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ORIGINAL

Microvasculature of Dental Pulp in a Rat Molar in an Occlusal Hypofunctional Condition

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ABSTRACT : In clinical orthodontics, hypofunctional teeth are a common problem as it is necessary to move these teeth to be able to restore the occlusal function. Previous studies reported that hypofunctional occlusion could lead to atrophic changes in the microvasculature of the periodontal ligament. However, there is a lack of empirical research on the relationship between hypofunctional occlusion and microvasculature of dental pulp. The purpose of this study is to elucidate details of changes in pulpal microvasculature in the occlusal hypofunctional condition.

Twenty-four 7-week-old male Wistar rats were divided into 4 groups: 1 week, 1 month, 3 months, and 6 months after extraction of opposing teeth. To establish an occlusal hypofunctional condition, maxillary left first and second molars were extracted. The mandibular left molar region was used as the experimental group and the opposite sides of the mandible in the same animals were used as the control group. Paraffin cross-sections of the mandibular first molars were stained with hematoxylin-eosin for light microscopic observations. In this study, the terms "Pulpal vascular area" and "Pulp cell nuclear area" refer to the total blood vessel cross-sectional area of the dental pulp and the total nuclear area of the dental pulp cells, respectively. The pulpal vascular area and the pulp cell nuclear area in the mesial half of the mandibular first molars were examined at 3 places: 1) in the pulp horn zone, 2) the middle zone, and 3) the root apex zone. All data were expressed as percentages of the measured area. Comparisons of the data in the two groups were performed.

The percentages of the vascular area and pulp cell nuclear area in the pulp horn zone were statistically significantly smaller in the experimental group than in the control group at 1 month, 3 months and 6 months, while there were no statistically significant differences between the two groups was observed in the middle and root apex areas.

The results suggest that occlusal stimulation affects the microvasculature and the cellular density of the dental pulp in the coronal area of teeth.

Key Words : microvasculature, dental pulp, occlusal, hypofunction, blood vessel

INTRODUCTION

In clinical practice, hypofunctional teeth (such as open bite incisors, non-opposing teeth, high-positioned canines, and bucco-version or linguo-version teeth) are one of the orthodontic problems that need to be corrected. Orthodontic forces are applied to non-

functional or hypofunctional teeth to establish their proper position for restoration of occlusal function.¹ From previous studies²⁻⁸ it is apparent that orthodontic forces can affect pulpal and periodontal blood circulations, resulting in degenerative changes in the pulp and periodontal tissues. During orthodontic tooth movement, blood vessels in the dental pulp are involved in the

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regulation of tissue remodeling in response to orthodontic forces. The role of the pulpal vascular system and blood circulation incident to orthodontic tooth movement has been shown by several studies.^{3, 4, 6, 9-11}

Dental pulp is soft tissue that is richly vascularized and innervated and has formative, nutritive, sensory, and reparative functions. Small blood vessels enter the pulp via the apical foramina in company with nerve bundles and pass up into the coronal pulp, where they give off numerous branches passing peripherally to form a plexus in the odontogenic region.^{1, 12, 13} This circulatory system is termed “microvasculature of the dental pulp”.

Hypofunctional occlusion can lead to atrophic changes in the periodontal ligament, such as narrowing of the periodontal ligament, disorientation of collagen fibers, and vascular constriction.^{1, 14, 15}

It is known that the blood vessels of both the pulp and the periodontal ligament arise from the same artery and are drained by the same veins in both the maxilla and mandible. The communication between vessels of the pulp and periodontal ligament in addition to the apical connections is further enhanced by connections through accessory canals.^{13, 16, 17} In other words, there is very close relationship between blood vessels of the pulp and the periodontal ligament. Thus, we hypothesized that the hypofunctional occlusion may cause changes in the microvasculature of the dental pulp.

However, most previous studies have been restricted to the periodontal ligament, alveolar bone, and the surrounding organs.^{1, 14, 15, 18-24} The relationship between the microvasculature of dental pulp and hypofunctional occlusion remains unclear. Consequently, it is essential to clarify this relationship contributing to a better understanding of this part. The purpose of the present study was to examine the changes in the pulpal microvasculature following removal the opposing teeth for establishing hypofunctional condition.

MATERIALS AND METHODS

Materials

Thirty-six male Wistar rats, 7-week-old and with a mean body weight of about 200 g, were used. The rats were randomly divided into four treated groups (6 rats in each). Twelve untreated rats were also used as untreated groups (3 rats in each; Table).

This experiment was approved by the Animal Care and Use Committee of Hokkaido University (09-0013) and was conducted with strict adherence to the

Table. Types of groups and numbers of animals in each group.

