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Original Article

Comparison of neurovascular relationships between human pulp and rat pulp

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Abstract: The purpose of this study was to reveal the relationships of nerves and blood vessels between human and rat. Human premolar teeth, extracted for orthodontic reason, and rat maxilla and mandible were placed in 4% paraformaldehyde. The presences of blood vessels (vascular endothelial growth factor VEGF), nerves (neurofilament heavy NFH) and myelinated nerve fiber (myelin basic protein MBP) were examined in the pulp by using immunohistochemistry (IHC). The pulp region was examined to determine the integral optical density (IOD) of blood vessels and nerves by using fluorescence microscopy. Morphology of human pulp was largely consistent with that of rat molar pulp. Nerve fibers were seen to run along the blood vessels in both human and rat dental pulps. Myelinated nerve fiber was in the human peripheral area of the dental pulp, but appeared at central area of the rat molar pulp. In addition, the distributions of human pulp nerves were appeared to vessel wall and perivascular space but the rat pulp only existed in vessel wall. There were significant differences in blood vessels between human and rat teeth at root and chamber ($P<0.05$), and the proportion of nerve fibers of human was significantly different from that of rat teeth at root and chamber ($P<0.05$). The distribution and proportion of blood vessels and nerve fibers in rat molar teeth were significantly different from that of the human teeth, which indicated that the rat molar teeth could not a valid model for human teeth research.

Keywords: Blood vessels, innervations, human pulp, rat pulp, suitable, animal model

Introduction

Dental pulp is a kind of highly vascularized and loose connective tissue, which contains cells, blood vessels and nerves. Dental pulp nerve fibers and blood vessels, which provide nutrients and oxygen, remove waste material and regulate inflammation, are in a state of dynamic balance. Dental pulp nerve fibers promote angiogenesis of immune cells and reinforce pulpal defense system [1]. At the same time, the human dental pulp blood vessel is controlled by nerve fibers [2]. Several studies [3-5] have described distribution of blood vessels and nerve fibers in human pulp, and nerve fiber bundles were along with vascular without branches in the root. However, nerve fibers gave off branches in the crown, which were accompanied by blood vessels in many instances, forming a fan-shaped structure. In the pulp horns, nerve bundles and blood vessels predominated formed a subodontoblastic plexus and capillaries. Blood vessels and nerve fibers not only play an important role in normal tooth function and self-repair, but also exert great significance on dental pulp tissue regeneration process [6-8].

All materials and devices used in dentistry may get in contact with the facial skin, oral mucosa, dental pulp or bone tissue. International Organization for Standardization (ISO) standard states clearly that only mammals are suitable species for animal research in order to ensure the safe use of the tested materials for humans [9]. However, rat teeth has never been tested by animal experiments concerning biocompatibility of dental materials and the rat has been used as an animal model in the dental pulp tissue experiments [10-12]. Sensory innervation patterns in rat incisor and molars of rats were similar in the early stages of development from a control of dental pulp microcirculation by neurons in rat incisor pulp, but different

in maturation [13]. Numerous immunoreactive nerve fibers with varicosities were observed along blood vessels in the center of the rat incisor pulp. In the periphery of the rat pulp, many nerve fibers were seen in the subodontoblastic region without extending into the predentin or dentin [14]. Previous study reported that the rat incisors simulating acute human pulpitis models were successful [15], but it is undeniable that rat incisor was absent from enamel at the pulp horns, nerve terminations in the dentine and subodontoblastic plexus. Further, rat incisor had special arrangement and development of the odontoblast [16]. In this case, the rat models simulating acute human pulpitis are still doubtful. Rat teeth were capped with different adhesive resin systems and calcium hydroxide preparation different quantity of mineralized dentin formation, which can not explicitly verify whether the same story happened in human dental pulp [11]. Further, the rat pulp demonstrated exceptional resilience and self-reparative capacity, which must be taken into account in the interpretation of experimental results [17]. Hence, certain specificities of the model and intrinsic problems have to be taken into consideration. Teeth of younger rat and older rat existed problem about technical aspects and physical changes. Cavities are more difficult to prepare in younger rats, older rats have smaller pulps [18]. Although 6-week-old rats were the easiest experimental animal model, it difficult to access the treatment area since the anatomic position of the molar teeth posterior to a small diastema [18]. To our best knowledge, no study has reported/proved dental pulp of rat is consistent with that of human. Would the distribution, the number of nerves and blood vessels in rat dental pulp tissue be same to those of humans? Is the rat model suitable to study various human pulp diseases?

The aim of the present study was firstly to undertake a semiquantitative assessment of the relationship of human pulpal blood vessels and nerve fibers and those of rats by using an immunocytochemcial approach. Secondly, we also provided experimental basis for dental pulp tissue engineering.

Materials and methods

Collection of samples

Thirty human premolar teeth were obtained from patients (15-40 years) who without hypertension, heart disease and any other systemic diseases or contagious diseases because of orthodontic reasons. The patient's consent was obtained. Teeth were extracted under local anesthesia without any signs of periodontal disease or caries at the Department of Orthodontics, Hospital of Stomatology, Lanzhou University. The tissue samples were fixed for 48 h in 4% paraformaldehyde at 4°C. Surface enamel and dentine of all human teeth were moved by high speed air turbine, making around the remaining dentin thickness of 2 mm.

