Received: 16 November 2021 | Revised: 7 April 2022 | Accepted: 10 May 2022

DOI: 10.1111/joor.13338

ORIGINAL ARTICLE

JOURNAL OF ORAL REHABILITATION WILEY

Distribution of mature and newly regenerated nerve fibres after tooth extraction and dental implant placement: An immunohistological study

Jian Li[1] | Shenghao Xue[1] | Zhongning Liu[1] | Dongyuan Yao[2] | Ting Jiang[1]

[1]Department of Prosthodontics, Peking University School and Hospital of Stomatology & National Center of Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Research Center of Oral Biomaterials and Digital Medical Devices & Beijing Key Laboratory of Digital Stomatology & Research Center of Engineering and Technology for Computerized Dentistry Ministry of Health & NMPA Key Laboratory for Dental Materials, Beijing, China

[2]Neurological Institute of Jiangxi Province and Department of Neurology, Jiangxi Provincial People's Hospital, Nanchang Medical College, Nanchang, China

Correspondence
Jian Li and Ting Jiang, Department of Prosthodontics, Peking University School and Hospital of Stomatology & National Center of Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Research Center of Oral Biomaterials and Digital Medical Devices & Beijing Key Laboratory of Digital Stomatology & Research Center of Engineering and Technology for Computerized Dentistry Ministry of Health & NMPA Key Laboratory for Dental Materials, Beijing, China.
Email: pkujianli@126.com (J.L.); jt_ketizu@163.com (T.J.)

Funding information
National Natural Science Foundation of China, Grant/Award Number: 81100771 and 81771045

Abstract

Background: The time-dependent peri-implant innervation needs to be elucidated in detail.

Objectives: To examine the distribution of mature and newly regenerated nerves around the implant with immunofluorescence during 28-day follow-up after implantation.

Methods: 35 male Sprague–Dawley rats were grouped into non-operated (n = 5), extraction (n = 5) and implant (n = 25) groups. For rats in the extraction and implant groups, three right maxillary molars were extracted. One month later, a titanium implant was placed into the healed alveolar ridge in the implant group. The implant group was further divided into 5 subgroups according to day 1, 3, 7, 14 or 28 after implantation, on which day serial histological sections were prepared for immunohistochemistry. On day 28, the serial sections were also prepared in the non-operated and extraction groups. Soluble protein-100 and growth-associated protein-43 were used to immunolabel mature and newly regenerated nerve fibres, respectively.

Results: In the peri-implant soft tissues, the number of both mature and newly regenerated nerves showed an increasing trend in 28 days. In the bone tissues, the number of mature or newly regenerated nerves in both areas at less than 100 μm and 100–200 μm from the implant surface on day 28 grew significantly compared with that on day 1 or 3. In addition, the closest distance from mature nerves to the implant surface decreased evidently.

Conclusion: The number of peri-implant nerves increased in 28 days since implantation. The innervation in the soft tissue took place faster than in the bone tissue. The mature nerves in the bone tissue approached the implant gradually.

KEYWORDS
dental implants, follow-up, mature nerves, newly regenerated nerves, peri-implant innervation

Jian Li and Shenghao Xue contributed equally to this study.

1 | INTRODUCTION

Periodontal mechanoreceptors provide essential tactile information to refine oral motor behaviours and play a key role in oral function.[1] After tooth extraction, due to the loss of these receptors and consequent disruption in the sensory-motor interaction, oral movement behaviours and natural biting function may be changed.[2] However, the patients with implant-supported prostheses not only felt comfortable wearing them, but also obtained an improved tactile sensation.[3] Neurophysiological studies have observed the activation of the primary sensorimotor cortex upon dental implant stimulation.[4,5] And clinical observations on patients with osseointegrated dental implants have confirmed a special type of sensory perception skill named 'osseoperception'.[6] Since the term 'Osseoperception' was coined by Prof. P-I Branemark, numerous studies have defined it in various ways. Now it has been generally accepted that osseoperception of implants not only originates from the peripheral nervous system[7] but also from the cerebral cortex.[8]

However, it was well reported that well-osseointegrated implants failed due to excessive biting force. The reason has already been brought to light that the tactile sensibility of oral implants is only 1/50 ~ 1/10 of natural teeth.[9,10] Without evidence on the source or the origin of osseoperception, improvement and enhancement of tactile sensibility of endosseous implants would be difficult. In the past decades, many studies endeavoured to investigate the biological basis of osseoperception in the peripheral nervous system, it was generally believed it rooted in two parts[11]: (i)activation of local receptors in the peri-implant tissues, such as bone, periosteum and soft tissue or (ii) activation of much remoter receptors, including the joint capsule, or the tension receptors of the masticatory muscles. So far, most studies have focused on peri-implant nerve fibres in soft tissue or bone tissue. The nerve fibres were revealed as histological evidence for osseoperception in the vicinity of osseointegration or the peri-implant epithelium through different biomarkers, such as protein gene product 9.5 (PGP9.5),[12] neurofilament protein (NFP) and soluble protein-100 (S100).[13] In addition, nerve fibres were identified to be densely populated in the peri-implant crestal gingiva and apical region, but less in the woven bone and osteons near the implant threads.[14] Moreover, both myelinated and unmyelinated nerve fibres were identified inside the Haversian canals under the screw threads, and the closest distance from the nerves to the implant surface was only 56 μm in a study that investigated nerve fibres around osseointegrated implants in humans.[15] Besides, the tactile-sensory ability of the soft tissues (gingiva and keratinised mucosa) around the implant was less than that of natural teeth but better than that of edentulous area,[16] which indicated there might be newly regenerated nerve fibres in the soft tissue around the implant. The newly regenerated nerve fibres could release neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P. The peptidergic nerve fibres coordinated the reconstruction of peri-implant microenvironments and affected the immune activity as well.[17,18]

Therefore, a hypothesis coming very naturally, the dental implants trigger for neural regeneration. Nevertheless, the quantitative observation of time-dependent changes of peri-implant innervation during the whole osseointegration process is rarely addressed or characterised histomorphologically. Hence the primary aim of the study was to observe the time-dependent changes of mature nerves and newly regenerated nerve fibres around implants in both soft tissues and bone tissues and to investigate their role in the development of osseoperception.

2 | MATERIAL AND METHODS

2.1 | Animals, study groups

All protocols and experimental procedures were approved by the Animal Care and Use Committee of Peking University Health Center (Beijing, China) in accordance with the Guide for the Care and Use of Laboratory Animals of the National institutes of Health (licence number: LA2017141).

