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Effect of decreased implant healing time on bone (re)modeling adjacent to plateaued implants under functional loading in a dog model

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Abstract

Objectives—The purpose of this study was to evaluate the effect of early functional loading to plateaued implants on bone formation and implant stability in a dog model.

Materials and methods—Early loading (EL), nonloading control, and delayed loading (DL) groups were compared using six beagle dogs under functional loading. The Periotest® values were measured dynamically for 6 weeks. Peri-implant bone architecture was evaluated qualitatively by microcomputed tomography (μ CT) and analyzed quantitatively by mineral apposition rates (MAR), bone-to-implant contact (BIC), and bone volumes (BV/TV) after the euthanasia at 3 and 6 weeks after loading.

Results—The EL implants showed poor stability at 1 week, but greater stability at 2 and 4 weeks after loading compared to DL implants. There was no significant difference between MAR of EL and unloaded implants at both time intervals. The EL implants displayed a significantly higher MAR when compared to DL implants at 3–5 weeks. A significantly higher BIC for the DL group was observed when compared to the EL group at 3 weeks following loading, however at 6 weeks; no significant difference between these groups was observed. The EL group gained a higher BIC than the no-treatment control group at 6 weeks.

Conclusions—For plateaued implant, the decreased healing time (1 week) displays a positive effect on peri-implant bone (re)modeling under functional loading during the early phase.

Clinical relevance—The early application of functional loading on plateaued implants can be used clinically to shorten the course of treatment and improve esthetics.

Keywords

Early loading; Bone (re)modeling; Plateaued implant; Implant stability

Introduction

The primary goal of osseointegrated dental implants is to replace load-bearing tooth organs by restoring their form and intraoral function [1]. Critical to this phenomenon is the ability

for biomaterials to mediate bone to implant contact at the surface of implanted materials. Over the past 40 years, basic and clinical researches have improved these interactions enabling faster treatment time frames and healing periods [2–5]. While substantial modifications have been made regarding implant surface biocompatibility, osseointegration, surface topography and hydrophilicity, less information has been devoted to early loading periods for plateaued implants.

Typically, the delayed loading protocol was used to ensure successful and predictable placement of dental implants. However, a number of clinical and experimental studies regarding early loading directly challenged this notion with convincing outcomes. The early loading protocol refers to load applied to implants between 2 days and 3 months postsurgery (between immediate loading and conventional loading) [6, 7]. Comparable or even better survival rates and less peri-implant bone loss for early loaded implants were achieved in a series of clinical trials [8–12].

In an experimental study, an intraoral hydraulic device was used to control in vivo load and healing time quantitatively to compare outcomes of early loaded implants and their unloaded counterparts. Both the biomechanical and histological analysis of the healing bone suggested that early loading protocol produced more favorable osseointegration [13]. Furthermore, De Smet et al. verified in a pig model that controlled loading can promote the maintenance of implant stability during the early critical healing period [14]. In their subsequent studies, it was found that early loading did not negatively affect the implant outcome [15] and the effect of controlled stimulation applied early on the peri-implant cortical bone of pigs depended on force amplitude/strain at low-frequency stimulation [16, 17].

Although many studies were conducted to determine the outcomes of early loading applied to threaded implants [13–17], few experimental studies exist that investigate peri-implant bone reactions under early loading applied to plateaued implants. The cylinder plateaued implant has a series of separate circumferential fins existing along the intraosseous portion of the implant, and a large space between the fins which have previously been described for blood clot formation and woven bone development [18]. It has been suggested that a relatively rapid bone healing and thus biomechanical stabilization of the implant could result from the vascularization and woven bone filling in this space [19]. The purpose of this study was to determine if plateaued implants could also achieve improved osseointegration and a higher implant stability under early functional loading by comparing plateaued implants after 1 week of healing (early loading (EL) group), control group receiving no loading, and delayed loading (DL) group under conventional loading in a dog model.

