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Effects of sealant and self etching primer on enamel decalcification in vivo

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EFFECTS OF SEALANT AND SELF ETCHING PRIMER ON ENAMEL DECALCIFICATION *IN VIVO*

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Thesis submitted to the
School of Dentistry
At West Virginia University
In partial fulfillment of the requirements
For the degree of

Master of Science
In
Orthodontics

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Morgantown, West Virginia
2004

ABSTRACT

EFFECTS OF SEALANT AND SELF ETCHING PRIMER ON ENAMEL DECALCIFICATION *IN VIVO*

Matthew A. Ghiz D.D.S.

Self etching primer (SEP) saves valuable time by eliminating the multiple steps required to place a sealant prior to bonding brackets. The purpose of this study is to compare the resistance to demineralization between SEP and a conventional sealant. 25 patients were bonded with either SEP or Sealant using the split arch technique. At the end of the observation period (18-24 months), the plaque index and decalcification scores were noted. Data was analyzed using ANOVA and Tukey Kramer test. Significantly higher decalcification score was found with the SEP group as compared with the Sealant Group ($p < 0.001$). Significant difference was also found between the level of hygiene compliance and decalcification ($p < 0.0001$). Patients with fair or poor hygiene compliance have higher decalcification scores with SEP as compared to Sealant. These results suggest that application of a self etching primer saves chair time but provides less resistance to enamel demineralization as compared to a conventional sealant.

DEDICATION

To my beautiful wife, **Becky:** My best friend and most important person in my life. Thank you for all that you have done and tolerated throughout our life together. Thank you for your unconditional support through the past seven years. You have always been patient and understanding in all that I needed and did. I love you with all that I have.

To **Cooper:** You are a joy to be around, always pleasant and enjoyable to be with. You were the reason that I decided to go back to school. I am proud to call you my son. Thank you and I love you very much.

To **Noah:** You always make me laugh, you are a loyal and passionate individual. You have had to deal with the most adjustments while being in school. Thank you for being my son, I love you very much and am always very proud of you.

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To **Ellie:** Thank you for being the only one in the family that really looks like me. You are a beautiful little girl with a darling personality already. I love you very much and can not imagine this family without you.

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CHAPTER I

INTRODUCTION

Fixed orthodontic appliances make it difficult for young patients to maintain adequate oral hygiene during orthodontic treatment. The tooth surface adjacent to bonded attachments is particularly susceptible to formation of caries. Several studies have found an increased amount of plaque around orthodontic appliances.(1;2) The adherence of plaque to the tooth surface initiates the decalcification process. Other studies have reported an increase in the number of *Streptococcus mutans* and *Lactobacillus* species in the oral cavity following placement of fixed orthodontic appliances.(3) A higher concentrations of these bacteria increase the risk of decalcification.(4) Plaque bacteria produce organic acids, which cause the dissolution of calcium and phosphate ions from the enamel surface. This dissolution results in the formation of a white spot or early carious lesion, which may form in a period of four weeks.(5-8) If the diffusion of ions away from the tooth surface continues, cavitation of the enamel surface will result.

Several methods have been implemented to prevent or reduce enamel decalcification during orthodontic treatment including fluoride application in various forms, enamel sealants, rigorous oral hygiene regimens and modified appliance designs.

Application of sealant after etching has been shown to prevent enamel decalcification *in vitro*.(9) The application is usually a two-step procedure involving etching of enamel with phosphoric acid, rinsing and application of a primer/sealant prior to the

orthodontic bracket being bonded to the tooth. A new product Transbond Plus Self-Etching Primer[®] (3M Unitek) was introduced on the market, which allows etching and priming of enamel to be done in one step. The advantage of this product is that it provides less chair time for the patients and the etching process is not as dramatic as traditional methods.(10;11) The disadvantage of such a product is the omission of the sealant layer. The purpose of this study is to determine the effects of a sealant compared with a self-etching primer on enamel decalcification around orthodontic brackets *in vivo*.

STATEMENT OF THE PROBLEM

Patients who undergo orthodontics are at an increased risk in developing enamel decalcification. This problem can lead to esthetic compromises and expensive restorative procedures in the future. Placement of sealant after etching of enamel gives protection against enamel decalcification. The use of self etching primers, which combines the steps of etching and priming, saves valuable chairtime but may not provide protection against enamel decalcification. The study will investigate two different bonding techniques and whether they may reduce the amount of decalcification present following orthodontic therapy, in the presence of a self-etching primer and sealant layer.

SIGNIFICANCE OF THE PROBLEM

The results of this *in vivo* study will help the clinicians determine whether sealant application after etching is necessary. This will allow clinicians to decide whether self-etching primer bonding system can be used as an alternative means to bonding orthodontic brackets without compromising the health of the patient's teeth.

HYPOTHESIS

1. There is no significant difference in the amount of decalcification between Light Bond, a conventional etch and seal adhesive system when compared to Transbond Plus, a one step self etching primer. when determining the amount of decalcification present.
2. There is no significant difference among hygiene groups and between Transbond Plus and a conventional sealant.
3. There is no significant difference between etch and sealant patterns of the two bonding agents when comparing surface morphology, utilizing a Scanning Electron Microscope.

DEFINITION OF TERMS

1. Bonding Material: materials used in orthodontic to attach the fixed appliances to teeth.
2. Decalcification: an early carious lesion in enamel, the loss or removal of minerals specifically calcium and phosphate, from calcified tissues. Clinically observed as a white opaque spot.
3. Dental Plaque: a sticky substance composed of saliva derived mucin, bacteria and bacterial products that accumulate on teeth.
4. Enamel Etchant: 35-40% phosphoric acid, 60-65% water (Reliance Orthodontic Products, Inc., Itasca, IL, lot # 908169)
5. Fixed Appliance: an orthodontic appliance that attaches to the enamel surface of teeth, by bonding or cementation. Most commonly referred to as brackets and bands.

6. Fluoride: the monovalent anion of fluorine which has a cariostatic effect.
7. Light Bond: a fluoride releasing light cure sealant resin system used for bracket placement (Reliance Orthodontic Products, Inc., Itasca, IL, lot # 912079).
8. Transbond Plus: a one step self-etching primer adhesive system used for bracket placement (3M Unitek, 3M Center, St. Paul Minnesota).

ASSUMPTIONS

1. Orthodontic bonding will increase enamel decalcification around the appliance.
2. The use of self etch primer system will decrease multiple steps and chair time for bonding.
3. The level of decalcification is related to the level of oral hygiene compliance.

LIMITATIONS

1. Few Patients in this study may not complete the study or orthodontic treatment.
2. Decalcification score may vary depending on oral hygiene compliance.
3. Patients may vary in decalcification score according to diet and frequency of consuming cariogenic food.
4. Assessment of decalcification will be clinical observation.

DELIMITATIONS

1. The only patients with a treatment time between 18 and 24 months were chosen.
2. Only two types of bonding techniques were used.
3. All teeth were cured with the same curing light and curing time according to manufactures suggestion.

4. A clinical exam (visual and tactile detection) will be performed to determine decalcification.
5. Patients will be given the same oral hygiene instruction, and will serve as their own control.

CHAPTER II

REVIEW OF LITERATURE

DECALCIFICATION

Decalcification is basically broken down mineral elements, specifically calcium and phosphate within the tooth structure, this most generally occurs when the oral environment is at a decreased pH.(12) Decalcification or demineralization is caused by ineffective oral hygiene and retention of bacterial plaque on the enamel surface for an extended period of time.(13) The reduction in the mineral content of the tooth structure is termed as a white spot lesion having a chalky appearance clinically.(14) This white spot lesion is the early stages of a carious lesion. The mineral content fluctuates as intra-oral conditions change in respect to the development and maturation of bacterial plaque. Decalcification is a back and forth process with alternating phases of demineralization and remineralization.(15) The influx and the efflux of minerals in the enamel is affected by several factors including the oral pH, the presence or absence of fluoride, the contents and concentration of saliva, the concentration and virulence of the oral bacteria, the frequency of sucrose ingestion and the duration of the pH conditions.(16) Demineralization and remineralization occur at the same time, at various depths in the early carious lesion during periods of high bacterial metabolic activity and low pH conditions.(17)

Decalcification may develop within a month of bracket placement this is due to prolonged accumulation and retention of plaque next to the brackets.(18) With the presence of orthodontic brackets and the bonding material can alter the oral environment, this alteration can support and initiate the formation of decalcification.(19) These orthodontic patients are at an increased risk of developing clinically detectable areas of decalcification as a consequence of plaque accumulation around orthodontic attachments.(20)

Decalcification can be found in both the orthodontic and non-orthodontic populations. Misrahi conducted a study of high school aged children and found that nearly 85% of the group had clinical evidence of enamel decalcification of unknown etiology.(21)

Many studies have shown that fixed orthodontic appliances complicate patients oral hygiene, it is difficult to achieve adequate plaque removal.(6;22-28) Patients are given instructions emphasizing the modification of oral hygiene, reduction in carbohydrate intake, and the administration of fluoride rinses. Even with these efforts, decalcification has been reported in 2-90% of patients depending on the study. The variability is a result of the interactions of the complex methods used to assess and quantify decalcification including lucency etiology and fluoride exposure.(29) In studies of cross sectional design, when comparing patients following orthodontic treatment and patients not treated orthodontically, it is difficult to distinguish between idiopathic white spot lesions and decalcification.(30) In an attempt to eliminate this problem, Zachrisson et al conducted a longitudinal study that recorded only new lesion formation. A large variance in the prevalence of white spot lesions were reported with 89% in 1971 and 15% in 1977.(31;32)

The formation of white spot lesions on the buccal surfaces of teeth is common during orthodontic fixed appliance therapy due to the accumulation of microbial plaque around

appliances.(33) Gorelick reported a significant increase in the incidence of white spot lesions following the placement of fixed orthodontic appliances when compared to a control group of untreated individuals. The authors also found no significant difference in susceptibility between banded or bonded teeth. This study also showed that 49.6% of the patients developed areas of decalcification.(34) Several studies report different findings concerning which teeth are most likely to show decalcification, Mizrahi cited the maxillary incisors and mandibular first molars as the most common teeth.(35) Ogaard reported the maxillary and mandibular first molars,(36) Trimpeneers found maxillary lateral incisors to be the most frequently involved teeth(37) and Gorelick reported that the maxillary lateral incisors were most often affected while no decalcification was found on the lingual surfaces of mandibular teeth.(38) The resistance of the mandibular segments to decalcification has been linked to the buffering capacity of saliva.(39;40) O'Reilly and Featherstone cited the following common areas for plaque accumulation: gingival margins under bands where the luting agents have washed away, resin flash around bonded appliances that was not removed,(41) and the junction of the bonding agent and etched enamel surface.(42) Despite the variance of the lesions location, it is evident that orthodontic patients are at an increased risk for decalcification regardless of the study.

Decalcification results from a highly localized drop in pH at the tooth surface. This local drop occurs as a result of metabolism within plaque communities rich in *Streptococcus Mutans* (*S. Mutans*) and *Lactobacillus*.(15) *S. Mutans* is the bacterial species most commonly associated with the initiation of caries due to their ability to rapidly produce acids from fermentable carbohydrates.(43) Caries rates are directly affected by an increase in the population of *S. Mutans*.(44;45) Generally accepted is the role of a high sucrose diet in the

increase of the *S. Mutans* population. *S. Mutans* is necessary to cause a significant smooth surface carious lesion.(46) Lundstrom and Krasse found an increase in both *S. Mutans* that is involved in the initiation of the carious lesion and *Lactobacillus* that is involved in the further development of the carious lesion after the fixation of orthodontic appliances.(47;48) Murray was able to determine that the most accepted etiological relationship of caries involves fermentable carbohydrate (sucrose source), enamel, acid-producing microorganisms and time.(49)

This bacterial plaque consists of a soft, translucent, sticky material that is composed of bacteria and bacterial by-products. Plaque accumulation is not a random process but rather a highly organized and ordered sequence.(15) These adherent bacteria attach to receptors within the acquired pellicle on the tooth surface and secrete a sticky matrix that permits them to cohere to each other.(50) The pellicle, which is formed from proteins and other salivary components, reforms almost immediately after a cleaned tooth is exposed to saliva.(51) This adhesion and cohesion allow the bacteria to colonize within minutes of the formation of the pellicle.(15) The pellicle, when in the presence of a fermentable carbohydrate, progresses into a thin plaque mass in approximately 24 hours. *S. Mutans* metabolizes sucrose in the production of energy resulting in the formation of lactic acid as a metabolic by-product.(52) As the level of lactic acid increases, the oral pH begins to decrease causing a shift in the mineral equilibrium favoring the net loss of minerals from the enamel surface. As the plaque layer thickens and the structural complexity increases, the various salivary components combine with extracellular particles secreted by bacteria. If the plaque mass is not removed, the pH will decrease below the critical level of approximately 5.5. This will allow for the dissolution of minerals away from the tooth surface.

Several studies report that in a period of four weeks a white spot lesion can develop under an orthodontic appliance when in the presence of a cariogenic challenge.(6;53-57) In a study using SEM, some enamel crystal dissolution was found after two days of plaque accumulation.(58) Thylstrup found that within eight to fourteen days following the elimination of plaque removal mechanisms, all of the participants demonstrated enamel decalcification despite the presence of a fluoridated community water supply. Balenseifen and Madonia reported that the presence of orthodontic appliances causes a change in the flora of the oral environment from acidiphobic to acidiphillic.(59) The authors found a significant decrease in the plaque pH as well as increases in the concentration of carbohydrates and microbial populations. This type of plaque is more cariogenic as a result of the high concentration of acid-producing bacteria on the surface. As the plaque mass matures, a change in the diversity of the bacterial species occurs with the appearance of more anaerobic and filamentous species.(60) Within the mass and near the tooth surface, the environment favors the growth of these more virulent bacteria while maintaining a low pH. The coupling of increased virulence (acidiphillic characteristics of bacteria) and low pH facilitates the continuation of the demineralization. The layer of plaque on the surface provides an ideal environment for bacterial colonization and acid production. This layer physically prohibits the acid from diffusing away from the surface as well as the calcium phosphate ions from reaching the surface to remineralize the enamel.(61)

Several studies link the initiation of enamel caries with the retention of plaque due to poor oral hygiene. This plaque is the source for acid production when it is coupled with a fermentable carbohydrate and it prevents remineralization of the affected enamel.(62) Orthodontic appliances complicate the removal of food debris and accumulated plaque.

Several studies have found an increased amount of plaque around orthodontic appliances.(63-65) Gwinnett and Ceen suggest that the presence of orthodontic appliances, wires, elastics and other attachments considerably affects an individual's ability to achieve optimal plaque removal.(66) Atack found that the increased plaque caused increased gingivitis, increased gingival bleeding, and increased probing depths. He found that the presence of orthodontic appliances leads to the increase in the population of cariogenic periodontal bacteria including *Actinobacillus*, *Bacteroides*, and *Prevotella*.(67) Increases in the quantity of plaque and the concentration of pathogenic bacteria contribute to an increased risk of decalcification in orthodontic patients.(68)

Associated with the increased plaque retention is the evidence of greater concentrations of bacteria (*S. Mutans* and *Lactobacillus*) and carbohydrates.(69) Lundstrom and Krasse conducted a study of the salivary contents following fixation of orthodontic appliances and found an increase in both *S. Mutans* and *Lactobacillus*.(70) *S. Mutans* is involved in the initiation of the carious lesion and *Lactobacillus* is involved in the further development of the carious lesion.(71) Gwinnett and Ceen proposed that the presence of excess bonding agent known as flash around the base of the bracket can allow for plaque accumulation.(72) The reduction in salivary access to tooth surfaces due to the appliance design may contribute to the accumulation of microorganisms and eventually decrease in the pH of the saliva.(73;74) This drop in pH of the saliva alters the local oral environment leading to decalcification.(75) Murray suggests that a diet consisting of frequent consumption of fermentable carbohydrates plays a significant role in the caries process.(49) Recent research suggests the microorganisms found in plaque may metabolize the polymeric matrix of bonding adhesives including acrylic composites thus creating channels within the

adhesive that increases porosity and pitting. The action of the bacteria weakens the bond at the adhesive-enamel junction and is considered to be a cause of premature debonding and eventually caries.(76) Mizrahi stated that cementing agents and food debris could contribute acid thus perpetuating the decalcification process. The author cited the evidence as presented by Lefkowitz and Bodecker that the liquid portion of zinc phosphate cement, which is 40% to 50% free phosphoric acid, may have a demineralizing effect on tooth surfaces beneath orthodontic bands. This phenomenon may occur with a thinned mix but not with a medium consistency mix.(77-80)

It is well known that sugars play an important role in the development of enamel caries. Due to several variables including tooth resistance, fluoride, salivary properties, and plaque composition, no direct relationship exists between sugar consumption and caries.(81) The relationship between sugar consumption and caries incidence shows that frequent consumption of sugars is directly associated with caries.(15;81-85) The acid produced by the fermentation of sugars results in a plaque pH drop, which initiates decalcification of the enamel. For several years, the major focus of research on constituents of diet associated with caries has been restricted to sugars, sucrose in particular. The close association of the frequency of ingestion of sucrose-containing foods, the duration that sugars are retained in the mouth, and the prevalence of dental caries have been reviewed exclusively.(15;81;86-89) The intake of dietary sucrose has two effects on plaque. First, the frequent ingestion of foods containing sucrose provides a stronger potential for colonization of *S. Mutans*, enhancing the caries potential of the plaque. And second, mature plaque exposed frequently to sucrose rapidly metabolizes it into organic acids, resulting in a profound and prolonged drop in plaque pH.(15)

