Increasing the amount of corticotomy does not affect orthodontic tooth movement or root resorption, but accelerates alveolar bone resorption in rats

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A short running title: Corticotomy accelerates alveolar resorption

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Summary

Objectives: The purpose of this study was to evaluate the relationships among the volume of bone cut during corticotomy, amount of tooth movement, volume of root resorption, and volume of the resultant alveolar bone resorption after tooth movement.

Methods: Ten-week-old female Wistar rats were distributed into the corticotomy groups and a control group that underwent sham corticotomy. Two experiments employing two different orthodontic forces (10 or 25 g) and experimental periods (14 or 21 days) were performed. The volumes of the bone cut by corticotomy were 0.1 mm^3, 1.0 mm^3, and 1.7 mm^3 in the 25-g groups, and 1.0 mm^3 and 1.7 mm^3 in the 10-g groups. Nickel-titanium closed-coil springs were set on the maxillary left first molars to induce mesial movement. After orthodontic tooth movement, the amount of tooth movement, volume of root resorption, and volume of alveolar bone resorption were measured.

Results: Despite differences in the volume of bone cut among the different corticotomy groups, there were not significant differences in the amount of tooth movement and volume of root resorption between the control group and any of the corticotomy groups. However, higher volume of bone cut during corticotomy was significantly related to the decreased alveolar bone volume — in particular, to the reduced height of the alveolar bone crest after tooth movement.

Conclusions: The volume of the alveolar bone cut during corticotomy does not affect tooth movement or root resorption in 10-week-old female Wistar rats; however, it may increase alveolar bone loss after tooth movement.

Introduction

According to clinical reports, the average duration of modern orthodontic treatment is 21–35 months (1, 2). Although orthodontic treatment generally provides satisfactory benefits to patients, prolonged treatment periods might be detrimental to the clinical outcome. The period of orthodontic treatment depends on many factors, including the severity of malocclusion, variations among individuals, orthodontic techniques, and patient cooperation. Prolonged orthodontic treatment periods can increase the risk of complications such as dental decalcification, gingivitis with alveolar bone loss, pulpal response, and root resorption (3-5), which might be troublesome for adult patients, whose numbers have increased dramatically in the last 20 years (6). Adult patients are generally at a higher risk of periodontal disease and alveolar bone loss than are younger patients. In addition, the metabolic activity of adults decreases with age, which might prolong the treatment period. These risks must be minimized in order to provide safe and reliable orthodontic treatment with results that are satisfactory to patients. Additionally, adult patients sometimes have private and social matters that restrict their treatment, and therefore, shortening the treatment period could help meet their specific needs.

Various mechanisms and appliances have been devised till date to shorten the treatment period. Many investigators have studied methods such as low-intensity pulsed ultrasound, magnetic force, electromagnetism, and low-level laser (7, 8). In addition, a method combining orthodontics with surgical treatment to accelerate tooth movement has been reported (9). To our knowledge, the first corticotomy-facilitated orthodontic treatment — an osteotomy to move teeth as a block of bone — was reported by Köle in 1959 (10).

A current method that is believed to accelerate tooth movement involves the application of surgical stress localized to the alveolar cortical bone (corticotomy). Bone remodeling has been reported to be accelerated after a fracture, osteotomy, or bone graft; this process was referred to as the “regional acceleratory phenomenon” (RAP) by Frost, who described the self-healing process after fracture (11, 12). Wilcko *et al.* (13, 14) published clinical reports indicating that, similar to the RAP, bone remodeling was accelerated by corticotomy, with increased tooth movement and decreased root resorption observed around the surgical site. However, existing literature lacks sufficient evidence to support the reliability of this novel and interesting method. In addition, most studies on corticotomy have focused on the rate of acceleration of tooth movement or the decrease of root resorption. The potential disadvantages of corticotomy need to be considered in addition to its benefits. Corticotomy might cause substantial inflammation, which includes pain, swelling, and subsequent alveolar bone loss (15). It is well known that the regeneration of horizontal bone loss through periodontal treatment is difficult (16, 17). Moreover, the volume of the cortical bone cut during corticotomy is not negligible. Therefore, it is necessary to determine the relationship between the amount of corticotomy and the final volume of alveolar bone loss.

The aim of this study was to evaluate and verify the relationships among the volume of alveolar bone cut by corticotomy, tooth movement, root resorption, and the resultant bone loss after tooth movement.

Materials and methods

This study was approved by the ****** Institutional Animal Care and Use Committee (approval number 1012090890).

Ten-week-old female Wistar rats (SLC, Shizuoka, Japan; body weight, 170–180 g) were used in this study. The rats were housed in plastic cages in a colony room and fed a standard pellet diet and water ad libitum. The rats were allowed a week for acclimatization before the experiments.

Experimental design

We performed two experiments with different orthodontic forces, volumes of bone cut, and experimental periods. In Experiment 1, a 25-g orthodontic force was applied for 14 days; in Experiment 2, a 10-g orthodontic force was applied for 21 days (Figure 1). In the previous study, an orthodontic force of 25 g applied to the upper first molar of rats — a rather heavy force, corresponding to approximately 500 g of force applied to a human upper first molar — clearly induced severe root resorption and tooth movement with high reproducibility, in 14 days (18). Therefore, we supposed that the experimental model subjected to a 25-g force might be suitable for evaluation of heavy root resorption. On the other hand, in the previous study, an orthodontic force of 10 g was found to induce much less root resorption, but with sufficient tooth movement, in 21 days, compared to the 25-g force (18). Moreover, a cycle of orthodontic tooth movement starts with the hyalinization of the periodontal ligament and is completed with the regeneration of the periodontal tissue in 21 days (19). Therefore, the 10-g force application for 21 days is considered to be suitable for evaluation of physiological tooth movement. We performed different types of corticotomy to study the effect of the volume of bone cut on tooth movement and root resorption (Figure 2). In Experiment 1, 40 rats were randomly divided into 4 groups; while 3 were subjected to different types of corticotomy (1 pit, 4 lines, and circumference of the tooth), the remaining group received no surgical operation (control). In Experiment 2, 30 rats were randomly divided into 3 groups; while 2 received corticotomy (mesial and circumference of the tooth), the remaining group received no surgical operation (control; Figure 2). At the beginning of the experiment, each group was composed of 10 rats. However, 14 rats were finally excluded from a total of 70 rats because of death or unfastening of the orthodontic appliance during the experiment period.

Corticotomy and appliance setting

Corticotomy, appliance setting, and micro-computed tomography (micro-CT) were performed under general anesthesia induced by intramuscular injection of 87 mg/kg ketamine hydrochloride (Ketalar 50, Sankyo, Tokyo, Japan) in combination with 13 mg/kg xylazine hydrochloride (Celactal 2%, Bayer-Japan, Tokyo, Japan). Corticotomy was performed as follows. Full-thickness flaps were elevated at the mesial site of the maxillary left first molar. The cortical bone was cut at a depth of 0.3 mm using a number 1/4 round bur and a dental handpiece at a low speed (100 rpm) under saline irrigation. The extent of corticotomy in the control (no operation) and the extent of the severest corticotomy (circumference of the tooth) were constant between experiments 1 and 2. The flaps were repositioned after surgery and secured with a nylon suture (size 4-0).

The design and volume of the cut cortical bone were varied in Experiments 1 and 2 (Figure 2). After corticotomy, a nickel-titanium closed-coil spring (Sentalloy, Tomy, Fukushima, Japan) was set to move the maxillary left first molar mesially. To fix the spring, a 0.008-inch stainless steel ligature was ligated around the cervical part of the maxillary left first molar on one side and through a transverse hole drilled in the right and left incisors. The occlusal surfaces of the molars, except for the maxillary left first molar, were raised using a self-curing resin (Super-Bond, Sun Medical, Shiga, Japan) to eliminate any occlusal force on the maxillary first molar that was moved by orthodontic force (Figure 3). Corticotomy and setting of the coil spring were performed on the same day. Because incisors in rodents grow continuously, the coil springs were reset every 7 days.

Micro-CT (RmCT, Rigaku, Tokyo, Japan) images were acquired on days 0, 3, 7, 14, and 21 (Figure 1). The image acquisition conditions were as follows: X-ray source voltage, 90 V; current, 100 mA; scanning time, 2 min; and resolution, 20 µm/pixel. The machine used for image acquisition was a cone-beam CT scanner, which can repeatedly acquire images of live animals under anesthesia. The rats were euthanized by overdosing with carbon dioxide on days 14 and 21 in Experiments 1 and 2, respectively.

