Original Article

Pulpal Microvasculature Changes During Orthodontic Loading: A Histomorphological Study in Humans

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Abstract

The objectives of this study were to perform quantitative and qualitative evaluations of the histological pulpal microvasculature changes and angiogenesis following orthodontic loading application in humans. Twelve third molars that were loaded with a 0.016-inch Sentalloy archwire for 1, 3, 4, and 8 weeks were used in the study. Following extraction, specimens were fixed, embedded, and stained with CD146, marker for endothelial cell lineage. The quantification of blood vessels in the histological sections (vascularity) was done with an image analyzer and the mean number of blood vessels was calculated. The results showed a significant increase in vascularity in the 1-week group compared to the other groups. However, the new blood vessels had small diameters and were congested with blood cells. In the 3-weeks and 4-weeks groups, the vascularity was similar to that of the control group. However, the blood vessels had a larger diameter than in the 1-week group with signs of congestion. In the 8-weeks group, the sizes of blood vessels were larger than in the 3-weeks and 4-weeks groups with no signs of congestion. In conclusion, angiogenesis is a critical aspect of dental pulp regeneration and homeostasis and can be observed histologically in the first week following orthodontic loading. In the 8-weeks group, the number of blood vessels was similar to that of the control group. However, dilatation and flaccid blood vessels with a thick layer of endothelial cells can be seen. Moreover, no congested blood vessel was seen.

Keywords: Angiogenesis, Dental pulp, Orthodontic loading, Vascular change

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Introduction

When a tissue is injured, an inflammatory process immediately occurs. The inflammatory process begins with vasodilation and the release of exudate, followed by leukocyte migration.[1] Chemokines are then produced to activate the down-regulated immune response.[2] During this time, the classic four cardinal signs, reported in many references found in the literature as rubor, dolor, calor, and tumor are present.[3]

Similarly, when the dental pulp tissue is injured, an inflammatory process occurs. Since dental pulp is enclosed within a mineralized hard tissue in the non-expandable pulpal chamber, any swelling caused by inflammation results in an increase in the intrapulpal pressure.[4]

Orthodontic force is one of the stimuli that can cause aseptic inflammatory reactions and necrosis of the pulpal tissue. Since blood supplies to the pulp arise from arterioles that make their way from the periodontium into the pulp via the apical foramen, any inflammation process increases the intrapulpal pressure followed by the decrease of pulpal blood flow.[5] As a result, the adaptation of pulpal blood flow via angiogenesis is necessary to avoid hypoxia and necrosis of the pulp.[6]

Currently, laser Doppler flowmetry is used as an accurate and reliable method for assessing microcirculatory function to detect blood flow changes clinically.[7] In this method, laser is transmitted to the pulp through a fiber optic probe. The scattered light beams from the moving red blood cells are frequency-shifted, whereas those from the static tissue remain unshifted in frequency.[8] Because this method is non-invasive and painless, it has been used to assess the blood flow changes during orthodontic tooth movement.[9] According to Sales *et al.* a significant decrease in the blood flow signal was verified during the initial phase of the treatment, followed by a complete recovery on day 30.[10]

Although the effect of orthodontic loading on blood flow changes has been extensively investigated, little is known about the histological aspect of pulpal microvasculature changes following orthodontic loading, such as angiogenesis. The histologic study can objectively describe the vascular cell changes, such as the shape, size, and number of cells, following orthodontic force application.[11]

Therefore, the purposes of the present study were to perform quantitative and qualitative evaluations of the histological pulpal microvasculature changes and angiogenesis following orthodontic loading application in humans.

Materials and Methods

In this present study, twelve patients (aged 18-32 years) at the Graduate Clinic, Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University, were referred for the removal of the third molars as part of an orthodontic treatment plan from January to September 2019. To be included in the study, the patients had to be healthy, and be free from pericoronitis and infection after the surgery. Patients were excluded if they had any missing data or missed the recall visits or if they reported the use of nontrivial drugs during the observation period. Informed consent was signed by all the patients before being included in this research.

The study was conducted in agreement with the Helsinki Declaration and ethical approval was obtained from the Bangkokthonburi University Research Ethics Committee (No. 11/2561). Detailed surgical procedures and potential risks were explained to each patient, who provided written and dated informed consent before the start of the study.