A total of 35 male Sprague–Dawley rats (Vital River Laboratory Animal Technology, Beijing, China) weighing 200 (±10) g on arrival were housed individually in a temperature 22 (±1)°C and humidity 50 (±5)% controlled environment on a 12-h light/dark cycle. After 7 days of acclimatisation, rats were randomly allocated into 3 main study groups namely non-operated group ($n = 5$), extraction group ($n = 5$), implant group ($n = 25$). The rats in the non-operated group received no treatment, in the extraction group had the right maxillary molar teeth extracted, healed naturally without further surgery, and in the implant group had the right maxillary molar teeth extracted and 1 month later received a second surgery involving the insertion of a dental implant into the healed extraction site of the right maxillary molar tooth, respectively. Since teeth extraction or implant surgery might impose some discomfort while biting hard diet, rats received mashed chow to ensure adequate feed intake in addition to water ad libitum. Animals were monitored daily to assess body weight, food consumption, general behaviour and any postoperative complications such as bleeding, inflammation as well as excessive grooming, scratching or biting of body parts.

Setting up a time baseline on the day of implants insertion, the implant group was divided into 5 subgroups according to day 1, 3, 7, 14 or 28 since the implant insertion day. Rats in each subgroup ($n = 5$) were sacrificed on a planned specific time point (day 1, 3, 7, 14, 28), which referred to key points of the osseointegration process based on previously published research.[19,20] Both the non- operated group and the extraction group were sacrificed on day 28.

2.2 | Anaesthesia

All surgical procedures were performed under general pentobarbital(1% , 0.5 ml/100 g)anaesthesia supplemented with local anaesthesia (0.1 ml, 2% lidocaine in 1 :100 000 epinephrine) of teeth and adjacent soft tissues. A heating pad was used to maintain the rat's body temperature at 37–37.5°C.

2.3 | Molar extraction and implant placement

The molar extraction and implant surgery were carried out under aseptic conditions. The gingival tissue around three right maxillary molars was detached with a probe and the teeth were luxated using Ventura forceps. One month later, prior to the implant surgery, the extraction sites were evaluated on gross examination for adequate soft tissue healing. Then, in the rats of the implant group, the healed edentulous alveolar crest was surgically exposed with a scalpel, then a bone cavity was prepared with a dental low-speed drill into the healed extraction site of the maxillary right first molar, and a titanium mini-screw implant (MCTBIO, Korea, MI; 1.2 mm in diameter, 3 mm in length) was inserted into the cavity. Primary implant stability (a prerequisite for successful bone healing around dental implants) was confirmed manually. The smooth cervix and head of implant was remained non-submerged and occluded with the mandibular first molar. Bilateral occlusion was checked to verify the existence of occlusal contact between the implant and the opposed tooth, as well as the occlusal contact on the contralateral side. Since increased occlusal load would interfere with bone healing around the dental implant, maxillomandibular occlusal contact was checked clinically every other day after placement to ensure no excessive force occurred on the implant.

Buprenorphine hydrochloride (0.05 mg/kg) and ketoprofen (5 mg/kg/d) were administered subcutaneously every 8–12 h during the first 3 postoperative days to reduce pain and inflammation. The implant stability or loss was checked clinically every other day in awake restrained rats. The rats with a mobile or lost implant were excluded from the study and new rats were supplemented.

2.4 | Animal euthanasia and histology

On euthanasia day, the rats were executed by excessive carbon dioxide. The entire maxilla was separated carefully. In the implant group, the specimen blocks with a thickness of 5 mm consisting of implants, peri-implant bone and gingiva tissue were retrieved and immersed in 10% formalin at 4°C for 24 h and then in 20% ethylenediaminetetraacetic acid (EDTA) at 37°C for 2 months to be fully decalcified, allowing the implants to be easily removed from the specimens using a surgical forceps. Following being dehydrated and embedded in paraffin, 4 μm thick sections were prepared serially in the mesial–distal direction along the central axis of the implant (Figure 1). Likewise, 4 μm thick sections were prepared serially for histomorphometry at the comparable position in the extraction socket in the extraction group and along the central axis of the natural tooth in the non-operated group (Figure 1).

2.5 | Immunohistochemistry of nerve fibres within the peri-implant area

The serial sections were dehydrated in gradient alcohol after deparaffinisation. Subsequently, the sections were immersed in 10 mmoL/L citrate buffer at 95°C, pH 6.0 for 12 min. Nonspecific binding sites were blocked with 10% normal donkey serum for 1 h. Then, in order to mark mature or newly regenerated nerve fibres, respectively, the sections were stained with primary antibody rabbit monoclonal S100 (1:100; Abcam, UK) or goat monoclonal growth-associated protein-43 (Gap-43, 1:100; Abcam, UK) (Mature nerves are wrapped by Schwann cells with myelin sheath and axons inside. S-100 can specifically label Schwann cells, to indirectly label mature nerves. Gap43 is a specific marker for germinating axons, so it is used to label newly regenerated nerves.) and incubated overnight at 4°C followed by incubation with fluorescence-conjugated secondary affinipure donkey anti-rabbit IgG (1:100, Proteintech, US) and affinipure donkey anti-goat IgG (1:100, Proteintech, US) for 1 h at room temperature. The sections were then washed in PBS and thereafter mounted on silane-coated slides (Sigma), air-dried and covered with 1–2 drops per slide of fluoromount™ aqueous mounting medium (Sigma, US) and cover-slipped.

Two regions of interest (ROI), that is two fixed 300 μm × 400 μm squares, delimited from each section were included in the analysis of structural characteristics and measurement of the number of nerve fibres. One ROI was in the soft tissue, and the other was in the bone tissue (Figure 1). In the non-operated group, one ROI included junctional epithelium with part of connective tissue below, the other was in the woven bone adjacent to the apical part of the root. In the extraction group, one ROI included keratinised gingival tissue over the extraction socket, and the other was located on the boundary of the extraction socket. In contrast, in the implant group, one ROI included long junctional epithelium, part of the sulcular epithelium and connective tissue below, and the other was in the apical bone region of the implant. Masson staining was also used to observe the bone remodelling around the implant. 10 serial sections were selected in each rat, which were stained with S100 and Gap-43 half and half. A computerised image analysis system (Image-pro plus, Media Cybernetics, USA) was used to measure the number of nerve fibres in two ROIs, and the average number of 5 ROIs in 5 serial sections were counted as the result for the soft tissue or the bone tissue, respectively, of each rat. Then the number of nerve fibres in the soft tissue (Figure 2) or in the bone tissue (Figure 3) was compared, respectively, among five time points in the implant group.

