Effect of Acidulated Fluoride Mouthrinse on Enamel Remineralization in Fixed Orthodontic Patients

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Thesis title	Effect of Acidulated Fluoride Mouthrinse on Enamel Remineralization in Fixed Orthodontic Patients
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ชื่อวิทยานิพนธ์	ผลของน้ำยาบ้วนปากฟลูออไรด์ที่มีฤทธิ์เป็นกรดต่อการคืนกลับของแร่ธาตุบนผิวเคลือบฟันในผู้ป่วยจัดฟันด้วยเครื่องมือจัดฟันชนิดติดแน่น
ผู้เขียน	นายปภินวิช วิพัฒนบวรวงศ์
สาขาวิชา	วิทยาศาสตร์สุขภาพช่องปาก
ปีการศึกษา	2552

บทคัดย่อ

วัตถุประสงค์ เพื่อวัดและเปรียบเทียบการคืนกลับของแร่ธาตุบนผิวเคลือบฟันในผู้ป่วยจัดฟันด้วยเครื่องมือจัดฟันชนิดติดแน่นก่อนและหลังบ้วนปากด้วยน้ำยาบ้วนปากฟลูออไรด์ชนิดที่เป็นกรดและชนิดที่เป็นกลาง **วัสดุและวิธีการวิจัย** ผู้เข้าร่วมวิจัยจำนวน 17 คน ประกอบไปด้วยฟันกรามน้อยที่จะต้องถูกถอนเพื่อการจัดฟันจำนวน 26 คู่ ถูกสุ่มเลือกจากผู้ป่วยที่เข้ามารับการรักษาทางทันตกรรมจัดฟัน ที่คลินิกจัดฟันของนักศึกษาหลังปริญญา ภายในโรงพยาบาลทันตกรรม คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ผู้ป่วยทุกคนได้รับการสอนการดูแลสุขภาพช่องปากก่อนติดเครื่องมือจัดฟัน และถูกแบ่งเข้ากลุ่มน้ำยาบ้วนปากฟลูออไรด์ที่เป็นกรด (n = 13 คู่) หรือกลุ่มน้ำยาบ้วนปากฟลูออไรด์ที่เป็นกลาง (n = 13 คู่) หลังจากติดเครื่องมือจัดฟันชนิดติดแน่นผ่านไป 30 วัน ผู้ป่วยทุกคนจะถูกสุ่มถอนฟันกรามน้อยหนึ่งข้าง และจะได้รับน้ำยาบ้วนปากตามชนิดของกลุ่มที่ผู้ป่วยสังกัดอยู่ ต่อมาอีก 30 วัน ผู้ป่วยจะถูกถอนฟันกรามน้อยข้างที่เหลือ ความแข็งพื้นผิวเคลือบฟันจะถูกวัดก่อนและหลังบ้วนน้ำยาบ้วนปากฟลูออไรด์ทั้ง 2 กลุ่ม โดยจะวัดที่ด้านใกล้ด้านบดเคี้ยว (เหนือต่อเหล็กจัดฟัน) และด้านใกล้เหงือก (ใต้ต่อเหล็กจัดฟัน) ต่อมาความแข็งพื้นผิวเคลือบฟันจะถูกคำนวณเป็นเปอร์เซ็นต์แร่ธาตุโดยปริมาตร การเปลี่ยนแปลงและความแตกต่างของการเปลี่ยนแปลงของความแข็งพื้นผิวเคลือบฟัน และเปอร์เซ็นต์แร่ธาตุโดยปริมาตร หลังจากบ้วนน้ำยาบ้วนปากฟลูออไรด์แต่ละชนิดและระหว่างสองชนิดจะถูกเปรียบเทียบโดยสถิติ paired *t*-test และ independence *t*-test ตามลำดับ โดยมีนัยสำคัญทางสถิติที่ $p \leq 0.05$ **ผล** น้ำยาบ้วนปากฟลูออไรด์ที่เป็นกรดทำให้ความแข็งพื้นผิวเคลือบฟัน และเปอร์เซ็นต์แร่ธาตุโดยปริมาตรเพิ่มขึ้นทั้งด้านใกล้ด้านบดเคี้ยวและด้านใกล้เหงือกอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.001$) ในขณะที่น้ำยาบ้วนปากฟลูออไรด์ที่เป็นกลางจะทำให้ความแข็งพื้นผิวเคลือบฟัน และเปอร์เซ็นต์แร่ธาตุโดยปริมาตรเพิ่มขึ้นทั้งด้านใกล้ด้านบดเคี้ยวและด้านใกล้เหงือก แต่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเฉพาะด้านใกล้เหงือก ($p \leq 0.05$) และน้ำยาบ้วนปากฟลูออไรด์ที่เป็นกรดจะมีความแข็งพื้นผิวเคลือบฟัน และเปอร์เซ็นต์แร่ธาตุโดยปริมาตร ที่ด้านใกล้ด้านบดเคี้ยวและด้านใกล้เหงือกเพิ่มขึ้นมากกว่าน้ำยาบ้วนปากฟลูออไรด์ที่เป็นกลางอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$) **สรุป** น้ำยาบ้วนปากฟลูออไรด์ที่เป็น

กรดทำให้เกิดการคืนกลับของแร่ธาตุบนผิวเคลือบฟันมากกว่าน้ำยาบ้วนปากฟลูออไรด์ที่เป็นกลาง ในผู้ป่วยจัดฟันด้วยเครื่องมือจัดฟันชนิดติดแน่น

คำสำคัญ รอยโรคสีขาวขุ่น. ความแข็งผิวเคลือบฟัน, เปอร์เซ็นต์แร่ธาตุบนผิวเคลือบฟัน

Thesis title	Effect of Acidulated Fluoride Mouthrinse on Enamel Remineralization in Fixed Orthodontic Patients
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Major program	Oral Health Sciences
Academic year	2009

ABSTRACT

Objectives: The objectives of this study were to measure and to compare the remineralization in the enamel of fixed orthodontic patients before and after rinsing with acidulated fluoride mouthrinse and neutral fluoride mouthrinse. **Research methodology:** Sixteen patients with twenty-six pairs of premolar teeth were randomly selected from the new patient pool at post-graduate orthodontic clinic in the dental hospital of the Faculty of Dentistry, Prince of Songkla University. They were received home care oral hygiene instructions before bracketing and were randomly allocated into acidulated fluoride mouthrinse (n = 13) or neutral fluoride mouthrinse (n = 13) group. After 30 days all samples were randomly extracted the one side of homolog premolar(s), they received the mouthrinse and the others side was extracted in 30 days later. Enamel microhardness was measured before and after rinsing at the occlusal and gingival directions to the premolar brackets in both groups and changed them to volume percent mineral. The changes of enamel microhardness and volume percent mineral after rinsing in both groups and the different between the groups were compared statistically using paired *t*-test and independence *t*-test respectively. The significance level was at $p \leq 0.05$. **Results:** The acidulated fluoride mouthrinse statistically significant increased enamel microhardness and volume percent mineral both occlusal and gingival directions to the bracket ($p \leq 0.001$). Whereas, the neutral fluoride mouthrinse increased enamel microhardness and volume percent mineral both occlusal and gingival directions to the bracket, but only the gingival direction to the bracket that increased statistically significant ($p \leq 0.05$). And the acidulated fluoride mouthrinse statistically significant increased enamel microhardness and volume percent mineral ($p \leq 0.05$) much more than neutral fluoride mouthrinse both occlusal and gingival directions to the bracket. **Conclusion:** The acidulated fluoride mouthrinse had better effect in remineralization than neutral fluoride

mouthrinse in fixed orthodontic patients.

Key words: White Spot Lesions (WSL), Enamel Microhardness, Enamel Mineral Content Percentage

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Pabhinvitch Vipatbovonwong

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LIST OF ABBREVIATIONS AND SYMBOLS

kg = Kilogram

L = Lower

mm^2 = Square millimeter

SD = Standard deviation

U = Upper

x = Multiply

µm = Micrometer

/ = Per

% = Percentage

” = Inch

√ = Square root

^{o}C = Degree Celsius

CHAPTER 1

INTRODUCTION

Background and rationale

At present, the number of patients treated with fixed orthodontic appliances in Thailand is much more than in the past[1]. Fixed orthodontic appliance patients were not likely to be the same as non-orthodontic patients. They were not only different in the oral equilibrium[2] but fixed orthodontic patients also promoted plaque accumulation at the tooth surface and around the brackets. Dental plaque will always lower the pH at the tooth surface. It causes decalcification of minerals in the enamel and then demineralization will occur followed by incipient caries or enamel caries. The clinical appearance of the demineralization is a white spot lesion. The prevalence of the white spot lesion in the fixed orthodontic treatment group is more frequent than in the untreated group[3]. Most of the lesions found were one third of the buccal enamel surface along the gingival margin[3]. Moreover, white spot lesions may progress to be cavitated dental caries.

Dental plaque control around orthodontic brackets will prevent remineralization, the cause of white spot lesion, on the tooth surface. Suitable tooth brushing and flossing technique can effectively control dental plaque around orthodontic brackets. However, tooth brushing and flossing may not completely remove dental plaque around orthodontic brackets, especially in patients who fail to brush properly. Mouthrinse is the best choice for removing dental plaque around orthodontic brackets that remains after tooth brushing. Mouthrinse containing neutral fluoride not only inhibits demineralization but also promotes remineralization of enamel too.[4] However, neutral fluoride mouthrinse just retards lesion development, it cannot completely reduce white spot lesions.[5,23] In contrast, a fluoride solution with low pH can completely inhibit the development of white spot lesions.[5,6] Therefore, in this study we tested the fluoride uptake in the enamel of fixed orthodontic patients using mouthrinse containing acidulated fluoride.

Review of the literature

Decalcification is defined as loss of calcified tooth substance and has been accepted as one of the hazards of orthodontic treatment.[7,8] It occurs when the pH of the oral environment diffuses calcium and phosphate ions out of the enamel. This dissolution follows the production of acid by bacteria plaque and results in an altered appearance of the tooth surface. The early lesion is an opaque white spot, which in active lesions appears chalky, and if mineral loss continues, cavitations may result.[9] Orthodontic fixed appliances hinder tooth cleaning and favour plaque and food retention, followed by an increase in the number of *Streptococcus mutans* and *Lactobacilli* that further develop into carious lesions and furthermore, their presence increases the risk of decalcification occurring.[10, 11] Caries development during treatment with fixed orthodontic appliances is a significant clinical problem despite fluoride therapy,[12-15] and non-cavitated lesions should receive preventive therapy aimed at arresting and remineralizing the lesion.[16]

Remineralization is the process whereby partially demineralized enamel is repaired through re-crystallization of tooth enamel by mineral salts[17]. A partial remineralizing effect may theoretically be reflected in a slowing or arrestment of the growth of caries lesions[18]. When oral fluids were used as the calcifying fluid, a small reduction in lesion size was found; however, these were limited to the surface of the lesion. The addition of fluoride ions at concentration of either 1 or 10 ppm produced no noticeable increase in remineralization. When the fluoride level was raised to 100 ppm, increases in remineralization were found and the superficial aspect of the body of the lesion was reduced from 8-11%[19]. Laboratory study shows that firstly, the high fluoride concentrations in the surface layer of the sound enamel were depleted during lesion formation and appeared to be redistributed into the subsurface body of the lesion. Secondly, the fluoride acquired from a topical application was greater in the lesion relative to the sound enamel, but was limited to the surface layers[19].

Fluoride is important in the prevention of dental decay[20] as discussed in the above paragraph. There are several methods of delivering fluoride, in addition to fluoridated toothpaste, to teeth in patients during orthodontic treatment. These include topical fluorides, for example, mouthrinse, gel, varnish and fluoride-releasing materials (e.g. adhesive and elastics[21]). In 2003, Marinho found a definite reduction in caries in children and adolescents who have regular supervised rinsing with a fluoride mouthwash. It has also been shown that fluoride may

reduce the number of white spots developing during brace treatment[22]. Geiger et al found a 30 percent reduction in the incidence of teeth affected by white spots when orthodontic patients used a fluoride mouthrinse in addition to regular brushing and flossing.[23]

In vitro studies have shown that the fluoride uptake from acidic phosphate fluoride solutions (pH < 4) was high in the surface layer of the enamel[24]. An acidulated mouthrinse that already contains a fixed combination of antiseptic essential oils has been shown to be effective in reducing supragingival plaque[25] and plaque acidogenicity[26], controlling plaque accumulation, and in helping to reduce gingivitis.[27, 28] Acidulated fluoride mouthrinse also has an effect in remineralization of artificial dental caries in vitro[29]. Neutral pH acidulated mouthrinse shifted the equilibrium of a solid phase hydroxyapatite to an aqueous phase hydroxyapatite and changed it into a calcium ion, phosphate ion and water; thereafter, when the pH, calcium ion and phosphate ion concentration were high the equilibrium shifted back to constitute hydroxyapatite and with the presence of fluoride it constituted fluorapatite or fluorhydroxyapatite. The mechanism of action of increased fluoride uptake by acidulated fluoride mouthrinse could be due to an increase in hydrogen fluoride concentration, an increase of diffusion rate[30], an increase in the precipitation of calcium fluoride on the surface that serves as a fluoride reservoir[31] or a change of enamel surface charge. We aimed to test whether fluoride in acidulated mouthrinse can diffuse and has an effect in the remineralization of enamel underneath the plaque occlusal and gingival to the brackets.

Objectives of the study

This study aims to measure and compare the remineralization in the enamel of fixed orthodontic patients before and after rinsing with acidulated fluoride mouthrinse and neutral fluoride mouthrinse, and to look at any comparison between types of mouthrinse.