Material and methods

Devices and surgery

Commercially pure-grade 2 titanium (SIO 5832/2) was chosen for the construction of these customized cylindrical plateaued Ti implants and abutments (Wuhan Tianhua Photoelectric Communication Co. Ltd., Wuhan, Hubei, China). The implants ($\phi 3 \times 8$ mm) were 2.2 mm in the internal diameter of plateau, and 3.0 mm in the external diameter of plateau (Fig. 1). The surface of all the implants were treated according to the previous methods [20, 21] as

follows: all implants were coarse grit blasted with 0.25–0.50 mm corundum grit at 5 bars for 1 min, and afterwards acid etched in hydrochloric acid/sulfuric acid (1:1) at 65 °C for 30 min. The implants were then ultrasonically cleaned with acetone, ethanol, and Milli-Q water each for two 15-min periods, and finally stored in Milli-Q water. A 1.5° locking taper was used to provide a stable connection between the implants and abutments. All implants and abutments were sterilized in a steam autoclaving machine for 20 min as described in the previous article [22] before the use to the animals.

The research protocol was approved by the Council of the Animal Protection of School & Hospital of Stomatology of Wuhan University, China. All surgical procedures were performed under the combination of general anesthesia induced with a 3 % solution of intravenously administered pentobarbital sodium (Merck, Darmstadt, Germany; 30 mg/kg) and local anesthesia with submucosally administered 4 % articaine (Acteon Pharma; 0.06 ml/kg). Each surgery was performed under sterile conditions. Food was withheld for 12 h before each surgery. The animals were administered intramuscular injections of penicillin (NCPC; 400,000 IU/ml, 0.1 ml/kgday) for anti-inflammation at the time of surgery and once daily for the next 5 days.

Six female beagle dogs (Anlu Ruikesen Experimental Animal Ltd., Anlu, Hubei, China), 1–1.5 years old and weighing about 13–15 kg each were used in this study. After the fabrication of an individualized impression, the second, third, and forth premolars (P2, P3, and P4) in both quadrants of the mandible were extracted for each dog using forceps without elevation of mucoperiosteal flap. The surgical sites were secured with interrupted nonabsorbent sutures. Postoperatively, the animals were checked daily and fed with a soft diet for 1 week. The sutures were removed 1 week after the extraction. Following a 10-week healing period, six implants were placed along the crestal alveolar ridge in each dog with the same submerged implant installation technique. The mucosal flaps were sutured interruptedly to cover the submerged implants. The sutures were removed after 1 week and a plaque-control program including cleaning of the remaining natural teeth and the implant-supported teeth two times per week was initiated. Hemi-hard food were provided for a week post-operation, and replaced by hard food.

Loading protocol

Three groups (EL, no-treatment control, and DL groups) were formed from 36 plateaued implants with six implants in each dog. Implants of all three groups were randomly located in the mandible side of each dog. Each group consisted of 12 implants. Timing of the loading depended on the implant's assigned group to simulate the early and delayed loading clinical settings as illustrated in Fig. 2.

For the EL group, loading began at 1 week after the implant installation. The abutment was inserted into the implant by manual tapping on the long axis. Single acrylic resin crowns were prepared at the day of abutment placement on a plaster model and were then placed onto the abutments. For the control group, incision and suture were performed at 1 week after the implant installation. However, no-loading procedures were carried out for this group until the death of animals. For the DL group, abutment and crown placements were

carried out 10 weeks after the implant installation. At weeks 3 and 6 of implant loading, three animals each were sacrificed and block specimens containing implants were dissected.

Stability measurements

The stability of implants was evaluated at the time of abutment placements, and at 2, 4, and 6 weeks of loading by Periotest® M (Medizintechnik Gulden, Bensheim Germany) for EL and DL groups. Twelve implants were included in the analysis of implant stability for each group and each time point. Periotest is an instrument designed to measure the damping capacity of the implant–bone continuum [23]. The measurement of damping capacity is expressed as a Periotest® value (PTV), which can range from –8 (very stable) to +50 (very mobile). The handpiece of the Periotest was placed parallel to the floor and perpendicular to the implant–abutment complex. In addition, measurements were taken with the rod of the Periotest immediately above the alveolar ridge from the abutment. The values were only accepted if two consecutive PTVs did not deviate more than one unit from each other. Three valid measurements per time point were obtained for each implant.

Histological preparation

Implants and surrounding tissues retrieved were immediately fixed in 4 % neutral-buffered formaldehyde for 72 h at room temperature and dehydrated in ascending concentrations of alcohols from 70 to 100 %, subsequently infiltrated for at least 3 weeks and embedded in methylmethacrylate resin (Techno-vit 9100; Heraeus Kulzer GmbH, Hanau, Germany) in vacuum drying oven (MMM Medcenter Einrichtungen GmbH, Germany). After polymerization, the specimens were used for microcomputed tomography (μCT) scanning, fluorescence and histomorphological analysis.