Organic acids, the metabolic byproducts of the bacterial contents of plaque, cause the dissolution of ions from the enamel.(90) The dissolution of ions from the tooth surface results in its altered appearance. The characteristic appearance of decalcification is described as a white spot lesion referring to the chalky opaque surface of the lesion. This appearance is an optical phenomenon attributed to the subsurface loss of calcified structure that is exaggerated by drying. Conclusions from several studies have been established that describe the characteristics of the early enamel carious lesion.(91-97) Evidence from these studies has supported the concept that the early enamel carious lesion consists of an intact overlying surface with a demineralized sub-surface area.(94) Two theories exist concerning the differing zones. One theory explains that the overlying surface remains intact due to its inherent resistance to dissolution. The other theory proposed by Andersen in 1926 suggests that the minerals from dissolution of the sub-surface area are deposited in the overlying surface zone resulting in remineralization.(94) The mineral-rich enamel surface area is slightly softer and more porous than surrounding enamel. In contrast, the sub-surface zone has a reduced mineral content of 10-70% volume.(15)

Sturdevant describes four zones present in the early carious lesion as seen by a polarized light microscope.(15) *Zone One* is known as the translucent zone. This zone is located in the deepest part and represents the advancing front of the enamel lesion. This zone is present in nearly 50% of lesions and may not extend the entire length of the front of the lesion.(96) This zone marks the initiation of demineralization and contains pores or voids that form along the enamel rod boundaries, cross striations and striae of Retzius. The increased spacing in the translucent zone is a result of the widening of the pores due to mineral loss. Nearly 1% of the volume of the zone is spacing or pores, which is ten times

greater than the pore volume of normal enamel. *Zone Two* is represented as the Dark Zone. This zone is located immediately superficial to the translucent zone and appears dark when viewed with polarized light. The dark zone is present in approximately 85-90% of lesions and has a total pore volume of 2-4%.⁽⁹⁶⁾ This zone appears dark due to the blockage of light by the numerous micropores that are too small to absorb the staining molecules. These pores are thought to be filled with air, which makes the zone appear opaque. It is thought that the dark zone may be formed by the deposition of ions into an area that previously contained only large pores. The loss of crystalline structure in the zone is the result of the demineralization and remineralization. The size of the dark zone may be indicative of the amount of recent remineralization. The presence of the dark zone appears to be related to speed of the lesion advancement through tooth structure.⁽⁹⁶⁾ *Zone Three* is known as the body of the lesion. This is the largest portion of the early carious lesion and is largely a demineralizing area. This zone is positioned between the dark zone and the surface zone. The cross striations and striae of Retzius are well marked indication the preferential loss of minerals along these areas of higher porosity. This zone has the largest pore volume, varying from 5% at its periphery increasing to 25% near the center.^(15;96) There exists several theories about the pathway of demineralization. Sturdevant⁽¹⁵⁾ stated that demineralization travels along the striae of Retzius and the cross striations and demineralizes the enamel rod cores preferentially. Gustafson proposed that it initially affected the periphery of the enamel rod despite his findings of core demineralization occurring first.⁽⁹⁸⁾ Crabb proposed that the demineralization process progresses in a wave-like manner and this causes an unmasking of the striae of Retzius and cross striations.⁽⁹⁹⁾ Several studies have reported that at approximately 300µm to 600µm into the body, the lesions have mineral losses from 25-

75%.(100) *Zone Four* known as the Surface Zone is relatively unaffected by the caries attack and is relatively intact while the degree of demineralization occurs at the subsurface level. This zone is approximately 20-30µm thick and has a radiopacity similar to that of the unaffected adjacent enamel. The surface morphology of the surface zone is comparable to that of normal sound enamel. This zone has a pore volume of less than 5% to nearly 1% and maintains a mineral content of approximately 83% by volume as compared to 87% mineral content of normal enamel.(96) Numerous theories exist that explain the preservation of the surface enamel and the continued demineralization of the subsurface structure. It has been found that the surface layer of enamel is more resistant to demineralization than the subsurface layers. It has been hypothesized that hypermineralization by contact with saliva, increased fluoride content or increased the amount of insoluble protein that may be responsible for the relative immunity of the surface enamel.(15;100;101) Others offer the explanation that the precipitation of minerals from the body of the lesion to the surface layer contributes to the surface zoned resistance to demineralization.(96;102) This theory proposes that the minerals lost from the body zone remineralize the surface zone and preserves this enamel.

The most common characteristic of the incipient lesion is the pattern of prismatic destruction. Initially the enamel prism sheaths enlarge followed by dissolution of the prism cores resulting in arcade-formed sheaths. Finally, the interprismatic material is destroyed. Frank and Nalbandian suggest that this is the reverse order of normal enamel development.(103) Arends and Christoffersen warn that artificially induced caries lesions are not identical to natural enamel lesions.(104) Goldberg suggests that microchannels are

found in artificially induced lesions and are absent in natural enamel lesions.(105) Therefore caution must be used when utilizing these lesions as experimental models.(94)

Decalcification is the basic result of an imbalance between demineralization and remineralization. If this the normal balance is disturbed and decalcification continues cavitation may occur.(106) The growth of the early carious lesions can be arrested or slowed when the diet sucrose level changes or bacterial plaque around the lesion is removed. Progression of the lesion may be slowed as the intact surface layer is broken down providing direct access to the cavitation for cleansing. Upon the loss of the surface layer, the lesion may clinically appear brown, black or yellow due to organic materials incorporated into the lesion. This staining is evidence of the lesion having been present for an extended period of time. Some staining may occur as the result of certain substances such as coffee, tea, or tobacco.(15)

Remineralization may occur if the pH is elevated for an extended period. This elevated pH will allow the influx of minerals from saliva or external sources into the enamel. Several studies suggest that a calcium fluoride surface layer forms when in contact with aqueous fluoride resulting in a layer that is less susceptible to demineralization than the original enamel surface.(96;107-109) It is proposed that a significant amount of time, between 16 to 24 hours, with an increased pH and in the presence of fluoride is necessary to favor an *in vitro* remineralization of enamel.(110) If the acidic challenge is removed in the early stages of demineralization, the remineralization process may cause the lesion to regress or even reverse.(111;112)

PREVENTION OF DECALCIFICATION

Currently several methods exist that will hinder/retard enamel decalcification including a thorough oral hygiene instruction and supervision, fluoride administration, sealant application and proper appliance selection and placement.

Oral hygiene can reduce or control the amount of plaque in contact with enamel which will result in the prevention of decalcification. Continuous reinforcement of oral hygiene habits and evaluation of the oral health status of the patient should be maintained throughout treatment.(113) The prevention or removal of plaque is logical, since in the absence of plaque neither caries nor periodontal disease is found.(114) Holmen(54) reported that professional oral plaque removal on a weekly basis could prevent the progression of an incipient lesion. Such measures are labor intensive and costly.(115) Mitchell suggests that plaque removal by mechanical or chemical means can be effective in the reduction of the rate of enamel decalcification.(116) Tooth brushing is the most common form of mechanical plaque removal and can be performed with manual and electric toothbrushes. Electronic toothbrushes have been found to be more effective in plaque removal than regular manual toothbrushes.(117;118) Specifically designed orthodontic toothbrushes seem to be more effective than regular toothbrushes in removing plaque deposits around brackets. Importance lies in the proper use and frequency of the cleaning. Other studies have shown that sonic toothbrushes are 20 to 47% more effective in removing supragingival plaque than manual toothbrushes.(119;120)

Oral irrigation devices, floss, and interdental cleaning aids may be included in the oral hygiene regimen as adjuncts and must not be considered to be substitutes for tooth brushing.(121) Gwinnett and Ceen recommend the use of a rigorous home care program that

can provide oral hygiene education and motivation for the patient to maintain an optimal level of plaque removal.(122) A chemical plaque removal agent (mouth rinse) is one that can reduce the oral flora by 99.9%(123) without disrupting the oral environment as well as being non-toxic. A varying array of antiseptic rinses is available with chlorhexidine being the most effective.(124) Hogg(123) attributes the effectiveness of chlorhexidine to its absorption onto the acquired pellicle resulting in substantivity. The use of chlorhexidine is associated with the deposition of a brown stain, which can be removed with a professional oral prophylaxis following appliance therapy.(125) Newer rinses that inhibit bacterial adhesion to tooth surfaces also appear promising, and it is suggested that more work on combinations of active ingredients is necessary.(126)

Fluoride administration has been proposed as a method of reducing enamel susceptibility to decalcification.(127) Many consider the use of fluoride to be an effective approach in the prevention of enamel caries in that it affects the caries process by enabling the formation of high quality fluorapatite that aids remineralization and inhibits glycolysis of plaque microorganisms.(128-130) Several manners of fluoride administration have been investigated including professionally applied gels, home rinses, and fluoride containing etchants, bonding agents, cementing media, and modules (elastics).(131) Fluoride rinses have been shown to be effective in reducing the incidence of white spot formation in orthodontic patients. Zachrisson found that professionally applied fluoride gel was beneficial in the prevention of enamel caries, however, it was not cost-effective.(132) In a study by O'Reilly and Featherstone it was found that the combined daily use of fluoride dentrifice and mouthrinse (0.05% sodium fluoride) provided optimal protection from enamel decalcification during orthodontic therapy.(133) Van der Linden and Dermaut(134) suggest that

decalcification may be reduced in orthodontic patients by employing a meticulous oral hygiene program including the use of fluoride. Geiger implied that a one-time fluoride application immediately prior to fixation of orthodontic appliances is ineffective in reducing the incidence of decalcification.(135) In this same study, decalcification was significantly reduced by 25% by the consistent use of a 0.05% sodium fluoride rinse during treatment. The authors also found only 50% compliance in their study despite extensive patient and parent education. If patient compliance is poor, plaque removal techniques and fluoride programs will be ineffective in preventing enamel decalcification. Therefore, clinicians have been focusing on techniques that reduce enamel susceptibility to decalcification while eliminating the requirement of patient compliance.

FLUORIDE VARNISH

Fluoride containing varnishes were developed during the late 1960s and early 1970s in an effort to improve shortcomings of existing fluoride vehicles, such as fluoride gels or mouth rinses, by prolonging contact of the fluoride with tooth enamel. By the 1980s, fluoride varnishes were widely used in European countries.(136) Fluoride varnish (Duraphat) contains 5% sodium fluoride (22,600 parts per million (ppm) F^- and an application of 0.3-0.5 milliliter would provide 6.8-11.3 milligrams of F^- .

Fluoride varnishes are not intended to adhere permanently to a tooth, but to remain in close contact with enamel for several hours. Tooth brushing may be sufficient to clean the teeth before application and prophylaxis is not required.(137) Fluoride varnish needs to be reapplied to maintain its caries-prevention effect.(138;139) Various application schedules have been proposed and semi annual application has been tested most often. It is also shown that Duraphat is most active if application is repeated after 3 months.(140)

With topical application of high concentration of fluoride (Duraphat), the main product deposited on the enamel surface and subsurface lesions is calcium fluoride.(141) Calcium fluoride may serve as a reservoir for fluoride ions to be redeposited as fluorapatite during remineralization.(142-144) Fluorapatite remains permanently bound within the crystalline structure of the enamel.(145) Numerous studies have been conducted to show the efficacy of Duraphat in the reduction of caries with results ranging from 18 to 77 percent , as reviewed by de Bruyn and Arends.(146) For studies utilizing a split mouth design, concern has been raised about possible crossover of fluoride varnish onto control teeth.(136;147) This effect would increase Type I error, that is, accept null hypothesis when, in fact, there is a difference in the preventive effect of varnishes compared with control.

For in vivo lesions induced under orthodontic bands, Oggard et al showed a 48% reduction of lesion depth with fluoride varnish.(148) Todd et al., evaluated the effect of fluoride varnish on demineralization adjacent to orthodontic brackets and found a 50 % less demineralization with the varnish.(149) Since demineralization occurs in about 50 % of patients undergoing orthodontic treatment, prophylactic use of fluoride varnish has been suggested. Bowman (2000) described the technique of application of fluoride varnish after bracket placement.(150) With lip retractors in place, teeth are air-dried, a thin layer of varnish is applied and after 5 minutes the retractors are removed. The patient should be instructed not to brush for 24 hours. The varnish has a fast setting time and sets up upon contact with saliva.

Despite fast setting time and the small dosage used, the risk exists that young child will ingest some of the product during placement. In addition, as fluoride is released from the varnishes after treatment, some fluoride will be ingested. Roberts and Longhurst reported that

a mean of 5.2 mg F⁻ (range, 0.7 to 14.5 mg F⁻) was applied.(151) According to the authors, no one received acute toxic levels (1 mg F⁻ per kilogram of body weight). Ekstrand and associates reported peak plasma fluoride concentration of 3.2 to 6.3 micromolar within two hours of treatment, followed by rapid two-hour decrease and a slower decrease thereafter.(152) These levels were comparable with those found after brushing with a fluoridated toothpaste (mean +/- standard deviation, 3.63 +/- 0.45 micromolar/L) or after ingesting a 1-mg F⁻ tablet (4.47 +/- 0.47 micromolar/L),(153) and were considerably lower than those reported for APF gels (16 to 76 micromolar/L).(154) Isakson et al. reported a case of dermatitis and another case of stomatitis.(155) These allergies were likely related to the colophony component of the varnish. The use of the product is contraindicated in patients with ulcerative gingivitis and stomatitis.

SEALANTS

Sealants, a protective chemical barrier on the tooth surface is an acceptable modality for reducing enamel decalcification.(156) Enamel sealants have been evaluated for effectiveness in inhibiting enamel decalcification during orthodontic treatment. In a study by Ceen(157) the thinness of the applied sealant layer was cited as the reason for the ineffectiveness in inhibiting enamel decalcification. It was suggested that the viscosity and filler load be increased to improve the effectiveness. Brant and Zachrisson stated that the effectiveness of a low viscosity resin sealant is due to its penetration into acid-etched enamel where it polymerizes and protects the enamel against a cariogenic challenge.(92) Another study found that decalcification was reduced by 13% when a viscous sealant was used.(158) Zachrisson testified that sealants provide protection, increase the resin bond strength, reduce need for strict moisture control during appliance placement and facilitate the appliance

debonding process.(159) Chemically cured sealants were shown to be ineffective as a result of incomplete polymerization of the thin sealant layers. The air-inhibited layer causes the incomplete polymerization. Chemically cured sealants also contained numerous voids within the thin sealant layer. Light cured sealants appear to have more complete polymerization and are more effective.(160) Even after the thin sealant layer has worn away, the sealed teeth were more resistant to demineralization. Joseph explained that the presence of resin tags in the enamel occupied potential sites of demineralization.

SELF ETCHING PRIMERS

Conventional adhesive systems use 2 different agents (an etchant conditioner and adhesive resin/sealant) in the process of bonding orthodontic brackets to enamel. A unique characteristic of some new bonding systems in operative dentistry is that they combine the conditioning and priming agents into a single acidic primer solution for simultaneous use on both enamel and dentin.(161;162) Combining conditioning and priming into a single treatment step result in improvement in both time and cost-effectiveness to the clinician and, indirectly, to the patient.(163) A single step adhesive system combines etchant and primer in one chemical compound, and are referred to as the sixth-generation adhesives. Differing from a traditional etch/seal system, lacking a sealant layer. One of these is Transbond Plus Self-Etching Primer[®] (3M Unitek) that employs this new chemistry.

A self-etch approach to bonding involves either a two or one step application procedure. The self-etch effect should be ascribed to monomers to which one or more carboxylic or phosphate acid groups are grafted.(164) Depending on etching aggressiveness, they can be subdivided into a “strong” and “mild” self-etch adhesive based on the pH.(165)

“Strong” self-etch adhesives usually have a pH of 1 or below. This high acidity results in rather deep demineralization effect. At the enamel the resulting acid-etch pattern resembles a phosphoric acid treatment following an etch and rinse approach.(164;166;167)

The chemistry of Transbond Plus Self-Etching Primer (pH = 1) is similar to that of phosphoric acid, with two primer chains that form a solid primer matrix upon curing.(168)

Transbond Plus The ingredients in the Transbond Plus self-etching primer consists of two groups; 75 – 85% by weight of a methacrylate ester derivative and 15 – 25% by weight of water. The component is then rubbed on the surface of the tooth causing a mass-transfer of product deep into the enamel, causing no damage to the enamel rods. The liquid begins to etch the enamel as soon as it is applied, but it changes to a primer once the two-hydroxide chains are converted and hydrogen is released. Because the monomers that cause the etching are also responsible for bonding, there is no need for rinsing and the depth of penetration of the monomers to be polymerized is exactly the same as the depth of demineralization, resulting in a complete hybrid layer.(10)

Sixth-generation adhesives were originally developed to adhere to dentin and reduce post-treatment sensitivity; since orthodontic bonding surfaces are only enamel, however, the etch patterns are consistently similar to those produced by etching with phosphoric acid.(169)

The unit-dose setup of Transbond Plus (3M Unitek) is designed for bonding an entire dental arch. After the teeth are pumiced, rinsed and dried as usual, the Transbond Plus is activated by popping two reservoirs and mixing the components together with an applicator, the applicator than gently rubbed onto each enamel surface for two to five seconds(11) with the microbrush contained in the package. As the pH rises, the etchant converts to the primer-matrix. The primer is then thinned with a burst of air, adhesive-coated brackets are placed

and any excess adhesive is removed with a scaler. After each bracket is light-cured interproximally for 10 seconds, the archwire can be tied in.

