20 μm. (c) Osteocyte parameters (e.g., object volume, surface area, number of vertices, ellipticity [oblate], and ellipticity [prolate]) were measured and compared among groups. All data are shown as means ± SDs (n = 15 ROIs, 5 rats). Significant differences were determined by one-way ANOVA. Sidak's multiple comparisons test was used for post hoc analysis. (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$).

Figure 3.

Quantitative three-dimensional analysis of osteocytes in parietal bone. (a) Schematic diagram demonstrating locations measured in parietal bone of the Sham + Vehicle group. (b) Representative images show osteocytes obtained from three different locations in parietal bone. Scale bars indicate 20 μm. (c) Osteocyte parameters (e.g., object volume, surface area, number of vertices, ellipticity [oblate], and ellipticity [prolate]) were measured and compared among groups. All data are shown as means ± SDs (n = 15 ROIs, 5 rats). Significant differences were determined by one-way ANOVA. Sidak's multiple comparisons test was used for post hoc analysis. (*: $p < 0.05$, **: $p < 0.01$, ***:

$p < 0.001$).

Figure 4.

Representative images of osteocytes obtained from mandibular bone in each of the four experimental groups. Scale bars indicate 20 μm.

Figure 5.

Quantitative three-dimensional analysis of osteocytes in mandibular bone. (a) Alveolar bone. (b) Buccal side. (c) Lingual side. All data are shown as means ± SDs (n = 25 ROIs, 5 rats). Significant differences were determined by one-way ANOVA. Sidak's multiple comparisons test was used for post hoc analysis. (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$).

Figure 6.

Representative images of osteocytes obtained from parietal bone in each of the four experimental groups. Scale bars indicate 20 μm.

Figure 7

Quantitative three-dimensional analysis of osteocytes in parietal bone. (a) Outer plate. (b) Middle plate. (c) Inner plate. All data are shown as means ± SDs (n = 25 ROIs, 5 rats). Significant differences were determined by one-way ANOVA. Sidak's multiple comparisons test was used for post hoc analysis. (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$).

Figure1

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Figure 1.