CHAPTER 2

MATERIALS AND METHODS

This study was composed of two parts. First, clinical study patients rinsed the acidulated fluoride mouthrinse in a test group and regular fluoride mouthrinse in a control group, then premolars were extracted for orthodontic treatment purposes and stored for second study. Second, a laboratory study was conducted to measure the enamel surface hardness using a Knoop microhardness testing machine.

1. A clinical study

Sample size

Sample size was calculated based on the previous study's mean of white spot lesions in an orthodontic full fixed edgewise appliance[32] with power of 0.85, where alpha = 0.05. The result indicated that at least 10 pairs of teeth in each group were needed. All patients were randomly selected from the new patient pool at the post-graduate orthodontics clinic, in the dental hospital of the Faculty of Dentistry, Prince of Songkla University.

The inclusion criteria for the study were as follows:

- Treatment with conventional fixed orthodontic appliances
- Extraction cases (pairs of upper or/and lower premolars) with similar malocclusion
- No restoration, discoloration and cracks on extracted teeth
- No decalcification on extracted teeth
- No abnormality regarding amaelogenesis
- No history of any fluoride mouthrinse sensitivity
- No diseases of abnormal saliva
- No systemic diseases

Eighteen patients scheduled to have homolog premolar extractions in upper and/or lower arches as part of their orthodontic treatment. They were randomly allocated into the test group that consisted of 15 pairs of homolog premolars and into the control group that consisted of 15 pairs of homolog premolars. The mean ages of all samples were 23.28 $\pm$ 5.31

years old. All patients and/or their parent(s) were advised of the purpose of this study. After they decided to participate the study, the consent forms were signed. Each patient was given a toothbrush, non-fluoride toothpaste, non-fluoridated dental floss, an interproximal-toothbrush and two types of 100 ppm mouthrinse depending on the sample group. All patients received precise home-care oral hygiene instructions on brushing with a toothbrush (modified Charter and Scrub techniques) and using non-fluoride containing toothpaste, an interproximal-toothbrush and non-fluoridated dental floss every day after their meals. The ability of the patients' oral hygiene care was evaluated by Visible Plaque Index (VPI) on the mesiobuccal surfaces of every bonded tooth, after rinsing and drying tooth surfaces (1 for visible plaque and 0 for non-visible plaque) before first homolog premolar(s) were extracted

All teeth in the mouth were polished with non-fluoridated pumice and bidimentional brackets (0.018" x 0.025" slot brackets for incisor teeth and 0.022" x 0.028" slot brackets for canines and posterior teeth) were full-mouth bonded including extracted teeth. Brackets were bonded using a standard etchant (Scothbond^{TM}, 3M ESPE, Seefeld, Germany) and non-fluoridated bonding materials (Assure^{R}, Reliance orthodontic products Inc., Itasca Illinois, USA). Special care was taken during the etching and bonding procedure to ensure that no excess of etchant and bonding material were excessed off the bracket placement areas, to avoid their influence on the enamel adjacent to the bracket. The initial main arch wires, 0.014" Nickel-Titanium, were applied to all patients for leveling and aligning the teeth. One side of the homolog premolar(s) was randomly selected for extraction as a reference for fluoride data in each group. Special care was taken during the extraction to ensure that the brackets on the buccal aspects of the premolars remained in the same position and were not displaced from their original positions. The patients were randomly allocated received two types of mouthrinse that consisted of the following:

1) The test group patients rinsed with acidulated fluoride mouthrinse (PH 4.24 at 25.8^{o}C) containing 100 ppm sodium fluoride at a dose of 20 ml. for 30 seconds twice daily in the morning and before bed time at night for 30 days[33].
2) The control group patients rinsed with neutral sodium fluoride mouthrinse (PH 6.53 at 25.8^{o}C) containing 100 ppm fluoride at a dose of 20 ml. for 30 seconds twice daily in the morning and before bed time at night for 30 days.

To ensure the patients compliance, the patients noted the amount and duration time of rinsing on a checklist twice every day. The other side of the homolog premolar(s) was extracted 30 days later. All extracted homolog premolars were stored separately in 0.02% thymol solution at a temperature of 4^{o}C to preserve the mineral properties of the dental enamel.[34] All of the patients continued to receive orthodontic treatment until their treatment was completed.

2. Laboratory study

Materials and methods

Roots of all extracted premolars were fixed in acrylic resin (Fig.1) for attaching in slow cutting machine (JeanWirtz CUTO I, Wirtz-Buehler GmbH, Germany) (Fig.2) at cutting speed 1,030 round per minute under water coolant. Teeth in acrylic resin were first vertically cut through the mid mesio-distal distance of the brackets resulted in mesial and distal sides of crown of the teeth that attached to their roots. Then the teeth were second sectioned cut at the cemento-enamel junction line of the buccal aspect of teeth (Fig.3), so the crowns with their brackets were separated from their roots. The samples were embedded in acrylic resin blocks with their cut faces exposed to the surfaces of the acrylic resin blocks (Fig.4). The surfaces were sequentially polished with sand paper grade 400 and 1,000 respectively (English abrasives, A unicom industries company, England) and a diamond polishing machine (JeanWirtz Pheonix 4000, Wirtz-Buehler GmbH, Germany) (Fig.5).

The fluoride uptake was indirectly measured by microhardness change parallel to the cutting surface of the enamel using a Knoop microhardness testing machine (Buehler Micromet II, Buehler, USA) (Fig.6). The indentations were made with the long axis of the indentor parallel to the outer enamel surface (Fig.7); 3 positions in the occlusal direction and 3 positions in the gingival direction. The first indentor was 100 μm away from bracket, the second and third indentors were 200 μm away from the first and second indentations respectively and not deeper than 50 μm from the anatomical enamel surface[40] having a loading force of 50 gram for 10 seconds (Fig.8). Means were obtained from each tooth in the test and control groups at the different positions. In each tooth consisted of two sides of crown, indentations were performed in 6 positions on both sides and the Knoop microhardness number (KHN, kilogram/millemeter^{2}) was calculated from each indentation length, and the mean of two sides at the same position in occlusal and gingival directions was recorded. The volume percent mineral (mineral content

Fig.1 Extracted premolar in acrylic resin

Fig.2 Slow cutting machine (JeanWirtz CUTO I, Wirtz-Buehler GmbH, Germany)

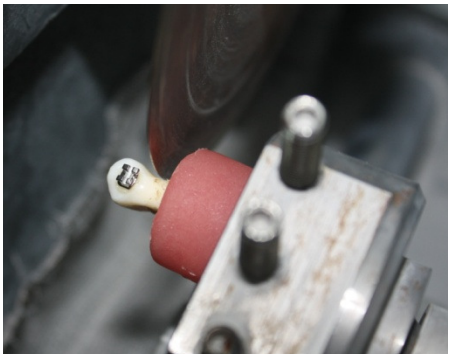

Fig. 3 The machine is cutting to separate the crown from the root

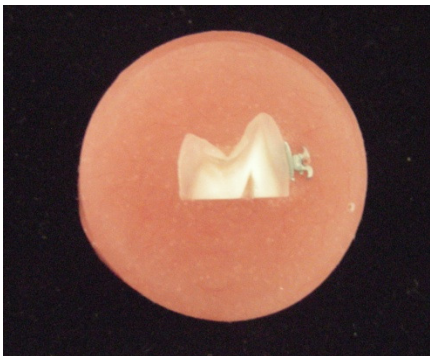

Fig. 4 Half crown in an acrylic resin block

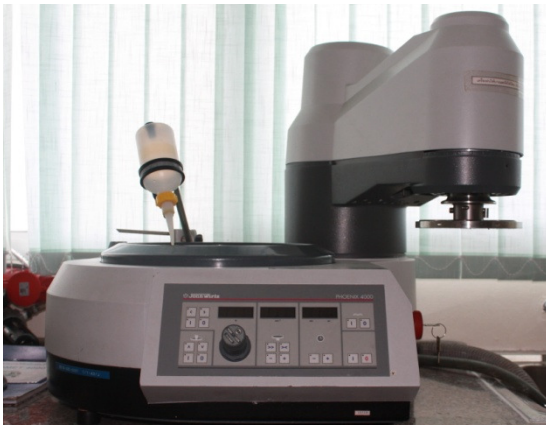

Fig. 5 Polishing machine (JeanWirtz Pheonix 4000, Wirtz-Buehler GmbH, Germany)

Fig.6 Knoop microhardness testing machine (Buehler Micromet II, Buehler, USA)

Fig. 7 Appearance under the Knoop microhardness tester at scale x100, bracket (upper left), acrylic resin (upper right) and enamel (lower)

Fig. 8 Acrylic resin (upper), enamel (lower) and indentations at scale x200 (arrow heads)

percentage) was calculated from: Volume percent mineral = $4.3(\sqrt{KHN}) + 11.3$.[35] The volume percent mineral could be interpreted as mineral loss or gain during demineralization and remineralization procedures. The means of the volume percent mineral of the 6 positions in the occlusal and gingival directions to the brackets were calculated to represent the occlusal and gingival mineral content. The means and standard deviations of enamel microhardness and volume percent mineral before and after rinsing were separately compared in the test and control groups, and the change of microhardness and percent mineral after rinsing between the test and control groups were also compared too.

Errors

Random error

The sample size was calculated based on the previous study's mean of the white

spot lesion in an orthodontic full fixed edgewise appliances[32] with a power of 0.85, where alpha = 0.05.

Systematic error

- **Confounding bias**

All samples were randomly selected from the new patient pool of the post-graduate orthodontics clinic, at the Dental hospital of the Faculty of Dentistry, Prince of Songkla University.

- **Measurement bias**

To reduce systematic error causes by measurement bias of this study, double blinded trials were performed by the subjects who were blind to the types of mouthrinse and whose the examiner was blind to the experimental groups.

- **Intra-examiner reliability**

Indentation length was performed by one examiner. Twelve teeth were randomly selected after first being measured at four weeks to repeat measurement for testing the intra-examiner reliability. The Dahlberg's formula[36] was performed and resulted in 0.32 at the mean, and the standard deviation of the indentation length was 49.9 $\pm$ 2.8 μm.

3. Statistical methods

Means of enamel microhardness and volume percent mineral were compared by Paired-samples *t*-test, before and after rinsing in each group and Independence-samples *t*-test, between the test and control groups. The significance level was determined at 95% and 99% confidence interval.

CHAPTER 3

RESULTS

There are 4 samples that had broken brackets during rinsing, 2 in the test group and the other in the control group. So there are 13 samples remaining in the test group (male = 4 patients, female = 5 patients) and 13 samples in the control group (male = 3 patients, female = 5 patients) as shown in Table 1. Compliance, assessed by returned checklists, appeared to be very good, every patient was rinsing under prescription. Ability of personal oral hygiene care was assessed with VPI and all of patients appeared to have acceptable score. Statistical significant difference was compared between the test and control groups as shown in Table 2. From independent-sample *t*-test, there was no statistical significant difference between the test and control groups at 95% confidence intervals (p = 0.682).

Table 1 Descriptive information

Group	Number of patients			Mean ages (years ± SD)	Pairs of teeth
	Male (%)	Female (%)	Total (%)		
Test	4 (44.4)	5 (55.6)	9 (100)	24.61 ± 3.93	13
Control	3 (37.5)	5 (62.5)	8 (100)	22.98 ± 6.05	13
Total	7 (41.2)	10 (58.8)	17 (100)	23.84 ± 4.95	26

Table 2 Visible plaque index (VPI score ± SD)

	Test group	Control group	p-value
Mean VPI score	8.22 ± 3.07	7.63 ± 2.77	0.682

Enamel microhardness

The result of the microhardness profile at each position was described as U1, U2, U3 and L1, L2, L3. U1's position was 100 μm from the bracket in the occlusal direction to the bracket. U2's position was 200 μm from the U1 in the occlusal direction to the bracket. U3's

position was 200 µm from the U2 in the occlusal direction to the bracket. L1's position was 100 µm from the bracket in the gingival direction to the bracket. L2's position was 200 µm from the L1 in the gingival direction to the bracket. L3's position was 200 µm from the L2 in the gingival direction to the bracket. Enamel microhardness profiles had a minimum value at the nearest position to the bracket both in the occlusal and gingival directions and increased in value with increasing distance from the bracket. (The data of enamel microhardness in all samples were described in appendices)

Table 3 Means (SD) of enamel microhardness (kg/mm^2)

Direction to bracket	**Test group (n=13)**			**Control group (n=13)**		
	Before rinsing	**After rinsing**	**Different**	**Before rinsing**	**After rinsing**	**Different**
Occlusal	228.94 (27.05)	271.04 ** (44.28)	42.09 † (39.49)	231.59 (35.33)	245.74 (40.66)	14.15 (34.62)
Gingival	214.69 (31.85)	268.22 ** (34.86)	53.52 † (31.48)	215.78 (33.89)	244.31 * (37.87)	28.53 (31.83)

*Significantly different from before rinsing, $p < .05$

**Significantly different from before rinsing, $p < .001$

†Significantly different from the control group, $p < .05$

Table 3 showed means and standard deviations of enamel microhardness, before rinsing, after rinsing and different between them in both test and control groups. The data in the test and control groups had a normal distribution ($p = 0.200$). Before rinsing, the enamel microhardness was no statistical significant different between the test and control both occlusal and gingival directions to the bracket ($p = 0.832$). Besides, the enamel microhardness in the occlusal direction to the bracket in both the test and control groups was higher than gingival direction to the bracket before rinsing. After rinsing the enamel microhardness increased both in the occlusal and gingival directions to the bracket and the occlusal direction to the bracket were still higher than gingival direction to the bracket in both groups. The increasing of enamel microhardness in the test group was much more than in the control group. In both groups, the

gingival direction to the bracket had higher microhardness increasing value than in the occlusal direction to the bracket.