Group	Number of Animals			
	Treated Group		Untreated Group	
	Left side (Exp)	Right side (Cont)	Left side	Right side
1 week	6	6	3	3
1 month	6	6	3	3
3 months	6	6	3	3
6 months	6	6	3	3
Total of animals	24		12	

Guidelines for Animal Experiments of Hokkaido University.

Experimental procedures

In all treated groups, the maxillary left first and second molars of each rat were extracted^{19, 25, 26} to establish hypofunctional occlusion on the mandibular left first and second molars (Figure 1) by using human root extraction forceps #69 (YDM Corporation, Tokyo) under general anesthesia with an intraperitoneal injection (0.25ml/100g of rat body weight) of 8% trichloroacetaldehyde monohydrate (Kanto Chemical Co., Inc., Tokyo). In the same rats, the left sides of the mandibles were used as the experimental group (Hypofunctional group) and the right sides were used as the control group. During the experimental period, the weights of rats subjected to tooth extraction were measured and compared with

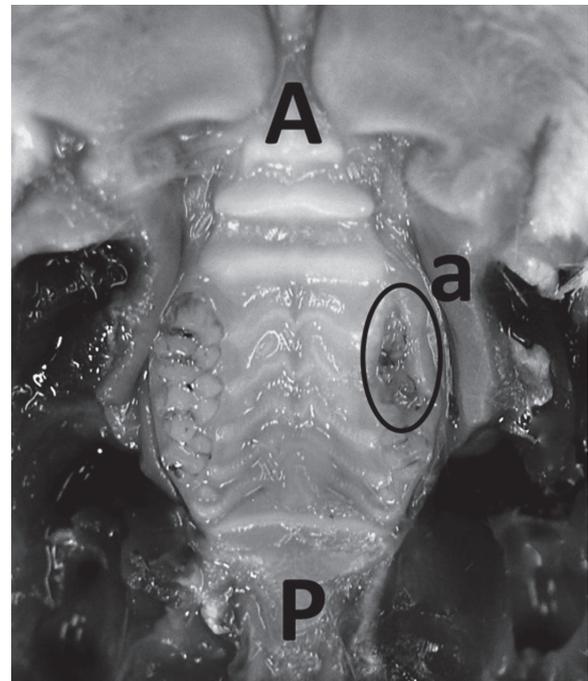


Figure 1. Extraction of maxillary left first and second molars. (a) Tooth extraction site. (A) Anterior part of the maxilla. (P) Posterior part of the maxilla.

those of untreated rats in the same experimental period.

At the end of the experimental period, the animals were sacrificed at 1 week (1W), 1 month (1M), 3 months (3M), and 6 months (6M). All animals were sacrificed by overdose of trichloroacetaldehyde monohydrate and then fixed by transcardial perfusion with 10% neutral buffered formalin (Kanto Chemical Co., Inc., Tokyo).

After perfusion fixation, the left and right mandibles were excised and immersed in 10% neutral buffered formalin as a fixative within 24 hours at 4 °C and then decalcified in 10% EDTA solution (Kanto Chemical Co., Inc., Tokyo), pH 7.4, at 4 °C for 4–6 weeks.²⁷ In the period of decalcification, X-rays of the specimen were taken using soft X-ray equipment (Softex CSM type, Nippon Softex Co., Ltd., Tokyo) before and after decalcification to ensure complete calcium removal. Finally, the specimens were embedded in paraffin by the conventional method. The paraffin-embedded specimens were sectioned (Retorotome REM-700, Yamato Kohki Industrial Co., Ltd., Tokyo) into 5- μm -thick serial sections perpendicular to the long axis of the mandibular left and right first molars and were mounted on slide glasses. The sectioned specimens were stained with hematoxylin-eosin (H&E) and examined under a light microscope (Eclipse 80i; Nikon, Tokyo).

Observation sites

The sites for observations were in the areas of the mesial pulp horn, root apex, and halfway between the pulp horn and root apex of the mesial root of the mandibular first molar (Figure 2). To define the observed sites, (1) the pulp horn area was examined at a

depth of 300 μm below the tip of the pulp horn (upper zone), (2) the root apex area was examined at a site 300 μm above the root-end opening (lower zone), and (3) the mid point between (1) and (2) was selected for observations (middle zone).

Histological measurements and data analysis

The stained specimens were viewed with a light microscope connected to a personal computer at a magnification of x350. For all specimens, the mesial pulp horn or root of the mandibular first molar was traced. The images were captured as digital files by a digital CCD color microscope camera (Micropublisher 5.0RTV, QImaging Co., British Columbia). Each of the digital images was analyzed with an image analysis software program (Image-Pro Plus ver.4.0, Planetron Inc., Tokyo). Measurements were performed at the three above-described observation sites in each group. Firstly, the pulp area was traced and measured in a unit of μm^2 . The total cross-sectional area of the pulpal blood vessels was measured and the percentage of the vascular area in dental pulp was then calculated. The total area of the pulp cell nucleus was also measured and the percentage of the pulp cell nuclear area was calculated in the same manner. Finally, the percentage of the vascular area and the percentage of the pulp cell nuclear were compared within the same experimental period and between different experimental periods.