20 male SD rats weighing 160~200 g were anesthetized by 10% chloral hydrate injected intraperitoneally. Maxillae and mandibule were removed after cardiac perfusion with 4% paraformaldehyde. Incisors and molars of rat, which were completely peeled from maxillae and mandibule, were fixed for in 4% paraformaldehyde 24 h at 4°C, and then rinsed with water for 1 h. The human and rat tooth were decalcified for 3 months in 15% EDTA at room temperature. EDTA were changed every two days and kept its volume 6 times more than that of all samples. The pulps of 15 chosen human teeth were carefully separated from hard structure with 11# surgical blade and tweezers, and then stored in 0.9% saline. Standards of complete decalcification were that all samples can be inserted with a 20# k-file without resistance. Moreover, all samples were pressed by tweezers and samples deformed without resistance, and then slowly returned it.

Sample preparation

Fixed and decalcified samples were dehydrated with the different concentration ethanol (70%, 75%, 85%, 95%, 100%) and treated with xylene. Then xylene was exchanged by molten paraffin wax (56-58°C) with 2 changes, 1.5 h each. Samples were embedded in fresh new paraffin. Paraffin blocks were cut with microtome to 3-5 μm thick sections and mounted on glass slides. One part of slides was stained with hematoxylin and eosin (H&E) and the other part of slides were submitted for immunohistochemical examination using the biotin-streptavidin system and tyramine signal amplification it. Blood vessels were labeled with vascular endothelial growth factor (VEGF, 1: 150, Bioss, Beijing, China). Nerve fibers were labeled with neurofilament protein (NFH200,

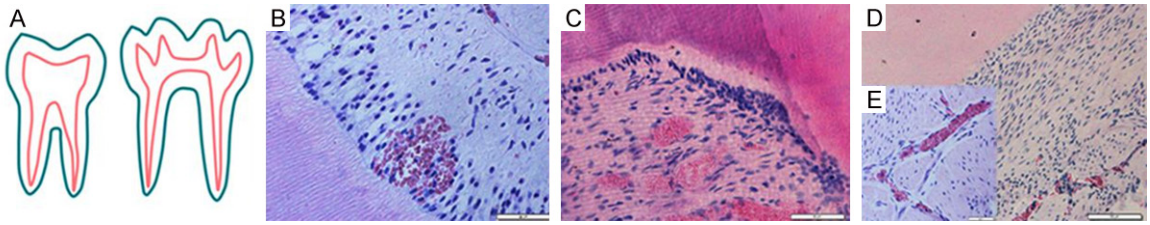

Figure 1. The contrast of human and rat dental histologic structure. Ideograph of human and rat tooth longitudinal section (A). In human dental pulp-dentin interface (B). In rat molar teeth pulp-dentin interface (C). In rat incisor teeth pulp-dentine interface (D). Inner of rat incisor teeth pulp (E). (B-E) is H&E staining. Scale bars: (B, C, E) 50 μm and (D) 100 μm.

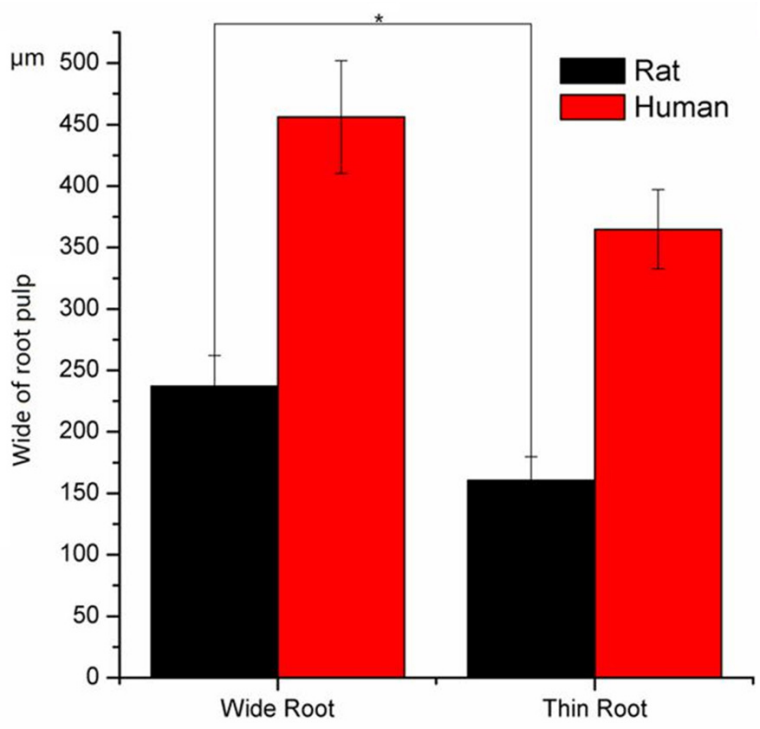

Figure 2. Comparation between the width of human root pulp and rat root pulp.

1:50, Bioss, Beijing, China) and myelinated nerve fibers were labeled with myelin basic protein (BMP, 1:50, Bioss, Beijing China). Citrate buffer pH 6 were added into a pressure cooker with slides and heated up to keep boiling for 2 min. Repeated this procedure after slides cooled down at room temperature. The sections were incubated for overnight with primary antibody at 4°C in blocking normal 1.5% goat serum. The secondary antibody (biotinylated goat anti-rabbit IgG) was applied for 30 min at 37°C and the slides were incubated with streptavidin-peroxidase complex. Slides were treated with a color reagent DAB for visualizing of positive signal and cellular nuclei were counterstained with hematoxylin.