Due to the fact that there were different of enamel microhardness between the occlusal and gingival directions to the bracket in the test and control groups, the enamel microhardness of the occlusal and gingival directions to the bracket were compared separately. Means of enamel microhardness at the occlusal and gingival directions to the bracket were compared statistically within each group by paired-samples *t*-test and between the test and control group by independent-samples *t*-test. The statistical comparisons between the test and control group at before and after rinsing and different between them of the enamel microhardness was shown in Table 3. In the test group, the enamel microhardness after rinsing at both the occlusal and gingival directions to the brackets had a statistical significant higher than before rinsing ($p < 0.001$). In the control group, the enamel microhardness after rinsing only at the gingival direction to the brackets had a statistical significant higher than before rinsing ($p < 0.05$). Comparison of different values of the enamel microhardness demonstrated statistical significant higher in the test group than the control group both occlusal and gingival directions to the bracket ($p < 0.05$).

Volume percent mineral (mineral content percentage)

The enamel microhardness was transformed to volume percent mineral because they were easily represent the mineral loss or gain than enamel microhardness. Table 4 demonstrated means and standard deviations of volume percent mineral, before rinsing, after rinsing and different between them in both test and control groups. (The data of volume percent mineral in all samples were described in appendices)

Table 4 demonstrated means and standard deviations of volume percent mineral, before rinsing, after rinsing and different between them in both test and control groups. The data in the test and control groups had a normal distribution ($p = 0.200$). Before rinsing, the volume percent mineral was no statistical significant different between the test and control both occlusal and gingival directions to the bracket ($p = 0.978$). Besides, the volume percent mineral in the occlusal direction to the bracket in both the test and control groups was higher than gingival direction to the bracket before rinsing. After rinsing the volume percent mineral increased both in the occlusal and gingival directions to the bracket and the occlusal direction to the bracket were still higher than gingival direction to the bracket in both groups. The increasing of volume percent

Table 4 Means (SD) of volume percent mineral (%)

Direction to bracket	Test group (n=13)			Control group (n=13)		
	Before rinsing	After rinsing	Different	Before rinsing	After rinsing	Different
Occlusal	76.37 (4.31)	82.63 ** (5.42)	6.26 † (5.45)	76.32 (5.18)	78.44 (5.67)	2.13 (5.13)
Gingival	73.91 (4.58)	81.58 ** (4.56)	7.67 † (4.45)	74.23 (5.13)	78.27 * (5.27)	4.04 (4.5)

*Significantly different from before rinsing, $p < .05$

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Due to the fact that there were different of volume percent mineral between the occlusal and gingival directions to the bracket in the test and control groups, the volume percent mineral of the occlusal and gingival directions to the bracket were compared separately. Means of volume percent mineral at the occlusal and gingival directions to the bracket were compared statistically within each group by paired-samples *t*-test and between the test and control group by independent-samples *t*-test. The statistical comparisons between the test and control group at before and after rinsing and different between them of the volume percent mineral was shown in Table 4. In the test group, the volume percent mineral after rinsing at both the occlusal and gingival directions to the brackets had a statistical significant higher than before rinsing ($p < 0.001$). In the control group, the volume percent mineral after rinsing only at the gingival direction to the brackets had a statistical significant higher than before rinsing ($p < 0.05$). Comparison of different values of the volume percent mineral demonstrated statistical significant higher in the test group than the control group both occlusal and gingival directions to the bracket ($p < 0.05$).

CHAPTER 4

DISCUSSION

In this study we controlled several factors that influenced to the enamel remineralization in each and between every orthodontic patient. These several factors included of individual oral physiology, dental plaque around orthodontic bracket and orthodontic brackets position. The factor that is difficult to control is individual oral physiology, for example, buffer capacity, saliva flow rate etc. So we designed the study to control this factor by split-mouth technique to investigate the effect of each type of mouthrinse to remineralization on the enamel in each subject. However, comparison of remineralization between types of mouthrinse may be affected by this factor. However, all subjects received the same home care oral hygiene instruction in order to control the dental plaque accumulation around the orthodontic brackets between the groups, which influence to the enamel remineralization. Moreover, we evaluated the oral hygiene care of every patient before rinsing by Visible Plaque Index (VPI). The VPI that represented the dental plaque accumulation showed that there was no different in dental plaque accumulation between the test and control groups. In case there was some different of dental plaque accumulation between the test and control groups before rinsing, VPI might have been the factor that influenced to the enamel remineralization and demineralization, the baseline value of enamel microhardness might be different and it could not be appropriately compared between the groups. As mentioned by Ogaard et al, their study showed that it had the correlation between the VPI and the remineralization- demineralization of enamel in fixed orthodontic patients.[37] The higher score in VPI, the higher in enamel demineralization. According to the comparison of VPI between test and control groups, there was no statistically significant difference between the groups. Therefore, it can be assumed that all patients could equally control dental plaque accumulation around the orthodontic brackets and so the changes of enamel mineralization in this study were almost based entirely on the effect of mouthrinse types. All of the subjects that were instructed the details of this study and would like to participate, their teeth were carefully etched and bracketed to prevent the bonding materials excess off the brackets. The bonding procedure for testing demineralization and remineralization around orthodontic brackets was followed the

guideline described by O'Reilly and Featherstone.[38] They proposed that orthodontic brackets were bonded to the buccal surfaces at a K distance of 4 millimeter from the occlusal surface using a standard acid-etch adhesive system and following the manufacturer's instructions. Particular care was taken during the etching procedure to ensure that only the area where the bracket would be placed would be etched and sealed. This was done to avoid the influence of the etching procedure upon the enamel adjacent to the bracket.[39] Because if there was an excess in etched and bonded material out of the orthodontic brackets, the enamel around orthodontic brackets would increased in demineralization. In this study we did not use the distance from the occlusal surface same to O'Reilly and Featherstone's study because it was a different in individual occlusion at premolars area. In some patient, when the bite closed, the buccal cusp of the upper premolar teeth could contact to the bracket on the opponent premolar teeth, leading to broken brackets or teeth. 30 days later, the first side of teeth was randomly extracted and patients were randomly allocated into test and control groups. The other side of teeth was extracted 30 days later. Afterwards extracted teeth were stored in a 0.02% thymol solution at 4^{o} C to preserve mineral properties of the dental enamel.[34] This solution could preserve the organic and inorganic mineral component in the human teeth after they were removed from the mouth.

Fluoride measurement could be performed by direct and indirect techniques. At first we tried to use a direct technique by using the electron microanalysis-quantitative analysis technique (SEM-EDS, JSM-5800 LV, JEOL, EDS: Oxford ISIS 300, Japan) to measure the amount of mineral in the enamel surface.[40] Because the sensitivity of our equipment was not enough to detect the small amount of fluoride on the enamel of extracted teeth, we changed the fluoride measurement method to measure the fluoride with fluoride electrode.[33] Unfortunately, the area to be sectioned was too small to cut by the slow cutting machine (JeanWirtz CUTO I, Wirtz-Buehler GmbH, Germany), so we had to change the fluoride measurement to the indirect technique by measuring the demineralization-remineralization process, using a Knoop surface microhardness testing machine. This technique was widely used in many studied because of its simplicity and widely accepted to represent the mineral lost or gained.[33, 38, 40] The samples were prepared for microhardness testing similarly to O'Reilly and Featherstone method.[38] They studied enamel microhardness of the bonded premolar teeth in different fluoride supplement types, by cutting them in half and performed the Knoop microhardness 500 μm away from the bracket both occlusal and gingival directions to the bracket and underneath the bracket at the different depths.

They showed that microhardness in the occlusal direction was higher than the gingival direction to the bracket and was higher when the measuring position was away from the bracket. So in this study, the enamel microhardness was tested at both occlusal and gingival directions to the brackets in 3 positions (100 μm, 300 μm and 500 μm) away from the brackets. In this study we did not measure the mineralization deeper than 50 μm from the anatomical enamel surface like they had done before, because the fluoride could only penetrate no deeper than 50 -100 μm into the enamel surface.[41] We changed the enamel microhardness to a mineral content percentage. Mineral content percentage was first described by Featherstone et al[35], they found the linear relationship between mineral content percentage determined by micro-radiography and the square root of the Knoop hardness number assessed by microhardness testing in the mineral range of 40 – 90 volume percent. They concluded that the enamel microhardness can represent the mineral gain or loss and when convert to mineral content percentage, it easier to indicate the mineral gain or loss in the enamel than microhardness value.[35]

Before rinsing, the enamel microhardness and mineral content percentage were not different between the test and control groups in both occlusal and gingival directions to the bracket. Therefore, we separately compared the enamel microhardness and mineral content percentage of the enamel at the occlusal and gingival directions to the bracket in both two groups. In the gingival direction, the mean of enamel microhardness and mineral content percentage was less than in the occlusal direction. Due to the gingival direction to the bracket being close to the marginal gingival, small amounts of food, debris and plaque can easily become struck these.[12] Moreover, it was difficult to clean by brushing alone or self-cleansing by salivary flow. So this area has higher chance to created enamel demineralization. Gorelick et al explained that the increase in initial demineralization was the result of higher plaque accumulation around the orthodontic brackets especially in the gingival direction to the orthodontic brackets.[12] They suggested that the factors behind the plaque and debris retention that made the initial demineralization of the bonded teeth were the access to the flow of saliva, the distance, and the tooth surface area between the gingival and the bracket. They assumed that the initial demineralization would be lesser, if there was a higher in salivary flow rate, larger in the tooth surface area, and longer distance between the gingival and the bracket.

Now, it was widely accept that fluoride enhanced enamel mineral uptake during continuous remineralization and inhibited mineral loss during demineralization in

demineralization and remineralization experiments.[41] In normal condition, enamel microhardness of human teeth was around 262 – 265 kg/mm^2.[42, 43] In this study, the enamel microhardness after bracketing was decreased below the normal. To measured microhardness of our sample in normal condition, we measured the enamel microhardness in the sample before rinsing at the site away from the bracket and the result showed that the enamel microhardness was 260 kg/mm^2. This result demonstrated that brushing with non-fluoridated toothpaste was not enough to prevent the teeth from demineralization around the bracket in fixed orthodontic patients. The reasons for decreasing of the enamel microhardness were that the fixed orthodontic appliances changed the oral equilibrium and the difficult to clean the teeth at the occlusal and gingival directions to the orthodontic bracket.[2, 12] The fixed orthodontic appliances shifted the oral equilibrium to the direction of the higher in oral bacteria and they also interfered the tooth-brushing at the occlusal and gingival directions to the bracket. These made the higher in plaque accumulation, enamel demineralization and the finally, decreased in enamel microhardness. After rinsing the value increased in both groups. We speculated that the increasing was the result of mineral uptake into the demineralized enamel. The mineral that uptake in demineralized enamel may re-crystallization of hydroxyapatite, fluorapatite or hydroxyfluorapatite. The first process, the re-crystallization of hydroxyapatite was the calcium and phosphate reformation into the enamel's pores.[46] The latter two processes were the uptake of fluoride into the demineralized enamel. Comparison among these 3 types of apatite, the fluorapatite had the highest microhardness value followed by the hydroxyfluorapatite and the lowest was the hydroxyapatite and its derivatives. The fluorides may play the important role for remineralization of the demineralized enamel. In this study, the patients rinsed with sodium fluoride mouthrinse with 100 ppm of fluoride concentration.[19] After rinsing in the control group, the enamel microhardness (245.74$\pm$40.66 kg/mm^2 at the occlusal direction to the bracket and 244.31$\pm$37.87 kg/mm^2 at the gingival direction to the bracket) still lower than the those of normal enamel condition, this revealed that the remineralization process may not be complete or the type of apatite may be hydroxyapatite which has lower hardness. In the test group, the enamel microhardness (271.04$\pm$44.28 kg/mm^2 at the occlusal direction to the bracket and 268.22$\pm$34.86 kg/mm^2 at the gingival direction to the bracket) increased much more than the enamel microhardness in the normal condition. So the apatite could be either the fluorapatite or the hydroxyfluorapatite that have high hardness due to the composition of fluoride in the apatite.

After rinsing, the mean of enamel microhardness and mineral content percentage in the gingival direction increased much more than occlusal direction, because demineralized enamel has a greater capacity to absorb fluoride.[18] Table 3, 4 showed that before rinsing, the mean of enamel microhardness and mineral content percentage of demineralized enamel in the gingival direction to the bracket had a value less than that in the occlusal direction for both groups. After rinsing the enamel microhardness and mineral content percentage in the gingival direction to the bracket, which demineralized more than in the occlusal direction, increased more than in the occlusal direction to the bracket for both groups.

The enamel microhardness and mineral content percentage in the test group increased much more than in the control group for both occlusal and gingival directions to the brackets. This could be explained by the fluoride diffusion rate in chemical reactions and the increase in calcium fluoride production. It had been shown in the diffusion measurements of Lodding et al that the acidity of the environment has a profound influence on the uptake and the rate of penetration of fluoride in enamel.[47] The coefficient of diffusion from the aqueous solution of sodium fluoride at pH 5 was three times above that at pH 7. The effect is thought to arise partly from chemical transformation of the apatite, partly from the occurrence of the hydrogen fluoride molecule as a mobile species. For calcium fluoride production, Ten cate showed that in the low pH situation there is more calcium fluoride production and this supersaturated calcium fluoride inhibited demineralization of enamel when added to an acid solution (pH 4-5).[48] Moreover, in vitro studies have shown that fluoride concentrations in 100 ppm at pH 5.0 are required for spontaneous precipitation of calcium fluoride.[49] Gray and Francis[50] suggested that acid dissolution may be inhibited by calcium fluoride forming a protective deposit on the enamel surface and acting as a diffusion barrier during acid attack.[51] This calcium fluoride formed on the enamel surface may be a potent reservoir, slowly releasing fluoride ions available for use in remineralization or redeposition into areas of demineralization.[52]

This result was supported by the recent study by Tufekci et al that performed in-vivo study to test the effectiveness of an essential oil mouthrinse in improving oral health in orthodontic patients. They concluded that essential oils mouthrinse with 100 ppm fluoride can reduce the amount of plaque index in patients undergoing orthodontic treatment. [53]

The limitations of this study included the variety of dining habits of patients, individual oral physiology, etching before bonding procedure and fluoride measurement method.

