



Peri-implant myelinated nerve fibers: Histological findings in dogs

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Abstract

Background and Objective: While osseointegration following various dental implant placement protocols has been extensively investigated, the neurohistological integration has received little attention. The primary aim of this study was to compare the myelinated nerve fibers density in peri-implant bone tissue following various implant placement protocols. The secondary aim assessed the effect of follow-up on peri-implant nerve fibers density.

Methods: Ten beagle dogs randomly received 68 commercially pure titanium implants in the mandibular premolar or molar region bilaterally following extraction utilizing one of the six treatment protocols: (a) immediate implant placement (IIP) and immediate loading (IL); (b) IIP and delayed loading (DL); (c) IIP and left unloaded (UL); (d) delayed implant placement (DIP) and IL; (e) DIP and DL; and (f) DIP and UL. Histomorphometric analysis of the peri-implant myelinated nerve fibers was performed in a 300 µm peri-implant zone at the cervical, middle, and apical level following implant placement. The follow-up assessment involved longitudinal observation at 3 months following each implant treatment protocol and at 6 months for IIP+IL and IIP+DL protocols.

Results: The influence of different treatment protocols, including the fixed effects of implant groups (IIP+IL, IIP+DL, IIP+UL, DIP+IL, DIP+DL, DIP+UL) and regions (cervical, middle, apical), was examined via a linear mixed model. The IIP+IL group showed a significantly higher myelinated nerve density compared to the IIP+UL and DIP+UL group. Peri-implant nerve re-innervation was significantly higher ($P = .002$) in the apical region compared to the cervical region. After immediate implant placement, the IL group showed a significantly ($P = .03$) higher density of myelinated nerve fibers compared to DL. No significant ($P = .19$) effect of follow-up on nerve density was observed.

Conclusion: The immediate implant placement and loading protocol showed most beneficial effect on peri-implant innervation with highest myelinated nerve density in the apical region. A longer loading time had no influence on the peri-implant nerve density.

KEYWORDS

dental implants, histology, immediate dental implant loading, myelinated, nerve fibers

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1 | INTRODUCTION

The periodontal mechanoreceptors are somatosensory receptors which provide an essential tactile information to refine oral motor behavior.^{1,2} While tooth extraction results in the loss of these receptors causing disruption in the sensory-motor interaction,³ dental implant rehabilitation allows partial restoration of the oral neurosensory function. Edentulous patients rehabilitated with bone-anchored oral prosthesis have been shown to have an improved tactile sensation. This phenomenon of partial tactile function recovery with improved sensory awareness is denoted as “osseoperception”.⁴ While psychophysical evidence confirms tactile threshold recovery following dental implant treatment,⁵⁻⁷ there is still a lack of evidence concentrating on the source of origin of these receptors.^{1,8}

Several histological studies evaluating the bone healing process following dental implant placement have verified the presence of mechanoreceptors and their associated nerve fibers in the gingiva, mucosa, muscle, and periosteum.⁹⁻¹¹ Nevertheless, nerve fibers found in the peri-implant region play the most vital role in restoration of the sensory function. The nerve density in peri-implant bone is less than that of the alveolar bone around natural teeth.¹² Previous animal experiments and clinical studies have shown that different implant placement and loading protocols exert a differential effect on the peri-implant nerve fiber regeneration.^{13,14} Some histological studies have indicated that the immediate implant placement and loading protocol shows a higher myelinated nerve fiber density compared to delayed and unloaded implants,¹⁴ while others have found no significant difference.¹³ Evidence also suggests inconsistent findings in relation to follow-up time after loading. Some studies indicated an increased nerve density following longer follow-up periods, whereas others suggested decreased number and density of nerve fiber following loading.^{15,16}

To address the ambiguity related to previous findings, the primary aim of this study was to histomorphometrically evaluate the impact of different implant placement and loading protocols on the myelinated nerve fibers density in peri-implant trabecular bone tissue. The secondary aim was to assess the effect of follow-up on peri-implant nerve density. The null hypothesis was that the nerve fibers density would be similar, regardless of the implant placement and loading protocol, and a longer follow-up period would show no difference in nerve density.