Image analysis

Results were observed under an immunofluorescence microscopy BX61-32FDIC-S08 (Olympus Japan). Images were processed for illustration purposes with Photoshop CS5. Quantification of width (diameter) of human and rat root pulp was done using a mathematical formula (width of root pulp = (cervical pulp width + mid-root pulp width + apical pulp width)/3). Four randomly selected visions per slides were evaluated and semi-quantitative analysis of the integral optical density (IOD) of VEGF, NFH and MBP was measured by image system. (Image Pro Plus 6.0) (IOD = density (mean)* area).

Statistical analysis

Statistical analysis was performed using SPSS version 19.0 for Windows. All data were presented as mean ± SE, and evaluated by Dunnett-T3 test. The *P* values less than 0.05 were considered statistically significant.

Results

Morphology of dental pulp between human and rat

Human molar was 2 cusps on the longitudinal section, but the frequency of cusp 4 was pres-

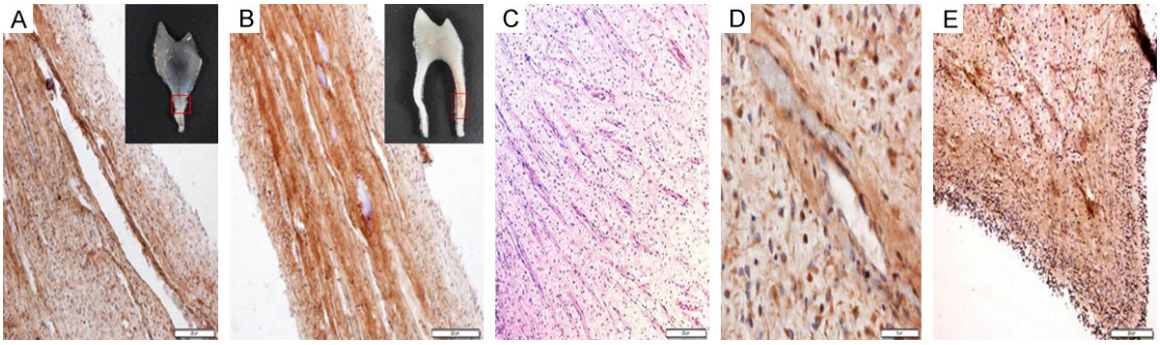

Figure 3. Blood vessels in human dental pulps express VEGF (A, B, D, E) and centre of pulp (C) showing the blood vessels by H&E staining. The single-root teeth of human (A). The double-roots teeth of human (B). The inset shows a representative human premolar tooth with the root pulp area delimitated in red. Chambers (C, D). Horns (E). Scale bars: (D) 50 μm and (A-C, E) 200 μm.

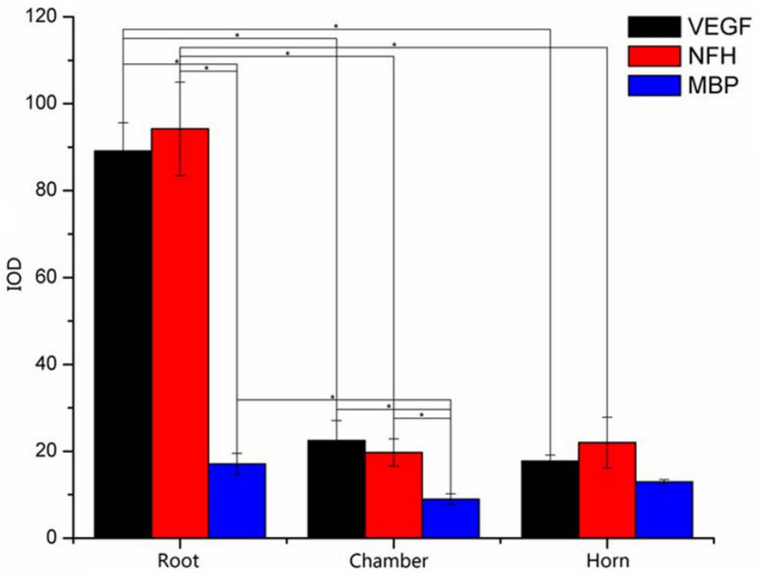

Figure 4. The contrast of VEGF, NFH, MBP IOD in different part of human pulp.

ent in rat molar. Compared with human tooth, rat cusp parts of the enamel were platform-shaped, with dumbbell-shaped chamber, which showed elongated in middle and triangles on both sides. The root and canal configuration of rat was similar to human (**Figure 1A**). Diameter of human premolar root pulp were not significantly different from that of rat ($P>0.05$), but quantification of diameter of rat root pulp revealed a highly significant difference ($P<0.05$, **Figure 2**). Rat root were classified into wide root and thin root based on pulp diameter. There were no significant differences on microscopic structure, cell morphology and pulp structure by the cross section of the dental pulp, the peripheral one contained dentin and predentin, the boundaries were not clear between a free-cell zone and a zone rich in cells in rat pulp although human pulp's four-layer structure was clearly visible (**Figure 1B**, **1C**). Rat incisor's structure was completely different from molars (**Figure 1D**). There was no obvious boundary in four-layer structure of rat incisor. Morphology of odontoblast was irregular in the central pulp with fibrous connective tissue, and fibroblasts and blood vessels. The main direction of the fiber in rat incisor was parallel with the long axis of the tooth. In addition, the connective tissue was surrounded by irregular squares, and blood vessels immersed within the fibrous tissue of the center of the rat incisor pulp (**Figure 1E**).

