Effects of the schedule and dosage of parathyroid hormone on bone regeneration

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Contents

Abstract

In this study, different schedules and dosages of parathyroid hormone (PTH) administration were evaluated on bone regeneration in critical-size bone defects in rat calvaria. Fifty rats were divided into five groups and critical-size bone defects were prepared in the calvaria. Each of those groups was treated with 15 μg/kg PTH daily (PTH-15), 35 μg/kg PTH three times in 7 days (PTH-35), 105 μg/kg once in 7 days (PTH-105-o) or 105 μg/kg three times in 7 days (PTH-105-t), and control animals were given vehicle every day. Bone regeneration was evaluated radiographically by micro-computed tomography (micro-CT) or histologically. The amount of newly generated bone was increased significantly in critical-size bone defects in the PTH groups compared to the control. The amount of newly regenerated bone did not differ significantly among groups PTH-15, PTH-35, and PTH-105-o, while group PTH-105-t showed significantly more new bone formation. In conclusion, the greater the dose of PTH, the more bone was regenerated in critical-size rat calvarial bone defects. These results also suggested that the total doses of PTH administration are significant in the bone regeneration in a defined period, indicating that a lower frequency of PTH administration could be possible in a protocol design of bone regeneration therapy.

Introduction

Various clinical protocols have been introduced to regenerate bone tissue (1,2). However, there have been few effective ones in reconstructing severe alveolar bone loss in periodontal diseases.

Parathyroid hormone (PTH) is a major regulator of bone remodeling and calcium homeostasis. PTH has both anabolic and catabolic effects in bone tissue. Continuous PTH treatment causes bone resorption, while intermittent PTH treatment promotes bone formation (3). Injecting rats with PTH increases the differentiation of osteoprogenitor cells into osteoblasts (4). The administration of PTH resulted in the inhibition of osteoblast apoptosis, causing prolonged, enhanced bone formation (5). The anabolic features of intermittent PTH administration are used in patients with severe osteoporosis, and increase bone mass and reduce the fracture rate (6).

The possibility of using PTH in regenerative applications, such as fracture healing and treating osseous defects, in craniofacial regions has attracted interest recently (7). However, little information is available in the literature on the effects of the duration or dosage of PTH on bone regeneration in bone defects. Thus, the purpose of this study was to evaluate the effects of various schedules and dosages of PTH on the regeneration of critical-size bone defects in the rat calvaria.

Materials and Methods

Animals

Fifty 12-week-old male Fischer rats weighing 250-300 g each were used. The animals were housed in metal cages in an animal room (temperature 22°C, 55% relative humidity, 12/12-h light/dark cycle) and fed a standard laboratory diet and water. This study was approved by the Animal Experimentation Committee of Nihon University School of Dentistry, Japan (AP13D018).

PTH

Synthetic human PTH (1-34 PTH; Asahi Kasei Pharma, Tokyo, Japan) was dissolved in 0.1 M Tris-HCl, at pH 7.5, containing 2% bovine serum albumin.

Surgical procedure

The animals were premedicated with isoflurane inhalation anesthetic and were generally anesthetized with injection of a mixture of 0.15 mg/kg dexmedetomidine hydrochloride, 2.0 mg/kg midazolam, and 2.5 mg/kg butorphanol tartrate intraperitoneal. The forehead of the rat was also anesthetized with a local injection of 0.5 mL of 1:8 diluted lidocaine (Xylocaine; Astra Zeneca, Osaka, Japan). The dorsal cranium was shaved and prepared for surgery aseptically. A horseshoe-shaped skin incision was made over the head, the parietal area was exposed under aseptic conditions, and the periosteum was elevated to expose the bone. Using a trephine burr constantly cooled with sterile saline, critical-size (5.0-mm diameter) bone defects were created on the right side of the parietal bone to avoid the sagittal suture (Fig. 1). Subsequently, the calvarial disk was removed carefully to avoid tearing the dura. After thoroughly rinsing the area with physiological saline to wash out any bone fragments, the skin was closed using 4-0 silk (Ethicon, Somerville, NJ, USA).