In this in vivo study the variation of samples could represent the population. In this study we tried to control the factors that influenced the results of this study by using visible plaque index to evaluate the home care oral hygiene during the testing interval but the oral physiology was very hard to control the salivary flow rate or buffer capacity of the saliva and the salivary quality had to be equal in every sample. The next limitation was the etching procedure at the buccal surface of the teeth. We always carefully etched every tooth surface but because of etchant consistency itself, the etchant solution could move out from the desired area. This may have influenced the baseline surface microhardness before rinsing if the samples were poor in salivary quality. The last limitation was the mineral measurement method. As aforementioned in this chapter, the direct measurement of mineral had a technical limitation. We used the surface microhardness measurement (demineralization-remineralization measurement) instead, because it is widely used and accepted in this method. The surface microhardness could represent the mineral gaining and the mineral losing of the enamel.

A further study could be a study of using direct fluoride measurement equipment to measure the amount and area that fluoride deposited on the enamel surface in fixed orthodontic patients to confirm our study.

CHAPTER 3

RESULTS

There are 4 samples that had broken brackets during rinsing, 2 in the test group and the other in the control group. So there are 13 samples remaining in the test group (male = 4 patients, female = 5 patients) and 13 samples in the control group (male = 3 patients, female = 5 patients) as shown in Table 1. Compliance, assessed by returned checklists, appeared to be very good, every patient was rinsing under prescription. Ability of personal oral hygiene care was assessed with VPI and all of patients appeared to have acceptable score. Statistical significant difference was compared between the test and control groups as shown in Table 2. From independent-sample *t*-test, there was no statistical significant difference between the test and control groups at 95% confidence intervals (p = 0.682).

Table 1 Descriptive information

Group	Number of patients			Mean ages (years ± SD)	Pairs of teeth
	Male (%)	Female (%)	Total (%)		
Test	4 (44.4)	5 (55.6)	9 (100)	24.61 ± 3.93	13
Control	3 (37.5)	5 (62.5)	8 (100)	22.98 ± 6.05	13
Total	7 (41.2)	10 (58.8)	17 (100)	23.84 ± 4.95	26

Table 2 Visible plaque index (VPI score ± SD)

	Test group	Control group	p-value
Mean VPI score	8.22 ± 3.07	7.63 ± 2.77	0.682

Enamel microhardness

The result of the microhardness profile at each position was described as U1, U2, U3 and L1, L2, L3. U1's position was 100 μm from the bracket in the occlusal direction to the bracket. U2's position was 200 μm from the U1 in the occlusal direction to the bracket. U3's

position was 200 μm from the U2 in the occlusal direction to the bracket. L1's position was 100 μm from the bracket in the gingival direction to the bracket. L2's position was 200 μm from the L1 in the gingival direction to the bracket. L3's position was 200 μm from the L2 in the gingival direction to the bracket. Enamel microhardness profiles had a minimum value at the nearest position to the bracket both in the occlusal and gingival directions and increased in value with increasing distance from the bracket. (The data of enamel microhardness in all samples were described in appendices)

Table 3 Means (SD) of enamel microhardness (kg/mm^2)

Direction to bracket	**Test group (n=13)**			**Control group (n=13)**		
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CHAPTER 4

DISCUSSION

In this study we controlled several factors that influenced to the enamel remineralization in each and between every orthodontic patient. These several factors included of individual oral physiology, dental plaque around orthodontic bracket and orthodontic brackets position. The factor that is difficult to control is individual oral physiology, for example, buffer capacity, saliva flow rate etc. So we designed the study to control this factor by split-mouth technique to investigate the effect of each type of mouthrinse to remineralization on the enamel in each subject. However, comparison of remineralization between types of mouthrinse may be affected by this factor. However, all subjects received the same home care oral hygiene instruction in order to control the dental plaque accumulation around the orthodontic brackets between the groups, which influence to the enamel remineralization. Moreover, we evaluated the oral hygiene care of every patient before rinsing by Visible Plaque Index (VPI). The VPI that represented the dental plaque accumulation showed that there was no different in dental plaque accumulation between the test and control groups. In case there was some different of dental plaque accumulation between the test and control groups before rinsing, VPI might have been the factor that influenced to the enamel remineralization and demineralization, the baseline value of enamel microhardness might be different and it could not be appropriately compared between the groups. As mentioned by Ogaard et al, their study showed that it had the correlation between the VPI and the remineralization- demineralization of enamel in fixed orthodontic patients.[37] The higher score in VPI, the higher in enamel demineralization. According to the comparison of VPI between test and control groups, there was no statistically significant difference between the groups. Therefore, it can be assumed that all patients could equally control dental plaque accumulation around the orthodontic brackets and so the changes of enamel mineralization in this study were almost based entirely on the effect of mouthrinse types. All of the subjects that were instructed the details of this study and would like to participate, their teeth were carefully etched and bracketed to prevent the bonding materials excess off the brackets. The bonding procedure for testing demineralization and remineralization around orthodontic brackets was followed the

guideline described by O'Reilly and Featherstone.[38] They proposed that orthodontic brackets were bonded to the buccal surfaces at a K distance of 4 millimeter from the occlusal surface using a standard acid-etch adhesive system and following the manufacturer's instructions. Particular care was taken during the etching procedure to ensure that only the area where the bracket would be placed would be etched and sealed. This was done to avoid the influence of the etching procedure upon the enamel adjacent to the bracket.[39] Because if there was an excess in etched and bonded material out of the orthodontic brackets, the enamel around orthodontic brackets would increased in demineralization. In this study we did not use the distance from the occlusal surface same to O'Reilly and Featherstone's study because it was a different in individual occlusion at premolars area. In some patient, when the bite closed, the buccal cusp of the upper premolar teeth could contact to the bracket on the opponent premolar teeth, leading to broken brackets or teeth. 30 days later, the first side of teeth was randomly extracted and patients were randomly allocated into test and control groups. The other side of teeth was extracted 30 days later. Afterwards extracted teeth were stored in a 0.02% thymol solution at 4^{o} C to preserve mineral properties of the dental enamel.[34] This solution could preserve the organic and inorganic mineral component in the human teeth after they were removed from the mouth.

Fluoride measurement could be performed by direct and indirect techniques. At first we tried to use a direct technique by using the electron microanalysis-quantitative analysis technique (SEM-EDS, JSM-5800 LV, JEOL, EDS: Oxford ISIS 300, Japan) to measure the amount of mineral in the enamel surface.[40] Because the sensitivity of our equipment was not enough to detect the small amount of fluoride on the enamel of extracted teeth, we changed the fluoride measurement method to measure the fluoride with fluoride electrode.[33] Unfortunately, the area to be sectioned was too small to cut by the slow cutting machine (IsomettJeanWirtz CUTO I, Wirtz-Buehler GmbH, Germany), so we had to change the fluoride measurement to the indirect technique by measuring the demineralization-remineralization process, using a Knoop surface microhardness testing machine. This technique was widely used in many studied because of its simplicity and widely accepted to represent the mineral lost or gained.[33, 38, 40] The samples were prepared for microhardness testing similarly to O'Reilly and Featherstone method.[38] They studied enamel microhardness of the bonded premolar teeth in different fluoride supplement types, by cutting them in half and performed the Knoop microhardness 500 μm away from the bracket both occlusal and gingival directions to the bracket and underneath the bracket at the different depths.

They showed that microhardness in the occlusal direction was higher than the gingival direction to the bracket and was higher when the measuring position was away from the bracket. So in this study, the enamel microhardness was tested at both occlusal and gingival directions to the brackets in 3 positions 100 µm, 300 µm and 500 µm away from the brackets. In this study we did not measure the mineralization deeper than 50 µm from the anatomical enamel surface like they had done before, because the fluoride could only penetrate no deeper than 50 -100 µm into the enamel surface.[41] We changed the enamel microhardness to a mineral content percentage. Mineral content percentage was first described by Featherstone et al[35], they found the linear relationship between mineral content percentage determined by micro-radiography and the square root of the Knoop hardness number assessed by microhardness testing in the mineral range of 40 – 90 volume percent. They concluded that the enamel microhardness can represent the mineral gain or loss and when convert to mineral content percentage, it easier to indicate the mineral gain or loss in the enamel than microhardness value.[35]

Before rinsing, the enamel microhardness and mineral content percentage were not different between the test and control groups in both occlusal and gingival directions to the bracket. Therefore, we separately compared the enamel microhardness and mineral content percentage of the enamel at the occlusal and gingival directions to the bracket in both two groups. In the gingival direction, the mean of enamel microhardness and mineral content percentage was less than in the occlusal direction. Due to the gingival direction to the bracket being close to the marginal gingival, small amounts of food, debris and plaque can easily become struck these.[12] Moreover, it was difficult to clean by brushing alone or self-cleansing by salivary flow. So this area has higher chance to created enamel demineralization. Gorelick et al explained that the increase in initial demineralization was the result of higher plaque accumulation around the orthodontic brackets especially in the gingival direction to the orthodontic brackets.[12] They suggested that the factors behind the plaque and debris retention that made the initial demineralization of the bonded teeth were the access to the flow of saliva, the distance, and the tooth surface area between the gingival and the bracket. They assumed that the initial demineralization would be lesser, if there was a higher in salivary flow rate, larger in the tooth surface area, and longer distance between the gingival and the bracket.

Now, it was widely accept that fluoride enhanced enamel mineral uptake during continuous remineralization and inhibited mineral loss during demineralization in

demineralization and remineralization experiments.[41] In normal condition, enamel microhardness of human teeth was around 262 – 265 kg/mm^2.[42, 43] In this study, the enamel microhardness after bracketing was decreased below the normal. To measured microhardness of our sample in normal condition, we measured the enamel microhardness in the sample before rinsing at the site away from the bracket and the result showed that the enamel microhardness was 260 kg/mm^2. This result demonstrated that brushing with non-fluoridated toothpaste was not enough to prevent the teeth from demineralization around the bracket in fixed orthodontic patients. The reasons for decreasing of the enamel microhardness were that the fixed orthodontic appliances changed the oral equilibrium and the difficult to clean the teeth at the occlusal and gingival directions to the orthodontic bracket.[2, 12] The fixed orthodontic appliances shifted the oral equilibrium to the direction of the higher in oral bacteria and they also interfered the tooth-brushing at the occlusal and gingival directions to the bracket. These made the higher in plaque accumulation, enamel demineralization and the finally, decreased in enamel microhardness. After rinsing the value increased in both groups. We speculated that the increasing was the result of mineral uptake into the demineralized enamel. The mineral that uptake in demineralized enamel may re-crystallization of hydroxyapatite, fluorapatite or hydroxyfluorapatite. The first process, the re-crystallization of hydroxyapatite was the calcium and phosphate reformation into the enamel's pores.[46] The latter two processes were the uptake of fluoride into the demineralized enamel. Comparison among these 3 types of apatite, the fluorapatite had the highest microhardness value followed by the hydroxyfluorapatite and the lowest was the hydroxyapatite and its derivatives. The fluorides may play the important role for remineralization of the demineralized enamel. In this study, the patients rinsed with sodium fluoride mouthrinse with 100 ppm of fluoride concentration.[19] After rinsing in the control group, the enamel microhardness 245.74$\pm$40.66 kg/mm^2 at the occlusal direction to the bracket and 244.31$\pm$37.87 kg/mm^2 at the gingival direction to the bracket still lower than the those of normal enamel condition, this revealed that the remineralization process may not be complete or the type of apatite may be hydroxyapatite which has lower hardness. In the test group, the enamel microhardness 271.04$\pm$44.28 kg/mm^2 at the occlusal direction to the bracket and 268.22$\pm$34.86 kg/mm^2 at the gingival direction to the bracket increased much more than the enamel microhardness in the normal condition. So the apatite could be either the fluorapatite or the hydroxyfluorapatite that have high hardness due to the composition of fluoride in the apatite.

After rinsing, the mean of enamel microhardness and mineral content percentage in the gingival direction increased much more than occlusal direction, because demineralized enamel has a greater capacity to absorb fluoride.[18] Table 3, 4 showed that before rinsing, the mean of enamel microhardness and mineral content percentage of demineralized enamel in the gingival direction to the bracket had a value less than that in the occlusal direction for both groups. After rinsing the enamel microhardness and mineral content percentage in the gingival direction to the bracket, which demineralized more than in the occlusal direction, increased more than in the occlusal direction to the bracket for both groups.

The enamel microhardness and mineral content percentage in the test group increased much more than in the control group for both occlusal and gingival directions to the brackets. This could be explained by the fluoride diffusion rate in chemical reactions and the increase in calcium fluoride production. It had been shown in the diffusion measurements of Lodding et al that the acidity of the environment has a profound influence on the uptake and the rate of penetration of fluoride in enamel.[47] The coefficient of diffusion from the aqueous solution of sodium fluoride at pH 5 was three times above that at pH 7. The effect is thought to arise partly from chemical transformation of the apatite, partly from the occurrence of the hydrogen fluoride molecule as a mobile species. For calcium fluoride production, Ten cate showed that in the low pH situation there is more calcium fluoride production and this supersaturated calcium fluoride inhibited demineralization of enamel when added to an acid solution pH 4-5.[48] Moreover, in vitro studies have shown that fluoride concentrations in 100 ppm at pH 5.0 are required for spontaneous precipitation of calcium fluoride.[49] Gray and Francis[50] suggested that acid dissolution may be inhibited by calcium fluoride forming a protective deposit on the enamel surface and acting as a diffusion barrier during acid attack.[51] This calcium fluoride formed on the enamel surface may be a potent reservoir, slowly releasing fluoride ions available for use in remineralization or redeposition into areas of demineralization.[52]

This result was supported by the recent study by Tufekci et al that performed in-vivo study to test the effectiveness of an essential oil mouthrinse in improving oral health in orthodontic patients. They concluded that essential oils mouthrinse with 100 ppm fluoride can reduce the amount of plaque index in patients undergoing orthodontic treatment.[53]

The limitations of this study included the variety of dining habits of patients, individual oral physiology, etching before bonding procedure and fluoride measurement method.

