THESIS

EFFECTS OF DIFFERENT ENAMEL PRETREATMENT METHODS AND TIMING OF APPLICATION ON SHEAR BOND STRENGTH OF ORTHODONTIC BRACKETS AND ENAMEL MICROHARDNESS AFTER DEMINERALIZATION: *AN IN VITRO STUDY*

BY

MISS USANEE PATTAMALAI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
(MAJOR IN ORTHODONTIC DENTISTRY)
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Chairman
D. Jotikasthira
(Prof. Dhirawat Jotikasthira, FRCDS)

Member and Advisor
(Asst. Prof. Sutiwa Benjakul, Ph.D)

Member
(Assoc. Prof. Awiruth Klaisiri, Ph.D)

Member
Nantawan Krajangta.
(Asst. Prof. Nantawan Krajangta, Ph.D)

Member
(Asst. Prof. Kanlaya Insee, FRCDS)

Dean
(Asst. Prof. Sutee Suksudaj, Ph.D)

Thesis Title	EFFECTS OF DIFFERENT ENAMEL PRETREATMENT METHODS AND TIMING OF APPLICATION ON SHEAR BOND STRENGTH OF ORTHODONTIC BRACKETS AND ENAMEL MICROHARDNESS AFTER DEMINERALIZATION: AN *IN VITRO* STUDY
Author	Miss Usanee Pattamalai
Degree	Master of Science
Major Field/Faculty/University	Major in Orthodontic Dentistry Faculty of Dentistry Thammasat University
Thesis Advisor	Assist. Prof. Sutiwa Benjakul
Thesis Co-Advisor	Assoc. Prof. Awiruth Klaisiri
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ABSTRACT

Objective: This *in vitro* study aimed to evaluate which enamel pretreatment methods and their timing of application with fluoride varnish, resin infiltration, flat resin sealant and S-PRG (Surface pre-reacted glass ionomer) coating material could prevent enamel demineralization on enamel surface without affecting the shear bond strength (SBS) of brackets in orthodontic patient who has high caries risk.

Materials and methods:

Part I: Enamel microhardness test: The teeth were randomized into 6 groups (n = 10). Group 1: no enamel pretreatment, Group 2: demineralization, Group 3-6 were treated with different enamel pretreatment methods; fluoride varnish (Duraphat^{TM}), resin infiltration (ICON^{TM}), flat resin sealant (Pro Seal^{TM}) and S-PRG (PRG Barrier Coat^{TM}). After enamel pretreatment procedure, group 3-6 were immersed in demineralizing solution for 96 hours. Vickers hardness (VH) was tested. Differences between Vickers hardness number (HV) were analyzed by Kruskal-Wallis followed by post-hoc multiple comparison. Statistically significant was set at $P < 0.05$.

Part II: SBS and ARI test: The teeth were randomized into 10 groups (n = 14). Group 1: no enamel pretreatment, Group 2: demineralization, Group 3-10 were treated with different enamel pretreatment methods; fluoride varnish (Duraphat^{TM}), resin infiltration (ICON^{TM}), flat resin sealant (Pro Seal^{TM}) and S-PRG (PRG Barrier Coat^{TM}) followed with immediate or delayed brackets bonding for 30 days, respectively. After bracket bonding, group 2-10 were demineralized. SBS and ARI were evaluated. Differences between SBS values were analyzed by two-way ANOVA followed by Tamhane. The ARI scores were analyzed using Fisher's Exact test. Statistically significant was set at $P < 0.05$.

Results:

Part I: There was significant difference in HV among all groups compared to control except Duraphat^{TM} group. The highest and the lowest HV among enamel pretreatment groups was found in Duraphat^{TM} and PRG Barrier Coat^{TM} groups, respectively. However, there was no significant difference in HV of Duraphat^{TM}, ICON^{TM} and Pro Seal^{TM} groups. In addition, HV of Duraphat^{TM} group was significantly higher than demineralization group.

Part II: The mean bracket SBS of all enamel pretreatment methods was in the clinically accepted values. There was no significant difference in SBS among all groups compared to control. The highest SBS was found in Pro Seal^{TM} with delayed bonding, which was significantly higher than demineralization and the immediate bonding of Duraphat^{TM}, Pro Seal^{TM} and PRG Barrier Coat^{TM}. The timing of application had no effect on SBS, excepted in Pro Seal^{TM}, which was increased in delayed group. The ARI scores were statistically significant difference between groups.

Conclusions: In order to achieve acidic enamel resistance in orthodontic patient who has high caries risk with adequate SBS of orthodontic brackets, enamel pretreatment with Duraphat^{TM} with delayed bracket bonding is recommended to be performed.

Keywords: Caries prevention, enamel pretreatment methods, orthodontics, shear bond strength, Vickers hardness, white-spot lesions

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Usanee Pattamalai

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LIST OF ABBREVIATIONS

Abbreviation	Term
SBS	Shear bond strength
ARI	Adhesive remnant index
HV	Vickers hardness number
VH	Vickers hardness
ANOVA	Analysis of Variance
WSLs	White spot lesions
CPP-ACP	Casein phosphopeptide amorphous calcium phosphate
S-PRG	Surface pre-reacted glass ionomer
S. mutans	Streptococcus mutans
PRG	Pre-reacted glass-ionomer
TEGDMA	Trietylene glycol dimetacrylate
MPa	Megapascal
µm	Micron
F-PRG	Full pre-reacted glass ionomer
BOB	Beauty Ortho Bond
GI	Glass ionomer
LED	Light emitting diode
NaF	Sodium fluoride
ES	Enamel solubility

CHAPTER 1
INTRODUCTION

1.1 Background

Enamel demineralization around orthodontic brackets and bands remains a concern to the orthodontists. Fixed orthodontic appliances can be attributed to the difficulties in tooth cleansing, leading to prolong plaque accumulation. The acidic byproducts of the bacteria in the plaque cause enamel demineralization and white spot lesions (WSLs). Enamel demineralization around orthodontic bracket can be arrested if treated properly or else they might progress to be cavitated carious lesions. As a result, debonding of brackets might occur, which complicated orthodontic treatment and prolong orthodontic treatment time.

The prevention of enamel demineralization can be done by improving patient oral hygiene, mechanical plaque removal, enhancing the enamel resistance to microbial acid by fluoride varnish, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and resin coating material containing surface pre-reacted glass ionomer fillers (S-PRG), or using protective barrier for enamel such as flat resin sealant and resin infiltration. Fluoride varnish is the fluoride high concentration leading to formation of calcium fluoride. It acts as a fluoride ions reservoir that release when pH is lowered during a caries attack. CPP-ACP promotes high rates of enamel remineralization by acting as a calcium and phosphate ions reservoir that release when intraoral acid attack had occurred. S-PRG coating material releases several ions which inhibit growth of Streptococcus mutans (S. mutans) and promotes remineralizing ability on the tooth. Flat resin sealant and resin infiltration are low-viscosity resins that can penetrate into enamel microporosities of etched enamel and serve as the physical barrier against acid.(1)

At present, many caries prevention methods are being used as enamel pretreatments before bracket bonding, in order to prevent enamel demineralization during orthodontic treatment. However, the pretreatment needs not only to prevent demineralization effectively, but also not to compromise shear bond strength (SBS) of brackets. The effects of enamel pretreatment on SBS of brackets have been studied on both sound enamel and also demineralized enamel to simulate bonding of brackets on teeth with WSLs. Previous studies shown that using of fluoride varnish as enamel pretreatment can compromise SBS of brackets. They suggested that bracket bonding should be delayed at least 15-30 days after fluoride varnish application, to reduce the negative effects on SBS of brackets.(2-4) On the other hand, the study of resin infiltration as enamel pretreatment before bracket bonding found that it should be done immediately, to improve the SBS of brackets.(5) For the flat resin sealants, there are some controversies about their effects on the SBS of brackets, which may be influenced from their compositions.(6, 7) Some flat resin sealant such as Pro Seal™ has fluoride-releasing ability, which may influence bracket bond strength. In addition, the study of PRG Barrier Coat™ before bracket bonding found that it has the negative effect on SBS of brackets and they suggested that the organic acid etching should be applied before PRG Barrier Coat™ to improve SBS of bracket values.(8) However, there are still some controversies about the effects of enamel pretreatment methods on SBS of brackets, which may be influenced from different parameters. To the best of our knowledge, there are not enough studies about enamel pretreatment materials especially that have the ion-release capability, which may be the factor that affects SBS of brackets and their timing of application may be the important factor influenced the bracket bond strength. In addition, there are no studies that compare their effects on SBS of brackets and also the prevention of enamel surface after demineralization.

This study aims to evaluate which enamel pretreatment methods and their timing of application with fluoride varnish, resin infiltration, flat resin sealant and S-PRG coating material could prevent enamel demineralization on enamel surface without affecting SBS of brackets in orthodontic patient who has high caries risk. It is hypothesized that certain enamel pretreatment methods with fluoride varnish, resin infiltration, flat resin sealant and S-PRG coating material with the proper timing of application can prevent demineralization of enamel surface without affecting the SBS of bracket bonding.

1.2 Research objective

The objective of this study is to evaluate which enamel pretreatment methods and timing of application with fluoride varnish, resin infiltration, flat resin sealant and S-PRG coating material could prevent enamel demineralization on enamel surface without affecting SBS of brackets in orthodontic patient who has high caries risk.

1.3 Research hypothesis

Certain enamel pretreatment methods can maintain enamel microhardness after an *in vitro* demineralization without compromising SBS of orthodontic brackets.

CHAPTER 2
REVIEW OF LITERATURE

2.1 White spot lesions (WSLs)

WSLs present as an opaque white chalky appearance when located on smooth tooth surface. These are area of local enamel decalcification without cavity formation. WSLs have white appearance due to an optical phenomenon caused by tooth surface mineral loss, enamel porosity, decrease of fluorescence radiance, resulting in greater visual enamel opacity when compared to healthy enamel surfaces.(9) WSLs as the first sign of caries lesion and can be noticed within 1 month of bracket placement. The incidence of WSLs during orthodontic treatment was 45.8% and the prevalence of lesions in patients undergoing orthodontic treatment was 68.4%.(9) Maxillary anterior teeth are the most commonly affected.(10) This is a clinical problem due to an unacceptable esthetic representation.(11) WSLs around orthodontic bracket can be arrested if treated properly or else they might progress to be cavitated carious lesions. In some severe cases, they require restorative treatment.(12) WSLs can be remineralized by saliva minerals. Enaia et al., 2011 reported 57.1% of WSLs in orthodontic patients showed the improvement without treatment, 26% of lesion remained unchanged and 16.7% detriorated after 1-year retention phase.(13) Nevertheless, Mattousch et al., 2007 reported WSLs did not disappear after debonding and 2 years follow-up. The majority of lesion were stable, 40% of lesion showed some improvement but 15% of lesion had worsened.(14) Al-Khateeb et al., 2000 demonstrated that WSLs could not be completely eradicated after using fluoride 1000 ppm for 10 weeks.(15)

2.2 Etiology of white spot lesions

Etiology of WSLs on the enamel surface during fixed orthodontic treatment is due to multiplicity factors such as cariogenic bacteria, host factors and enviromental factors. Acidogenic bacteria have long been identified as the primary cause in the dental caries development. *S. mutans* and *Lactobacilli* are the primary microbial agents that ferment carbohydrates and create an acidic environment leading to development of dental caries.(16,17) The individual host factors including salivary flow and composition, enamel solubility, diet, and oral hygiene affect to individual caries risk. In addition, fixed orthodontic treatment can be attributed to the difficulties in mechanical plaque removal and prolong plaque deposition on the tooth surfaces. These lead to a decrease in pH that shifts the demineralization-remineralization balance toward mineral loss, which can lead to WSLs development.(9,17) Gwinnett and Ceen, 1979 found that fixed orthodontic appliances influence a rapid increase of dental plaque.(18)

2.3 Prevention of WSLs in orthodontic treatment patients

2.3.1 Fluoride varnish

Fluoride varnish is a professionally applied adherent material which consists of high concentration of fluoride. Fluoride varnish was first introduced in 1964 in Europe; Duraphat^{TM}. Nowadays, several brands of fluoride varnish with different compositions such as Duraphat^{TM} (5% NaF), Fluor Protector^{TM} (1% difluorosilane and 0.1% NaF) and Duraflor^{TM} (5% NaF) are available.