PTH administration

The rats were divided into five groups. PTH was administered by subcutaneous injection at 15 μg/kg PTH daily (PTH-15), 35 μg/kg PTH three times in 7 days (PTH-35), 105 μg/kg PTH once in 7 days (PTH-105-o) or 105 μg/kg PTH three times in 7 days (PTH-105-t). The

control animals were given only vehicle (sterile saline) each day during the experiment (Fig. 2). The first day of PTH administration and surgery was designated as day 0.

Measurement of serum calcium (Ca^{2+}) concentration and alkaline phosphatase (ALP) activity

Under ether anesthesia, ~80 μL of blood were obtained from the subclavian vein every day for 1 week after surgery. The blood was centrifuged and the serum was stored at -80°C. Total serum Ca^{2+} and ALP were determined using a detection kit with automated clinical chemistry analyzers (7180 Hitachi automated analyzer, Ibaraki, Japan).

Micro-computerized tomography (micro-CT) analysis

A micro-CT (R_mCT; Rigaku, Tokyo, Japan) was utilized to take images on days 0, 7, 14, 21, and 28 to examine the bone regeneration *in vivo*. The images were reconstructed on a personal computer using the *i*-View software (Rigaku, *i*-view Image Center, Tokyo, Japan). On day 0, a screen cylinder was drawn to define the initial bone defect. The bone volume (BV) was measured as the bone tissue within the cylinder on voxel images using BV measurement software (Kitasenjyu Radist Dental Clinic, *i*-View Image Center, Tokyo, Japan), which calculates the gray values and corresponding number of voxels in the regions of interest.

Histological and histometric analyses

The animals were sacrificed at 28 days after the surgery. The skin was dissected, and the defect sites were removed, along with the surrounding bone and soft tissues. Then, the specimens were fixed in 10% neutral buffered formalin, after which they were decalcified with a 10% EDTA for 1 week and embedded in paraffin. Coronal sections (5 μm in thickness) were prepared through the center of circular defect, stained with hematoxylin and eosin, and examined under a light microscope equipped with a morphometric system (Model BHF-142; Olympus, Tokyo, Japan).

Closure of bone defect was determined by measuring the distance between the defect margins, and was expressed as a percentage of the width of the original bone defect. The osteoblast-like cells were counted in four areas at the edge of the original defects and expressed as averages (Fig. 3).

Statistical analysis

Means and standard deviations were calculated for the reossification ratio on days 7, 14, 21, and 28. The mean BV, defect closure, and the numbers of osteoblasts-like cells were compared among groups using the Kruskal-Wallis rank test. The level of statistical significance was set at 0.05. The statistical analyses were performed using the SPSS software (Ver. 16.0J for Windows; SPSS, Inc., Chicago, IL, USA).

Results

Concentration of serum Ca^{2+} and activity of serum ALP

There were no significant differences in the serum Ca^{2+} levels among the PTH and control groups. The PTH groups tended to have higher serum ALP levels than the control group, but these differences were not significant (Figs. 4, 5).

Micro-CT analysis

In the micro-CT images, the radiopacity increased gradually in a time-dependent manner in all groups. The outline of the bone defect in the PTH groups was blurred and an irregular high-density region was observed in the defect area. In the PTH groups, there was significantly greater radiopacity at 21 and 28 days, compared with the control group (Fig. 6).

The BV was significantly higher in the PTH groups compared with the controls at days 21 and 28. The BV did not differ significantly among groups PTH-15, PTH-35, and PTH-105-o, while group PTH-105-t had significantly more new bone at day 28 (Fig. 7).