2 | MATERIAL AND METHODS

The study protocol was approved by the ethical committee of Dalian Medical University (Protocol No. 21100370000896). A randomized design using six dental implant treatment protocols in 10 healthy adult male beagle dogs was used (Figure 1). Randomization was performed using the Rand function in Microsoft Excel (Microsoft Excel 2016, Microsoft Corp.) To reduce the number of animals used in the experiment, a sample size calculation was carried out based on a previous study on dogs with similar design.^{14,17} A priori power analysis

in G*power 3.1 suggested a minimum sample size of 4 specimens per treatment protocol when assuming 80% power and a significance level of 5% ($\alpha = .05$). All dogs (weight: 10.0-14.0 kg) were individually housed and were free of any oral lesion or systemic disease. The dogs were fed according to the general feeding program at the Experiment Animal Center of Dalian Medical University, China.

2.1 | Surgical procedure

The dogs received 1 week of intramuscular prophylactic antibiotic (gentamicin sulfate 1 600 000 U/d, Lingrui Pharmaceutical Co., Ltd.). A 12-hour fasting period was applied to prevent possible vomiting during surgery. All surgical procedures were performed under general anesthesia with application of xylazine hydrochloride (Lumianlin 0.1 mL/kg, Changchun Military Academy of Medical Sciences, Changchun, China) and local anesthesia (2-4 mL lidocaine 2% with epinephrine 1:100 000, Tianjin Pharmaceutical Co., Ltd) at the surgical site. Following tooth extraction and implant placement, all animals were administered with intramuscular (IM) antibiotics (gentamicin sulfate 1 600 000 U/d, Tianjin Pharmaceutical) and ibuprofen (IM, 5-8 mg/kg, Tianjin Pharmaceutical) for a period of 3 days to control post-operative infection and pain.

Following anesthesia, bilateral extraction of mandibular 2nd, 3rd, and 4th premolar and 1st molar was performed with closure of the extraction site using a 5.0 resorbable suture under sterile conditions. Periapical radiographs were taken before tooth extraction to observe the root shape, after tooth extraction to ensure that no roots tips were left behind and following implant placement to confirm correct positioning of dental implant. Sixty-eight titanium implants (3.8 mm × 8 mm for 2nd premolars, 3.8 mm × 10 mm for 3rd and 4th premolars, 4.5 mm × 12 mm for 1st molars, DIO implant system, Dong Seo Ltd. company) were randomly assigned to one of the six treatment protocols (Figure 1): (a) immediate implant placement and immediate loading (IIP+IL); (b) immediate implant placement and delayed loading (3 months after implant placement) (IIP+DL); (c) immediate implant placement and unloaded (IIP+UL); (d) delayed implant placement (3 months after extraction) and immediate loading (DIP+IL); (e) delayed implant placement (3 months after extraction) and delayed loading (DIP+DL); and (f) delayed implant placement (3 months after extraction) and unloaded (DIP+UL). Table S1 describes the random allocation of treatment protocol for each site in individual dogs.

One experienced surgeon (HZ) performed the surgical procedure. All implant sites were prepared with a starter drill followed by drill depths of 2.5 mm × 8 mm and 3 mm × 8 mm. The final preparation was made with 3.5 mm and 4.2 mm width increasing drills. Implants were placed using a low-speed protocol (800 rpm) while being cooled with sterilized saline. A torque of 45-50 N/cm was applied to ensure a good primary stability with an implant shoulder at the marginal bone level. Nickel-titanium (NiTi) alloy-based crowns were placed on the loading site using resin cement (RelyX, Unicem, RX, 3M ESPE). The crowns involved in the immediate loading