Moreover, in the interest of the time-dependent changes in the closest distance from nerves to the implant surface in the apical region of the implant, in the ROI two areas with the same size of 100 μm × 100 μm were selected (Figure 3), one of which was at less than 100 μm from the implant surface, the other of which was at 100-200 μm from the implant surface. Then a computerised image analysis system (Image-pro plus, Media Cybernetics, USA) was also used to measure the number of nerve fibres in these two areas and the distance from five closest staining-positive areas of mature nerve fibres to the calibration line (indicating the implant surface). In the non-operated group and extraction group (Figure 4), the corresponding measurement was performed with the calibration line indicating the dental root surface or the extraction socket boundary. Five measurement data were aggregated and averaged to indicate the closest nerve-implant distance (CNID) (Figure 5).

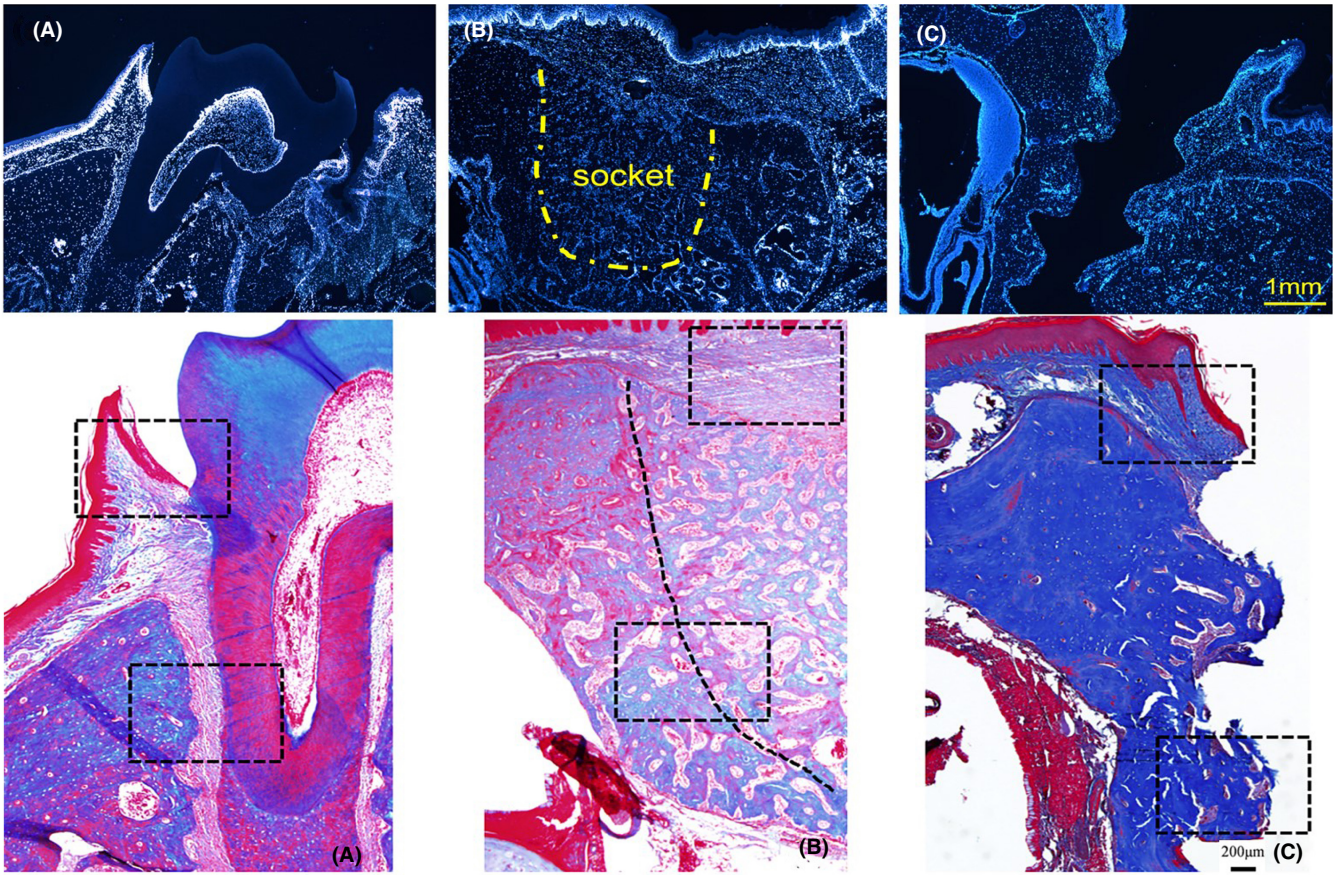

FIGURE 1 The fluorescent staining of the nuclei by DAPI (upper row) and the Masson staining sections showed how to choose the regions of interest (ROIs) (lower row). The serial sections (4 μm thick) prepared in the mesial–distal direction along the central axis of the tooth in the non-operated group (A), the socket in the extraction group (B) and the implant in the implant group (C)

2.6 | Statistical analysis

Experimental data were presented as mean ± standard deviation. The differences in the numbers of mature nerves and newly regenerated nerves in soft tissue or bone tissue among 5 time points in the implant group, and the closest distance from mature nerves to the surface of tooth or implant or the boundary of extraction socket on day 28, were assessed using a dedicated statistical software (SPSS V27.0; IBM). Firstly, the homogeneity of variance was tested using Bartlett's test. If the variance was homogeneous, ANOVA was performed, otherwise Kruskal-Wallis (rank-sum test). Furtherly the differences in the variables between two time points or two groups were tested by SNK (Student-Neuman-Keuls) method. The significance detection level was $p < .05$.

3 | RESULTS

3.1 | Mature nerves and newly regenerated nerves in peri-implant soft tissue

It showed that in the non-operated group, around the natural tooth, there were lots of mature nerve bundles in the connective tissue under the sulcular epithelium and periodontal ligament with some newly regenerated nerve fibres scattering around (Figure 4). But in the extraction group, there were a few residual mature nerve fibres in the extraction socket, but few newly regenerated nerve fibres on the day 28, nearly 2 months after teeth extraction (Figure 4).

After implantation, the number of newly regenerated nerve fibres in peri-implant soft tissue (long junction epithelium together with part of connective tissue below) was significantly increased on day 14 compared with day 1, 3 and 7. Also, the number of both mature nerves and newly regenerated nerves showed significant increase on day 28 compared with day 1, 3, 7 and 14 (Figure 2). Meanwhile, after merging the staining pictures of mature or newly regenerated nerves of the same selected view, it was clearly found that the newly regenerated nerves in the soft tissue were always closer to the implant surface than the mature (Figure 2).