Mechanisms of action of the fluoride varnish including;

1. Fluoride varnish acts by a slow release of fluoride ion to the underlying tooth structure. The availability of fluoride in the liquid phase around the apatite crystallites blocks crystalline dissolution and decreases the rate of demineralization.
2. Fluoride varnish acts as a fluoride ion reservoir and prevents caries by inhibition of mineral loss when oral pH is decreased leading to formation of calcium fluoride. A continuous layer of calcium fluoride is formed to protect the enamel against acidic byproducts of the bacteria in the plaque, since calcium fluoride is less soluble than fluoroapatite.(19)

Fluoride varnish is recommended as the treatment of choice for preventing dental caries in high- risk patients especially for children who are younger than 6 years old in terms of safety and ease application. One of the advantage of fluoride varnish is that it works in the presence of plaque; thoroughly cleaning the tooth surface is unnecessary before fluoride varnish application.(17) The application of fluoride varnish could significantly reduce WSLs around orthodontic bracket.(3) Bichu et al., 2013 reported that the application of fluoride varnish (Fluor Protector^{TM}) on the enamel surface provided the greatest protection of enamel demineralization in term of reduction of lesion depth than CPP-ACP (GC Tooth Mousse^{TM}), CPP-ACP combined with fluoride (GC Tooth Mousse Plus^{TM}), calcium sodium phosphosilicate (SHY-NM paste^{TM}) and non-coated enamel groups.(20) The application of fluoride varnish could significantly reduce WSLs around orthodontic brackets.(5,21,22) Montasser et al., 2014 showed that there was no significant difference in the enamel microhardness between the fluoride varnish group (Clinpro^{TM}) and the resin infiltration group (ICON^{TM}) after artificial carious lesions. They concluded that, the application of fluoride varnish and resin infiltration on the enamel surfaces could increased enamel resistance to demineralization.(23) There are several studies about the effects of fluoride on the bracket bond strength. Meng et al., 1998 studied the effect of acidulated phosphate fluoride (APF) application after acid etching on the bracket bond strength of sound enamel and reported that SBS of orthodontic brackets was compromised.(21) Al-Kawari and Al-Jobair, 2014 compared the effects of the application of 5% NaF (Fluoraphat^{TM}), CPP-ACP (MI paste^{TM}) and CPP-ACPF (MI paste plus^{TM}) before and after acid etching on the bracket bond strength of sound enamel. They reported that the SBS before and after acid etching of Fluoraphat^{TM} were lowest.(22) Triwardhani et al., 2020 reported that even though fluoride varnish (Fluor Protector^{TM}) pretreatment could repair enamel demineralization, it caused the very low SBS of the bracket.(5) An *in vivo* split mouth study by Talic, 2011 assessed the effect on the clinical bond failure rates of using fluoridated prophylaxis paste compared with plain pumice before bracket bonding. He showed that enamel pretreatment with fluoridate prophylaxis paste resulted in a significantly increase in the failure rate and decreased the survival times of the brackets. He concluded that the preparation of teeth with plain pumice before bracket bonding is recommended.(24) However, previous studies have shown that fluoride varnish enamel pretreatment should be applied and left at least for 15-30 days before the bracket bonding, to reduce the negative effects on SBS of brackets.(2-4) In addition, it should be re-applied every 3 months for WSLs prevention efficiency.(3) Choi et al., 2010 showed that the pretreatment with APF (Sultan Care 4^{TM}) decreased the surface roughness and the formation of microporosities in the enamel. They reported that the acid-etching procedure 2 weeks after performing an APF pretreatment is recommended to obtain the maximum enamel adhesion of a resin composite.(4) Perrini et al., 2016 reported that application of fluoride varnish (Duraphat™) to the vestibular enamel around the brackets after bracket bonding simultaneously *in vivo* and re-application of 3- month interval after appliance fitting could significantly reduce WSLs around orthodontic brackets than untreated group.(3) Cossellu et al., 2017 reported that the application of fluoride varnish (Fluor Protector™) enamel pretreatment before bracket bonding for 15 days was significantly lower the SBS values, while it returned to an optimal value after fluoride varnish pretreatment for 30 days. These researchers concluded that the optimal timing of bracket bonding after application of the fluoride varnish in order to obtained an optimal bond strength of the bracket to the enamel was more than 15 days.(2)

2.3.2 Resin infiltration

Resin infiltration is a new approach that shows potential to improve the appearance of WSLs that involves filling, reinforcing and protecting demineralizing enamel. Resin infiltration was first introduced in 2009 in Germany; ICON™. This technique uses a light-cured low-viscosity resin monomer, trietylene glycol dimetacrylate(TEGDMA) as an active ingredient to fill porosities on the enamel surface resulting to reinforce and inhibit the progression of enamel demineralization without causing the loss of healthy hard tissue.(25) This creates a refractory index that is more similar to healthy enamel, resulting to an improvement in appearance of the lesion. Jia et al., 2012 reported that the application of the resin infiltration prior to bracket bonding did not impair the SBS of bracket on sound or demineralized enamel. In addition, SBS was increased when self-etching agent was used.(26) Simunovic et al., 2020 concluded that the application of resin infiltration with different adhesives on demineralized enamel prior to bracket bonding did not impair SBS of orthodontic brackets when compared to sound enamel. In addition, the SBS values in the Assure Plus™ group was significantly higher than Transbond XT™ and Scotchbond™ as well as the control group.(27) Taher et al., 2011 compared the effects of resin infiltration and fissure sealant on the surface microhardness and roughness of sound enamel. They reported that enamel microhardness before and after application of resin infiltration was not significant difference, but those treated with fissure sealant was significantly decrease. The microhardness of enamel surfaces treated by resin infiltration showed significantly higher than those treated with fissure sealant (244.0±79.8 and 37.5±14.2 respectively). However, no difference in surface roughness was found between before and after application of either material.(28) Similarly to the studies by Elhiny,2016 found that the SBS of orthodontic brackets and surface microhardness of enamel surfaces before and after application of resin infiltration were not significant difference.(29) Torres et al., 2012 demonstrated that the surface microhardness of enamel surfaces of initial enamel carious lesions after application of resin infiltration had increased significantly than those treated with 0.05% fluoride solution and 2% neutral fluoride gel but the final microhardness after a new acid challenge was similar in the specimens infiltrated with resin and treated with 0.05% daily fluoride solution.(30) On the other hand, studies done by Triwardhani et al., 2020 found that the SBS of orthodontic brackets in enamel pretreatment with resin infiltration was significantly higher than application of fluoride varnish and CPP-ACP varnish (9.3312±0.9854, 5.7242±1.5869 and 1.0324±0.5089 MPa respectively). They concluded that the application of resin infiltration as the enamel pretreatment before bracket bonding is recommended.(5)

2.3.3 Pre-reacted glass-ionomer (PRG)

PRG filler has been developed since 1996.(31) It is fabricated from fluoroaluminosilicate glass and polyalkenoic acids via acid-base reaction in the presence of water to form a wet siliceous hydrogel and freeze-drying procedure. Then the desiccated xerogel was milled and silanized to form pre-reacted glass-ionomer filler in a specific size.

There are two types of PRG fillers which depend on degree of reaction of the glass-ionomer.

3.4.1 S-PRG: The reaction is detected on the surface, so it is called surface reaction type (S-PRG fillers)

3.4.2 F-PRG: The reaction performs throughout of the material, certainly it is called full reaction type (F-PRG fillers)

PRG fillers promote rapid fluoride release through ligand exchange within the pre-reacted hydrogel.(32) F-PRG releases a large amount of fluoride as the core of the particle is thoroughly reacted which is dissimilar to S-PRG, so F-PRG degrades faster than S-PRG. S-PRG fillers release six ions including aluminium, borate, sodium, silicate, strontium and fluoride ion. Borate and fluoride ion demonstrate the strongest inhibitory effect on the growth of *S. mutans*(1) and have the remineralizing ability.(33) Ma et al., 2012 evaluated the ability of a coating material containing S-PRG filler (PRG Barrier Coat™) to protect the root against demineralization *in vitro*. They reported that the application of PRG Barrier Coat™ produced a barrier coating layer with the thickness approximately 200 µm and inhibited the bacteria-induced drop in pH at the interface between the material and the bacteria. They concluded that PRG Barrier Coat™ could completely protect root dentin surface from acidic demineralization.(34) Arita et al., 2016 reported that the SBS of S-PRG coating materials (approximately 8-12 MPa) with flowable resin to dentin were significantly higher than glass ionomer coating material (approximately 4-6 MPa) but the lesion depths of dentin surface in group of glass ionomer coating materials (approximately 110-180 µm) was shallower than S-PRG coating material (approximately 200-260 µm).(35) Alsayed et al., 2016 reported that S-PRG coating material (PRG Barrier Coat™) on the enamel could superficially protect the adjacent area as well as the coated area by ions releasing.(36)

Although, there are several studies about the effects of PRG on the prevention of tooth surface demineralization, the use of PRG as enamel pretreatment before orthodontic bracket bonding is not extensively discussed. To the best of our knowledge, there is only one study from Kobayashi et al., 2021 which compared the effects of four enamel surface treatment systems on the SBS of orthodontic brackets bonded to sound enamel. They reported that, resin-modified glass-ionomer cements (RMGICs; Fuji Ortho LC™) system exhibited a significantly higher SBS (11.7±1.8 MPa) than the resin composite cement (Beauty Ortho Bond™; BOB™) system combined with the self-etching primer (9.6±1.8 MPa), BOB system combined with the resin coating material (PRG Barrier Coat™) after the organic acid etching agent (9.7±1.6 MPa) and BOB system combined with the PRG Barrier Coat™ coating (7.0±1.3 MPa). They concluded that, SBS values of resin composite cement system combined with the resin coating material after the organic acid etching agent was comparable to that combined with the self-etching primer. In addition, they suggested that resin coating material using with organic acid etching and resin composite cement system can serve as alternative for orthodontic bracket bonding.(8) Although, PRG Barrier Coat™ has been proved that it can protect the tooth from acidic demineralization, there are not enough studies to conclude their effects on SBS of brackets when using as enamel pretreatment before bracket bonding. In addition, these materials have the ions releasing property that may interfere with bracket bond strength and their timing of application may be the important factor impacted on the SBS of brackets.

2.3.4 Flat surface sealant

Flat surface sealant was developed for prevention of dental caries around orthodontic brackets. The main ingredient is dimethacrylate monomers but it also contains fluorides. There are 2 types of sealants depend on mode of activation. The chemical cured sealants do not effectively seal smooth enamel surface because of oxygen inhibition of polymerization. On the contrary, light-cured sealants have been proven to cure completely on smooth enamel surfaces and prevent enamel demineralization effectively *in-vitro*. In addition, different fillers were added for different sealant properties such as glass ionomer (GI) fillers were added because of the fluoride-releasing characteristic. Bishara et al., 2005 reported that the application of Pro Seal™ , a highly filled light-cured fluoride-releasing sealant , prior bracket bonding application did not affect the SBS of the adhesive.(6) Soliman et al., 2006 reported that fluoride ions in Pro Seal™ had the sustained release but significantly decrease in the first week. Pro Seal™ had the recharge ability with fluoride ions introduced from a foaming solution of acidulated phosphate fluoride but significantly decrease on the eight week.(37) Paschos et al., 2009 reported that application of unfilled flat surface sealant (Light Bond®) and filled sealant (Pro Seal™) prior bracket bonding application decreased enamel lesion depth when compared to non-pretreatment group but not significant difference.(38) Shinaishins et al., 2011 studied the efficacy of filled flat resin sealant (Pro Seal™) in preventing enamel decalcification and compared its effect with fluoride varnish (Duraflor™) and unfilled sealant (Light Bond Sealant™) using atomic force microscopy. The researchers concluded that the use of Duraflor™, Light Bond Sealant™ and Pro Seal™ showed significantly lower roughness height with no signs of surface demineralization. Although, there was no significant difference between the three groups, the surface roughness of Pro Seal™ was the lowest.(39) There is another highly filled resin sealant composed of 38% GI fillers and nano fillers, Opal Seal™. It had claimed to provide long-lasting coverage with fluoride releasing and recharging ability. Kolstad et al., 2020 reported that Pro Seal™ and Opal Seal™ sealants were not significant difference on bracket SBS of sound enamel from each other (16.5±4.8 and 15.7±3.9, respectively) but exhibited lower SBS than adhesive primer (20.1±6.0 MPa). In addition, Opal Seal™ sealant showed significantly greater ARI scores, indicating that more adhesive remained on the teeth after debonding.(7) Coordes el al., 2017 compared six different fluoride releasing products of the three smooth surface sealants (Pro Seal™, Alpha Glaze™, Seal&Protect™) and three fluoride varnishes (Tiefenfluorid™, Protecto™, Fluor Protector™) in terms of their effectiveness on the protection against chemical, thermal and mechanical loading *in vitro*. They concluded that only Pro Seal™ demonstrated significant protection against decay under the test condition. The tooth surfaces treated with the other products showed significant demineralizations after treatment.(40) Pro Seal™ was recommended to have additional local fluoridation after a 17-week period, due to the decreasing fluoride release over time.(37) In addition, Pro Seal™ was color instability and discoloration.(40-42)

Although, there are several studies about the effectiveness of flat resin sealant on the reduction of tooth surface demineralization and their effects on the brackets bond strength when using as enamel pretreatment before bracket bonding, there are still some controversies about their effects which may be influenced from different parameters including their compositions and abilities. The ion-release capability of the materials may be the factor that affects SBS of brackets and their timing of application may be the important factor influenced the bracket bond strength. However, there are not enough studies about flat resin sealant enamel pretreatment and their timing of application on bracket bond strength and enamel microhardness after demineralization.

2.4 Enamel microhardness

Enamel is composed with 96 wt.% inorganic material and 4 wt.% organic material and water. This inorganic material is mainly composed by a calcium phosphate related to the hexagonal hydroxyapatite, whose chemical formula is $Ca_{10}(PO_4)_6 \cdot 2(OH)$. (43) Demineralization is damage to tooth enamel integrity that can be stopped and/or reversed. In dental research microhardness indentation measurements to assess de- and remineralization phenomena have been employed by numerous authors.(44-48) This method is easy, quick, and requires only a flat tiny surface area of specimen for testing.

This measurement has been used to get information on enamel softening and tooth mineral loss after immersion in acidic induced lesion.(45,47,49,50) The average hardness value of sound enamel is in the range from 250-360 HV.(44) Arends et al.,1980 investigated the relation between the average lesion depth of artificial carious lesions and the length of microhardness indentations on both human and bovine enamel. They reported that there was an empirical linear relationship between lesion depth of artificial carious lesions and the length of microhardness indentations. Linearity has been found for decalcification periods 2-8 days and pH values 4.5 and 4.0.(49) Colly et al., 1993 investigated changes at the enamel surfaces of surface-softened enamel and surface-etched enamel with calcifying solutions. They found that the rehardening of surface demineralized enamel increased resistance to indenter penetration into the test surface.(51) Sorvari et al., 1994 evaluated the effect of fluoride varnish and solution on enamel erosion with acidic beverage (pH 2.6). They reported that both fluoride treatments statistically significant increase in enamel microhardness when compared with untreated part. Scanning electron micrographs revealed that untreated enamel erosion was seen in vary characteristics such as honeycombed pattern, more pitted and irregular surfaces. On the test side with fluoride treatment, remnants of varnish were observed either covering the large surface or small patched between the eroded areas.(45) Attin et al., 2003 studied the effect of mineral supplements to citric acid (pH 2.21) on enamel erosion. They reported that addition of calcium, phosphate and fluoride resulted in significantly lower hardness loss compared the untreated group. The enamel loss of specimens was quantitatively with the profilometric analysis. The significantly highest enamel loss was recorded for untreated group compared to all other samples. (44)

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Conceptual framework

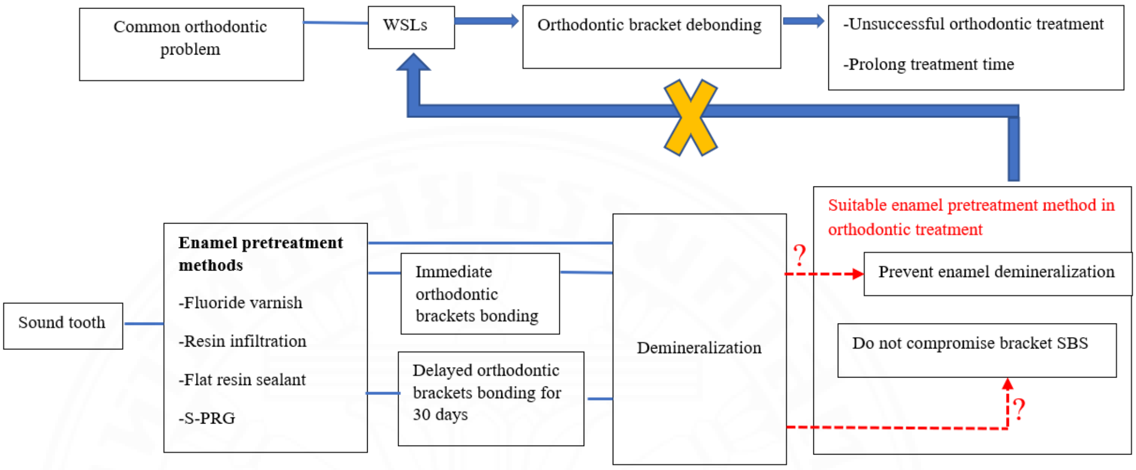


Figure 3.1 Conceptual framework in term of the enamel microhardness and shear bond strength

3.2 Experimental design

This study was an *in vitro* study. The test was performed at laboratory of Faculty of Dentistry, Thammasat University.