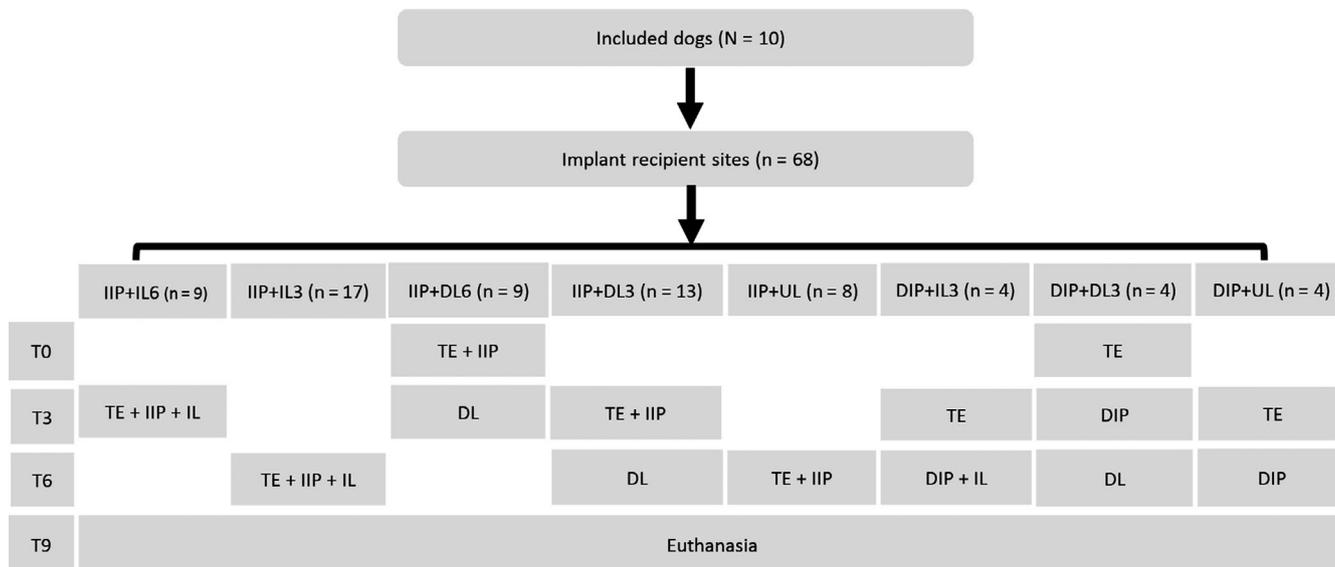


FIGURE 1 Flowchart of the implant placement and loading protocols. DIP, delayed implant placement; DL, delayed loading; DL3 or IL3, 3 mo loading protocol; DL6 or IL6, 6 mo loading protocols; IIP, immediate implant placement; IL, immediate loading; T0, baseline; T3, 3 mo; T6, 6 mo; T9, 9 mo; TE, tooth extraction; UL, unloaded

protocols were set within 2 hours and for the delayed loading protocols 3 months after implant placement. For the dogs having a delayed loading treatment protocol, the restoration procedure was performed under general anesthesia with the application of xylazine hydrochloride (Lumianlin 0.1 mL/kg, Changchun Military Academy of Medical Sciences) 3 months after implant placement.

In the group IIP+IL3 and DIP+IL3, the implant was placed and loaded at 6 months. The IIP+DL3 and DIP+DL3 treatment protocols involved implant placement at the third month and loading at sixth month. In group IIP+UL and DIP+UL, the implant was placed at the sixth month without loading. In group IIP+IL6, the implant was placed and loaded at the third month, whereas, in group IIP+DL6, the implant was placed at the baseline time point and loaded at third month. All animals were euthanized at the same time point of 9 months. The aforementioned treatment strategies at various time points before euthanization allowed the follow-up assessment at a 3-months period for six treatment protocols (IIP+IL3, IIP+DL3, IIP+UL, DIP+IL3, DIP+DL3, DIP+UL) and 6-months only for two treatment protocols (IIP+IL6 and IIP+DL6).