Traditionally, orthodontists still use the acid-etch technique when bonding brackets to patients. This system was introduced to orthodontic bonding by Newman in 1965.(170) Many different bonding systems and techniques have evolved since its conception.(171-173) Light Bond (Reliance) incorporates the acid-etch technique into its system. Following etching with 37% phosphoric acid, the tooth is thoroughly washed and dried. The sealant/primer (Light Bond) is then applied and light cured for 10 seconds. The sealant/primer provides a chemical protective barrier and adhesive system, as mentioned earlier, adhesive coated brackets are then placed and any excess is removed with a scaler prior to final cure.

Transbond Plus would benefit the patient in time saved, as well minimal etching required to achieve the optimal results of bonding. All the exposed enamel rods are immediately filled with the primer solution leaving no unexposed enamel rods that can be subjected to oral insult following bonding.

A recent *in vitro* study (Tanna et al) comparing demineralization between self-etching primer and conventional sealant showed there is a significant difference in the incidence of lesion formation between a self etching primer and a conventional sealant when exposed to an *in vitro* acidic challenge. This same study showed self etching primer shows no resistance to demineralization and resulted in a 100% incidence of lesions formation *in vitro*.(174) This study will use self-etching primers *in vivo* to determine whether decalcification is influenced by these new sixth-generation adhesives systems.

IN VIVO DECALCIFICATION STUDIES

Decalcification studies that are done *in vivo* are different than *in vitro* decalcification studies, in that the specimen cannot be extracted for microscopic evaluation following sectioning of the tooth. Photographic interpretation has been used as a mean to qualify decalcification. Photographic data has shown that both the individual variability of enamel surfaces and the variability of a single tooth can be determined.(175) Millet et al. used photographic interpretation in a study in 1996.(176) Photographic data used in this study gives an enlarged permanent record which can be rescored as many times as required. Allowing for longitudinal comparison, it was found that the lesions appearing clinically as white spots may also comprise microcavities. The enamel surface changes were classified at a magnification of 20X by three observers. At debonding and after 1 and 2 years, tooth surface conditions were recorded photographically and visually inspected as before. Classification was made according to a modification of a scoring by Geiger et al.(177) as follows: 1 = no white spot formation, 2 = slight white spot formation, 3 = severe white spot formation, 4 = excessive white spot formation with cavitation. In case of disagreement between the observers, the concordant classification between the three observers holds good.

A search of the literature revealed that earlier studies on the etiology of caries employed photographs to assess and quantify the extent of enamel surface changes.(178) This approach was modified in Mitchell's study as it gives a magnified permanent record, which can be rescored. In addition, this method allows the size of the lesions to be quantified. A Nikon F2 camera plus a 135-mm Nikon lens was used with a bellows extension of 190mm. A specially adapted light source was mounted on a pair of head phones to minimize reflection on the tooth surface as this would mask any enamel surface change.

The photographic technique used a specially adapted light source below the level of the teeth to minimize surface reflection. For an accurate assessment of the extent of any decalcification the photographs needed to be taken perpendicular to the tooth surface. Depending upon the curvature of the upper labial segment, three to six views were needed to accurately record upper right canine to upper left canine. Each patient was photographed before and after treatment, and where possible after 3 months post-treatment observation. Kodak plus X black and white film was used and the prints enlarged to 6X to show maximum detail. The pre and post-treatment photographs were examined in light microscope, thus idiopathic enamel opacities present at pre-treatment could be discounted.(179) Initially, a qualitative assessment of the presence or absence of decalcification was made. Any affected areas were scored after: 0 = no enamel surface change, 1 = grayish discoloration, 2 = distinct white decalcification, 3 = cavitation. A reproducibility study was carried out on 24 teeth to determine the accuracy of assessing the presence or absence of decalcification and the quantification of the changes seen. The reproducibility study on 24 teeth showed 100% agreement on the presence or absence of the decalcification. Five of the 24 teeth showed evidence of enamel surface change and when the decalcification was expressed as a percentage of the area of the tooth surface, a paired t-test showed no significant difference between the two sets of measurements. No significant difference was found between the two sets of measurements. No significant difference was found between the numbers of test and control teeth with decalcification.(180)

A reproducible photographic method would provide three major advantages according to Hill et al.(181) Number 1) It would reduce the examination time to a few minutes. 2) The size as well as the intensity of the areas of altered enamel could be recorded. 3) The

scoring could be double blind (the examiner not knowing from which subject and at what stage of the experiment the photograph was taken) with multiple examiners and thus greatly increase the accuracy of the test.

Geiger et al.(182) recorded the date each tooth was debonded and the labial enamel surface was visually examined in an illuminated and relatively dry field. White spot formation, as reported previously, was scored. For purposes of simplicity, the data were analyzed according to the absence versus presence of white spots. At the termination of active treatment and debonding of remaining brackets, an evaluation of oral hygiene status was recorded for each patient as poor, acceptable, or excellent on the basis of the treatment record.

Most clinical studies of lesion progression have relied on visual inspection of the loss of translucency and the extent of the opaque area. In Linton's study,(183) three in vivo lesion measuring methods were explored, namely, caries index, subtraction radiography and optical instruments. Pilot experimentation proved that subtraction radiography of smooth surface lesions to be insensitive to minute changes in mineral content of the lesions. An experimental optical instrument producing light scattering phenomena appears to be promising as an in vivo method, in that it is nondestructive and shows good correlation with mineral loss, but this method has not yet reached clinical application. The third method caries index was used as a visual inspection, such that as the photographic interpretation, and where able to qualify their measurements with clinical appearance.

Gorelick et al.(184) examined the presence of white spots on the labial surface of individual teeth and scored the lesions as follows: 1 = No white spot formation, 2 = slight white spot formation, 3 = excessive white spot formation, 4 = white spot formation with

cavitation. After pumicing for a few seconds and drying with a stream of compressed air for 10-20 seconds the carious state of the vestibular enamel of premolars, canines and incisors in both jaws was recorded under direct illumination from a dental lamp. The following scoring system was used: (A) Caries index system as proposed by Fehr in 1961 for the assessment of the opacity of the carious white spots. Score 0 = surface appears intact, 1 = limited grayish tinge, with or without accentuated perikymata, 2 = perikymata well accentuated, in some areas confluent into grayish white spots, 3 = pronounced white decalcification. Desintergration of the enamel surface was registered as cavitation. (B) A modification of the scoring system proposed by Gorelick for assessment of the extension of carious white spots. Score 0 = no white spot formation, 1 = white spot lesion involves less than one third of the vestibular enamel surface area outside the area covered by the bracket and bonding material during treatment, 2 = white spot lesion involves more than one third, but less than two thirds of the vestibular enamel surface area in question, 3 = white spot lesion involves more than two thirds of the vestibular enamel surface area in question. Scorings were performed on mesial, distal, gingival and incisal aspects of each vestibular tooth surface. Evaluation was performed jointly by the authors, in a few cases of disagreement the scores were arrived after discussion. The reproducibility of the measurements for opacity and extension of the carious lesions were tested. Eleven patients were randomly selected from one test group and re-examined after one week. The mean score per tooth for each patient was then calculated. Pearson's product moment correlation coefficients were calculated between first and second measurement of extension of the lesions. The difference between double measurements never exceeded one score unit. On four of the 206 teeth re-examined a carious white spot was diagnosed as present at one examination, and not at another.(185) The mean score per

tooth per subject was calculated for both scoring systems. Mann-Whitney's non-parametric test was conducted to determine any statistically significant differences in score between the groups. In addition, the number of teeth with carious white spots, regardless of localization, opacity and extension, was calculated for each person. Chi-square test was used to determine any statistically significant differences among the groups in distribution of patients with teeth affected by carious white spots.(185)

SCANNING ELECTRON MICROSCOPE

The scanning electron microscope (SEM) is a research tool often employed in dental research.(186) The SEM allows the examination of the entire surface of an object at both low and very high magnifications, whereas the whole surface cannot be examined with the light and transmission electron microscopes. In addition, the depth of focus of the SEM is 300 times greater than that of conventional instruments.(187)

Electron microscopes were developed due to the limitations of light microscopes which are limited by the physics of light to 500x or 1000x magnification and a resolution of 0.2 micrometers. The Transmission Electron Microscope (TEM) was the first type of electron microscope to be developed and is patterned exactly on the light transmission microscope except that a focused beam of electrons is used instead of light to see through the specimen. Scanning Electron Microscopes (SEM) debuted in 1942 with the first commercial instruments around 1965.(188)

Conventional light microscopes use a series of glass lenses to bend light waves and create a magnified image. An SEM shows very detailed 3-dimensional images that are created without light waves and are rendered in black and white. Samples have to be prepared carefully to withstand a vacuum within the microscope. Specimens are dried in a

special way that prevents distortion of the object. Because the SEM illuminates them with electrons, the specimens have to be made to conduct electricity. This is done by coating the object in a very thin layer of gold by a machine called a sputter coater. The sample is then placed inside the microscope's vacuum column through an air-tight door. After the air is pumped out of the column, an electron gun (at the top) emits a beam of high energy electrons. The beam travels downward through a series of magnetic lenses designed to focus the electrons to a very fine spot. Near the bottom of the SEM, a set of scanning coils moves the focused beam back and forth across the sample, row by row. As the electron beam hits each spot on the sample, secondary electrons are knocked loose from its surface. A detector counts these electrons and sends the signals to an amplifier. The final image is built up from the number of electrons emitted from each spot on the sample.(189)

Poole and Johnson studied the effects of formic, lactic, hydrochloric, and ethylene diamine tetra-acetic acids on enamel surfaces by SEM.(190) They pointed out that the natural surfaces of enamel showed minimal recognizable structure before etching because of adherent plaque. They also pointed out that the acids "dissolved the axial portions of prism heads so that etched surfaces transverse to prisms had a honeycomb appearance, etched surfaces parallel with prism direction showed troughs and ridges.(190) Hoffman et al evaluated the SEM for ultrastructural studies of tooth enamel and found it well suited for such studies.(191) Hoffman agreed with Poole and Johnson that acid etching causes demineralization of prism cores.

One can hope that the application of an SEM in a study of the effect of pumicing, surface treatment, and bonding to tooth surfaces would open avenues of knowledge to

improve the selection and properties of an adhesive as well as suggest supplementary techniques.(191)

CHAPTER III

MATERIALS AND METHODS

INTRODUCTION

This chapter describes the samples, methods of data collection, statistical analysis, materials, method of error, and protocol that was used in this *in vivo* study.

SAMPLE DESCRIPTION

Twenty five sequential patients who meet the criteria for selection at the Department of Orthodontics, West Virginia University School of Dentistry, were assigned for this study. The criteria of selection will included: 1) patients with permanent dentition in both, maxillary and mandibular arches, 2) no previous orthodontic treatment, 3) comprehensive orthodontic treatment, with fixed appliances, completed between 18 and 24 months, 4) no presence of decalcification on the surface of the tooth to be bonded in this study, 5) teeth to be bonded are maxillary and mandibular 2nd premolar to 2nd premolar.

METHODOLOGY

The patients chosen for this study first met the criteria for selection as previously stated. A split mouth technique process was employed in this study, allowing each patient to serve as their own treatment and control groups. Alternating opposing arches were assigned to the patients, in sequential order, to randomize the study. Patient data for analysis at

completion of treatment consisted of the following groups; hygiene compliance score, bonding agent, location of bonding agent, and amount of decalcification.

Plaque scores were taken according to the O'Leary Plaque Index(192), to determine oral hygiene compliance throughout treatment. An initial plaque index was taken prior to treatment. Four periodic plaque scores were done during treatment, as well as one at the completion of treatment. Each patient will have a total of 6 plaque scores. Each plaque score is recorded in percentage by summing the total # of surfaces with plaque and dividing by the total # of surfaces examined then multiplying by 100. Plaque scores were then averaged to determine the patient's final hygiene score. Based on the final hygiene score, a patient was assigned a hygiene grade of excellent (E), good (G), fair (F) and poor (P). (E = 0-20% plaque, G = 21-40%, F = 41-60%, P = 61-100%). Plaque scores will be recorded on treatment bonding forms located in Appendix B.

All patients received the same oral hygiene instructions following bonding procedures, and at periodic times during treatment when needed. This instruction included, flossing and brushing with fluoridated toothpaste.

Plaque Score Technique

1. Suction out all saliva.
2. Apply disclosing solution to all surfaces.
3. Gently rinse and suction saliva and water out mouth.
4. Record disclosed surfaces on chart below (excluding all molars).
5. Divide total surfaces with plaque by total # of surfaces examined.
6. Multiply by 100 to get a percentage.
7. Record percentage.

Two different bonding techniques were used for this *in-vivo* study; 1) a conventional etch-sealant system, see Figure 1 (Enamel etchant and Light Bond, Reliance Orthodontic Products, Inc., Itasca, IL) and 2) a self etching primer, containing no sealant layer, see Figure 2 (Transbond Plus, 3M Unitek, Monrovia, CA).

Figure 1. Conventional etch-seal bonding system by Reliance

Figure 2. Self-etching Primer by 3M Unitek

Bonding techniques will be done according to manufactures recommendations.

Bonding Technique – Conventional Etch/Sealant System (CES)

1. Prophyl teeth with non-fluoride oil-free pumice.
2. Air dry thoroughly using oil and moisture-free air source.
3. Isolate the teeth to be bonded.
4. Apply etching agent with applicator (37% phosphoric acid).
5. Allow 30 seconds for etching, rinse each tooth for 10 seconds.
6. Dry each tooth thoroughly, area should appear frosty white.
7. Apply thin coat of primer/sealant to tooth to be bonded.
8. Light cure sealant for 10 seconds.
9. Apply bracket with composite to tooth, wipe off excess composite and cure each side of bracket for 10 seconds.

Bonding Technique – Self Etching Primer (SEP)

1. Prophyl teeth with non-fluoride oil-free pumice.
2. Hold sheath of disposable applicator in one hand. Use the thumb and index finger of the other hand to completely squeeze the liquid out of the black reservoir towards the disposable applicator.
3. Completely empty the contents of the black reservoir into the white (middle) reservoir by squeezing the liquid forward and carefully fold back the package at the interface of the black reservoir, keep the black reservoir tightly squeezed with thumb and index finger while bending the package.

4. Starting at the far end squeeze the liquid from the white (middle) reservoir into the purple (smallest) reservoir of the blister package using controlled pressure.
5. Once the liquid was transferred to the purple (smallest) section of the blister pack, churn and swirl the applicator inside purple reservoir for 5 seconds to completely mix chemicals and thoroughly coat applicator tip.
6. Remove applicator from the reservoir. The applicator tip must have a light yellow color. If not yellow, re-squeeze contents from outer reservoir to middle reservoir to small reservoir and mix well using a churning and swirling motion.
7. Rub the saturated tip of applicator onto tooth surface. Continue rubbing liquid onto enamel while applying some pressure for a minimum of 3-5 seconds per tooth.
8. Redip applicator into reservoir to saturate tip before rubbing it onto next tooth.
9. Repeat steps 6 through 8 for each tooth.
10. When all teeth on one arch are primed, use an oil and moisture-free air source to deliver a gentle air burst to each tooth to dry primer into a thin film. Direct air stream away from gingival. One Transbond Plus self etching primer unit will etch and prime all teeth on one arch only.
11. Proceed immediately with bonding. If bonding was delayed, another coat was applied, delivered a gentle air burst to dry primer into a thin film and bonded bracket.
12. Light cured.

Twelve patients were bonded with the conventional etch/seal system (CES) on the maxillary arch and the self etching primer (SEP) on the mandibular arch, while the other thirteen patients had the SEP on the maxillary arch and the CES system on the mandibular arch. Documentation of the location of bonding agent was recorded on the pre-treatment bonding sheet shown in Appendix B.

The criteria for selection of patients in this study presented with no pre-treatment decalcification. Once patients have completed treatment, teeth decalcification scoring was followed using the scoring system as described by Geiger et al.(193) Clinical exams will be performed using both tactile and visual senses to determine the score of the designated tooth.

Geiger used photographic interpretation in his study to determine decalcification in his study. A Nikon F2 camera plus a 135mm Nikon lens was used with a bellows extension of 190mm.(194) The 190mm extension can produce almost double the size of print. Due to the inability to obtain the similar lens, it was decided to use tactile and intra-oral examination as the method for scoring.

Decalcification was scored based on amount and severity of condition of selected teeth. The scoring system followed: Score 1 = no white-spot formation/decalcification, Score 2 = slight white-spot formation/decalcification (one area), Score 3 = severe white-spot formation or multiple areas of decalcification, Score 4 = Excessive white-spot formation, cavitation. Scoring was recorded on the post-treatment bonding form seen in Appendix B.

Following completion of treatment all patients will have a hygiene score, type and location of bonding agent, and decalcification score. All groups will have the same criteria and correlate to the type and location of bonding; the level of hygiene compliance based on

their plaque score; the decalcification score and location of the bonding agent. Subjects were then analyzed according to the following groups.

Group #1) Decalcification lesion by teeth. This showed a selective distribution in the *occurrence* of white spots for teeth.

Group #2) Decalcification lesion by location. This showed a comparison of maxillary and mandibular arches.

Group #3) Decalcification according to hygiene compliance. This showed a distribution according to occurrence and severity based on hygiene grade of poor (P), fair (F), good (G), and excellent (E).

Group #4) Decalcification according to the bonding agent. This showed a distribution of occurrence and severity based on the type of bonding agent that was used.