μCT scanning and analysis

A μCT imaging system (μCT50, Scanco Medical, Basersdorf, Switzerland) was used to scan and reestablish the peri-implant bone of all embedded samples. Scanning was performed at 90 kV and 155 μA with a thickness of 15 μm per slice in medium-resolution mode and 1,200 ms integration time. A Gaussian filter (sigma=0.8 and support =1) was used to remove noise. The mineralized bone was differentially segmented to exclude the titanium and the nonmineralized tissue with a fixed threshold (200–800). Peri-implant bone architectures were evaluated by means of three-dimensional reconstruction.

Confocal laser scanning microscope analysis

During the healing period, the fluorescence tracer calcein green was intravenously injected with 10 mg/kg at 3 weeks prior to sacrificing animals. Alizarin red was intravenously injected with 35 mg/kg at 1 week before sacrificing animals in order to label newly mineralized bone or new bone formation as previously described [24]. All dyes were prepared immediately before use.

The specimens were sectioned with a diamond saw blade along the implant axis at a thickness of 70 μm and analyzed by confocal laser scanning microscope (Leica TCS Sp2 AOBs MP confocal microscope system, Heidelberg, Germany). The polished sections were viewed at a magnification of ×100 under a mercury lamp microscope for evidence of

fluorochrome double-labeled trabeculae at the bone–implant interface. Five measurements were made along the span of each double-label by measuring the distance between two parallel fluorescent labels. The thickness of three double-labels was measured for each implant. The thickness of the newly mineralized bone layer at the active bone forming surface was averaged from the total of 3×5 measurements. The mineral apposition rate (MAR), expressed in unit of microns per day, was determined by dividing the thickness of the newly mineralized bone layer by the 14-day labeling interval. Six implants were included in the analysis of MAR for each group and each time interval (0–2 and 3–5 weeks after loading).

Histomorphology

Following confocal microscopy scanning, sections were stained with methylene blue and basic fuchsin [21] and grounded to a thickness of 30–50 µm. A light microscope (DM4000B, Leica, Germany) equipped with an image analysis system (Q-win, Leica) was used for histological evaluation. All sections (two sections per sample) were used for the quantitative measurements. An area with a height of 3.0 and 2.5 mm below the implant shoulder was defined as the region of interest for each implant. In this area, bone-to-implant contact (BIC; in percentage) were calculated and the quantification of bone volume fraction (BV/TV; in percentage) were performed in the regions of 0–100, 100–200, and 200–300 µm away from both buccal and lingual implant surfaces. Six implants were involved in the histomorphometrical analysis for each group and each time point (3 and 6 weeks of loading).

Statistical analysis

Data were statistically analyzed by means of SPSS 16.0 for windows. Independent-samples *t* tests were applied for comparison of PTV between EL and DL groups. One-way analysis of variance at 95 % level of significance and Bonferroni tests were run for the comparisons of MAR and histomorphological parameters of different groups with the same loading period.

Results

All animals survived well during the course of the experiment. Two crowns were displaced during the loading period and the restorations were performed again on the following day. All other implants integrated well, without any signs of complication.

Stability of implants

Implant stability of EL and DL groups expressed as the average PTV for the different time points are presented in Fig. 3. Significant differences were observed between EL and DL groups at 0, 2, and 4 weeks after abutment placement (Table 1). Although there was no significant difference for EL and DL groups 6 weeks after abutment placement, the course during these 6 weeks varied substantially. The EL implants showed a sharp descent during the first 2 weeks after abutment placement, the mean PTV at 2 weeks was 9.3 units lower than at abutment placement. A gradual increase of PTV for EL group was observed during the third and fourth weeks after abutment placement. The PTV of EL group decreased again during the last 2 weeks, which resulted to its lowest values at 6 weeks post-abutment

placement. The PTV of the DL group showed an increase during the first 2 weeks and a gradual decrease during the following weeks.

Fluorescence analysis

Fluorescence images were captured and analyzed to assess the volume of newly formed bone determined by the relative area of the green (calcein green) and red (alizarin) labels, which were administered at 3 and 1 weeks prior to sacrificing animals (Fig. 4). For EL group, control group and DL groups, new bone formation at 0–2 weeks (Fig. 4a–c; bar= 300 μm), and at 3–5 weeks (Fig. 4d–f; bar=300 μm) after abutment placement were observed. Active trabecular bone formation was observed around the implants in all groups. For the period of 0–2 weeks, few green labels administered at the time of abutment placement were observed in the notch, but red labels administered 2 weeks later were observed both by green labels and also in the notch, which was indicative of early bone formation (Fig. 4a,b).