2.2 | Histological preparation

All animals were euthanized with an excess amount of xylazine hydrochloride intramuscularly and immediately perfused with 4% paraformaldehyde and 0.0125% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4) through the external carotid arteries. The jawbones were removed, defleshed, and trimmed into bone blocks with a bone width of 3–5 mm from the dental implant surface in the mesial and distal regions. Samples were decalcified in 0.5 mol/L ethylenediaminetetraacetic acid (EDTA) and phosphate-buffered saline (pH 7.4) for 10 months at 4°C; thereafter, the implants were easily removed

using surgical forceps. The specimens were neutralized with 5% sodium sulfate, dehydrated by a graded series of increasing ethanol concentration, and were then embedded in paraffin. Tissue blocks were serially cut into 4–6 µm thick sections using a microtome in a vertical plane perpendicular to the long axis of the implant-removed socket. Sections were stained with hematoxylin and eosin staining (H&E staining).

2.3 | Histomorphometric analysis

Each bone block was separated into three parts (cervical, middle, apical), and three serial sections from each sample were digitized using MiraxScan (Carl Zeiss) at ×100 magnification. All digital slide files were viewed on a 17-inch LCD monitor (Dell) using an image software package (Panoramic Viewer) at three root levels (1, 5, and 9 mm from the apex, respectively). One experienced observer, who was blinded to treatment protocol allocation, analyzed the myelinated nerve fibers density (N/mm², number of myelinated nerve fibers per area) with an automated digital image analysis system linked to histomorphometry software (Leco Instruments) following the protocol based on a previous study.¹⁸ This system displayed the microscopic image on a video monitor calibrated to 0.125 mm/pixel. Each section was measured three times at an interval of 1 week between measurements, and a mean value was generated for minimizing the intra-observer variability. The histomorphometric analysis of myelinated nerve density was based on a protocol previously described by Huang et al,¹⁴ which involved assessment of a 300 µm wide peri-implant zone at 2 mm height for three regions of interests (ROIs), that is, cervical, middle, and apical levels (Figure 2). A 300 µm wide peri-implant zone was selected as this region is most widely influenced by the dental implant's loading transmission.¹⁹ The single

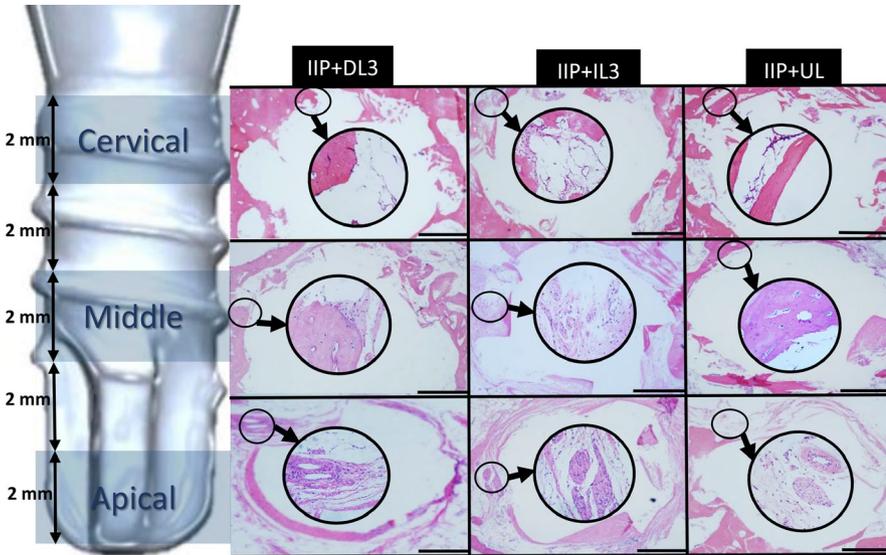


FIGURE 2 Illustration of comparative histological analysis for three treatment protocols. The histological slices present myelinated nerve fibers magnified (×20) in the circle (black). The Bar is 500 μm