Group #5) Decalcification according to the bonding agent used and the occurrence and severity based on a hygiene grade of poor, fair, good and excellent.

SCANNING ELECTRON MICROSCOPE

Four extracted 3rd molars will be analyzed by a SEM to compare surface irregularities associated with the different bonding agents employed in this study. Preparations for the teeth are done accordingly; Tooth #1 - prophy tooth with non-fluoride oil-free pumice, rinse with water and allow 24 hours to dry; Tooth #2 – prophy tooth with non-fluoride oil-free pumice, rinse with water, apply 37% phosphoric acid etchant for 30 seconds, rinse with water and allow 24 hours to dry; Tooth #3- prophy tooth with non-fluoride oil-free pumice, rinse with water, apply 37% phosphoric acid etchant for 30 seconds, rinse with water 10 seconds and dry, apply thin coat of sealant according to manufactures protocol (see previous bonding

technique for CES), light cure and allow 24 hours to dry; and Tooth #4 - prophy tooth with non-fluoride oil-free pumice, rinse with water and dry, follow instructions for SEP bonding technique as stated above, light cure for 30 sec and allow to dry for 24 hours.

Once teeth have been prepared, each tooth will be individually analyzed with a 1500x magnification factor, by the JEOL scanning electron microscope, model # JSM-6400, manufactured in Tokyo, Japan. While in the SEM an X-ray spectrum analysis will be done on the CES and SEP surfaces using the Princeton Gamma Tech X-ray spectrometer, manufactured in Princeton New Jersey, all images will be used for the purpose of discussing surface differences.

STATISTICAL ANALYSIS

Statistical significance in the difference of frequencies of decalcification among the groups will be determined by using ANOVA, one-way analysis of variance, Tukey Kramer multiple comparison procedure, and reliability coefficient to determine the method of error. Statistical significance was set at $p < 0.05$. Analysis will be figured according to groups listed below.

SYSTEM OF ERROR

Determining the method of error was done by rescoring 226 surfaces. This was done between 1 and 2 months following the initial decalcification score. The operator was unaware of the location of bonding agent, the previous hygiene score, and previous decalcification score for that surface. The surfaces were then compared to the original score and a reliability coefficient was created for this model.

CHAPTER 4

RESULTS AND DISCUSSION

RESULTS

As mentioned previously, Geiger used photographic interpretation in his study to determine decalcification in his study. A Nikon F2 camera plus a 135mm Nikon lens was used with a bellows extension of 190mm.(195) The 190mm extension can produce almost double the size of print. Providing the clinician with up close visual detection projected on a large screen. Due to the inability to obtain the similar lens, it was decided to use tactile and intra-oral examination as the method for scoring decalcification, while continually following the clinical methodology developed by Geiger(196), we were able to obtain the following results.

Decalcification Score of Teeth

When scoring individual teeth as a whole, there were a total of 469 teeth studied. Taking no other categories into consideration except the decalcification score; it was found that 371 (79%) teeth presented with a score =1 (no white spot formation), 61 (13%) teeth presented with a score = 2 (slight white spot formation/decalcification), 33 (7%) teeth presented with a score = 3 (severe white spot formation or multiple small areas of decalcification), 4 (1%) teeth presented with a score = 4 (cavitation).

Decalcification Score by Location

There were 237 teeth represented in this study that were bonded on the mandibular arch and 232 teeth on the maxillary arch. Although there was a slight difference in the overall decalcification between maxillary and mandibular arches, the difference was not found to be significant ($p = 0.85$). The mean score for the decalcification on the mandibular arch and maxillary arch was 1.29 and 1.30 respectively.

Refer to Figure 3.

Figure 3. Decalcification score according to location

Decalcification Score by Hygiene Compliance

Of the 469 teeth in this current study, it was shown that 32.4% reported excellent hygiene; 40.3% for good hygiene; 12.4% for fair hygiene; and 14.9% teeth were associated with poor hygiene. The relationship between decalcification score and hygiene score was investigated.

A significant difference was found between the hygiene status and decalcification score ($p < 0.0001$). When using a Tukey-Kramer multiple comparisons test, there was no significant difference between excellent and good hygiene. However, significant differences were found comparing all other groups; E and F groups, E and P groups, G and F groups, G and P groups, and groups F and P.

The mean decalcification score of all patients based on level of hygiene increased in score as hygiene level changed from excellent to poor. Refer to Figure 4.

Figure 4. Decalcification score according to level of hygiene compliance

Decalcification Score by Bonding Agent Groups

When scoring total number of teeth with regards to bonding agent, of the 236 teeth examined using the SEP, 171 (72.4%) teeth bonded presented with a score of 1 (no white spots); 41 (17.4%) teeth with a score of 2 (slight white spots); 21 (8.9%) with a score of 3 (multiple areas of white spot formation); 3 (1.3%) teeth with a score of 4 (cavitation). With 233 teeth examined using CES, 200 (85.6%) teeth scored a 1; 20 (8.6%) teeth scored a 2; 12 (5.4%) teeth with a score of a 3; and 1 (0.4%) tooth with a score of a 4. Refer to Figure 5.

Figure 5. Decalcification score according to bonding agent

When decalcification according to teeth, was analyzed according to the bonding agent used, it was shown that the SEP group recorded nearly double the amount of teeth (65 teeth) affected by decalcification, compared to the CES group (33 teeth). A one-way analysis of variance showed that there was a significant difference between the bonding agents ($p = 0.001$). The mean score of all teeth recorded in the SEP group was 1.39 and the CES was

1.20. See figure 6.

Figure 6. Overall decalcification score according to bonding agent

Decalcification Score by Bonding Agent and Hygiene Interaction

Decalcification score, bonding agent, (CES or SEP), hygiene level (E, G, F, or P), and location of agent were correlated, analysis of variance (ANOVA) showed that there was a significant hygiene level (E, G, F, or P) by bonding agent (CES or SEP) interaction according to decalcification score ($p < 0.0001$). The least square mean for hygiene level decalcification scores were as follows: Excellent Hygiene Group, bonding with CES was 0.99 and with SEP was 1.02: For Good Hygiene Group, bonding with CES was 1.04, while 1.17 with SEP: For the Fair Hygiene Group, bonding with CES was 1.42, while bonding with the SEP was 1.91: and for the Poor Hygiene Group bonding was 1.85 with the CES, while 2.40 when bonding with the SEP. Refer to Figure 7.

Figure 7. Decalcification score according to agent and hygiene interaction

The different decalcification score between poor and excellent hygiene levels in bonding agent CES is less than the difference in bonding agent SEP. Figure 8 indicates that as the patient's hygiene compliance decrease, there was a change in the difference in mean decalcification score between CES and SEP groups.

Figure 8. Mean decalcification score differences between groups

System of Error

The method of error for this study was done by rescoring 226 surfaces. This was done between 1 and 2 months following the initial decalcification score. The operator was unaware of the location of bonding agent, the previous hygiene score, and previous decalcification score for that surface. The surfaces were then compared to the original score and a reliability coefficient was created for this model. The reliability coefficient of 0.903, indicating that there was no significant difference ($p < 0.0001$) that would affect the original data.

Scanning Electron Microscope Images

The accompanying photomicrographs of an area on the labial surfaces of pretreated extracted teeth represent the surface changes that occurred on the samples studied. Although individual teeth as a whole may present with slightly different appearances, the tooth differences described here can be considered as representative.

Figure 9 demonstrates the tooth surface after pumicing (non-fluoride oil-free pumice) with a hand piece and rubber cup. With the disappearance of the organic (soft) material, the patterned appearance of enamel rods, with the enamel prisms still intact and the cores not exposed with accentuation of lamella and pits being visible.

Figure 9. SEM of pumiced enamel surface (1,500x).

Figure 10 demonstrates tooth surface morphology following treatment with 37% phosphoric acid for 30 seconds. This type of etch pattern corresponds with Silverstone's classification pattern Type I.(197) The *Central Etch Type* shows a preferred dissolution of central prism portions, thus constituting a honeycomb-like image. The etch pattern exhibits hollowing of the prism cores with intact peripheral borders. The various prism heads have crest-like peripheries. Their jagged contours vary from the circular basic shape, adopting an arcade-like, pointed, or double to triple-peaked appearance. Each prism head is surrounded by a fine marginal cleft of about $0.2\mu\text{m}$.(197)

Figure 10. SEM of etched enamel surface (1,500x)

Figure 11 demonstrates the surface appearance following a 37% phosphoric acid treatment as seen in Figure 10 and the application of a cured sealant layer. Note the properly polymerized sealant layer covering the previously exposed enamel rods (Figure 10). Sealant penetrates enamel prisms prior to curing, creating sealant tags, which are beneficial to the retention of the sealant layer. The continuity of the sealant layer filling the deep tags, left by the etching process, along with the presence of the filler in the sealants are also evident.

Figure 11. SEM of CES coated enamel surface (1,500x).

Figure 12 is the surface appearance of a self-etching primer following the pumicing process seen in Figure 9. Note the scattered appearance of the enamel surface with no real regular pattern, lacking the characteristic honeycomb pattern, presenting with differing sizes of opacities and lucent areas. The surface appears to be lightly covered by a thin layer of polymerized primer. Surface remains somewhat porous.

Figure 12. SEM of SEP coated enamel surface (1,500x).

Figure 13 demonstrates the x-ray spectrum calcium peaks associated with the SEM images taken on the SEP and CES surfaces. The two calcium peaks are identical with both images.

Figure 13. Calcium peaks of SEP and CES taken from an SEM.

DISCUSSION

This study compared two different bonding agents, a conventional etch/seal (CES) and a self-etching primer (SEP) which lacks a sealant/adhesive agent, and the amount of decalcification that may result with using them to bond orthodontic brackets in patients during treatment.

The study's population consisted of 25 patients totaling 469 teeth bonded with orthodontic appliance. These teeth were followed between 18 and 24 months. 20.9% of all teeth had some form of decalcification present after treatment. When teeth with decalcification were broken down into treatment groups (CES and SEP), the CES group (control) had 13.9% of the teeth with some form of white spot formation, compared to 27.5% in the SEP group. This is higher than previous studies investigating the significance of white spot formation following bonding by Geiger in 1988(198) and Gorlick in 1982(199). However, the incidence of decalcification is slightly lower than a study by Millett et al in 1999.(200)

Millett et al reported that the mean number of teeth affected per patient increased by about 30%. Geiger et al found that of the entire sample that only 7.5% of the teeth presented with white spot formation. However, his sample included in office and home fluoride application procedures with acidulated phosphate gel. Gorlick et al's study, which did not include a fluoride treatment, reported 11.3% of white spot formation, which was similar to what we found in the control sealant group (CES). The main difference could be equated to the large sample size and the fact that Gorlick included molars in his study. Sonis and Snell(201) reported a 13% incidence of decalcification in the control group in their study, which corresponds to 13.9% of decalcification in our control group. Geiger et al does not

state the type of adhesive system employed in his study. It can be speculated that the quality of the sealant 15 years ago may not be the same today.

The SEP group, which reported 27.5% of teeth affected with some form of white spot formation, doubling the occurrence of incidence present in the CES group in this study and other studies control group's.(202;203) Difference in decalcification incidence between the SEP and CES could be due to the fact that the SEP contains only etch and priming agents but lacking a sealant layer. This observation is substantiated in an *in vitro* investigation comparing demineralization between SEP and conventional sealant. Tanna et al reported that an application of a sealant provided resistance to demineralization and in 50% of the samples where SEP provided no resistance to demineralization (100% incidence of lesion formation).(174)

The location of decalcification was not a significant factor in this study, although there was a slight difference in the mean decalcification score between maxillary and mandibular arches. This is in agreement with the findings by Gorelick et al.(204) The author found no differences in the incidence of white spot formation between the right and left sides in the maxilla. In the mandible, the right posterior segment and the left anterior segment showed a greater incidence than their contra-lateral sides, but the findings were not significant.(205) This was in agreement with the study reported by Gaworski et al who also showed no significant difference between contralateral sides.(206)

This study presented with a significant correlation between the hygiene level and the amount of white spot formation quantified as decalcification score. Geiger et al (1988) divided his patients according to their hygiene status as non-compliers and compliers. A significant association between white spot formation and compliance was found among

subjects and teeth.(207) The findings agreed with our that a significant difference existed when comparing; E and F groups, E and P groups, G and F groups, G and P groups, and groups F and P. The decalcification score of all teeth increased as the hygiene level changed from excellent to poor.

Analysis of bonding agent's used in this study indicated that there is a significant difference between CES and SEP groups ($p=0.001$). *In vitro* and *in vivo* studies that used a traditional sealant agent as control, have shown an incidence of 20 to 23% demineralization following the use of sealants.(208;209). Similarly, Frazier in an *in vitro* study reported a 20% incidence of demineralization following the application of a sealant.(210) An *in vivo* investigation by Banks' found a 23% incidence of demineralization with a non-viscous light cured sealant and found no significant difference when compared to the control, a traditional sealant.(211) The current study (CES group) reported only 13.9% incidence by lesion. This is slightly lower than Banks study. This difference could be due to the design that used a modified decalcification index and direct clinical observation. As previously stated the SEP group in this study reported 27.5% of teeth affected with some form of decalcification. The SEP used in this study combines an etchant with a primer but does not have a resin component to provide a protective outer layer against demineralization from an acidic challenge. The low pH of 1 (212) in the SEP renders a continuous acidic challenge, with the absence of a sealant layer, this could explain why the incidence of white spot formation is doubled in the SEP group.

A current *in vitro* study by Tanna(174)comparing the same SEP in this study and a conventional sealant reported that lesions occurred in the sealant group only when there was a break in the sealant layer. This was in agreement with findings in other studies which have

shown that demineralization occurs if there is a break in the sealant layer integrity or at the periphery of the sealant material and slowly advances below the sealant layer.(96;213)

Tanna's (174) study was the first to investigate enamel demineralization when SEP was used and the findings suggest that SEP provides no protection against demineralization and that it was no better than the control when no surface treatment was rendered. 50% of the sealant group showed incidence of lesion, but only when there was a break in the sealant layer after 2 minutes of simulated brushing.(174) Tanna was also able to show that the SEP and no surface treatment control groups had similar extent of lesions.(174) As previously stated there was a significant difference between the CES and SEP groups ($p=0.001$), with the SEP group showing an increase in white spot formation in every category. Of the teeth studied 33 teeth with the CES treated surfaces reported white spot formation compared to 65 teeth treated with SEP.

When considering all groups; decalcification score, bonding agent, hygiene status and location of agent, it was shown that there was a significant hygiene status by bonding agent interaction according to decalcification score ($p<0.0001$). When comparing the data there was not a significant difference between both agents in the excellent hygiene category. However as the patient's hygiene compliance decreased, the amount of difference between the CES and SEP groups mean decalcification score increased.

When comparing decalcification scores among hygiene levels it can be seen that comparing the fair and poor hygiene level patients, the mean score of the SEP group in the fair hygiene compliance group (1.91) is higher than the mean score of the CES group (1.85). It implies that the particular surface treatment before bonding is irrelevant if the hygiene is

good to excellent, however, CES should be used if patients hygiene can be predicted as compromised.

An attempt was made to use SEM to compare the surface characteristics created by the etching process of the two bonding systems. The etching process in the SEP group is such that it is automatically converted to a primer, making it impossible to determine the surface change by the etchant. Many studies have shown that with 37% phosphoric acid, the selective solubility of the enamel, acid-etching patterns which exposes the prism structure and roughens the surface creating deeply penetrating microclefs.(214;215). The etchant pattern in the CES group, Figure 10, is consistent with Silverstone's Classification Pattern 1, as mentioned previously.(197) Information concerning the type of etching pattern created by an SEP is limited. Visual detection of the etch pattern with a SEM is difficult due to the primer component incorporated in the component.

The self-etch effect should be ascribed to monomers to which one or more carboxylic or phosphate acid groups are grafted.(164) Depending on etching aggressiveness, they can be subdivided into a "strong" and "mild" self-etch adhesive based on the pH.(216) Van Meerbeek et al (2003) was able to show that depending on the acidity of the self-etching primer, the strong self-etch adhesives relatively intensively interacted with enamel up to a depth of 5 μ m which is similar to 37% phosphoric acid. While the mild self-etch adhesives penetrate to a maximum of 2 μ m, and do not have similar 37% phosphoric acid affect.(164)

This can be substantiated by viewing the calcium peaks on an x-ray spectrum done in this study seen in Figure 13. It was shown that when comparing the CES and SEP calcium peaks that there was no change in the amount of calcium present on the surface. Indicating

the amount of calcium removed by the etching process of both could be considered comparable.

“Strong” self-etch adhesives usually have a pH of 1 or below. While a “Mild” self-etch adhesives has a higher pH. The SEP in this current study is classified as a strong self-etch with a pH of 1.(217) This high acidity results in rather deep demineralization effect. At the enamel the resulting acid-etch pattern resembles a phosphoric acid treatment following an etch and rinse approach.(164;218;219) Miller (2001) was able to show that the SEP is similar to that of phosphoric acid, with two primer chains that form a solid primer matrix upon curing.(10) See Figure 14.

Figure 14. Chemical comparison of phosphoric acid to self etching primer.

When comparing the SEM images of the CES and SEP groups differences in the surface morphology can be found. The SEP image lacks the characteristic honeycomb appearance, also showing no continuity along the surface, the surface appears to be lightly covered by a thin layer of polymerized primer with the surface remaining somewhat porous.