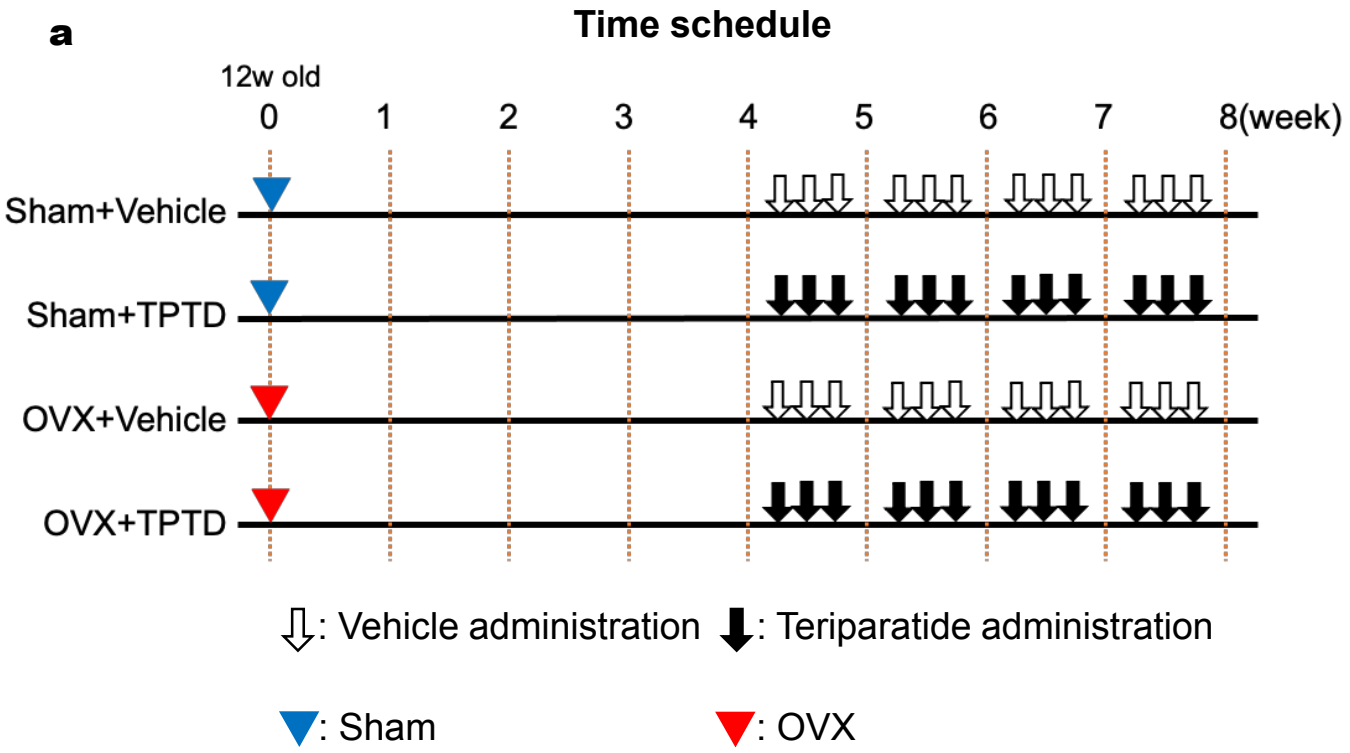
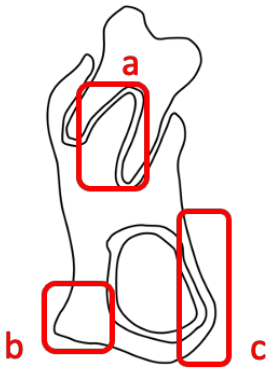