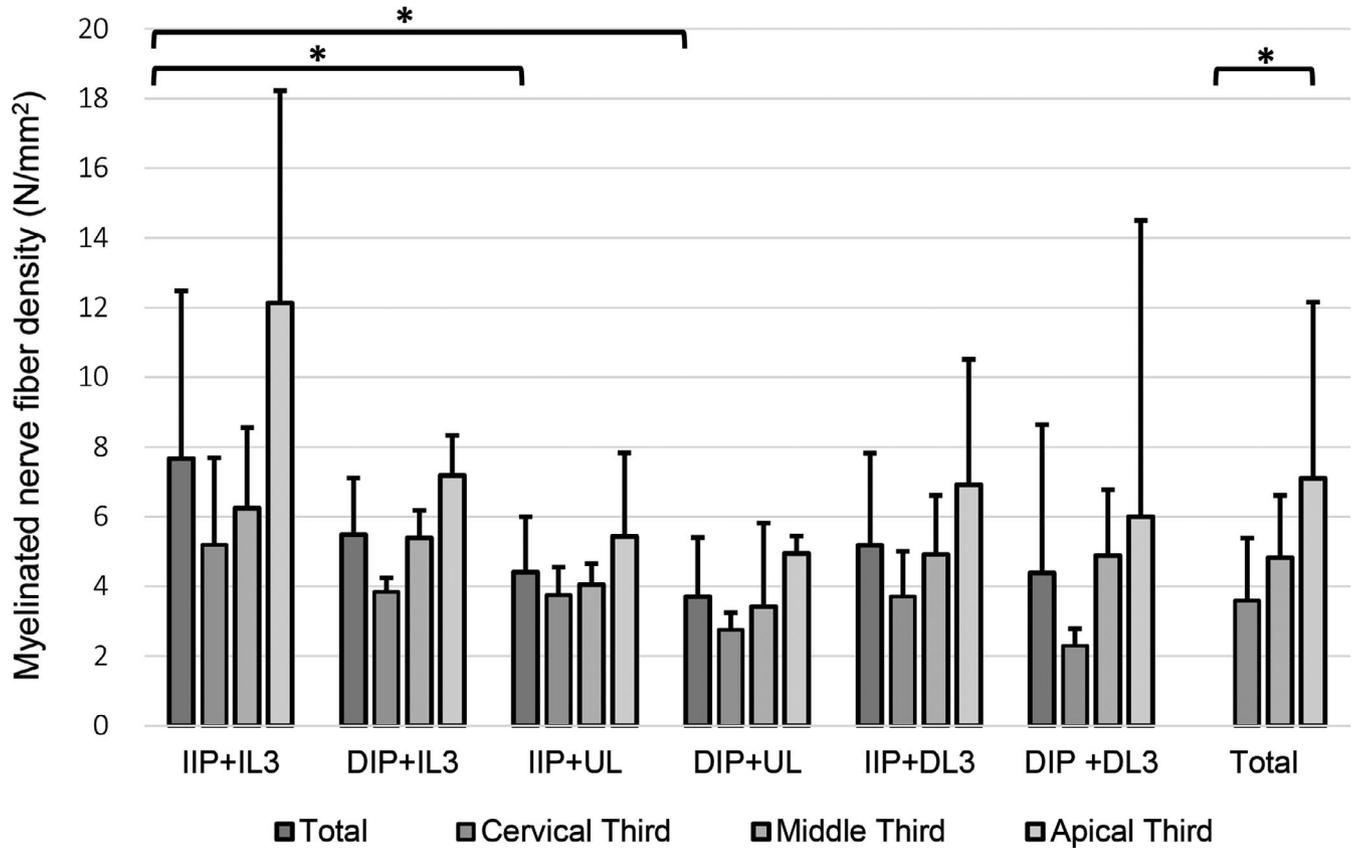


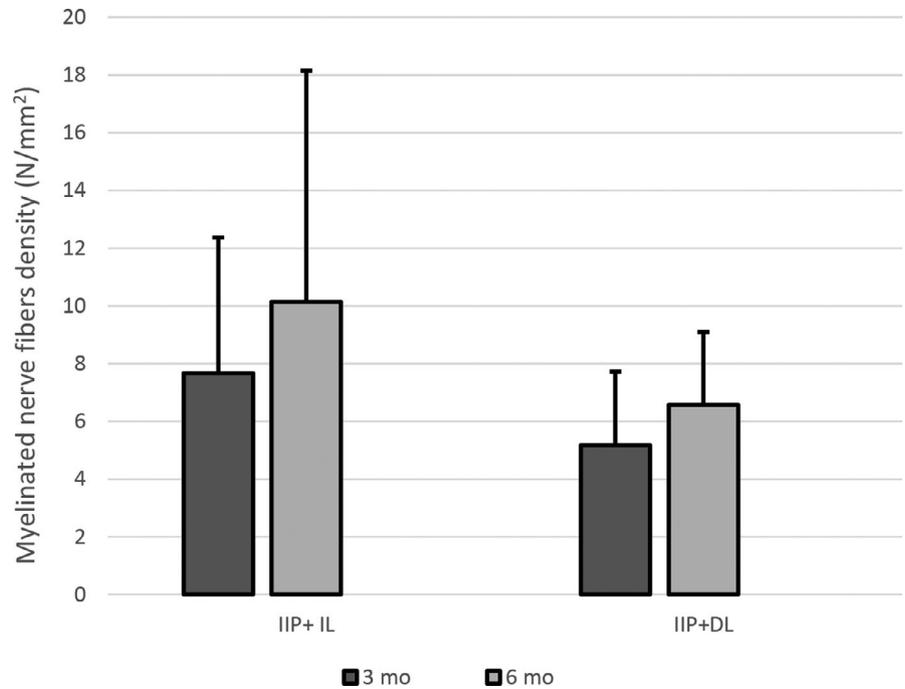
FIGURE 3 Chart showing myelinated nerve fiber density of treatment protocols in relation to region of interest. **P* < .05 shows the significant difference

myelinated nerve fibers were measured, which always existed individually and were situated mostly in the peri-implant bone. A single myelinated nerve axon was defined as the nerve axon around which there was no more than one axon in a 20 μm range. The partial fibers at the borderline of an ROI were excluded. An interleaved gap of 1 mm for the 8 mm implants and 2 mm for the 10 mm implants between the 3 ROIs was set.

2.4 | Statistical analysis

Two separate linear mixed models were defined to fully consider the effect of the randomized design. The first model was applied to examine the influence of the different treatment protocols, including the fixed effects of implant groups (IIP+IL, IIP+DL, IIP+UL, DIP+IL, DIP+DL, DIP+UL), regions (cervical, middle, apical), their two-way interaction

FIGURE 4 Mean density of myelinated nerve fibers at 3- and 6-month follow-up in IIP+IL and IIP+DL groups. DL: delayed loading; IIP: immediate implant placement; IL: immediate loading.



effect, and the random effects of dogs. The second model evaluated the effect of follow-up, including group (IIP+IL, IIP+DL), follow-up time (3 months, 6 months), their two-way interaction effect, and the random effect of dogs. Bonferroni-corrected post hoc *t* tests were used to examine significant main and interaction effects. All statistical testing was performed in SPSS (IBM) at a significant level of $\alpha = .05$.

3 | RESULTS

All animals remained in good health throughout the whole experimental period. Six implants failed due to local peri-implantitis (4 implants: IIP+DL3 group, 1 implant: IIP+DL6 group, 1 implant: IIP+IL3 group) and were excluded from further analysis.

3.1 | Effect of implant placement and loading protocols

There was a main effect of treatment protocol ($F = 4.1$; $P = .01$) and region ($F = 6.9$; $P = .002$; Figure 3). Post hoc test showed significant differences between the IIP+IL3 group and IIP+UL ($P = .02$), and DIP+UL ($P = .010$; Figure 3). Myelinated nerve fiber density was significantly higher in the apical region compared to the cervical region ($P = .002$; Figures 2 and 3). There was no interaction between treatment protocol and region ($P = .77$).