Relationships between vasculature and the surrounding tissue

Human pulp: In single-root teeth of human, there was the largest located blood vessel in the central portion of the pulp with some capillaries running around (**Figure 3A**). In double-roots teeth, dental pulp displayed the extensive arrangement of medium-size blood vessels without large blood vessels immersed within two roots (**Figure 3B**). Reticular struc-

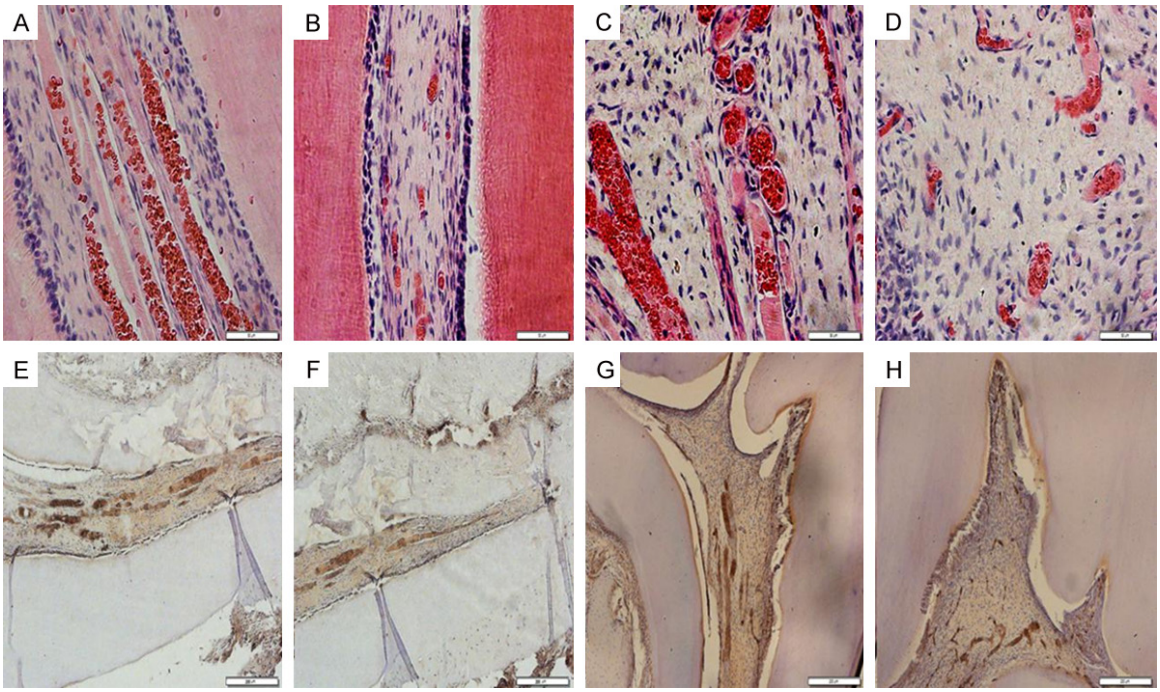

Figure 5. H&E staining (A-D) and immunocytochemical staining demonstrating VEGF-positive blood vessels in rat pulp (E-H). Wide root (A, E). Thin root (B, F), Large part of pulp chamber (C, G). Small part of rat pulp chamber (D, H). Scale bars: (A-D) 50 μm and (E-H) 200 μm.

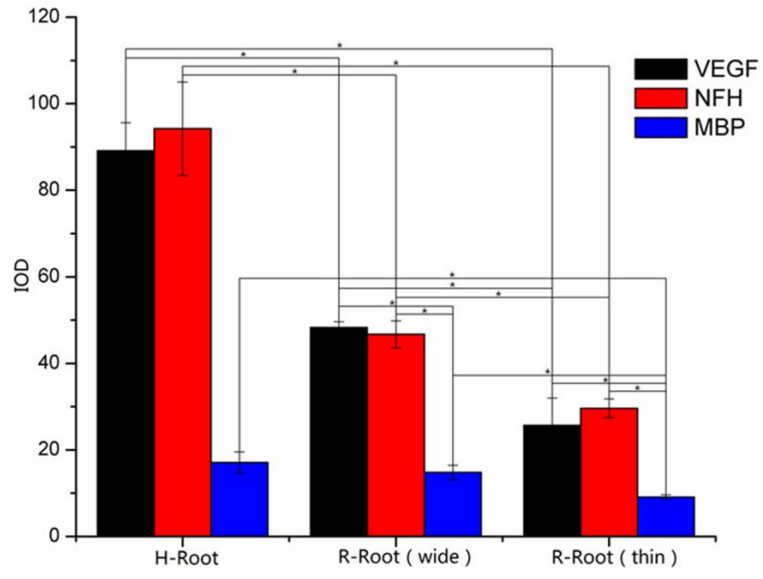

Figure 6. The comparison IOD of VEGF, NFH, MBP between human root pulp and rat root pulp. H-Root: human root, R-Root: rat root.

ture was formed by disorder blood vessels in the central area of the dental pulp (**Figure 3C**, **3D**). Under the pulp horn regions, capillaries gathered passing through the odontoblast layer, when reaching the pulp horn, capillaries gathered toward the horn (**Figure 3E**). At the same time, the greatest IOD of blood vessels were found in the pulp root region, compared with chamber and horn ($P<0.05$, **Figure 4**).