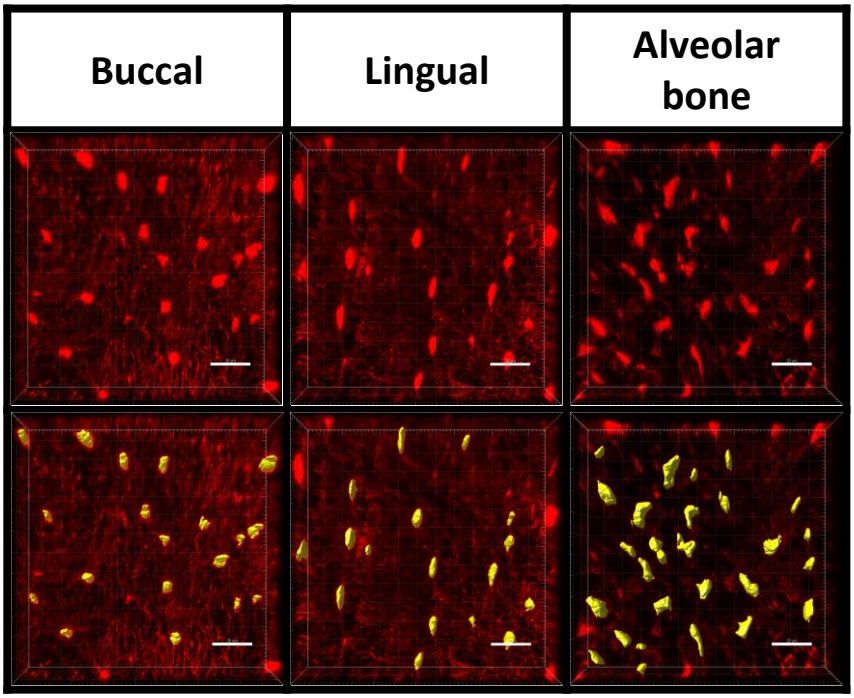
Figure 2

a



a. Alveolar bone
b. Lingual side
c. Buccal side

b



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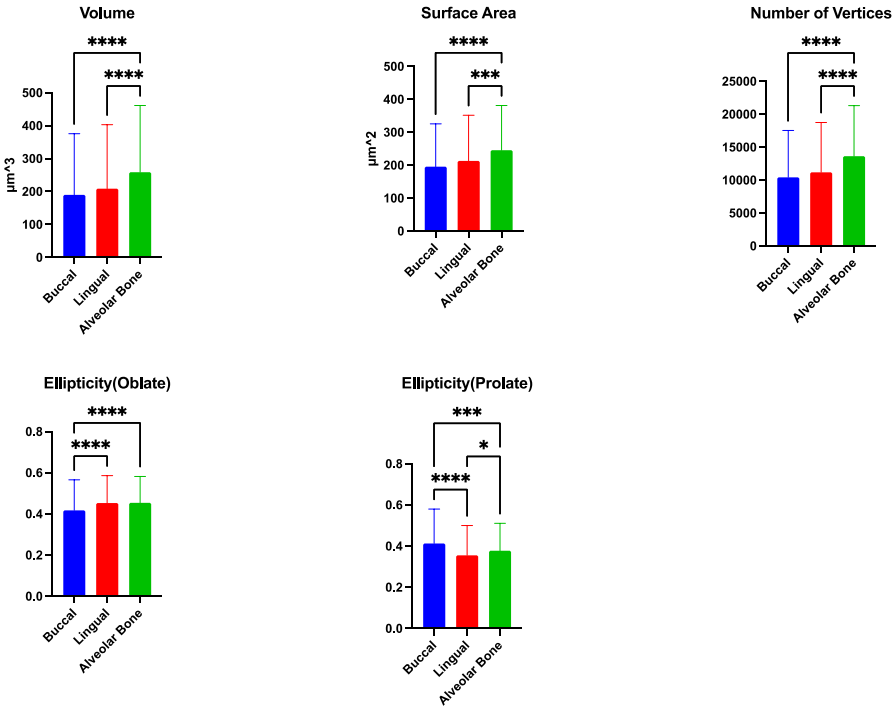

Figure 3

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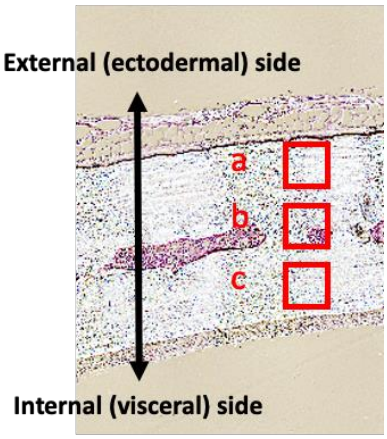
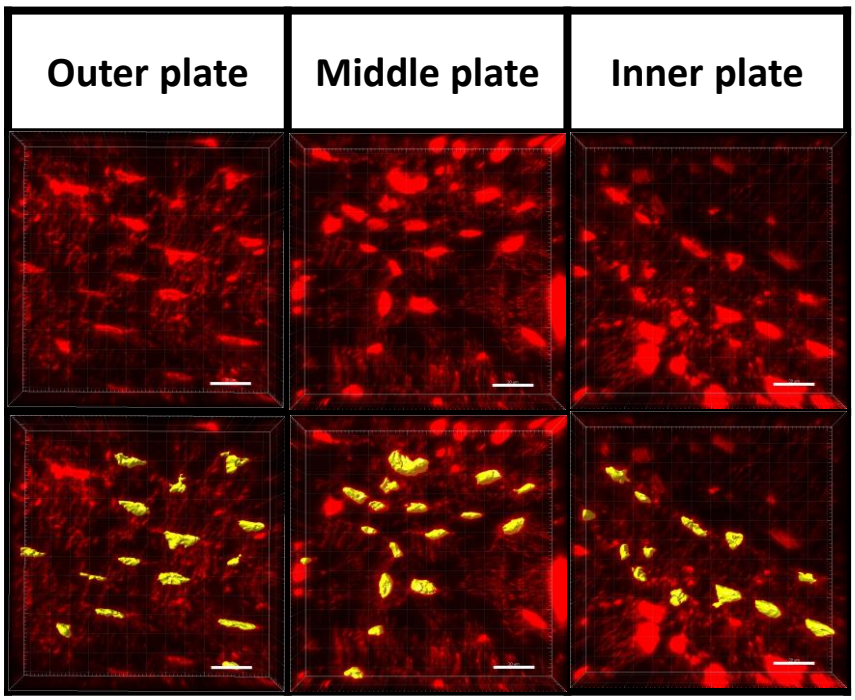