In the present study, 12 third molars were applied orthodontic loading for 1, 3, 4, and 8 weeks before the extractions. Three contralateral third molars that were not loaded served as a control. The orthodontic loading was performed using a 0.016-inch improved superelastic nickel-titanium alloy wire (Sentalloy®, Tomy International, Inc., Tokyo, Japan) that was engaged to a 0.018 x 0.025-inch

slot buccal tube. Following extractions, samples were immediately fixed in 10% neutral buffered formalin for 24 hours. A longitudinal groove on the teeth at the depth of 1 mm was made with a high-speed diamond bur under constant water spray coolant. Then, a chisel and a hammer were used to separate the teeth and the pulp tissue was collected and immediately fixed in 10 % neutral buffered formalin for additional 24 hours. The tissue preparing process was done according to the method of Tantiwetruangdet *et al.*[12] Briefly, pulp tissues were dehydrated with graded concentrations of ethanol and embedded in paraffin. Serial 5-micron longitudinal sections were cut. Slides were deparaffinized in xylene and rehydrated through graded concentrations of ethanol to distilled water. Antigen retrieval with EDTA and immunohistochemistry staining were done using the UltraVersion Quanto Detection System. CD 146 was used as a primary antibody and then scanned with the Panoramic MIDI digital slide scanner (3DHISTECH, Hungary). A photomicrograph was taken at pulp proper of the coronal pulp randomly 3 times with 20x and 40x magnification. The number of blood vessels was counted and the mean number of blood vessels was calculated.

Statistical Analysis

Data were analyzed using SPSS statistical software (version 19.0; SPSS Inc, Chicago, Ill). Kruskal-Wallis test was used to compare the number of blood vessels among each group followed by a post hoc test (Dunnett's test). Significance was set at $p < 0.05$. The results were presented as means and standard errors of the mean.

Results

In the 1-week group, the results showed a significant increase in the number of blood vessels when compared to the other groups ($p = 0.0006$) (Fig. 1). Blood vessels were smaller in size when compared to the control group. Moreover, we found a congested blood vessel with red blood cells (Fig. 2).

In the 3-weeks and 4-weeks groups, the number of blood vessels was decreased to the same level as in the control group, but the size of blood vessels was increased. Some congested blood vessels were observed.

In the 8-weeks group, the number of blood vessels was similar to the control group. However, dilatation and flaccid blood vessels with a thick layer of endothelial cells could be seen. Moreover, no congested blood vessel was seen.

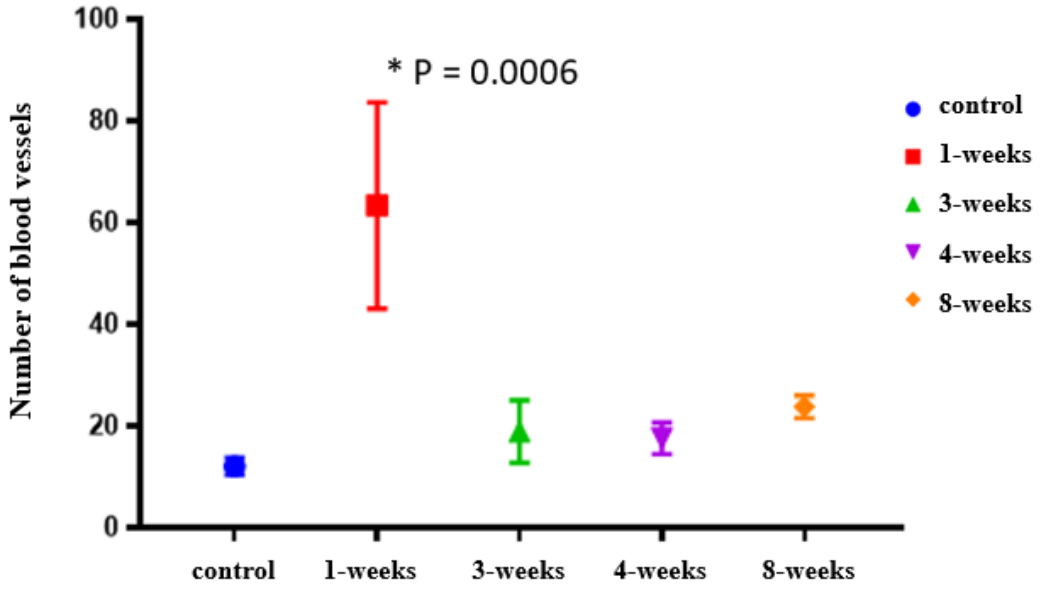

Figure 1 *Mean number of blood vessels for the control and the experimental groups*