Rat pulp: The rat blood vessels at root were paralleled to the long axis of the tooth (**Figure 5A**, **5B**). The pulp chamber vessels arranged at random with a three-dimensional network structure (**Figure 5C**, **5D**). The results of immunological histological chemistry were almost same as those obtained by HE staining. The wide root was rich with extensive medium-diameter blood vessels and few single large-size blood vessel, while the blood vessels were sparse in the thin root (**Figure 5E**, **5F**). There was significant difference between wide root and thin root ($P<0.05$) based on semi-quantitative analysis (**Figure 6**). In the rat pulp

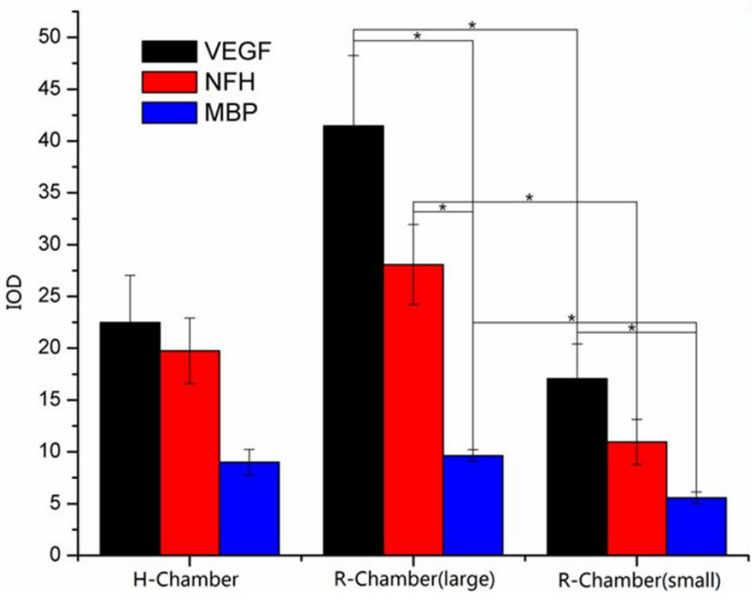

Figure 7. The comparison IOD of VEGF, NFH, MBP between human chamber and rat chamber. H-Chamber: human chamber, R-Chamber: rat chamber.

chamber, the vessels were mainly located in the near root instead of middle pulp chamber. Mild expression of VEGF was observed in the rat pulp horns and capillaries localized in the odontoblast layer. In large part of rat pulp chamber, the quantity of blood vessels was rich, but numbers of blood vessels were scarce in small part of rat pulp chamber (**Figure 5G**, **5H**). The vascularity at large part of pulp chamber was significantly greater than that of small part of pulp chamber ($P<0.05$, **Figure 7**).

The relationships between human and rat nerve fibers and the surrounding tissues

Human pulp: Nerve fibers shape in human tooth was long fibrous shape observed from classical histological examinations of the H&E stained. Neuron nucleus were fusiform (**Figure 8A**). In order to analyze the distributed regularity of nerve fibers and myelinated nerve fibers, longitudinal paraffin sections were stained using NFH and MBP antibodies in human teeth and rat teeth. NFH-positive nerves were mainly found at the blood vessel wall and perivascular region in human pulp (**Figure 8C**). Density of nerve fibers was high in the immunostained in the mesial region of the root pulp (**Figure 8B**), while nerves ramified into a complex network at chamber. Nerve fibers at the horn with high intensity were located in the peripheral pulp and centre region of human pulp horn with slightly stained (**Figure 8D**). Myelinated nerve fibers were sparse in the peripheral of the pulp tissue obtained by MBP immunolabeling at pulp root (**Figure 8E**), the pulp chamber (**Figure 8G**) and pulp horn (**Figure 8H**). Large segmental and nodular unmyelinated nerve fibers were associated with small myelinated nerve fibers and unmyelinated fibers were wrapped in myelin sheath with discontinuity (**Figure 8F**). Integral optical density of nerve fibers was different among root, chamber and horn of human teeth ($P<0.05$, **Figure 4**). There were significant differences in myelinated nerve fibers between root and chamber of human pulp ($P<0.05$, **Figure 4**). The number of myelinated nerve fiber and nerve fiber in human pulp root was the most (**Figure 4**).

Rat pulp: The rat pulp was mainly innervated by unmyelinated fibers. High levels of NFH expression was found at the central portion of the root and near root rather than the middle chamber by immunoreactivity of healthy rat pulp. The distribution of rat pulp nerve fibers was same as the distribution of blood vessels, mostly around the vessel wall (**Figure 9A-D**). Homogeneous MBP immunoreactivity of low intensity was evident at pulp and myelinated fibers were often seen in close proximity to vessels (**Figure 9E-H**). Expression of NFH and VEGF were found not only wide root, thin root, but also large part of chamber, small part of chamber, and IOD of NFH and VEGF were not different in different part of rat pulp ($P>0.05$, **Figures 6**, **7**). NFH-expressing nerve fibers and MBP-expressing myelinated fibers were significantly different between wide root and thin root ($P<0.05$, **Figure 6**). There were significant differences in expression of NFH and MBP between large part of chamber and small part of chamber ($P<0.05$, **Figure 7**). In addition, expression of NFH and MBP which had significantly differences can be observed in wide root and in thin root ($P<0.05$, **Figure 6**). Further, there were significant differences in expression of NFH and MBP in large part of chamber, while there were no significant differences in expres-