a. Outer plate
b. Middle plate
c. Inner plate

b



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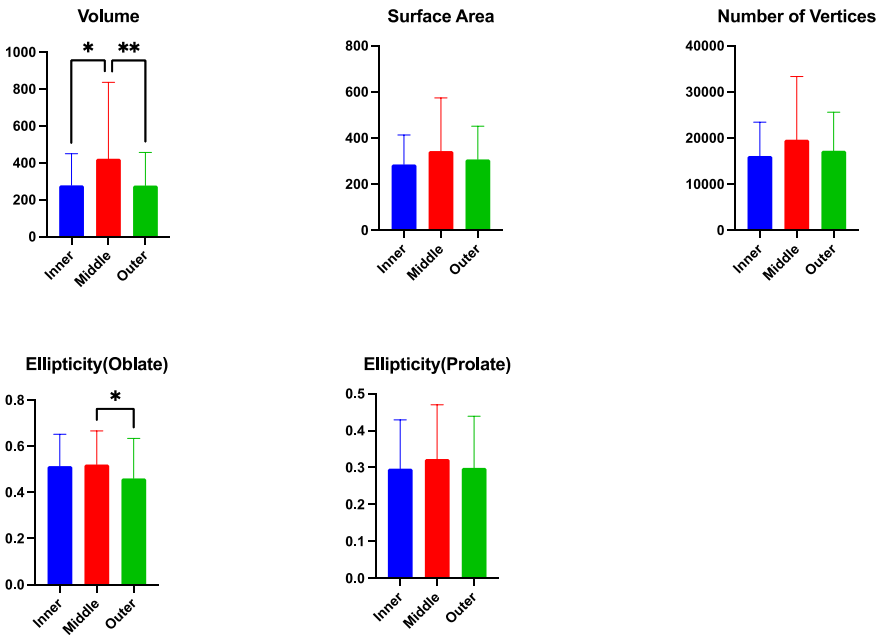