Measurement of tooth movement

The amount of orthodontic tooth movement was measured on the micro-CT images acquired on days 3, 7, and 14 in Experiment 1 and on those acquired on days 3, 7, 14, and 21 in Experiment 2, using a three-dimensional (3D) image reconstruction software (i-view, J. Morita, Kyoto, Japan). The following three parameters were defined to measure tooth movement (Figure 4).

1. Distance between contact points — the distance between the contact points of the maxillary left first and second molars identified on the image on day 0.
2. Shortest distance — the shortest distance between the distal surface of the maxillary left first molar and the mesial surface of the maxillary left second molar.
3. Angle of tooth inclination — the change in tooth inclination defined by the mesial

root of the maxillary left first molar and the occlusal plane.

The amount of eruption of the incisors and the change in the angle of the coil spring caused by eruption were also measured.

Measurement of the volume of root resorption

The volume of root resorption was measured as described previously (18). In short, five roots of the first molars were divided into 3 parts (mesial, middle, and distal) using diamond disks, and the mesial, distobuccal, and distopalatal roots were further evaluated in this study. The root resorption craters on the apical third region were not evaluated since the root apexes surrounded by cellular cementum continuously change their shape and dimension in rats because of changes by the secondary cementum, as described previously (20). The resorption craters on the mesial surface of the mesial, distobuccal, and distopalatal roots were evaluated using a scanning electron microscope (SEM; TM-1000, Hitachi, Tokyo, Japan) and a 3D-laser scanning microscope (LSM; VK-8500, Keyence, Kyoto, Japan). Because the resorption craters on the distal surface of the roots were scarcely detectable, they were excluded from this study. The surface area of the resorption craters was measured on the images captured from the SEM using a commercial software (Mimics, Materialise, Leuven, Belgium). The mean depth of the root resorption craters was calculated using a laser microscope software program (ImageJ, National Institutes of Health, Bethesda, Maryland, USA). The volume of root resorption was calculated by multiplying the resorption area by the depth.

Measurement of the volume of bone cut and the alveolar bone resorption

A 3D image analysis software (TRI-BONE, Ratoc System Engineering, Tokyo, Japan) was used to measure the volume of the cut bone, height of the alveolar bone crest, and bone resorption. The micro-CT images acquired before and after corticotomy on day 0 and the one acquired on the last day were superimposed over the palatal bone, maxillary left second and third molars, and zygoma. The volume of the bone cut by corticotomy was calculated by measuring the bone volume on the 3D images before and after corticotomy. The height of the alveolar bone crest at the mesial bone ridge of the tooth was measured. Bone resorption was calculated by subtracting the bone volume on the last day from the initial bone volume after corticotomy (Figure 5).

The same investigator (****) performed all of the measurements, which were repeated three times. The mean value of the measurements was used as the final measurement.

Statistical analysis

Statistical analysis was performed using the EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (21). The orthodontic tooth movement, root resorption, volume of cut bone, and alveolar bone resorption were compared among the groups using the Kruskal-Wallis tests. The Mann-Whitney tests and Bonferroni correction were used as post hoc tests for inter-group comparison ($p < 0.05$).

Results

Amount of eruption of the incisors

The average amount of eruption of the incisors was 0.51 mm in 7 days and the average change of the angle of the coil spring was 4.77° for 7 days. There were no significant differences among the groups in terms of either of these parameters (Figure 6).

Amount of tooth movement

On day 3, all of the measurements (distance between the contact points, shortest distance, and angle of tooth inclination) were found to have increased in all of the groups, and they were further increased in all of the groups from day 3 through the respective last days of each of the experiments. The median values of the distance between the contacts was 0.24 mm in the control group, 0.27 mm in the 1-pit group, 0.17 mm in the 4-lines group, and 0.19 mm in the circumference group on day 14 of Experiment 1 (Figure 7A and Table 1). In Experiment 2, the median values of the distance between the contacts was 0.43 mm in the control group, 0.38 mm in the mesial group, and 0.34 mm in the circumference group on day 21 (Figure 7D and Table 1). The distances between the contact points in the corticotomy groups were not significantly different from that in the corresponding control group in either Experiments 1 or 2.

The values of the shortest distance between the first and second molars were slightly lower than the distance between the contact points throughout the experimental period; however, there were no significant differences among the groups in either Experiments 1 or 2. (Figures 7B and E and Table 1).

The mean angle of tooth inclination was 1.3° on day 3 and 11° on day 21 (Figures 7C and F and Table 1). The angle of tooth inclination gradually increased in all of the groups throughout the experimental period, but there were no statistically significant differences between any of the groups.

Volume of root resorption

In the SEM images, the apical thirds of the roots were covered with thick cementum with a rough and irregular surface that occasionally contained resorption craters. Isolated lacunae as well as wide, shallow, and deep resorption craters were found in all of the experimental groups. The small isolated lacunae were mainly scattered on the mesial roots, on the cervical half of their mesial surfaces. The wide, shallow, and deep resorption craters were scattered mainly on the cervical and middle portions of the distal roots.

In Experiment 1, there were no differences in the root resorption areas of the three separated roots (mesial, M; distobuccal, DB; and distopalatal, DP) among any of the groups. Similarly, there were no differences in the total root resorption area among any of the groups. Furthermore, we did not find any differences in the root resorption depth or volume among the groups (Figures 8A, B, and C). Similar results were obtained in Experiment 2; there were statistically significant differences among the groups neither in terms of the root resorption areas, depths, or volumes of the three roots nor in the total root resorption volume (Figures 8D, E, and F).

The volume of bone cut and alveolar bone resorption

The volume of the cortical bone cut was distinctly different in every group (Figures 9A and D).

The height of the alveolar bone crest after tooth movement decreased in proportion to the volume of the bone cut by corticotomy. The largest decrease of the alveolar bone crest was observed in the circumference groups of both experiments ($p < 0.05$). In Experiment 1, the median value of the decrease of the alveolar bone crest in the circumference group (0.55 mm) was significantly higher than that in the control group (0.24 mm; Figure 9B and Table 2). Similar results were obtained in Experiment 2 (Figure

9E and Table 2). The value of the decrease of the alveolar bone crest in the control groups was almost the same in both Experiments 1 and 2.

Similarly, despite the difference in the period of force application, the volume of alveolar bone resorption around the tooth increased depending on the volume of the bone cut in both Experiments 1 and 2 (Figures 9C and F and Table 2). These results were in contrast to those of root resorption.

Discussion

In this study, the shape and volume of the bone cut by corticotomy were quantified using micro-CT images, and the damaged cortical bone was clearly quantified. Studies have reported that an inflammatory reaction is induced by corticotomy, a surgical procedure performed on the cortical bone, following the activation of bone remodeling by the RAP (13, 14, 22, 23). Such reactions in the periodontal tissue did not occur in the present study. Similar to our results, Lee *et al.* (24) found no evidence of increase of tooth movement following corticotomy in rats. Furthermore, Murphy *et al.* (25) also reported no differences in tooth movement in the presence or absence of corticision using orthodontic forces of 10 g or 100 g in rats; they also reported no differences in the number of osteoclasts and osteoblasts. These minority papers strongly support the results in this study. At this point, Fanelli et al. reported that papers are less likely to be published and to be cited if they report negative results, therefore, if publication pressures increase scientific bias, the frequency of positive results in the literature should be higher in the more competitive academic environments. (26)

Tooth movement

A small amount of orthodontic tooth movement was observed on days 3 and 7 in all of the experimental groups in the present study. The initial orthodontic tooth movement might have primarily been caused by the compression of the periodontal ligament soon after the application of orthodontic force. On the other hand, some amount of tooth movement also was observed on days 14 and 21. Tooth movement after 10 days was the result of bone remodeling since bone resorption was obviously observed in the micro-CT images. On days 14 and 21, there were no differences in tooth movement between the corticotomy and control groups. The theory behind the acceleration of tooth movement by corticotomy is believed to be that surgical stress induces a response in the alveolar bone that causes demineralization around the dental roots. Orthodontic tooth movement is site-specific bone remodeling involving bone resorption and bone formation. Tooth movement begins with periodontal stress and is followed by the production of inflammatory mediators around the periodontal ligament, appearance of osteoclasts on the compressed side with subsequent bone resorption, and appearance of osteoblasts on the tension side with subsequent bone formation (27). Thus far, many studies have reported on the acceleration of tooth movement by corticotomy; however, Patterson et al. opined that the quality of the body of evidence was not high owing to the presence of multiple methodology issues, high risks of bias, and heterogeneity in the reviewed literatures (28). Though it is not clear why many contradictory results have been reported, as Fanelli mentioned (26), plural and complicated factors may have been involved.