Statistical analysis

Data are expressed as means with standard deviation (SD). Comparisons of means of each parameter between the experimental group (occlusal hypofunction) and the control group within the same animal were performed by the paired-samples t-test using statistical analysis software (SPSS 14.0 for Windows; SPSS Inc., Chicago). The independent samples t-test was used for comparison between the treated groups and untreated groups. Statistical significance was defined as $P < .05$.

RESULTS

Body weight

Body weights of rats in the treated and untreated groups were measured once a week and are shown in Figure 3. From the start until the end of experiments, the body weights of animals in both groups gradually increased. However, there was no significant difference in body weight between the groups.

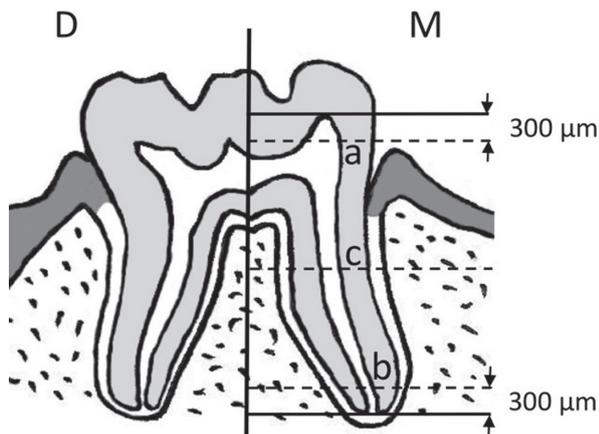


Figure 2. Sites for histological observation. (a) Upper zone. (b) Lower zone. (c) Middle zone. (M) Mesial half. (D) Distal half.

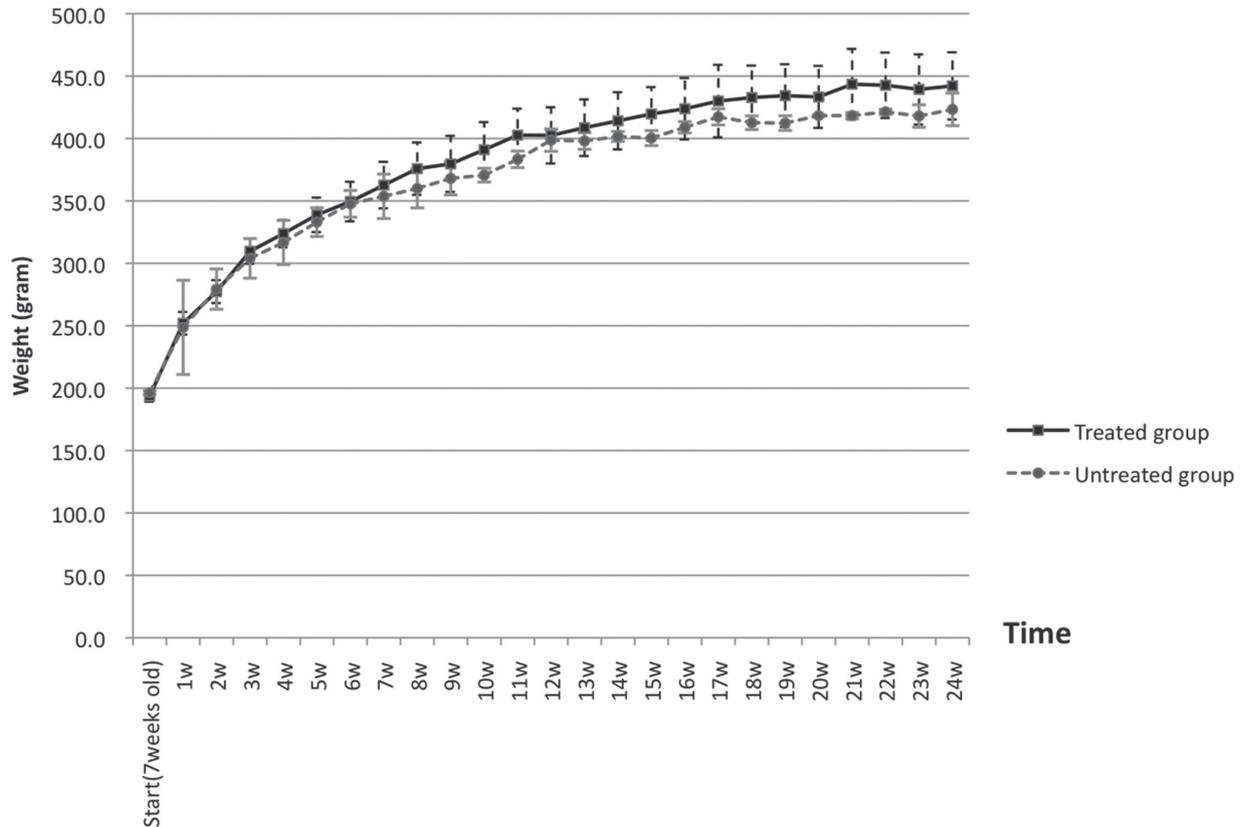


Figure 3. Comparison of body weights in the treated group and untreated group. Each point and vertical bar indicate the mean and SD of animal weight.