The CES image shows the continuity of the sealant layer filling the deep tags, left by the etching process, along with the presence of the filler in the sealants. Findings in Tanna and Frazier et al suggested that decalcification occurred only when there was a break in the conventional sealant layer.(174;220) Tanna's study was also able to show that the entire surface of the SEP group exhibited demineralization, and that the entire surface of the SEP is susceptible to acidic challenge by the acid polymer. The lack sealant resin in the SEP group, could lead to further demineralization when the tooth is placed in an artificial acidic environment.(174)

Gwinnett and Buonocore were the first authors to describe resin tags inside the enamel as filamentous resin projections, similar to enamel prisms and approximately 10 μm in length.(221) Soares de Menezes and Chevitarese and others have described the role played by the resin tags as a factor responsible for the adhesive retention process for the sealants.(222) Surface appearance of the CES group, Figure 11, is consistent with the previous studies mentioned. It can be assumed that the CES group produces the same resin tag formation that other studies have shown with similar etching techniques.(221-223) It is unclear whether the SEP group produces tag formation. However, Van Meerbeek et al was able to show that a strong acid self-etch adhesives system will produced comparable etch to phosphoric acid.(164)

Self-etching primers have been proven to intensely interact with enamel up to a depth of 5 μm , which is similar to regular etching.(164) SEP system lacks a sealant/adhesive layer, making the primer solution the only component that could possible protect and penetrate into the etched enamel. The primer could possibly have a potential to wash-out adjacent to the

bonded appliance, or just not be strong enough to withstand normal everyday oral influences, creating a more susceptible environment to the decalcification process.

CLINICAL IMPLICATIONS

The formation of white spots (decalcification) during orthodontic treatment is a concern for clinicians. Past research has shown that the incidence of lesions can be as high as 50% during orthodontic treatment. With the addition of a sealant, one can reduce the incidence of decalcification up to 80%.(224;225) This study has shown that bonding of orthodontic appliances with conventional methods, applying sealant, is still the treatment of choice with regards to prevention of decalcification.

Our findings suggest that although the SEP is convenient and fulfills the purpose of improving the efficiency related to bonding, it offers little if no resistance against the formation of decalcification on the surfaces bonded. A SEP agent would be ideal, if one would incorporate a sealant component. In the mean time, if one chooses to employ an SEP in ones practice, additional preventive measures may be necessary. Preventive measures may include but not limited to a periodic fluoride varnish application, additional home care fluoride treatments, or a fluoride releasing sealant around the bracket of the patients that may be determined as having poor or fair hygiene status and at a high risk for decalcification.

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY

The purpose of this *in vivo* study was to evaluate the amount of white spot formation (decalcification) associated with a self etching primer (SEP) as compared to the application of traditional bonding technique, which includes a sealant layer (CES). In addition, the effect of patient's individual hygiene level and how one's oral hygiene compliance affects the level of decalcification associated with the bonding agents.

This study investigated two different bonding techniques and whether they may reduce the amount of decalcification present following orthodontic therapy in the presence of a self-etching primer or conventional etch and rinse plus sealant layer. 25 subjects were bonded using two different agents, 12 patients were bonded with a self-etching primer (SEP) on the maxillary arch(Transbond Plus, 3M Unitek) and a traditional sealant layer (CES) on the mandibular arch, while in the other 13 patients, this process was reversed. A Scanning Electron Microscope will be used to compare surface differences among the groups. At the end of treatment (treatment time ranged between 18 and 24 months) subjects were broken down into groups according to their oral hygiene compliance throughout treatment (E-excellent, G-good, F-fair, and P-poor). As well as the amount of decalcification present on the subject's dentition. Once the teeth were all scored and recorded the decalcification score was analyzed according to the following groups; bonding agent, location of bonding agent,

the level of hygiene compliance and then interactions. Statistical analysis, with the significance set at $p < 0.05$, was done using ANOVA and one-way analysis of variance. This showed that there is no difference when comparing maxillary and mandibular arches'. There is a significant difference when comparing the type of bonding agent as this relates to decalcification $p < 0.001$. A significant difference exist between the level of hygiene compliance and decalcification $p < 0.0001$. When using Tukey Kramer multiple comparisons test it was found that there is no significant difference in decalcification score between the excellent and good hygiene groups, but there is a significant difference between the excellent, good, fair and poor as they relate to each other. It was shown that self-etching primers when compared with traditional sealant layers have significantly more decalcification present. While comparing hygiene compliance with decalcification, a self-etching primer presents with more decalcification in all groups. There is no significant difference in the location of the bonding agent as it relates to maxillary or mandibular bonding. When comparing surface characteristics among the SEM images, it was shown that the typical honeycomb appearance, associated with the traditional etch and rinse pattern (CES) differs quite significantly when comparing it to the SEP. A porous polymer covered surface is apparent in the SEP compared to the continuous coverage with sealant in CES. It is suggested that when using a self-etching primer other remineralizing means should be used to help fight the occurrence of decalcification.

CONCLUSIONS

The following conclusions were made:

1. There is a significant difference between the hygiene level and the related decalcification score ($p < 0.0001$).
2. Significant differences in decalcification was found when comparing hygiene compliance groups; excellent and fair group; excellent and poor group; good and fair group; good and poor group; and fair and poor group.
3. No significant difference in decalcification score was found between the hygiene compliance level in the excellent and good group.
4. The mean decalcification score of all patients based on level of hygiene compliance increased in score as hygiene level changed from excellent to poor.
5. There was a significant difference in decalcification score between bonding agents ($p < 0.001$).
6. No significant difference existed with regards to the location of the bonding agent.
7. The amount of teeth affected by decalcification was doubled when compared to the conventional bonding group (CES).
8. When comparing the bonding agent used, hygiene level and decalcification score, it was shown that there was a significant hygiene compliance level by bonding agent interaction in decalcification score ($p < 0.0001$).
9. The SEP group significantly presented with a higher decalcification score in all hygiene compliance levels.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are offered. It would be beneficial to perform an *in vitro* and *in vivo* study to evaluate what product is best suited to help fight decalcification while using a self etching primer (SEP). Also since *in vivo* quantification can be difficult to see demineralization with the naked eye, a study design that utilizes teeth that are treatment planned for extraction may offer more insight with microscopic evaluation.

While the self etch primers may vary in acidic strength and etching aggressiveness, incorporating dissolved hydroxyapatite crystals and smear layer remnants within the bond may weaken the bond integrity and bond strength. As well could influence surface breakdown leading to further decalcification. Long-term success of using an SEP as the bonding agent of choice needs further investigation.

The clinician needs to evaluate the differences between a “Strong” pH SEP and a “Mild” pH SEP and how decalcification and bond strength may be influenced by each.

REFERENCE LIST

Reference List

1. **Gwinnett AJ, Ceen RF.** Plaque distribution on bonded brackets: a scanning microscope study. *Am.J.Orthod.* 1979; 75: 667-77.
2. **Mizrahi E.** Enamel demineralization following orthodontic treatment. *Am.J.Orthod.* 1982; 82: 62-7.
3. **Balenseifen JW, Madonia JV.** Study of dental plaque in orthodontic patients. *J.Dent.Res.* 1970; 49: 320-4.
4. **Schwaninger B, Vickers-Schwaninger N.** Developing an effective oral hygiene program for the orthodontic patient: review, rationale, and recommendations. *Am.J.Orthod.* 1979; 75: 447-52.
5. **Diedrich P.** Enamel alterations from bracket bonding and debonding: a study with the scanning electron microscope. *Am.J.Orthod.* 1981; 79: 500-22.
6. **Glatz EGM FJ.** Demineralization related to orthodontic bands and brackets. *Am.J.Orthod.* 1985; 87.
7. **O'Reilly MM, Featherstone JD.** Demineralization and remineralization around orthodontic appliances: an in vivo study. *Am.J.Orthod.Dentofacial Orthop.* 1987; 92: 33-40.
8. **Ogaard B, Rolla G, Arends J.** Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am.J.Orthod.Dentofacial Orthop.* 1988; 94: 68-73.
9. Patel, Pranav. Surface Morphology and Film Thickness of Sealant Following Abrasion and Exposure to Acidic Environments: an In Vitro Study. 1998.
Ref Type: Thesis/Dissertation
10. **Miller RA.** Laboratory and clinical evaluation of a self-etching primer. *J Clin Orthod* 2001; 35: 42-5.
11. Hansen, J. Application technique of a new self-etching primer, in vitro study conducted by 3M Unitek. 2001.
Ref Type: Report
12. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.

13. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
14. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
15. **Sturdevant C RTHHSJ.** *The Art and Science of Operative Dentistry.* (Mosby-Year Book, Inc.:St. Louis): 1995.
16. **Underwood ML, Rawls HR, Zimmerman BF.** Clinical evaluation of a fluoride-exchanging resin as an orthodontic adhesive. *Am.J.Orthod.Dentofacial Orthop.* 1989; 96: 93-9.
17. **Underwood ML, Rawls HR, Zimmerman BF.** Clinical evaluation of a fluoride-exchanging resin as an orthodontic adhesive. *Am.J.Orthod.Dentofacial Orthop.* 1989; 96: 93-9.
18. **Van Palenstein Helderman W.H., Matee M.I., van der Hoeven JS, Mikx FH.** Cariogenicity depends more on diet than on the prevailing mutan streptococcal species. *J Dent Res* 1996; 75: 535-45.
19. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
20. **Sinha PK, Nanda RS, Duncanson MG, Jr., Hosier MJ.** In vitro evaluation of matrix-bound fluoride-releasing orthodontic bonding adhesives. *Am.J.Orthod.Dentofacial Orthop.* 1997; 111: 276-82.
21. **Mizrahi E.** Enamel opacities in primary and high school pupils. *J.Dent.* 1982; 10: 28-38.
22. **Balenseifen JW, Madonia JV.** Study of dental plaque in orthodontic patients. *J.Dent.Res.* 1970; 49: 320-4.
23. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
24. **Gwinnett AJ, Ceen RF.** Plaque distribution on bonded brackets: a scanning microscope study. *Am.J.Orthod.* 1979; 75: 667-77.
25. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
26. **O'Reilly MM, Featherstone JD.** Demineralization and remineralization around orthodontic appliances: an in vivo study. *Am.J.Orthod.Dentofacial Orthop.* 1987; 92: 33-40.

27. **Ogaard B, Rolla G, Arends J.** Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am.J.Orthod.Dentofacial Orthop.* 1988; 94: 68-73.
28. **Younis O, Hughes DO, Weber FN.** Enamel decalcification in orthodontic treatment. *Am.J.Orthod.* 1979; 75: 678-81.
29. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
30. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
31. **Zachrisson BU, Zachrisson S.** Caries incidence and oral hygiene during orthodontic treatment. *Scand.J.Dent.Res.* 1971; 79: 394-401.
32. **Zachrisson BU.** Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. *Am.J.Orthod.* 1976; 69: 285-300.
33. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
34. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
35. **Mizrahi E.** Surface distribution of enamel opacities following orthodontic treatment. *Am.J.Orthod.* 1983; 84: 323-31.
36. **Ogaard B.** Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. *Am.J.Orthod.Dentofacial Orthop.* 1989; 96: 423-7.
37. **Trimpeneers LM, Dermaut LR.** A clinical evaluation of the effectiveness of a fluoride-releasing visible light-activated bonding system to reduce demineralization around orthodontic brackets. *Am.J.Orthod.Dentofacial Orthop.* 1996; 110: 218-22.
38. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
39. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
40. **Trimpeneers LM, Dermaut LR.** A clinical evaluation of the effectiveness of a fluoride-releasing visible light-activated bonding system to reduce demineralization around orthodontic brackets. *Am.J.Orthod.Dentofacial Orthop.* 1996; 110: 218-22.
41. **O'Reilly MM, Featherstone JD.** Demineralization and remineralization around orthodontic appliances: an in vivo study. *Am.J.Orthod.Dentofacial Orthop.* 1987; 92: 33-40.

42. **Gwinnett AJ, Ceen RF.** Plaque distribution on bonded brackets: a scanning microscope study. *Am.J.Orthod.* 1979; 75: 667-77.
43. **Underwood ML, Rawls HR, Zimmerman BF.** Clinical evaluation of a fluoride-exchanging resin as an orthodontic adhesive. *Am.J.Orthod.Dentofacial Orthop.* 1989; 96: 93-9.
44. **Loesche WJ.** Role of Streptococcus mutans in human dental decay. *Microbiol.Rev.* 1986; 50: 353-80.
45. **Svanberg M, Loesche WJ.** The salivary concentration of Streptococci mutans and Streptococci sanguis and their colonization of artificial tooth fissures in man. *Arch.Oral Biol.* 1977; 22: 441-7.
46. **Tanzer JM.** Essential dependence of smooth surface caries on, and augmentation of fissure caries by, sucrose and Streptococcus mutans infection. *Infect.Immun.* 1979; 25: 526-31.
47. **Lundstrom F, Krasse B.** Streptococcus mutans and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. *Eur.J.Orthod.* 1987; 9: 109-16.
48. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
49. **Murray JJ.** *The prevention of Dental Disease.* Oxford University Press.; 1989.
50. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
51. **Loesche WJ.** Role of Streptococcus mutans in human dental decay. *Microbiol.Rev.* 1986; 50: 353-80.
52. **Loesche WJ.** Role of Streptococcus mutans in human dental decay. *Microbiol.Rev.* 1986; 50: 353-80.
53. **Diedrich P.** Enamel alterations from bracket bonding and debonding: a study with the scanning electron microscope. *Am.J.Orthod.* 1981; 79: 500-22.
54. **Holmen L, Mejare I, Malmgren B, Thylstrup A.** The effect of regular professional plaque removal on dental caries in vivo. A polarized light and scanning electron microscope study. *Caries Res.* 1988; 22: 250-6.
55. **O'Reilly MM, Featherstone JD.** Demineralization and remineralization around orthodontic appliances: an in vivo study. *Am.J.Orthod.Dentofacial Orthop.* 1987; 92: 33-40.

56. **Ogaard B, Rolla G, Arends J.** Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am.J.Orthod.Dentofacial Orthop.* 1988; 94: 68-73.
57. **Thylstrup A, Boyar RM, Holmen L, Bowden GH.** A light and scanning electron microscopic study of enamel decalcification in children living in a water-fluoridated area. *J.Dent.Res.* 1990; 69: 1626-33.
58. **Thylstrup A, Boyar RM, Holmen L, Bowden GH.** A light and scanning electron microscopic study of enamel decalcification in children living in a water-fluoridated area. *J.Dent.Res.* 1990; 69: 1626-33.
59. **Balenseifen JW, Madonia JV.** Study of dental plaque in orthodontic patients. *J.Dent.Res.* 1970; 49: 320-4.
60. **Atack NE, Sandy JR, Addy M.** Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. *J.Periodontol.* 1996; 67: 78-85.
61. **Atack NE, Sandy JR, Addy M.** Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. *J.Periodontol.* 1996; 67: 78-85.
62. **Atack NE, Sandy JR, Addy M.** Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. *J.Periodontol.* 1996; 67: 78-85.
63. **Gwinnett AJ, Ceen RF.** Plaque distribution on bonded brackets: a scanning microscope study. *Am.J.Orthod.* 1979; 75: 667-77.
64. **Huser MC, Baehni PC, Lang R.** Effects of orthodontic bands on microbiologic and clinical parameters. *Am.J.Orthod.Dentofacial Orthop.* 1990; 97: 213-8.
65. **Mizrahi E.** Enamel demineralization following orthodontic treatment. *Am.J.Orthod.* 1982; 82: 62-7.
66. **Gwinnett AJ, Ceen RF.** Plaque distribution on bonded brackets: a scanning microscope study. *Am.J.Orthod.* 1979; 75: 667-77.
67. **Atack NE, Sandy JR, Addy M.** Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. *J.Periodontol.* 1996; 67: 78-85.
68. **Mizrahi E.** Enamel demineralization following orthodontic treatment. *Am.J.Orthod.* 1982; 82: 62-7.
69. **Atack NE, Sandy JR, Addy M.** Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. *J.Periodontol.* 1996; 67: 78-85.

70. **Lundstrom F, Krasse B.** Streptococcus mutans and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. *Eur.J.Orthod.* 1987; 9: 109-16.
71. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
72. **Gwinnett AJ, Ceen RF.** Plaque distribution on bonded brackets: a scanning microscope study. *Am.J.Orthod.* 1979; 75: 667-77.
73. **Balenseifen JW, Madonia JV.** Study of dental plaque in orthodontic patients. *J.Dent.Res.* 1970; 49: 320-4.
74. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
75. **Balenseifen JW, Madonia JV.** Study of dental plaque in orthodontic patients. *J.Dent.Res.* 1970; 49: 320-4.
76. **Matasa CG.** Microbial attack of orthodontic adhesives. *Am.J.Orthod.Dentofacial Orthop.* 1995; 108: 132-41.
77. **Docking AR, Newbury CR.** The effect of orthodontic cement on tooth enamel. *Aust Dent J* 1953; 57: 139-49.
78. **Lefkowitz W, Dodecker CF.** Concerning the vitality of the calcified dental tissues. II. The permability of enamel. *J Dent Res* 1938; 17: 453-63.
79. **Seniff RW.** Enamel surface change caused by oxyphosphate cement. *Am.J.Orthod.* 1962; 48: 219-20.
80. **Wisth PJ.** The role of zinc phosphate cement in enamel surface changes on banded teeth. *Angle Orthod.* 1970; 40: 329-33.
81. **Gopinath VK, Tandon S, Shirwaikar A.** The effect of chewing gums on acidogenicity of plaque after a sucrose challenge. *J.Clin.Pediatr.Dent.* 1997; 22: 77-81.
82. **Bowen WH.** Food components and caries. *Adv.Dent.Res.* 1994; 8: 215-20.
83. **Gaffar A, Blake-Haskins JC, Sullivan R, Simone A, Schmidt R, Saunders F.** Cariostatic effects of a xylitol/NaF dentifrice in vivo. *Int.Dent.J.* 1998; 48: 32-9.
84. **Isotupa KP, Gunn S, Chen CY, Lopatin D, Makinen KK.** Effect of polyol gums on dental plaque in orthodontic patients. *Am.J Orthod.Dentofacial Orthop.* 1995; 107: 497-504.
85. **Levine RS.** Briefing paper: xylitol, caries and plaque. *Br.Dent.J.* 1998; 185: 520.