3.2 | Mature nerves and newly regenerated nerves in peri-implant bone tissue

In the bone tissue, at the early stage after implant placement (day 1 and 3), there were some nerve fibre bundles beside the

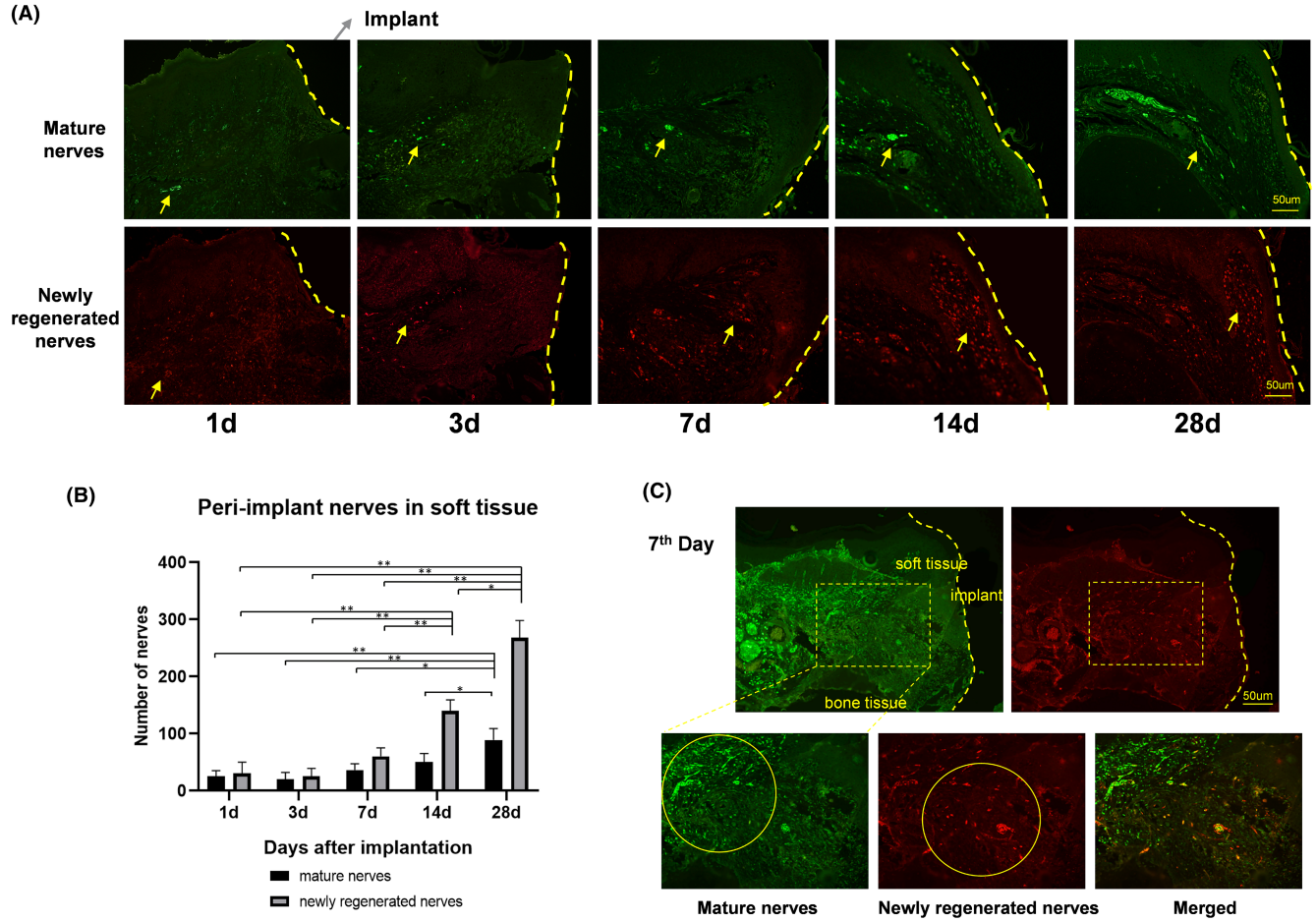

FIGURE 2 (A) Distribution of mature and newly regenerated nerves in the soft tissues on five time points after implantation. The region of interest (ROI) in peri-implant soft tissue, a fixed 300 μm × 400 μm square, included long junctional epithelium, part of the sulcular epithelium and connective tissue below. (B) The time-dependent changes in the number of peri-implant nerves in soft tissues. On day 28, the number of both mature and newly regenerated nerves increased significantly compared with that on day 1, 3, 7 or 14. The number of newly regenerated nerve fibres also increased significantly on day 14 compared with that on day 1, 3 and 7. Data are shown as mean ± SD; '*' means $p < .05$; '**' means $p < .01$. (C) The stained mature or newly regenerated fibres in the implant group on day 7. There were few mature nerves in the connective tissue near the long junction epithelium, while many newly regenerated nerves scattered in this area and were closer to the implant surface

thread of implant, with some newly regenerated nerve fibres scattered around. As the bone remodelling progressed, the closest distance from the mature nerves to the implant surface showed a decreasing trend: on day 1 and 3, it was 100–200 μm; but on day 28, mature myelinated nerve fibres with a diameter of 7–12 μm could be seen at 15–25 μm from the implant surface (Figure 3).

In the area at less than 100 μm from the implant surface, the quantity of mature and newly regenerated nerves gradually increased over time within 28 days (Figure 3). In both areas at a distance of less than 100 μm or 100–200 μm, on day 28, the number of both mature and newly regenerated nerves increased significantly compared with those on day 1 or 3. On day 14, the amount of both kinds of nerves in both areas increased significantly compared with that on day 1. In the area at a distance of 100–200 μm, on day 14, the amount of both kinds of nerves also increased significantly compared with that on day 3. In the area at a distance of less than 100 μm, on day 7 or 14, the number of newly regenerated nerves increased significantly compared with that on day 3.

Finally, looking into the closest distance from mature nerves to the surface of tooth or implant or the boundary of extraction socket on day 28 (Figure 5), the distribution pattern was significantly different among three groups. It could be seen the closest distance was the least in the non-operated group, and farther in the implant group, and the farthest in the extraction group. In the implant group, the mature nerves distributed closely to the implant surface, which was similar to those in the periodontal ligament of natural teeth in the non-operated group, but did not form a regularly layered innervation, which was seen along the surface of natural dental root.