Quantification analysis of average mineral apposition rates were assessed for EL, control, and DL groups at both time intervals (Fig. 5). There was no statistical significance between the mineral apposition rates of any two of the three groups during the first 2 weeks after loading. EL implants demonstrated a significantly higher mineral apposition rates than DL implants due to the drop of DL implants during 3–5 weeks postloading (2.04 vs. 1.71 $\mu\text{m}/\text{day}$, $p<0.01$). The mineral apposition rates of control group was also higher than that of DL group (1.94 vs. 1.71 $\mu\text{m}/\text{day}$, $p<0.05$). There was no statistical significance between mineral apposition rates of EL and control groups.

μCT imaging

By means of 3D reconstruction of the μCT images at 3 and 6 weeks of healing, the peri-implant bone architectures of three groups were compared (Fig. 6). A wound healing chamber was formed between the trough of implant and osteotomy. The healing bone in the chamber grew more densely at 6 weeks of healing when compared to that at 3 weeks for all groups by observing the images of longitudinal sections. For EL and control groups at 3 weeks, fewer bone existed in the wound healing chamber. For DL groups at 3 weeks and all groups at 6 weeks, large areas of chamber had been occupied with mineralized bone. Both longitudinal and cross-sections of EL and DL groups showed thin trabeculi at 3 weeks of loading. However, the bony struts surrounding EL and DL groups were relatively thick with less intertrabecular space when compared to the control group at 6 weeks.

Histomorphometry

Following 3 weeks of healing, the EL and control groups contained newly formed bone in various degrees of maturity and numerous spindle-shaped osteoblasts lying on the front of the bone matrix (a1, a2, b1, and b2 of Fig. 7). Most areas of the troughs between the plateau tips for EL and control groups at 3 weeks were filled with unmineralized tissue. Fewer newly formed bone extending from the surface of surrounding bone to the trough and lower bone-to-implant contact were observed. At 6 weeks, new bone with uniform maturity and fewer cuboidal osteoblasts were observed, especially for delayed loading implants at both 3 and 6 weeks (c1, c2, d1, d2, e1, e2, f1, and f2 of Fig. 7). Specifically, more areas of troughs were occupied by mineralized bone with obviously higher bone-to-implant contact. At 6

weeks of healing, large areas of the implant surface appeared to be in direct contact with newly formed bone for nonloaded implants. The newly formed bone for EL and DL groups was seen to occupy most of the surface of the implant.

The bone-to-implant contact and bone volumes in the regions of 0–100, 100–200, and 200–300 μm from the surfaces of implants were estimated from 3 and 6 weeks sections (Fig. 8). The DL implants had significantly higher bone-to-implant contact compared to the EL group at 3 weeks following loading (70.09 vs. 8.29 %, $p<0.01$). At 6 weeks, there was no significant difference between the EL and DL groups, and the EL group gained higher bone-to-implant contact than the no-treatment control group (62.84 vs. 38.20 %, $p<0.05$). In addition, the EL group showed significantly lower bone volumes in both regions of 0–100 and 100–200 μm from the surfaces of implants compared with the DL group 3 weeks after abutment placement (14.11 vs. 62.28 %, $p<0.01$; 36.31 vs. 62.17 %, $p<0.01$), but showed comparable values 6 weeks postloading. No significant differences were observed for bone volumes between any two groups in the region of 200–300 μm .

Discussion

The installation of implants in the alveolar process elicits a peri-implant bone wound healing process, which involves new bone formation, modeling, and remodeling adjacent to the implant surface [25, 26]. Achieving predictable hard and soft tissue integration to re-establish function with the implant-supported prosthesis in a shortened healing period has been a highly studied topic in the field of implant dentistry for almost a decade. In the present study, plateaued implants in EL, control, and DL groups were tested for their ability for osseointegration under functional loading during the early healing period. The PTV, mineral apposition rates, and histological parameters were investigated objectively and quantitatively to compare the effect of EL and DL protocols on osseointegration at different levels.