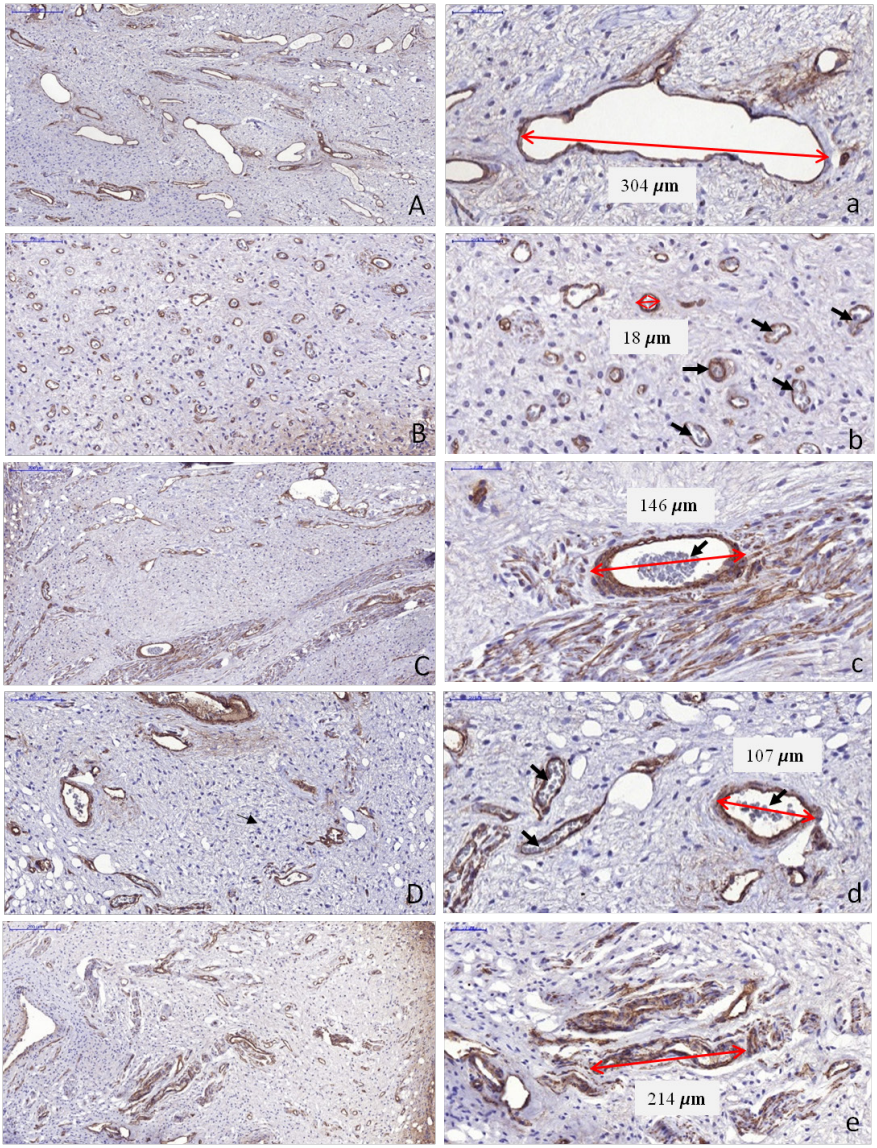

Figure 2 *Immunohistochemical staining of CD146 in pulpal vascular endothelial cells in the control group (A, a) showed large sized blood vessels. In the 1-week group (B, b) micro-blood vessels were significantly increased and displayed congested blood vessels (black arrow). In the 3-weeks group (C, c) and 4-weeks group (D, d) the number of blood vessels was decreased. The sizes of blood vessel were larger than in the 1-week group. Some congested blood vessels were observed (black arrow). In the 8-weeks group (E), the sizes of blood vessels were larger than in the 3-weeks and 4-weeks groups with no signs of congestion.*

Discussion

The application of orthodontic loading generates inflammatory responses in the tooth pulp, which raise intrapulpal pressure and reduce pulpal blood flow.[4] To avoid hypoxia and pulp necrosis, pulpal blood flow must be adapted through angiogenesis or neovascularization.[6]

The number of blood vessels in the 1-week group was raised fivefold, even though the size blood vessels was 15 times smaller than in the control group. The results showed that angiogenesis started within 1 week after force application. The possible mechanism for angiogenesis in the pulp is the sprouting angiogenesis.[13] In the sprouting angiogenesis, new blood vessels are formed from the existing blood vessels via endothelial sprouting.[14]