In this in vivo study the variation of samples could represent the population. In this study we tried to control the factors that influenced the results of this study by using visible plaque index to evaluate the home care oral hygiene during the testing interval but the oral physiology was very hard to control the salivary flow rate or buffer capacity of the saliva and the salivary quality had to be equal in every sample. The next limitation was the etching procedure at the buccal surface of the teeth. We always carefully etched every tooth surface but because of etchant consistency itself, the etchant solution could move out from the desired area. This may have influenced the baseline surface microhardness before rinsing if the samples were poor in salivary quality. The last limitation was the mineral measurement method. As aforementioned in this chapter, the direct measurement of mineral had a technical limitation. We used the surface microhardness measurement demineralization-remineralization measurement instead, because it is widely used and accepted in this method. The surface microhardness could represent the mineral gaining and the mineral losing of the enamel.

A further study could be a study of using direct fluoride measurement equipment to measure the amount and area that fluoride deposited on the enamel surface in fixed orthodontic patients to confirm our study.

CHAPTER 5

CONCLUSIONS

The acidulated fluoride mouthrinse and neutral fluoride mouthrinse increased enamel microhardness and remineralization. The acidulated fluoride mouthrinse increased enamel microhardness and remineralization much more than neutral fluoride mouthrinse in fixed orthodontic patients.

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APPENDICES

ใบเชิญชวน

ขอเชิญเข้าร่วมโครงการวิจัยเรื่อง ผลของน้ำยาบ้วนปากฟลูออไรด์ที่เป็นกรดต่อการดูดซึมของฟลูออไรด์บนผิวเคลือบฟันในผู้ป่วยจัดฟันด้วยเครื่องมือจัดฟันชนิดติดแน่น

เรียน ท่านผู้อ่านที่นับถือ

ข้าพเจ้า ทพ.ปภินวิช วิพัฒนบวรวงศ์ กำลังศึกษาระดับปริญญาโท สาขาทันตกรรมจัดฟัน ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ใคร่ขอเล่าถึงโครงการวิจัยที่กำลังทำอยู่ และขอเชิญชวนท่านเข้าร่วมโครงการดังนี้ โครงการวิจัยนี้จะทำการศึกษาการดูดซึมของฟลูออไรด์บนผิวเคลือบฟันหลังบ้วนปากด้วยน้ำยาบ้วนปากโซเดียมฟลูออไรด์ที่เป็นกรด โดยเปรียบเทียบระหว่างน้ำยาบ้วนปากโซเดียมฟลูออไรด์ที่เป็นกรด และน้ำยาบ้วนปากโซเดียมฟลูออไรด์ทั่วไป เป็นเวลา 14 วัน มีรายงานการวิจัยว่าความเป็นกรดเล็กน้อยจะช่วยให้ฟลูออไรด์เข้าสู่ผิวฟันได้มากขึ้น น้ำยาบ้วนปากที่ใช้ในงานวิจัยนี้มีความเป็นกรดเล็กน้อยและน้อยกว่าผลไม้บางประเภทเช่น สัปปะรด, ส้ม ทั้งนี้น้ำลายในช่องปากยังมีความสามารถในการช่วยลดความเป็นกรดของน้ำยาบ้วนปากอีกด้วย น้ำยาบ้วนปากทุก ชนิดที่ใช้ในการวิจัยนี้เป็นน้ำยาบ้วนปากที่ปลอดภัย ไม่ก่อให้เกิดอันตรายใดๆ ต่อเนื้อเยื่อในช่องปาก และได้รับการรับรองความปลอดภัยจากองค์การอาหารและยาแห่งประเทศไทยแล้ว ผู้เข้าร่วมวิจัยจะได้รับน้ำยาบ้วนปากในระหว่างการ วิจัยฟรี หลังจากสิ้นสุดการวิจัยแล้ว ผู้เข้าร่วมการวิจัยจะได้รับการรักษาทางทันตกรรมจัดฟันด้วยวิธีการตามปกติต่อไป

ถ้าท่านตัดสินใจเข้าร่วมโครงการนี้จะมีขั้นตอนของการวิจัยที่เกี่ยวข้องกับท่านคือ ท่านจะได้รับการติดเครื่องมือจัดฟันชนิดติดแน่นเป็นเวลา 30 วัน และจะถอนฟันกรามน้อยหนึ่งซี่เพื่อนำไปใช้ประเมินผลในการวิจัย หลังจากนั้นท่านจะได้รับน้ำยาบ้วนปากไปใช้ที่บ้านตอนเช้าและก่อนนอนทุกวันติดต่อกัน เป็นเวลา 14 วัน และจะถอนฟันกรามน้อยหนึ่งซี่เพื่อนำไปใช้ประเมินผลในการวิจัยเช่นเดียวกัน ซึ่งการถอนฟันกรามน้อยนั้นเป็นการถอนฟันเพื่อการจัดฟันอยู่แล้ว ทั้งนี้ผู้เข้าร่วมการวิจัยจะต้องแปรงฟัน, ใช้แปรงซอกฟัน, ใช้ไหมขัดฟัน และบ้วนน้ำยาบ้วนปากตามวิธีใช้ที่กำหนดให้อย่างสม่ำเสมอ ระหว่างบ้วนน้ำยาบ้วนปากมักพบอาการแสบในช่องปาก ขณะบ้วนคือการที่ปลายประสาทถูกกระตุ้นเหมือนตอนทานอาหารรสเผ็ดหรือขณะอมน้ำแข็งซึ่งไม่มีอันตรายใดๆ และอาการจะหายไปภายในเวลาไม่กี่นาทีหลังบ้วนน้ำยาบ้วนปากทิ้ง ถ้าพบว่าผู้ป่วยแพ้น้ำยาบ้วนปากโซเดียมฟลูออไรด์ที่เป็นกรดซึ่งจะมีการหลุดลอกของเนื้อเยื่อในช่องปาก หรือมีอาการเสียวฟันอันเนื่องมาจากการบ้วนน้ำยาบ้วนปากโซเดียมฟลูออไรด์ที่เป็นกรด ทางผู้วิจัยจะให้หยุดบ้วนและออกจากโครงการวิจัยนี้ทันที แต่ผู้ป่วยยังคงได้รับการรักษาทางทันตกรรมจัดฟันตามปกติต่อไปจนเสร็จสิ้นการรักษา และทางผู้วิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายในการรักษา

ไม่ว่าท่านจะเข้าร่วมในโครงการวิจัยนี้หรือไม่ ท่านจะยังคงได้รับการรักษาเช่นเดียวกับผู้ป่วยคนอื่นๆ และถ้าท่านต้องการที่จะถอนตัวออกจากการศึกษานี้เมื่อใด ท่านก็สามารถกระทำได้อย่างอิสระ

ถ้าท่านมีคำถามใดๆ ก่อนที่จะตัดสินใจก่อนเข้าร่วมโครงการนี้ โปรดซักถามคณะผู้วิจัยได้อย่างเต็มที่

ขอขอบคุณเป็นอย่างสูง

ทพ.ปภินวิช วิพัฒนบวรวงศ์

หมายเหตุ: กรุณาอ่านข้อความให้เข้าใจก่อนเซ็นชื่อยินยอมเข้าร่วมโครงการ

แบบยินยอมเข้าร่วมการศึกษา

โครงการวิจัยเรื่อง ผลของน้ำยาบ้วนปากฟลูออไรด์ที่เป็นกรดต่อการดูดซึมของฟลูออไรด์
บนผิวเคลือบฟันในผู้ป่วยจัดฟันด้วยเครื่องมือจัดฟันชนิดติดแน่น

วันที่_____เดือน_____

พ.ศ._____

ข้าพเจ้า_____อายุ_____ปี อาศัยอยู่บ้านเลขที่_____ หมู่_____ ถนน_____ตำบล_____อำเภอ_____ จังหวัด_____ ได้รับการอธิบายถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย อันตรายที่อาจเกิดขึ้นจากการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

หากข้าพเจ้ามีข้อสงสัยประการใด หรือเกิดผลข้างเคียงจากการวิจัยจะสามารถติดต่อกับ ทพ.ปภินวิช วิพัฒนบวรวงศ์ ได้ที่ภาควิชาทันตกรรมป้องกัน คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ หมายเลขโทรศัพท์ 074-429876 หรือเมื่อมีปัญหาใดๆ เกิดขึ้นเนื่องจากการทำวิจัยในเรื่องนี้ ข้าพเจ้าสามารถร้องเรียนได้ที่คณบดี คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อ.หาดใหญ่ จ.สงขลา 90112 หมายเลขโทรศัพท์ 074-287510

หากผู้วิจัยมีข้อมูลเพิ่มเติมทั้งทางด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ ผู้วิจัยจะแจ้งให้ข้าพเจ้าทราบอย่างรวดเร็วโดยไม่มีปิดบัง

ข้าพเจ้ามีสิทธิ์ที่จะของดการเข้าร่วมโครงการวิจัย โดยจะแจ้งให้ทราบล่วงหน้า โดยการงดการเข้าร่วมการวิจัยนี้จะไม่มีผลต่อการได้รับบริการหรือการรักษาที่ข้าพเจ้าจะได้รับแต่อย่างใด

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวกับตัวข้าพเจ้าเป็นความลับ จะไม่เปิดเผยข้อมูลหรือผลการวิจัยของข้าพเจ้าเป็นรายบุคคลต่อสาธารณชน จะเปิดเผยได้ในรูปที่เป็นสรุปผลการวิจัย หรือการเปิดเผยข้อมูลต่อผู้มีหน้าที่ที่เกี่ยวข้องกับการสนับสนุนและกำกับดูแลการวิจัย

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ โดยผู้วิจัยได้ให้สำเนาแบบยินยอมที่ลงนามแล้วกับข้าพเจ้าเพื่อเก็บไว้เป็นหลักฐานจำนวน 1 ชุด

ลงชื่อ……..……....ผู้ยินยอม
( )

ลงชื่อ……ผู้รับผิดชอบโครงการวิจัย
(ทันตแพทย์ปภินวิช วิพัฒนบวรวงศ์)

ลงชื่อ……..……...……บิดา/ผู้ใช้อำนาจปกครอง
( )

ลงชื่อ……..……...……มารดา/ผู้ใช้อำนาจปกครอง
( )

ลงชื่อ……..……...……พยาน
( )

ลงชื่อ……..……...……..……..……พยาน
( )

ชื่อ-นามสกุล.....**กลุ่ม**.....

