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Letter from President of The Dental Association of Thailand

It is an honor for me to address in the opening chapter of this proceeding of the 18th International Scientific Conference of the Dental Faculty Consortium of Thailand (DFCT2021).

Dental Faculty Consortium of Thailand (DFCT) has developed tremendous progresses in developing the effective curriculums and directives of dental education since the establishment in the year 1983 (B.E. 2526).

On behalf of the President of The Dental Association of Thailand I would like to express my sincere appreciation for all the efforts done by DFCT through the passing 38 years. Generation after generation of all Dental educators and administrators, DFCT has been placed at the forefront of their roles in creating thousands of newly qualified dental graduates with highly clinical proficiency for the Dental Society and for Thais.

The 18th International Scientific Conference of the Dental Faculty Consortium of Thailand is another big step od DFCT to promote the awareness and importance of regular participation in the Scientific conference. Thus, will enable all the faculties to refresh and produce newly interesting topics in Dental Sciences and enhances the high capabilities of all faculty members, especially the young generation.

To conduct such an important event, the staffs and team work of the host are the crucial issue that will create success of the conference. I would like to congratulate the Faculty of Dentistry, Chiang Mai University for the efforts done in creating this event. With highly energetic staffs and faculty members, this conference will be the hall mark for all members of the Chiang Mai University.

Dr. Chavalit Karnjanaopaswong
President of The Dental Association of Thailand

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Original Article

Original Article

Effect of Bleaching Methods on Surface Roughness of Resin Impregnated Area of Tooth

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Abstract

The aim of this study was to evaluate the surface roughness of resin impregnated area of tooth before and after bleaching with different procedures. Buccal surfaces of extracted maxillary premolars were luted with orthodontic resin cement and polished until smooth with tungsten carbide bur. Initial surface roughness of the polished area was measured. Teeth were divided into 4 groups as follows: group 1) bleached with 10 % carbamide peroxide 8 hours per day (15 days), group 2) bleached with 20 % carbamide peroxide 8 hours per day (9 days), group 3) bleached with 40 % hydrogen peroxide 3 cycles (2 cycles at day 1 and 1 cycle at day 6) and group 4) bleached with 40 % hydrogen peroxide 2 cycles at day 1 and with 10 % carbamide peroxide 8 hours per day (9 days). Mean initial surface roughness between groups was not statistically different. After bleaching, the surface roughness was measured again. Data were analyzed using pair *T*-test and F-test one way ANOVA ($p<0.05$). Results revealed that all bleaching methods significantly increased the surface roughness of the resin impregnated areas. However, there was no significant difference between groups.

Keyword: Resin impregnated area, Surface roughness, Tooth bleaching

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Introduction

Tooth bleaching is a conservative treatment for whitening the tooth color. Currently, there are various approaches of tooth bleaching either in dental clinic or at patient's home with low and high concentrations of bleaching agents.[1–3] Mechanism of vital tooth bleaching is based on hydrogen peroxide or chemical agents containing peroxides due to their high oxidizing capability. The process starts when hydrogen peroxide bursts into oxygen, water and free radicals. These small radical molecules will diffuse through enamel and dentin to oxidize chromophores inside the tooth. Double bonds of the color molecules are broken into single bonds leading to brightened color.[2,4,5] Carbamide peroxide is another tooth bleaching agent that decomposes to 30% hydrogen peroxide and 70% urea. Hence, 10 % and 20% carbamide peroxide provide 3-3.5% and 6-7% of hydrogen peroxide, respectively.[1]

Tooth surface morphology could be affected by bleaching process. Increasing surface roughness and decreasing surface hardness were reported in previous studies which used hydrogen peroxide and carbamide peroxide.[1–3] High concentration of bleaching agents induced more changes of the enamel surface.[4] This surface alteration can lead to biofilm formation and tooth staining.[6]

In case of fixed orthodontic treatment, adhesive cement still remains on tooth surface after bracket is debonded. It will normally be polished out from the tooth surface, but impregnated cement layer approximately 30-50 microns is left in the enamel.[7] Surface of this hybrid layer can be stained and appears as a spot on the tooth.[8,9] Typically, this layer can finally be abraded out later by brushing. However, some orthodontic patients need to brighten their teeth immediately after the appliances are removed. This cement-enamel layer acts as a barrier, preventing the bleaching agent to diffuse inside the tooth.[5-7] Bleaching on the resin impregnated area results in delayed color changes compared to sound tooth structure.[10] Nantanapiboon and Maneenut[10] used low- and high-concentration bleaching agents as well as several bleaching methods for resin impregnated tooth bleaching. They reported that prolongation of bleaching time was needed in resin impregnated site. The mismatched color between the resin impregnated area and the surrounding area was initially observed in the first week of bleaching and declined at the later weeks. Time used for blending the color of resin impregnated area and the color of surrounding tooth depended on the bleaching agent concentration. The period of mismatched color was reduced by using high concentration of bleaching agents.

At present, there is no data about the effect of bleaching agent on resin impregnated area on tooth surface whether there will be less or more roughness after bleaching. Therefore, the aim of this study was to investigate whether different bleaching methods and different concentrations of bleaching agents could affect surface roughness of resin impregnated area of the tooth.

Materials and methods

Extracted human maxillary premolars were collected. Teeth were cleaned using dental scaler and polished with fine pumice slurry using a low-speed handpiece. They were inspected for signs of cracks, decay and restoration by stereomicroscope (Stereo Microscope SZ61, Olympus, Japan). Forty sound teeth were included into the experiment. The selected teeth were stored in 0.1% thymol solution at 37 degrees Celsius.

Teeth were mounted on acrylic resin blocks and individual silicone jig, 1 mm thick, was prepared. Circular hole, 6 mm in diameter, was made on the silicone jig at buccal surface of the tooth for locating the experimental area. Bleaching tray with 1 mm space at buccal aspect was also fabricated. Positioning of silicone jig and bleaching tray on each tooth could be repeated. (Fig. 1)

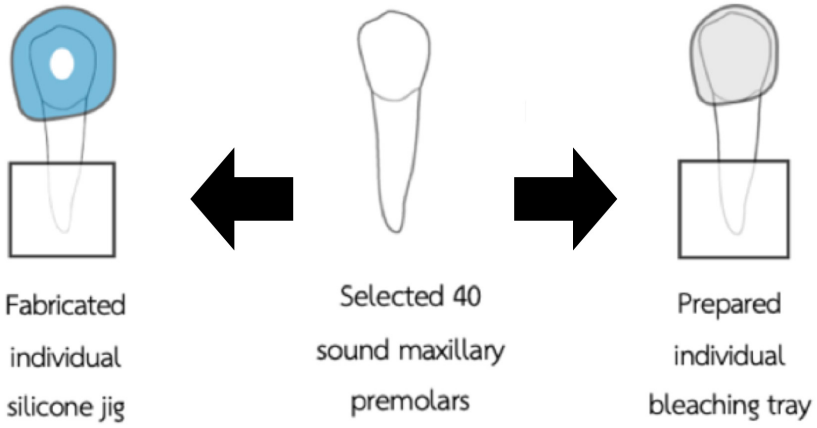

Figure 1 *Silicone jig and bleaching tray preparation*

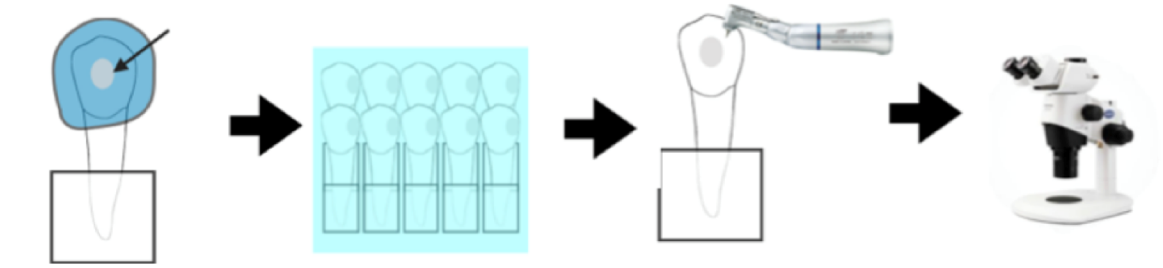

Figure 2 *Resin Impregnated layer preparation*

Resin Impregnated layer preparation (Fig. 2)

The silicone jig was placed on the tooth and buccal area in the hole was etched with 37% phosphoric acid for 30 seconds, rinsed with spray-water for 30 seconds, completely air-dried with air spray for 10 seconds, primed and luted with clear light-cured orthodontic adhesive cement (Transbond XT, 3M/Unitek, Monrovia, CA, USA) about 0.5 mm thick. The cement was pressed against tooth surface using a transparent cylindrical crystal, 6 mm in diameter and 2 mm in height. Light (1,100-1,330 mW/mm^2) from light curing unit (DemiTM Plus, Kerr, USA) was applied through the crystal for 40 seconds. The cylindrical crystal and silicon jig were removed from the tooth and light from light curing unit was applied on cement for 20 seconds. Specimen was stored in distilled water at 37 degrees Celsius for 24 hours.

The on-surface cement polishing was carried out with a slow speed 30-fluted tungsten carbide bur (Shofu dental corporation, Japan) without water.[11] The bur was changed after polishing 5 specimens. The polishing was stopped when the cement was reduced to the same level of tooth surface and its smoothness was checked by dental explorer. The specimen was polished with non-fluoride pumice for 30 seconds and rinsed with 20 ml of distilled water. The resin impregnated area was confirmed using stereomicroscope (Stereo Microscope SZ61, Olympus, Japan) at X40 magnification. (Fig. 3)

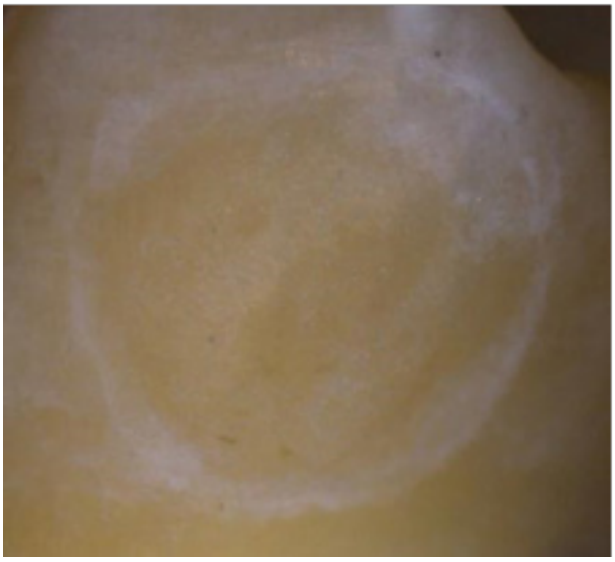

Figure 3 *Resin impregnated area*

Initial surface roughness at the polished area was measured using a non-contact surface roughness tester (Infinite Focus SL, Alicona. Austria). Roughness average (Ra) value was calculated from 3 measurements. Surface texture measurement (Sa) value was also calculated. Teeth were divided into 4 groups, means initial surface roughness of which were not statistically different.

Bleaching protocols

Teeth in each group were subjected to 4 bleaching methods, according to the previous study results.[10] (Table 1)

Table 1 *Bleaching protocols*

Group	Bleaching agent	Bleaching time	Teeth
1	Opalescence 10% carbamide peroxide, Ultradent, USA (Home-bleaching)	8 hours per day, 15 days	10
2	Opalescence 20% carbamide peroxide, Ultradent, USA (Home-bleaching)	8 hours per day, 9 days	10
3	Opalescence Boost 40% hydrogen peroxide, Ultradent, USA (In-office bleaching)	2 cycles at day 1 and repeated 1 cycle at day 6	10
4	Opalescence Boost 40% hydrogen peroxide, Ultradent, USA (In-office bleaching) and Opalescence 10% carbamide peroxide Ultradent, USA (Home-bleaching)	2 cycles of in-office bleaching at day 1, followed by 8-hour home bleaching per day for 9 days	10

For group 1 and 2, bleaching tray with gel was applied to the tooth for 8 hours per day for 15 days and 9 days, respectively. After 8-hour bleaching on each day, tray was removed, gel was rinsed out and tooth was stored in non-fluoride artificial saliva (Artificial saliva, Faculty of Dentistry, Chulalongkorn University, Thailand) at 37 degrees Celsius for 16 hours.