Root resorption

We found no differences in any of the root resorption measurements among any of the corticotomy or control groups in both experiments (1 and 2). The root resorption cavity of the mesial root was mild, and that of the distal root was severe. The location, contour, and surface of the root resorption cavities did not differ among the groups. Brudvik *et al.* (29) reported that the administration of prostaglandin, a well-known chemical mediator of inflammation, increased root resorption, although the difference was not statistically significant. Corticotomy might induce a considerable amount of inflammatory mediators around the periodontal tissue; however, this inflammatory reaction on the surface of the cortical bone might not cause an obvious increase in root resorption or tooth movement.

Alveolar bone resorption

Although a few studies have examined the bone density after corticotomy, none has evaluated the relationship between the volume of the bone cut and the subsequent loss of alveolar bone. In the present study, the precise amount of alveolar bone change was measured, and the bone volume was found to have decreased obviously around the corticotomy site following surgery. Surgical stresses such as bone cuts induce inflammation, causing the activated cells at the inflammatory site to produce inflammatory cytokines. The inflammatory cytokines are known to enhance osteoclast differentiation and induction and induce severe bone resorption (30). In the present study, the alveolar bone volume after corticotomy was found to have decreased in proportion to the volume of bone cut, although the tooth movement showed no changes. This decrease of the alveolar bone volume would have resulted from the inflammatory reaction at the invasive surgical site, as suggested by previous studies.

Horizontal bone loss is clinically regarded as being rather unrecoverable in periodontics (16, 17). Corticotomy surrounding the tooth poses the risk of decrease in the height of the alveolar ridge, which might not return to its original position after long-term orthodontic treatment. Bone loss should be avoided following orthodontic treatment should be avoided when corticotomy is performed. Bone grafting with corticotomy for bone augmentation, as advocated by Wilco *et al.* (13, 14) be an option. Further study is necessary to elucidate the suitability of corticotomy for orthodontic treatment.

Conclusions

The volume of bone cut during corticotomy does not affect tooth movement or root resorption in 10-week-old female Wistar rats; it may, however, jeopardize the alveolar bone height after tooth movement.

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FIGURE LEGENDS

Figure 1. Experimental protocol. Corticotomy was performed and the orthodontic

appliance was set on day 0 (open triangle). Micro-CT images were acquired on days 0, 3, 7, 14, and 21 (filled triangle).

Figure 2. A, Corticotomy design. The figures on top show the micro-CT 3D-reconstructed images of the site of bone cut of the maxillary left first molar; the figures on the bottom show the schematic drawings. White lines in the schematic drawings indicate bone cuts. B, intraoral photograph of a rat after corticotomy, but before suturing of the gingiva, representing the animals of the 1-pit group from Experiment 1.

Figure 3. Appliance design. Figures on the left, micro-CT 3D-reconstructed images; figures on the right, drawings of the buccal and occlusal views of the appliance. Arrows indicate the direction of orthodontic force (F).

Figure 4. Measurement of tooth movement. Axial and sagittal micro-CT images after tooth movement and schematic view of the incisor eruption. A, distance between the contact points (arrowheads). B, the shortest distance between the upper first and second molars. Arrowheads indicate the closest points between the first and second molars. C, Change of the mesial root inclination of the first molar before and after tooth movement, in the sagittal images. D, length of the incisor eruption. E, the amount of change in the direction of coil spring.

Figure 5. Micro-CT superimposition images. Micro-CT images acquired on the first and

last days (red and green, respectively) of the experimental period are superimposed. White-dashed boxes indicate the measurement areas of the alveolar bone volume (from the top — alveolar crests: mesial, 1 mm anterior to the first molar mesial root; distal, distal edge of the first molar before tooth movement; height, 3 mm; width, 3 mm at the center of mesial root). Crest decrease indicates the decrease of the alveolar bone crest of the mesial ridge.

Figure 6. Box plot of the eruption of the incisor. A, length of the incisor eruption in Experiment 1. B, length of the incisor eruption in Experiment 2. C, the amount of change in the direction of coil spring in Experiment 1. D, the amount of change in the direction of coil spring in Experiment 2.

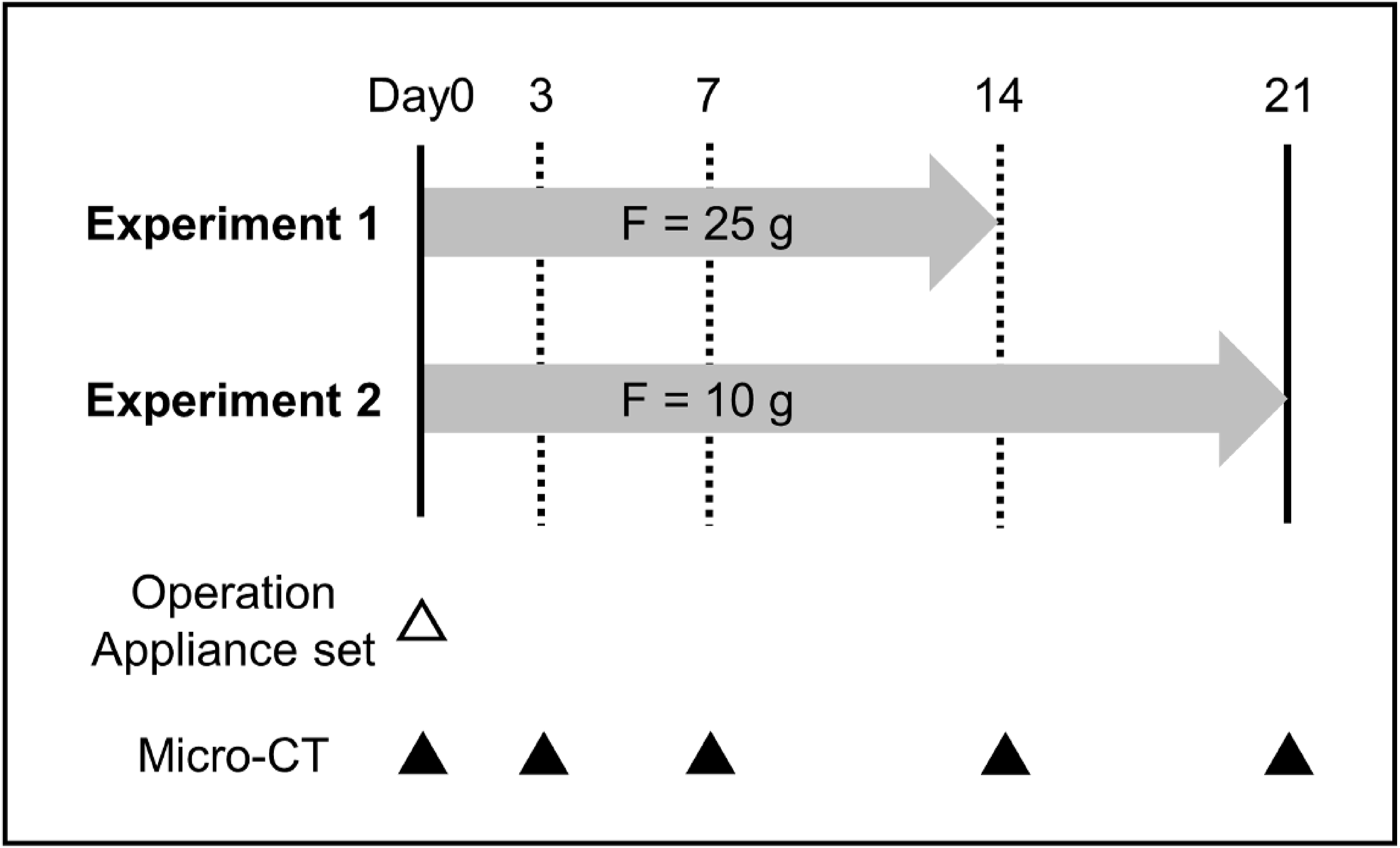
Figure 7. Box plot of tooth movement. A, distance between the contact points in Experiment 1. B, shortest distance between the upper first and second molars in Experiment 1. C, tooth inclination in Experiment 1. D, distance between the contact points in Experiment 2. E, shortest distance between the upper first and second molars in Experiment 2. F, tooth inclination in Experiment 2.

Figure 8. Box plot of root resorption. A, root resorption area in Experiment 1. B, root resorption depth in Experiment 1. C, root resorption volume in Experiment 1. D, root resorption area in Experiment 2. E, root resorption depth in Experiment 2. F, root

resorption volume in Experiment 2.

M; mesial root, DB; distobuccal root, DP; distopalatal root; Total, sum of the three roots; Average, average of the three roots.