Distribution of pulpal microvasculature

In the upper zone, the number of blood vessels in the experimental groups tended to be less than that in the control groups (Figure 4). Differences were observed at 1M, 3M and 6M (Figure 4). However, no apparent difference between the groups was seen in the middle and lower zones (Figure 5).

In comparison among the different experimental periods, dental pulps at 1W and that at 1M were highly vascularized with a network of small blood vessels and several capillaries passing within the pulp core and periphery. In dental pulp at 3M and that at 6M, the blood vessels were less numerous and more concentrated in the core of the pulp (Figure 4, 5).

Quantitative analysis

1. Percentage of vascular area

Percentages of vascular area in the experimental (hypofunctional side) and control (occluded side) groups are summarized in Figure 6a-c; there were significant differences between the experimental groups and the control groups in the upper zone of the pulp at 1M, 3M and 6M. Analysis of data from all groups

demonstrated that the percentages of vascular area in dental pulp were lower in the experimental groups than in the control groups in the upper zone. However, in the middle and lower zones, no significant differences were found throughout the experimental period.

In comparison of all experimental period groups, the percentage of vascular area in the upper zone of the control group was significantly greater at 6M than that in other groups (Figure 6d), while there were no significant differences among all experimental period groups in the experimental group. In the middle and lower zones, the significant reductions in the percentages at 6M were observed in the two groups except the lower zone of the experimental group (Figure 6e,f).

Noticeably, the percentage of vascular area was always lower in the upper zone (ranging from 0.02–0.97%) than in the middle zone (3.66–11.95%) and lower zone (2.07–10.99%) regardless of age.

Comparison of the percentages of vascular area in the control groups and untreated groups is shown in Figure 7a-c. There were no significant differences between the groups when compared within the same experimental period.

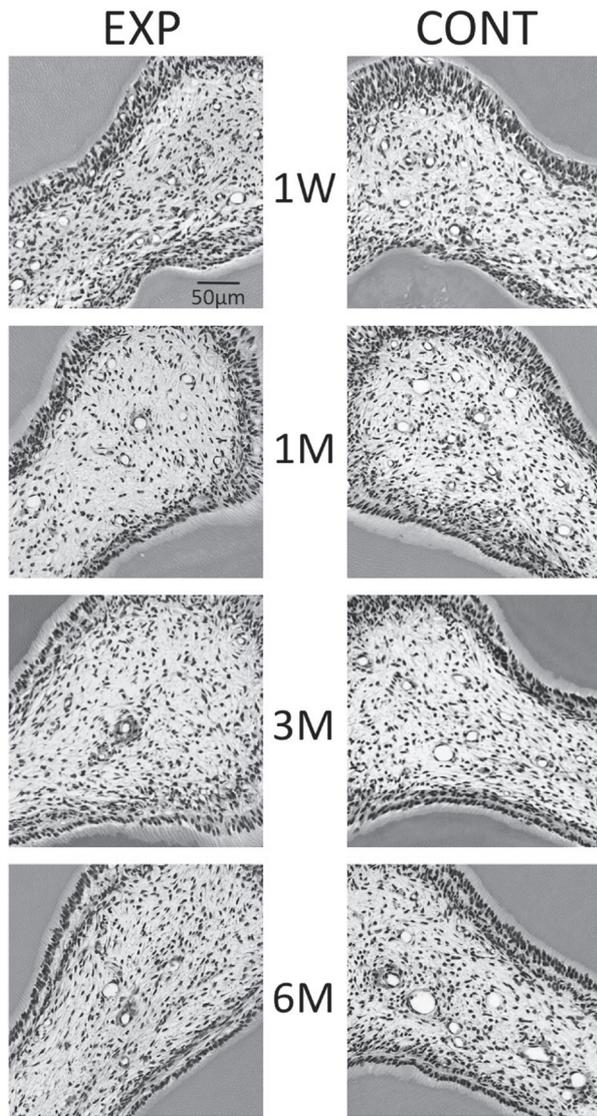


Figure 4. H&E staining of the dental pulp at the upper zone showing the greater vascular area in the control groups (CONT) compared to that in the experimental groups (EXP) at 1M, 3M and 6M. Bar = 50µm.