The results are in accordance with previous studies that evaluated the effects of the orthodontic force on the intrapulpal inflammatory process.[15] The

intrapulpal inflammation produces vasodilation and fluid exudation followed by swelling of the pulp tissue.[16] This process increases the intrapulpal pressure and decreases pulpal blood flow, thus leading to hypoxia.[6] The hypoxia triggers angiogenesis by activating hypoxia-inducible transcription factors and up-regulate angiogenic genes including the vascular endothelial growth factor, a major regulator of angiogenesis.[17]

The results are in agreement with previous clinical studies that evaluated the blood flow changes after orthodontic loading application with the Laser Dropper flowmetry.[5] According to Sabuncuoglu and Ersahan's study, the pulpal blood flow decreased after applying orthodontic loading on day 3 due to the increased intrapulpal pressure. Then, the pulpal blood flow gradually increased at 1 week and returned to the baseline level again at 4 weeks.[5] The results of the present study allow us to assume that orthodontic loading stimulates angiogenesis within 1 week in an attempt to reduce hypoxia and increase the pulpal blood flow.

The results are in agreement with the histological studies of the pulp reaction to the orthodontic loading.[18, 19] Santamaria *et al* observed the initial blood vessels congestion and widening after 6 hours of molar protraction in rats. The blood vessel was congested from 24 hours to 72 hours.[19] Mostafa et al., demonstrated that blood vessel dilation and congestion occurred in 1 week and continued up to 4 weeks.[18] However, dilated blood vessels were not observed in the 1-week group.

In the 3- and 4-weeks groups, a reduced number of blood vessels, similar to the control group was observed. These blood vessels had large diameters, a thick layer of endothelial cells, and were congested with blood cells. The explanation for the reduced number of blood vessels is the occurrence of anastomoses.[20]

Following the angiogenesis and maturation of the blood vessels, our results imply that anastomoses and stabilization of blood vessels occur after the 3-weeks force applications. Consequently, the decrease in the number and the increase in the diameter of the blood vessels can be seen. However, at weeks 3 and 4, the blood vessels were still congested with leucocytes, and erythrocytes with no dilated blood vessels, thus indicating the increased intrapulpar pressure with low blood flow.[19]

The results are not in agreement with previous clinical studies using laser Dropper flowmetry studies. Sabuncuoglu and Ersahan demonstrated that after the initial decrease of the blood flow following the orthodontic loading, a progressive increase in the blood flow similar to the baseline levels at the week 4 occurred.[5] In our study, it can be assumed that at week 4, the pulpal blood flow started to increased to values similar to the baseline levels since the blood vessels were anastomosed and increased in size. However, the blood flow did not return to normal baseline homeostasis values, due to the presence of congested blood vessels.

In week 8, the number of blood vessels was similar to the control group. However, dilatation and flaccid blood vessels with a thick layer of endothelial cells could be seen. Moreover, no congested blood vessel was seen, thus indicating the reduced intrapulpar blood pressure and increased blood flow. It is assumed that at week 8, the intrapulpar pressure returns to the baseline values, therefore achieving the homeostasis values.

From clinical studies using laser Dropper flowmetry, it is possible to conclude that the non-invasive laser Dropper flowmetry measured the recovery in the blood flow at 4 weeks.[5] Contrastingly, our results showed the actual and complete recovery at the histomorphometric levels did not occur until 8 weeks. This is a new finding of the present study. However, further studies combining the laser dropper flowmetry and histomorphometric studies are necessary.

Histological studies performed in humans are limited due to the invasiveness.[21, 22] However, the possibility of using the third molars as a histological model for the assessment of the orthodontic loading to provide clear quantitative and qualitative analyses of the cellular changes following the orthodontic loading application is advantageous.

The sample size is one of the limitations of this study due to fully erupted third molars might be extracted

before the patients come for orthodontic treatment. Moreover, the impacted third molars in which brackets can not be placed in the proper positions, must be excluded from our study. In the process of dental pulp collection, some samples might be lost due to the small size of the pulp. Due to the aforementioned reasons, our sample size is limited.

Another limitation of the present study is the use of light leveling forces. Further studies with higher force magnitude and directions, such as intrusion, extrusion, and protraction, should be performed in the future studies.

Conclusion

Angiogenesis is a critical aspect of dental pulp regeneration and homeostasis and can be observed histologically in the first week following orthodontic loading.

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Conflict of interest

The authors declare no conflict of interest.

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