Figure 9. Box plot of the alveolar bone measurements: A, volume of the bone cut during corticotomy in Experiment 1. B, decrease of the alveolar crest height in Experiment 1. C, volume of alveolar bone resorption after corticotomy in Experiment 1. D, volume of the bone cut in experiment 2. E, decrease of the alveolar crest height in Experiment 2. F, volume of alveolar bone resorption after corticotomy in Experiment 2. $^{*}p < 0.05$.


Day0
3
7
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Experiment 1
F = 25 g
Experiment 2
F = 10 g
Operation
Appliance set
△
Micro-CT
▲
▲
▲
▲
▲

Figure 2

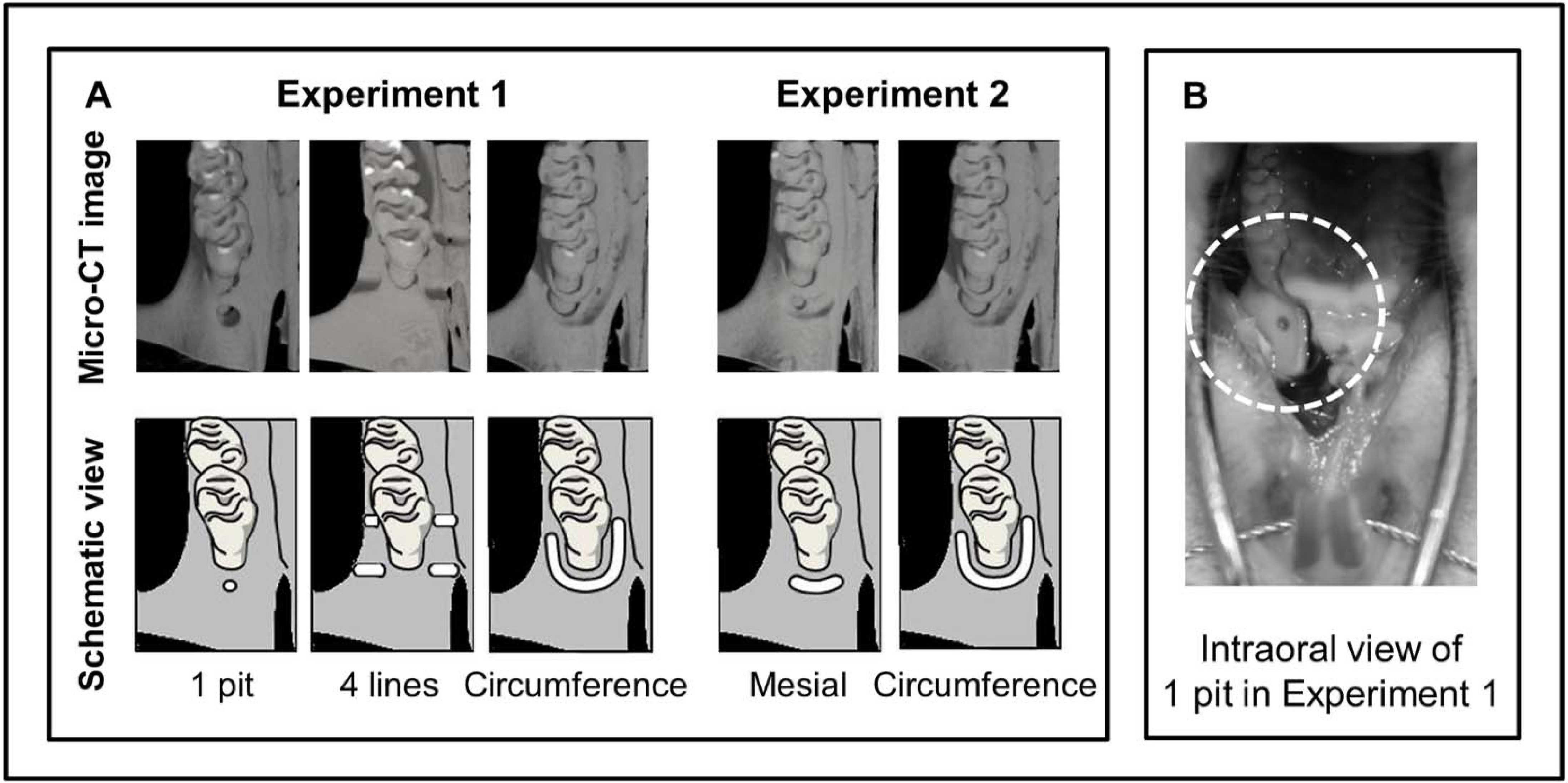
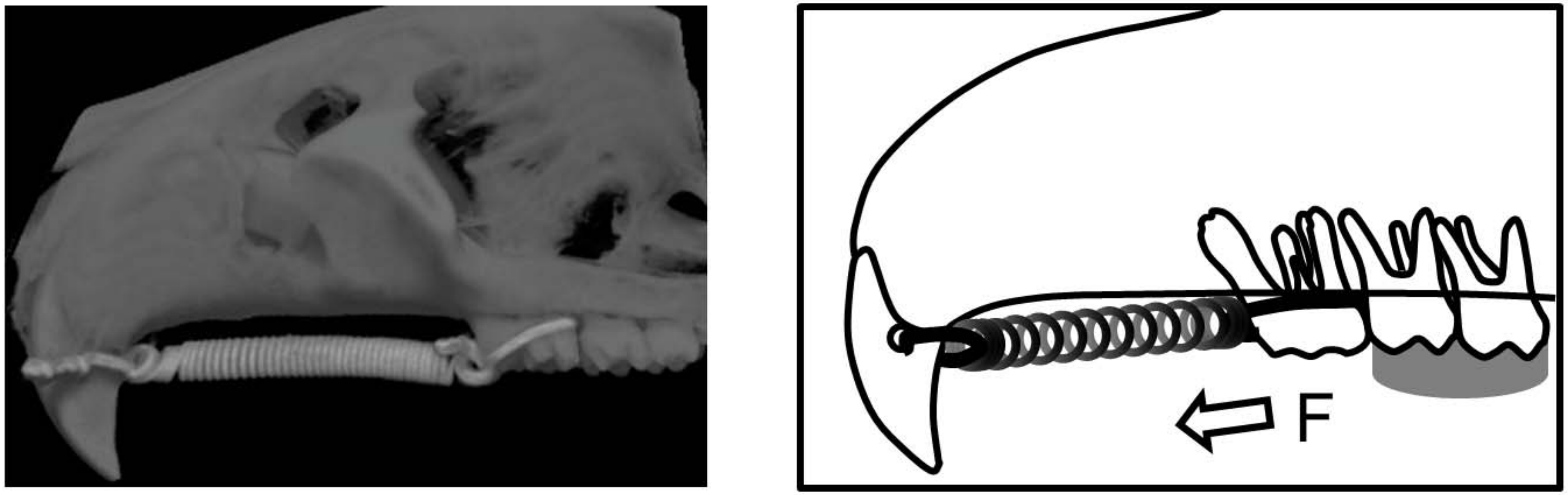

A
Experiment 1
Experiment 2
Micro-CT image
Schematic view
1 pit
4 lines
Circumference
Mesial
Circumference
B
Intraoral view of
1 pit in Experiment 1