2. Percentage of pulp cell nuclear area

Percentages of the pulp cell nuclear area in the experimental groups and control groups are shown in Figure 8a-f. The percentage of the pulp cell nuclear area in the experimental groups was significantly lower than that in the control group in the upper zone from 1 month, but there was no significant difference between the groups at 1W. In the middle zone and lower zone, remarkable differences were not observed throughout the experimental period.

When compared among the experimental periods, there were significant reductions in percentages in the upper zone and lower zone of the experimental groups: 1) between the 1W group and 3M group in the upper zone

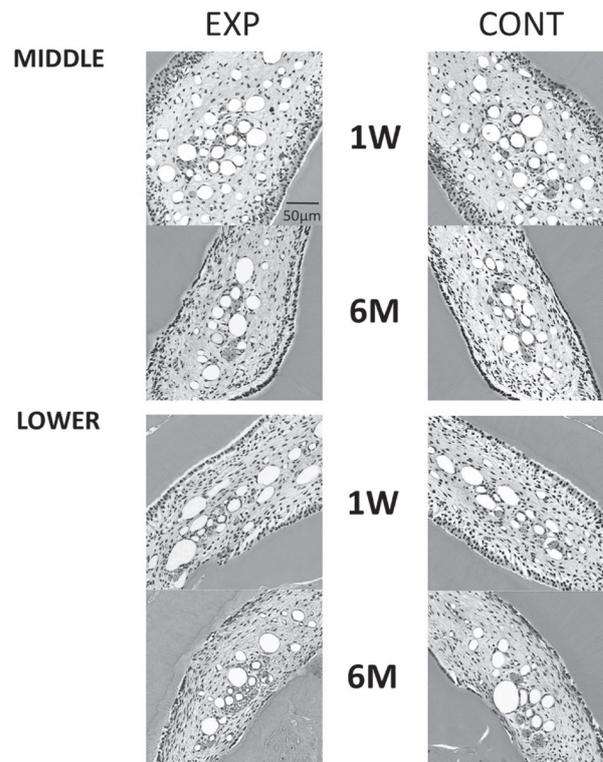


Figure 5. Representative photomicrographs showing comparison of the distribution of microvasculature in the experimental and control groups at the middle and lower zones. Bar = 50µm.

and 2) between the 1M group and the 3M or 6M group in the middle zone. In the lower zones of the experimental and control groups, percentages of the pulp cell nuclear area tended to decrease with increase in age with significant reductions between those at 1W and 6M.

DISCUSSION

There are three main methods for establishing occlusal hypofunction in an animal model: by resection of the crown of the opposing teeth^{1, 28}, by attachment of a bite plate on the incisors^{20, 29-32}, and by extraction of antagonists^{25, 33-35}. In this study, the tooth extraction method was used to examine the effects of occlusal hypofunction on rat dental pulp. This method is simple and easy way to exclude following effects of antagonist elongation or occlusal contact recurrence. Although it is considered to be harmful to animals, this study showed that the recovery for almost complete healing takes only a few days. The animals had gained their weight in normal rate compared to untreated group within 1 week after tooth-extraction.