Histological and histometric observations

The PTH groups showed significant formation of new bone throughout the defects, compared with the control group. The PTH-105-t group showed considerably thick lamellar bone. The control groups showed limited bone formation with fibrous connective tissue (Fig. 8).

The closure of bone defects was enhanced significantly in the PTH groups compared to the control group. The PTH-105-t group exhibited significantly greater defect closure than the other groups (Table 1).

More osteoblast-like cells were observed in the edge of the original bone defect in the PTH groups. The greatest number of osteoblast-like cells was present in the PTH-105-t group (Table 2).

Discussion

The intermittent administration of PTH has an anabolic effect in promoting bone defect healing. The results of this study indicated that the amount of newly generated bone increased significantly in critical-size bone defects in the PTH groups, when compared with the control group. Moreover, bone defect healing was faster in the PTH groups than in the control group.

Various dosages of PTH have been used to promote new bone regeneration. PTH administered at 60 μg/kg increased bone deposition in rat parietal critical-size bone defects (8). Moreover, 25 μg/kg PTH accelerated new bone formation in rabbit mandible critical-size bone defects (9). Yun et al. (10) and Stancoven et al. (11) showed that 15 μg/kg PTH administration increased bone formation in rat calvarial bone defects. Thus, the lowest reported dose of 15 μg/kg PTH was used in this study. However, the optimal dose and schedule for enhancing bone defect healing remain unclear. In the present study, bone regeneration was enhanced significantly in group PTH-105-t compared with other three groups, PTH-15, -35, and PTH-105-o, suggesting the greater the dose of PTH, the more bone was regenerated in rat critical-size bone defects. In contrast, the PTH-15, -35, and PTH-105-o groups did not show significant difference within these groups in bone regeneration. Because cumulative dosage of PTH administered to these three groups was the same amount, it is possible to explain that the total amount of PTH in the experimental period is significant in the regenerative bone healing.

A daily intermittent subcutaneous administration of PTH enhanced bone regeneration (12, 13). However, daily PTH injection is never welcomed to patients. Thus, the optimal effective schedule for PTH administration to enhance bone regeneration needs to be considered. The amount of bone regenerated in PTH-15, -35, and PTH-105-o groups was approximately the same level, but the total frequency of PTH administration was different in these groups. Namely, fewer frequency of PTH administration with a larger dosage, and more frequency with a small dosage could be both equally effective for an expected amount of bone regeneration, as long as the total cumulative amount of PTH is identical. This idea would useful for designing a better schedule of PTH administration.

PTH is a peptide hormone that regulates calcium and phosphate metabolism. A continuous infusion of PTH induced catabolic effects in bones, resulting in hypercalcemia (14). In the present study, blood Ca^{2+} levels did not increased in the experimental groups

compared to the control group. This finding indicates that the dose and frequency of PTH administration in this study exerted anabolic effects. On the other hand, serum ALP levels showed a tendency of increase, suggesting active bone turnover, because serum ALP levels and bone turnover are known to correlate to each other (15).

The histological observations showed that PTH stimulated new bone formation with increasing number of osteoblasts around the existing bone. A previous study suggested that this increase in the number of osteoblasts was not dependent on the proliferation of osteoprogenitor cells or osteoblastogenesis; rather, PTH affected existing cells (16). These lining cells might undergo transition to active form and resume matrix synthesis in response to PTH treatment.

Conclusion

The greater the dose of PTH, the more bone was regenerated in critical-size rat calvarial bone defects. These results also suggested that the total doses of PTH administration is significant in the bone regeneration in a defined period, indicating that a fewer frequency of PTH administration could be possible in a protocol design of bone regeneration therapy.