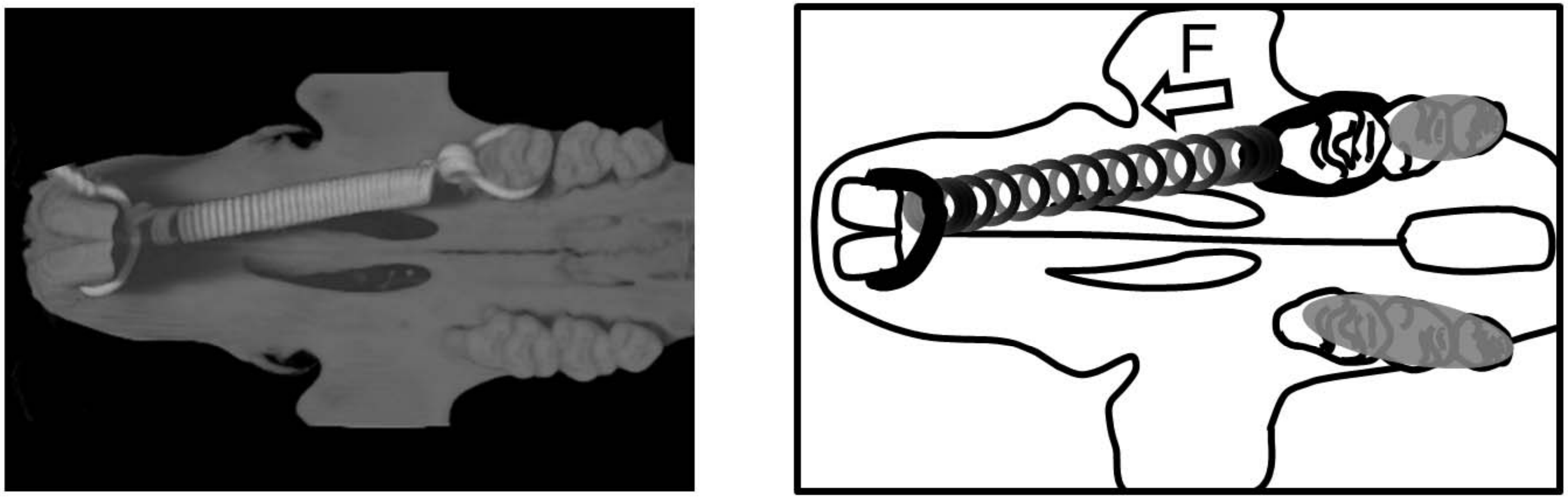
Figure 3

Appliance design



Micro-CT buccal view

Buccal view



Micro-CT occlusal view

Occlusal view

Figure 4

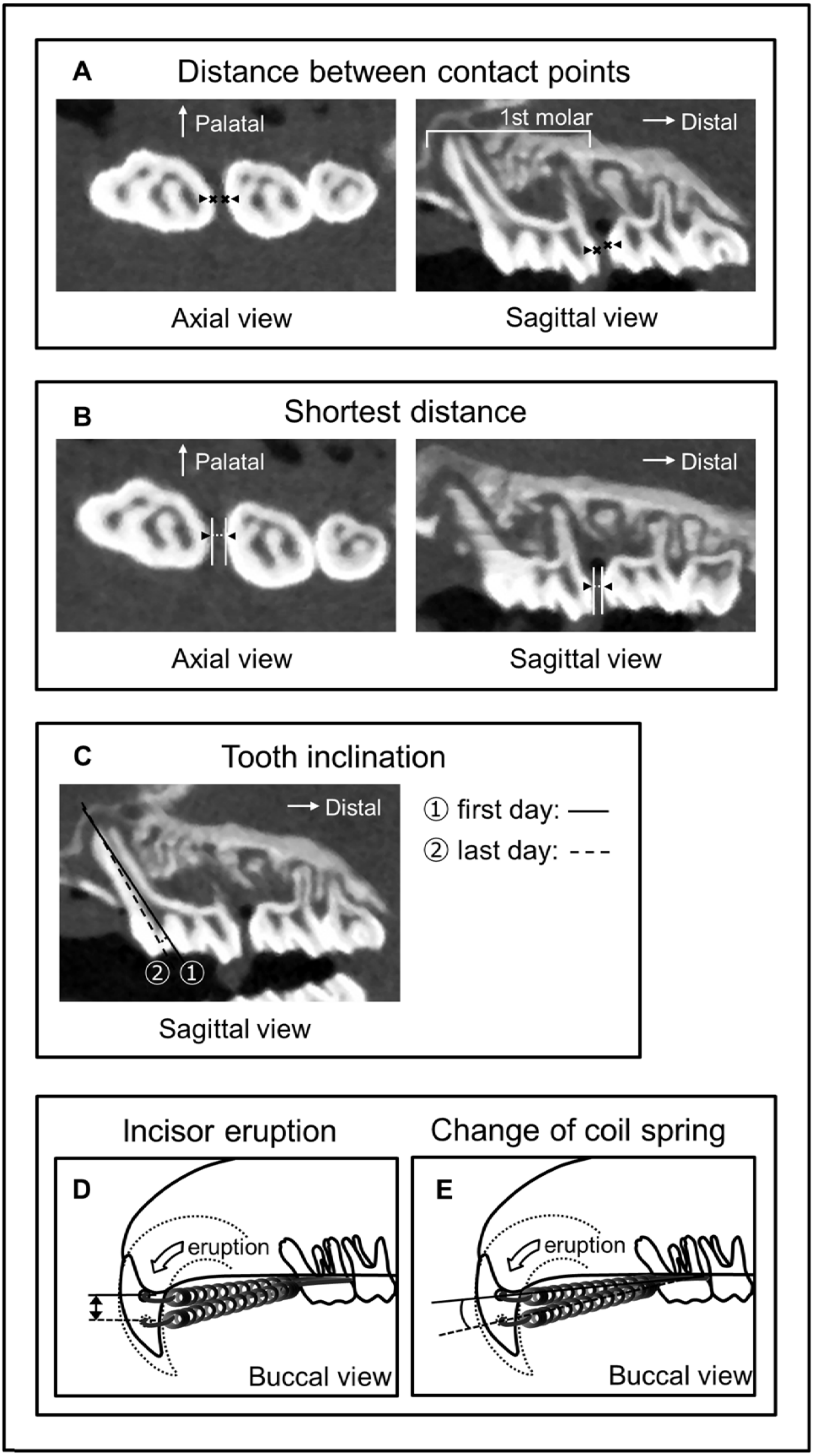
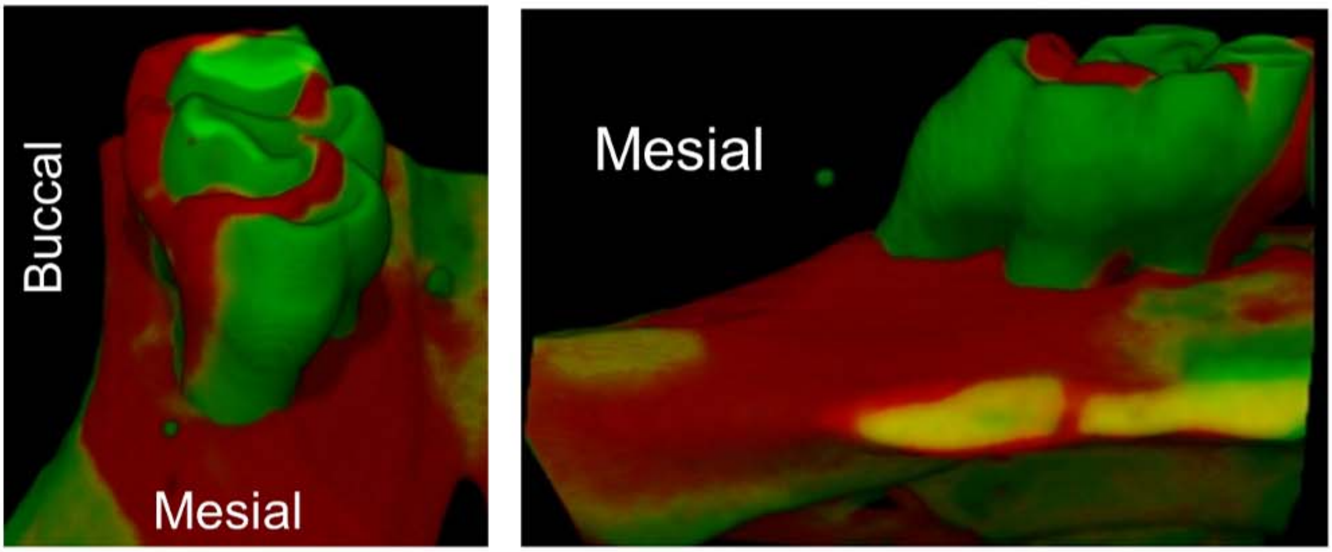

A
Distance between contact points
Palatal
Axial view
1st molar
Distal
Sagittal view
B
Shortest distance
Palatal
Axial view
Distal
Sagittal view
C
Tooth inclination
Distal
② ①
Sagittal view
① first day: ——
② last day: - - -
Incisor eruption
Change of coil spring
D
eruption
Buccal view
E
eruption
Buccal view