3.3 List of variables

3.3.1 Independent variables: enamel pretreatment methods and timing of application

3.3.2 Dependent variables: SBS of bracket, ARI and enamel microhardness

3.3.3 Controlled variables: type of bracket and adhesive used, force in bonding bracket, pH and temperature during immersed in solution

3.4 Materials and methods

Lower pH in the oral cavity of orthodontic patient who has high caries risk was simulated by setting the demineralization procedure.

To control confounding factors such as orthodontic bracket bonding method which may affect to enamel microhardness values or microhardness measurement process. The method was divided into two parts.(52,53) The first part was examined the effect of different enamel pretreatment methods on enamel microhardness after demineralization. The second part was investigated the effect of different enamel pretreatment methods and timing of application on enamel SBS and ARI after demineralization.

3.4.1 Enamel microhardness test

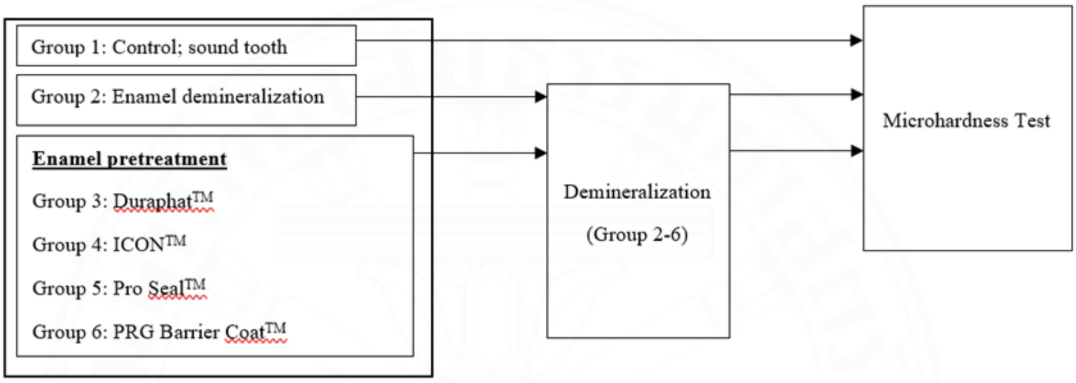


Figure 3.2 The experimental design of enamel microhardness test

3.4.1.1 Sample size calculation

The sample size was calculated using G*Power 3.1 Software base on previous study.(50) Effect size = 0.56, Power = 0.8, alpha = 0.05 and number of groups = 6. The samples were increased by 20%. The total samples were 60 (Figure 3.3).

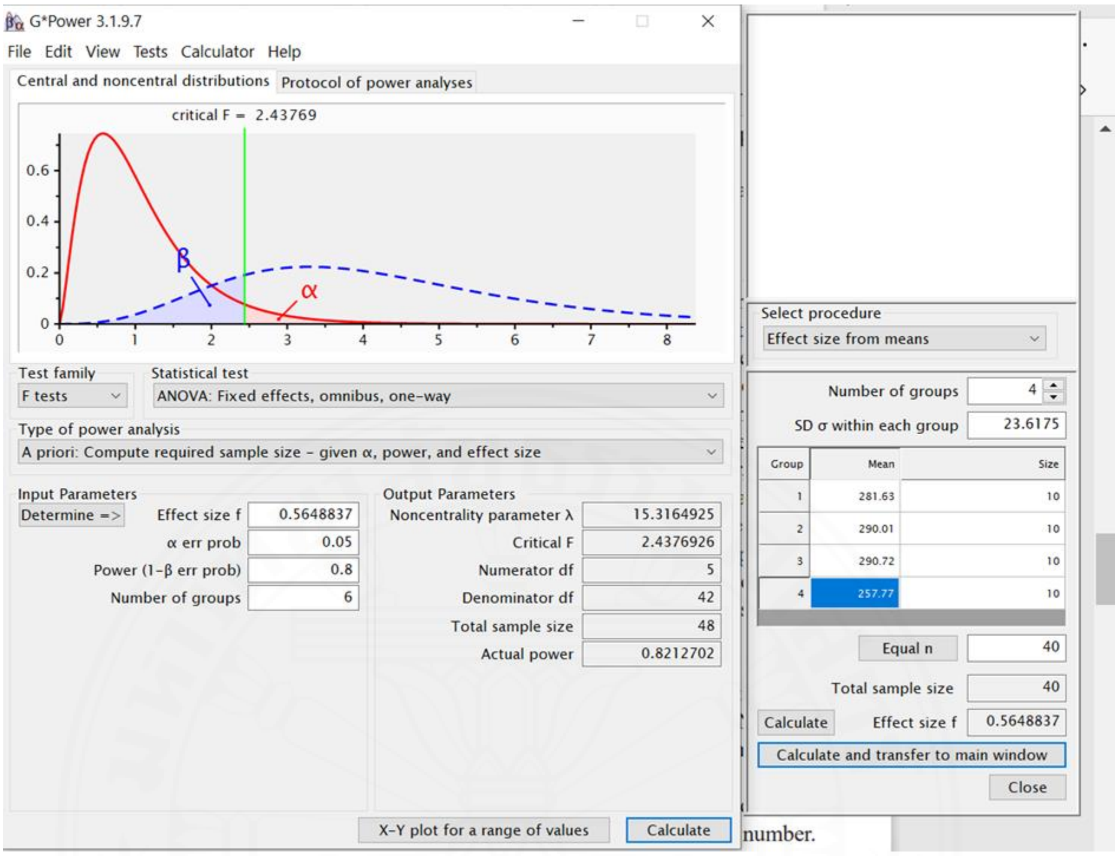
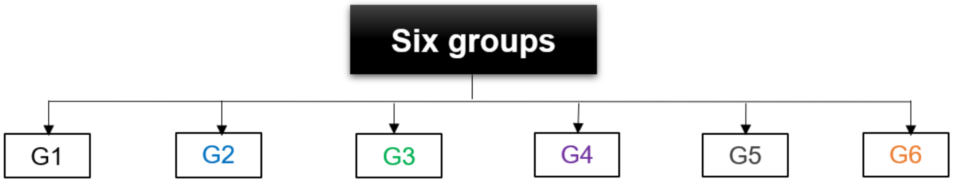


Figure 3.3 Sample size calculation of enamel microhardness test

3.4.1.2 Specimen preparation

1. The sixty maxillary first premolars without any structural defects on the buccal surface, which were extracted due to orthodontic treatment, were collected. The teeth were stored in 0.1% thymol solution until used. These teeth were cleaned and verified for any defected under stereomicroscope. Then the root was cut at 2 mm under the cervical line.

2. The collected teeth were randomized in to 6 groups (n=10/group); control group, enamel demineralization group and 4 groups of different enamel pretreatment methods (Duraphat™, ICON™, Pro Seal™ and PRG Barrier Coat™) (Figure 3.4). The materials used in this study were listed in Table 3.1.



G1 : Control; No pretreatment, no demineralization
G2 : Enamel demineralization
G3: Duraphat™
G4 : ICON™
G5 : Pro Seal™
G6 : PRG Barrier Coat™

Figure 3.4 The 6 groups of different enamel pretreatment methods for enamel microhardness test

Table 3.1 The materials were used in this study

Material	Brand	Composition	Manufacture
Fluoride varnish	Duraphat™	5% sodium fluoride	Colgate Oral Pharmaceuticals Inc, Massachusetts, USA
Resin infiltration	ICON Etch™ ICON-Dry™ ICON-Infiltrant™	Hydrochloric acid, Pyrogenic silicic acid 99% Ethanol TEDMA70-95% Camphoro Quinone < 2.5%	DMG, Hamburg, Germany
Flat resin sealant	Pro Seal™	Ethoxylated bisphenol-A-diacrylate (10–50%) Urethane acrylic ester (10–40%) Polyethylene glycol diacrylate (10–40%) Fluoridated glass frits (5–40%) Fluorescent agents Photoinitiator Lucerin	Reliance Orthodontic Products Inc, Illinois, USA)
S-PRG coating material	PRG Barrier Coat™	Base: Fluoroaluminosilicate glass, distilled water, Methacrylic acid monomer and others	Shofu Inc, Kyoto, Japan

		Active: Phosphonic acid monomer, Methacrylic acids monomer, Bis-MPEPP, Carboxylic acid monomer, TEGDMA, Polymerization initiator and others	
Enamel etchant	Scotchbond etchant™	35% Phosphoric acid	3M Unitek, California, USA
Orthodontic adhesive	Transbond XT Primer™ Transbond XT light Cure Adhesive™	Bis-GMA, TEGDMA, dimethylamino-benzene ethanol, DL-camphorquinone, hydrochinone Silane-treated quartz, bis-GMA, bis-EMA, silane-treated sililca, diphenyliodonium hexa-fluorophosphate	3M Unitek, California, USA
LED light curing unit	Mini LED™	Light source: LED Light intensity: 2,000 mW/cm^2 Wavelength: 420–480 nm	Acteon, Merignac, France

3. These teeth were embedded in cylindrical blocks with self-curing acrylic resin by positioning the convex buccal surface of the clinical crown projection up from acrylic surface in order to facilitate testing.

4. The enamel surface of the specimens were polished with 800, 1200, 1500-grit silicon carbide papers under water coolant to obtain flat enamel surfaces.(44, 47, 49, 51, 54) Two layers of nail varnish were applied to cover the enamel surface(51), leaving a window of about 3×3 mm^2 for demineralization and enamel pretreatment processes.

5. The polished surface of the teeth were cleaned with non-fluoride pumice, rinsed with oil free air-water spray for 30 seconds, air dried with air-water spray.

6. Enamel pretreatment methods used were as follows in Table 3.2.

Table 3.2 The application procedures of each enamel pretreatment methods according to manufacturer's recommendation for enamel microhardness test.(55-58)

Groups	Enamel pretreatment methods	Application procedures
Group 1	No enamel pretreatment and demineralization	No
Group 2	No enamel pretreatment with demineralization	No
Group 3	Duraphat™	Use microbrush to apply and spread out over surface and leave 4 hours.
Group 4	ICON™	1. Etch with 15% HCl (ICON Etch™) for 2 minutes, rinsed with oil free air-water spray for 30 seconds, air dried with air-water spray. 2. Dry with ethanol drying agent (ICON-Dry™) for 30 seconds. 3. Apply with resin infiltration (ICON-Infiltrant ™), let fully penetration for 3 minutes, remove excess material with microbrush and light cured with LED light curing unit for 40 seconds. 4. Reapply and leave for 1 minute, removed excess material with microbrush and light cured for 40 seconds.
Group 5	Pro Seal™	1. Etch with 35% phosphoric acid gel for 30 seconds, rinsed with oil free air-water spray for 30 seconds, air dried with air-water spray. 2. Apply Pro Seal™ to the enamel surface using a brush to ensure the application of a uniform layer and light cured for 10 seconds.

Group 6	PRG Barrier Coat™	Activation and mixing Add 1 drop of activator to base container and mix with disposable brush provided. Use the material within 2 minutes after mixing. Application Apply a thin layer of the mixture on the dried tooth surface and leave undisturbed for 5 seconds and light cured for 10 seconds.

7. Demineralization procedure: The specimens were immersed in 20 ml of demineralization solution for 96 hours in the incubator at 37 °C. The demineralization solution contained 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4, 0.05M acetic acid. The pH was adjusted to 4.4 with 1 M KOH. The pH was adjusted to 4.4 with 1 M KOH. The solution was renewed daily. The content of the solution was used in this study is the same that was used by Kumar et al.,2007, which produced artificial caries lesion as deep as 120–200 μm.(59) To confirmed the effective of demineralization procedure, The WSL was shown after demineralization**.** Then the Micro-CT (The SkyScan 1275™; Bruker Corporation, Billerica, Massachusetts, USA) revealed the demineralization of the enamel produced artificial caries lesion as deep as 120–200 μm (Figure 3.5).