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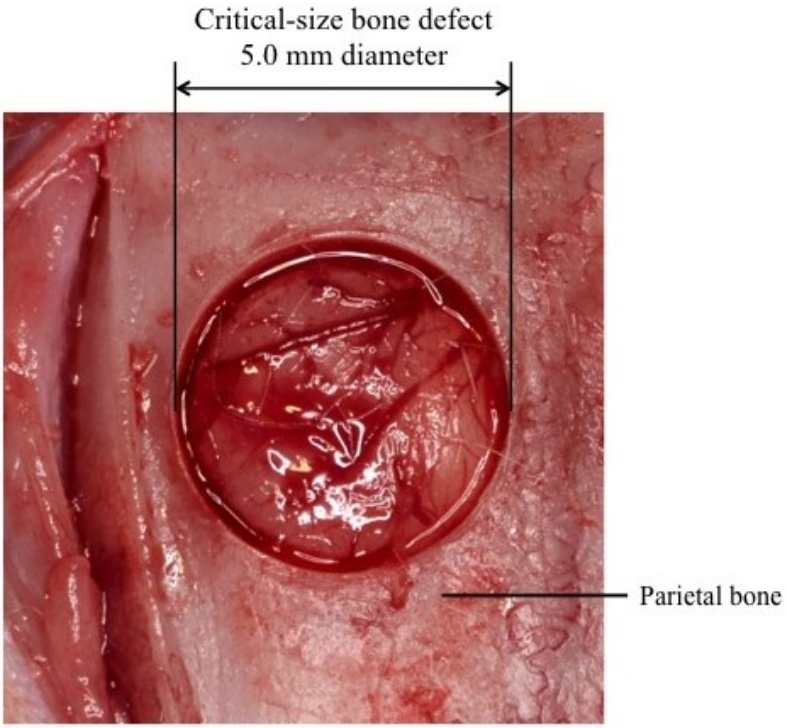

Fig. 1 Critical-size calvarial bone defects (5-mm diameter) were created in the dorsal part of the right parietal bone.

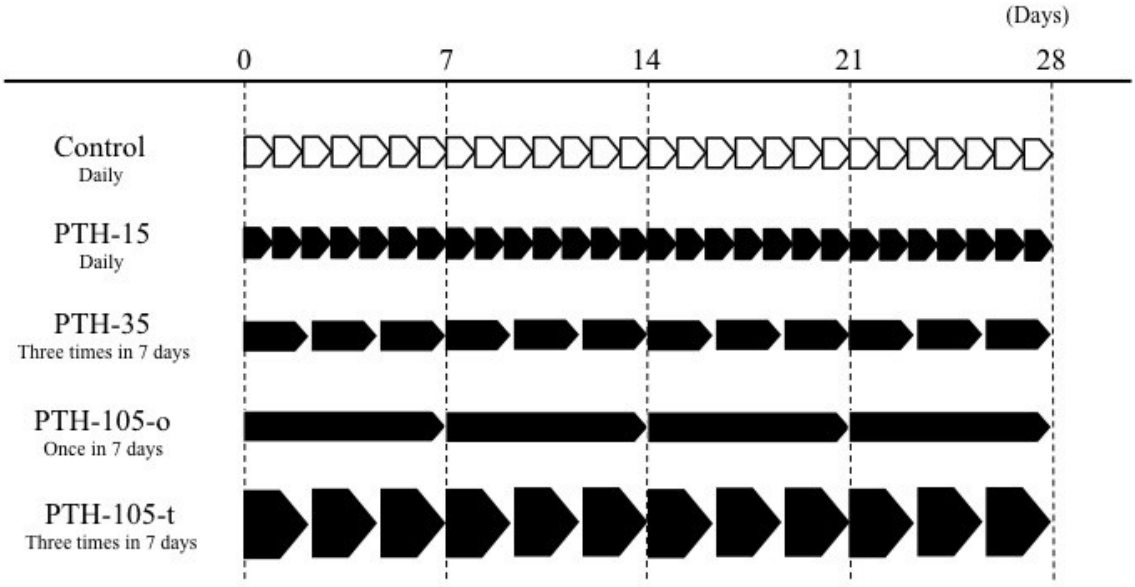

Fig. 2 Schedule of PTH administration.