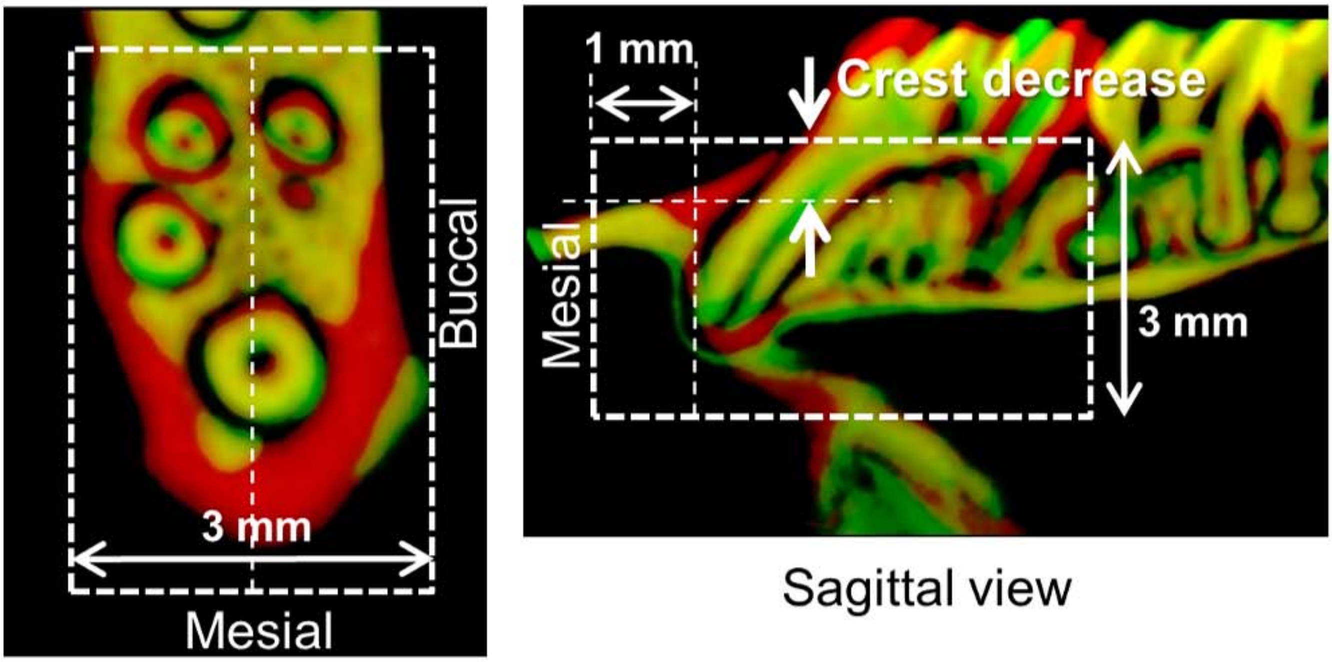
Figure 5



Micro-CT Superposition image:
Around the maxillary left first molar

Occlusal view

Palatal view



Measurement area of bone resorption

Axial view

Sagittal view

Figure 6

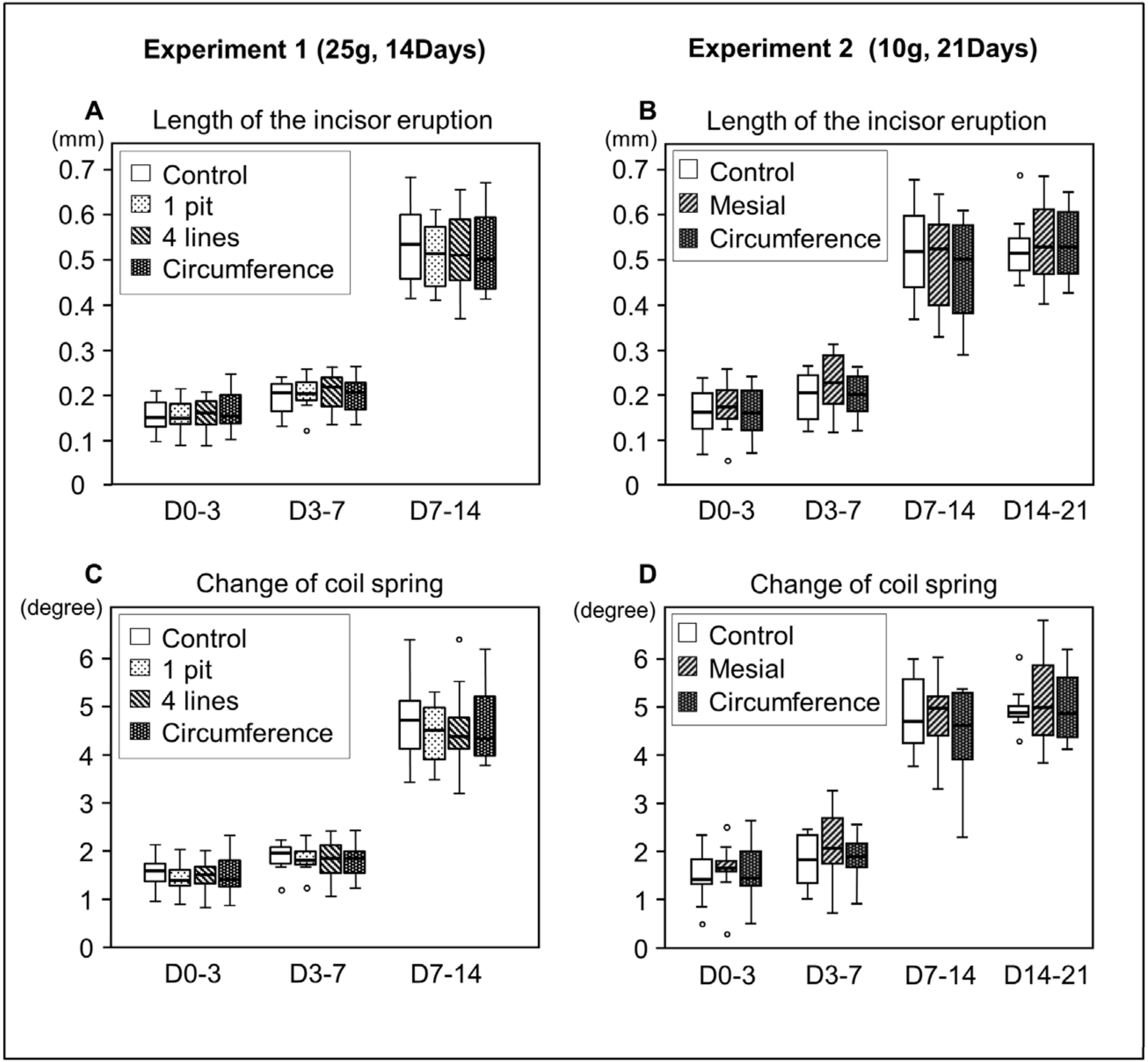

Experiment 1 (25g, 14Days)
Experiment 2 (10g, 21Days)
A
Length of the incisor eruption
(mm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
1 pit
4 lines
Circumference
D0-3
D3-7
D7-14
B
Length of the incisor eruption
(mm)
Control
Mesial
Circumference
D0-3
D3-7
D7-14
D14-21
C
Change of coil spring
(degree)
6
5
4
3
2
1
0
Control
1 pit
4 lines
Circumference
D0-3
D3-7
D7-14
D
Change of coil spring
(degree)
Control
Mesial
Circumference
D0-3
D3-7
D7-14
D14-21