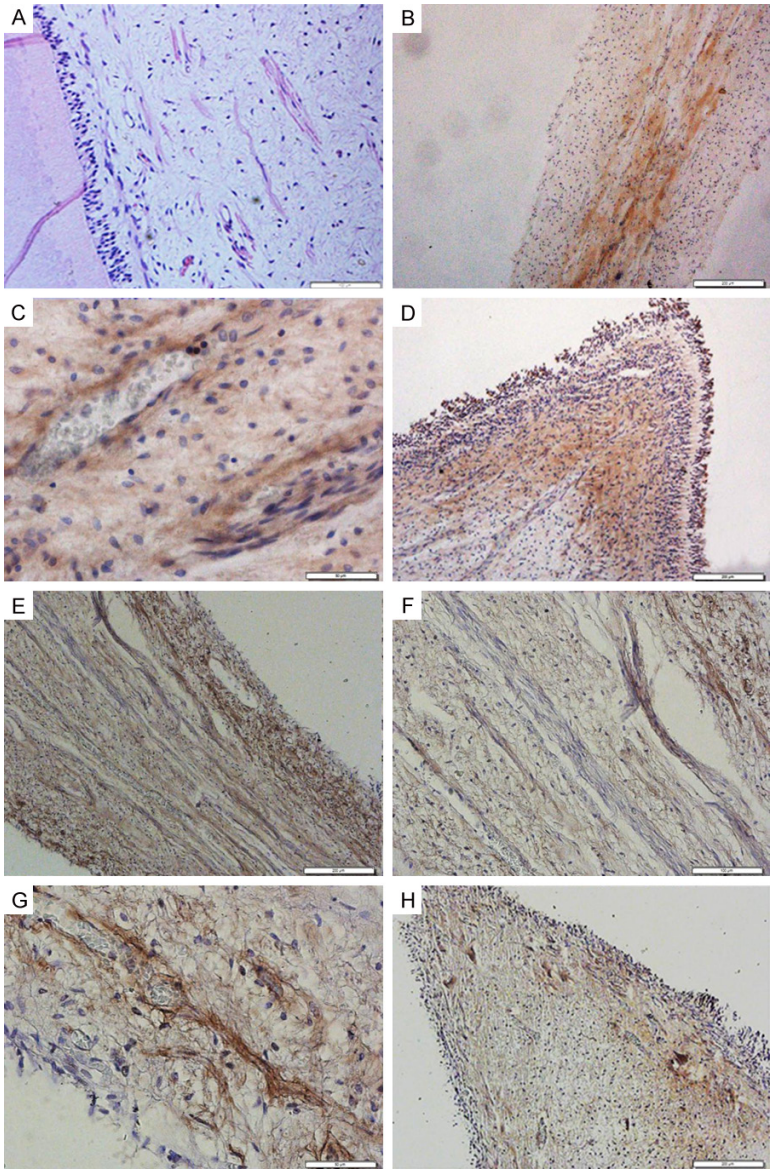

Figure 8. Expression of NFH (B-D) in nerve fibers, and MBP (E-H) in myelinated fibers in human pulp as shown by IHC. H&E staining showed nerve fibers (A). The double root (B, E). Chambers (C, F, G). Horns (D, H). Scale bars: (C, G) 50 μm, (F) 100 μm and (A, B, D, E, H) 200 μm.

sion of NFH and MBP in small part of chamber by electron microscopy (**Figure 7**).

Comparison with human and rat pulp: The integral optical density of VEGF-positive blood vessels did not reveal a significant difference between human chamber and rat chamber (P>0.05, **Figure 7**). On the contrary, there existed significant differences between human root and rat root (P<0.05, **Figure 6**). Myelinated fibers expression within NFH of human chamber was not significantly different from that of and rat chamber (P>0.05, **Figure 7**), but the IOD of NFH on human root were significant different from that of rat root pulp (P<0.05, **Figure 6**). MBP identified unmyelinated fibers were not significantly different between human chamber and rat chamber (P>0.05, **Figure 7**), and there were not significant differences in expression of MBP between human root and rat wide root (P>0.05), while MBP revealed significant differences between human root and rat thin root (P<0.05, **Figure 6**).

Discussion

The association between pulpal nerves and the vasculature has been discussed in human teeth [3-5, 19-21]. However, it has not been described the relationship of nerve fibers and pulpal blood vessels in the rat tooth pulp. We first described the distribution and number of nerve fibers and pulpal blood vessels in the human and rat tooth pulp, which could prove that rats were not suitable species for animal research in order to study human pulp tissue and assess the biocompatibility of dental materials.

The dental pulp is a loose connective tissue developed from the ectomesenchyme. Pulp sensitive fibers and blood vessels penetrate through the apical foramen and gradually fan out branches. Our data showed the nerves were always accompanied by blood vessels and not only presented in the blood vessels wall, but also proximity to the vessels in human pulp. The dental pulp was mainly innervated by unmyelinated nerve fibers, which were widely distributed at the peripheral of pulp tissue. Small myelinated nerve fibers were closely associated with large unmyelinated nerve fibers from our experiment. Myelinated nerve