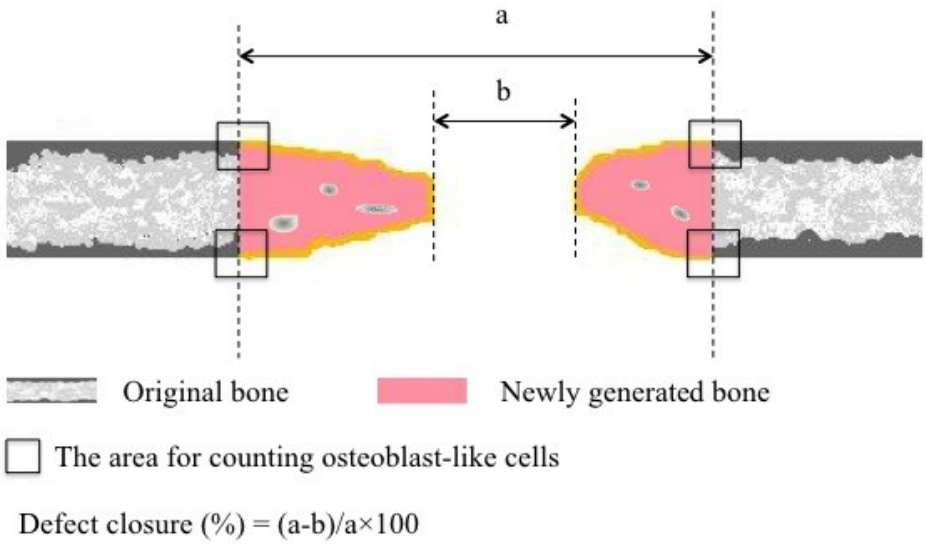

Fig. 3 Schematic drawing of regenerative bone in calvarial defects and histometric parameters.