Figure 7

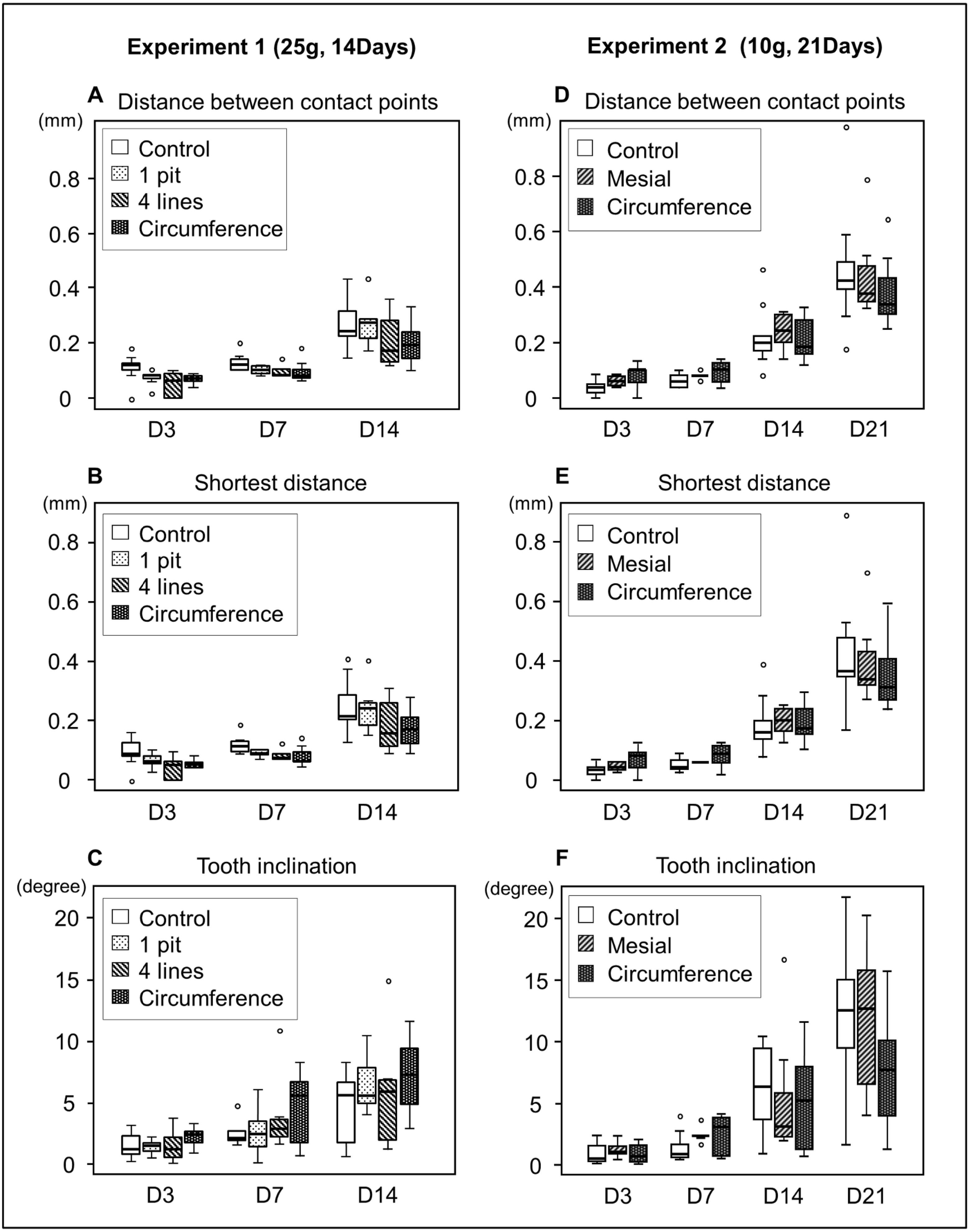
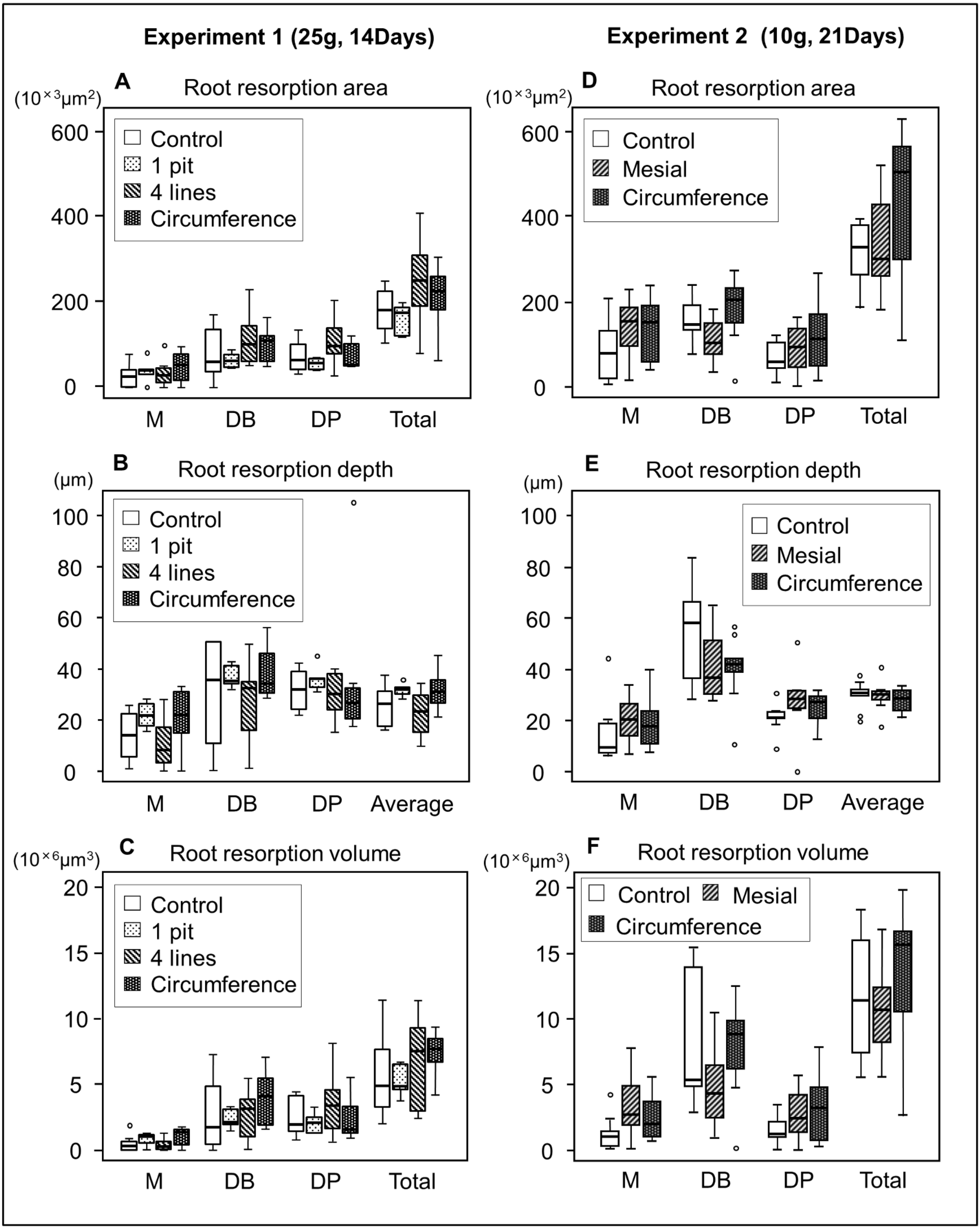

Experiment 1 (25g, 14Days)
Experiment 2 (10g, 21Days)
A
Distance between contact points
(mm)
0.8
0.6
0.4
0.2
0
Control
1 pit
4 lines
Circumference
D3
D7
D14
D
Distance between contact points
(mm)
0.8
0.6
0.4
0.2
0
Control
Mesial
Circumference
D3
D7
D14
D21
B
Shortest distance
(mm)
0.8
0.6
0.4
0.2
0
Control
1 pit
4 lines
Circumference
D3
D7
D14
E
Shortest distance
(mm)
0.8
0.6
0.4
0.2
0
Control
Mesial
Circumference
D3
D7
D14
D21
C
Tooth inclination
(degree)
20
15
10
5
0
Control
1 pit
4 lines
Circumference
D3
D7
D14
F
Tooth inclination
(degree)
20
15
10
5
0
Control
Mesial
Circumference
D3
D7
D14
D21

Figure 8


Experiment 1 (25g, 14Days)
Experiment 2 (10g, 21Days)
A
Root resorption area
(10×3μm2)
600
400
200
0
Control
1 pit
4 lines
Circumference
M
DB
DP
Total
D
Root resorption area
(10×3μm2)
600
400
200
0
Control
Mesial
Circumference
M
DB
DP
Total
B
Root resorption depth
(μm)
100
80
60
40
20
0
Control
1 pit
4 lines
Circumference
M
DB
DP
Average
E
Root resorption depth
(μm)
100
80
60
40
20
0
Control
Mesial
Circumference
M
DB
DP
Average
C
Root resorption volume
(10×6μm3)
20
15
10
5
0
Control
1 pit
4 lines
Circumference
M
DB
DP
Total
F
Root resorption volume
(10×6μm3)
20
15
10
5
0
Control
Mesial
Circumference
M
DB
DP
Total