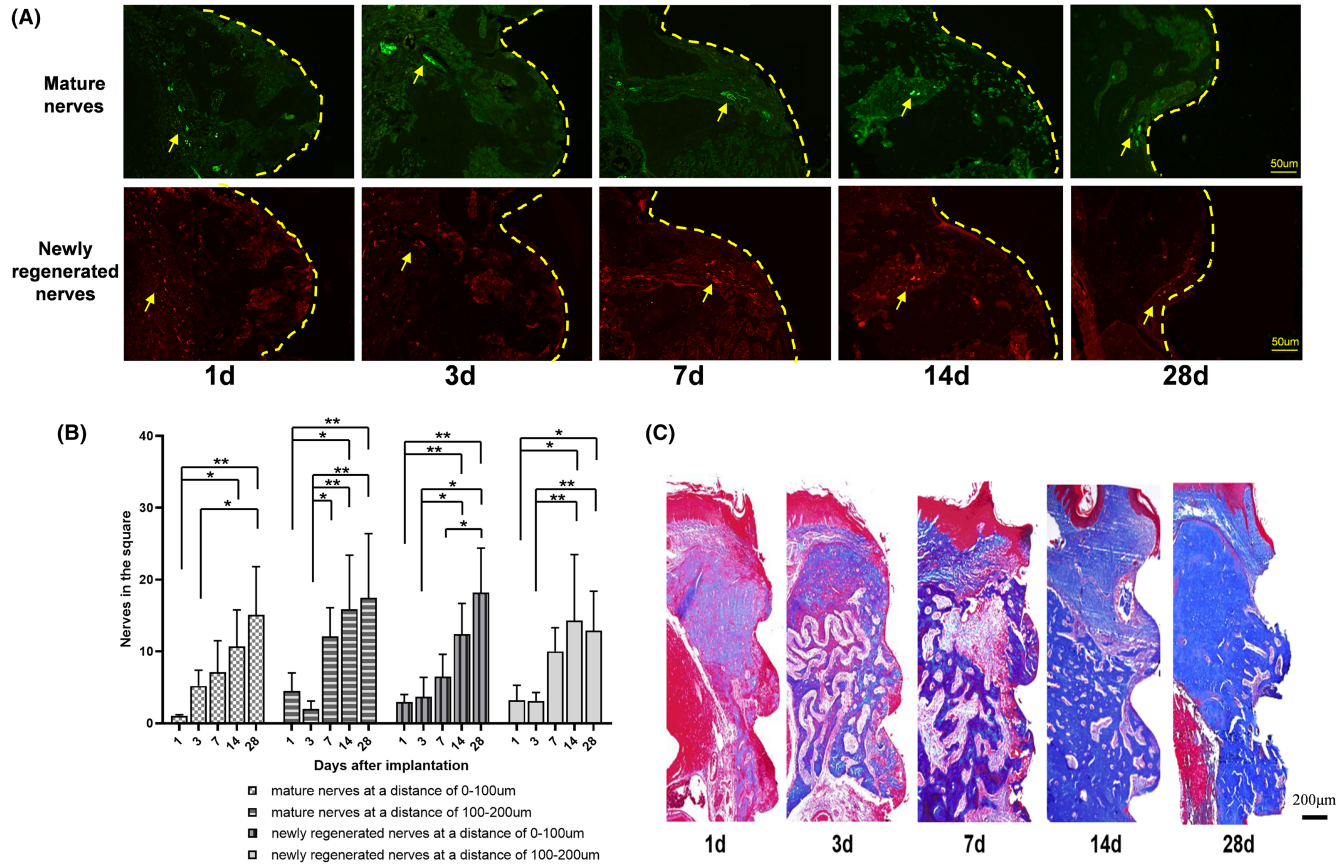

FIGURE 3 (A) Distribution of mature nerves and newly regenerated nerves in bone tissue on different days after implantation. The region of interest (ROI) in peri-implant bone tissue, a fixed 300 μm × 400 μm square, was in the apical region of the implant. Moreover, in the interest of the time-dependent changes in the closest distance from nerves to the implant surface in the apical region of the implant, two areas with the same size of 100 μm × 100 μm in the forementioned square were selected. On day 1 and 3, the closest distance of mature nerve fibre to implant surface was 100–200 μm, but on day 28, mature nerves could be seen at a distance of 15–25 μm from the surface of the implant with some newly regenerated nerves scattered around. (B) The time-dependent changes in the number of peri-implant nerves in bone tissue. In the area at less than 100 μm from the implant surface, the amount of mature and newly regenerated nerves gradually increased over time within 28 days. In two areas at the distance of less than 100 μm and 100–200 μm, on day 28, the number of both mature and newly regenerated nerves increased significantly compared with those on day 1 or 3. On day 14, the quantity of both kinds of nerves in both areas increased significantly compared with that on day 1. In the area at 100–200 μm, on day 14, the number of both kinds of nerves also increased significantly compared with those on day 3. In the area at 0–100 μm, on day 7 or 14, the quantity of newly regenerated nerves increased significantly compared with that on day 3. Data are shown as mean ± SD; '*' means $p < .05$; '**' means $p < .01$. (C) Masson staining of the peri-implant tissue. The surrounding bone gradually remodelled from inflammatory fibrous tissue to mature bone tissue from day the 1 to 28 after implantation

4 | DISCUSSION

Learning the time-dependent changes of peri-implant innervation in soft tissue and bone tissue is believed to be essential to deepen the cognition of tactile sensibility of dental implants. However, the nerve regeneration and distribution in the implant osseointegration process has not been elucidated so far. By analysing the phenomenon of peripheral nerve regeneration of implants, this experiment hoped to explore the origin of osseoperception and laid a foundation for clinical strategies to promote the tactile-sensory ability of implants in near future. The basic data obtained in this experiment made up for the blank of the research on the distribution of peripheral nerve during the process of implant osseointegration, and would have guiding significance for the follow-up experiment.