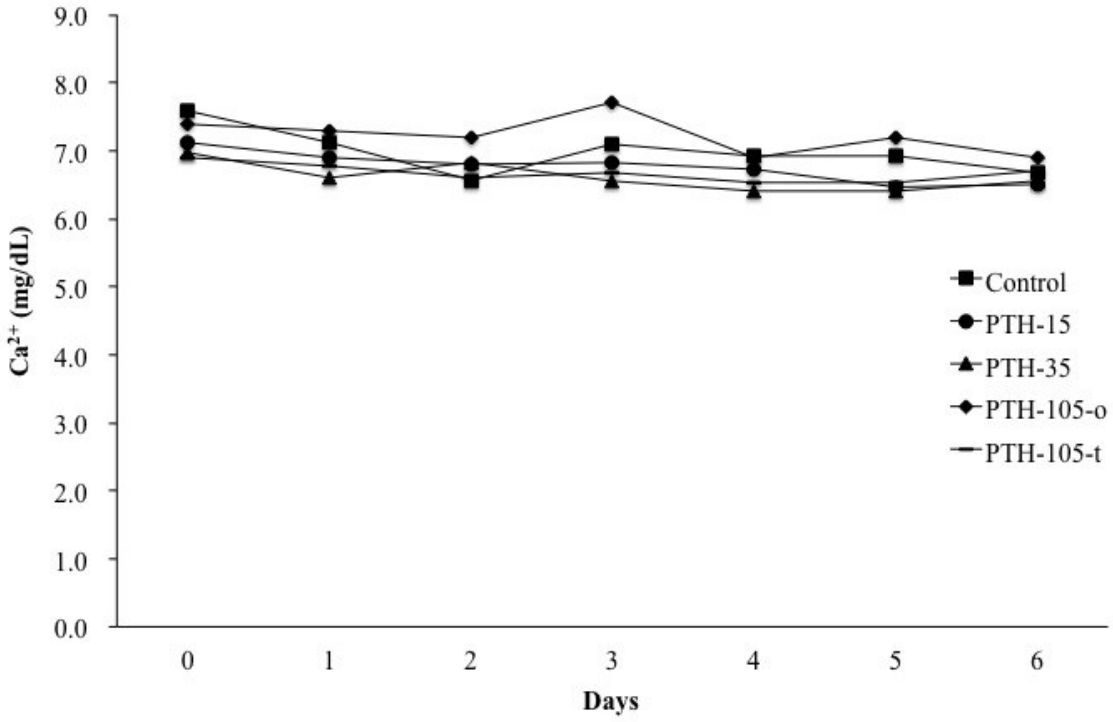

Fig. 4 Concentration of serum Ca^{2+}.

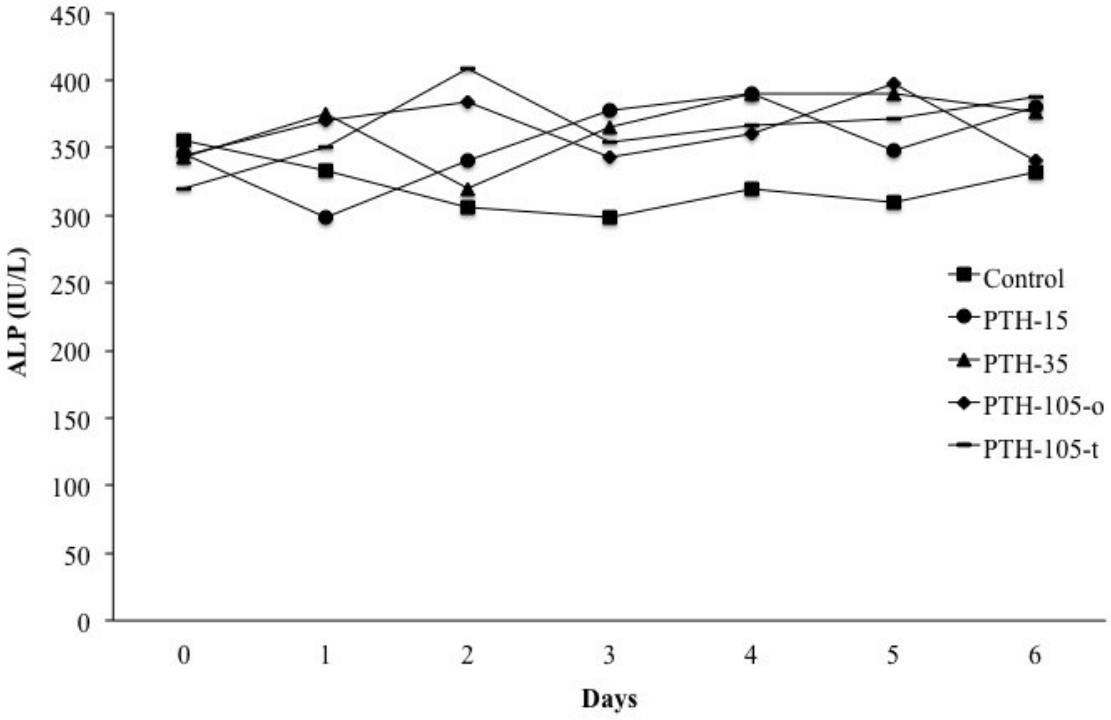

Fig. 5 Activity of serum ALP.