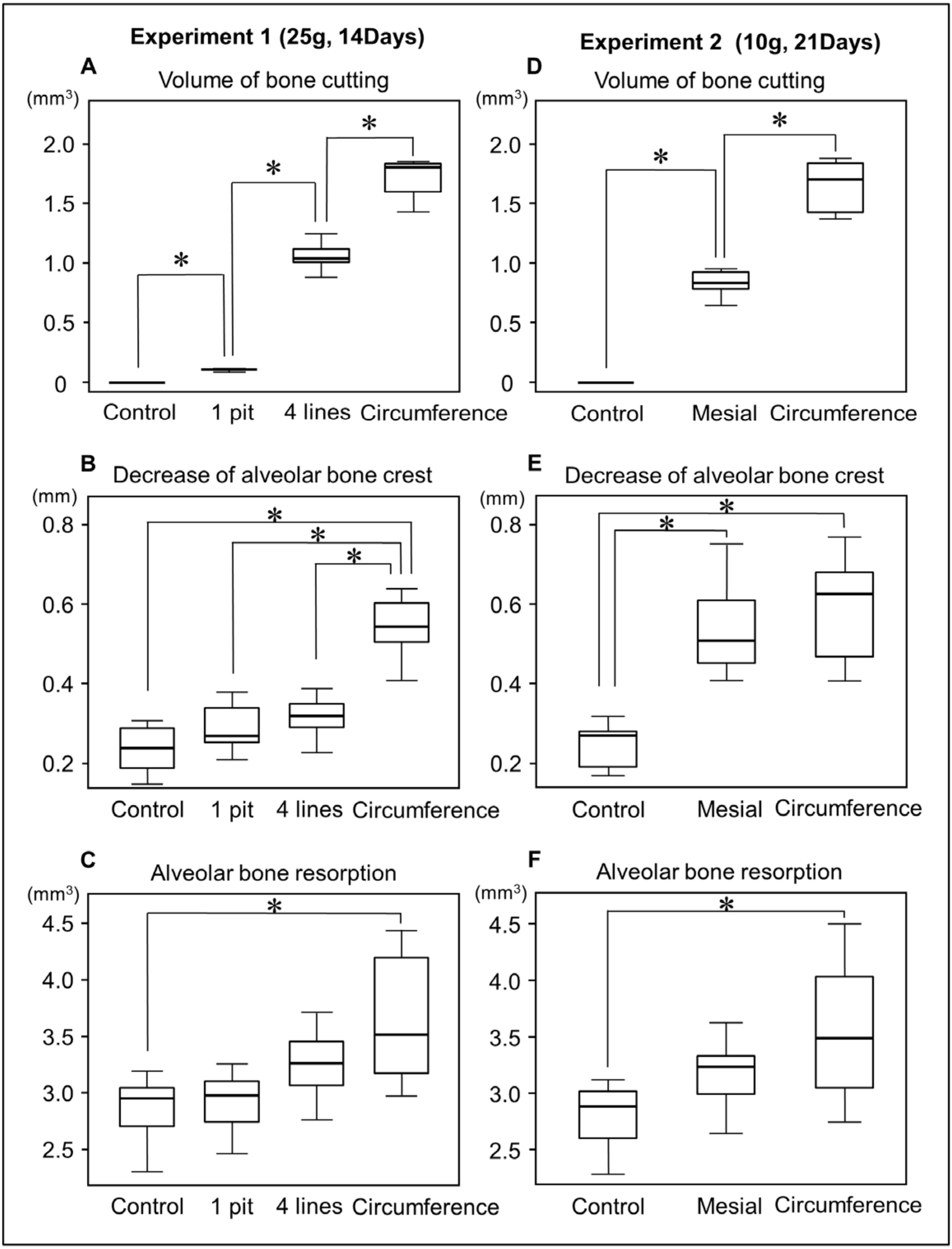

Experiment 1 (25g, 14Days)
Experiment 2 (10g, 21Days)
A
Volume of bone cutting
(mm^3)
2.0
1.5
1.0
0.5
0
*
*
*
*
Control
1 pit
4 lines
Circumference
D
Volume of bone cutting
(mm^3)
2.0
1.5
1.0
0.5
0
*
*
Control
Mesial
Circumference
B
Decrease of alveolar bone crest
(mm)
0.8
0.6
0.4
0.2
*
*
*
Control
1 pit
4 lines
Circumference
E
Decrease of alveolar bone crest
(mm)
0.8
0.6
0.4
0.2
*
*
Control
Mesial
Circumference
C
Alveolar bone resorption
(mm^3)
4.5
4.0
3.5
3.0
2.5
*
Control
1 pit
4 lines
Circumference
F
Alveolar bone resorption
(mm^3)
4.5
4.0
3.5
3.0
2.5
*
Control
Mesial
Circumference

Fig 9

Table 1. Amount of the tooth movement (Experiment 1, 2)

	Experiment 1				Experiment 2			
	D3	D7	D14		D3	D7	D14	D21
Distance between contact points（mm）								
Control (n = 9)				Control (n = 9)				
Mean	0.11	0.13	0.28		0.04	0.06	0.22	0.46
SD	0.05	0.03	0.09		0.03	0.02	0.11	0.23
Median	0.12	0.12	0.24		0.04	0.06	0.20	0.43
IQR	0.02	0.04	0.09		0.03	0.04	0.05	0.10
1 pit (n = 7)				Mesial (n = 7)				
Mean	0.07	0.10	0.27		0.06	0.08	0.24	0.45
SD	0.03	0.02	0.08		0.02	0.01	0.07	0.16
Median	0.08	0.10	0.27		0.06	0.08	0.24	0.38
IQR	0.01	0.03	0.07		0.02	0.00	0.10	0.13
4 lines (n = 8)				Circumference (n = 8)				
Mean	0.05	0.10	0.21		0.08	0.10	0.21	0.38
SD	0.04	0.02	0.09		0.04	0.03	0.08	0.13
Median	0.06	0.08	0.17		0.09	0.10	0.19	0.34
IQR	0.09	0.02	0.14		0.05	0.03	0.10	0.09
Circumference (n = 8)								
Mean	0.07	0.10	0.20					
SD	0.02	0.04	0.07					
Median	0.07	0.08	0.19					
IQR	0.02	0.02	0.07					
Shortest distance（mm）								
Control (n = 9)				Control (n = 9)				
Mean	0.09	0.11	0.25		0.03	0.05	0.19	0.42
SD	0.05	0.03	0.09		0.03	0.02	0.09	0.20
Median	0.09	0.11	0.22		0.04	0.05	0.16	0.37
IQR	0.05	0.04	0.08		0.03	0.03	0.06	0.13
1 pit (n = 7)				Mesial (n = 7)				
Mean	0.07	0.09	0.24		0.05	0.06	0.20	0.41
SD	0.03	0.01	0.08		0.02	0.00	0.05	0.14
Median	0.06	0.09	0.24		0.05	0.06	0.20	0.34
IQR	0.03	0.02	0.08		0.03	0.00	0.08	0.11
4 lines (n = 8)				Circumference (n = 8)				
Mean	0.04	0.08	0.18		0.07	0.09	0.19	0.35
SD	0.04	0.02	0.08		0.04	0.04	0.06	0.12
Median	0.05	0.07	0.16		0.09	0.09	0.18	0.32
IQR	0.06	0.02	0.14		0.04	0.05	0.06	0.09
Circumference (n = 8)								
Mean	0.05	0.08	0.17					
SD	0.02	0.03	0.06					
Median	0.05	0.06	0.17					
IQR	0.02	0.03	0.07					
Tooth inclination（degree）								
Control (n = 9)				Control (n = 9)				
Mean	1.47	2.23	4.67		0.99	1.49	6.41	12.41
SD	0.96	1.70	2.91		0.82	1.21	3.54	6.02
Median	1.19	1.71	5.62		0.57	0.93	6.38	12.57
IQR	1.47	2.29	4.95		1.24	1.09	5.67	5.47
1 pit (n = 7)				Mesial (n = 7)				
Mean	1.39	2.53	6.54		1.26	2.49	5.49	11.70
SD	0.58	1.09	2.39		0.66	0.61	5.42	6.16
Median	1.48	2.12	5.59		1.07	2.43	3.18	12.73
IQR	0.67	0.74	2.97		0.65	0.13	3.59	9.29
4 lines (n = 8)				Circumference (n = 8)				
Mean	1.47	3.33	5.73		0.93	2.52	5.19	7.63
SD	1.29	1.82	4.44		0.76	1.59	3.92	4.73
Median	1.20	3.71	5.92		0.74	3.12	5.28	7.77
IQR	1.14	1.96	4.63		1.33	2.95	6.30	5.91
Circumference (n = 8)								
Mean	2.23	3.76	7.22					
SD	0.76	3.00	3.02					
Median	2.41	2.90	7.29					
IQR	0.75	1.11	3.63					

SD: standard deviation IQR: interquaritile range

Table 2. Amount of the decrease of alveolar bone crest and the alveolar bone resorption (Experiment 1, 2

Group	Experiment 1				Experiment 2		
	Control (n = 9)	1 pit (n = 7)	4 lines (n = 9)	Circumference (n = 8)	Control (n = 9)	Mesial (n = 9)	Circumference (n =10)
Decrease of alveolar crest height（mm）							
Mean	0.24	0.29	0.31	0.54	0.24	0.54	0.58
SD	0.06	0.06	0.05	0.08	0.06	0.12	0.13
Median	0.24	0.27	0.32	0.55	0.27	0.51	0.63
IQR	0.35	0.36	0.39	0.93	0.42	0.32	0.89
Alveolar bone resorption（mm^3）							
Mean	2.85	2.91	3.26	3.64	2.80	3.16	3.55
SD	0.32	0.29	0.32	0.56	0.31	0.30	0.61
Median	2.96	2.98	3.27	3.51	2.88	3.23	3.48
IQR	0.35	0.36	0.39	0.93	0.42	0.32	0.89

SD, standard deviation; IQR, interquaritile range