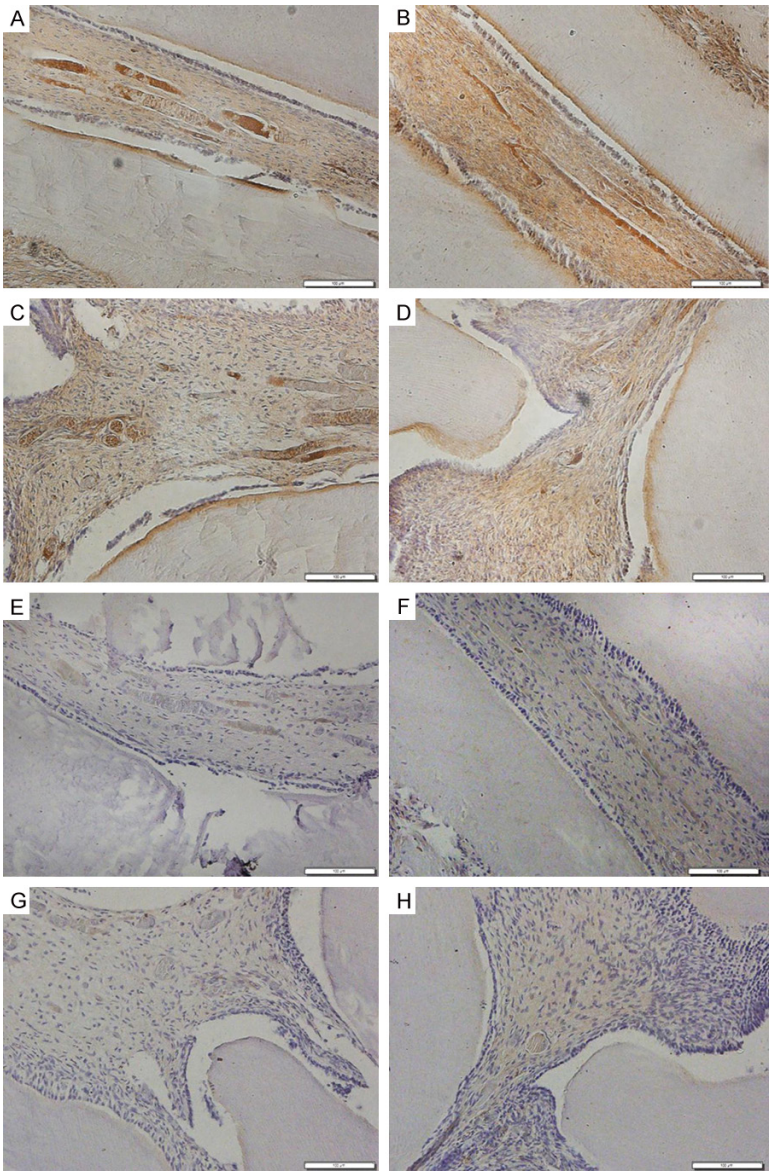

Figure 9. Expression of NFH (A-D) in nerve fibers, and MBP (E-H) in myelinated fibers in rat pulp as shown by IHC. Wide root (A, E). Thin root (B, F). Large part of pulp chamber (C, G). Small part of pulp chamber (D, H). Scale bars: (A-H) 100 μm.

fibers with myelin sheath presented the segmental and incomplete shape, especially in the chamber. These phenomena were supported by other previous research [19-25]. Expression of protein gene product 9.5 (PGP9.5) was observed in the pulp roots, crowns and below the odontoblast layer. Nerve fibers with few ramifications were accompanied by larger vessels but branched into small bundles and passed through the odontoblast layer under the cell-rich zone [21]. NF-positive nerve bundles were distributed in the apical and crown, which were associated with blood vessels in many instances. In the coronal parts of the pulp, nerve fibers fanned out, and divided into smaller branches that entered into the pulp dentine interface. S100B labelling was also detected in the region of apical, coronal and odontoblasts. NSE immunoreactive nerve fibers were present in mainly apical, coronal and odontoblastic parts of the pulp [19]. β III-tubulin immunoreactivity was localized to nerve fiber endings, which were mostly seen in the pulp and predentin-dentin interface forming a terminal network [22]. Most nerve fibers including myelinated and unmyelinated fibers within dental pulp expressed NFH and myelin sheaths were generally positive for MBP immunolabelling using confocal microscopic and the nerve fibers with myelin were prominent in coronal and peripheral region, TH positive axons, in close proximity to blood vessels, were also observed [20]. Nerve fiber bundles were generally positive for S100 immunolabelling with high intensity in the radicular pulp, and associated with neighbouring blood vessels, while in the coronary pulp they were repeatedly branching passing through odontoblastic layer and projecting into the dentinal tubules with low intensity, which could present interruptions [24]. However, not all nerve bundles accompanied arterioles in the pulp chamber and in the root canals and venules were not associated with nerve bundles [26]. Furthermore, our experiment showed there were not significant differences in IOD of NFH and VEGF in human pulp (root, chamber, horn, $P>0.05$), but there were significant differences in IOD of NFH and MBP in human pulp (root and chamber, $P<0.05$), indicating nerve fibers were mostly seen in close association with blood

vessels and the numbers of nerves and blood vessels could match well. Myelinated nerve fibers were rare. The greatest numbers of nerve fibers, vascular and myelinated fibers were found in the pulp root region because nerves and blood vessel entered the tooth via the apical foramina, ascended towards the coronal region and gave off branches en route. Vascular and nerve fiber density were high at horns in human pulp. There were not significant differences in IOD of VEGF, NFH and MBP in human pulp horns ($P>0.05$). We speculate it might be caused by their relatively small space, unclear borderline and measuring error.