For group 3, the bleaching tray with gel was applied to the tooth for 2 cycles (20 minutes per cycle) at day 1.[12] The tray was removed, gel was rinsed out and the tooth was stored in non-fluoride artificial saliva at 37 degrees Celsius for 5 days. The other bleaching cycle was done at day 6. In group 4, 2 bleaching cycles (20 minutes per cycle) were done at day 1 followed by home bleaching for 8 hours per day for 9 days. After each bleaching session, the tray was removed, gel was rinsed out and the tooth was stored in non-fluoride artificial saliva at 37 degrees Celsius.

After bleaching procedure was done, the surface roughness of resin impregnated area in all groups was re-measured.

The research proposal was approved by Ethics Committee of the Faculty of Dentistry, Chulalongkorn University. The study code was HREC-DCU2020-081.

Statistical Analysis

The data was analyzed by SPSS version 26.0 with a 95 % confidence interval to indicate the significant difference using paired *t*-test and F-test one way ANOVA.

Results

F-test one way ANOVA revealed that before bleaching, resin impregnated surface roughness (Ra and Sa values) of all groups were not significantly different ($p>0.05$) (Table 3). After bleaching, pair *T*-test revealed that surface roughness of all groups was significantly increased (Table 2).

However, F-test one way ANOVA showed that the increasing of roughness was not statistically different among groups (Table 3).

Surface roughness images revealed smooth resin impregnated surface of all groups before bleaching. The surfaces were rougher after bleaching (Fig. 4).

Table 2 *Surface roughness, before and after bleaching*

Material	Ra			Sa		
	Before bleaching	After bleaching	*p* - value	Before bleaching	After bleaching	*p* - value
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Group 1 10% Carbamide peroxide	514.44 nm. (92.89)	946.11 nm. (107.85)	0.013*	985.89 nm^2 (93.69)	1526.54 nm^2 (249.21)	0.003*

Table 2 *Surface roughness, before and after bleaching (cont.)*

Material	Ra			Sa		
	Before bleaching	After bleaching	*p* - value	Before bleaching	After bleaching	*p* - value
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)	
Group 2 20% Carbamide peroxide	532.16 nm. (61.07)	950.98 nm. (138.42)	0.025*	998.93 nm2 (89.27)	1602.47 nm^2 (270.09)	0.041*
Group 3 40% Hydrogen peroxide	535.17 nm. (37.60)	937.75 nm. (187.67)	0.050*	1009.40 nm2 (76.46)	1492.93 nm^2 (325.57)	0.047*
Group 4 40% Hydrogen peroxide follow by 10% Carbamide peroxide	514.19 nm. (60.77)	809.88 nm. (97.44)	0.011*	982.88 nm2 (88.30)	1384.48 nm^2 (297.26)	0.031*

** Means of surface roughness before and after bleaching for each group*

Table 3 *Demonstrated the p-value of Ra and Sa before and after bleaching*

	Ra		Sa	
	Before bleaching (*p*–value)	After bleaching (*p*–value)	Before bleaching (*p*–value)	After bleaching (*p*–value)
Between groups	0.670	0.204	0.893	0.454

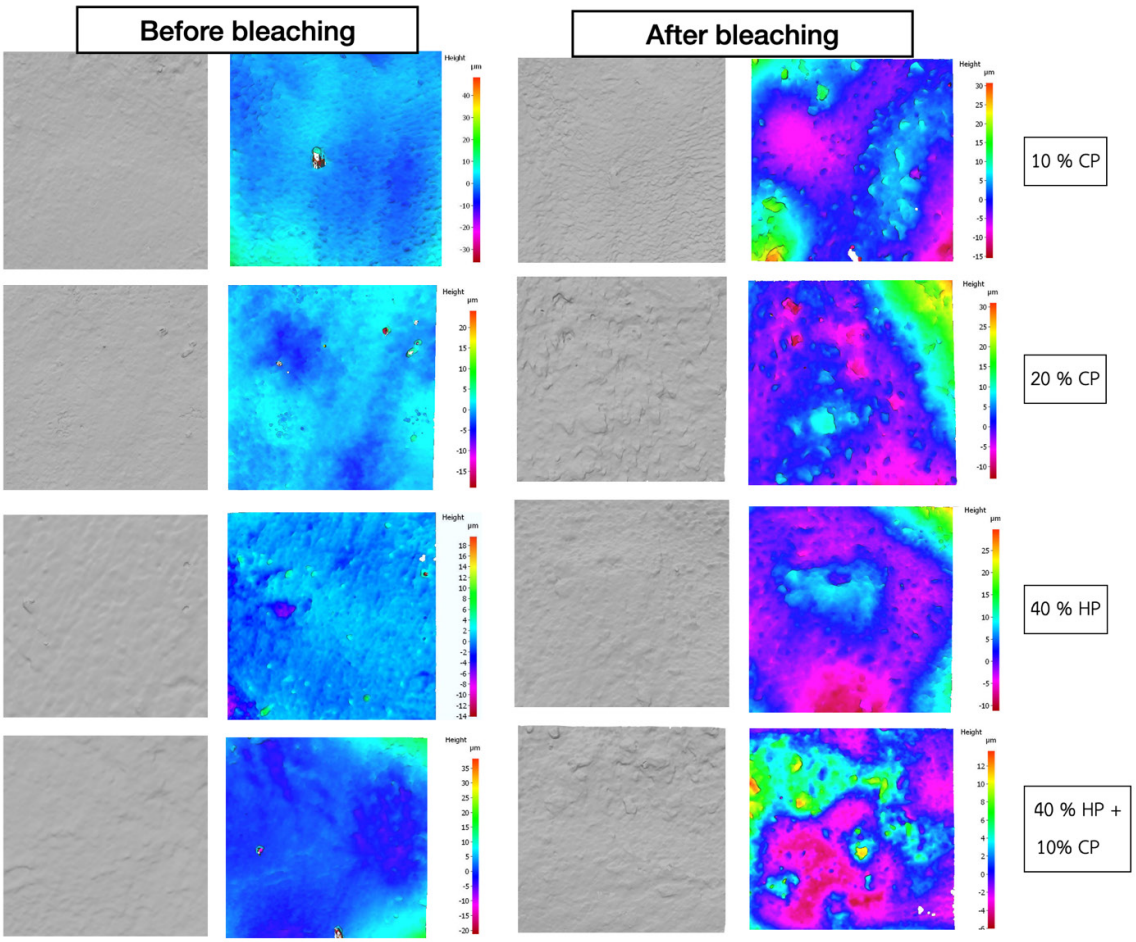

Figure 4 *Represented images of resin impregnated surface area, before and after bleaching*

Discussion

Using the non-contact surface roughness tester in this study could avoid the scratched surface of the specimen and could measure the roughness at the deep level. Apart from the scale of roughness average (Ra) and surface texture value (Sa) reported, 3D image of the surface could be generated. The principle of this tester is that laser light emits from the light source to the measured surface and reflects to the charge-coupled device camera to assess the roughness of the specimen.[12]

This present study found that using either 10% or 20% carbamide peroxide or 40% hydrogen peroxide or 40% hydrogen peroxide combined with 10% carbamide peroxide increased the roughness of resin impregnated surface. Without SEM picture of the polished surface, it could be speculated that after on-surface cement was polished to the level of enamel, the surface of this resin impregnated layer should consist of exposed enamel and resin. Orthodontic resin cement's components are similar to those of resin composite filling material. The matrix part comprises bisphenol A-glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA) and camphorquinone. The fillers are inorganic silica 77 % by weight and also contain phosphorous hexafluoride.[13] Reaction to bleaching of the surface may be similar to that of sound tooth structure and resin composite. Previous study revealed that the effect of both 10% CP and 40% HP increased the surface roughness of resin composite.[14] This may be accounted for by that the peroxide containing compound decomposing from the bleaching agent oxidized the remaining double bonds of the polymer chains. According to the study of Durmer and colleagues[15], they discovered that hydrogen peroxide reacted with not only the double bonds, but also the single bonds in resin composite polymer. This may cause resin matrix degradation and dissolution. This phenomenon may be increased in the present study due to the immersion of the tooth sample in artificial saliva for a period of time during bleaching. Water sorption of the resin impregnated layer could degrade the resin matrix.[16] Moreover, free radicals derived from the peroxide compound affected at the resin matrix–filler interface resulting in the dislodgement of the filler. The stress occurred from the water absorption of resin composite was another factor for the dislodgement of resin the filler. On the other hand, Londono[17] studied the effect of vital tooth bleaching on the solubility and roughness of dual-cured and self–adhesive resin cement (Rely X ARC and Rely X Unicem) for crown cementation (Rely X ARC and Rely X Unicem) and found that the in-office bleaching agent, 38% hydrogen peroxide, and the home bleaching agent, 20% carbamide peroxide, did not affect the resin cement surface roughness. The authors explained that the self-cured resin cement would create the rigid and stable network so the oxidation reaction of bleaching agent could not proceed.

Bleaching agents have the capability to increase surface roughness of the intact enamel. Previous study[18] revealed that higher concentration of the bleaching agent created more roughness after using 35% carbamide peroxide. The chemical agents in bleaching gel could lead to a loss of calcium and alter the Ca to P ratio on the tooth surface.[19] Bleaching agents might also enlarge gapsbetween enamel prisms, creating invasive tract to the surface. However, the damages have not been detected macroscopically or clinically visible. In contrast, 10% carbamide peroxide agent does not affect the enamel surface.

Tooth bleaching agents normally have neutral pH.[20] The differences among them are only the chemicals and concentrations that are used for initiating the free radicals. Duration of the bleaching procedures in this present study was from the result of the previous study[10], which was different for each group. According to the manufacturers' recommendation, the high concentration of bleaching agent, such as 40% hydrogen peroxide, has less tooth-contact time than the lower concentrations, i.e. 10% and 20% carbamide peroxide. The increase in roughness (Sa and Ra) of all groups in this study were not statistically significant (Table 3). This finding implies that the total amount of free radicals released from each group was comparable and

affects the surface roughness in the same rate. Nevertheless, if the contact time of all the groups had been the same, some differences would have occurred.

This study used the customized tray to carry the bleaching agent for the in-office bleaching, called "Sealed in office bleaching". This method was introduced to control the amount of peroxide in the oral situation[21] as in this study to control the amount of gel for each tooth. Previous study showed that using a customized tray with 35% hydrogen peroxide in the in-office method did not affect the level of sensitivity reported by the patient during the procedures. However, this technique may increase the chances and intensity of tooth sensitivity for the first 24 hours after the completion of the procedure.[21]

Nowadays, many bleaching approaches have been provided to achieve the most effective way of tooth whitening. The combination between in-office and home-bleaching methods (group 4) is recommended. Tooth whitening starts with in-office bleaching followed by continuous home-bleaching. Previous studies found that the combination of both methods reduced the risk of tooth sensitivity and gingival irritation. Moreover, the final whitened tooth appeared faster than using single individual mean.[22]

The present study did not evaluate the amount of peroxide penetrating into the tooth. The previous study by Benetti and colleagues.[23] revealed that the bleaching agents could enter the pulp through enamel and dentin after bleaching for 60 minutes. However, there was no report of penetration of bleaching agents through both resin composite and dentin into deeper area of the tooth. Since this resin impregnated layer is hybrid layer which has different properties from enamel and resin cement. Therefore, the outcome of bleaching on this layer may not be similar to that on the enamel or resin composite

Tooth bleaching process contributed to tooth surface changes[24–26] and altered the surface characteristic of resin composite and glass ionomer cement.[27] From the present study, the surface roughness of resin impregnated area was increased after bleaching which may lead to more biofilm and bacterial accumulation. Previous studies showed that the rougher surface of resin composite and resin-modified glass ionomer cement promoted cariogenic bacteria (i.e., *Streptococcus mutans* and *Streptococcus sanguinis*) biofilm adhesion after tooth bleaching. All of these bacteria are the pathogenic bacteria that may cause dental caries.[14,28,29]

In a clinical situation, the use of high concentration of hydrogen peroxide may cause the patient discomfort or tooth sensitivity during bleaching procedure. This side effect seems to be higher in hydrogen peroxide treated teeth compared to carbamide peroxide treated teeth due to the ability of hydrogen peroxide in producing more free radicals.[1,3-5] The result of the present study showed that there was no significant difference of roughness among groups of different methods used as shown in figure 4. Therefore, the use of a non-aggressive bleaching agent such as 10% carbamide peroxide seems to be more appropriate regarding tooth sensitivity. Moreover, it is recommended to re-polish the resin-impregnated surface after bleaching with fine and superfine polishing discs to decrease the roughness of the surface.