Figure 4

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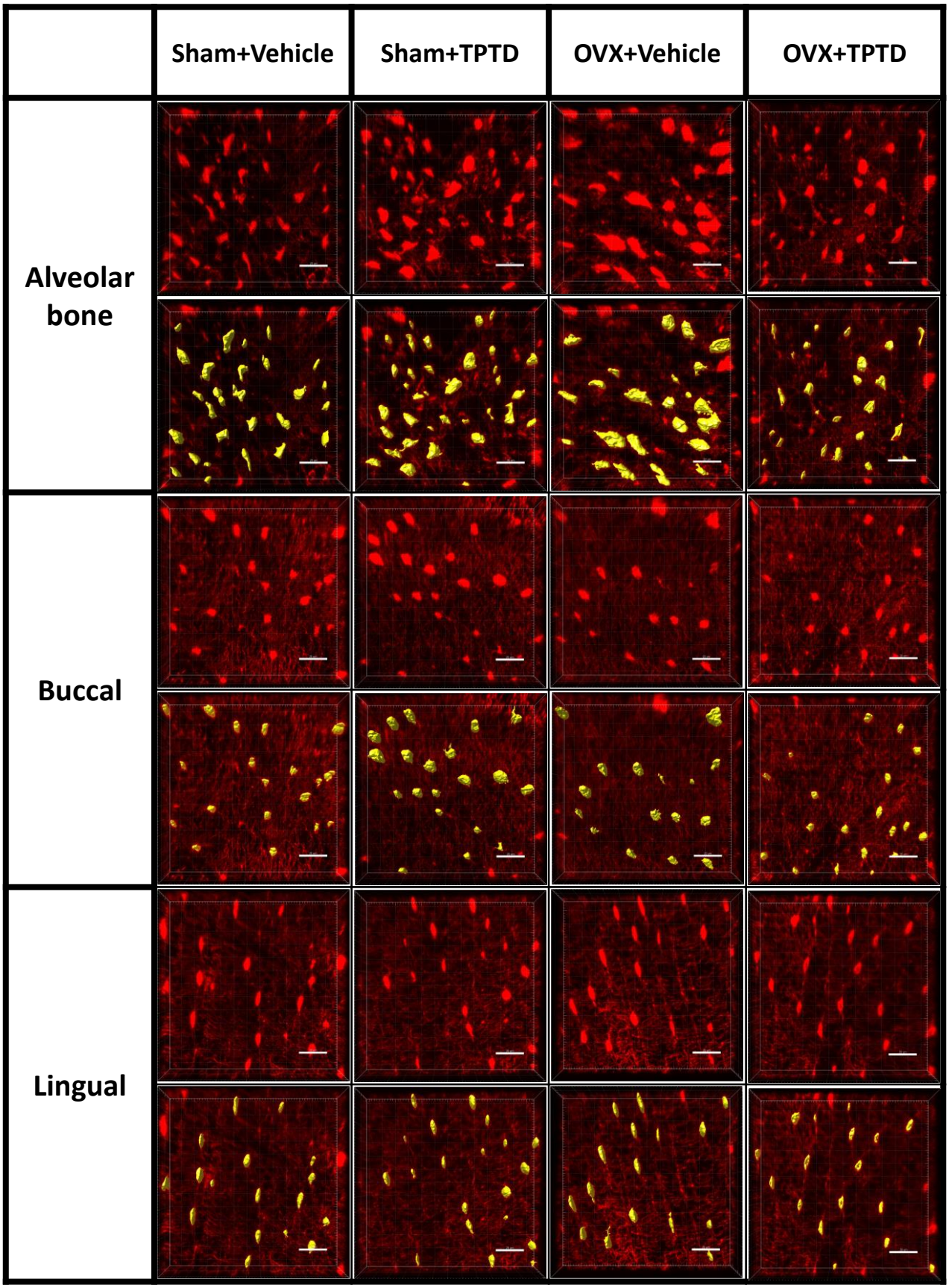