Previously, Berglundh et al. have investigated different phases of wound healing adjacent to screw implants with circumferential troughs that established a series of separate wound compartments in a dog model [27]. It was observed that the chambers of implants were occupied with a provisional matrix, part of which contained newly formed woven bone 1 week after implantation. In addition, portions of the newly formed bone in direct contact with the surface of their implants was observed. Thus, we chose a time point of 1 week to begin applying load in the EL group and studied its subsequent effects on peri-implant bone (re)modeling.

Periotest was introduced to measure tooth and implant movement by Schulte et al. [28], and was demonstrated to be useful for the assessment of implant stability [29–31]. In our study, the EL implants showed poor stability during abutment placement, but greater stability at 2 and 4 weeks following loading when compared to DL implants by means of PTV. This increase in stability during the initial healing phase differed from previous results comparing PTV and ISQ of screw implants, in which the PTVs maintained their stability [32]. A possible reasoning for this difference could be due to the external diameter of circumferential fins coinciding with that of implant beds which may have resulted in no

interlocking relationship between fins and the wall of implant bed, and thus relatively poor primary stability. Both the higher stability of EL implants compared to DL implants at 2 and 4 weeks of loading and comparable stability at 6 weeks support the hypothesis that early loading could also achieve osseointegration for plateaued implants.

The fluorescent label demarcates the mineralization front at the time of administration and can be detected in histological sections without any further staining or decalcification [24]. We observed that the mineral apposition rates of plateaued implants for EL, control, and DL groups in this model were similar to those reported adjacent to immediately loaded and unloaded screw implants in a mini-pig model [33]. The findings that EL implants demonstrated a significantly higher mineral apposition rates than DL implants at 3–5 weeks after loading and that new bone formation became slower over time for DL implants suggest that EL implants may be an effective alternative to conventional DL implants.

By means of μ CT scanning and evaluation, newly formed bone of all samples was assessed to compare peri-implant bone architecture in different groups. The trabeculi of EL group grew more densely and thicker as loading time increased. Denser trabeculi of EL group were detected compared to control group at 6 weeks. Also, the density of EL implants was comparable to DL implants at the same time point. All these findings indicated that much more dense bone was deposited in response to the early functional loading.

The bone-to-implant contact in this study (62.84 %) for EL implants after 6 weeks of loading is close to the previously reported data investigating threaded implants under immediate/early loading protocol during longer periods [33–36]. In addition, the bone-to-implant contact in our study can also be compared to a reported 66.91 ± 13.36 % for plateaued commercial implants inserted in dogs' tibia after a 4-week unloaded period [37]. However, a relatively lower bone-to-implant contact of implants exposed to early functional loading at 3 weeks of healing was observed. A possible reason for this lower bone-to-implant contact is suggested to be the variance of the implant coating. Nevertheless, the quickly growing bone-to-implant contact of EL implants from 3 to 6 weeks of loading indicated that there seems to be no negative effect of early loading on osseointegration of plateaued implants in the early healing period, which supported the quantitative measurements of bone volumes and qualitative observation of μ CT.

An interesting finding from our study demonstrated that the DL implants showed a descent in bone-to-implant contact and bone volumes in the regions of 0–100 and 100–200 μ m from the implant surface over time, which was consistent with the decreased mineral apposition rates for DL implants. It supports the reported finding that no matter what the initial percentage of bone-to-implant contact, about 58–60 % contact will be achieved finally when a balance is reached by bone remodeling in a 2D finite element model [38].

Conclusions

The present study demonstrate that a decreased healing time of 1 week displayed positive effects on peri-implant bone remodeling under early functional loading. EL implants showed greater stability at 2 and 4 weeks, a higher mineral apposition rates at 3–5 weeks and a

higher bone-to-implant contact at 6 weeks following loading when compared to DL implants. These findings suggest that early loading promote more favorable osseointegration than delayed loading for plateaued implants during the early phase following loading. Yet, the effect of early loading during longer phase will have to be furthermore carefully investigated.

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Fig. 1.
The cylinder plateaueed implant ($\phi 3 \times 8$ mm) has a series of separate circumferential fins with the same external diameter along the bone-implant interface

Fig. 2.