ใบบันทึกการบ้วนน้ำยาบ้วนปาก

วันที่	เช้า		ก่อนนอน		บันทึกอาหารรสเปรี้ยวหรือเครื่องดื่มรสเปรี้ยวที่ทาน
	ปริมาตร (ซีซี)	เวลา (วินาที)	ปริมาตร (ซีซี)	เวลา (วินาที)	
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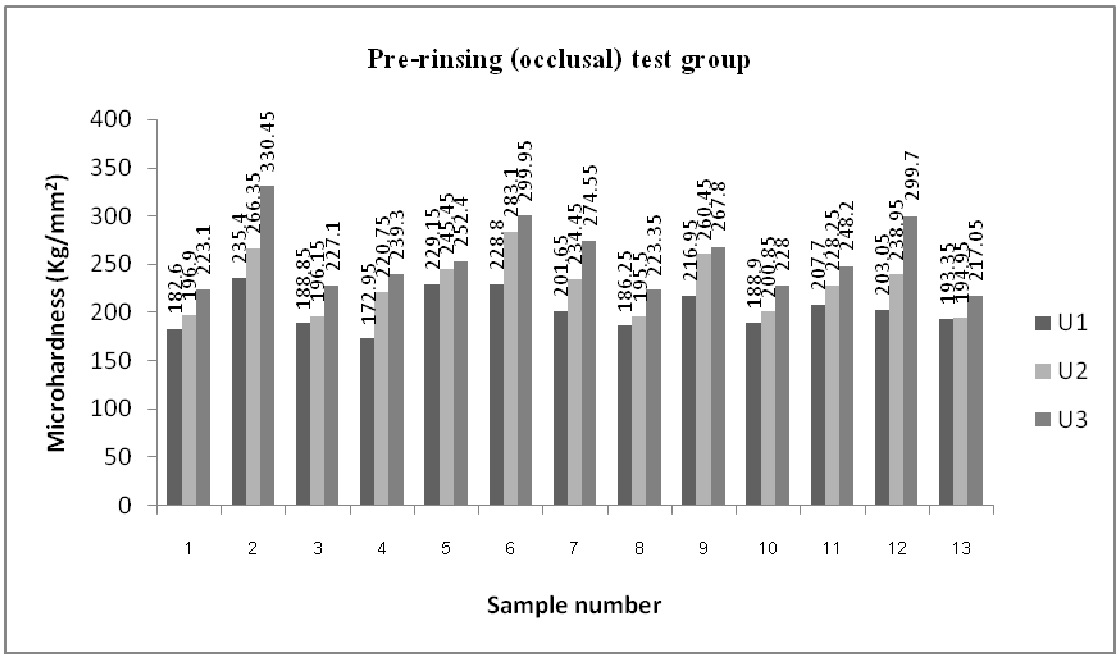
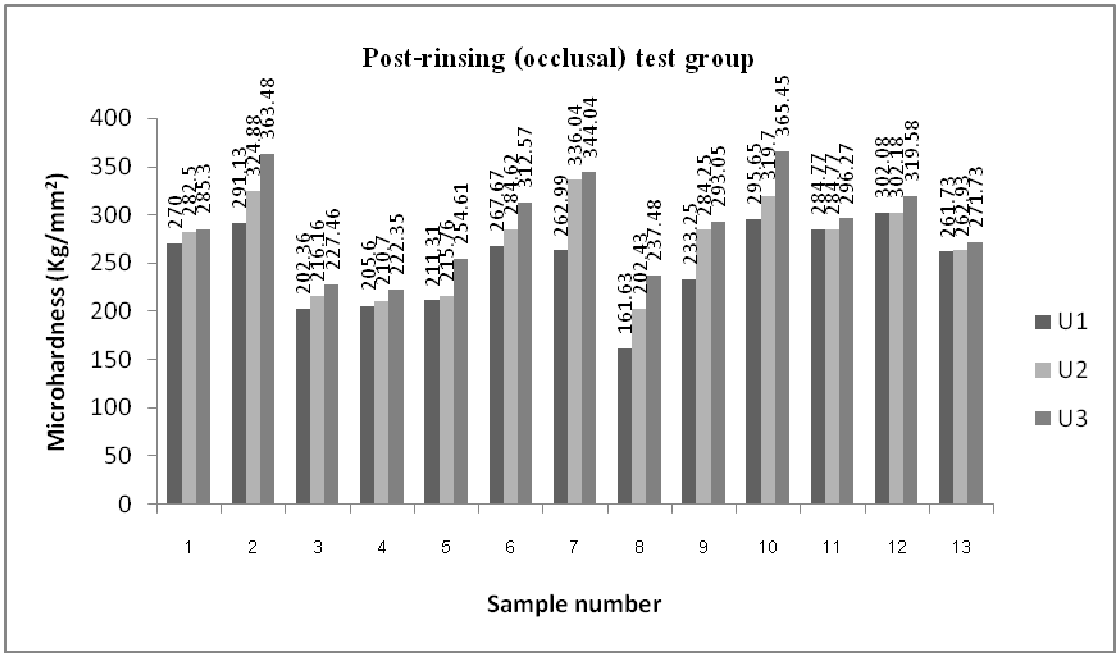
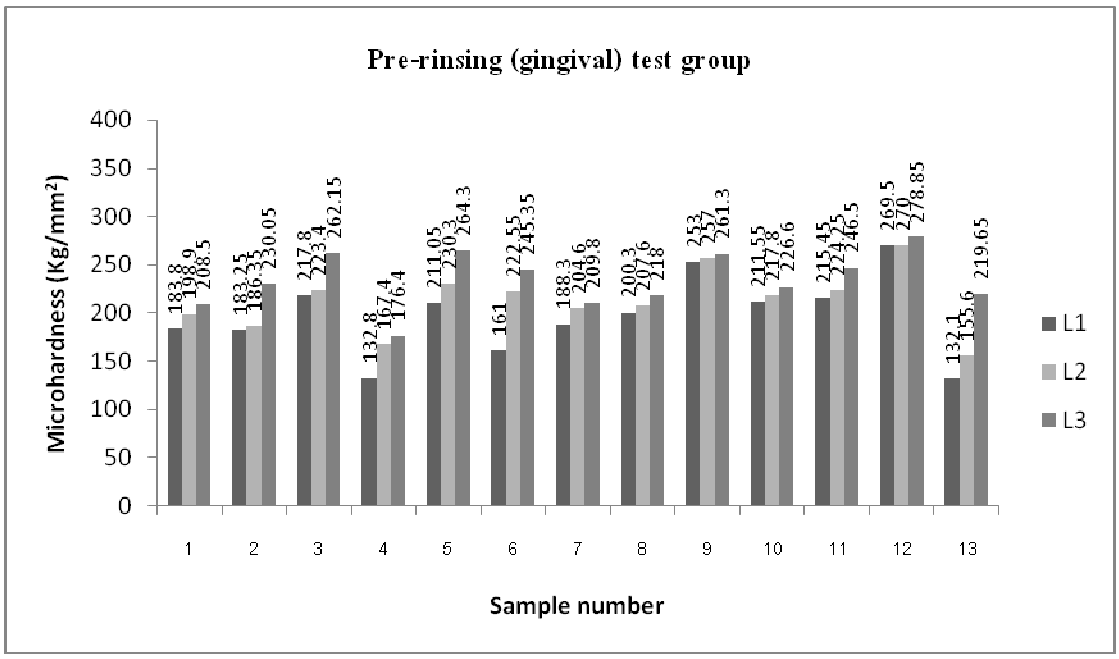
Raw data



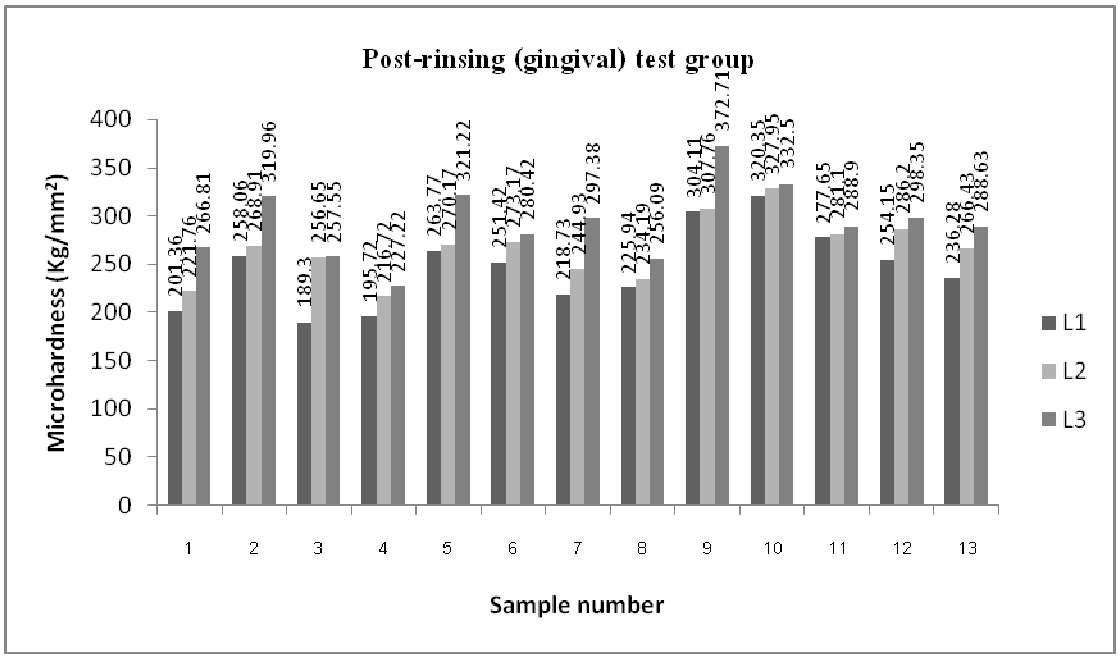
Microhardness of the test group before rinsing at occlusal to the bracket



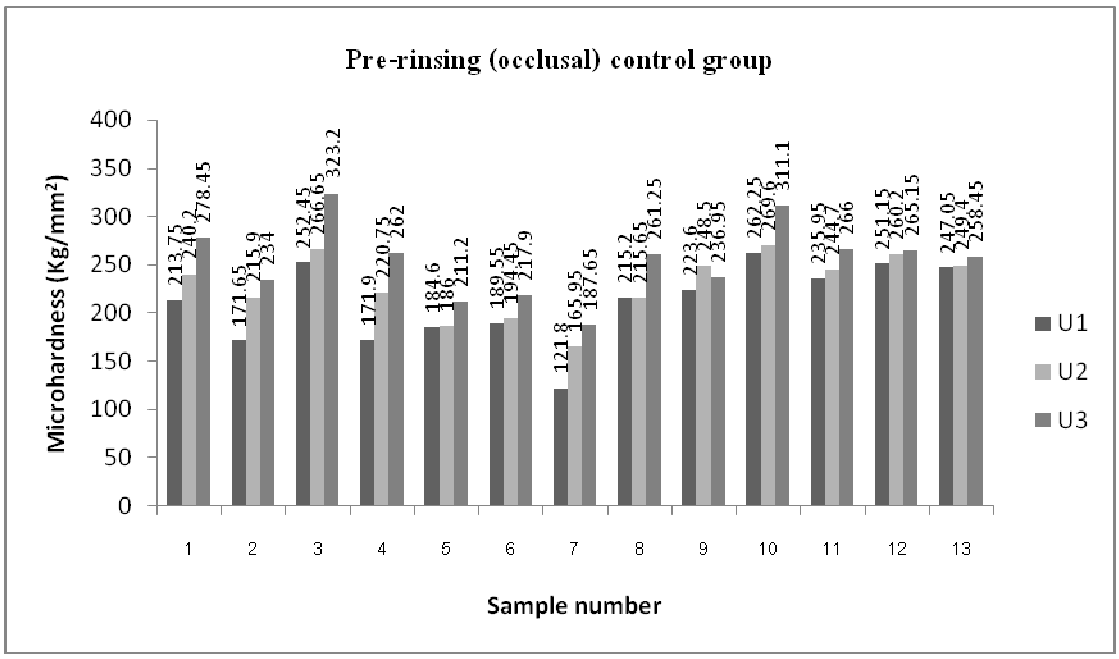
Microhardness of the test group after rinsing at occlusal to the bracket



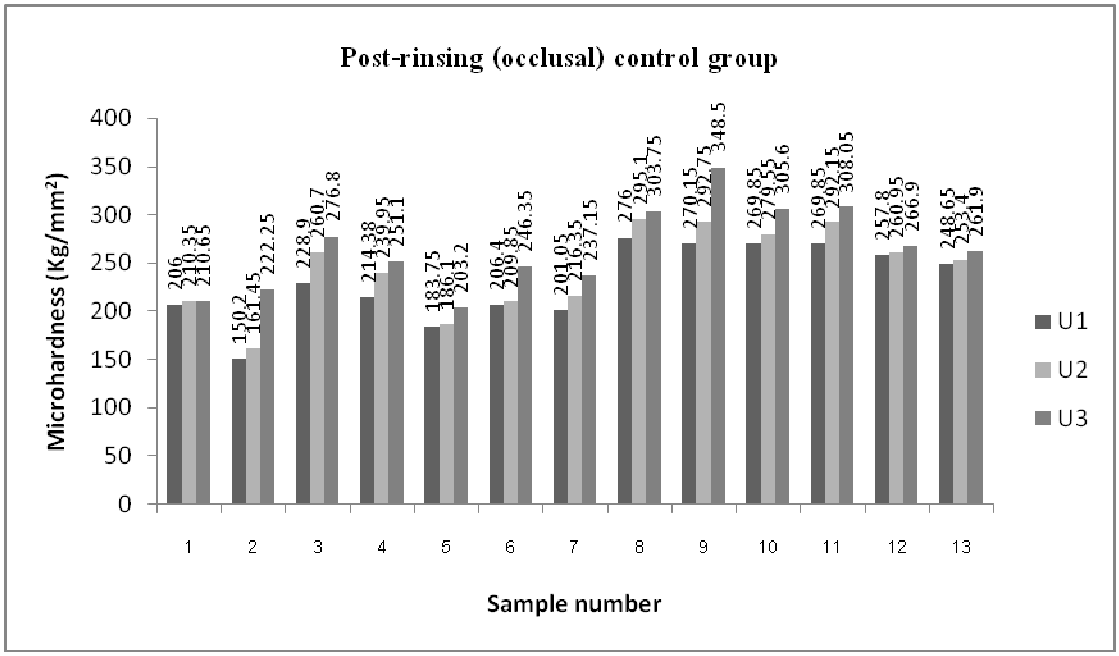
Microhardness of the test group before rinsing at gingival to the bracket



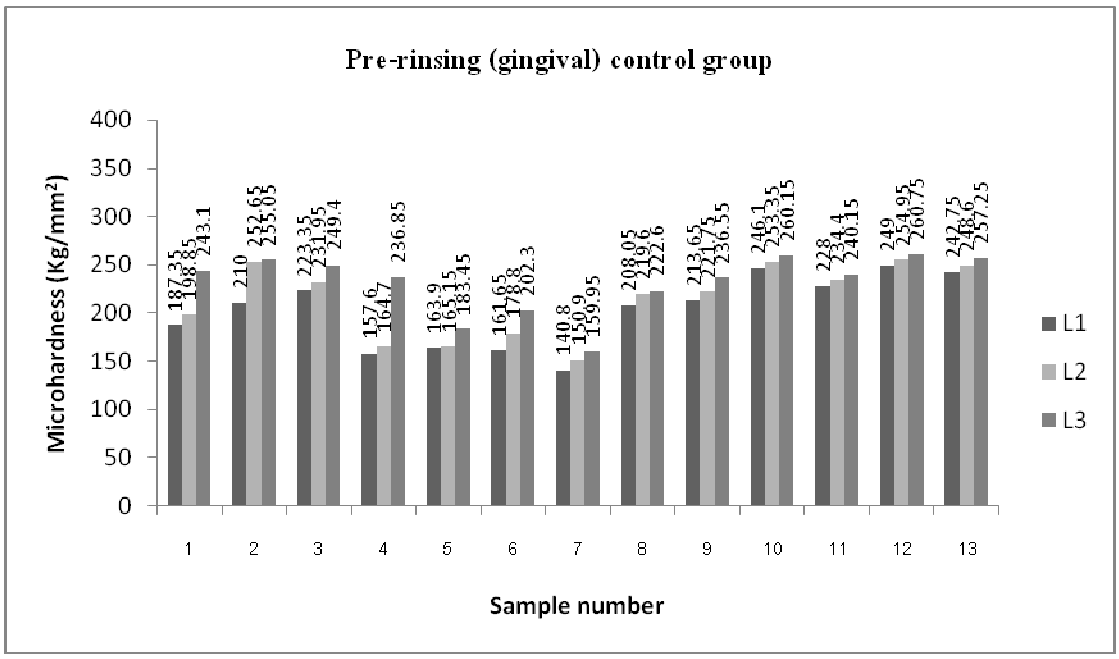
Microhardness of the test group after rinsing at gingival to the bracket