Conclusion

The surface roughness of resin impregnated area of tooth is influenced by bleaching procedures. All bleaching methods induce significantly increased roughness, but the final roughness is not significantly different between methods.

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Original Article

Comparison Study of Periotest M and AnyCheck for Tooth Stability Measurement at the Incisal Edge of the Crown During Active Orthodontic Treatment: A Suggested Protocol

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Abstract

Assessment of tooth stability (TS) during orthodontic treatment provides relevant information regarding the biomechanical behavior of the periodontium. Therefore, the purpose of the present study was 1) to compare the performance of the Periotest M and the AnyCheck in assessing tooth stability, 2) to compare the measurement of TS values obtained from the middle and the incisal edges, and 3) to develop a protocol of tooth stability measurement during the active phase of orthodontic treatment. Comparison of reliability of the Periotest M (Medizintechnik Gulden, Modautal, Germany) and the AnyCheck (IMT-100, DMS Co., LTD. Gangwon-do, Korea) was performed on 20 participants. Both devices are designed to provide objective measurements by assessing the damping capacity. Since the periotest values are displayed in PTV values and AnyCheck displayed in the iST scale (Implant Stability Test), a conversion equation to convert PTV into IST values was developed. A comparison of tooth stability values obtained from the middle and the incisal edge was performed to allow measurements during the active orthodontic treatment. Data was collected and analyzed statistically. Significant differences in TS measurements between the middle and incisal sites were observed. The Periotest produced the largest discrepancies (42.2%, ± 22.2%) between the middle and incisal readings. (p<0.001) Measurements of the posterior teeth were not possible with the Periotest due to the bulky head size. The AnyCheck produced reduced discrepancies between the middle and incisal readings (6.8%, SD 1.9%) with no significant changes in the posterior teeth. Relatively simple measurements were possible with AnyCheck. The correlation coefficient between the mean Periotest M and AnyCheck values was 0.870 (P<0.01). A strong correlation between the Periotest M and AnyCheck values was observed. The use of incisal edge for tooth stability measurements provided reliable and consistent tooth stability measurements. Moreover, it allows measurement during the active phase of orthodontic treatment.

Keywords: AnyCheck, Orthodontic tooth movement, Periotest M, Tooth stability

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Introduction

Assessment of tooth stability has shown to be an important clinical indicator of the health status and biomechanical behavior of the periodontium during orthodontic tooth movement.[1,2] The continuous remodeling of the periodontal tissues during orthodontic tooth movement promotes the increase in tooth mobility.[1] Therefore, the assessment of tooth mobility changes can be used as an important evaluation tool for the evaluation of the biomechanical characteristics of the periodontium.[2] Consequently, the assessment of TS values can be used as a clinical indicator of the tooth movement and treatment duration. Moreover, it is commonly accepted that tooth mobility increases during orthodontic treatment and is gradually restored to baseline levels after completion of orthodontic treatment.[3,4] Therefore, the assessment of tooth stability changes during orthodontic treatment and at the retention period has been investigated.[1-5] Tanaka *et al.* had performed the longitudinal measurements of tooth mobility during orthodontic treatment using a Periotest.[4] However, measurements were performed only on the anterior teeth.

Several studies had been performed to assess the values of tooth stability in permanent dentition using different approaches.[6-10] However, their acceptance has been limited because of the subjectivity associated with their use.[6] The Periotest is a non-invasive, electronic device that provides an objective measurement of the reaction of the periodontium to a defined impact load applied to the tooth crown. Consequently, the assessment of tooth stability with the Periotest as a special test for assessing the periodontal status of teeth in children that have suffered trauma has been broadly used.[7-10]

This method has been described as an efficient and reliable method to assess tooth mobility.[11] The Periotest measures the mobility and damping of natural teeth by measuring the acceleration in response to an applied impact.[2-4] The periotest values are displayed in PTV values (-8 to +50), with a higher scale representing lower stability or higher mobility. The Periotest values are related to clinical tooth mobility through a simple correlation.[6]

Recently, a new measuring device, AnyCheck (IMT-100, DMS Co., LTD. Gangwon-do, Korea) has been introduced to assess the stability of dental implants.[12,13] This device uses the tapping method which measures the time the tapping rod of the device contacts the implant fixture. The result of measurement is displayed in the iST (Implant Stability Test) scale (1 to 99) with a higher scale representing greater stability or lower mobility.

Both the Periotest M and the AnyCheck devices are dynamic devices designed to provide objective measurement of tooth mobility and implant stability by assessing their damping characteristics. However, the AnyCheck device has not been tested for the measurement of tooth stability. Moreover, according to the manufacturer's instructions, the handpiece must be oriented perpendicular to the tooth's long axis with the tapping rod being placed towards the middle of the anatomical crown.[6-11] However, the middle of the anatomical crown is often the selected place for the orthodontic buccal brackets placement. Consequently, monitoring tooth stability with such devices during the active phase of orthodontic treatment is not possible.

To avoid these limitations, the authors propose an alternative measurement method by modifying the point of impact of the tapping rod to the incisal edge of the anatomical crown, consequently allowing the measurement of tooth mobility throughout the orthodontic treatment. However, the impact of these changes on the reliability of the measurements has not been investigated.

Therefore, the purpose of the present study was 1) to compare the performance of the Periotest M and the AnyCheck in assessing tooth stability, 2) to compare the measurement of TS values obtained from the middle and the incisal edge, and 3) to develop a protocol of tooth stability measurement during the active phase of orthodontic treatment.

Materials and Methods

Assessment of Tooth Mobility

Periotest M vs AnyCheck

In the first part of the study, the selection of the best equipment for tooth stability measurement was made. Therefore, the comparison of Periotest M (Medizintechnik Gulden, Modautal, Germany) and the AnyCheck (IMT-100, DMS Co., LTD. Gangwon-do, Korea) in assessing tooth stability was performed on 560 teeth of 20 volunteer participants. (Fig. 1 A-C) Measurements were performed of all maxillary and mandibular teeth.

Tooth stability assessment was performed following the instructions of the manufacturer. Measurements were performed with the participants seated in the dental chair in an upright position with a stable headrest. The tapping rod of the measurement device was placed in the middle of the anatomical crown. For the Periotest M device, the tapping rod was placed in a horizontal position 0.5–2 mm away from the tooth surface. Measurements are performed with the handpiece positioned perpendicular to the long axis of the tooth. (Fig. 1D-G) Measurements were performed by two trained examiners. Each measurement was performed twice for each tooth and was averaged for analysis.

Conversion Formulas

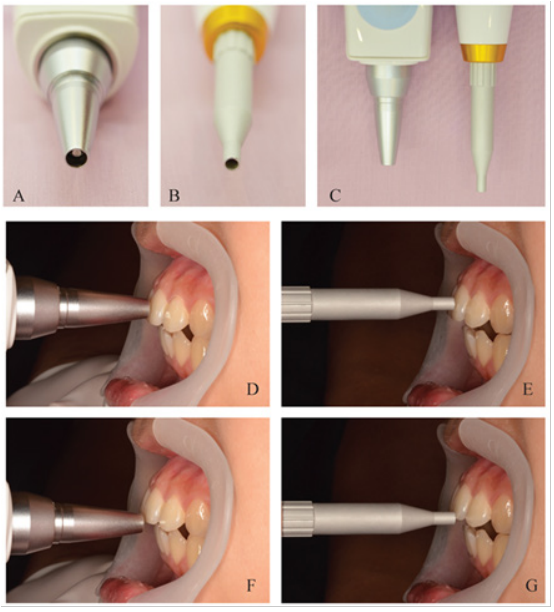

Figure 1 *Close-up pictures of the tips of the AnyCheck and Periotest M devices. Measurement devices were placed in the middle and the incisal edge of the tooth crown*

Both the Periotest M and the AnyCheck are dynamic devices designed to provide objective measurement of tooth stability by assessing damping characteristics of the periodontium. The periotest values are displayed in PTV values (-8 to +50), with a higher scale representing lower stability or higher mobility. In contrast, the AnyCheck values are displayed in iST (implant stability test) values (1 to 99) with a higher scale representing higher stability or lower mobility. Therefore, to allow the comparison of the standard deviations of the two devices, a conversion formula was created for both converting the PTV values into iST values. Moreover, since the Periotest M was designed to provide tooth stability values and the AnyCheck was designed to provide stability values, the conversion formula was proposed to represent the stability values.

The conversion of the PTV values into the iST values to assess stability was performed using the following equation: iST = 99 – ((PTV+8) * 99/58)

In this formula, the PTV values, which range from -8 to +50, thus containing a 58-unit scale, were converted into a 99-unit scale. The 0 to 99 scale is used for the iST assessment.

In this formula, the higher PTV scales represent the lower stability or higher mobility, while the higher iST scales represent higher stability and lower mobility.

Alternative Target Point for Tapping. (Middle versus Incisal edge)

In the second part of the study, the selection of an alternative target point for the tapping rod was performed to allow consistent and repeatable measurements during the active phase of orthodontic treatment.

For the conventional measurement for TM, the tapping rod of the measurement device is positioned at the middle of the anatomical crown perpendicular to the tooth's long axis. (Fig 2.) However, this position interferes with measurements during the active phase of orthodontic movement since this position coincides with the site where the orthodontic bracket is placed. Therefore, an alternative target point for the tapping rod was performed to allow

consistent and repeatable measurements during the active phase of orthodontic treatment.

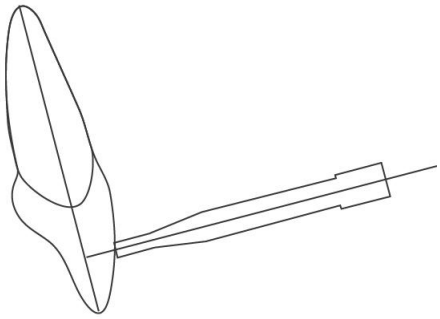

Figure 2 *Illustration of the tapping position perpendicular to the tooth's long axis*

All measurements were performed using the Periotest M and the AnyCheck device. The periotest values were converted into iST values using the proposed formula to allow comparison between devices.

Therefore, the selected point for the anterior incisors, canines, and premolars was the incisal edge perpendicular to the long tooth axis. For the molars, the selected point was the incisal edge of the mesial cusp. (Fig. 3)

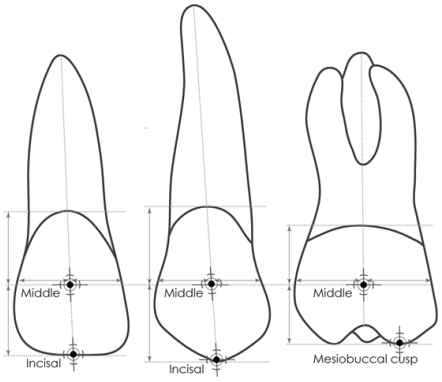

Figure 3 *Illustration of the middle and the incisal edge target sites for tapping*

The selected target point provides a reliable reference for tooth stability measurements during all phases of Orthodontic treatment, including at the baseline active and retention periods. (Fig. 4)

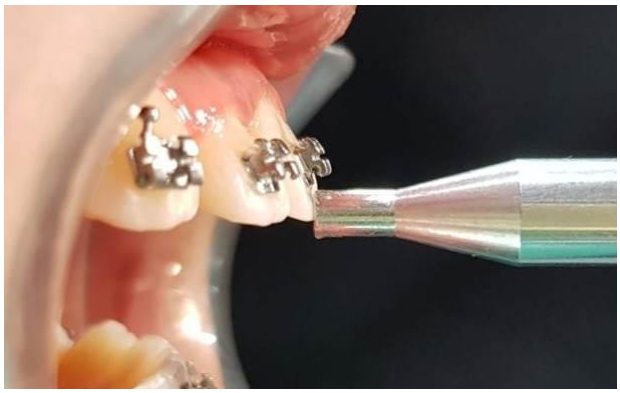

Figure 4 *Assessment of tooth stability during active orthodontic treatment*

Measurements performed at the middle and the incisal edge of the dental crown were performed to detect the differences between the different sites.