Study design: grouping and loading protocol. Thirty-six implants were assigned to three groups: early loading group (EL group), no-loading control group (control group), and delayed loading group (DL group). Implants of all three groups were randomly located in the mandible side of each dog. Each group consisted of 12 implants. Loading began at 1 week after the implant installation for the EL group and at 10 weeks after the implant installation for the DL group. No loading procedures were carried out for control group until the death of animals. At weeks 3 and 6 of implant loading, three animals each were sacrificed and block specimens containing implants were dissected

Fig. 3.

Mean PTV for EL and DL implants at 0, 2, 4, and 6 weeks after abutment placement. The PTV for EL group descended sharply during the first 2 weeks after abutment placement, increased gradually during the third and fourth weeks and decreased again during the last two weeks, which resulted to its lowest values at 6 weeks of healing. The PTV of the DL group showed an increase during the first 2 weeks and a gradual decrease during the following weeks

Fig. 4. Basic confocal microscopy of folded images stained with calcein green (*green*) and alizarin (*red*) to assess new bone formation. For EL, control, and DL groups, new bone formation at 0–2 weeks (**a–c**, *bar*=300 μm), and at 3–5 weeks (**d–f**, *bar*= 300 μm) after abutment placement was observed

Fig. 5.

Quantification analysis of new bone formation expressed as MAR. No statistical significance between the MAR of any two of the three groups during the first 2 weeks after loading was observed. EL implants demonstrated a significantly higher MAR than DL implants due to the drop of DL implants during 3–5 weeks postloading (2.04 vs. 1.71 µm/day, ** $p < 0.01$). The MAR of control group was also higher than that of DL group (1.94 vs. 1.71 µm/day, * $p < 0.05$). There was no statistical significance between MAR of EL and control groups

Fig. 6. μ CT images of peri-implant bone architectures of EL, control, and DL groups at 3 and 6 weeks of healing (bar=300 μ m). For each group at each time point, three images, a longitudinal section (*n1*) and two cross-sections at different levels (*n2* and *n3*) were displayed. A wound healing chamber was formed between the trough of implant and osteotomy. The healing bone in the chamber grew more densely at 6 weeks of healing when compared to that at 3 weeks for all groups. At 3 weeks of loading, the peri-implant trabeculi were thin for EL and control groups. However, the bony struts surrounding EL and DL implants were relatively thick with less intertrabecular space when compared to the unloaded implants at 6 weeks. It seemed that much more dense bone was deposited in response to the early functional loading

Fig. 7.

Ground sections of peri-implant bone following EL, control, and DL group after 3 and 6 weeks of loading with methylene blue and basic fuchsin stainings. *LB* lamellar bone, *OW* osteotomy wall, *WB* woven bone, *NM* noncalcified matrix, *OB* osteoblasts. Calcified bone stains as *bright red*, with variations in intensity depending on the maturity of the bone. Noncalcified bone and osteoid stain as *blue*. Three-week healing tissue for the EL group and control group (*a1*, *a2*, *b1*, and *b2*) consisted of newly formed bone in various degrees of maturity, with numerous osteoblasts lying on the front of bone matrix. Less newly formed bone extended from the surface of surrounding bone to the trough with much lower BIC. Large areas of chambers were filled with noncalcified matrix. The tissue of DL group at 3 weeks and of all three groups at 6 weeks (*c1*, *c2*, *d1*, *d2*, *e1*, *e2*, *f1*, and *f2*) contained new bone with relatively uniform maturity, especially for delayed loading implants at both 3 and 6 weeks. Specifically, more areas of roughs were occupied by mineralized bone with obviously higher BIC. At 6 weeks of loading, the newly formed bone for EL and DL groups was seen to occupy most area of the surface of implant

Fig. 8.

Quantification analysis of histomorphometric parameters including bone-to-implant contact (BIC, in percentage; **a**) and bone volume fraction (BV/TV, in percentage) performed in the regions of 0–100 µm (**b**), 100–200 µm (**c**), and 200–300 µm (**d**) from the buccal and lingual implant surfaces (* $p < 0.05$, ** $p < 0.01$)

Table 1

Mean PTV of EL and DL at 0, 2, 4, and 6 weeks after abutment placement expressed as mean (standard deviation)

Loading period (week)	EL group	DL group	<i>p</i> value
0	10.0 (2.7)	3.1 (2.5)	<0.01
2	0.7 (0.9)	4.9 (2.5)	<0.01
4	1.1 (1.8)	4.4 (2.3)	<0.05
6	-0.4 (1.5)	2.6 (3.0)	>0.05