Figure 5

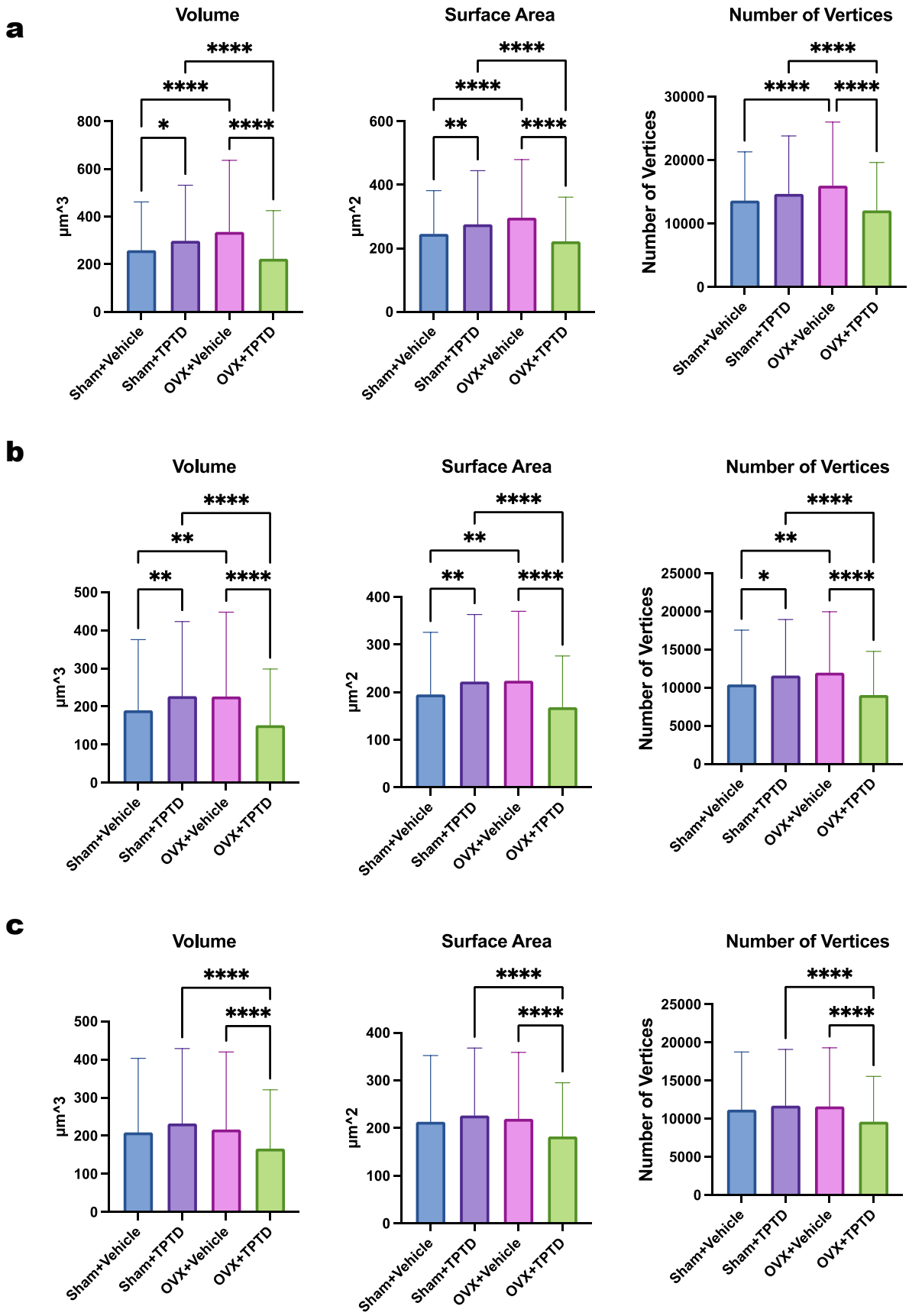

Figure 6