Participants

Assessment of tooth stability was performed on 560 teeth from 20 pre-orthodontic patients at the Graduate Clinic, Department of Orthodontics, Faculty of Dentistry, Bangkokthonbuti University between Jan 2018 – Jun 2018. This study was approved by the Human Experimentation Committee, Faculty of Dentistry, Bangkokthonburi University (Approval Number: 26/2561). Informed consent was obtained from all participants before the initiation of the study.

Inclusion and exclusion criteria

The overall inclusion criteria were; participants with good general health, excellent oral hygiene with sound teeth with normal shape and size, no periodontal disease nor bone loss visible on panoramic radiographs. Also, they should have no history of dental trauma nor previous orthodontic treatment with an absence of large restorative treatment such as large filling or crowns as well as no missing teeth except for the third molars.

Inter and intraindividual calibration

For the reproducibility and reliability of the measurements, inter-and intraindividual reliabilities were

performed using the intraclass correlation coefficients (ICC). Tooth stability was conducted twice at the incisal edge and the middle of the dental crown. For the middle of the dental crown measurements, the ICC was 0.850 and 0.915 for the inter-and intraindividual reliabilities, respectively. Whereas for the incisal edge of the dental crown measurements, the ICC was 0.801 and 0.844 for the inter-and intraindividual reliabilities, respectively.

Statistical Analysis

SPSS version 27.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis of the results. The paired *t*-test was used to compare the Periotest M and AnyCheck measurements at the middle and incisal edges. The agreement between the Periotest M and AnyCheck values measurements was evaluated with Pearson's correlation and coefficient and Bland-Altman analysis. The level of significance was set at 95% ($P<0.05$).

Results

Periotest M vs AnyCheck

Results of tooth stability measurements using the Periotest M (PTV values were converted in iST*) and AnyCheck values (iST) are shown in Table 1. There were no observed significant differences in the tooth stability values between both measurements. However, the Periotest M device could not perform measurements in the posterior molar area due to the large head. The Anychek device, presenting a longer and thinner tip for measurement, allowed simple measurement in both the anterior and the posterior teeth. The correlation coefficient between the mean Periotest M and AnyCheck values was 0.870 ($P<0.01$). Figure 5 Bland-Altman analysis demonstrated good agreement between the Periotest M and AnyCheck measurements. The results indicate that there is no consistent bias of one approach versus the other. (Fig. 6)

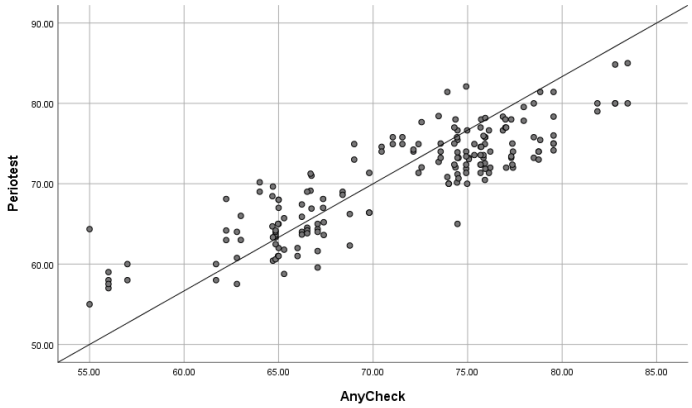

Figure 5 *Correlation of the Periotest M and AnyCheck values*

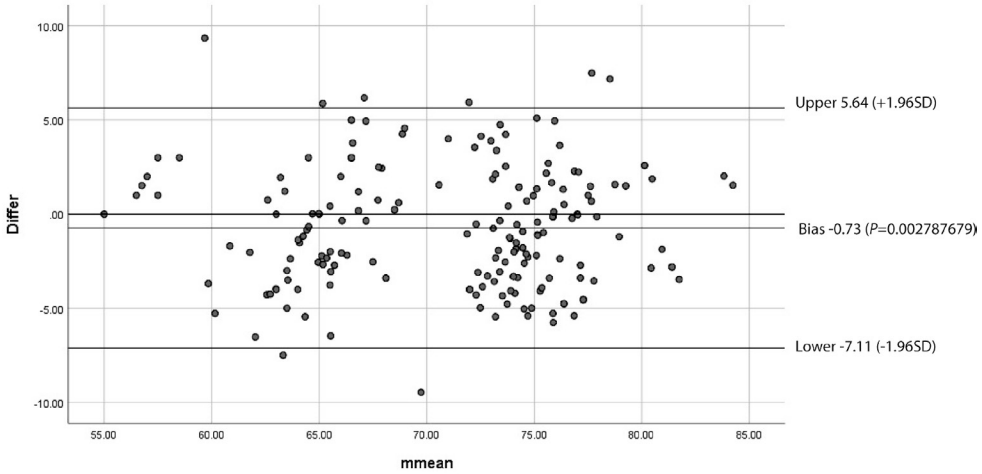

Figure 6 *Bland-Altman analysis to compare the reliability of the two measurements*

Table 1 *Assessment of tooth stability at the incisal edge using Periotest M and AnyCheck*

		Periotest M (PTV)				AnyCheck (iST)		*P* value
		PTV		iST*		iST		
		Mean	SD	Mean	SD	Mean	SD	
Maxilla	Central Incisor	14.2	2.3	61.1	5.9	66.2	4.7	0.335
	Lateral Incisor	12.5	2.2	64.0	4.7	64.7	4.3	0.464
	Canines	4.8	2.3	77.1	4.9	73.5	4.2	0.715
	First Premolar	9.5	2.2	69.1	4.0	72.6	4.0	0.468
	Second Premolar	5.7	3.7	75.6	5.9	71.5	4.2	0.406
	First Molar	4.1	5.3	78.3	5.1	76.2	3.9	0.626
	Second Molar	n/a				73.9	4.7	n/a
Mandible	Central Incisor	15.0	2.5	59.7	4.3	62.0	3.7	0.457
	Lateral Incisor	13.8	2.7	61.8	5.8	64.4	4.3	0.476
	Canines	7.7	3.0	72.1	5.1	73.1	5.0	0.696
	First Premolar	7.8	2.6	72.0	4.6	74.9	4.4	0.732
	Second Premolar	8.0	4.1	71.6	5.3	74.4	4.9	0.484
	First Molar	2.3	6.7	81.4	9.9	80.1	3.5	0.665
	Second Molar	n/a				76.2	3.9	n/a

PTV values were converted into iST values using the conversion equation*

Paired t-test, significant at P<0.05. n/a = Not applicable

Alternative Tapping Point (Middle versus Incisal)

Comparisons of tooth stability measurements between the middle and the incisal edge of the tooth's crown with Periotest M and AnyCheck are presented in Tables 2 and 3. Significant differences in tooth stability between both sites were observed.

For the Periotest M, a significant increase in the overall incisal readings (42.2%, SD 22.2%) was observed (p<0.001). The largest differences were observed in the anterior teeth. In Table 2, a moderate correlation (0.421) between the middle and incisal edge measurements was observed. (P<0.01) (Table 4)

For the AnyCheck, although an overall decrease in all incisal readings (6.8%, SD 1.9%) was observed, no significant changes in the tooth stability readings in the posterior teeth were observed. Table 3 A strong correlation (0.868) between the middle and incisal edge measurements was observed. (P<0.001) (Table 4)

Table 2 *Comparison of tooth stability values between middle and incisal sites using Periotest M*

		Periotest M (PTV)							
		Middle		Incisal		Diff		(%)	*P* value
		Mean	SD	Mean	SD	Mean	SD		
Maxilla	U1	7.37	2.73	14.2	2.3	6.8	-0.5	93.0 %	<0.001***
	U2	9.15	3.19	12.5	2.2	3.3	-1.0	36.4 %	<0.001***
	U3	4.01	2.50	4.8	2.3	0.8	-0.2	20.0 %	0.002**
	U4	5.72	1.79	9.5	2.2	3.8	0.4	66.7 %	<0.001***
	U5	4.41	1.00	5.7	3.7	1.3	2.7	29.5 %	0.002**
	U6	3.67	1.39	4.1	5.3	0.4	3.9	12.0 %	0.004**
	U7	n/a		n/a					

Table 2 *Comparison of tooth stability values between middle and incisal sites using Periotest M (cont.)*

		Periotest M (PTV)							
		Middle		Incisal		Diff		(%)	*P* value
		Mean	SD	Mean	SD	Mean	SD		
Mandible	L1	11.05	1.07	15.0	2.5	4.0	1.4	36.2 %	<0.001***
	L2	10.87	1.94	13.8	2.7	2.9	0.7	26.9 %	0.005**
	L3	5.91	1.32	7.7	3.0	1.8	1.7	31.0 %	0.006**
	L4	5.64	1.91	7.8	2.6	2.2	0.6	38.7 %	0.008**
	L5	5.43	2.34	8.0	4.1	2.6	1.7	48.3 %	<0.001***
	L6	1.47	0.91	2.3	6.7	0.8	5.8	56.1 %	0.004**
	L7	n/a		n/a					
	Mean	6.2	1.8	8.8	3.3	2.6	1.4	41.2 %	<0.001***

*Paired t-test, * P< 0.05, **P < 0.01, ***P < 0.001. n/a = not applicable*

Table 3 *Comparison of tooth stability values between middle and incisal sites using AnyCheck*

		AnyCheck (iST)							
		Middle		Incisal		Diff		(%)	*P* value
		Mean	SD	Mean	SD	Mean	SD		
Maxilla	U1	71.7	4.3	66.2	4.7	5.4	-0.4	7.6 %	0.042*
	U2	71.5	5.7	64.7	4.3	6.8	1.3	9.5 %	0.048*
	U3	78.4	5.5	73.5	4.2	4.9	1.3	6.2 %	0.025*
	U4	78.4	4.2	72.6	4.0	5.9	0.2	7.5 %	0.036*
	U5	76.2	4.2	71.5	4.2	4.6	0.0	6.1 %	0.124
	U6	81.4	4.4	76.2	3.9	5.2	0.6	6.4 %	0.126
	U7	77.1	5.5	73.9	4.7	3.2	0.8	4.2 %	0.133
Mandible	L1	68.6	6.1	62.0	3.7	6.6	2.4	9.6 %	0.048*
	L2	70.5	4.6	64.4	4.3	6.1	0.3	8.7 %	0.040*
	L3	79.8	3.7	73.1	5.0	6.6	-1.3	8.3 %	0.137
	L4	78.9	3.1	74.9	4.4	4.0	-1.3	5.1 %	0.234
	L5	79.4	3.2	74.4	4.9	5.0	-1.7	6.3 %	0.244
	L6	83.1	2.0	80.1	3.5	3.0	-1.5	3.6 %	0.246
	L7	80.2	4.7	76.2	3.9	4.0	0.7	5.0 %	0.181
	Mean	76.8	4.4	71.7	4.3	5.1	0.1	6.7 %	0.056

*Paired t-test, * P< 0.05, n/a = not applicable*

Table 4 *Correlation between measurements at the incisal edge and middle of the crown using Periotest M and AnyCheck*

	Incisal and Middle	*P*-value
Periotest M	0.421	0.007**
AnyCheck	0.868	<0.001***

Pearson correlation coefficient, significant at P< 0.01 and **P < 0.001*

Discussion

The orthodontic force applied to teeth generates specific compressive and tensile mechanical loading patterns that create complex biological responses in the periodontal tissues surrounding the loaded teeth.[14] As a result, the remodeling of the alveolar bone occurs accompanied by the widening of the periodontal ligament to allow the dental movement towards the compressive direction.[15,16] These sequential events play an important role in tooth stability. Therefore, the accurate determination of the tooth stability values at the baseline, and the changes during the active and retentive phases of orthodontic treatment provides relevant information regarding the biomechanical behavior of the periodontium.[1-6]

However, limited information is available regarding the tooth mobility at the baseline and the changes during the active orthodontic treatment. Since most of the measurement devices use the middle of the clinical anatomical crown, monitoring tooth stability with such devices during the active phase of orthodontic treatment with conventional buccal appliances is not possible.

In the present study, the authors describe a protocol for the measurement of tooth mobility and stability that can be applied during the active phase of the orthodontic treatment.