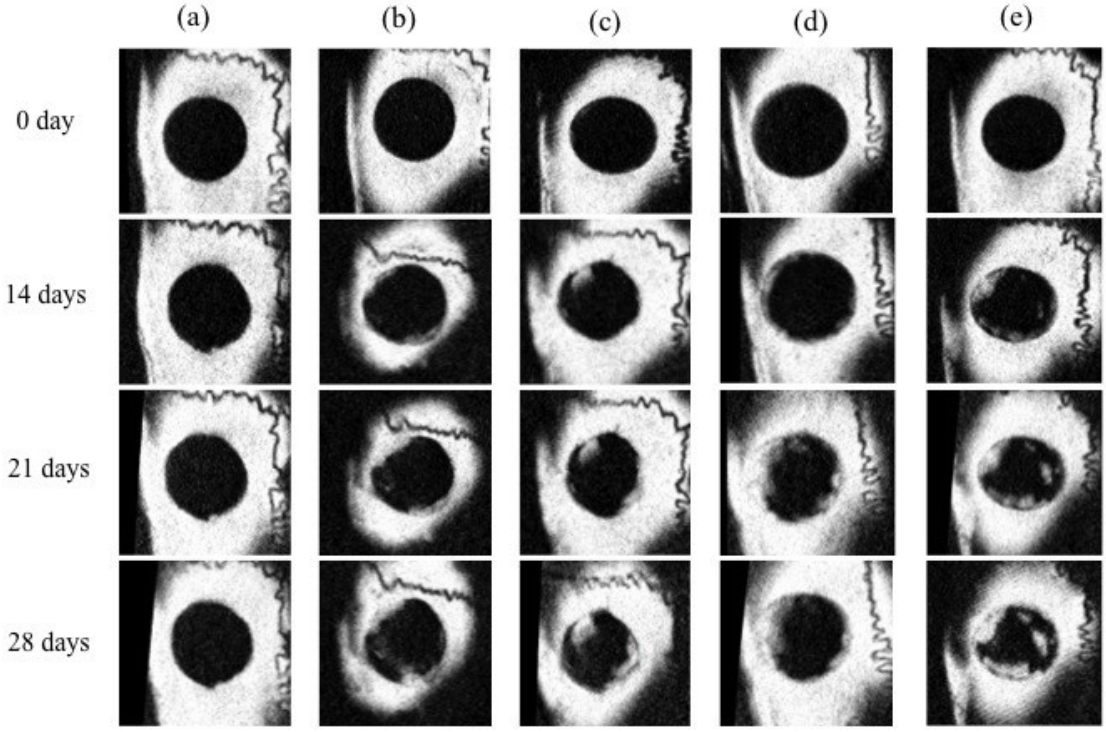

Fig. 6 Micro-CT images of critical-size bone defects at days 7-28:
(a) Control, (b) PTH-15, (c) PTH-35, (d) PTH-105-o , and (e) PTH-105-t.