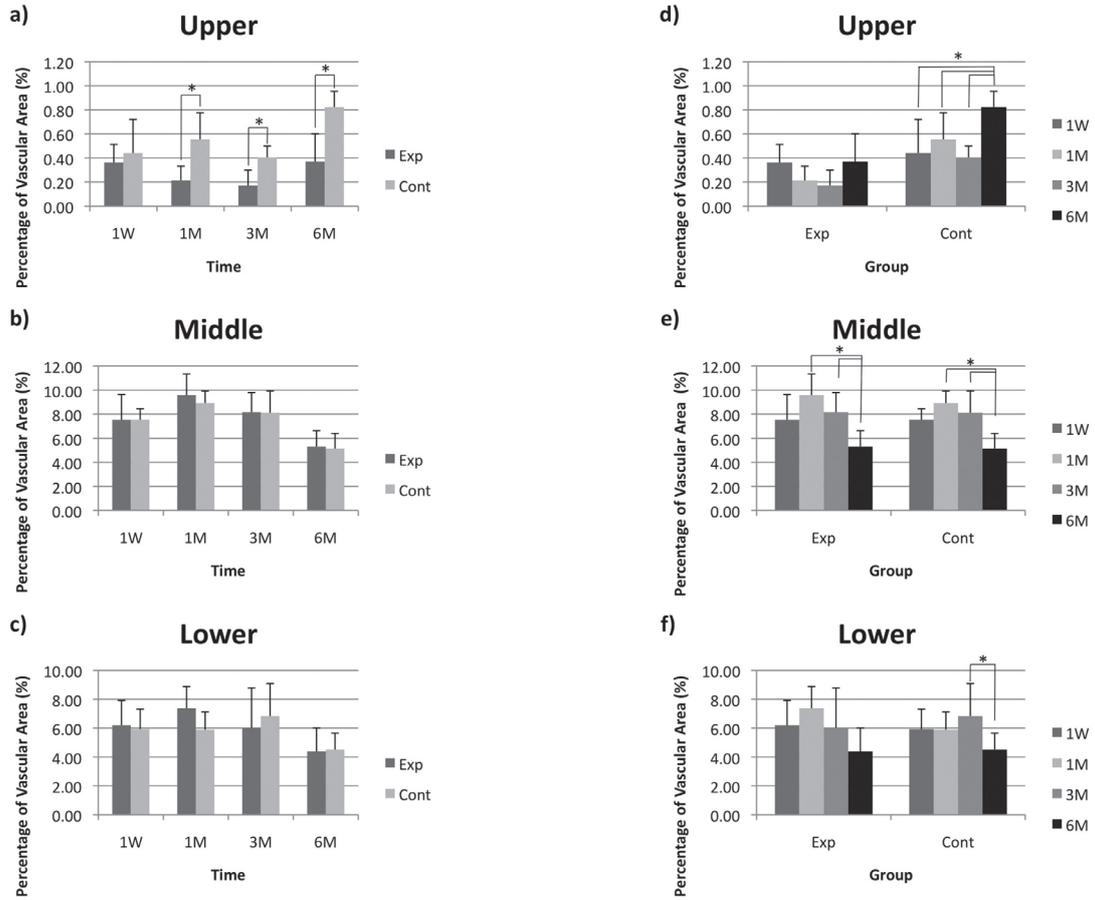


Figure 6. Comparison of percentages of vascular area. a) -c) show comparisons between the experimental and control groups. d) -f) show comparisons among experimental periods.

* Significant difference (P<.05)

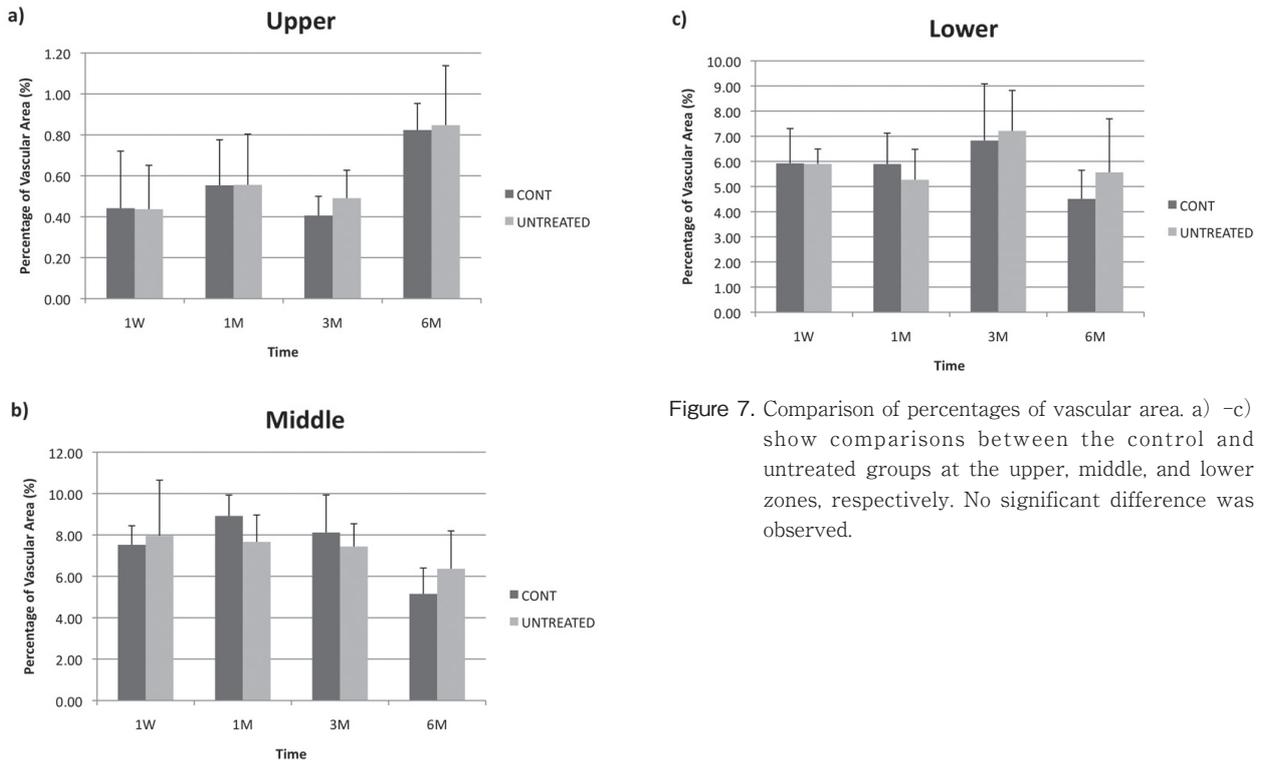


Figure 7. Comparison of percentages of vascular area. a) -c) show comparisons between the control and untreated groups at the upper, middle, and lower zones, respectively. No significant difference was observed.