In this randomised controlled animal experiment, in order to minimise the potential random errors and researchers' bias among experimental animals, the consistency of positions measured and ranges of field counted among groups were strictly followed to ensure strong comparability. The time-axis was limited to 28 days because the osseointegration time of rodents is about 4 weeks.[19]

With a comparison of the distribution of different kinds of nerves at a different distance of less than 100 μm or 100–200 μm from the implant, this research found the nerves moved towards the implant. Both the mature and the newly regenerated nerves approached the implant over time within 28 days. Based on the results from the Masson staining of the peri-implant tissues, it could be speculated that during the bone remodelling process, nerves grew into the inflammatory tissue along with the blood vessels, and participated

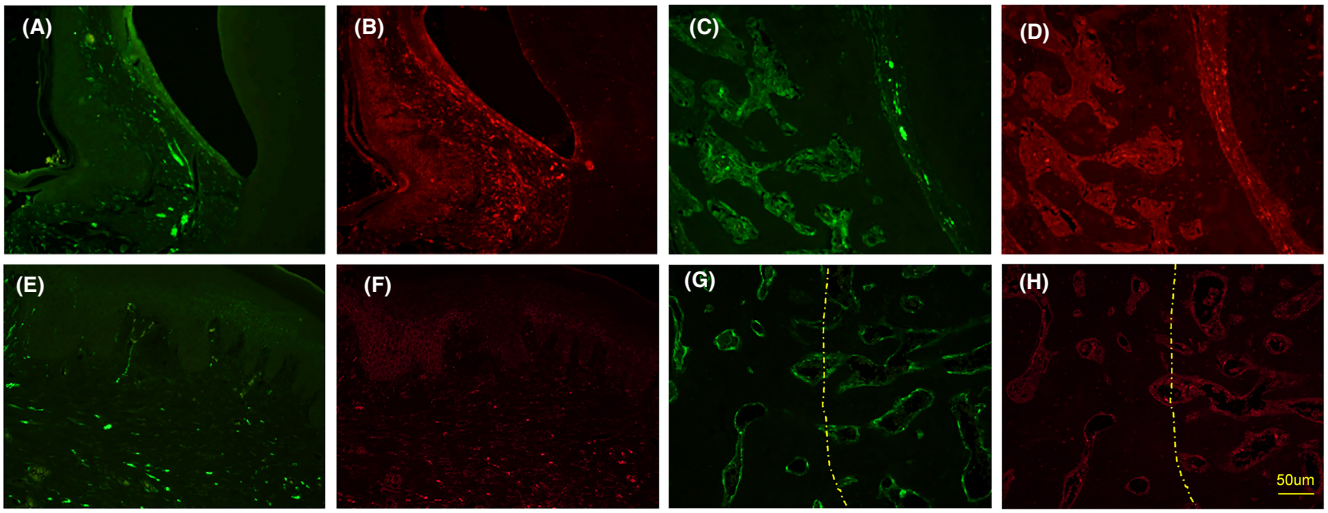

FIGURE 4 Distribution of nerve fibres in the junctional epithelium and the adjacent connective tissue of natural teeth (A, B) and in the bone tissue along the root (C, D). The distribution of nerve fibres in the mucosa (E, F) and in the bone tissue of extraction socket (G, H). A, C, E and G showed the staining of mature nerves (green fluorescence); B, D, F and H showed the staining of newly regenerated nerves (red fluorescence)

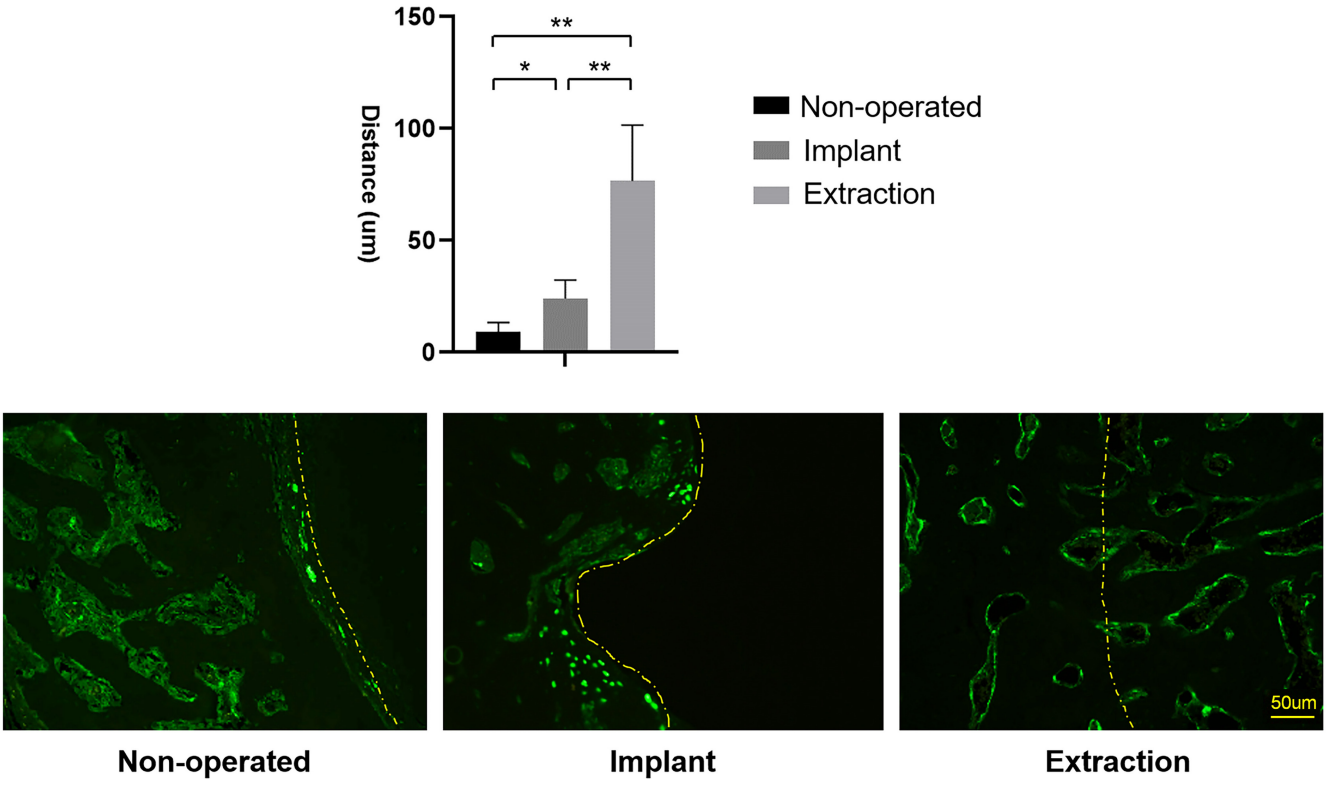

FIGURE 5 Comparison of the closest distance from mature nerves to the surface of tooth or implant or the boundary of extraction socket on day 28. The calibration line is the surface of dental root in the non-operated group, or the surface of implant in the implant group, or the boundary of socket in the extraction group. The closest distance was the least in the non-operated group, and farther in the implant group, and the farthest in the extraction group. In the implant group, the mature nerves distributed closely to the implant surface, which was similar to those in the periodontal ligament of natural teeth in the non-operated group, but did not form a regularly layered innervation, which was seen along the surface of natural dental root

in the remodelling process. Moreover, on day 28 the distribution of nerves around the implant was similar to that in the periodontal ligament of natural teeth but did not form a regularly layered innervation, which was seen along the surface of natural dental root. It was believed that the implant had an impact on the surrounding microenvironment while restoring mastication function and induced

the peripheral nerves to return to their original innervation. During this period, it could be speculated that the bite force conducted from implants played an important role in nerve regeneration, similar to natural teeth.[2]