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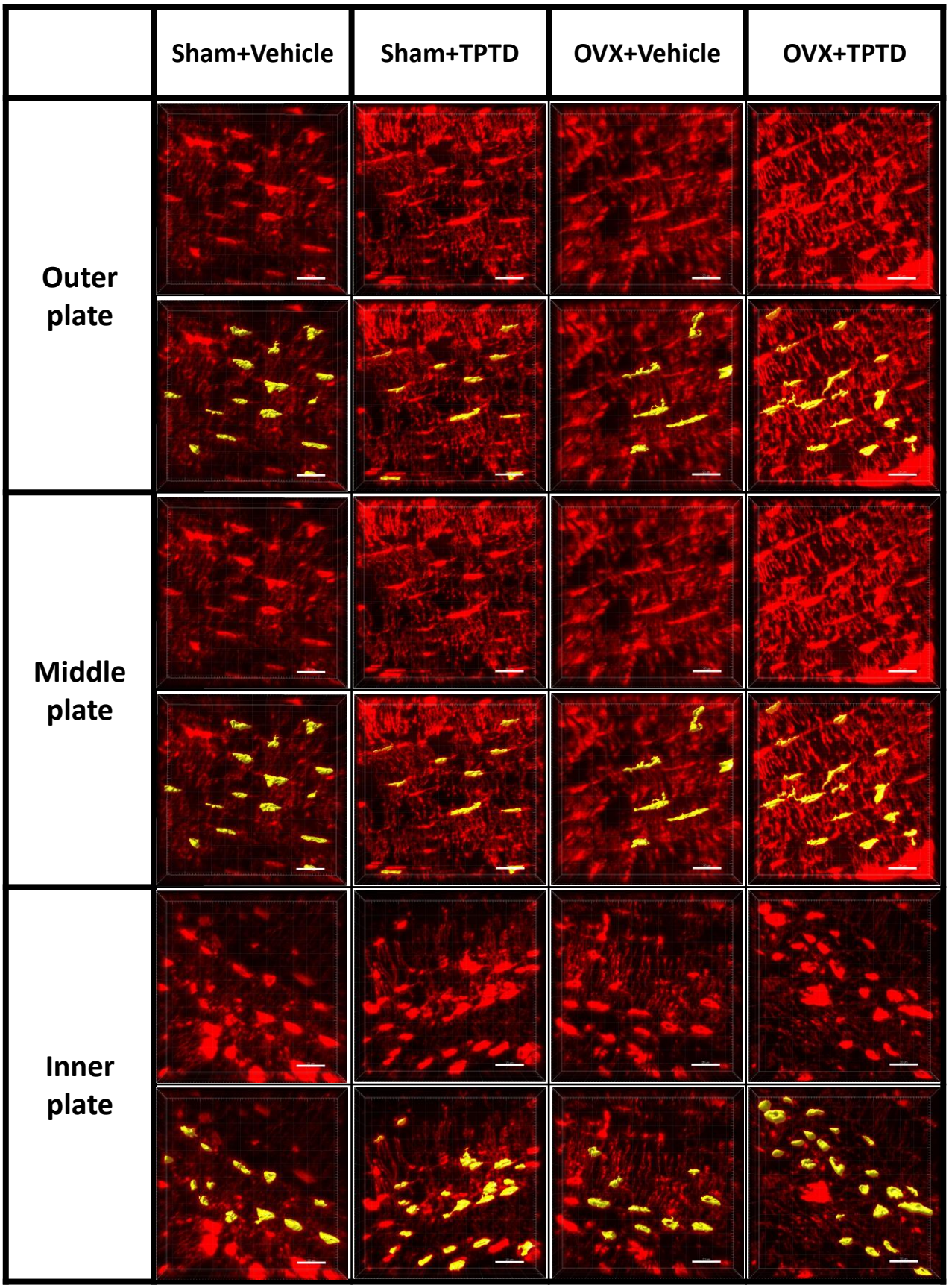