86. **Bowen WH.** Food components and caries. *Adv.Dent.Res.* 1994; 8: 215-20.
87. **Gaffar A, Blake-Haskins JC, Sullivan R, Simone A, Schmidt R, Saunders F.** Cariostatic effects of a xylitol/NaF dentifrice in vivo. *Int.Dent.J.* 1998; 48: 32-9.
88. **Isotupa KP, Gunn S, Chen CY, Lopatin D, Makinen KK.** Effect of polyol gums on dental plaque in orthodontic patients. *Am.J Orthod.Dentofacial Orthop.* 1995; 107: 497-504.
89. **Levine RS.** Briefing paper: xylitol, caries and plaque. *Br.Dent.J.* 1998; 185: 520.
90. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
91. **Arends J, Christoffersen J.** The nature of early caries lesions in enamel. *J.Dent.Res.* 1986; 65: 2-11.
92. **Brant ZB.** Dr. Bjorn Zachrisson on iatrogenic damage in orthodontic treatment (Part 2). *JCO* 1978; 208-20.
93. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
94. **Haikel Y, Frank RM, Voegel JC.** Scanning electron microscopy of the human enamel surface layer of incipient carious lesions. *Caries Res.* 1983; 17: 1-13.
95. **Ogaard B, Rolla G, Arends J.** Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am.J.Orthod.Dentofacial Orthop.* 1988; 94: 68-73.
96. **Silverstone LM.** Structure of carious enamel, including the early lesion. *Oral Sci.Rev.* 1973; 3: 100-60.
97. **Silverstone L.** Remineralization and enamel caries: significance of fluoride and effect of crystal diameter. In: Leach SA, Edgar WM, eds. *Demineralization and remineralization of teeth.* Oxford: IRL Press, Ltd.; 1983: 185-205.
98. **Gustafson G.** The histopathology of caries of human enamel with special reference to the division of the lesion into zones. *Acta Odontol.Scand.* 1957; 15: 13.
99. **Crabb HS.** Enamel caries. Observations on the histology and pattern of progress of the approximal lesion. *Br.Dent J* 1966; 121: 115-29.
100. **Brudevold F.** A study of the phosphate solubility of the human enamel surface. *J Dent Res* 1984; 320.
101. **Isaac S, Brudevold F, Smith FA, Gardner DE.** Solubility rate and natural fluoride content of surface and subsurface enamel. *J Dent Res* 1958; 37: 254.

102. **ten Cate JM.** In vitro studies on the effects of fluoride on de- and remineralization. *J.Dent.Res.* 1990; 69 Spec No: 614-9.
103. **Frank RM, Nalbandian J.** *Ultrastructure of amelogenesis in mile, structural and chemical organiation of teeth.* New York, 1967.
104. **Arends J, Christoffersen J.** The nature of early caries lesions in enamel. *J.Dent.Res.* 1986; 65: 2-11.
105. **Goldberg M, Arends J, Septier D, Jongebloed WL.** Microchannels in the surface zone of artificially produced caries-like enamel lesions. *J Biol.Buccale* 1981; 9: 297-314.
106. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
107. **Arends J, Christoffersen J.** The nature of early caries lesions in enamel. *J.Dent.Res.* 1986; 65: 2-11.
108. **Goldberg M, Arends J, Septier D, Jongebloed WL.** Microchannels in the surface zone of artificially produced caries-like enamel lesions. *J Biol.Buccale* 1981; 9: 297-314.
109. **ten Cate JM.** In vitro studies on the effects of fluoride on de- and remineralization. *J.Dent.Res.* 1990; 69 Spec No: 614-9.
110. **Arends J, Christoffersen J.** The nature of early caries lesions in enamel. *J.Dent.Res.* 1986; 65: 2-11.
111. **Backer DO.** Post-eruptive changes in dental enamel. *J Dent Res* 1966; 45: 502-11.
112. **Von der Fehr FR LHTE.** Experimental caries in man. *Caries Res* 1970; 131-48.
113. **Schwaninger B, Vickers-Schwaninger N.** Developing an effective oral hygiene program for the orthodontic patient: review, rationale, and recommendations. *Am.J.Orthod.* 1979; 75: 447-52.
114. **Adams D, Addy M.** Mouthrinses. *Adv.Dent Res* 1994; 8: 291-301.
115. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
116. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
117. **Boyd RL, Murray P, Robertson PB.** Effect on periodontal status of rotary electric toothbrushes vs. manual toothbrushes during periodontal maintenance. I. Clinical results. *J Periodontol.* 1989; 60: 390-5.

118. **Wilcoxon DB, Ackerman RJ, Jr., Killoy WJ, Love JW, Sakumura JS, Tira DE.** The effectiveness of a counterrotational-action power toothbrush on plaque control in orthodontic patients. *Am.J.Orthod.Dentofacial Orthop.* 1991; 99: 7-14.
119. **Ho HP, Niederman R.** Effectiveness of the Sonicare sonic toothbrush on reduction of plaque, gingivitis, probing pocket depth and subgingival bacteria in adolescent orthodontic patients. *J.Clin.Dent.* 1997; 8: 15-9.
120. **White LW.** Efficacy of a sonic toothbrush in reducing plaque and gingivitis in adolescent patients. *J.Clin.Orthod.* 1996; 30: 85-90.
121. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
122. **Gwinnett AJ, Ceen RF.** Plaque distribution on bonded brackets: a scanning microscope study. *Am.J.Orthod.* 1979; 75: 667-77.
123. **Hogg SD.** Chemical control of plaque. *Dental Update* 1990; 330-3.
124. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
125. **Brightman LJ, Terezhalmay GT, Greenwell H, Jacobs M, Enlow DH.** The effects of a 0.12% chlorhexidine gluconate mouthrinse on orthodontic patients aged 11 through 17 with established gingivitis. *Am.J.Orthod.Dentofacial Orthop.* 1991; 100: 324-9.
126. **Adams D, Addy M.** Mouthrinses. *Adv.Dent Res* 1994; 8: 291-301.
127. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
128. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
129. **Schwaninger B, Vickers-Schwaninger N.** Developing an effective oral hygiene program for the orthodontic patient: review, rationale, and recommendations. *Am.J.Orthod.* 1979; 75: 447-52.
130. **Zachrisson BU.** Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. *Am.J.Orthod.* 1976; 69: 285-300.
131. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
132. **Zachrisson BU.** Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. *Am.J.Orthod.* 1976; 69: 285-300.

133. **O'Reilly MM, Featherstone JD.** Demineralization and remineralization around orthodontic appliances: an in vivo study. *Am.J.Orthod.Dentofacial Orthop.* 1987; 92: 33-40.
134. **van der Linden RP, Dermaut LR.** White spot formation under orthodontic bands cemented with glass ionomer with or without Fluor Protector. *Eur.J.Orthod.* 1998; 20: 219-24.
135. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.
136. **Seppa L.** Studies of fluoride varnishes in Finland. *Proc.Finn.Dent.Soc.* 1991; 87: 541-7.
137. **Seppa L.** Effect of dental plaque on fluoride uptake by enamel from a sodium fluoride varnish in vivo. *Caries Res.* 1983; 17: 71-5.
138. **Seppa L, Tuutti H, Luoma H.** Post-treatment effect of fluoride varnishes in children with a high prevalence of dental caries in a community with fluoridated water. *J.Dent.Res.* 1984; 63: 1221-2.
139. **Seppa L.** Fluoride content of enamel during treatment and 2 years after discontinuation of treatment with fluoride varnishes. *Caries Res.* 1984; 18: 278-81.
140. **Modeer T, Twetman S, Bergstrand F.** Three-year study of the effect of fluoride varnish (Duraphat) on proximal caries progression in teenagers. *Scand.J.Dent.Res.* 1984; 92: 400-7.
141. **Arends J, Schuthof J.** Fluoride content in human enamel after fluoride application and washing - an in vitro study. *Caries Res.* 1975; 9: 363-72.
142. **Koulourides T.** Summary of session II: fluoride and caries process. *J Dent Res* 1990; 558.
143. **Ogaard B, Rolla G, Helgeland K.** Alkali soluble and alkali insoluble fluoride retention in demineralized enamel in vivo. *Scand.J.Dent.Res.* 1983; 91: 200-4.
144. **Ogaard B, Rolla G, Helgeland K.** Fluoride retention in sound and demineralized enamel in vivo after treatment with a fluoride varnish (Duraphat). *Scand.J Dent Res* 1984; 92: 190-7.
145. **Caslavska V, Moreno EC, Brudevold F.** Determination of the calcium fluoride formed from in vitro exposure of human enamel to fluoride solutions. *Arch.Oral Biol.* 1975; 20: 333-9.
146. **de Bruyn H AJ.** Fluoride varnishes- a review. *J Biol Buccale* 1987.

147. **Clark DC.** A review on fluoride varnishes: an alternative topical fluoride treatment. *Community Dent.Oral Epidemiol.* 1982; 10: 117-23.
148. **Ogaard B, Duschner H, Ruben J, Arends J.** Microradiography and confocal laser scanning microscopy applied to enamel lesions formed in vivo with and without fluoride varnish treatment. *Eur.J.Oral Sci.* 1996; 104: 378-83.
149. **Todd MA, Staley RN, Kanellis MJ, Donly KJ, Wefel JS.** Effect of a fluoride varnish on demineralization adjacent to orthodontic brackets. *Am.J.Orthod.Dentofacial Orthop.* 1999; 116: 159-67.
150. **Bowman S.J.** Use of a fluoride varnish to reduce decalcification. *J Clin Orthod* 2000.
151. **Roberts JF, Longhurst P.** A clinical estimation of the fluoride used during application of a fluoride varnish. *Br.Dent.J.* 1987; 162: 463-6.
152. **Ekstrand J, Koch G, Petersson LG.** Plasma fluoride concentration and urinary fluoride excretion in children following application of the fluoride-containing varnish Duraphat. *Caries Res* 1980; 14: 185-9.
153. **Ekstrand J, Koch G, Petersson LG.** Plasma fluoride concentrations in pre-school children after ingestion of fluoride tablets and toothpaste. *Caries Res.* 1983; 17: 379-84.
154. **Ekstrand J, Koch G, Lindgren LE, Petersson LG.** Pharmacokinetics of fluoride gels in children and adults. *Caries Res.* 1981; 15: 213-20.
155. **Isaksson M, Bruze M, Bjorkner B, Niklasson B.** Contact allergy to Duraphat. *Scand.J.Dent.Res.* 1993; 101: 49-51.
156. **Mitchell L.** Decalcification during orthodontic treatment with fixed appliances--an overview. *Br.J.Orthod.* 1992; 19: 199-205.
157. **Ceen RF, Gwinnett AJ.** White spot formation associated with sealants used in orthodontics. *Pediatr.Dent.* 1981; 3: 174-8.
158. **Banks PA, Richmond S.** Enamel sealants: a clinical evaluation of their value during fixed appliance therapy. *Eur.J.Orthod.* 1994; 16: 19-25.
159. **Zachrisson BU.** Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. *Am.J.Orthod.* 1976; 69: 285-300.
160. **Joseph VP, Rossouw PE, Basson NJ.** Some "sealants" seal--a scanning electron microscopy (SEM) investigation. *Am.J.Orthod.Dentofacial Orthop.* 1994; 105: 362-8.
161. **Chigira H, Koike T, Hasegawa T, Itoh K, Wakuumoto S, Hyakawa T.** Effect of the self etching dentin primers on the bonding efficacy of dentine adhesive. *Dent Materials J* 1989; 8: 86-92.

162. **Nishida K, Yamauchi J, Wada T, Hosoda H.** Development of a new bonding system (abstract). *J Dent Res* 1993; 72: 137.
 163. **Bishara SE, VonWald L, Laffoon JF, Warren JJ.** Effect of a self-etch primer/adhesive on the shear bond strength of orthodontic brackets. *Am.J.Orthod.Dentofacial Orthop.* 2001; 119: 621-4.
 164. **Van Meerbeek B, De Munck J, Yoshida Y et al.** Adhesion to Enamel and Dentin Current: Current Status and Future Challenges. *Operative Dentistry* 2003; 28: 215-35.
 165. **Tay FR, Pashley DH.** Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent.Mater.* 2001; 17: 296-308.
 166. **Pashley DH, Tay FR.** Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. *Dent.Mater.* 2001; 17: 430-44.
 167. **Tay FR, Pashley DH.** Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent.Mater.* 2001; 17: 296-308.
 168. **Tay FR, Pashley DH.** Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent.Mater.* 2001; 17: 296-308.
 169. **Garcia-Goday F.** Bond strength of Prompt L-Pop to enamel and dentin, report to ESPE. *University of Texas Health Science Center* 2001.
 170. **Newman GV.** Epoxy adhesives for orthodontic attachments: progress report. *Am.J Orthod* 1965; 51: 901-12.
 171. **Greenlaw R, Way DC, Galil KA.** An in vitro evaluation of a visible light-cured resin as an alternative to conventional resin bonding systems. *Am.J Orthod Dentofacial Orthop.* 1989; 96: 214-20.
 172. **Reynolds R.** A review of direct orthodontic bonding. *Br J Orthodontics* 1975; 2: 171-8.
 173. **Smith RT, Shivapuja PK.** The evaluation of dual cement resins in orthodontic bonding. *Am.J Orthod Dentofacial Orthop.* 1993; 103: 448-51.
 174. **Kao E, Tana N, and Ngan P.** A comparison of demineralization between self etching primer and conventional sealant: An in vitro and in vivo study. *J Dent Res* 83(Spec Issue). 2004. West Virginia University.
- Ref Type: Abstract
175. **Chang HS, Walsh LJ, Freer TJ.** Enamel demineralization during orthodontic treatment. Aetiology and prevention. *Aust Dent J* 1997; 42: 322-7.
 176. **Millet DT, McCabe JF.** Orthodontic bonding with glass ionomer cement-a review. *European Journal of orthodontics* 1996; 18: 325-99.

177. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.
178. **Hill B, Geddes DA.** A photographic approach to recording pre-carious changes on teeth in situ. *Med.Biol.Illus.* 1975; 25: 79-88.
179. **Mitchell L.** An investigation into the effect of a fluoride releasing adhesive on the prevalence of enamel surface changes associated with directly bonded orthodontic attachments. *Br.J.Orthod.* 1992; 19: 207-14.
180. **Mitchell L.** An investigation into the effect of a fluoride releasing adhesive on the prevalence of enamel surface changes associated with directly bonded orthodontic attachments. *Br.J.Orthod.* 1992; 19: 207-14.
181. **Hill B, Geddes DA.** A photographic approach to recording pre-carious changes on teeth in situ. *Med.Biol.Illus.* 1975; 25: 79-88.
182. **Geiger AM, Gorelick L, Gwinnett AJ, Benson BJ.** Reducing white spot lesions in orthodontic populations with fluoride rinsing. *Am.J.Orthod.Dentofacial Orthop.* 1992; 101: 403-7.
183. **Linton JL.** Quantitative measurements of remineralization of incipient caries. *Am.J Orthod Dentofacial Orthop.* 1996; 110: 590-7.
184. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
185. **Artum J, Brobakken B.O.** Prevalence of carious white spots after orthodontic treatment with multibonded appliances. *Eur J Orthod* 1986; 8: 229-34.
186. **Newman GV, Facq JM.** The effects of adhesive systems on tooth surfaces. *Am.J Orthod* 1971; 59: 67-75.
187. **Newman GV, Facq JM.** The effects of adhesive systems on tooth surfaces. *Am.J Orthod* 1971; 59: 67-75.
188. **CMRA.** Scanning Electron Microscopes. 2-26-2004.
Ref Type: Internet Communication
189. **Science Learning Network.** Scanning Electron Microscopes. 2-26-2004.
Ref Type: Internet Communication
190. **Poole DFG, Johnson NW.** The effects of different demineralizing agents on human enamel surfaces studied by scanning electron microscopy. *Arch.Oral Biol.* 1967; 12: 1621-34.

191. **Hoffman S, McEwan WS, Drew DM.** Scanning electron microscope studies of dental enamel. *J.Dent.Res.* 1969; 48: 242.
 192. **O'Leary TJ, Drake RB, Naylor JE.** The plaque control record. *J Periodontol* 1972; 43: 38.
 193. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.
 194. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.
 195. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.
 196. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.
 197. Silverstone, L. M. The acid etch technique: In vitro studies with special reference to the enamel surface and the enamel-resin interface, Proceedings of an Internationalsymposium on the Acid Etch Technique. 13-39. 1975. St. Paul, North Central Publishing Company.
- Ref Type: Report
198. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.
 199. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
 200. **Millet DT, Nunn JH, Welbury RR, Gordon PH.** Decalcification in relation to brackets bonded with glass ionomer cement or a resin adhesive. *Angle Orthod.* 1999; 69: 65-70.
 201. **Sonis AL, Snell W.** An evaluation of a fluoride releasing visible light activated bonding system for orthodontic bracket placement. *Am J Orthod Dentofac Orthop* 1989; 95: 306-11.
 202. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.

203. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
204. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
205. **Gorelick L, Geiger AM, Gwinnett AJ.** Incidence of white spot formation after bonding and banding. *Am.J.Orthod.* 1982; 81: 93-8.
206. **Gaworski M, Weinstein M, Borislow AJ, Braitman LE.** Decalcification and bond failure: A comparison of a glass ionomer and a composite resin bonding system in vivo. *Am J Orthod Dentofac Orthop* 1999; 116: 518-21.
207. **Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG.** The effect of a fluoride program on white spot formation during orthodontic treatment. *Am.J.Orthod.Dentofacial Orthop.* 1988; 93: 29-37.
208. **Banks PA, Richmond S.** Enamel sealants: a clinical evaluation of their value during fixed appliance therapy. *Eur.J.Orthod.* 1994; 16: 19-25.
209. **Frazier MC, Southard TE, Doster PM.** Prevention of enamel demineralization during orthodontic treatment: an in vitro study using pit and fissure sealants. *Am.J.Orthod.Dentofacial Orthop.* 1996; 110: 459-65.
210. **Frazier MC, Southard TE, Doster PM.** Prevention of enamel demineralization during orthodontic treatment: an in vitro study using pit and fissure sealants. *Am.J.Orthod.Dentofacial Orthop.* 1996; 110: 459-65.
211. **Banks PA, Richmond S.** Enamel sealants: a clinical evaluation of their value during fixed appliance therapy. *Eur.J.Orthod.* 1994; 16: 19-25.
212. **Tay FR, Pashley DH.** Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent.Mater.* 2001; 17: 296-308.
213. **Frazier MC, Southard TE, Doster PM.** Prevention of enamel demineralization during orthodontic treatment: an in vitro study using pit and fissure sealants. *Am.J.Orthod.Dentofacial Orthop.* 1996; 110: 459-65.
214. **Diedrich P.** Enamel alterations from bracket bonding and debonding: a study with the scanning electron microscope. *Am.J.Orthod.* 1981; 79: 500-22.
215. **Galil KA, Wright GZ.** Acid etching patterns on buccal surfaces of permanent teeth. *Pediatr.Dent.* 1979; 1: 230-4.
216. **Tay FR, Pashley DH.** Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent.Mater.* 2001; 17: 296-308.

217. **Tay FR, Pashley DH.** Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent.Mater.* 2001; 17: 296-308.
218. **Pashley DH, Tay FR.** Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. *Dent.Mater.* 2001; 17: 430-44.
219. **Tay FR, Pashley DH.** Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent.Mater.* 2001; 17: 296-308.
220. **Frazier MC, Southard TE, Doster PM.** Prevention of enamel demineralization during orthodontic treatment: an in vitro study using pit and fissure sealants. *Am.J.Orthod.Dentofacial Orthop.* 1996; 110: 459-65.
221. **Gwinnett AJ, Buonocore MG.** Adhesives and caries prevention, a preliminary report. *Br Dent J* 1965; 119: 77-80.
222. **Soares de Menezes LF, Chevitaese O.** Sealant and resin viscosity and their influence on the formation of resin tags. *Angle Orthod.* 1994; 64: 383-8.
223. **Newman GV, Facq JM.** The effects of adhesive systems on tooth surfaces. *Am.J Orthod* 1971; 59: 67-75.
224. **Banks PA, Richmond S.** Enamel sealants: a clinical evaluation of their value during fixed appliance therapy. *Eur.J.Orthod.* 1994; 16: 19-25.
225. **Frazier MC, Southard TE, Doster PM.** Prevention of enamel demineralization during orthodontic treatment: an in vitro study using pit and fissure sealants. *Am.J.Orthod.Dentofacial Orthop.* 1996; 110: 459-65.

APPENDIX A
IRB APPROVAL FORMS

Application for Exemption

You must receive approval from the IRB staff prior to beginning the research described below. Please type all responses and submit this form with original signatures. All investigators must complete Ethics Training before an approval will be granted.

1. Title of study: Effects of Sealant and Self-Etching Primer on Enamel Decalcification In Vivo

2. Investigators (list all investigators, principal investigator first; attach additional sheets if necessary):

Name	Signature	Dept/Collage	Address	Tel No
Matthew A. Gliz		Dentistry/Orthodontics	PO Box 9480	293-5217
Name or initials				
M.G.		YES if training has been completed	Ethics Training ¹	HIPAA Training ²
			Yes	Yes
		¹ Ethics training: http://www.wvu.edu/~rc/irb/ethi_tra.htm		
		² HIPAA info: http://www.wvu.edu/~rc/irb/index.htm		

3. Estimated period of human subject involvement: Starting date: November 1, 03 Ending date: July 1, 04

4. Reason for conducting research ☐ Professional ☐ Dissertation ☒ Thesis
☐ Class Assignment ☐ Other

5. Source of funding (if applicable) WVU Foundation

6. Number of projected subjects 25 Number of projected records or data files 25

7. This research involves (check all that apply—see attached "Exempt Research" page):

- ☒ a Collection or study of existing data, documents, records or specimens, recorded without identifying information
- ☐ b Normal educational practices conducted in established or commonly accepted educational settings
- ☐ c Educational tests (cognitive, diagnostic, aptitude, achievement)
- ☐ d Observation of public behavior
- ☐ e Surveys, interviews or hand-outs for subjects over 18
 (use age ranges, not actual age, for demographic information):
☐ Mail ☐ Telephone ☐ Person-to-person
- ☐ f Any possibility of identifying a subject (discuss in cover letter)
- ☐ g The possibility that the subject's responses or conduct (if they became public) may place the subject at risk of criminal or civil liability or be damaging to the subject's financial standing or employability
- ☐ h Sensitive aspects of personal behavior (for example: illegal conduct, drug use, sexual behavior or use of alcohol)
- ☐ i Investigator's participation in activities being observed
- ☐ j Only surveys or interviews of elected or appointed public officials or candidates for public office
- ☐ k Audiotaping
- ☐ l Children under age 18 (see Chapter II of the Guidelines)
 Note: Interviews and surveys with children are never exempt.
- ☐ m Food tasting and evaluation
- ☐ n Research and demonstration projects
- ☐ o Access to protected health information (PHI) (See HIPAA requirements: <http://www.wvu.edu/~rc/irb/index.htm>)

8. Goal of research

This Study will investigate two different types of bonding techniques currently used at West Virginia University, School of Dentistry, Dept. of Orthodontics. This will allow us to determine whether they may reduce the amount of decalcification present following orthodontic therapy, in the presence of a self-etching primer and sealant layer.

9. Explanation of procedures involved in research

Patient records, including intra-oral photographs, oral hygiene and post-treatment finding, will be examined. Initial records as well as final records, following orthodontic therapy will be looked at to determine the amount of decalcification present. The data of involved teeth only will be sent to determine statistical significance.

10. Explanation of known risks to human subjects

Records have already been completed, as required by normal practices in the department of orthodontics.

There are no known risks of anykind to the patients.

11. Explanation of how records will be kept

I will keep my information by a numbering system in which I am the only one that will have the information as to whom the data belongs.

This information will be kept in a locked location.

A cover letter addressed to respondents must accompany any survey or questionnaire. The cover letter must be on *your WFU* departmental letterhead and must include the following:

- 1 a statement that the project is research being conducted in partial fulfillment of the requirements for a course, master's thesis, dissertation, etc.
- 2 purpose of study
- 3 a statement that subjects' responses will be kept anonymous or confidential (explain extent of confidentiality if subjects' names are requested)
- 4 if audiotaping, a statement that subject is being audiotaped (explain how tapes will be stored or disposed of during and after the study)
- 5 a statement that subjects do not have to answer every question
- 6 a statement that subject's class standing, grades, or job status (or status on an athletic team, if applicable) will not be affected by refusal to participate or by withdrawal from the study
- 7 a statement that participation is voluntary

Attachments:

- ☐ questionnaire/survey to be used
- ☐ telephone text (including introductory remarks as in a cover letter—see above)
- ☐ cover letter
- ☐ permission from external institution, on their letterhead (if applicable)

I have reviewed the above information and recommend this study for exemption.

Dean or Director

Peter Ngan D.M.D.
Department Chair

Faculty Advisor Chris Martin D.D.S., M.S.

APPENDIX B
***IN VIVO* DATA COLLECTION FORMS**

Pre-Treatment Bonding Data Sheet

Case #	Patient Name	Chart #	Max. Bond Date	Man. Bond Date

O'Leary Plaque Index

of surfaces with plaque divided by total # of surfaces X 100 = _____ %

Excellent hygiene: 0-20%

Good hygiene: 21-40%

Fair hygiene: 41-60%

Poor hygiene: 61-100%

Bonding Scheme

Circle the bonding agent used.

Maxillary Arch CES SEP
Mandibular Arch CES SEP

Post-Treatment Bonding Data Sheet

Case #	Patient Name	Chart #	Max. Bond Date	Man. Bond Date

O'Leary Plaque Index

of surfaces with plaque divided by total # of surfaces X 100 = _____ %

Excellent hygiene: 0-20%

Good hygiene: 21-40%

Fair hygiene: 41-60%

Poor hygiene: 61-100%

Decalcification Score:

Score 1

Score 2

Score 3

Score 4

Score 1 = No decalcification

Score 2 = Slight decalcification (one area)

Score 3 = Large area or multiple areas

Score 4 = Cavitation present

Individual scoring of teeth: Insert score in box accordingly.

5	4	3	2	1	1	2	3	4	5
5	4	3	2	1	1	2	3	4	5

APPENDIX C

IN VIVO RAW DATA

Patient	Patient Hygiene	Agent	Tooth #	Decalcification Score
1	P	A	UR1	2
1	P	A	UR2	4
1	P	A	UR3	3
1	P	A	UR5	3
1	P	A	UL1	2
1	P	A	UL2	3
1	P	A	UL3	3
1	P	A	UL5	2
1	P	B	LR1	1
1	P	B	LR2	1
1	P	B	LR3	2
1	P	B	LR5	2
1	P	B	LL1	1
1	P	B	LL2	1
1	P	B	LL3	1
1	P	B	LL5	1
2	E	A	UR1	1
2	E	A	UR2	1
2	E	A	UR3	1
2	E	A	UR5	2
2	E	A	UL1	1
2	E	A	UL2	1
2	E	A	UL3	1
2	E	A	UL5	1
2	E	B	LR1	1
2	E	B	LR2	1
2	E	B	LR3	1
2	E	B	LR5	1
2	E	B	LL1	1
2	E	B	LL2	1
2	E	B	LL3	1
2	E	B	LL5	1
3	G	A	UR1	1
3	G	A	UR2	2
3	G	A	UR3	1
3	G	A	UR4	1
3	G	A	UR5	1
3	G	A	UL1	1
3	G	A	UL2	1
3	G	A	UL3	1
3	G	A	UL4	1
3	G	A	UL5	1
3	G	B	LR1	1
3	G	B	LR2	1
3	G	B	LR3	1
3	G	B	LR4	1
3	G	B	LR5	1

3	G	B	LL1	1
3	G	B	LL2	1
3	G	B	LL3	1
3	G	B	LL4	1
3	G	B	LL5	1
4	E	A	UR1	1
4	E	A	UR2	1
4	E	A	UR3	1
4	E	A	UR4	1
4	E	A	UR5	1
4	E	A	UL1	1
4	E	A	UL2	1
4	E	A	UL3	1
4	E	A	UL4	1
4	E	A	UL5	1
4	E	B	LR1	1
4	E	B	LR2	1
4	E	B	LR3	1
4	E	B	LR4	1
4	E	B	LR5	1
4	E	B	LL1	1
4	E	B	LL2	1
4	E	B	LL3	1
4	E	B	LL4	1
4	E	B	LL5	1
5	G	A	UR1	1
5	G	A	UR2	1
5	G	A	UR3	1
5	G	A	UR4	1
5	G	A	UR5	1
5	G	A	UL1	1
5	G	A	UL2	1
5	G	A	UL3	1
5	G	A	UL4	1
5	G	A	UL5	1
5	G	B	LR1	1
5	G	B	LR2	1
5	G	B	LR3	1
5	G	B	LR4	1
5	G	B	LR5	1
5	G	B	LL1	1
5	G	B	LL2	1
5	G	B	LL3	1
5	G	B	LL4	1
5	G	B	LL5	1
6	G	A	UR1	1
6	G	A	UR2	2
6	G	A	UR3	1
6	G	A	UR4	1
6	G	A	UR5	1

6	G	A	UL1	1
6	G	A	UL2	1
6	G	A	UL3	1
6	G	A	UL4	2
6	G	A	UL5	2
6	G	B	LR1	1
6	G	B	LR2	1
6	G	B	LR3	1
6	G	B	LR4	1
6	G	B	LR5	1
6	G	B	LL1	1
6	G	B	LL2	1
6	G	B	LL3	1
6	G	B	LL4	1
6	G	B	LL5	1
7	E	A	UR1	1
7	E	A	UR2	1
7	E	A	UR3	1
7	E	A	UR4	1
7	E	A	UR5	1
7	E	A	UL1	1
7	E	A	UL2	1
7	E	A	UL3	1
7	E	A	UL4	1
7	E	A	UL5	1
7	E	B	LR1	1
7	E	B	LR2	1
7	E	B	LR3	1
7	E	B	LR4	1
7	E	B	LR5	1
7	E	B	LL1	1
7	E	B	LL2	1
7	E	B	LL3	1
7	E	B	LL4	1
7	E	B	LL5	1
8	E	A	UR1	1
8	E	A	UR2	1
8	E	A	UR3	1
8	E	A	UR4	1
8	E	A	UR5	1
8	E	A	UL1	1
8	E	A	UL2	1
8	E	A	UL3	1
8	E	A	UL4	1
8	E	A	UL5	1
8	E	B	LR1	1
8	E	B	LR2	1
8	E	B	LR3	1
8	E	B	LR4	1
8	E	B	LR5	1

8	E	B	LL1	1
8	E	B	LL2	1
8	E	B	LL3	1
8	E	B	LL4	1
8	E	B	LL5	1
20	E	A	UR1	1
20	E	A	UR2	1
20	E	A	UR3	1
20	E	A	UR4	1
20	E	A	UR5	1
20	E	A	UL1	1
20	E	A	UL2	1
20	E	A	UL3	1
20	E	A	UL4	1
20	E	A	UL5	1
20	E	B	LR1	1
20	E	B	LR2	1
20	E	B	LR3	1
20	E	B	LR4	1
20	E	B	LR5	1
20	E	B	LL1	1
20	E	B	LL2	1
20	E	B	LL3	1
20	E	B	LL4	1
20	E	B	LL5	1
21	G	A	UR1	1
21	G	A	UR2	1
21	G	A	UR3	1
21	G	A	UR4	1
21	G	A	UR5	2
21	G	A	UL1	1
21	G	A	UL2	1
21	G	A	UL3	1
21	G	A	UL5	2
21	G	B	LR1	1
21	G	B	LR2	1
21	G	B	LR3	1
21	G	B	LR4	1
21	G	B	LR5	1
21	G	B	LL1	1
21	G	B	LL2	1
21	G	B	LL3	1
21	G	B	LL4	1
21	G	B	LL5	1
22	P	A	UR1	4
22	P	A	UR2	3
22	P	A	UR3	3
22	P	A	UR5	3
22	P	A	UL1	3
22	P	A	UL2	3

22	P	A	UL3	3
22	P	A	UL5	3
22	P	B	LR1	3
22	P	B	LR2	3
22	P	B	LR3	3
22	P	B	LR4	3
22	P	B	LL1	2
22	P	B	LL2	2
22	P	B	LL3	2
22	P	B	LL4	3
23	E	A	UR1	1
23	E	A	UR2	1
23	E	A	UR3	1
23	E	A	UR4	1
23	E	A	UR5	1
23	E	A	UL1	1
23	E	A	UL2	1
23	E	A	UL3	1
23	E	A	UL4	1
23	E	A	UL5	1
23	E	B	LR1	1
23	E	B	LR2	1
23	E	B	LR3	1
23	E	B	LR4	1
23	E	B	LR5	1
23	E	B	LL1	1
23	E	B	LL2	1
23	E	B	LL3	1
23	E	B	LL4	1
23	E	B	LL5	1
24	G	A	UR1	1
24	G	A	UR2	1
24	G	A	UR3	1
24	G	A	UR4	1
24	G	A	UL1	1
24	G	A	UL2	1
24	G	A	UL3	1
24	G	A	UL4	1
24	G	B	LR1	1
24	G	B	LR2	1
24	G	B	LR3	1
24	G	B	LR5	1
24	G	B	LL1	1
24	G	B	LL2	1
24	G	B	LL3	1
24	G	B	LL5	1