The Periotest M method has been described as an efficient and reliable method to assess tooth mobility.[11] Consequently, most studies involving assessment of tooth mobility utilize the Periotest M device to obtain reliable data. Recently, a new stability-measuring device, AnyCheck, has been introduced in the field of dental implantology. [12,13] Similar to the Periotest M device, the AnyCheck device measures fixture stability by using damping capacity analysis. Comparison of the sensitivity and reliability of the Periotest M and the AnyCheck for the assessment of the stability of dental implants have demonstrated a strong correlation between measurements.[12,13] Lee *et al.,* observed a strong correlation between Periotest M and AnyCheck values in an *in vitro* study.[12] Later, Lee *et al.* observed similar results in an *ex vivo* experiment.[13]

In the present study, the comparison of the sensitivity and reliability of the Periotest M and the AnyCheck for clinical assessment of tooth stability have demonstrated a strong correlation between measurements.

To the author's knowledge, the clinical use of the AnyCheck for assessing tooth stability values has not been performed. Moreover, to allow the comparison of tooth stability values obtained by both devices, a conversion formula was elaborated to convert PTV values into iST values.

In the present study, Bland-Altman analysis was performed to compare the reliability of the two measurements. No significant difference was found between the Periotest M and AnyCheck readings in the incisal sites. Moreover, a significantly strong correlation between both measurements was observed. The results are in agreement with previous studies that compared the Periotest M and AnyCheck values of implant stability.[12,13]

However, the Periotest M could not perform adequate and reliable measurements on posterior teeth. Repetition of several measurements was needed to obtain final tooth stability readings. This difficulty became more evident in the measurement of the posterior teeth. This difficulty was also reported by previous studies due to the difficulty of positioning the device as per the manufacturer's manual.[11]

Moreover, the Periotest M was hard to handle and measurements were time-consuming with several tooth measurements readings and the assessment of the second molars was not possible. The main reason for this difficulty was the large number of tapping times required for measurements, and relatively heavy tapping forces applied to the tooth. Moreover, the bulky size of Periotest M tips (large and short) and the need to maintain a constant clearance distance (0.5 to 2.0 mm) from the tooth surface to allow measurements, including difficult measurements with the Periotest M.

In contrast, the AnyCheck device was relatively simple and easy to handle. It allowed for relatively more simple and easy measurements of tooth stability in both

the anterior and posterior sites. Therefore, compared to the Periotest M, the AnyCheck device is more "user-friendly".

Comparison of different sites of tooth stability between the middle and incisal edge of the tooth's crown showed contrasting results between the Periotest M and the AnyCheck results.

For the Periotest M, a large discrepancy between the middle and incisal edge measurements was observed. The incisal edge site produced the largest tooth mobility values compared to the middle sites. The higher differences were observed more in the anterior teeth, in particular to the maxillary incisors. However, only a moderate correlation between reading between the middle and the incisal edge measurements was observed. Such discrepancies might be explained by the differences in the distances from the tooth's center of resistance, which is located in the middle third of the roots.

For the AnyCheck device, the significant differences between the middle and incisal edge readings were observed only with the anterior teeth. Moreover, the differences between the middle and incisal edge readings were eight times smaller than the differences observed with the Periotest M readings. In the posterior area, no significant differences in the middle and incisal edge readings were observed. This might be explained by the relatively short clinical crown observed in the posterior teeth and the relatively small distances between the middle and incisal edges observed in the posterior teeth.

Therefore, based on the results of this study, the AnyCheck device might be considered as an alternative equipment for evaluating the damping capacity of tooth stability.

A limitation of the present study might be the presence of inter-individual variation, such as the skeletal pattern, gender, and age. Therefore, further studies are necessary to assess factors related to the differences in tooth mobility.

In the present study, the authors had proposed an alternative measurement protocol for tooth stability by using the AnyCheck device and by modifying the point of impact of the tapping rod to the incisal edge of the tooth's anatomical crown. Such modifications have provided reliable and consistent tooth stability measurements. Consequently, the assessment of tooth stability throughout the active phase of the orthodontic treatment can be easily and consistently performed following this protocol.

Although it is generally known that an increase in tooth mobility occurs during orthodontic treatment, limited information regarding the amount of tooth mobility changes or the limits of safe tooth mobility values during active orthodontic treatment is available. Moreover, the possibility of using the tooth mobility analysis for predicting quantitatively the amounts of tooth movement might allow the construction of algorithms to precisely predict the overall optimum treatment duration. Therefore, further studies to assess the physiological values of the tooth mobility at the baseline, during the active and the retention phases of the orthodontic treatment, should be investigated in future studies.

Conclusions

1. A strong correlation between Periotest M and AnyCheck values in clinical measurements was observed.

2. The use of the incisal edge for tooth stability measurements provided reliable and consistent tooth stability measurements. Moreover, it allows for measurement during the active phase of orthodontic treatment.

3. The AnyCheck device allowed for relatively more simple and easy measurements of tooth stability in both anterior and posterior sites. Therefore, it might be considered as an alternative and reliable equipment for evaluating the damping capacity of tooth stability.

4. A protocol of tooth stability measurement using the incisal edge of the tooths' crown during the active orthodontic treatment with the AnyCheck device has been presented.

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Original Article

Self-awareness of Individuals with Severe Periodontitis in Thai Adults

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Abstract

This cross-sectional study aimed to estimate the level of self-awareness and its associated factors among Thai adults with severe periodontitis. This study comprised of 619 participants from employees of the Electricity Generating Authority of Thailand (EGAT) who had completed medical examinations, periodontal examinations, and an interview with self-reported periodontal status questions. All included participants had severe periodontitis. Periodontitis self-awareness was determined by one-on-one interviews of what they thought of their current periodontal status. The answer would be no problem or having gum disease/periodontitis. The prevalence of severe periodontitis with and without self-awareness was estimated. Binary logistic regression was used to identify associated factors with self-awareness. Results showed low awareness of Thai adults with severe periodontitis. Among the participants with the disease, only 24.9 % (95% CI, 21.5, 28.3) reported having periodontitis. The percentage of the participants aware significantly decreased with older age, lower education level, and lower income level. However, it significantly increased with disease severity. The multivariate logistic regression suggested a significant association between the unaware participants and education level of less than bachelor's degree with the adjusted OR of 1.7. In conclusion, this study shows that periodontitis self-awareness in Thai adults was poor. Older individuals with a lower education level, and lower income were more likely to be unaware of periodontitis. Therefore, periodontal health promotion needs to be emphasized.

Keywords: Awareness, Epidemiology, Periodontitis, Self-report

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Introduction

Periodontitis is the most common oral disease affecting the global population.[1] According to Global Burden Disease Study[2], severe periodontitis was the sixth-most prevalence condition which affected 11.2 % of the population worldwide. According to a national oral health survey of Thailand in 2017[3], approximately 25.9 % of adults and 36.3 % of the elderly had periodontitis with a periodontal pocket depth of ≥4 mm. The hallmarks of periodontitis are gingival inflammation and alveolar bone destruction. If left untreated, it could eventually result in tooth loss. These pathological changes influence daily life functions such as masticatory functions, speech problems, esthetic problems, and an impaired quality of life.[4] Therefore, it is of paramount importance that participants be aware of the presence of their periodontal diseases, which may enable them to seek proper periodontal treatment.[5,6]

However, the course of periodontal disease is slow and relatively painless, participants were usually unaware of its presence and progression.[7] Not until it is at an advanced stage and symptoms such as gingival swelling, pus discharge or tooth mobility occur, that participants notice the presence of periodontal disease. In most of the studies[8-13], less than half of the participants were aware of having periodontal disease. The National Health and Nutrition Examination Survey in the United States (NHANES) demonstrated only 25 % of the participants with periodontitis were aware of having the disease.[9] The survey also showed that age, race, and educational level had an influence on the extent of periodontal awareness. Other previous studies found that 60 to 80 % of the participants lacked knowledge concerning the cause, symptoms, and treatment of periodontal disease. Most of them had never been informed about periodontal disease by a dentist even for participants who regularly visit the dentist.[8,14,15] This could imply that self-awareness of periodontitis was quite low, as many patients did not realize their condition. Additionally, studies indicated the need to improve periodontal awareness and the need to promote knowledge concerning periodontitis for better self-care and disease detection.

The recognition and awareness of existing periodontal disease by patients is the first crucial step of treatment. Patients who noticed periodontal disease are more likely to seek periodontal treatment. Knowing to what extent patients are aware of their periodontal condition will be of significant use for oral health care providers in planning patients' education and motivation regarding periodontal disease especially in groups with low disease awareness. Therefore, the aims of this study were to estimate the level of self-awareness and its associated factors among Thai adults with severe periodontitis.

Materials and Methods

This cross-sectional study accessed and utilized the secondary data from a 2013 health survey among employees of the Electricity Generating Authority of Thailand (EGAT).[16] EGAT employees in Bangkok and the surrounding area were randomly selected by simple randomization with a specific age range. All participants underwent a routine health examination and oral examination. In addition, demographic data, underlying diseases, health behaviors and self-report periodontal status were assessed by the interview and questionnaires. This study protocol was approved by The Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2020-020).

Study population

The EGAT employees that met the inclusion criteria were those who registered for the 2013 health survey (EGAT 2/4) and completed both the periodontal examination, and the interview of a self-reported periodontal status question. Participants were excluded from the study if they were fully edentulous or had contraindication for periodontal examination including: a high-risk group of infectious endocarditis according to the American Heart Association or had an indication of antibiotic prophylaxis prior to periodontal examination.[17] Only participants with severe periodontitis, having ≥2 proximal sites with loss of clinical attachment level (CAL) ≥6 mm (not on same tooth) and ≥1 proximal site with probing depth (PD) ≥5 mm[18], were included in this study.

Periodontal examination

Dental examinations were carried out by eight experienced periodontists from the Department of Periodontology, Faculty of Dentistry, Chulalongkorn University in mobile dental units. Standardization for periodontal measurements were performed among the eight examiners before the survey. The weighted kappa (within ±1 mm) of the inter-examiners and intra-examiner ranged from 0.74-1.00, and 0.87-1.00, respectively. The details of periodontal examination were reported elsewhere.[19] In brief, the examination included the number of teeth remaining, plaque score, bleeding on probing (BOP), PD and recession (RE). The standard full-mouth periodontal examination protocol was performed. The PD and RE were measured in all teeth except the third molars and retained roots by UNC-15 periodontal probe at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual). Then, the severity of periodontal disease was classified by the 2012 Centers for Disease Control/American Association of Periodontology (CDC/AAP) periodontitis case definition.[18]

Self-reported periodontal status question

Self-report periodontitis status question wording was simplified for easy understanding and the question was carried out in trials on dental assistants and lay persons. The question was tested for reliability by asking the same question to the same person twice one week apart. The kappa coefficient of the test-retest reliability in this question was 0.97. The interviewer was trained to provide related information for the question. Participants were interviewed one-on-one by the interviewer with a self-reported periodontitis status question prior to periodontal examination. Participants were asked the question concerning what they thought of their current periodontal status. The answer would be whether there was no problem or having gum disease/periodontitis. The interview was performed in Thai language without time constraints. In case that participants were uncertain, further relevant information about periodontal diseases would be provided by the interviewer.

Statistical analysis

Demographic data and clinical variables were presented using descriptive statistics. Participants with severe periodontitis who self-reported having a gum disease/periodontitis would be assumed to be individuals with awareness. The point estimation and 95 % confidence interval (95% CI) of self-awareness level were assessed from the proportion of aware participants among the total of all the participants. Characteristics between aware and unaware participants were compared by the Pearson Chi-square test. Periodontal parameters, which were considered as continuous data, were tested for normality by the Kolmogorov–Smirnov test. Then, the differences between the groups of aware and unaware participants were identified using the *t*-test or the Mann-Whitney *U* test, where appropriate. Moreover, the association between co-variables and periodontitis self-awareness was determined using binary logistic regression. Age, gender, marital status, education level, income, smoking habits, diabetes, dental visit frequency, and severity of periodontitis were considered as covariates in the binary logistic regression. The covariates with a *p*-value <0.2 in the univariate analysis were simultaneously considered in the multivariate analysis. Odds ratios (ORs) and their 95% CI were also estimated. All analyses were performed using the STATA version 14.2. The *p*-value <0.05 was considered statistically significant.