Figure 7

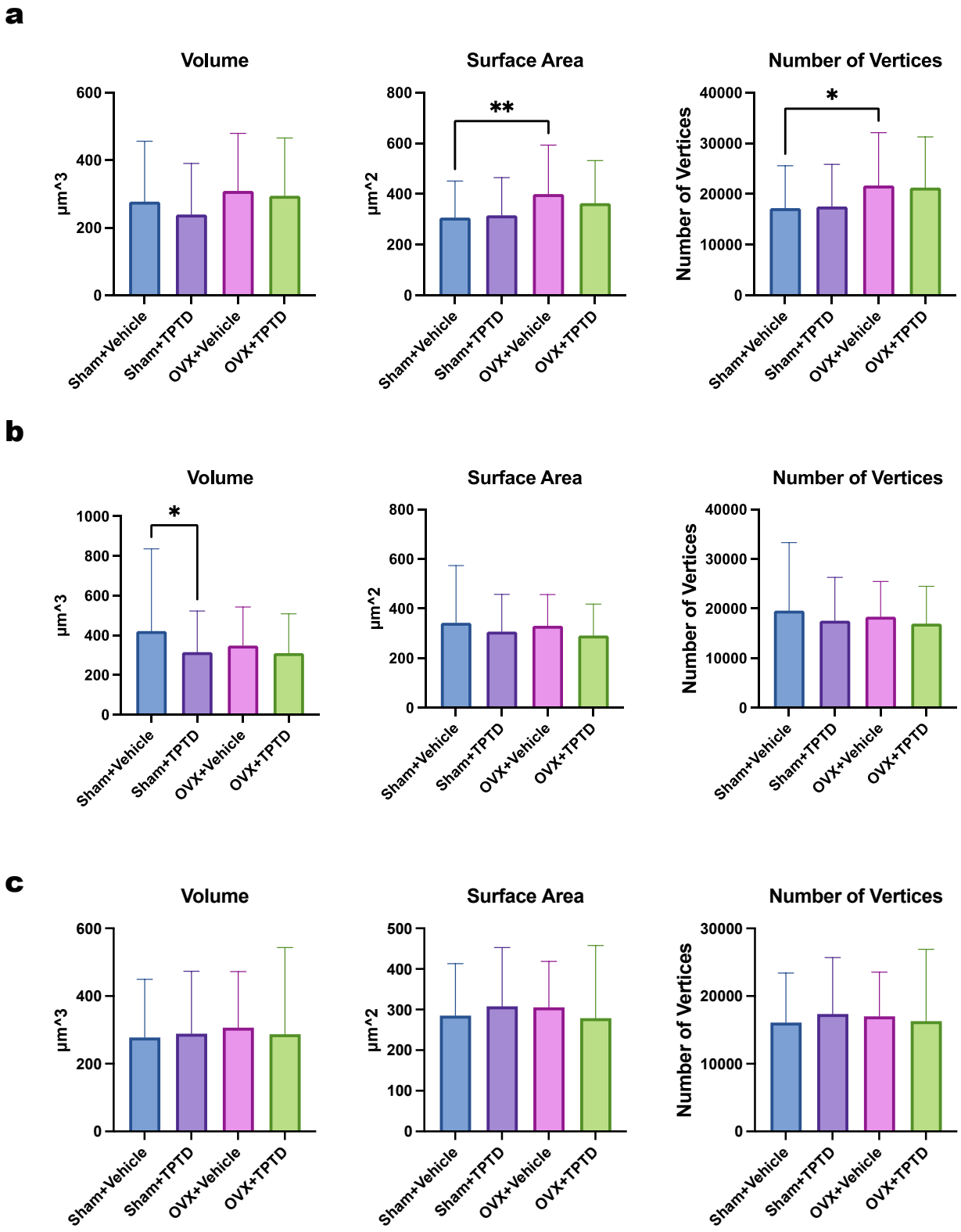

Table 1

Table 1.

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Table1. Dosing regimen settings of this study

Group	Part	Ope	Treatment	Dose μg・Kg^{-1} perweek	Dose μg・Kg^{-1}	Frequency	n
1	Mandibular bone	Sham	Vehicle	30	90	3/week	5
2	Mandibular bone	Sham	TPTD	30	90	3/week	5
3	Mandibular bone	OVX	Vehicle	30	90	3/week	5
4	Mandibular bone	OVX	TPTD	30	90	3/week	5
5	Parietal bone	Sham	Vehicle	30	90	3/week	5
6	Parietal bone	Sham	TPTD	30	90	3/week	5
7	Parietal bone	OVX	Vehicle	30	90	3/week	5
8	Parietal bone	OVX	TPTD	30	90	3/week	5