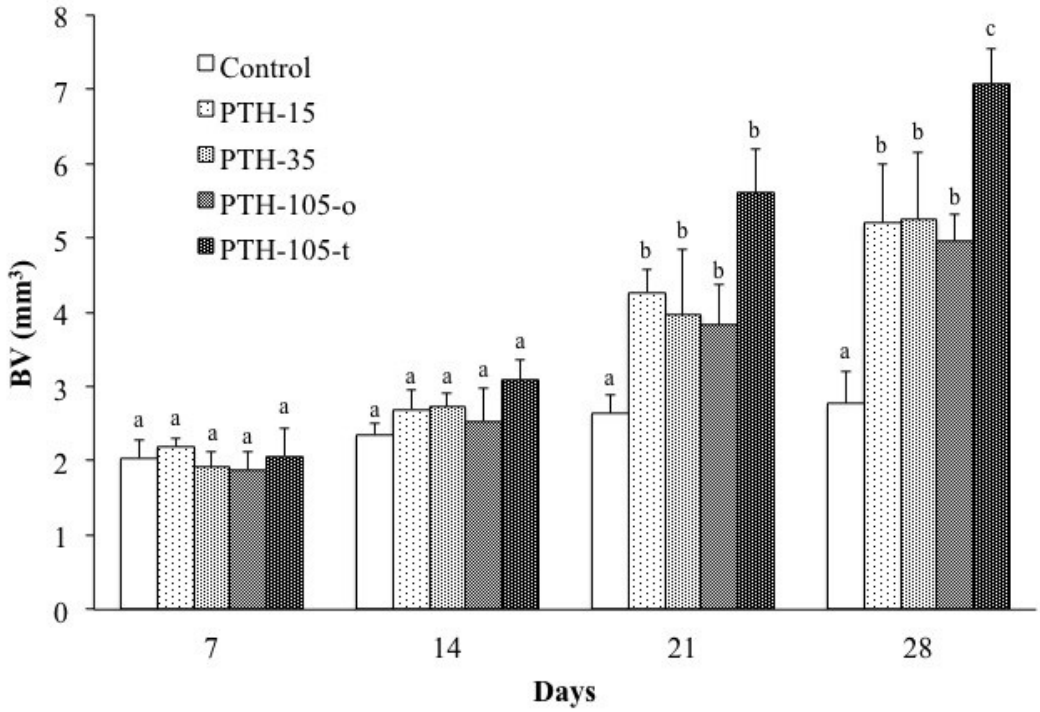

Fig. 7 Regenerative bone volume in critical-size bone defects. The same lower case letters in the same days indicate no statistical difference, Kruskal-Wallis rank test ($P > 0.05$).

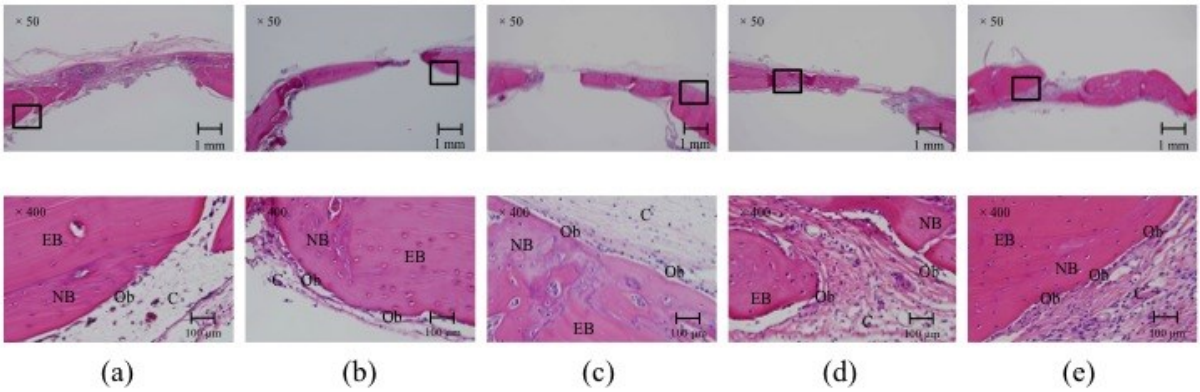

Fig. 8 Histological sections at 28 days in critical-size bone defects, showing boxed area is high magnification histology, (a) Control, (b) PTH-15, (c) PTH-35, (d) PTH-105-o, and (e) PTH-105-t.

Table 1 Percentage of bone closure rate

Group	Closure of bone defects (%)
Control	10.3 ± 7.3 [a]
PTH-15	40.3 ± 15.2 [b]
PTH-35	48.9 ± 12.4 [b]
PTH-105-o	55.3 ± 4.2 [b]
PTH-105-t	66.2 ± 9.3 [c]

The values with the same superscript letters indicate no statistical difference, group means ± SD, Kruskal-Wallis rank test ($P > 0.05$).

Table 2 Number of osteoblast-like cells observed around the edge of the existing bone

Group	Number of cells
Control	70 ± 8 [a]
PTH-15	121 ± 30 [b]
PTH-35	146 ± 22 [b]
PTH-105-o	129 ± 29 [b]
PTH-105-t	244 ± 35 [c]

The values with the same superscript letters indicate no statistical difference, group means ± SD, Kruskal-Wallis rank test ($P > 0.05$).