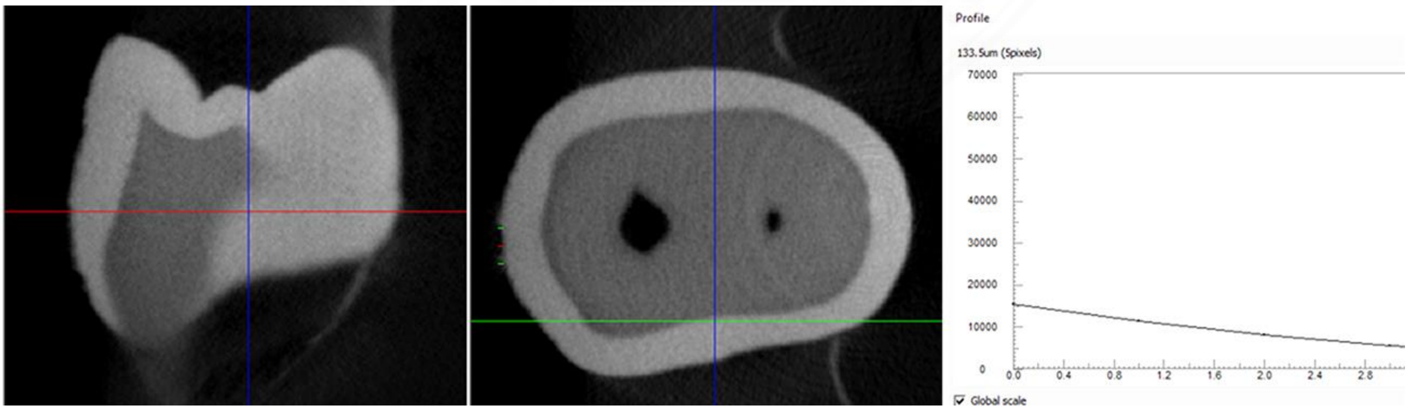


Figure 3.5 Lesion depth presented in this study

3.4.1.3 Vickers microhardness test:

All specimens were tested with a FM-800 microhardness tester™ (FUTURE-TECH CORP, Kanagawa, Japan). Indentation test was performed by impressing a Vickers diamond pyramid with indentation load 300 grams for 15 seconds on the enamel surface.(60) Each specimen was repeated indentation test 4 areas (4 positions according to the number of sides of the window). The diagonals'length of the indentations was measured. Vickers values were converted into microhardness values. Vickers hardness number (HV) was then calculated using the following equation:

$$HV = \frac{1.854\ F}{d^2} \quad \frac{(KgF)}{(mm^2)}$$

Where P is the load in Kgf, and d is the length of the diagonals in mm.(23)

3.4.2 Enamel shear bond strength and acrylic remnant index (ARI)

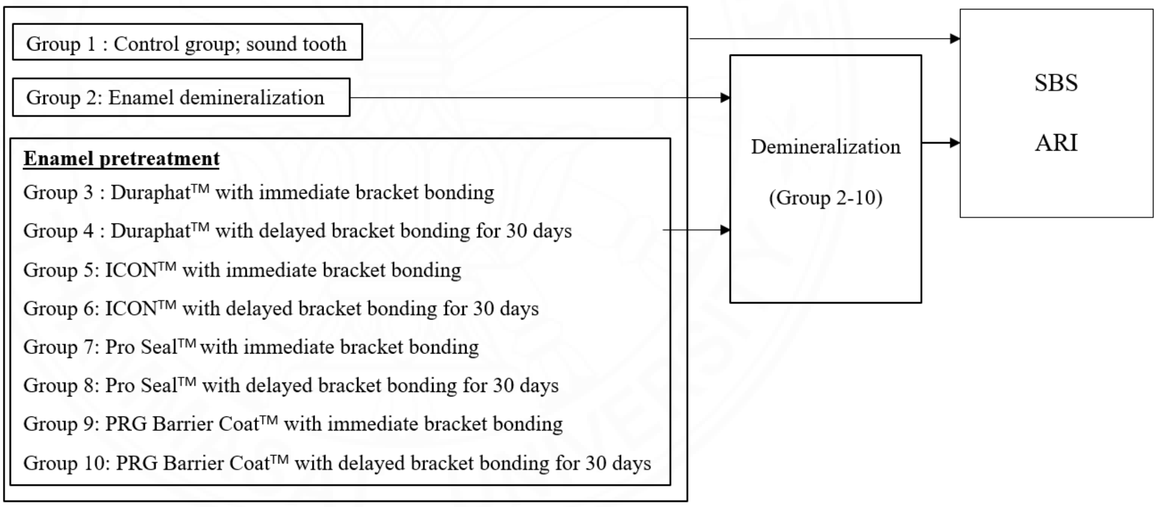


Figure 3.6 Experimental design of enamel shear bond strength and adhesive remnant index test

3.4.2.1 The sample size calculation

The sample size was calculated using G*Power 3.1 Software base on previous study.(7) Effect size = 0.4, Power = 0.8, alpha = 0.05 and number of groups = 10. The samples were increased by 20%. The total sample were 140 (Figure 3.7).

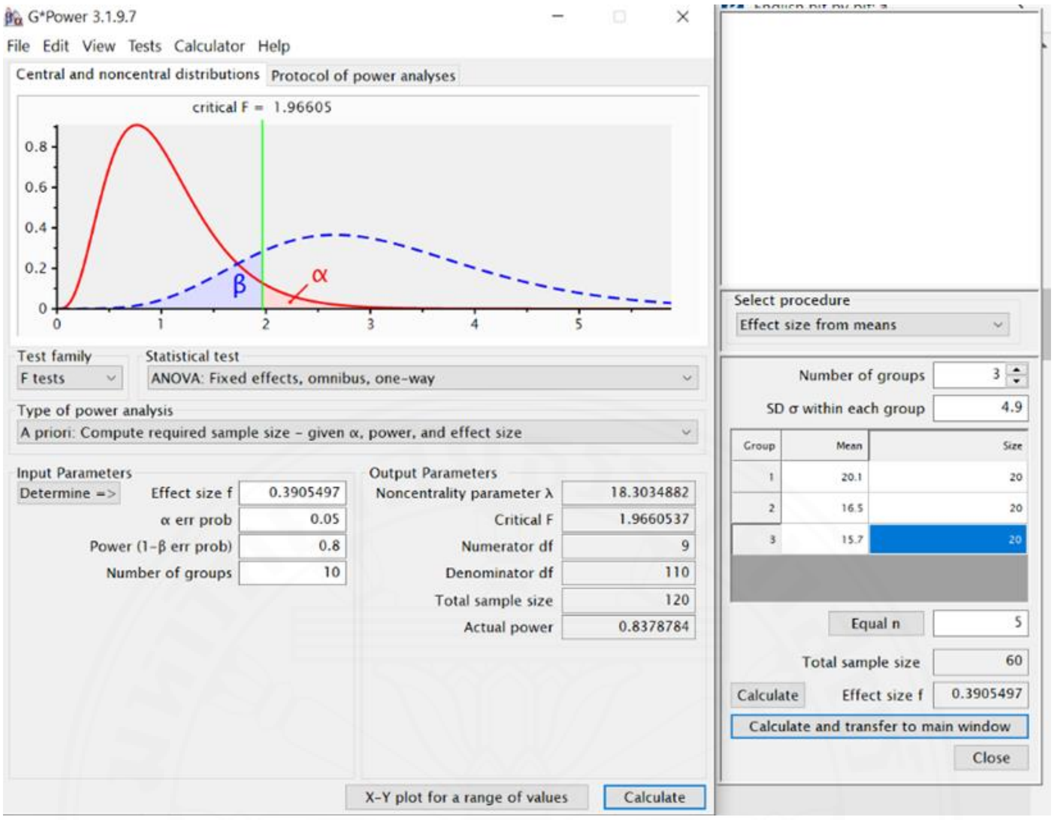


Figure 3.7 Sample size calculation of enamel shear bond strength and adhesive remnant index test

3.4.2.2 Specimen preparation

1. The one hundred and forty maxillary first premolars without any structural defects on the buccal surface, which were extracted due to orthodontic treatment, were collected. The teeth were stored in 0.1% thymol solution until used. These teeth were cleaned and verified for any defected under stereomicroscope. Then the root was cut at 2 mm under the cervical line.

2. The collected teeth were randomized in to 10 groups (n= 14/ group); control group, enamel demineralization group and 8 groups of different enamel pretreatment methods; Duraphat^{TM}, ICON^{TM}, Pro Seal^{TM} and PRG Barrier Coat^{TM} with different timing of application (immediate bracket bonding and delayed bracket bonding for 30 days) as shown in Figure 3.8. The materials used in this study were listed in Table 3.1 All treatments were performed according to the manufacturer's instruction as shown in Table 3.3. The specimens were immersed in distilled water until the bracket bonding procedure were conducted.

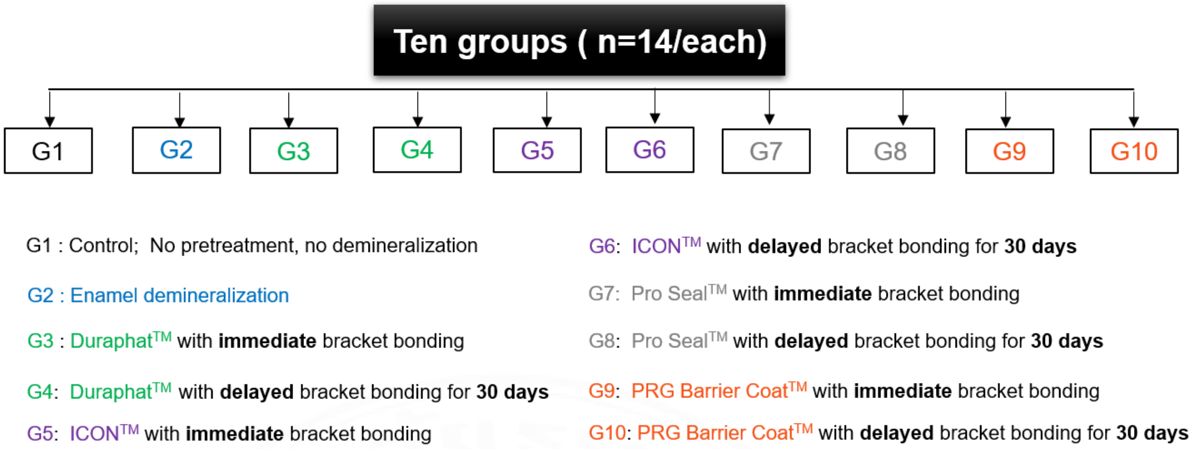


Figure 3.8 The 10 groups of different enamel pretreatment methods and timing of application

Table 3.3 The application procedures of each enamel pretreatment methods according to manufacturer's recommendation and timing of bracket bonding for enamel shear bond strength test.(55-58)

Groups	Enamel pretreatment methods	Procedure
Group 1	No enamel pretreatment and demineralization	1. Etch with 35% phosphoric acid for 15 seconds, rinsed with oil free air- water spray for 15 seconds, air dried with air-water spray. 2. Bracket bonding and embedding procedure as described in Bonding procedure.
Group 2	No enamel pretreatment with demineralization	1. Etching, bracket bonding and embedding procedure as described in Group 1. 2. Immerse in 20 ml of demineralizing solution as described in Demineralization procedure.
Group 3	Duraphat™ with immediate bracket bonding	1. Etch with 35% phosphoric acid for 15 seconds, rinsed with oil free air- water spray for 15 seconds, air dried with air-water spray. 2. Apply Duraphat™ as describe in Table 3.2 and leave undisturbed for 4 hours.

		3. Bracket bonding, embedding and demineralization procedure.
Group 4	Duraphat™ with delayed bracket bonding for 30 days	1. Apply Duraphat™ as describe in Table 3.2 and leave undisturbed for 4 hours. 2. Immersed in distilled water for 30 days. 3. Etching, bracket bonding, embedding and demineralization procedure.
Group 5	ICON™ with immediate bracket bonding	1. Apply ICON™ as describe in Table 3.2. 2. Bracket bonding, embedding and demineralization procedure.
Group 6	ICON™ with delayed bracket bonding for 30 days	1. Apply ICON™ as describe in Table 3.2. 2. Immersed in distilled water for 30 days. 3. Etching, bracket bonding, embedding and demineralization procedure.
Group 7	Pro Seal™ with immediate bracket bonding	1. Etch with 35% phosphoric acid for 15 seconds, rinsed with oil free air- water spray for 15 seconds, air dried with air-water spray. 2. Apply Pro Seal™ as describe in Table 3.2. 3. Bracket bonding, embedding and demineralization procedure.
Group 8	Pro Seal™ with delayed bracket bonding for 30 days	1. Etch with 35% phosphoric acid for 15 seconds, rinsed with oil free air- water spray for 15 seconds, air dried with air-water spray. 2. Apply Pro Seal™ as describe in Table 3.2. 3. Immersed in distilled water for 30 days. 4. Etching, bracket bonding, embedding and demineralization procedure.
Group 9	PRG Barrier Coat™ with immediate bracket bonding	1. Etch with 35% phosphoric acid for 15 seconds, rinsed with oil free air- water spray for 15 seconds, air dried with air-water spray. 2. Apply PRG Barrier Coat™ as describe in Table 3.2. 3. Bracket bonding, embedding and demineralization procedure.

Group 10	PRG Barrier Coat™ with delayed bracket bonding for 30 days	1. Apply PRG Barrier Coat™ as describe in Table 3.2. 2. Immersed in distilled water for 30 days. 3. Etching, bracket bonding, embedding and demineralization procedure.

3. Bonding procedure: The brackets (GEMINI™, 3M Unitek, California, USA) were bonded onto the teeth using Transbond XT™ light cure bonding agent. The excessive adhesive was removed by explorer. Then, curing with an LED light-curing unit from 4 directions, each for 10 seconds. The teeth were embedded in self-curing acrylic resin in PVC blocks by taking the buccal axis of the clinical crown parallel to horizontal plane and the bracket bases were compensated by plastic sheet and held in position with orthodontic wire and O-ring.(61) The labial surfaces were parallel to the force during the SBS test (Figure 3.9 A,B). After that, they were immersed in distilled water until demineralization procedure. Finally, the SBS and ARI testing were performed.

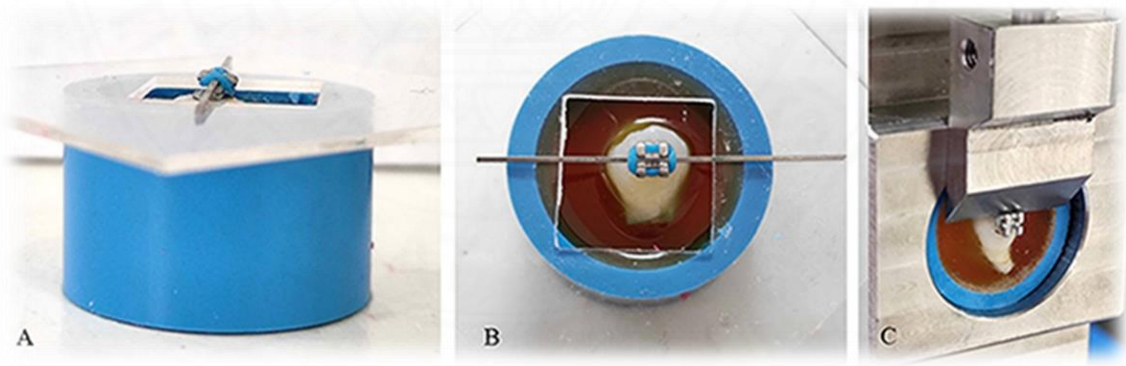


Figure 3.9 The method of sample preparation. (A,B) Orthodontic bracket attached parallel to the buccal surface of a tooth. (C) Blade of SBS testing was positioned at the interface between bracket and tooth surface.