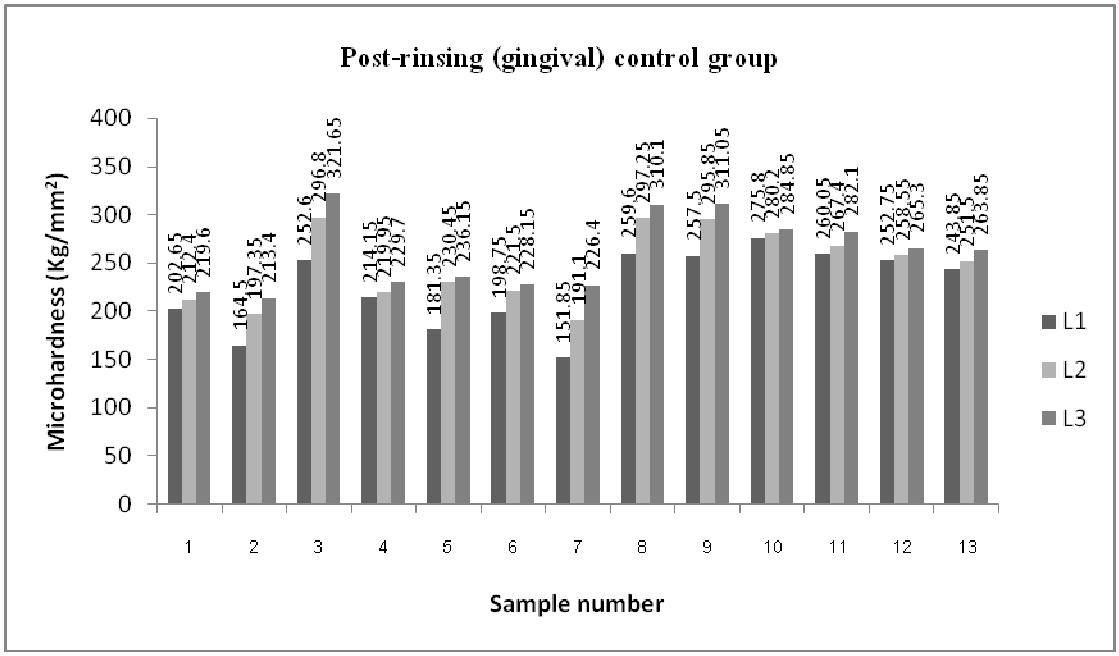
Microhardness of the control group before rinsing at occlusal to the bracket



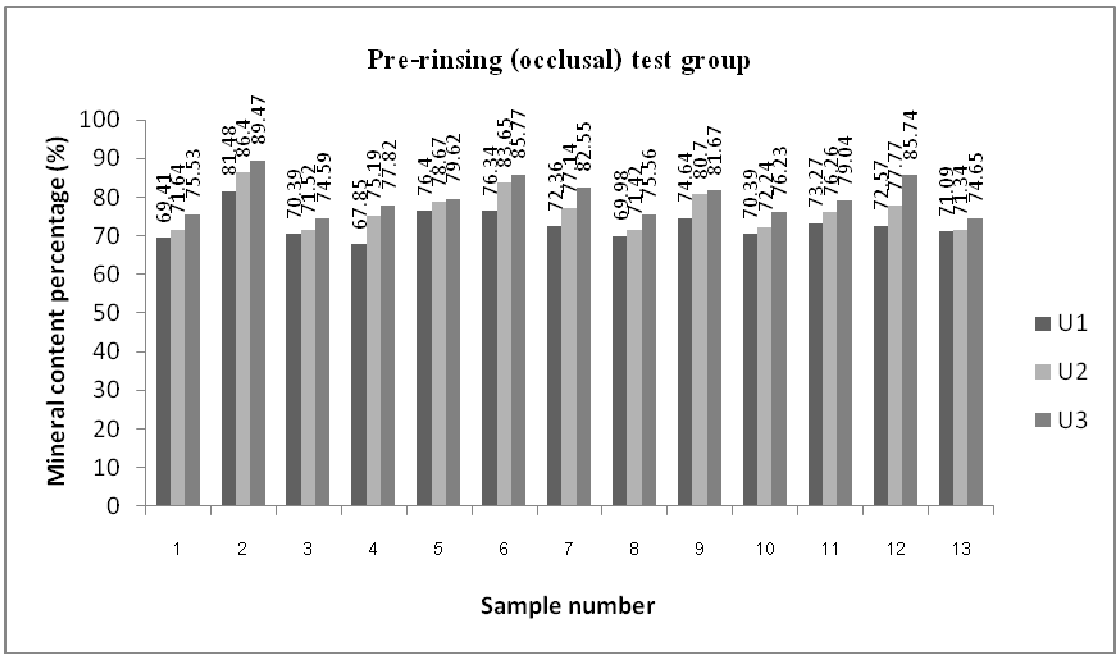
Microhardness of the control group after rinsing at occlusal to the bracket



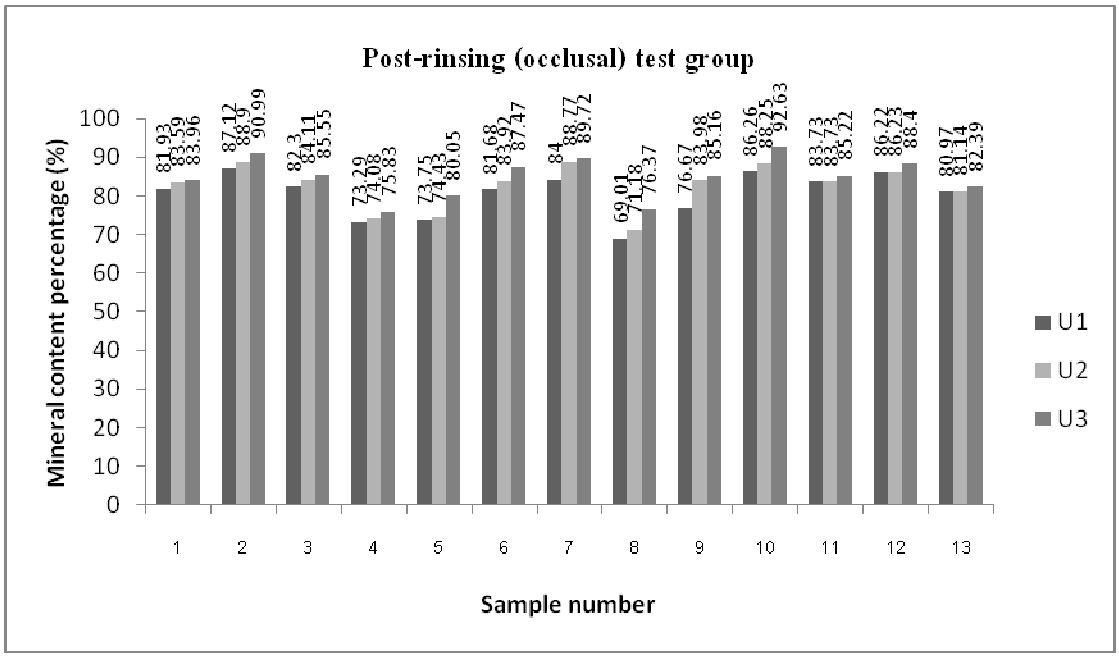
Microhardness of the control group before rinsing at gingival to the bracket



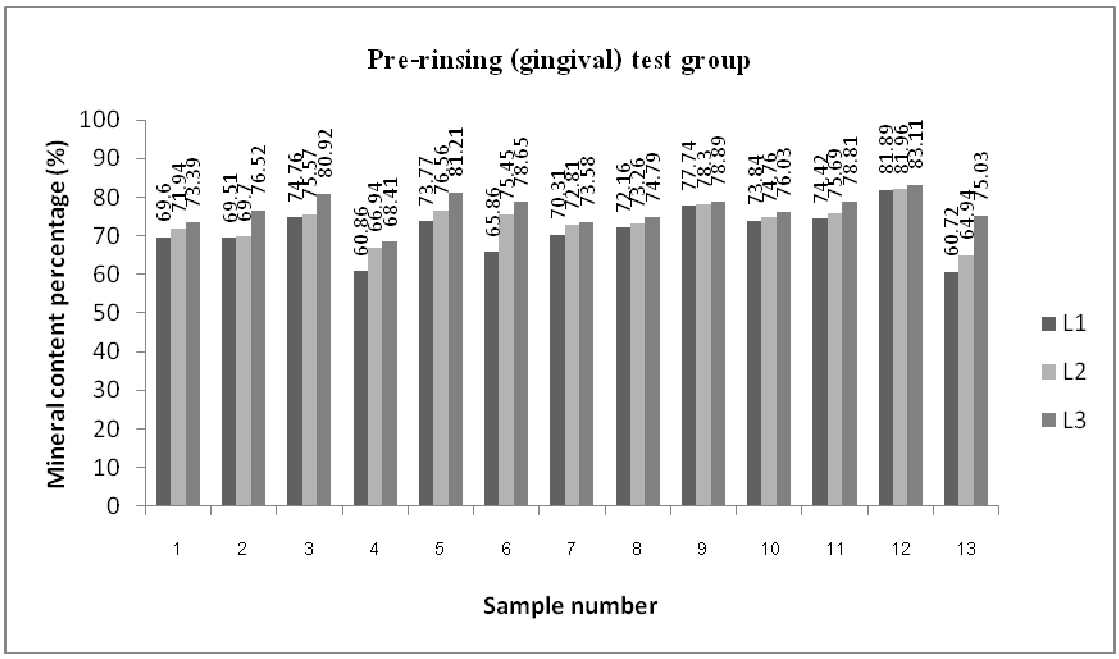
Microhardness of the control group after rinsing at gingival to the bracket



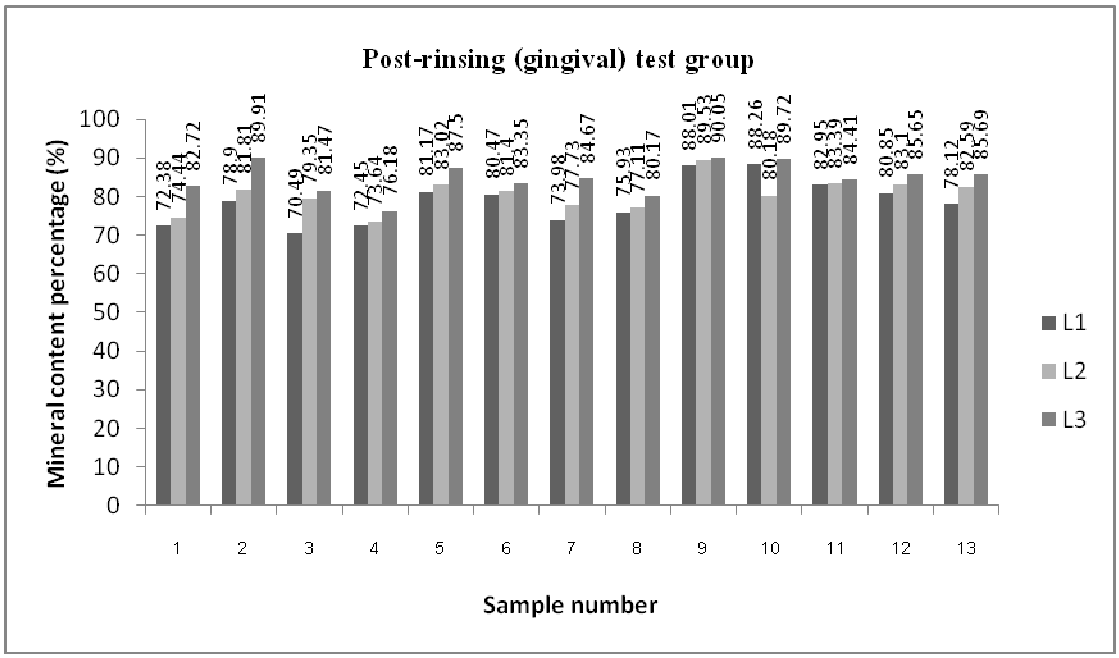
Mineral content percentage of the test group before rinsing at occlusal to the bracket



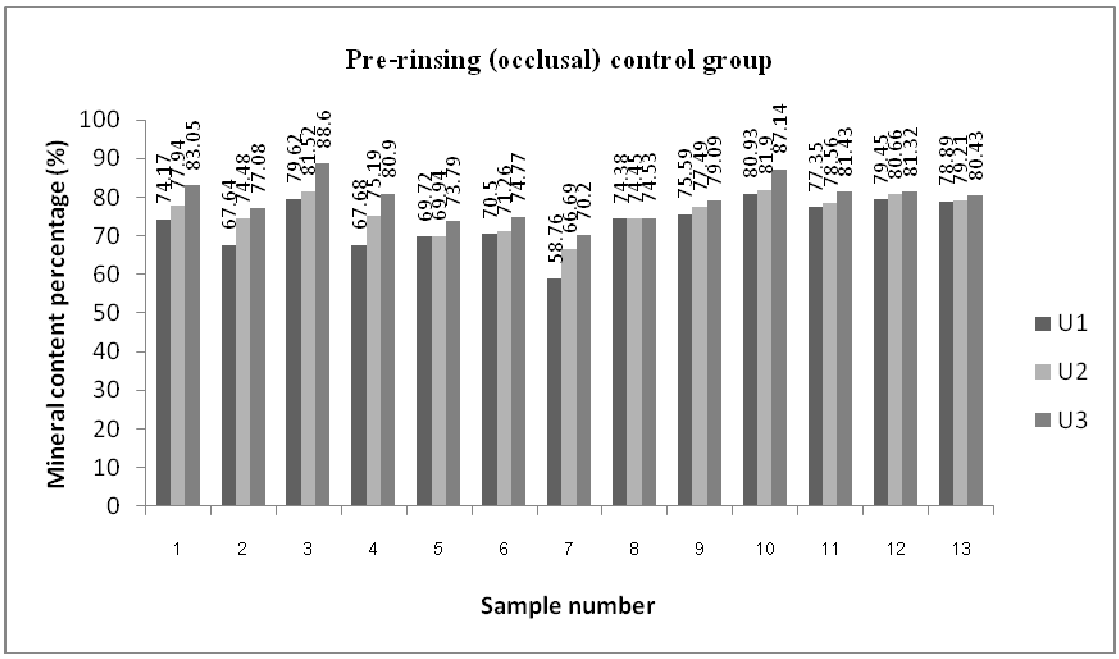
Mineral content percentage of the test group after rinsing at occlusal to the bracket