Patient	Patient Hygiene	Agent	Tooth #	Decalcification Score
9	G	B	UR1	1
9	G	B	UR2	1
9	G	B	UR3	1
9	G	B	UR4	2
9	G	B	UR5	1
9	G	B	UL1	1
9	G	B	UL2	1
9	G	B	UL3	1
9	G	B	UL4	1
9	G	B	UL5	1
9	G	A	LR1	2
9	G	A	LR2	1
9	G	A	LR3	2
9	G	A	LR4	1
9	G	A	LR5	1
9	G	A	LL1	2
9	G	A	LL2	1
9	G	A	LL3	1
9	G	A	LL4	1
9	G	A	LL5	1
10	G	B	UR1	1
10	G	B	UR2	1
10	G	B	UR3	1
10	G	B	UR5	1
10	G	B	UL1	1
10	G	B	UL2	1
10	G	B	UL3	1
10	G	B	UL5	1
10	G	A	LR1	1
10	G	A	LR2	1
10	G	A	LR3	2
10	G	A	LR4	2
10	G	A	LR5	1
10	G	A	LL1	1
10	G	A	LL2	1
10	G	A	LL3	2
10	G	A	LL4	1
10	G	A	LL5	1
11	E	B	UR1	1
11	E	B	UR2	1
11	E	B	UR3	1
11	E	B	UR4	1
11	E	B	UL1	1
11	E	B	UL2	1
11	E	B	UL3	1
11	E	B	UL4	1
11	E	A	LR1	1
11	E	A	LR2	1
11	E	A	LR3	1

11	E	A	LR5	1
11	E	A	LL1	1
11	E	A	LL2	1
11	E	A	LL3	1
11	E	A	LL5	1
12	G	B	UR1	1
12	G	B	UR2	1
12	G	B	UR3	1
12	G	B	UR5	1
12	G	B	UL1	1
12	G	B	UL2	1
12	G	B	UL3	1
12	G	B	UL5	1
12	G	A	LR1	1
12	G	A	LR2	1
12	G	A	LR3	1
12	G	A	LR5	1
12	G	A	LL1	1
12	G	A	LL2	1
12	G	A	LL3	2
12	G	A	LL5	1
13	F	B	UR1	1
13	F	B	UR2	1
13	F	B	UR3	1
13	F	B	UR5	1
13	F	B	UL1	1
13	F	B	UL2	1
13	F	B	UL3	1
13	F	B	UL5	1
13	F	A	LR1	1
13	F	A	LR2	2
13	F	A	LR3	2
13	F	A	LR4	1
13	F	A	LR5	2
13	F	A	LL1	1
13	F	A	LL2	2
13	F	A	LL3	2
13	F	A	LL4	2
13	F	A	LL5	2
14	G	B	UR1	1
14	G	B	UR2	1
14	G	B	UR3	1
14	G	B	UR4	1
14	G	B	UR5	1
14	G	B	UL1	1
14	G	B	UL2	1
14	G	B	UL3	2
14	G	B	UL4	2
14	G	B	UL5	2
14	G	A	LR1	1

14	G	A	LR2	1
14	G	A	LR3	1
14	G	A	LR4	1
14	G	A	LR5	1
14	G	A	LL1	1
14	G	A	LL2	1
14	G	A	LL3	1
14	G	A	LL4	1
14	G	A	LL5	1
15	F	B	UR1	1
15	F	B	UR2	1
15	F	B	UR3	1
15	F	B	UR4	1
15	F	B	UR5	1
15	F	B	UL1	1
15	F	B	UL2	1
15	F	B	UL3	1
15	F	B	UL4	1
15	F	B	UL5	1
15	F	A	LR1	2
15	F	A	LR2	1
15	F	A	LR3	1
15	F	A	LR4	1
15	F	A	LR5	1
15	F	A	LL1	2
15	F	A	LL2	1
15	F	A	LL3	1
15	F	A	LL4	1
15	F	A	LL5	1
16	G	B	UR1	1
16	G	B	UR2	1
16	G	B	UR3	1
16	G	B	UR4	1
16	G	B	UR5	1
16	G	B	UL1	1
16	G	B	UL2	1
16	G	B	UL3	1
16	G	B	UL4	1
16	G	B	UL5	1
16	G	A	LR1	2
16	G	A	LR2	1
16	G	A	LR3	2
16	G	A	LR4	1
16	G	A	LR5	1
16	G	A	LL1	1
16	G	A	LL2	1
16	G	A	LL3	2
16	G	A	LL4	1
16	G	A	LL5	1
17	P	B	UR1	1

17	P	B	UR2	1
17	P	B	UR3	3
17	P	B	UR4	1
17	P	B	UR5	1
17	P	B	UL1	1
17	P	B	UL2	1
17	P	B	UL3	3
17	P	B	UL4	1
17	P	B	UL5	2
17	P	A	LR1	1
17	P	A	LR2	1
17	P	A	LR3	3
17	P	A	LR4	2
17	P	A	LR5	2
17	P	A	LL1	1
17	P	A	LL2	1
17	P	A	LL3	3
17	P	A	LL4	2
17	P	A	LL5	2
18	P	B	UR1	1
18	P	B	UR2	3
18	P	B	UR3	3
18	P	B	UR4	2
18	P	B	UR5	1
18	P	B	UL1	2
18	P	B	UL2	3
18	P	B	UL3	3
18	P	B	UL5	1
18	P	A	LR1	1
18	P	A	LR2	1
18	P	A	LR3	2
18	P	A	LR4	4
18	P	A	LR5	3
18	P	A	LL1	2
18	P	A	LL2	2
18	P	A	LL3	2
18	P	A	LL5	2
19	E	B	UR1	1
19	E	B	UR2	1
19	E	B	UR3	1
19	E	B	UR4	1
19	E	B	UR5	1
19	E	B	UL1	1
19	E	B	UL2	1
19	E	B	UL3	1
19	E	B	UL4	1
19	E	B	UL5	1
19	E	A	LR1	1
19	E	A	LR2	1
19	E	A	LR3	1

19	E	A	LR4	1
19	E	A	LR5	1
19	E	A	LL1	1
19	E	A	LL2	1
19	E	A	LL3	1
19	E	A	LL4	1
19	E	A	LL5	1
25	F	B	UR1	2
25	F	B	UR2	4
25	F	B	UR3	2
25	F	B	UR4	2
25	F	B	UR5	2
25	F	B	UL1	2
25	F	B	UL2	3
25	F	B	UL3	2
25	F	B	UL4	2
25	F	B	UL5	2
25	F	A	LR1	2
25	F	A	LR2	3
25	F	A	LR3	3
25	F	A	LR4	3
25	F	A	LR5	2
25	F	A	LL1	3
25	F	A	LL2	3
25	F	A	LL3	3
25	F	A	LL4	3
25	F	A	LL5	2

APPENDIX D

IN VIVO STATISTICAL ANALYSIS RESULTS

Decalcification Score By U/L

Crosstabs

		U/L		
		L	U	
Decalcification Score	Count			
	Total %			
	Row %			
	Col %			
	Col %			
1	185	186	371	
	39.45	39.66	79.10	
	49.87	50.13		
	78.08	80.17		
2	36	25	61	
	7.68	5.33	13.01	
	59.02	40.98		
	15.19	10.78		
3	15	18	33	
	3.20	3.84	7.04	
	45.45	54.55		
	6.33	7.76		
4	1	3	4	
	0.21	0.64	0.85	
	25.00	75.00		
	0.42	1.29		
	237	232	469	
	50.53	49.47		

Tests

Source	DF	-LogLikelihood	RSquare (U)
Model	3	1.63174	0.0051
Error	465	316.39676	
C Total	468	318.02850	
Total Count	469		

Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	3.263	0.3526
Pearson	3.206	0.3609

Warning: 20% of cells have expected count less than 5. Chi-squares suspect

Agent By Decalcification Score

Crosstabs

		Decalcification Score			
		1	2	3	4
Agent	Count				
	Total %				
	Row %				
	Col %				
	Col %				
A	171	41	21	3	236
	36.46	8.74	4.48	0.64	50.32
	72.46	17.37	8.90	1.27	
	46.09	67.21	63.64	75.00	
B	200	20	12	1	233
	42.64	4.26	2.56	0.21	49.68
	85.84	8.58	5.15	0.43	
	53.91	32.79	36.36	25.00	
	371	61	33	4	469
	79.10	13.01	7.04	0.85	

Tests

Source	DF	-LogLikelihood	RSquare (U)
Model	3	6.58096	0.0202
Error	465	318.49547	
C Total	468	325.07643	
Total Count	469		

Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	13.162	0.0043
Pearson	12.932	0.0048

Warning: 20% of cells have expected count less than 5. Chi-squares suspect

Response: Decalcification Score

Summary of Fit

RSquare	0.453694
RSquare Adj	0.444193
Root Mean Square Error	0.472271
Mean of Response	1.296375
Observations (or Sum Wgts)	469

Lack of Fit

Parameter Estimates

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Patient Hygiene	3	3	75.973773	113.5429	<.0001
Agent	1	1	7.219813	32.3700	<.0001
Location	1	1	0.640192	2.8703	0.0909
Patient Hygiene*Agent	3	3	4.850451	7.2490	<.0001

Whole-Model Test

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Model	8	85.20547	10.6507	47.7524	
Error	460	102.59837	0.2230		
C Total	468	187.80384			<.0001

Patient Hygiene

Profile Plot

Effect Test

Sum of Squares	F Ratio	DF	Prob>F
75.973773	113.5429	3	<.0001

Least Squares Means

Level	Least Sq Mean	Std Error	Mean
E	1.006578947	0.0383062391	1.00658
F	1.665476190	0.0620491259	1.67241
G	1.106150063	0.0343553722	1.10582
P	2.128571429	0.0564471851	2.12857

Response: Decalcification Score

Summary of Fit

RSquare	0.427867
RSquare Adj	0.421688
Root Mean Square Error	0.481737
Mean of Response	1.296375
Observations (or Sum Wgts)	469

Lack of Fit

Parameter Estimates

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Patient Hygiene	3	3	76.204435	109.4557	<.0001
Agent	1	1	4.031803	17.3732	<.0001
Location	1	1	0.002471	0.0106	0.9179

Whole-Model Test

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Model	5	80.35502	16.0710	69.2504	
Error	463	107.44882	0.2321		
C Total	468	187.80384			<.0001

Patient Hygiene

Profile Plot

Effect Test

Sum of Squares	F Ratio	DF	Prob>F
76.204435	109.4557	3	<.0001

Least Squares Means

Level	Least Sq Mean	Std Error	Mean
E	1.006578947	0.0390740595	1.00658
F	1.669293457	0.0632648687	1.67241
G	1.105365631	0.0350432596	1.10582
P	2.128571429	0.0575786273	2.12857

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
A	236	1.38983	0.702989	0.04576
B	233	1.20172	0.539473	0.03534

Means Comparisons

Dif=Mean[i]-Mean[j]	A	B
A	0.000000	0.188114
B	-0.18811	0.000000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q *		
1.96506		
Abs(Dif)-LSD	A	B
A	-0.11344	0.074306
B	0.074306	-0.11417

Positive values show pairs of means that are significantly different.

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
A	236	59119.5	250.506	3.517
B	233	51095.5	219.294	-3.517

2-Sample Test, Normal Approximation

S	Z	Prob> Z
51095.5	-3.51740	0.0004

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
12.3755	1	0.0004

Median Test (Number of Points Above Median)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
A	236	65	0.275424	3.560
B	233	33	0.141631	-3.560

2-Sample Test, Normal Approximation

S	Z	Prob> Z
33	-3.55951	0.0004

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
12.6701	1	0.0004

Decalcification Score By Agent

Oneway Anova

Summary of Fit

RSquare 0.022092
 RSquare Adj 0.019998
 Root Mean Square Error 0.627109
 Mean of Response 1.296375
 Observations (or Sum Wgts) 469

t-Test

	Difference	t-Test	DF	Prob> t
Estimate	0.188114	3.248	467	0.0012
Std Error	0.057916			
Lower 95%	0.074305			
Upper 95%	0.301923			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Model	1	4.14893	4.14893	10.5500	
Error	467	183.65491	0.39327		
C Total	468	187.80384	0.40129		0.0012

Means for Oneway Anova

Level	Number	Mean	Std Error
A	236	1.38983	0.04082
B	233	1.20172	0.04106

Std Error uses a pooled estimate of error variance

Tests that the Variances are Equal

Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
A	236	0.7029893	0.5649239	0.3898305
B	233	0.5394734	0.3462948	0.2017167

Test	F Ratio	DF Num	DF Den	Prob>F
O'Brien[.5]	5.4807	1	467	0.0199
Brown-Forsythe	10.5500	1	467	0.0012
Levene	32.5529	1	467	<.0001
Bartlett	16.1255	1	*	<.0001

Welch Anova testing Means Equal, allowing Std's Not Equal

F Ratio	DF Num	DF Den	Prob>F
10.5850	1	440.28	0.0012
t-Test			3.2535

Decalcification Score By Location

Oneway Anova

Summary of Fit

RSquare	0.00007
RSquare Adj	-0.00207
Root Mean Square Error	0.63413
Mean of Response	1.296375
Observations (or Sum Wgts)	469

t-Test

	Difference	t-Test	DF	Prob> t
Estimate	-0.01058	-0.181	467	0.8567
Std Error	0.058566			
Lower 95%	-0.12567			
Upper 95%	0.104503			

Assuming equal variances

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Model	1	0.01314	0.013135	0.0327	
Error	467	187.79070	0.402121		
C Total	468	187.80384	0.401290	0.8567	

Means for Oneway Anova

Level	Number	Mean	Std Error
L	237	1.29114	0.04119
U	232	1.30172	0.04163

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
L	237	1.29114	0.599828	0.03896
U	232	1.30172	0.667357	0.04381

Means Comparisons

Dif=Mean[i]-Mean[j]	U	L
U	0.000000	0.010585
L	-0.01058	0.000000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

q *		
1.96506		
Abs(Dif)-LSD	U	L
U	-0.1157	-0.1045
L	-0.1045	-0.11447

Positive values show pairs of means that are significantly different.

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
L	237	56084.5	236.643	0.374
U	232	54130.5	233.321	-0.374

2-Sample Test, Normal Approximation

S	Z	Prob> Z
54130.5	-0.37396	0.7084

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
0.1402	1	0.7081

Median Test (Number of Points Above Median)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
L	237	52	0.219409	0.562
U	232	46	0.198276	-0.562

2-Sample Test, Normal Approximation

S	Z	Prob> Z
46	-0.56223	0.5740

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
0.3161	1	0.5740

Tests that the Variances are Equal

Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
L	237	0.5998283	0.4545212	0.2911392
U	232	0.6673567	0.4837990	0.3017241

Test	F Ratio	DF Num	DF Den	Prob>F
O'Brien[.5]	0.9153	1	467	0.3392
Brown-Forsythe	0.0327	1	467	0.8567
Levene	0.5552	1	467	0.4566
Bartlett	2.6484	1	+	0.1037

Welch Anova testing Means Equal, allowing Std's Not Equal

F Ratio	DF Num	DF Den	Prob>F
0.0326	1	459.53	0.8568

t-Test
0.1805

Decalcification Score By Patient Hygiene

Oneway Anova

Summary of Fit

RSquare	0.406318
RSquare Adj	0.402488
Root Mean Square Error	0.489669
Mean of Response	1.296375
Observations (or Sum Wgts)	469

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Model	3	76.30810	25.4360	105.0828	
Error	465	111.49574	0.2396		
C Total	468	187.80384	0.4013		<.0001

Means for Oneway Anova

Level	Number	Mean	Std Error
E	152	1.00658	0.03972
F	58	1.67241	0.08430
G	189	1.10582	0.03562
P	70	2.12857	0.05853

Std Error uses a pooled estimate of error variance

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
E	152	1.00658	0.081111	0.00658
F	58	1.67241	0.781091	0.10256
G	189	1.10582	0.308424	0.02243
P	70	2.12857	0.915588	0.10943

Means Comparisons

Dif=Mean[i]-Mean[j]	P	F	G	E
P	0.00000	0.45616	1.02275	1.12199
F	-0.45616	0.00000	0.56659	0.66583
G	-1.02275	-0.56659	0.00000	0.09924
E	-1.12199	-0.66583	-0.09924	0.00000

Alpha= 0.05

Comparisons for all pairs using Tukey-Kramer HSD

Abs(Dif)-LSD	P	F	G	E
P	-0.21341	0.231980	0.846099	0.939621
F	0.231980	-0.23445	0.377074	0.470974
G	0.846099	0.377074	-0.12988	-0.03831
E	0.939621	0.470974	-0.03831	-0.14483

Positive values show pairs of means that are significantly different.

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
E	152	28486	187.421	-7.426
F	58	17499.5	301.612	5.640
G	189	39474	208.857	-4.841
P	70	24759.5	353.707	11.207

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
186.0318	3	<.0001

Median Test (Number of Points Above Median)

Level	Count	Score Sum	Score Mean	(Mean-Mean0)/Std0
E	152	1	0.006579	-7.457
F	58	29	0.500000	5.816
G	189	20	0.105820	-4.509
P	70	48	0.685714	10.626

1-way Test, Chi-Square Approximation

ChiSquare	DF	Prob>ChiSq
175.4321	3	<.0001

Tests that the Variances are Equal

Level	Count	Std Dev	MeanAbsDif to Mean	MeanAbsDif to Median
E	152	0.0811107	0.0130713	0.0065789
F	58	0.7810908	0.6724138	0.6724138
G	189	0.3084243	0.1892444	0.1058201
P	70	0.9155885	0.7828571	0.7571429

Test	F Ratio	DF Num	DF Den	Prob>F
O'Brien[.5]	74.0067	3	465	<.0001
Brown-Forsythe	133.1347	3	465	<.0001
Levene	169.9976	3	465	<.0001
Bartlett	191.5028	3	*	<.0001

Weich Anova testing Means Equal, allowing Std's Not Equal

F Ratio	DF Num	DF Den	Prob>F
53.4378	3	137.46	<.0001

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