3.4.2.3 Enamel shear bond strength (SBS) and adhesive remnant index (ARI) test

All specimens were tested with a Universal Testing Machine

(Shimadzu™, Tokyo, Japan). A knife-edge chisel was applied at the interface between the tooth and the bracket (Figure 3.9 C). The load was applied at the speed of 1 mm/min until the bracket was detached.(62-64) The maximal shear force was recorded. SBS was then calculated using the following equation.(65)

$$SBS = \frac{F}{A} \quad \left(\frac{N}{mm^2}\right)$$

Where F was a force of bracket failure in Newtons and A was the surface area of the bracket base in mm^2.(65) The surface area of the bracket base was 10.48 mm^2.

Then, the ARI was analyzed by examining the residual adhesive on the bracket base at 10 times magnification of stereomicroscope.(67) The ARI classification was modified from Artün and Bergland.(68) The ARI scores represented the amount of adhesive remaining on the bracket base after debonding. The ARI was scored according to the following:

Score 0 = All the cement remained on bracket

Score 1 = More than half of the cement remained on bracket

Score 2 = Less than half of the cement remained on bracket

Score 3 = No cement remained on bracket

3.5 Statistical analysis

All data were analyzed using SPSS (IBM, Illinois, USA).

3.5.1 Vickers microhardness value

Vickers hardness number (HV) were presented as mean± SD. Kolmogorov-Smirnov test were used to confirm the normality. The data were not normally distributed. Then the Kruskal-Wallis test follow by multiple comparison using Dunnett's test was used. Statistically significant was set at $P < 0.05$.

3.5.2 Shear bond strength and ARI value

SBS values were presented as mean± SD. Kolmogorov-Smirnov and Levene's test were used to confirm the normality and equal variance of data. The data were normally distributed. Two-way ANOVA follow by Tamhane multiple comparison test were used. For the ARI scores, Fisher's Exact Test was used to compare the differences between groups. Statistically significant was set at $P < 0.05$.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Enamel microhardness

Figure 4.1 showed images of indentation in each groups. The data of HV were not normally distributed. Kruskal-Wallis followed by post-hoc multiple comparison test were performed to analyze the effect of type of enamel pretreatment methods on the HV of all groups. Kruskal-Wallis showed that type of enamel pretreatment methods had a statistically significant effect on the Vickers hardness. The mean HV were presented in Figure 4.2. There were significant difference in HV among groups compared to control. The highest and lowest HV was found in control (390.91±6.21) and PRG Barrier Coat™ (125.41±16.43). The HV of the control group was significantly higher than Demineralizing, ICON™, Pro Seal™ and PRG Barrier Coat™ groups (390.91±6.21, 154.61±15.88, 249.11±19.39, 244.18±11.77, 125.41±16.43), respectively. The HV of the demineralization group (154.61±15.88) was significantly lower than control group (390.91±6.21) and Duraphat™ group (331.79±25.80). The HV of the PRG Barrier Coat™ group was significantly lower than the control, Duraphat™, ICON™ and Pro Seal™ groups (125.41±16.43, 390.91±6.21, 331.79±25.80, 249.11±19.39, 244.18±11.77), respectively.

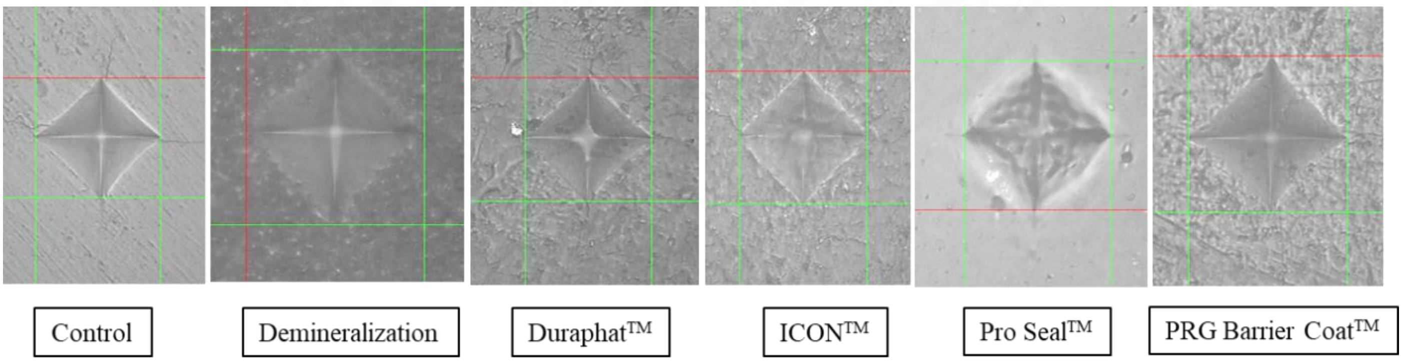


Figure 4.1 Images exhibit indentation of samples

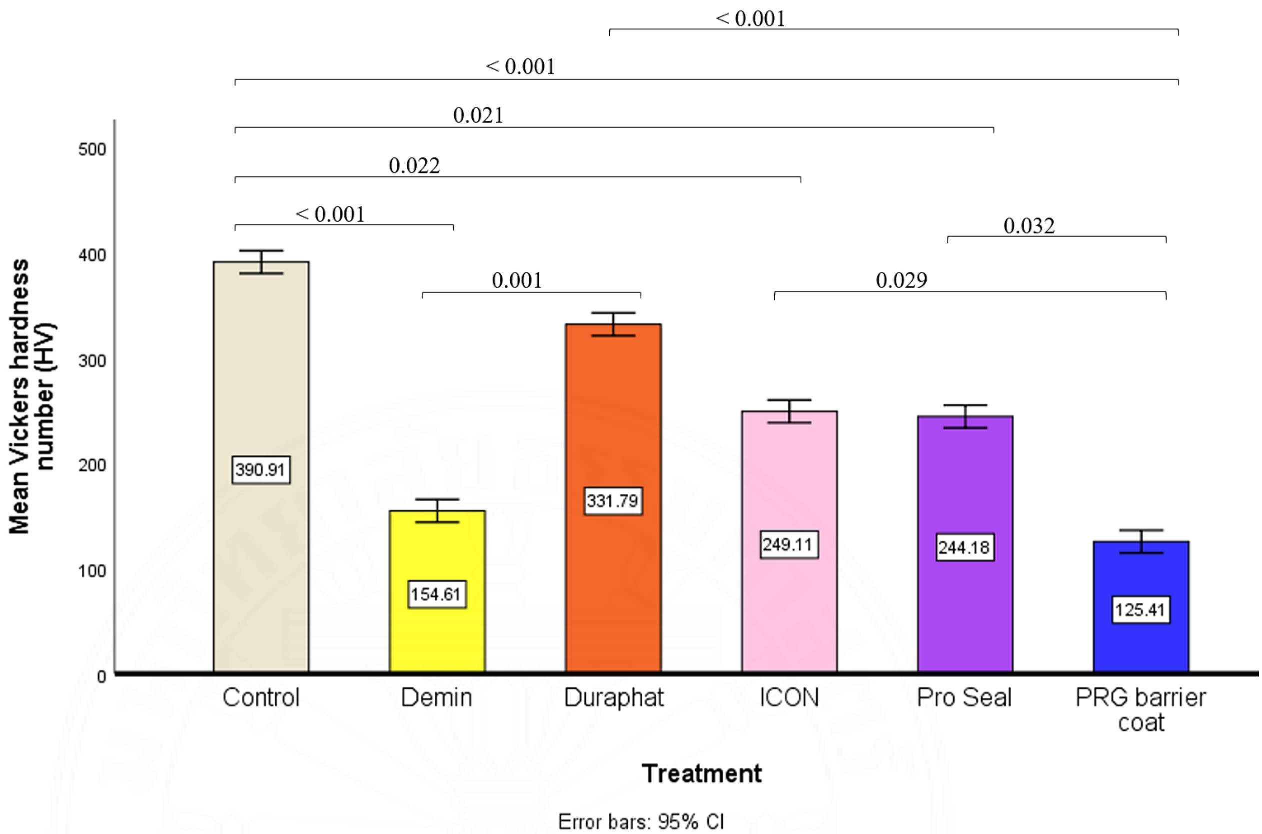


Figure 4.2 Bar chart of Vickers hardness number of all groups. The lines indicated significant difference between groups ($p < 0.05$). Error bars were 95% CI ($n = 14$).

4.1.2 Enamel Shear Bond Strength (SBS) and Adhesive Remnant Index (ARI) Score

4.1.2.1 Enamel Shear Bond Strength

The data were normally distributed but non-homogeneity. Therefore, Two-way ANOVA followed by post-hoc Tamhane multiple comparison test were performed to analyze the effect of type of enamel pretreatment methods, timing of application as well as by the interaction between the two factors as shown in Table 4.1.

The mean SBS values were presented in Figure 4.3. There was no significant difference in SBS among all groups compared to control. The highest SBS was found in Pro Seal^{TM} with delayed bonding (17.46±5.02 MPa) and the lowest SBS was found in PRG Barrier Coat^{TM} with immediate bonding (7.33±4.50 MPa). Pro Seal^{TM} with delayed bonding was significantly higher than demineralization and the immediate bonding of Duraphat^{TM}, Pro Seal^{TM} and PRG Barrier Coat^{TM} (17.46±5.02 MPa, 9.72±3.47 MPa, 8.16±4.30 MPa, 10.46±2.38 MPa, 7.33±4.50 MPa), respectively.

ICON™ with delayed bonding (13.71±4.00 MPa) was significantly higher than immediate bonding of PRG Barrier Coat™ (7.33±4.50 MPa). However, within the same agent groups, significant difference was found between the applications of Pro Seal™ with immediate bracket bonding (10.46±2.38 MPa) and delayed bracket bonding group (17.46±5.02 MPa).

Table 4.1 The results from Two-way ANOVA test investigating the effects of the two explanatory factors (Enamel pretreatment methods and timing of application) and the interaction between them on SBS

Source	Mean square	F	P
Enamel pretreatment method	140.37	6.49	0.000
Timing of application	173.31	8.02	0.005
Enamel pretreatment methods × timing of application	115.011	5.32	0.002

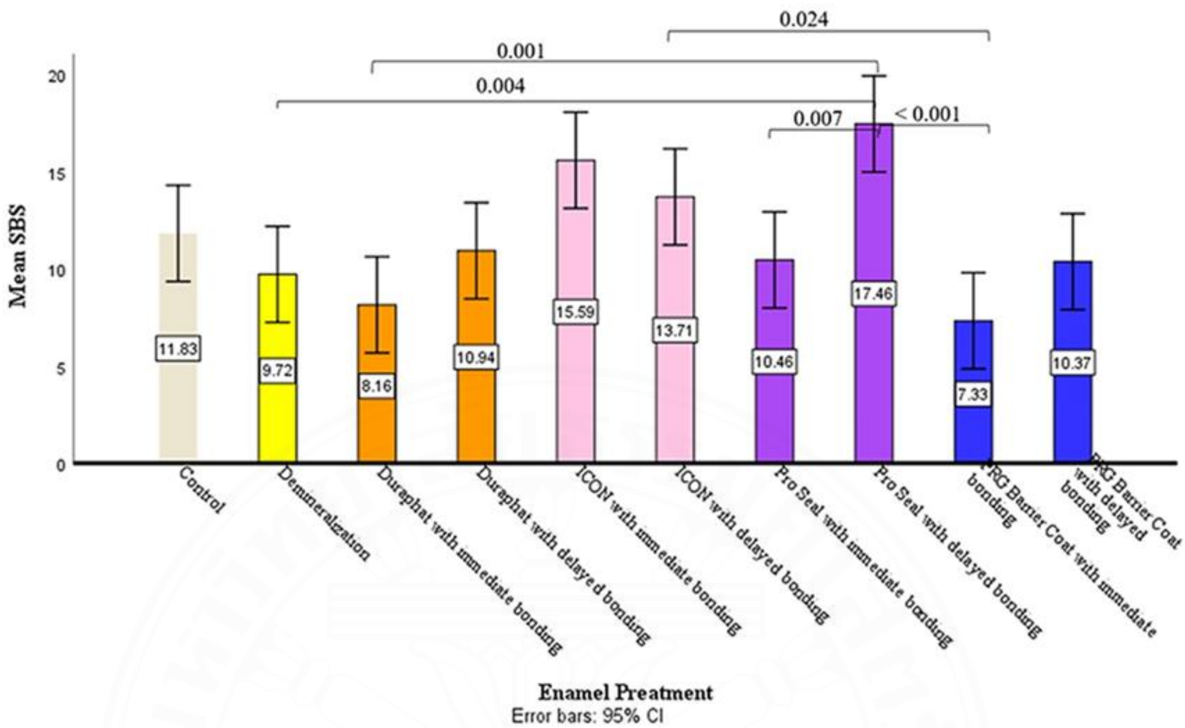


Figure 4.3 Bar chart of enamel shear bond strength of all groups. The lines indicated significant difference between groups ($p < 0.05$). Error bars are 95% CI (n=14).

4.1.2.2 Adhesive Remnant Index (ARI)

Figure 4.4 presented remained adhesive on bracket base at 10 times magnification of stereomicroscope, ARI values were presented in Table 4.2 and Figure 4.5. The most common ARI scores observed for the experimental materials were 1 and 2. While, ARI score 0 was commonly observed in immediate bonding of Duraphat^{TM} (57.14%) and PRG Barrier Coat^{TM} (42.86%). A score of 3 was only detected for Duraphat^{TM} with delayed bonding group. Th e distribution of the ARI score among each group was significantly different ($P < 0.05$).