Results

Of all 2,037 employees registered for the health survey, 45 participants denied periodontal examination, another three were excluded due to systemic contraindication for periodontal examination, and 19 were fully edentulous participants. Out of 1,970 participants with periodontal examination, 1,317 non-severe periodontitis participants were excluded. Thirty-four participants had incomplete records on the periodontal self-awareness questionnaire. Therefore, the total number of 619 participants with severe periodontitis were included for the analysis. The flow of included participants is shown in Figure 1.

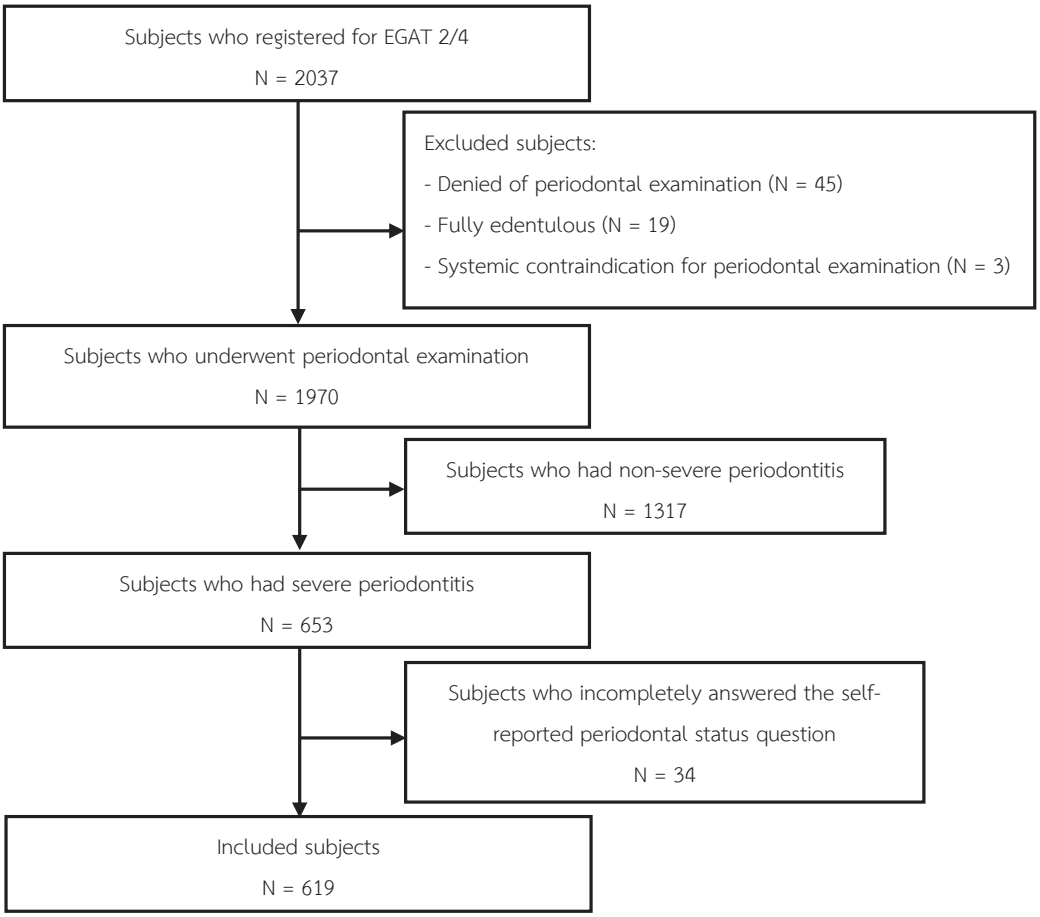

Figure 1 *Flow of included subjects*

The demographic data is presented in Table 1. Out of 619 participants, 111 were females and 508 were males with an age range from 49 to 70 years old, and a mean age of 57.8 ± 4.8 years old. The majority of the participants were married. The education level was mostly diploma or higher degrees with an income of ≥50,000 baht/month. Approximately a quarter of the participants were current smokers. Only 15 % of the participants had never had a dental visit in the last five years, while 40 % had regular annual dental visits.

The proportion from self-reported showed that only 24.9 % of participants with severe periodontitis reported having periodontitis (95% CI: 21.5, 28.3) when comparing the characteristics between the aware and the unaware groups (Table1). the percentage of the aware participants significantly decreased with older age, lower education level, and lower income.

Table 1 *Distribution of baseline demographic characteristics according to periodontitis self-awareness*

Characteristics	Total N* (%)	Unaware	Aware	*p*-value
Age				0.017
<60	422 (68.2)	305 (72.3)	117 (27.7)	
≥60	197 (31.8)	160 (81.2)	37 (18.8)	
Gender				0.109
Female	111 (17.9)	90 (81.1)	21 (18.9)	
Male	508 (82.1)	375 (73.8)	133 (26.2)	

Table 1 *Distribution of baseline demographic characteristics according to periodontitis self-awareness (cont.)*

Characteristics	Total N* (%)	Unaware	Aware	*p*-value
Marital status				0.414
Single	23 (3.8)	16 (69.6)	7 (30.4)	
Married	513 (84.2)	382 (74.5)	131 (25.5)	
Divorced	73 (12.0)	59 (80.8)	14 (19.2)	
Education level				0.047
≤High school	188 (30.9)	148 (78.7)	40 (21.3)	
Diploma	233 (38.2)	180 (77.2)	53 (22.8)	
≥Bachelor's degree	188 (30.9)	129 (68.6)	59 (31.4)	
Income				0.019
<20,000 Baht/month	87 (14.3)	75 (86.2)	12 (13.8)	
20,000 – 49,999 Baht/month	103 (16.9)	80 (77.7)	23 (22.3)	
≥50,000 Baht/month	418 (68.8)	302 (72.2)	116 (27.8)	
Smoking status				0.546
Non-smoker	238 (39.0)	180 (75.6)	58 (24.4)	
Quit	227 (37.2)	174 (76.7)	53 (23.3)	
Smoker	145 (23.8)	104 (71.7)	41 (28.3)	
Diabetes mellitus				0.460
No	492 (79.6)	367 (74.6)	125 (25.4)	
Yes	126 (20.4)	98 (77.8)	28 (22.2)	
Dental visit frequency				0.279
Never in last 5 years	85 (15.0)	70 (82.4)	15 (17.6)	
When having symptoms	254 (44.8)	189 (74.4)	65 (25.6)	
Regularly every year	228 (40.2)	169 (74.1)	59 (25.9)	

** Total number in study sample may vary depending on missing values*

Participants' proportions were distributed evenly regardless of their marital status, smoking status, and medical condition. Female participants and ones who have never visited a dentist demonstrated slightly lower periodontitis awareness, although these associations were not statistically significant.

A comparison of periodontal parameters between the periodontitis awareness and unawareness participants is shown in table 2.

The periodontal status among the aware participants was more advanced than the unaware. The mean PD and CAL were significantly higher in the aware participants. The extent of disease represented by the percent sites with PD ≥6 mm and CAL ≥5 mm was also significantly higher in the group.

From the co-variable selection processes, age, gender, education level, income, and severity of periodontitis were included in the multivariable analysis, Table 3.

Table 2 *Comparison of periodontal parameters between periodontitis aware and unaware participants*

Periodontal parameters	Aware	Unaware	*p*-value
Number of remaining teeth (teeth)	23.02 ± 6.18	23.25 ± 6.40	0.697*
Mean PD (mm)	3.24 ± 0.87	3.01 ± 0.75	0.004*
Mean CAL (mm)	4.21 ± 1.41	3.95 ± 1.33	0.040*
% site PD ≥6 mm (%)	4.36 (0, 75.0)	2.47 (0, 58.33)	<0.001**
% site CAL ≥5 mm (%)	29.68 (3.33, 97.73)	20.18 (1.39, 100)	0.007**

** t-test*
*** Mann-Whitney U test*

Table 3 *Multivariable analysis*

Factors	OR adjusted	95% LCI	95% UCI	*p*-value
Age				
<60	1			
≥60	1.483	0.906	2.426	0.117
Gender				
Female	1.376	0.802	2.361	0.247
Male	1			
Education level				
≤High school	1.703	1.016	2.854	0.043
Diploma	1.757	1.119	2.758	0.014
≥Bachelor's degree	1			
Income				
<20,000 Baht/month	1.834	0.870	3.868	0.111
20,000 – 49,999 Baht/month	1.057	0.596	1.876	0.849
≥50,000 Baht/month	1			
Periodontal severity				
(%site with PD ≥6 mm; continuous)	0.963	0.945	0.981	<0.001

The results of binary logistic regression showed that age, gender, and income were not associated with the level of periodontitis awareness. However, education level and the extent of severe periodontitis was statistically significantly associated with the perception of the participants. Compared with higher education individuals, ones who graduated with less than a bachelor's degree were 1.7 times more likely to be unaware of their periodontitis. In addition, for periodontitis extent, the adjusted OR of 0.96 (95%CI: 0.95, 0.98) indicated that the increase of each percentage of sites with PD ≥6 mm reduced the likelihood of being periodontitis unaware participants by 4 %. In other words, the awareness relatively increased with the severity of periodontitis.

Discussion

This was the first study on self-awareness of individuals with severe periodontitis conducted in Thailand. This study aimed to estimate self-awareness level and indicated its associated factors. The results showed that among Thai adults, only a quarter of participants with severe periodontitis recognized their periodontitis presence. The

low level of education and periodontitis severity were associated with the perception of patients.

Our study showed that the level of self-awareness was low for severe periodontitis, demonstrating that only 24.9 % of participants with severe periodontitis reported having periodontitis whereas 75.1 % thought that they had no gingival problems. Our results agreed with most of the previous studies done in many countries, such as the USA[9,12,20], the UK[11], Spain[21,22], Norway[14,23], and Brazil.[24] They found that a small percentage of periodontal participants were aware of having the disease. The level of awareness varied from 14 to 40 % in different studies depending on characteristics and number of included participants, methods of data collection, and definition of periodontal diseases. Interestingly, studies conducted in the specific groups of health educated participants or participants during dental care, resulted in a high level of awareness of more than 60 %[25,26], which could have been related to the knowledge of oral health care.

Examples of responses to this study question, "In your opinion, how is your current periodontal status?", were "I don't feel anything wrong about my gums. I think it is healthy.", "I think I must have a gum disease because I have painful and swollen gums.", "I have periodontitis. A dentist told me so.", and "I am not sure, but my gums might hurt sometimes.". Most of the answers were simple, clear, and direct to the point. They usually gave additional reasons why they think so. In case that the interviewees were uncertain, the interviewer would explain that periodontitis is a chronic inflammatory destructive disease of the gums and bone that surround and support the teeth, with symptoms such as painful gums, halitosis, bleeding on brushing, tooth mobility, pus from the gums, or swollen gums, so that they could give a definitive answer. These answers showed that participants could understand the question well. However, a lot of them might be unable to perceive the problem or unconcerned about their periodontal health or lack sufficient knowledge to be aware of it.

Previous studies used a variety of questions to assess individuals' periodontitis self-awareness. Apart from a question of self-perception of periodontal condition, questions regarding self-reported periodontal symptoms were also used.[27-29] Besides, questions regarding history of periodontal treatment, previous periodontal diagnosis, and oral hygiene behavior may be used in addition to self-perception of periodontal status and symptoms. However, most studies found that using self-reported parameters was ineffective, participants were frequently unaware of periodontal symptoms.[27,28]

The 2012 CDC/AAP periodontitis case definitions classified periodontal disease into gingivitis, mild, moderate, and severe periodontitis. However, the difference between gingivitis and especially between mild and moderate periodontitis according to these criteria was very modest. Moreover, the early stage of disease exhibited relatively mild symptoms which could lower the self-detection of participants. Therefore, our study chose to assess the self-awareness specifically in participants with severe periodontitis, and excluding gingivitis, mild and moderate periodontitis cases.

It was observed from this study that participants who were older, had a lower education level, and lower income were more frequently unaware of having periodontitis. The characteristics were quite in concordance with the previous findings[9,29] which found that age, race, education level, and systemic conditions had an influence on periodontal awareness. In addition, Alshammary *et al.*[30] found that females who had a higher education, and higher income all contribute to a positive perception of oral health, whereas increasing age has the opposite effect. These could be explained by the fact that these factors led to the lack of access to dental care and oral health-related knowledge which were the key factors to periodontal self-awareness. The elderly may have low self-awareness because they recognized the deterioration of their health conditions as a normal part of the aging or adaptation process. Moreover, individuals with higher education and income may have a better attitude toward

oral hygiene practice and be more attentive to their oral health status. Hence it was confirmed from this study that participants with older age and low socioeconomic status (SES) should be the top priority for the periodontal health promotion campaign.