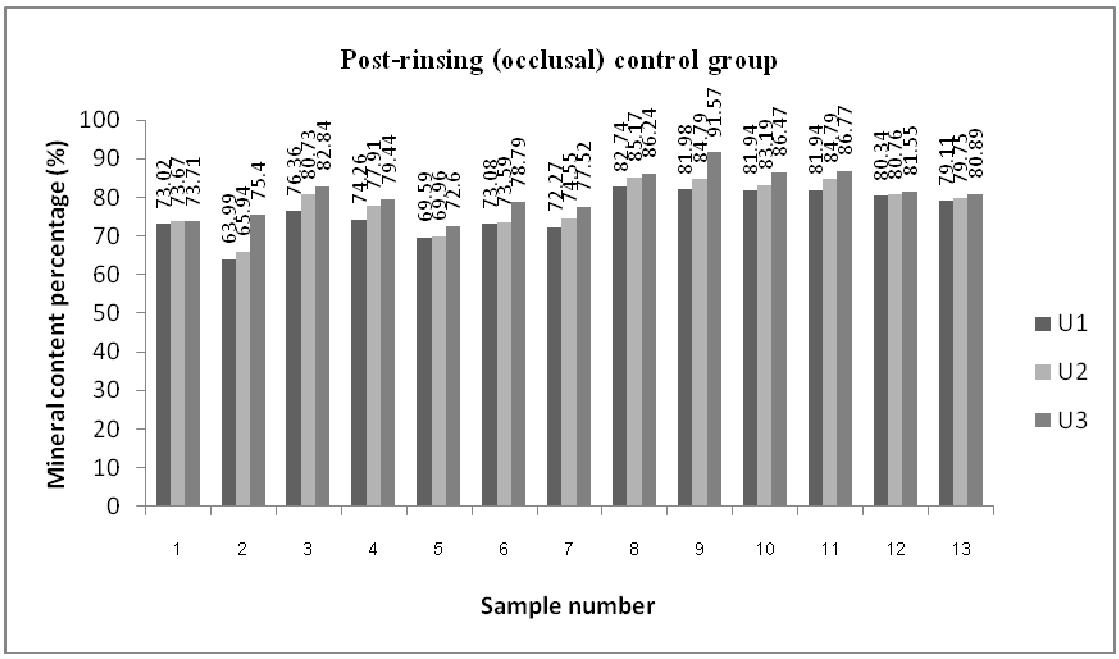
Mineral content percentage of the test group before rinsing at gingival to the bracket



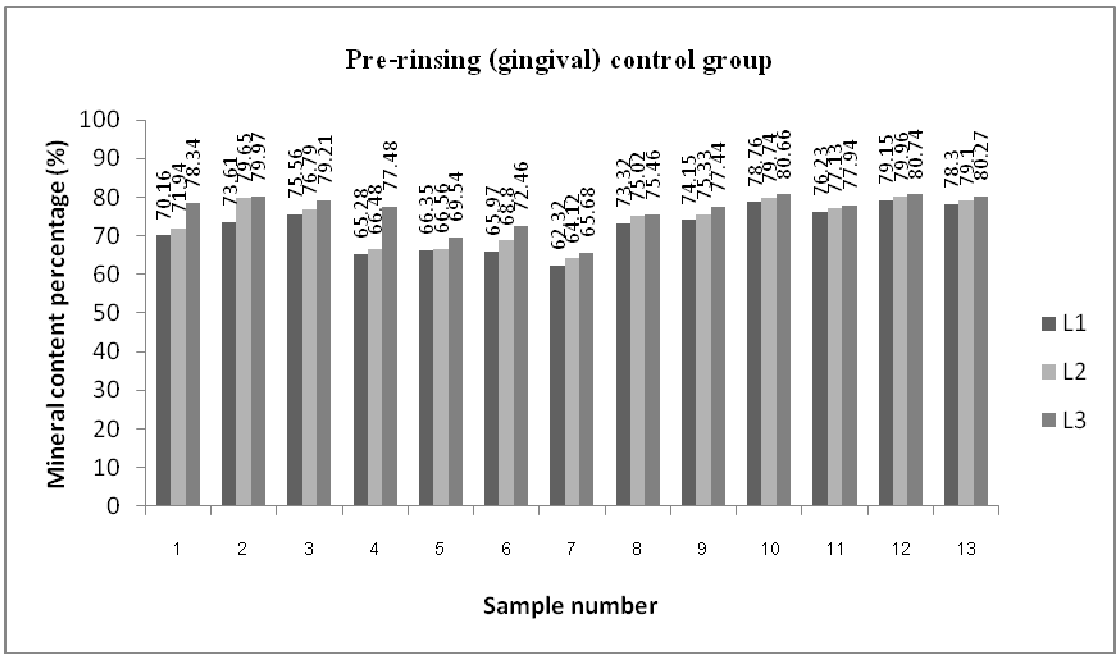
Mineral content percentage of the test group after rinsing at gingival to the bracket



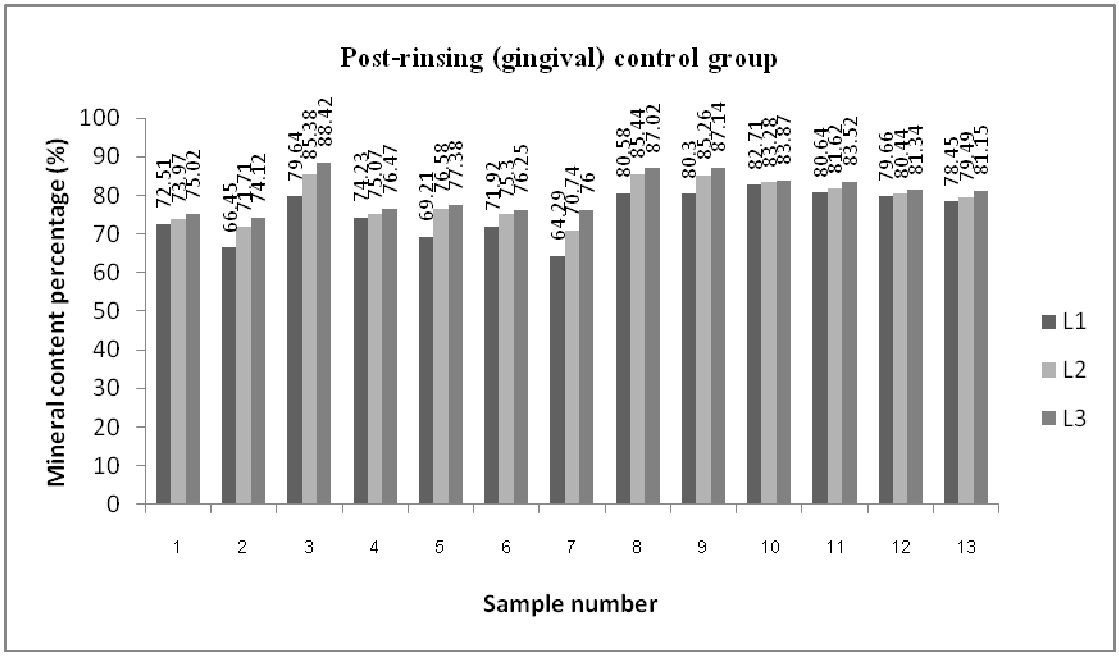
Mineral content percentage of the control group before rinsing at occlusal to the bracket



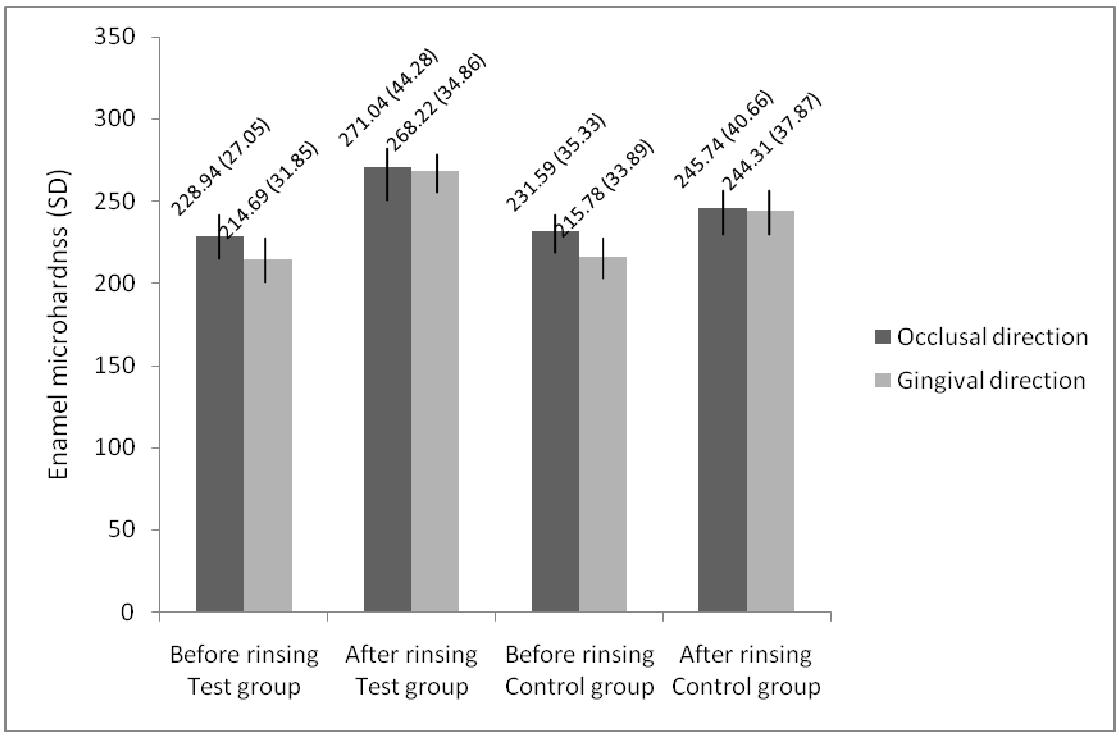
Mineral content percentage of the control group after rinsing at occlusal to the bracket



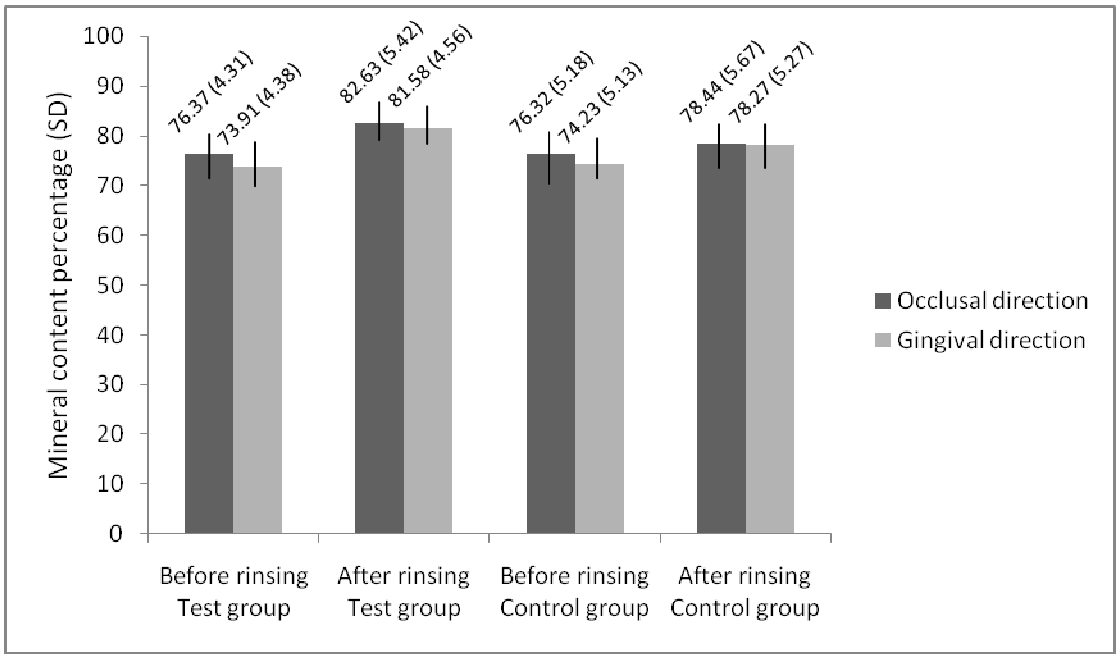
Mineral content percentage of the control group before rinsing at gingival to the bracket



Mineral content percentage of the control group after rinsing at gingival to the bracket



Enamel microhardness of test and control groups in occlusal and gingival directions



Mineral content percentage of test and control groups in occlusal and gingival directions

Visible plaque index score (VPI)

	Test group									Control group							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
VPI score	12	7	12	5	7	8	7	4	12	8	8	11	12	7	5	6	4

VITAE

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