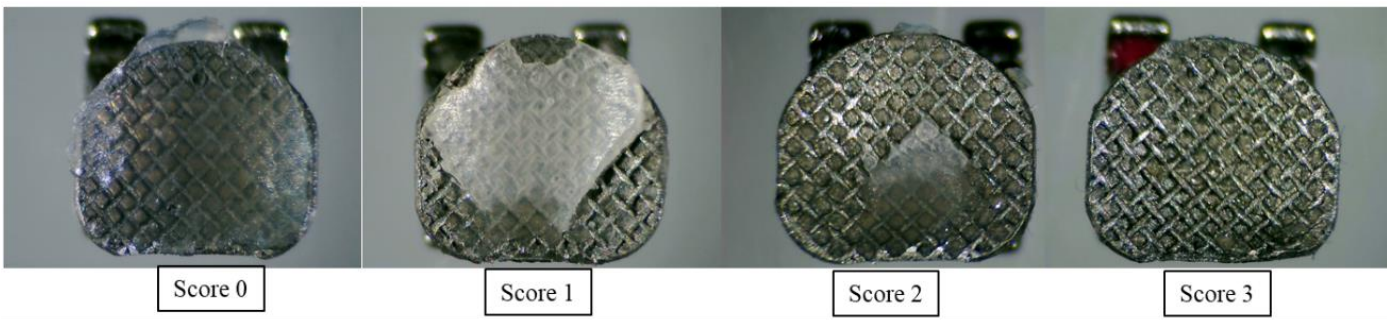


Figure 4.4 ARI score was observed from this experiment.

Table 4.2 Frequency of distribution of ARI scores and Fisher's Exact Test comparison between the groups tested. ($p < 0.001$)

Group (n = 14)	ARI score				P value
	0 n (%)	1 n (%)	2 n (%)	3 n (%)	
1	1 (7.14)	9 (64.29)	4 (28.57)	0 (0)	< 0.001
2	0 (0)	10 (71.43)	4 (28.57)	0 (0)	
3	8 (57.14)	4 (28.57)	2 (14.29)	0 (0)	
4	1 (7.14)	10 (71.43)	2 (14.29)	1 (7.14)	
5	0 (0)	7 (50)	7 (50)	0 (0)	
6	0 (0)	8 (57.14)	6 (42.86)	0 (0)	
7	0 (0)	4 (28.57)	10 (71.43)	0 (0)	
8	0 (0)	13 (92.86)	1 (7.14)	0 (0)	
9	6 (42.86)	6 (42.86)	2 (14.28)	0 (0)	
10	3 (21.43)	10 (71.43)	1 (7.14)	0 (0)	

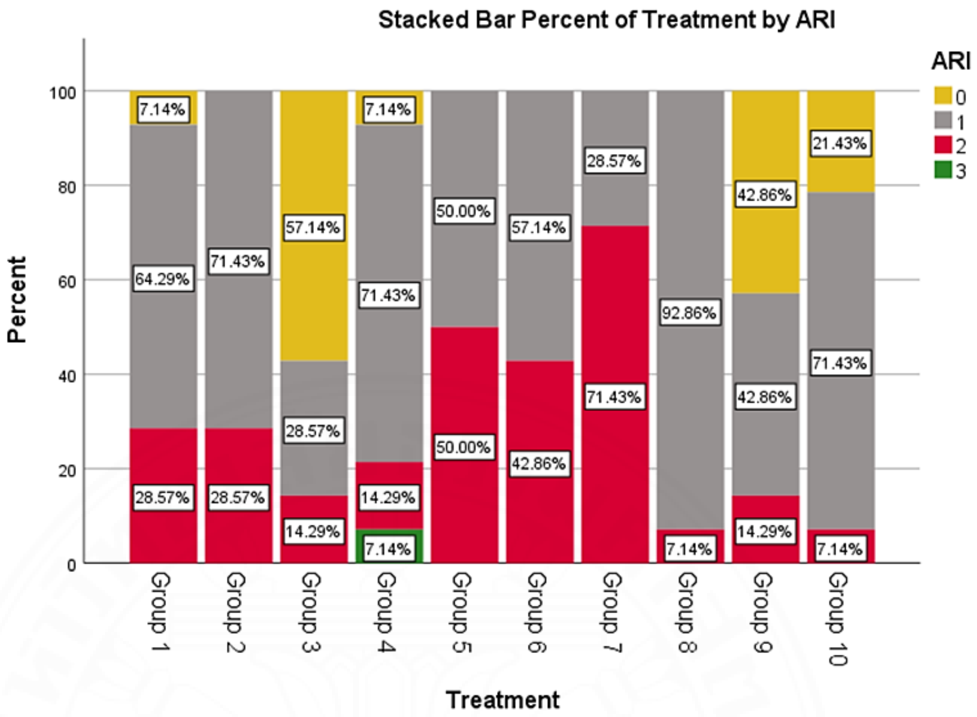


Figure 4.5 Stacked bar of ARI score observed after SBS test

4.2 Discussions

This study aimed to analyse which enamel pretreatment methods and their timing of application could prevent demineralization of enamel surface without affecting the SBS of bracket bonding in orthodontic patient who has high caries risk. We simulated lower pH in the oral cavity of orthodontic patient who has high caries risk by setting the demineralization procedure after bracket bonding in our experiment.

4.2.1 Enamel microhardness test

In dental research microhardness indentation measurements have been used to evaluate de- and remineralization events.(44,45,54) This method is easy, quick, and requires only a flat tiny surface area of specimen for testing. This measurement has been used to get information on enamel softening and tooth mineral loss after immersion in acidic induced lesion.

According to our study, immersion of teeth in the demineralizing solution significantly reduced enamel microhardness when compared to the control group. This result corresponds to the several studies that reported demineralizing solution has effect on enamel microhardness. The enamel microhardness after immersed in acidic beverages such as fruit juice, energy drinks for 3 minutes to 4 weeks was significant decreased.(69-72) Meurman et al.,1990 investigated the effects of sports drinks on bovine enamel by Profilometric analysis and surface hardness, an *in vitro* study. The pH of the beverages was range 3.05-8.55. They found that dental hydroxyapatite dissolved at pH 5.0 but increased aggressively with decreasing pH.(71) Meurman et al.,1996 found that the longer immersions of human and bovine teeth in cola-type drinks, the more was the dissolution of enamel prism cores and interprismatic areas.(72)

Applying fluoride varnish to the enamel before demineralization could preserve the enamel microhardness to a certain extent. Fluoride pretreatment in this study was Duraphat^{TM}. It composes of 5%NaF (2.26% F,22.6 mg/ml F or 22,600ppm). In the presence of high fluoride concentration (F > 100 ppm), calcium fluoride is formed and acts as a fluoride reservoir on the tooth surface. Enamel microhardness of fluoride pretreatment with artificial carious lesion in this study was significantly higher than the demineralization group. This finding coincided with previous studies.(20,73,74) These event was described by demineralization remineralization procedure. When enamel is subjected to demineralization procedure with fluoride present, the remineralization process occurs. The fluoride application on enamel forms fluoroapatite-like mineral. It improves the ability of the enamel to resist acid challenge.(73,74) Bichu et al.,2013 reported that the pretreatment of Fluor Protector^{TM} on the enamel surface could significantly reduce in the lesion depths of enamel under polarized light microscopy after immersed in ten Cate demineralizing solution for 96 hours.(20)

In our experiment, there was no significant difference in enamel microhardness between the ICON^{TM} and Duraphat^{TM} groups. This result corresponds to the several studies that reported there was no significant different in the enamel microhardness between fluoride varnish and resin infiltration enamel pretreatment.(23,30,75) Montasser et al.,2014 investigated the potential protection effect of different enamel pretreatment methods ; fluoride varnish (Clinpro^{TM}), resin infiltration (ICON^{TM}) and self-etch primer system (Transbond Plus Self-Etch Primer^{XT}); after immerse in artificial saliva and carious lesion for 21 days. They found that there was no significant different in the enamel microhardness between the Clinpro^{TM} and the ICON^{TM} groups after artificial carious lesions. They concluded that, the application of fluoride varnish and resin infiltration on the enamel surfaces could increased enamel resistance to demineralization.(23) Dhillon et al.,2020 compared enamel microhardness and enamel solubility (ES) of the enamel pretreatment with resin infiltration, fluoride varnish, and CPP-ACP after immersed in demineralizing solution pH 4.4 for 3 days. They found that there was no significant difference in enamel microhardness between fluoride varnish and resin infiltration groups at the end of day 3. The mean enamel microhardness of fluoride varnish group further decreased at day 2, and gradually stabilized by day 3. There was no statistically significant difference in enamel microhardness values between baseline to day 3, which represented continuous and sustained fluoride release to the enamel surface lesions leading to rehardening of the enamel surface. On the contrary, the mean enamel microhardness of the resin infiltration group decreased with the increase in number of acid exposures. There was significant difference in enamel microhardness between day 1 and day 3. These may be cause by enamel dissolution due to incompletely impregnated of the adhesive resin matrix or resin shrinkage during light curing procedure.(75) These results in leakage and consequently reduction of acid resistance and crack dissolution on the surface as stated by Torres et al.,2012.(30) Maximum ES was found in negative control group. The amount of calcium ion (ppm) leach out of the fluoride varnish, resin infiltration and CPP-ACP decreases from day 1 to day 3. Fluoride varnish group has least ES and highest enamel microhardness compared to other remineralizing agents.(75)

Our study found that, enamel microhardness of Pro Seal™ group was higher than demineralization group. This represented the benefit of flat resin sealant for protecting enamel from demineralizing agent. It showed acidic resistance ability and could protect the enamel to develop WSLs. This experimental result corresponds with the study of Premaraj et al.,2017. They evaluated the ability to resist acid penetration of 2 flat resin sealants (Pro Seal™ and Opal Seal™) after immersed in lactic acid (pH=4.5) for 4 weeks. Both sealants showed statistically significant difference in the depth of acid penetration compared to unsealed control side. However, Pro Seal™ released fluoride significantly greater than Opal Seal™.(76) From our study, enamel microhardness of Pro Seal™ group was similar to ICON™ group. This finding agreed with Provenzano et al.,2023. They studied the preventive effect of two resin materials, Pro Seal™ and ICON™, on lesion depth by compare the areas treated and exposed to the artificial demineralization procedure. There was no significant difference in lesion depth between ICON™ and Pro Seal™ group. However, the Pro Seal™ group showed the lower demineralization index.(77)

This study revealed that PRG Barrier Coat™ group showed significantly lower enamel microhardness than the others. These demonstrated that, acid resistance ability of S-PRG was not recognized in this study. On the contrary, previous studies reported PRG Barrier Coat™ might be effective to arrest caries.(33,34) MA et al.,2012 found no sign of demineralization which was observed from the three-dimensional images reconstructed after micro-CT scanning of PRG Barrier Coat™ pretreatment group after immersed in demineralizing solution pH 4.5 for 3 days. Conversely, they found radiolucent concave-shaped superficial lesion with 200 µm depth of control group.(34)

Hosoya et al.,2013 could not observe the progression of demineralization on the enamel at PRG Barrier Coat^{TM} area by scanning electron microscopy. There was no significantly lower enamel microhardness of PRG Barrier Coat^{TM} area and non- PRG Barrier Coat^{TM} area after immersion in demineralized solution for 1 week. They discussed that PRG Barrier Coat^{TM} might have been dissolved by demineralizing solution and might affected on the non-PRG Barrier Coat^{TM} area also.(33) Since the relatively low enamel microhardness result of PRG Barrier Coat^{TM} from this study, additional study concerning the effects of PRG Barrier Coat^{TM} on acid resistance of enamel would be required. The timing of immersion in demineralizing solution may be a factor involved.

4.2.2 Enamel Shear Bond Strength (SBS) and Adhesive Remnant Index (ARI) Score

To simulate lower pH in the oral cavity of orthodontic patient who has high caries risk, we set the demineralization procedure in our experiment. For the demineralization group, the SBS was reduced. However, there was no significant difference compared to control group. Demineralization trends to decrease SBS and may cause bracket failure.(78,79) Triwardhani et al.,2020 evaluated the enamel surface morphology after enamel demineralization at 50 magnification of scanning electron microscopy. They found that, the demineralized enamel surface was coarser, whitish and deeper porosities than control group. More enamel crack was found.(5) It is suggested that enamel pretreatment should be done in patients who has high caries risk to prevent the enamel demineralization and to enhance SBS of bracket.

All enamel pretreatment methods in this study showed higher SBS than the clinically accepted values.(80) Suggesting that they can be used for caries prevention before bracket bonding without compromising the bracket SBS. However, it should be done carefully with caution, because the higher SBS may has the side effects causing enamel damage in the debonding procedure. In addition, these results came from an *in vitro* study which was not actually representative intraoral condition. Further studies in the clinical situation are needed to confirm the results.

According to our study, the SBS of orthodontic bracket of fluoride varnish pretreatment with immediate bracket bonding was lower than fluoride varnish with delayed bracket bonding. However, there was no significant difference. Several studies reported that, fluoride pretreatment reduced SBS of orthodontic brackets.(5,21,22,24) Since fluoride reacts with enamel to form calcium fluoride. It enhances remineralization of the enamel, less soluble and increases its resistance to crystalline dissolution.(19,81,82) It is recommended to avoid using fluoride shortly before bonding.(2,82) Choi et al.,2010 found that an acid-etching after pretreatment with APF gel decreased the surface roughness and the formation of microporosities in the enamel. They suggested that an acid-etching should be performed 2 weeks after APF pretreatment in order to obtain the maximum enamel adhesion of a resin composite (4). Cossellu et al.,2017 reported that the application of Fluor Protector™ pretreatment before bracket bonding for 15 days was significantly lowered the SBS values, while it returned to an optimal value after fluoride varnish pretreatment for 30 days.(2) Based on the available evidence, we suggest that the bracket bonding should be delayed after enamel pretreatment with fluoride varnish in order to decrease possibility of orthodontic bracket detachment in patient who required high SBS of bracket.