It has already been reported that there is an increase in peripheral mature nerve fibres during implant healing.[21] Peri-implant nerve re-innervation was also significantly higher in the apical region than that in the cervical region.[14] After immediate implant placement, the immediate loading group showed a significantly higher density of myelinated nerve fibres than the delay loading.[22] In human experiments, myelinated and unmyelinated nerve fibres were identified inside the Haversian canals of the woven bone near the implant threads. Besides, it was assumed that there was also nerve regeneration in soft tissues (gingiva and keratinised mucosa) around implants.[5] For the bone tissue and soft tissue near the thread of osseointegrated implant, nerve fibres with various markers including neurofilament protein,[13] PGP-9.5, calcitonin gene-related peptide, etc. have been reported. These nerve fibres were speculated to be directly involved in the development of osseoperception. Based on the aforementioned results, this study focused on exploring the difference in innervation patterns of peripheral nerves between in the soft tissue and in the bone tissue.

In the soft tissue, there were always several thick nerve bundles around the implant with some newly regenerated nerve fibres scattered around, which were closer to the implant surface than the mature nerves. It could be assumed the newly regenerated nerve fibres probably regenerated from the original mature nerve bundles. But in peri-implant bone tissue, the innervation showed a different pattern. In the early stage of the osseointegration, such as 1st to 3rd day after implantation, there were more fibrous tissue and trabecular bone under the thread of implant, especially in the process of mineralisation of fibrous tissue changing into mature bone, more mature nerves and new nerve fibres scattered around the implant. The mature nerves being situated about 200 μm away from the implant surface were assumed to be residual nerves in the original extraction socket, from which the newly regenerated nerve fibres stained with Gap-43 may regenerate. On day 28, when the osseointegration was about to complete, the bone tissue in the area about 200 μm away from the implant surface became much denser but with fewer mature nerves formed, and accompanied by significantly lessened newly regenerated nerves. At the same time, the mature nerves in the bone tissue gradually approached the thread surface, which were accompanied by some newly regenerated nerves scattered around. Since the implant thread was found to be a concentrated area where the bite force distributed,[23] the relationship between the type of regenerated nerves and original mechanoreceptors in the periodontal tissue still needs further exploration.

Looking into the time-dependent difference of nerves distribution between in soft tissue and in bone tissue, it was obvious that the regeneration of nerves in the soft tissue showed a more regular pattern and took place faster than in the bone tissue. It could be concluded the long junction epithelium and surrounding connective tissue might play a leading role in the establishment of osseoperception. This was of great significance for follow-up research. More concern should be put on the nerve regeneration in peri-implant soft tissue in order to promote implant tactile sensitivity.

The sensory nerve fibres in the periodontal tissue are mainly myelinated type A fibres and unmyelinated type C fibres. As S100-labelled mature nerves in bone tissue around implant had a diameter of 7–12 μm, it was reasonable to speculate they were similar to Aβ fibres, which are a mechanoreceptor of skin tactile pressure.[24] The somatosensory evoked potentials (SEPs) were recorded with a relatively low stimulus acted on the dental implant in the bone, which was believed to be derived from Aβ nerve fibre.[25] Studies have also shown that during the process of osseointegration and nerve regeneration, axons in the bone are gradually myelinated to form mature nerves.[26,27] Since the S100-labelled nerves in the experiment were wrapped in myelin sheath, and the diameter of the nerves fibres was about 10 μm, which was in the range of Aβ fibres, the S100-labelled nerves were speculated to be mature nerves that participated in tactility afference of dental implants.

5 | CONCLUSIONS

This study revealed the innervation in the soft tissue took place faster than in the bone tissue and the mature nerves in the bone tissue approached the implant gradually, based on which some effective strategies would be possible to be developed to boost the sensory tactile ability of implants. At the present stage just from the point of the promotion of nerve growth, it was reasonable to pay close attention to the primary healing of soft tissue after the insertion of implants. The accelerating methods of innervation in the peri-implant soft tissue or bone tissue possess the priority to be studied to ameliorate the rehabilitation results of implant prostheses.

6 | LIMITATIONS

Although the SD rat model with which dental implants are inserted in the residual ridges has already been verified, there is still some difference in jaw bone remoulding after implantation between rats and human beings, which is influenced by bite force distribution.[28] In order to avoid the influence from internal secretion tied up with physiological cycle in female rats, the present results just obtained the data of time-dependent peri-implant innervation from the male SD rats. So, the findings were still of limited convertibility to be used in humans. But the effect of peripheral hormone on peri-implant innervation has raised concern and would be further studied. In addition, large-scale animal studies and longer follow-up periods are needed to confirm the findings.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or in revising it critically for the important intellectual content, and all authors read and approved the final version to be published. All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Jian Li and Ting Jiang conceived the ideas and designed the study; Jian Li, Shenghao Xue and Zhongning Liu collected the data; Jian Li, Shenghao Xue, Ting Jiang and Dongyuan Yao analysed and interpreted the data; and Jian Li and Shenghao Xue led the writing.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (NO. 81100771 and 81771045). The authors are deeply thankful to Ms. Du Ning (Technician of Morphology and Histology, Laboratory Center, Peking University School and Hospital of Stomatology) for her technical assistance in immunofluorescence study.

CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest to this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/joor.13338.