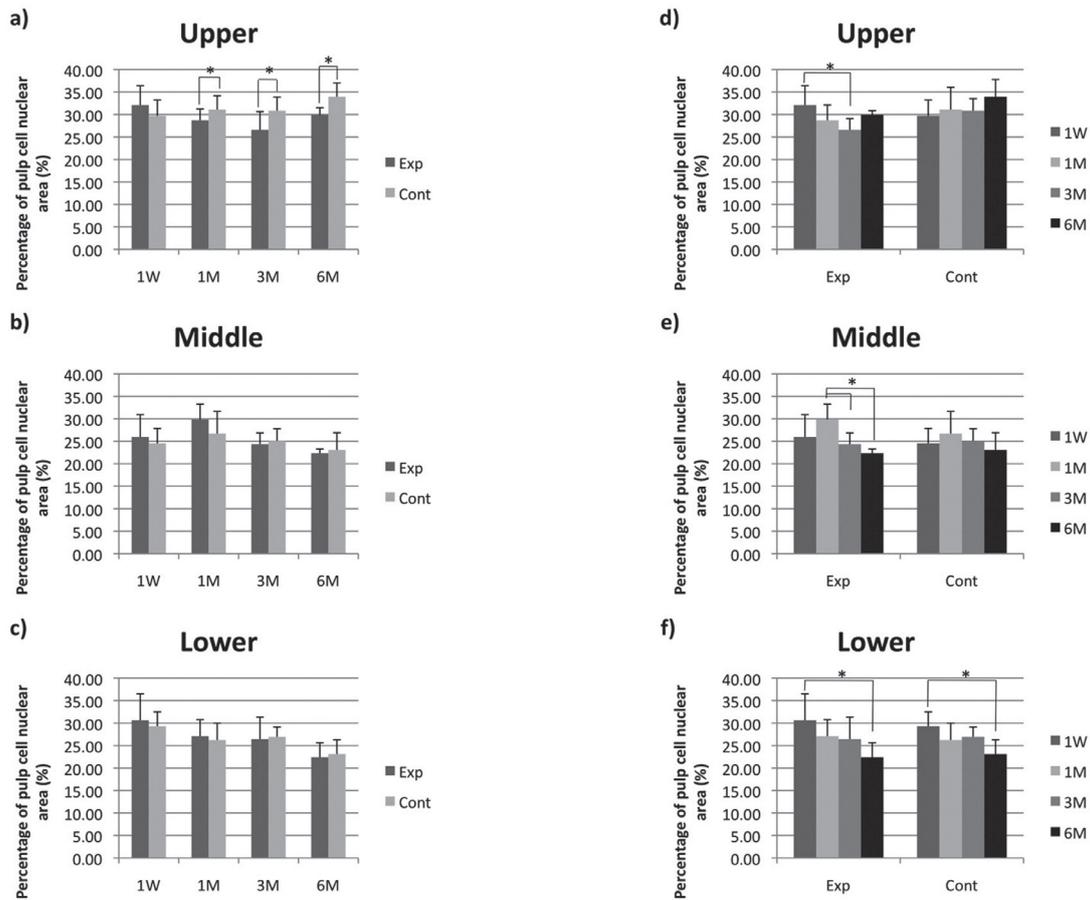


Figure 8. Comparison of percentages of pulp cell nuclear area. a) -c) show comparisons between the experimental and control groups. d) -f) show comparisons among experimental periods. * Significant difference (P<.05)

In order to avoid individual variations in vascular and cellular density of the dental pulp, we assigned the mandibular right first molar in the same rat for the control group. However, it is thought that this molar may receive higher occlusal force than normal as a result of impairment of occlusion on the contralateral side. Thus, the untreated group was used to determine whether the mandibular left first molar was appropriate for use as a control. The results showed that there was no difference in histology or percentage of the vascular area between the groups. Moreover, we also extracted only the maxillary left first and second molars and kept the left third molars in their intact occlusion to ameliorate the side effects of complete unilateral tooth loss.

According to previous studies³⁶⁻³⁸, overall vascular structures are diminished with loss of the peripheral capillary plexus and the primary vessels converge centrally in aged pulp. This is in agreement with Bernick's finding³⁹ that there is a remarkable diminution in the number of blood vessels supplying coronal pulp and only the main large vessels persist in

the core of the pulp. The present study confirmed that the distribution of pulpal microvasculature is affected by aging regardless of alteration in occlusal function.