In our experiment, the SBS of ICON™ with immediate bonding was higher than the other groups, and also as increased as Pro Seal™ with delayed bonding. This finding agrees with previous studies.(5,83-85) Since ICON™ has a hydrophilic property which may enable their penetration into the tooth surface, which results in a direct contact to this surface. Besides the high percentage of TEGDMA in ICON™ facilitates penetration and induces the formation of a thicker oxygen inhibition layer on the surface, which increases the polymerization reaction.(86) The application of ICON™ did not compromise the SBS of orthodontic bracket, conversely it was enhanced. Whereas some studies found that, SBS of orthodontic brackets before and after application of ICON™ were not significant difference.(23,29) However, the SBS of the ICON™ with delayed bracketing trended to decrease compared to ICON™ with immediate bonding group, but not significant difference. Costenoble et al.,2016 studied the effect of ICON™ on SBS with immediate and 1- month delayed bracket bonding on eroded enamel. They found that ICON™ with delayed bracket bonding produced lower SBS than ICON™ with immediate bracket bonding, but no significant difference. The SEM image of ICON™ with immediate bonding group showed homogeneous infiltrant penetration covered the enamel surface and well copolymerized with the adhesive. On the contrary, ICON™ with delayed bonding group presented a homogeneous infiltrant penetration without covering the enamel surface. In addition, the gaps were observable between the infiltrant and the adhesive. These event may be described by the aging.(87) From the presented evidence, in patient who requires high bracket SBS, we suggest that orthodontic bracket bonding should be done immediately or shortly after resin infiltration.

Our study found that Pro Seal™ with delayed bonding had highest SBS compared to the other groups. Moreover, it was significantly higher than demineralization and Pro Seal™ with immediate bonding groups. This result corresponds to the several studies that reported Pro Seal™ has no negative effect on SBS of orthodontic bracket.(6,7,88,89). In addition, a systematic review and meta-analysis of SBS after using sealant before orthodontic bracket bonding reported the highest SBS values found in Pro Seal™ before bracket bonding, which were in the range of 10-15 MPa.(90) However, there was no statistically significant between sealant and control groups in our investigation. Similarly, Lowder et al.,2008 found no significant different in mean SBS values of the Transbond XT/ProSeal™ compared to Transbond XT reference group.(88) In addition, there was reported that Pro Seal™ achieves 100% polymerization without a residual oxygen inhibited layer(6) and has the ability of resistant to wear(89), which may not decrease SBS of bracket. However, the timing of application of Pro Seal™ might affect the bracket SBS. From our study, the SBS of Pro Seal™ with delayed bracket bonding was significantly higher than Pro Seal™ with immediate bracket bonding group. This may come from the effects of the fluoride composition in Pro Seal™, which may interfere the bracket SBS. Soliman et al.,2006 reported that fluoride ions in Pro Seal™ had the sustained release and significantly decreased at the end of the 17^{th} week.(37) In cases that require high SBS of bracket such as high musculature force or reduced occlusal clearance that increase the risk of bracket dislodgement. We recommend the delayed bracket bonding after pretreatment with Pro Seal™ at less 30 days to reduce the effects of fluoride releasing and enhance the bracket SBS.

For the PRG Barrier Coat™, the SBS of immediate bracket bonding group was lowest compared to the other groups. While, it trended to increase in delayed bonding group compared to the immediate bonding group, but no statistically significant different. A probable reason why the SBS of PRG Barrier Coat™ with immediate bracket bonding was decreased is that PRG fillers promote rapid fluoride release through ligand exchange within the pre-reacted hydrogel(32,91), which may interfere the SBS of bracket. Base on the available evidence, we suggest that bracket bonding to the tooth that has pretreatment with PRG Barrier Coat™ should be delayed to reduce the effects of fluoride releasing that may reduce the SBS of brackets. However, with the limited of data, this review points out the need for further investigations to get additional data.

ARI was evaluated the residual adhesive on the tooth surface after the SBS test with a stereomicroscope at 10 times magnification. Generally, the most required clinical situation is a low ARI scores with less composite remaining on the enamel surface. On the contrary, higher ARIs scores disallow orthodontists to clean residual adhesive material faster with tendency of enamel damage. In this experiment, ARI scores were detected higher in ICON™ and Pro Seal™ groups. Since chemical bond between resin in these two groups and resin in bonding agent occurred, there were resin tag to penetrate deeper into porosity after acid etching in these enamel pretreatment process. Conversely, fluoride varnish and S-PRG consist of fluoride which may interfere with the etching on remineralized enamel surface caused lower ARI scores.(5)

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

The brackets SBS of all pretreatment methods in this study was in the clinical acceptance. They can be used for caries prevention before bracket bonding without compromising bracket The decision of the orthodontists regarding the material of choice for preventing demineralization of enamel surface in orthodontic patient who has high caries risk must be considered.

Within the limitation of the present study, fluoride varnish with delayed bracket bonding for 30 days could prevent enamel demineralization on enamel surface without affecting SBS of orthodontic brackets. Moreover it was considered favorable since there was adequate ARI score, less adhesive to remove from the tooth surface and, thus, less risk of iatrogenic damage during debonding.

Furthermore, laboratory design simulated oral conditions or *in vivo* evaluations should be planned to verify these findings.

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APPENDICES

APPENDIX A

SPSS OUTPUT OF THE ENAMEL MICROHARDNESS TEST

Figure 1 The descriptive statistics of the enamel microhardness

Descriptives

	Treatment			Statistic	Std. Error
Hardness	Control	Mean		390.9088	1.96274
		95% Confidence Interval for Mean	Lower Bound	386.4687	
			Upper Bound	395.3488	
		5% Trimmed Mean		391.1185	
		Median		390.4450	
		Variance		38.523	
		Std. Deviation		6.20672	
		Minimum		379.60	
		Maximum		398.45	
		Range		18.85	
		Interquartile Range		10.49	
		Skewness		-.470	.687
		Kurtosis		-.384	1.334
	Demin	Mean		154.6087	5.02294
		95% Confidence Interval for Mean	Lower Bound	143.2461	
			Upper Bound	165.9714	
		5% Trimmed Mean		154.2233	
		Median		152.2000	
		Variance		252.299	
		Std. Deviation		15.88394	
		Minimum		129.59	
		Maximum		186.57	
		Range		56.98	
		Interquartile Range		16.02	
		Skewness		.665	.687
		Kurtosis		1.075	1.334
	Duraphat	Mean		331.7888	8.15910
		95% Confidence Interval for Mean	Lower Bound	313.3316	
			Upper Bound	350.2459	
		5% Trimmed Mean		333.4724	
		Median		343.0038	

		Variance		665.709	
		Std. Deviation		25.80134	
		Minimum		282.68	
		Maximum		350.59	
		Range		67.91	
		Interquartile Range		32.11	
		Skewness		-1.522	.687
		Kurtosis		.797	1.334
	ICON	Mean		249.1088	6.13192
		95% Confidence Interval for Mean	Lower Bound	235.2374	
			Upper Bound	262.9801	
		5% Trimmed Mean		247.9449	
		Median		244.0800	
		Variance		376.004	
		Std. Deviation		19.39083	
		Minimum		227.91	
		Maximum		291.26	
		Range		63.35	
		Interquartile Range		26.28	
		Skewness		1.303	.687
		Kurtosis		1.390	1.334
	Pro Seal	Mean		244.1790	3.72129
		95% Confidence Interval for Mean	Lower Bound	235.7609	
			Upper Bound	252.5971	
		5% Trimmed Mean		244.3486	
		Median		246.9400	
		Variance		138.480	
		Std. Deviation		11.76775	
		Minimum		219.88	
		Maximum		265.43	
		Range		45.56	
		Interquartile Range		11.50	
		Skewness		-.419	.687
		Kurtosis		2.033	1.334
	PRG barrier coat	Mean		125.4091	5.19430
		95% Confidence Interval for Mean	Lower Bound	113.6587	
			Upper Bound	137.1594	
		5% Trimmed Mean		125.0283	

	Median	123.0938	
	Variance	269.808	
	Std. Deviation	16.42582	
	Minimum	98.95	
	Maximum	158.73	
	Range	59.78	
	Interquartile Range	16.57	
	Skewness	.548	.687
	Kurtosis	1.219	1.334

Figure 2 Kolmogorov-Smirnov test of Normality

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	Statistic	df	Sig.	Statistic	df	Sig.
Hardness	Control	.158	10	.200*	.933	10	.475
	Demin	.193	10	.200*	.951	10	.681
	Duraphat	.345	10	.001	.706	10	.001
	ICON	.220	10	.188	.886	10	.155
	Pro Seal	.192	10	.200*	.927	10	.418
	PRG barrier coat	.170	10	.200*	.960	10	.784

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Figure 3 Kruskal-Wallis followed by post-hoc multiple comparison

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Hardness is the same across categories of Treatment.	Independent-Samples Kruskal-Wallis Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .050.

Independent-Samples Kruskal-Wallis Test Summary

Total N	60
Test Statistic	54.953[a]
Degree Of Freedom	5
Asymptotic Sig.(2-sided test)	.000

a. The test statistic is adjusted for ties.

Pairwise Comparisons of Treatment

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
PRG barrier coat-Demin	8.000	7.810	1.024	.306	1.000
PRG barrier coat-Pro Seal	24.000	7.810	3.073	.002	.032
PRG barrier coat-ICON	24.200	7.810	3.098	.002	.029
PRG barrier coat-Duraphat	38.800	7.810	4.968	.000	.000
PRG barrier coat-Control	49.000	7.810	6.274	.000	.000
Demin-Pro Seal	-16.000	7.810	-2.049	.041	.608
Demin-ICON	-16.200	7.810	-2.074	.038	.571
Demin-Duraphat	-30.800	7.810	-3.944	.000	.001
Demin-Control	41.000	7.810	5.250	.000	.000
Pro Seal-ICON	.200	7.810	.026	.980	1.000
Pro Seal-Duraphat	14.800	7.810	1.895	.058	.871
Pro Seal-Control	25.000	7.810	3.201	.001	.021
ICON-Duraphat	14.600	7.810	1.869	.062	.924
ICON-Control	24.800	7.810	3.175	.001	.022
Duraphat-Control	10.200	7.810	1.306	.192	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

APPENDIX B

SPSS OUTPUT OF THE ENAMEL SHEAR BOND STRENGHT TEST

Figure 1 The descriptive statistics of the shear bond strength

Descriptives

	Treatment			Statistic	Std. Error
SBS	Group 1	Mean		11.826994427	.7370207431
		95% Confidence Interval for Mean	Lower Bound	10.234757914	
			Upper Bound	13.419230939	
		5% Trimmed Mean		11.679543257	
		Median		11.123652325	
		Variance		7.605	
		Std. Deviation		2.7576791075	
		Minimum		8.5936743	
		Maximum		17.7144357	
		Range		9.1207614	
		Interquartile Range		2.8509923	
		Skewness		1.328	.597
		Kurtosis		1.181	1.154
	Group 2	Mean		9.724360407	.9264868547
		95% Confidence Interval for Mean	Lower Bound	7.722807246	
			Upper Bound	11.725913569	
		5% Trimmed Mean		9.654108644	
		Median		9.031986452	
		Variance		12.017	
		Std. Deviation		3.4665963836	
		Minimum		5.1884935	
		Maximum		15.5247591	
		Range		10.3362656	
		Interquartile Range		5.0752361	
		Skewness		.841	.597
		Kurtosis		-.505	1.154
	Group 3	Mean		8.157118323	1.1486429274
		95% Confidence Interval for Mean	Lower Bound	5.675626145	
			Upper Bound	10.638610501	
		5% Trimmed Mean		7.932758446	

	Median		7.596703559	
	Variance		18.471	
	Std. Deviation		4.2978282940	
	Minimum		3.6453392	
	Maximum		16.7073752	
	Range		13.0620361	
	Interquartile Range		6.3643379	
	Skewness		.852	.597
	Kurtosis		-.279	1.154
Group 4	Mean		10.937812640	1.3008464341
	95% Confidence Interval for Mean	Lower Bound	8.127504777	
		Upper Bound	13.748120503	
	5% Trimmed Mean		10.646156991	
	Median		9.083870814	
	Variance		23.691	
	Std. Deviation		4.8673216694	
	Minimum		5.8514073	
	Maximum		21.2740197	
	Range		15.4226123	
	Interquartile Range		6.5112680	
	Skewness		1.117	.597
	Kurtosis		.219	1.154
Group 5	Mean		15.588891462	1.9281709589
	95% Confidence Interval for Mean	Lower Bound	11.423331358	
		Upper Bound	19.754451566	
	5% Trimmed Mean		15.205215407	
	Median		12.656235090	
	Variance		52.050	
	Std. Deviation		7.2145551112	
	Minimum		8.8502910	
	Maximum		29.2336609	
	Range		20.3833699	
	Interquartile Range		10.6000429	
	Skewness		.941	.597
	Kurtosis		-.423	1.154
Group 6	Mean		13.707190562	1.0684109740
	95% Confidence Interval for Mean	Lower Bound	11.399028981	
		Upper Bound	16.015352142	

	5% Trimmed Mean		13.587258349	
	Median		14.517603280	
	Variance		15.981	
	Std. Deviation		3.9976278129	
	Minimum		8.3558153	
	Maximum		21.2173457	
	Range		12.8615304	
	Interquartile Range		6.5596007	
	Skewness		.264	.597
	Kurtosis		-.887	1.154
Group 7	Mean		10.462317527	.6369493321
	95% Confidence Interval for Mean	Lower Bound	9.086272154	
		Upper Bound	11.838362900	
	5% Trimmed Mean		10.325272715	
	Median		9.842271728	
	Variance		5.680	
	Std. Deviation		2.3832461733	
	Minimum		7.7690774	
	Maximum		15.6223643	
	Range		7.8532869	
	Interquartile Range		2.9282869	
	Skewness		1.033	.597
	Kurtosis		.470	1.154
Group 8	Mean		17.455218288	1.3422838963
	95% Confidence Interval for Mean	Lower Bound	14.555390230	
		Upper Bound	20.355046346	
	5% Trimmed Mean		17.281545747	
	Median		16.394475720	
	Variance		25.224	
	Std. Deviation		5.0223664558	
	Minimum		10.8019273	
	Maximum		27.2346150	
	Range		16.4326877	
	Interquartile Range		8.3075088	
	Skewness		.358	.597
	Kurtosis		-.866	1.154
Group 9	Mean		7.334846730	1.2030208180
		Lower Bound	4.735878262	