3.2 | Effect of follow-up after IIP+IL and IIP+DL

Myelinated nerve density after immediate loading was significantly higher compared to delayed loading ($F = 5.0$; $P = .03$; Figure 4). The

immediate loading group showed a 53% higher amount of myelinated nerve fibers compared to delayed loading after immediate implant placement. While there was a tendency that the myelinated nerve fibers density increased slightly over time in both IIP+IL group and IIP+DL group (Figure 4), no significant difference was found between 3 and 6 months of follow-up ($F = 1.7$; $P = .19$).

4 | DISCUSSION

The present study was carried out to assess the effect of different implant placement protocols on nerve density at the cervical, middle, and apical peri-implant region. In addition, the influence of follow-up period after loading was also investigated in a randomized controlled animal experiment. These findings might be of importance for further understanding the underlying mechanism of osseoperception and for guiding a treatment design. In our study, immediate implant placement and loading protocols showed increased peri-implant re-innervation with highest nerve density in the apical region, and a longer follow-up led to an increase in density of myelinated nerves. Therefore, the null hypothesis was rejected.

In order to minimize the potential bias related to implant position and dynamic loading forces among experimental animals, a randomized design was applied. Each of the six treatment protocols was randomly distributed in both anterior and posterior mandibular region. Following 10 months of complete decalcification process, the presence of myelinated nerve fibers in peri-implant bone was confirmed. The myelinated nerve fibers were histologically observed as rounded structures with dark-blue color at the periphery and light blue at the center, concentrated under the screw thread, bone marrow, and haversian canals in the ROIs.

In the present study, density of myelinated nerve fibers at a region of 300 μm from the implant interface was found to be highest in the IIP+IL group, while lowest in DIP+UL group. These results suggested that the immediate implant placement and immediate loading were more beneficial in relation to improved regeneration of nerve fibers compared to delayed implant placement and delayed loading. Our findings were in accordance with Huang et al's study, which also showed increased mean nerve density of myelinated axons in the IIP+IL group compared to DIP+DL and DIP+IL protocols.¹⁴ The loaded group showed increase in nerve fibers compared to the unloaded group which was also consistent with the previous findings.^{13,19} Our findings suggested that the ability of the nerve fibers regeneration is linked to the time point of when the implant is inserted and the prosthesis is loaded.

Previous studies showed high concentration of nerve fibers in the apical region of natural teeth, as the periodontal ligament in this region receives the most loading.^{18,20} Based on this fact, our findings suggested increased nerve fiber density in the apical region of dental implant. Wada et al¹³ also found proliferation of the neurofilament protein (NFP)-positive nerve fibers in apical region of implant.

The present study indicated no beneficial effect of longer post-experimental loading time on the density of nerve fibers. Although the density of nerve fibers increased slightly at 6-month follow-up, there was no significant difference when compared with 3-month follow-up. The re-innervation in the peri-implant bone after 3 months tended to be stable. This outcome was consistent with the findings of Zhu and Lin,²¹ which also showed an improvement of nerve fiber regeneration within 3-month post-loading. This evidence proved that early implant stimulation was beneficial for peri-implant nerve fibers regeneration. Upon implant loading, the signals from activated nerves and/or dedifferentiated Schwann cells might promote the regeneration of peri-implant nerve fibers.

The limitations of the present study included failure of six dental implants due to peri-implantitis and non-osseointegration during the healing period. The most susceptible site for implant failure was second premolar region potentially related to a thin cortex. However, based on a randomized design with implant recipient site and protocol allocation, implant failure did not exert a negative effect on the results.

It should also be mentioned that the present study assessed neurohistology, but this does not enable us to link the current histological findings to the function and origin of the nerve fibers. Further studies should focus on discovering the origin of the regenerating nerve fibers and combining the accumulated neurophysiological, psychophysical, clinical, and histological evidence to better understand the neurosensory function as a keystone in osseoperception, oral function, and neurophysiological integration of the implant in the bone.

In conclusion, while immediate implant placement resulted in increased nerve fiber density, immediate loading contributed to a further increase as compared to delayed implant loading. Also, peri-implant innervation had the highest myelinated nerve density in the apical region. No significant difference was observed between nerve densities and between 3- and 6-month follow-up of implant loading.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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