The results of present study was in accordance with several previous studies [27, 28], which reported that rat dental pulp nerve was linked closely with the blood vessels, and the distribution of nerve generally followed a similar pattern as blood vessels. In the rat root pulp, nerve fiber bundles and blood vessels were parallel to the long axis of the tooth without obvious branches, ramified in the coronal parts, and were present in an abundant fiber and vascular network. In addition, our study also demonstrated that the number between rat pulp blood vessels and nerves using semi-quantitative analysis. There was significant difference in IOD of VEGF, NFH and MBP expression in all parts of rat pulp, including wide root, thin root, large chamber and small chamber, suggesting the number of nerve fibers and blood vessels in wide root and large part of chamber was higher ($P<0.05$). The density of nerve fibers and blood vessels was not statistical significance in rat root ($P>0.05$), while the density of nerve fibers and myelinated fibers was statistical significant ($P<0.05$), indicating that nerve fibers accompanied vessels and the majority of the nerve fibers in the rat root pulp were unmyelinated fibers. Rat pulp express MBP, a protein commonly expressed myelinated fibers, was in small numbers. Similarly, the IOD of nerve fibers and blood vessels was not statistical significance in rat chamber ($P>0.05$), number of nerve fibers and blood vessels was basically consistent. The fraction of NFH-expressing nerve fibers was higher than MBP-expressing myelinated fibers in large part of chamber ($P<0.05$). At variance with large part of chamber, the proportion of nerve fibers was no significantly difference from myelinated fibers in small large part of chamber ($P>0.05$). Nerve fibers were mainly unmyelinated fibers in large part of chamber and myelinated fibers in small part of chamber showed a distinct distribution pattern on rat chamber, which may be proven that different innervation take place in different areas of the chamber. This hypothesis requires further experiments to testify.

The results from our HE staining experiment showed there were big structure differences between human teeth and rat incisor, but there was subtle difference from four microscopic zones of molar teeth between human and rat. Histological structure of rat molar teeth was unclear, although some authors suggested the biological responses of rats were consistent with the reaction of human due to their similar molar dental pulp tissue structure and anatomical structure of rat [29]. The majority of nerve fibers in human dental pulp were presented in central of human pulp. MBP was expressed in the human myelinated nerve fibers presented at the peripheral area. Both unmyelinated and myelinated fibers in the human dental were located blood vessels wall and perivascular regions. However, nerve fibers and myelinated nerve fibers were located in central parts of rat pulp, the expression of MBP in myelinated nerve fibers accompanied by blood vessels, while unmyelinated nerve fibers in rat pulp were mainly observed at the vascular walls. We also found that the number of blood vessels and nerve fibers in the human root of pulp was higher than those in rat root of pulp ($P<0.05$), while the number of blood vessels and nerves in the human chamber and rat chamber had no obvious difference ($P>0.05$), showed that radicular regions in human pulp were rich in blood supply and innervation, change of blood vessels and nerves was similar in human and rat pulp, suggesting different distribution and number of nerve fibers may reflect the difference in vascular supply nutrition for pulp tissue, nerve responses may regulate blood flow, they influenced each other [2, 30]. In the human and rat pulp, distribution of myelinated nerve fibers which were scarce was different.

In the rat, pituitary adenylate cyclase-activating polypeptide (PACAP)-immunoreactive (IR) nerve fibers contained calcitonin gene-related peptide (CGRP)-IR and originated from the trigeminal ganglion. PACAP-IR nerve fibers in the human tooth pulp contained VIP-IR and originated

from the superior cervical ganglion [31]. In the rat incisor, nerve fibers did not form plexus in the sub-odontoblast layer and not enter the dentin [32]. In the early stages of development, the sensory innervation pattern was different between rat incisor and molar pulp, but change in maturity, which might account for rat incisors are continuously growing [33]. Sensory nerve fibers of the rat incisor pulp involved in the vasoregulatory function, which made it a model distinct from other teeth model for studying neurovascular interactions [14]. The proportion of large myelinated fibers and unmyelinated fibers was significantly different between molar and incisor within the parent axons. The fraction of large myelinated fibers was significantly higher for the molar-than incisor-parent axons, while the fraction of unmyelinated fibers was significantly higher for the incisor-than molar-parent axons [34]. Current experimental samples were dominated by small animals, which were a limitation in the study and recommended that it was necessary to use large mammals [35]. So, distribution and the number of nerve fibers and blood vessels in the human dental pulp were different from rat tooth pulp may be due to species differences in the oral environment, function and the state of engagement through tooth development. This suggests that the rat model for studying teeth is still controversial.

Regenerative endodontic treatment, which is the most perfect treatment of dental pulp necrosis, allows not only continuation of root development and apical closure, but also restoration of immune and sensory functions. However, this clinical technology has not yet been approved. Further investigation is needed to find a valid model to explore it [36]. The dental pulp tissue regeneration of experimental animal model is mainly rat [10-12, 37]. However, the present study showed that distribution and number of blood vessels and nerve fibers in rat molar teeth and the human teeth had a dramatic difference. In general, lower animals (rabbits, SD rats) had stronger regenerative capacity than higher animals, on account of the former with poor structural and functional organization and the latter with strong defense capability [38-40]. Therefore, rat molar teeth were not suitable for biological testing of human pulp tissue.

This study demonstrated that there was a significant difference in the distribution and number of blood vessels and nerve fibers in rat molar teeth and the human teeth by IHC, which supported why rats are not listed as suitable animals in ISO standard 7405, despite of similarity of their histological structure.

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Disclosure of conflict of interest

None.

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