This study also found that self-awareness was not associated with the frequency of dental visits. Among participants who had regular dental visits at least once a year, the level of self-awareness of periodontitis was only 26 %. Limited communication between dentists and patients, as well as negligence of proper periodontal examination by dentists might be implied from this unpleasant circumstance.[8,14,15] As a result, it could be suggested that there is a need for oral health care providers to not only do dental and periodontal examinations more carefully but also to improve patient education concerning periodontal disease.

Interestingly, in contrast to previous studies[28,31,32] which found that smokers and participants with diabetes mellitus were more likely to be aware of periodontitis as they had more prevalent and severe periodontitis, both factors were not associated with the self-awareness level in our study. Due to vasoconstriction induced by tobacco, the clinical signs of inflammation, such as gingival redness and gingival bleeding, could be reduced. Thus, smokers may miss early signs of periodontal disease.[29] In regard to our diabetes mellitus participants, most of them were well controlled or borderline diabetes, which might have less pronounced periodontal symptoms. In addition, the number of smokers and diabetics in ours was low which may have insufficient power in statistical testing leading to biased results. This indicated the need for further investigation in large well-designed diagnostic studies in various Thai populations.

Besides the advantages of a large sample size, our study also has other strengths. Attaining the answers to the questions was done in the native language of the participants by trained interviewers. The questions had been well designed, easy to understand, and the test-retest for reliability was performed.[21,24] Among the method used for collecting information from self-reported questions, the one-on-one interviewing demonstrated the highest validity and reliability.[20] Moreover, the gold standard full-mouth periodontal examination was done by the trained and calibrated periodontists instead of using some of the indexed teeth to represent the whole mouth.

However, there are some limitations to this study. The study was conducted with a specific group of Thai population, most of them were moderate to high SES. Moreover, our definition of low income[19] may not accurately reflect "low income" in Thailand, which is likely to be lower. Therefore, the generalization might be limited. Next, most of the included participants received the dental services at the provided health section of the EGAT enterprise. The reported association between self-awareness and dental visit might be biased. Finally, to justify self-awareness based on only one question. Even though this study used interview question, the perception of participants could be different from the interviewers, mis-interpretation of the question was still possible.

Conclusion

This study showed that periodontitis self-awareness in Thai adults was poor. Individuals with older age, lower educational level, and lower income were more likely to be unaware of periodontitis. Periodontal health promotion should be emphasized in Thailand, especially for older people with low SES.

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Original Article

Effect of Orthodontic Loading on Periodontal Ligament Proliferation: A Preliminary Study

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Abstract

The preapplication of orthodontic loading enhances the proliferation of the periodontal ligament (PDL) and is beneficial for tooth autotransplantation (TAT). However, the changes in the PDL thickness following loading application are unknown. The purpose of the present study was to determine the changes of remaining periodontal ligament (PDL) thickness on the root surface of extracted premolars following orthodontic loading. Twenty-four premolars were divided into control and preloaded (4, 8, and >12-weeks) groups. Premolars were extracted, fixed, and stained with toluidine blue for the assessment of the remaining PDL. The radicular portion was sectioned into apical, middle, and coronal thirds. Images of the sections were recorded under a stereomicroscope and the PDL thickness was measured with ImageJ software. Data was collected and analyzed statistically. The preloading groups (4, 8, and >12-weeks) showed a significant increase in the overall PDL thickness compared to the control (0.123 ± 0.01 mm) (P<0.01). The 8-weeks group (0.198 ± 0.022 mm) provided the highest increase in overall PDL thickness among the preloading groups (P<0.05). No statistical difference in the PDL thickness between the 4-weeks (0.153 ± 0.014 mm), >12-weeks (0.157 ± 0.019 mm) groups was observed. The 8-weeks orthodontic preloading duration provided the highest increase in the PDL thickness. After this period, the PDL thickness rebounded, therefore indicating the rebound of the PDL. An increase in PDL thickness is advantageous for the success of TAT.

Keywords: Orthodontic loading, PDL thickness, Tooth autotransplantation

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Introduction

Tooth autotransplantation (TAT) has been an alternative approach in orthodontic practice to replace missing or hopeless teeth.[1] Various treatment protocols for autogenous tooth autotransplantation have been described and high success rates were reported.[2] Successful periodontal ligament (PDL) healing after tooth replantation can be expected if damage to the PDL of the donor's tooth during extraction is reduced and limited.[1,3] The prognosis of TAT

has been greatly improved by preserving the supporting tissues using careful surgical strategies to preserve the PDL around the extracted root surface.

The PDL is a soft cellular connective tissue richly vascularized surrounding the root of the teeth and joining the root cementum with alveolar bone.[4] The periodontal ligament is responsible for orthodontic tooth movement and transmits orthodontic appliance force to the alveolar bone. When the orthodontic loading is applied to a tooth, a proliferation of the PDL occurs followed by bone remodeling and tooth migration.[5] The proliferation of the PDL combined with bone remodeling results in a radiographic finding characterized by the radiolucent space between the lamina dura and the tooth root, or the widening of the PDL space.[6] The widening of the PDL causes changes in the biomechanical characteristics of the periodontium and an increase in tooth mobility.[7] Therefore, the widening of the PDL plays an important role in the physiological aspect of tooth movement.[5]

The presence of intact and viable PDL cells on the root surface of the donor's tooth decreases complications after transplantation including ankylosis. Clinical and experimental studies have suggested that the preapplication of mechanical stimuli to the donor's teeth increases the PDL and eases the extraction.[1,4,8] In a study with animals, preloading of light orthodontic force for seven days before extraction significantly increased the PDL space as well as the width of the alveolar socket, resulting in rich PDL tissues attached to the root surface of the extracted teeth.[1]

In a study with humans, Nakdilok *et al.,*[8] found that the optimal duration for stimulating the PDL proliferation and ease of extraction was 4 weeks of orthodontic preloading force. However, the assessment of the PDL enhancement was performed two-dimensionally based on the percentage of stained PDL on the root surface. Moreover, no information regarding the thickness of the remaining PDL on the root surface of an extracted tooth was provided.

A classic article from Coolidge in 1937[9] measured the PDL space in humans. Specimens were obtained from cadavers and histologic sections of the PDL space at the alveolar crest, middle and apical sites were used for measurements. The average normal width of the PDL space ranged from 0.15 mm to 0.21 mm, which decreased with age and function. The thinnest width of the PDL was located at the middle portion.

Recently, measurement of the PDL space width was performed using imaging obtained from the CBCT images of dry human mandibular bone. The PDL space widths ranged from 0.16 mm and 0.28 mm.[10] The authors concluded that the CBCT images obtained at 0.075 mm voxel size are preferred for the accurate measurement of PDL space.

Although the preapplication of orthodontic loading enhances the proliferation of the PDL and facilitates the simple extraction of the donor's tooth, no information regarding the thickness of the remaining PDL on the root surface of an orthodontically preloaded donor tooth, as well as the optimum loading duration was available.

Therefore, the purpose of the present study was to determine the changes of the remaining PDL thickness on the root surface of extracted premolars following orthodontic preloading and to define the optimum preloading period.

Materials and Methods

Participants

In the present study, twenty-four premolars of six patients (age 21.5 ± 4 years), at the Graduate Clinic, Department of Orthodontics, Faculty of Dentistry, Bangkokthonburi University were used. Premolars were referred for removal as part of the orthodontic treatment planning from January to September 2019. To be included in the study sample, patients had to be healthy and free of periodontitis. The patients were excluded as study participants if they had any missing data or recall visits or they reported the use of nontrivial drugs during the observation period.

The study was conducted in agreement with the Helsinki Declaration and ethical approval was obtained from the Bangkokthonburi University Research Ethics Committee (No. 3/2564). Detailed procedures and potential

risks were explained to each patient, who provided written and dated informed consent before the study.

Orthodontic Preloading

In the present study, each patient had their premolar orthodontics loaded for 4, 8, and >12 weeks before the extraction. A contralateral premolar that was not loaded served as a control. The orthodontic preloading was performed using a 0.016-inch improved superelastic nickel-titanium alloy wire (Sentalloy®, Tomy International, Inc., Tokyo, Japan) that was engaged to the fixed appliances. The orthodontic loading was provided by the Sentalloy archwire that was connected to the brackets on the buccal surface of the premolars, thus generating buccal-lingual force vectors. The 0.016" Sentalloy archwire generates light and continuous force (100 g).[8]

Toluidine Blue Staining

Staining with toluidine blue was performed to determine the amount of PDL tissue with proliferative cells on the root surface. The extracted teeth were washed gently in phosphate-buffered saline (PBS), pH7.4, and fixed with 10% buffered formalin solution in 50-ml tubes (Corning, Inc., Corning, NY, USA) for 24 h. The teeth were stained with 0.04% (w/v) toluidine blue (Sigma-Aldrich, St. Louis, MO, USA) for one min, and de-stained with 4 ml of PBS, which was changed daily for two days. After destaining, the teeth were photo recorded and sectioned.

Measurement of the Stained PDL Area on the Root Surface

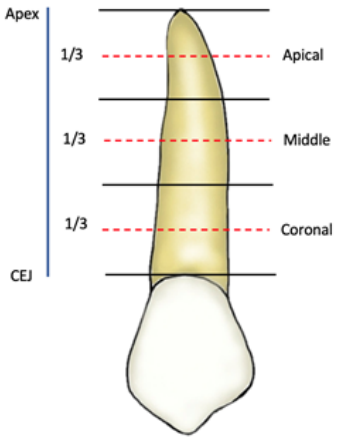

Figure 1 *The radicular portion was divided equally according to its length into apical, middle, and coronal thirds*

After staining, the radicular portion was sectioned by thirds into the apical, middle, and coronal using a diamond disk. (Fig 1.) Each sample was sectioned perpendicular to the tooth's long axis and then digitally photographed under a stereomicroscope (Earkin FHD3860 (0.7-4.5x); Shenzhen Juaiqu Electronic Co., Ltd., Shenzhen, China). Images were analyzed using ImageJ software version 1.51r (National Institute of Health, Bethesda, MD, USA), and the stained area indicating the PDL thickness was measured. (Fig 2.) Each section was subdivided into; buccal, lingual, mesial and distal surfaces. Each surface was equally subdivided into 10 sites. The measurements of PDL thickness were performed in these 10 sites and averaged.

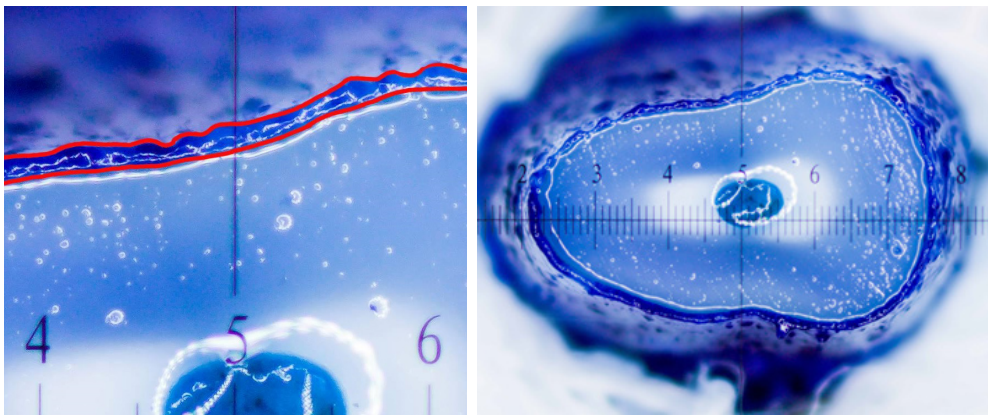

Figure 2 *A. The 4.0 x magnifying image of the PDL thickness. B. The apical section of a preloaded tooth.*

Statistical analysis

Data were analyzed using SPSS statistical software (version 19.0; SPSS Inc, Chicago, Ill). The data normal distribution was confirmed by the Shapiro-Wilk test. Differences in the PDL thickness between the control and the preloading were assessed by two-way repeated measures ANOVA. Comparison of PDL thickness within groups was performed with one-way ANOVA followed by multiple comparisons using the Bonferroni and the Dunnett T3 test. The results were considered statistically significant for $P<0.05$.