The percentage of pulpal vascular area in the pulp horn was lower in experimental group than that in control group. Differences were observed at 1M, 3M and 6M, while there was no significant difference at 1W. These results demonstrate that occlusal hypofunction is likely to induce a decrease in the volume of the pulpal microvasculature in the chronic stage. However, the percentages of vascular area in the middle and apex zones showed no significant change due to occlusal hypofunction. The pulpal microvasculature in the root part might receive less influence from occlusal stimuli. In other words, the microvasculature in the pulp horn, which is subjacent to occlusal stimuli, is affected by alteration of occlusal function. In contrast to our findings, Shibutani et al. (2010)²⁹, who studied effects of occlusal stimuli on pulpal microvasculature in rat molar roots, reported that the luminal area of the arterioles decreased at 7 days and 14 days after loss of occlusal stimuli. This

discrepancy may be due to the fact that we examined pulpal blood vessels, including arterioles and venules, while only arterioles were examined in their study, and also that there was a long experimental period of more than 1 month in our study.

Reduction of periodontal blood vessels caused by occlusal hypofunction has been reported. When occlusal stimuli are diminished, mechanical stress toward the periodontal ligament decreases, leading to a decrease in periodontal blood vessels in the chronic state.¹ This change in the periodontal ligament is similar to the results of our study showing decreases in the pulpal vascular area in the coronal pulp of the hypofunctional tooth at 1M, 3M and 6M. Hence, the previous and present data suggest that occlusal stimuli play a role in inducing long-term adaptation of the coronal pulp and periodontal ligament. It might be considered as a phenomenon of disuse atrophy.²³ Moreover, this probably means that the alteration of microvasculature of the dental pulp is not only directly affected by occlusal stimuli, but also relates to the alteration of microvasculature of the periodontal ligament.

A number of studies⁴⁰⁻⁴³ on mechanical stimuli and pulp cells, particularly odontoblasts, have been performed to clarify the mechanism of pulp in response to mechanical forces. The density of pulp cells has implications for the reparative capacity of pulp tissue to regenerate lost or damaged dentinal matrix. Following dentinal injury by trauma, caries, or cavity preparation, secretion of tertiary dentin is often necessary to protect the tooth pulp from infection and the chemical or cytotoxic effects of dental materials.⁴⁴ The pulp cell density is a critical factor indicating the potential of pulp reparative responses and thus the loss of cells will impair pulp reparative capacity.^{45, 46}

In this study, we assigned the percentage of pulp cell nuclear area to represent pulp cell density. The results obtained in this study indicate that the percentage of pulp cell nuclear area tends to decrease when occlusal stimuli are diminished. Changes in pulp cell density can be observed at 1 month after loss of occlusal function. Our results imply that the dentinal secretory activity and reparative capacity of an occlusal hypofunctional tooth may be diminished or delayed in comparison with those of a normal functional tooth.

The primary functions of the pulpal microvasculature are to deliver nutrient substances, gases, metabolites and water to pulp cells and then to collect carbon dioxide from

pulp cells that has passed through the capillary network to maintain pulp homeostasis.^{47, 48} Our results showed that the percentages of pulpal vascular area and pulp cell nuclear area changed similarly in response to alteration of occlusal stimuli. These changes are considered as the mutual relation between microvasculature and pulp cells. However, we have not elucidated whether change in pulpal microvasculature or change in pulp cell density takes place first.

Change in the percentages of vascular area and pulp cell nuclear area showed similar tendencies with increase in age. Interestingly, the pattern of change in the pulp horn, which is adjacent to occlusal stimuli, was found to be different from that in the radical pulp. In the pulp horn, the vascular area and pulp cell nuclear area tended to be increased at 6M, while those in the radical pulp gradually decreased with increase in age. These findings suggest that there are regional differences in pulp adaptation, which is influenced by intrinsic and extrinsic factors.

The significance of comprehending the effects of occlusal hypofunction on pulpal microvasculature is to provide a clinical indication for orthodontic treatment of hypofunctional teeth. Owing to the reduction of pulpal microvasculature and pulp cells in hypofunctional teeth, it is important to treat them by restoring occlusion.

Further studies are required to investigate the ultrastructure of the pulpal microvasculature under the condition of occlusal hypofunction for clearly understanding changes in morphological characteristics. Moreover, it would be of interest to clarify the pulp responses when an occlusal hypofunctional tooth is restored to its normal functional occlusion⁴³.

CONCLUSIONS

Our results suggest that occlusal stimulation affects the microvasculature and cellular density of dental pulp in the coronal area of teeth.

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