ORCID

Jian Li https://orcid.org/0000-0002-1044-0128

REFERENCES

1. Bakshi PV, Thakur S, Kulkarni S. Perception by osseointegrated dental implants supporting a fixed prosthesis: a prospective study. *Int J Oral Maxillofac Implants*. 2017;32(6):1346-1350.
2. Avivi-Arber L, Lee JC, Sood M, et al. Long-term neuroplasticity of the face primary motor cortex and adjacent somatosensory cortex induced by tooth loss can be reversed following dental implant replacement in rats. *J Comp Neurol*. 2015;523(16):2372-2389.
3. Enkling N, Heussner S, Nicolay C, Bayer S, Mericske-Stern R, Utz KH. Tactile sensibility of single-tooth implants and natural teeth under local anesthesia of the natural antagonistic teeth. *Clin Implant Dent Relat Res*. 2012;14(2):273-280.
4. Yan C, Ye L, Zhen J, Ke L, Gang L. Neuroplasticity of edentulous patients with implant-supported full dentures. *Eur J Oral Sci*. 2008;116(5):387-393.
5. Habre-Hallage P, Dricot L, Jacobs R, van Steenberghe D, Reychler H, Grandin CB. Brain plasticity and cortical correlates of osseoperception revealed by punctate mechanical stimulation of osseointegrated oral implants during fMRI. Eur. *J Oral Implantol*. 2012;5(2):175-190.
6. Klineberg I, Calford MB, Dreher B, et al. A consensus statement on osseoperception. *Clin Exp Pharmacol Physiol*. 2005;32(1-2):145-146.
7. Mishra SK, Chowdhary R, Chrcanovic BR, Brånemark PI. Osseoperception in dental implants: a systematic review. *J Prosthodont*. 2016;25(3):185-195.
8. Avivi-Arber L, Martin R, Lee JC, Sessle BJ. Face sensorimotor cortex and its neuroplasticity related to orofacial sensorimotor functions. *Arch Oral Biol*. 2011;56(12):1440-1465.
9. Weiner S, Sirois D, Ehrenberg D, Lehrmann N, Simon B, Zohn H. Sensory responses from loading of implants: a pilot study. *Int J Oral Maxillofac Implants*. 2004;19(1):44-51.
10. Abarca M, Van Steenberghe D, Malevez C, Jacobs R. The neurophysiology of osseointegrated oral implants. A clinically underestimated aspect. *J Oral Rehabil*. 2006;33(3):161-169.
11. Klineberg I, Murray G. Osseoperception: sensory function and proprioception. *Adv Dent Res*. 1999;13:120-129.
12. Suzuki Y, Matsuzaka K, Ishizaki K, Tazaki M, Sato T, Inoue T. Characterization of the peri-implant epithelium in hamster palatine mucosa: behavior of Merkel cells and nerve endings. *Biomed Res*. 2005;26(6):257-269.
13. Wang YY, Gong P, Zhang J. Effects of platelet-derived growth factor on nerve regeneration around implant in rats. *Hua Xi Kou Qiang Yi Xue Za Zhi*. 2019;37(4):350-354.
14. Huang Y, van Dessel J, Martens W, et al. Sensory innervation around immediately vs. delayed loaded implants: a pilot study. *Int J Oral Sci*. 2015;7(1):49-55.
15. Corpas Ldos S, Lambrichts I, Quirynen M, et al. Peri-implant bone innervation: histological findings in humans. *Eur J Oral Implantol*. 2014;7(3):283-292.
16. Habre-Hallage P, Abboud-Naaman NB, Reychler H, van Steenberghe D, Jacobs R. Perceptual changes in the peri-implant soft tissues assessed by directional cutaneous kinaesthesia and graphaesthesia: a prospective study. *Clin Implant Dent Relat Res*. 2011;13(4):296-304.
17. Xiang L, Ma L, Wei N, et al. Effect of lentiviral vector overexpression α-calcitonin gene-related peptide on titanium implant osseointegration in α-CGRP-deficient mice. *Bone*. 2017;94:135-140.
18. Yamaza T, Kido MA, Wang B, et al. Distribution of substance P and neurokinin-1 receptors in the peri-implant epithelium around titanium dental implants in rats. *Cell Tissue Res*. 2009;335(2):407-415.
19. Fujii N, Ohnishi H, Shirakura M, Nomura S, Ohshima H, Maeda T. Regeneration of nerve fibres in the peri-implant epithelium incident to implantation in the rat maxilla as demonstrated by immunocytochemistry for protein gene product 9.5 (PGP9.5) and calcitonin gene-related peptide (CGRP). *Clin Oral Implants Res*. 2003;14(2):240-247.
20. Irie K, Hara-Irie F, Ozawa H, Yajima T. Calcitonin gene-related peptide (CGRP)-containing nerve fibers in bone tissue and their involvement in bone remodeling. *Microsc Res Tech*. 2002;58(2):85-90.
21. Song D, Liang X, Zheng H, et al. Peri-implant myelinated nerve fibers: histological findings in dogs. *J Periodontal Res*. 2020;55(4):567-573.
22. Song D, Huang Y, Van Dessel J, et al. Effect of platelet-rich and platelet-poor plasma on peri-implant innervation in dog mandibles. *Int J Implant Dent*. 2019;5(1):40.
23. Rand A, Stiesch M, Eisenburger M, Greuling A. The effect of direct and indirect force transmission on peri-implant bone stress - a contact finite element analysis. *Comput Methods Biomech Biomed Engin*. 2017;20(10):1132-1139.
24. Iba K, Yamashita T. Control of bone remodeling by nervous system. Nerve distribution and pain in bone tissue. *Clin Calcium*. 2010;20(12):1793-1799.
25. Van Loven K, Jacobs R, Swinnen A, Van Huffel S, Van Hees J, van Steenberghe D. Sensations and trigeminal somatosensory-evoked potentials elicited by electrical stimulation of endosseous oral implants in humans. *Arch Oral Biol*. 2000;45(12):1083-1090.

26. Naot D, Musson DS, Cornish J. The activity of peptides of the calcitonin family in bone. *Physiol Rev*. 2019;99(1):781-805.
27. Dubový P, Klusáková I, Hradilová-Svíženská I, Joukal M. Expression of regeneration-associated proteins in primary sensory neurons and regenerating axons after nerve injury-an overview. *Anat Rec (Hoboken)*. 2018;301(10):1618-1627.
28. Du Z, Lee RS, Hamlet S, Doan N, Ivanovski S, Xiao Y. Evaluation of the first maxillary molar post-extraction socket as a model for dental implant osseointegration research. *Clin Oral Implants Res*. 2016;27(12):1469-1478.

How to cite this article: Li J, Xue S, Liu Z, Yao D, Jiang T. Distribution of mature and newly regenerated nerve fibres after tooth extraction and dental implant placement: An immunohistological study. *J Oral Rehabil*. 2022;49:796-805. doi: 10.1111/joor.13338