		95% Confidence Interval for Mean	Upper Bound	9.933815198	
		5% Trimmed Mean		6.845919980	
		Median		5.963820247	
		Variance		20.262	
		Std. Deviation		4.5012917301	
		Minimum		3.1949241	
		Maximum		20.2754508	
		Range		17.0805267	
		Interquartile Range		5.4537616	
		Skewness		1.953	.597
		Kurtosis		4.838	1.154
	Group 10	Mean		10.373464263	1.6385952708
		95% Confidence Interval for Mean	Lower Bound	6.833494400	
			Upper Bound	13.913434127	
		5% Trimmed Mean		9.951505788	
		Median		9.539185446	
		Variance		37.590	
		Std. Deviation		6.1310620989	
		Minimum		2.0494609	
		Maximum		26.2927202	
		Range		24.2432592	
		Interquartile Range		5.2823085	
		Skewness		1.286	.597
		Kurtosis		2.740	1.154

Figure 2 Kolmogorov-Smirnov test of Normality

Tests of Normality

	Treatment	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
SBS	Group 1	.188	14	.193	.841	14	.017
	Group 2	.173	14	.200*	.859	14	.029
	Group 3	.165	14	.200*	.888	14	.076
	Group 4	.210	14	.093	.867	14	.038
	Group 5	.224	14	.055	.839	14	.016
	Group 6	.135	14	.200*	.939	14	.409
	Group 7	.175	14	.200*	.897	14	.103
	Group 8	.170	14	.200*	.935	14	.356
	Group 9	.182	14	.200*	.796	14	.004
	Group 10	.190	14	.182	.893	14	.090

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Figure 3 Two-way ANOVA followed by post-hoc Tamhane multiple comparison test

Levene's Test of Equality of Error Variances[a,b]

		Levene Statistic	df1	df2	Sig.
SBS	Based on Mean	2.797	9	130	.005
	Based on Median	1.770	9	130	.080
	Based on Median and with adjusted df	1.770	9	82.161	.087
	Based on trimmed mean	2.560	9	130	.010

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Dependent variable: SBS

b. Design: Intercept + Treatment + Time + Treatment * Time

Tests of Between-Subjects Effects

Dependent Variable: SBS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1233.689[a]	9	137.077	6.342	.000
Intercept	17804.153	1	17804.153	823.689	.000
Treatment	701.844	5	140.369	6.494	.000
Time	173.311	1	173.311	8.018	.005
Treatment * Time	345.032	3	115.011	5.321	.002
Error	2809.969	130	21.615		
Total	22426.659	140			
Corrected Total	4043.658	139			

a. R Squared = .305 (Adjusted R Squared = .257)

Multiple Comparisons

Dependent Variable: SBS

Tamhane

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 1	Group 2	2.102634020	1.1838823707	.984	-2.252299832	6.457567871
	Group 3	3.669876104	1.3647637709	.454	-1.426970996	8.766723204
	Group 4	.889181787	1.4951257542	1.000	-4.757578317	6.535941891
	Group 5	-3.761897035	2.0642293531	.983	-11.849185003	4.325390932
	Group 6	-1.880196135	1.2979605483	1.000	-6.699227980	2.938835710
	Group 7	1.364676900	.9741170501	1.000	-2.205713500	4.935067301
	Group 8	-5.628223861	1.5313150016	.065	-11.428881357	.172433635
	Group 9	4.492147697	1.4108361578	.179	-.798082046	9.782377440
	Group 10	1.453530164	1.7967175730	1.000	-5.483746874	8.390807201
Group 2	Group 1	-2.102634020	1.1838823707	.984	-6.457567871	2.252299832
	Group 3	1.567242084	1.4757230318	1.000	-3.857414092	6.991898261
	Group 4	-1.213452233	1.5970533295	1.000	-7.128928303	4.702023837
	Group 5	-5.864531055	2.1392104007	.448	-14.072549851	2.343487741
	Group 6	-3.982830154	1.4141711004	.342	-9.165529510	1.199869201
	Group 7	-.737957120	1.1243141659	1.000	-4.913366480	3.437452241
	Group 8	-7.730857881*	1.6309825107	.004	-13.786054544	-1.675661217
	Group 9	2.389513677	1.5184324089	.998	-3.206100629	7.985127984
	Group 10	-.649103856	1.8823847517	1.000	-7.759822761	6.461615049
Group 3	Group 1	-3.669876104	1.3647637709	.454	-8.766723204	1.426970996

	Group 2	-1.567242084	1.4757230318	1.000	-6.991898261	3.857414092
	Group 4	-2.780694317	1.7353910279	.997	-9.136953918	3.575565284
	Group 5	-7.431773139	2.2443760427	.138	-15.868879265	1.005332986
	Group 6	-5.550072238	1.5687200464	.067	-11.288794254	.188649777
	Group 7	-2.305199204	1.3134249222	.988	-7.275813707	2.665415299
	Group 8	-9.298099965*	1.7666653992	.001	-15.775666082	-2.820533848
	Group 9	.822271593	1.6633218760	1.000	-5.260236514	6.904779700
	Group 10	-2.216345940	2.0010934601	1.000	-9.636978868	5.204286988
Group 4	Group 1	-.889181787	1.4951257542	1.000	-6.535941891	4.757578317
	Group 2	1.213452233	1.5970533295	1.000	-4.702023837	7.128928303
	Group 3	2.780694317	1.7353910279	.997	-3.575565284	9.136953918
	Group 5	-4.651078822	2.3259502772	.931	-13.301776430	3.999618785
	Group 6	-2.769377922	1.6833607618	.995	-8.951973124	3.413217281
	Group 7	.475495113	1.4484149602	1.000	-5.071934284	6.022924510
	Group 8	-6.517405648	1.8692050459	.076	-13.351836083	.317024787
	Group 9	3.602965910	1.7718522889	.911	-2.879539374	10.085471194
	Group 10	.564348377	2.0921749226	1.000	-7.132580046	8.261276799
Group 5	Group 1	3.761897035	2.0642293531	.983	-4.325390932	11.849185003
	Group 2	5.864531055	2.1392104007	.448	-2.343487741	14.072549851
	Group 3	7.431773139	2.2443760427	.138	-1.005332986	15.868879265
	Group 4	4.651078822	2.3259502772	.931	-3.999618785	13.301776430
	Group 6	1.881700901	2.2043922645	1.000	-6.461348039	10.224749841
	Group 7	5.126573936	2.0306520377	.644	-2.921469706	13.174617577
	Group 8	-1.866326826	2.3493763651	1.000	-10.583006855	6.850353204
	Group 9	8.254044732	2.2726861497	.065	-.254117867	16.762207332
	Group 10	5.215427199	2.5303829173	.899	-4.064767131	14.495621529
Group 6	Group 1	1.880196135	1.2979605483	1.000	-2.938835710	6.699227980
	Group 2	3.982830154	1.4141711004	.342	-1.199869201	9.165529510
	Group 3	5.550072239	1.5687200464	.067	-.188649777	11.288794254
	Group 4	2.769377922	1.6833607618	.995	-3.413217281	8.951973124
	Group 5	-1.881700901	2.2043922645	1.000	-10.224749841	6.461348039
	Group 7	3.244873035	1.2438675415	.523	-1.430778514	7.920524583
	Group 8	-3.748027726	1.7155838853	.830	-10.058643601	2.562588149
	Group 9	6.372343832*	1.6089627397	.024	.480144330	12.264543333
	Group 10	3.333726298	1.9561432644	.992	-3.961812049	10.629264646
Group 7	Group 1	-1.364676900	.9741170501	1.000	-4.935067301	2.205713500
	Group 2	.737957120	1.1243141659	1.000	-3.437452241	4.913366480
	Group 3	2.305199204	1.3134249222	.988	-2.665415299	7.275813707

	Group 4	-.475495113	1.4484149602	1.000	-6.022924510	5.071934284
	Group 5	-5.126573936	2.0306520377	.644	-13.174617577	2.921469706
	Group 6	-3.244873035	1.2438675415	.523	-7.920524583	1.430778514
	Group 8	-6.992900761*	1.4857424103	.007	-12.700434410	-1.285367112
	Group 9	3.127470797	1.3612360340	.776	-2.046931789	8.301873383
	Group 10	.088853264	1.7580383708	1.000	-6.788980491	6.966687018
Group 8	Group 1	5.628223861	1.5313150016	.065	-.172433635	11.428881357
	Group 2	7.730857881*	1.6309825107	.004	1.675661217	13.786054544
	Group 3	9.298099965*	1.7666653992	.001	2.820533848	15.775666082
	Group 4	6.517405648	1.8692050459	.076	-.317024787	13.351836083
	Group 5	1.866326826	2.3493763651	1.000	-6.850353204	10.583006855
	Group 6	3.748027726	1.7155838853	.830	-2.562588149	10.058643601
	Group 7	6.992900761*	1.4857424103	.007	1.285367112	12.700434410
	Group 9	10.120371558*	1.8024941461	.000	3.521085212	16.719657904
	Group 10	7.081754025	2.1181880275	.111	-.698799504	14.862307554
Group 9	Group 1	-4.492147697	1.4108361578	.179	-9.782377440	.798082046
	Group 2	-2.389513677	1.5184324089	.998	-7.985127984	3.206100629
	Group 3	-.822271593	1.6633218760	1.000	-6.904779700	5.260236514
	Group 4	-3.602965910	1.7718522889	.911	-10.085471194	2.879539374
	Group 5	-8.254044732	2.2726861497	.065	-16.762207332	.254117867
	Group 6	-6.372343832*	1.6089627397	.024	-12.264543333	-.480144330
	Group 7	-3.127470797	1.3612360340	.776	-8.301873383	2.046931789
	Group 8	-10.120371558*	1.8024941461	.000	-16.719657904	-3.521085212
	Group 10	-3.038617533	2.0327945174	.999	-10.552233966	4.474998899
Group 10	Group 1	-1.453530164	1.7967175730	1.000	-8.390807201	5.483746874
	Group 2	.649103856	1.8823847517	1.000	-6.461615049	7.759822761
	Group 3	2.216345940	2.0010934601	1.000	-5.204286988	9.636978868
	Group 4	-.564348377	2.0921749226	1.000	-8.261276799	7.132580046
	Group 5	-5.215427199	2.5303829173	.899	-14.495621529	4.064767131
	Group 6	-3.333726298	1.9561432644	.992	-10.629264646	3.961812049
	Group 7	-.088853264	1.7580383708	1.000	-6.966687018	6.788980491
	Group 8	-7.081754025	2.1181880275	.111	-14.862307554	.698799504
	Group 9	3.038617533	2.0327945174	.999	-4.474998899	10.552233966

Based on observed means.

The error term is Mean Square(Error) = 21.857.

*. The mean difference is significant at the .05 level.

APPENDIX C

SPSS OUTPUT OF THE ADHESIVE REMNANT INDEX TEST

Figure 1 Frequency of distribution of ARI scores

Treatment * ARI Crosstabulation

Count

		ARI				Total
		0	1	2	3	
Treatment	Control	1	9	4	0	14
	Demin	0	10	4	0	14
	Duraphat 1d	8	4	2	0	14
	Duraphat 30d	1	10	2	1	14
	Icon 1d	0	7	7	0	14
	Icon 30d	0	8	6	0	14
	Proseal 1d	0	4	10	0	14
	Proseal 30d	0	13	1	0	14
	PRG 1d	6	6	2	0	14
	PRG 30d	3	10	1	0	14
Total		19	81	39	1	140

Figure 2 Chi-Square and Fisher's Exact test

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Monte Carlo Sig. (2-sided)			Monte Carlo Sig. (1-sided)		
					99% Confidence Interval			99% Confidence Interval	
				Significance	Lower Bound	Upper Bound	Significance	Lower Bound	Upper Bound
Pearson Chi-Square	77.899[a]	27	.000	.000[b]	.000	.000			
Likelihood Ratio	70.424	27	.000	.000[b]	.000	.000			
Fisher's Exact Test	64.150			.000[b]	.000	.000			
Linear-by-Linear Association	1.087[c]	1	.297	.307[b]	.295	.319	.152[b]	.142	.161
N of Valid Cases	140								

a. 30 cells (75.0%) have expected count less than 5. The minimum expected count is .10.

b. Based on 10000 sampled tables with starting seed 299883525.

c. The standardized statistic is -1.043.

ETHICAL REQUIREMENT

Collecting extracted teeth was approved by the to the Human Research Ethics Committee of Thammasat University No. 002, date of issue: 1 Febuary 2022).

ScF 03_02 (En

The Human Research Ethics Committee of Thammasat University (Science), (HREC-TUSc)
Room No. 110, Piyachart Building, 1st Floor, Thammasat University Rangsit Campus,
Prathumthani 12121, Thailand, Tel: 0-2986-9213 ext.7358 E-mail: ecsctu3@tu.ac.th

COE No. 002/2565

Certificate of Exemption

Project No. : 010/2565
Protocol Title : Effects of Different Enamel Pretreatment Methods and Timing of Application on Shear Bond Strength of Orthodontic Brackets and Enamel Microhardness after Demineralization, an In Vitro Study
Principle Investigator : Usanee Pattamalai
Place of Proposed Study/Institution: Faculty of Dentistry, Thammasat University

The Human Research Ethics Committee of Thammasat University (Science), Thailand, has approved the above study project, in accordance with the compliance to the Declaration of Helsinki, the Belmont report, CIOMS guidelines and the International practice (ICH-GCP).
The Human Research Ethics Committee of Thammasat University (Science), decided to exempt the above study. These decision has been reported in 003/2022 meeting.

Signature:..
(Assoc. Prof. Jinda Wangboonskul, Ph.D.)
Chairman of The Human Research Ethics Committee of Thammasat University (Science)

Signature: ....
(Assoc. Prof.Laksana Laokiat, Ph.D.)
Secretary of The Human Research Ethics Committee of Thammasat University (Science)

Date of issue: February 1,2022

The approval documents including

1) Research proposal
2) Principal investigator's Curriculum Vitae

BIOGRAPHY

Name	Miss Usanee Pattamalai
Educational Attainment	2003: DDS degree (Doctor of Dental Surgery): Srinakharinwirot University
	2010 Certificate Residency Training in Prosthodontics Mahidol university.