Results

The preloading groups (4, 8, and >12-weeks) showed a significant increase in the overall PDL thickness compared to the control (0.123 ± 0.01 mm) ($P<0.01$). The 8-weeks group (0.198 ± 0.022 mm) provided the highest increase in overall PDL thickness among the preloading groups ($P<0.05$). No statistical difference in the PDL thickness between the 4-weeks group (0.153 ± 0.014 mm), and the >12-weeks group (0.157 ± 0.019 mm) was observed. (Fig 3.)

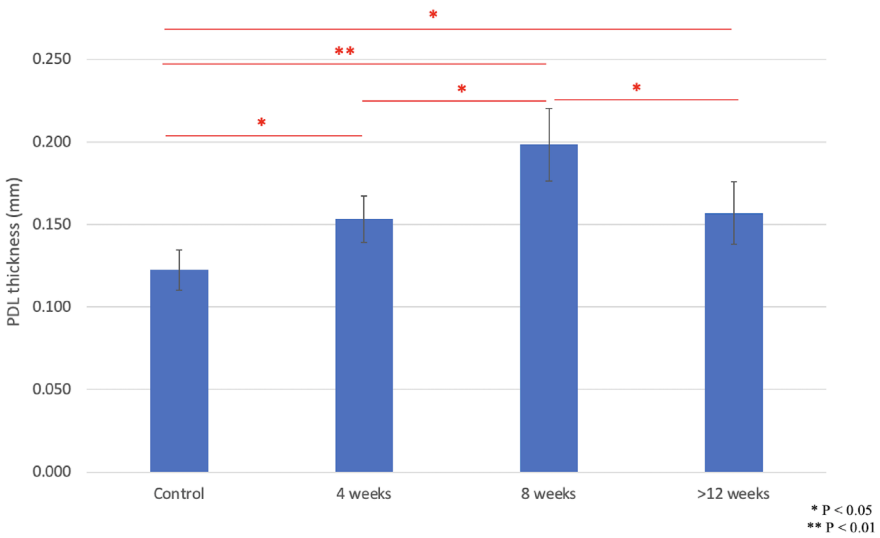

Figure 3 *Changes in the PDL thickness following orthodontic preloading*

Discussion

The preapplication of orthodontic loading on the donor's tooth increases the proliferative and metabolic activities of the PDL cells and eases the extraction, thus reducing the risks of PDL tissue damage.[1,4,8] Consequently, common complications such as root resorption and ankylosis following the TAT can be reduced.[2,3]

In the present study, the assessment of remaining PDL thickness on the root surface of extracted premolars following orthodontic preloading was performed to define the optimum preloading period.

The results have confirmed the overall increase in PDL thickness in the preloading groups (4, 8, and >12 weeks) compared to the control group. This is the result of the orthodontic loading on the PDL which causes the bone resorption through the osteoclastic activity, thus creating irregular cavities in the bone with the simultaneous increase in the PDL thickness.[11] The results are in agreement with previous studies that investigate the effects of orthodontic loading on PDL proliferation.[1,8,12] Suzaki *et al.,*[1] in a histomorphological study had reported the increase in the PDL thickness following the application of orthodontic loading in rats. Nakdilok *et al.,*[8] concluded that a 4-weeks period was sufficient to provide a significant increase of the overall percentage of stained PDL on the surface of the root of the preloaded tooth.[8] Promchaiwattana *et al.,*[13] confirmed the PDL enhancement following the application of Smart

Springs for the orthodontic extraction of mesio-angulated mandibular third molars. However, in these studies, only a two-dimensional topographic analysis of the root surface with no information regarding the PDL thickness was performed. Therefore, the results of the present study provide complementary insight into the PDL proliferative characteristics.

In the present study, the 8-weeks group exhibited the highest amounts of increased PDL thickness among the preloading groups. This is a new finding of the study since most of the studies that investigated PDL proliferation reported four weeks for the maximum expression of the PDL enhancement.[12] Therefore, the presented results are not in agreement with the study of Phutinart *et al.,*[12] who evaluated the expressions of bone biomolecules in the increased PDL volumes. In their study, the authors had concluded that orthodontic preloading for four weeks enhances the amounts of PDL tissue together with the RUNX2 and ALP expressions and the RANKL/OPG ratio in the PDL, suggesting that this loading period is suitable for successful TAT. However, the observation period used by these authors was limited to four weeks. Consequently, the following PDL proliferation analysis was not performed. The findings of the present study suggest that the maximum expression of the biomolecules in the increased PDL volumes occurs within four weeks, while the maximum PDL thickness occurs in eight weeks. Therefore, further studies to assess the effects of the biomolecules and the PDL proliferation in three dimensions should be performed in future studies.

Results of the present study indicated that after the highest increase of PDL thickness at eight weeks, a significant decrease in the PDL thickness was observed in the >12-weeks periods. Whereas, no statistical difference in the PDL thickness between the 4 and >12-weeks groups was observed. The increase and decrease in the PDL thickness are interpreted as the rebound of PDL. The rebound of the PDL is the direct effect of its elastic characteristics combined with the continuous and progressive alveolar bone remodeling in an attempt to achieve homeostasis.[11] The results are in agreement with previous studies that investigated the effect of orthodontic loading on periodontium.[11,13]

The effect of the orthodontic loading on the PDL resulted in the initial bone resorption combined with a simultaneous increase in PDL thickness that was measured in the 4-weeks group.[13] The maximum expression of the PDL thickness was achieved in the 8-weeks period. Following this period, the surrounding alveolar bone was filled by newly formed bone owing to osteoblast activity resulting in the rebound of the PDL. Consequently, the PDL thickness was significantly reduced in the >12-weeks groups.

To the author's knowledge, the assessment of the increase and rebound of the PDL thickness as a result of orthodontic loading has never been investigated. Consequently, the results obtained in the present study can be considered a new finding and deserves further investigation.

Although the PDL thickness varies along the root surface with the thinnest PDL in the middle portion, comparison of PDL thickness between apical, middle and coronal sections were not performed due to the reduced sample number. This is the main limitation of the present study. Therefore, further studies with an increased number of samples must be performed.

A limitation of the present study was the reduced number of participants and the possibility of intraindividual variation. Therefore, further studies with an increased number of samples must be performed.

The present study has demonstrated that a period of 8-week duration of orthodontic preloading is sufficient to adequately enhance the PDL and atraumatic extraction of the donor's tooth; both outcomes are beneficial for successful TAT.[14]

Conclusion

The 8-weeks orthodontic preloading duration provided the highest increase in the PDL thickness. After this period, the PDL thickness rebounded, therefore indicating the rebound of the PDL.

Acknowledgements

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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.

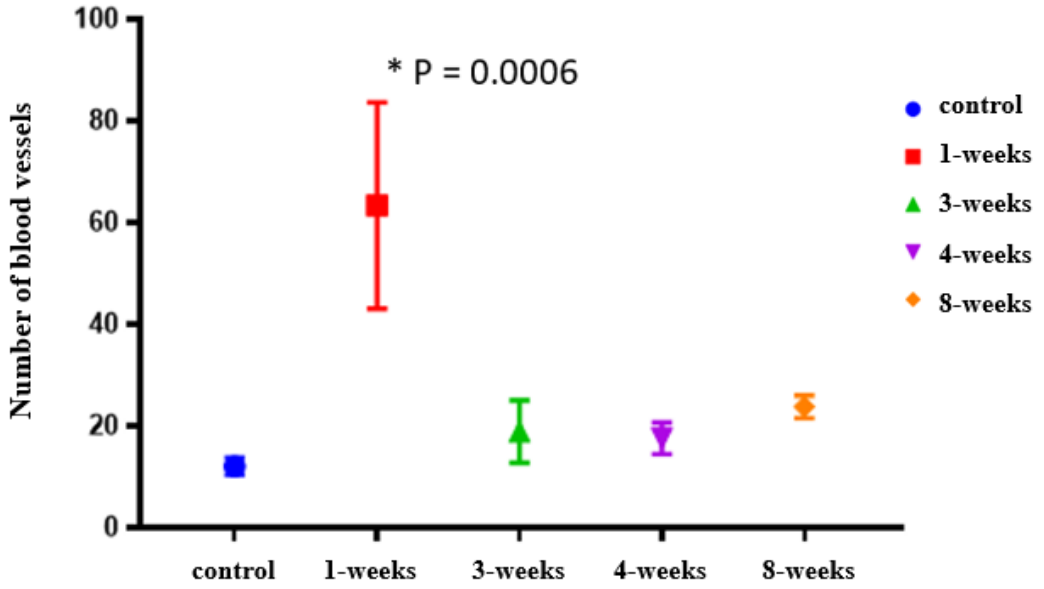

Figure 1 *Mean number of blood vessels for the control and the experimental groups*

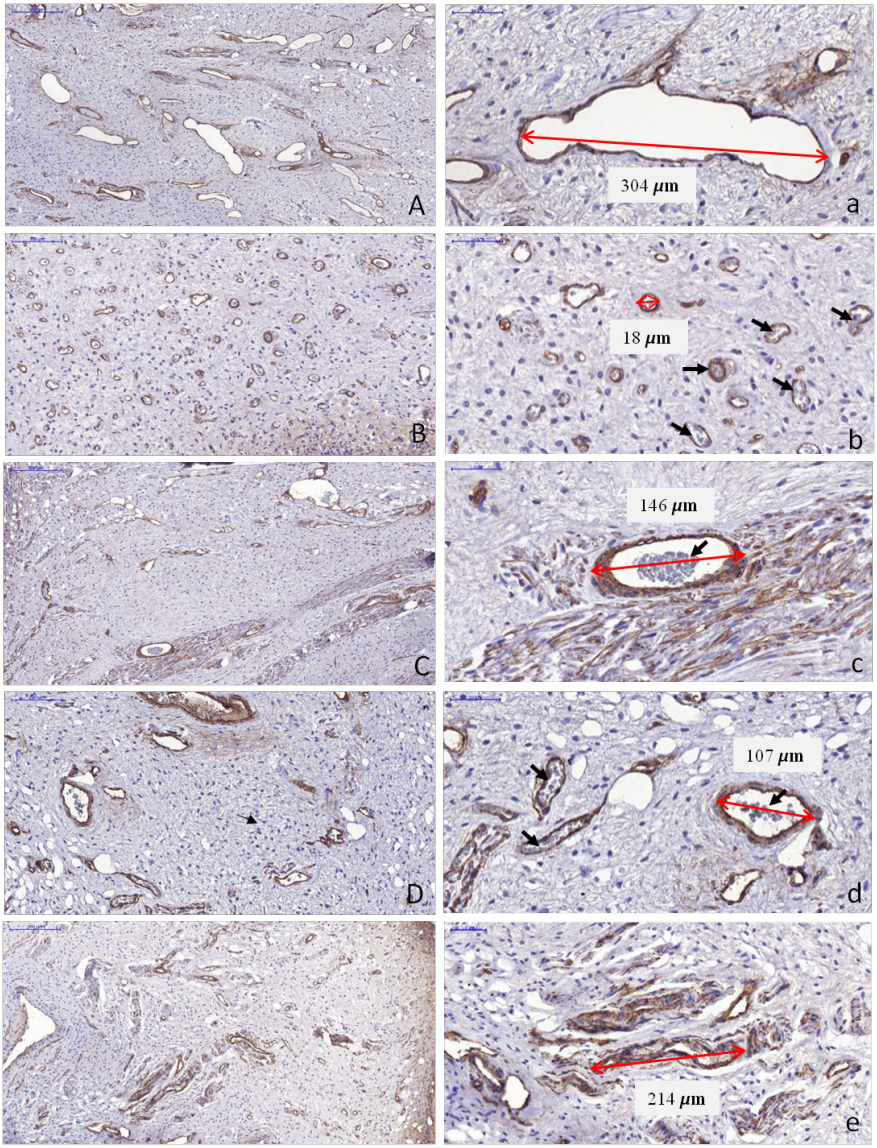

Figure 2 *Immunohistochemical staining of CD146 in pulpal vascular endothelial cells in the control group (A, a) showed large sized blood vessels. In the 1-week group (B, b) micro-blood vessels were significantly increased and displayed congested blood vessels (black arrow). In the 3-weeks group (C, 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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