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School of Dentistry  
Virginia Commonwealth University

This is to certify that the thesis prepared by Daniel Ryan Pennella, D.M.D., entitled The Effects of a Fluoride Releasing Orthodontic Primer on Demineralization around Brackets: An in-vivo study has been approved by his committee as satisfactory completion of the thesis requirement for the degree of Master of Science in Dentistry.

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The Effects of a Fluoride Releasing Orthodontic Primer on Demineralization around Brackets:  
An in-vivo study

**A thesis submitted in partial fulfillment of the requirements for the degree of Master of  
Science in Dentistry at Virginia Commonwealth University.**

**By**

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Abstract**The Effects of a Fluoride Releasing Orthodontic Primer on Demineralization around Brackets: An in-vivo study**

By Daniel Ryan Pennella, D.M.D.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

Virginia Commonwealth University, 2011

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The purpose of this study was to investigate the effects of a fluoride releasing orthodontic primer on demineralization adjacent to brackets. Twenty-two patients were recruited for this study. One premolar was randomly chosen as the experimental tooth, the contralateral tooth was the control. Teeth were visually analyzed for white spot lesions (WSLs). Knoop microhardness was used to determine hardness. Visual examination results showed no significant difference in the number of WSLs observed between Opal Seal and Transbond XT over the duration of this study. Solid conclusions could not be drawn from the results of microhardness testing. Therefore, it cannot be concluded that there is a difference in enamel hardness between Opal Seal and Transbond XT. However, prior to 90 days, teeth showed a significant difference in WSLs. Suggesting a protective effect of Opal Seal that diminished with time. Future studies are necessary to determine the clinical performance of this product.

## Introduction

Demineralization around orthodontic brackets is a common problem during fixed appliance therapy when oral hygiene is poor. Lesions become first clinically visible as white spots, due to an optical phenomenon that is caused by mineral loss in the surface or sub-surface enamel.<sup>1-3</sup> Previous studies on the mechanical and crystallographic characteristics of these incipient carious lesions have shown that there is a 10-50% reduction in mineral content.<sup>4,5</sup> White spot lesions (WSLs) have been previously reported to develop within 4 weeks of band/bracket placement. The prevalence of WSLs in orthodontic patients has been reported in the range of 50-96%.<sup>2,5,6</sup> The increased prevalence of enamel demineralization is largely due to patients' inability to perform effective oral hygiene measures in the presence of brackets and to the increased plaque retention around orthodontic attachments.<sup>1,7</sup>

It has been previously shown that patients with fixed orthodontic appliances have increased levels of acidogenic bacteria present in plaque, most notably *S. mutans* and *Lactobacilli*.<sup>8</sup> The elevated levels of bacteria are responsible for decreasing the pH of plaque in orthodontic patients more than that of non-orthodontic patients.<sup>9</sup> The plaque layer on the enamel surface provides a source of acid production as the bacteria produce hydrogen ions from the metabolic breakdown of fermentable carbohydrates. As hydrogen ions are released, the acids diffuse through the plaque into the adjacent enamel. Once enamel is exposed to a critical pH around 5.5, it begins to dissolve causing demineralization of hydroxyapatite tooth structure.<sup>10</sup> The layer of plaque also acts as a physical barrier by limiting the diffusion of acid away from the tooth surface. Therefore, the potential for remineralization from the available exogenous calcium and phosphate ions in the patients' saliva is greatly reduced in the presence of plaque.<sup>11</sup> The

resulting demineralization and prevention of remineralization leads to the development of WSLs that often persist and cause long term esthetic problems.<sup>3,12</sup>

Many published studies and review articles advocate management of orthodontic WSLs with preventive strategies that include patient education, routine professional prophylaxis, and appropriate preventive medicaments such as topical fluorides.<sup>1,13-16</sup> In response to the prevalent problem of WSLs, fluoride mouthrinses and fluoride releasing cements have also been advocated for orthodontic patients. Numerous studies have reported that fluoride regimens can reduce caries during orthodontic treatment with fixed appliances.<sup>17-22</sup>

The scientific basis for the use of fluoride to prevent demineralization has been well established.<sup>23,24</sup> Fluoride can be incorporated into the crystalline lattice of the mineral of dental hard tissues, resulting in a mineral phase that is less soluble and more acid resistant. Calcium fluoride is the major reaction product of topical fluoride treatment of enamel and it has been found to be a major factor in the cariostatic mechanism of fluoride. Calcium fluoride can persist in dental plaque on the enamel surface for several weeks after topical application, and it can be incorporated into the lattice structure of the tooth as fluorapatite. This fluorapatite formation has been shown to be successful in reducing enamel demineralization during fixed appliance therapy.<sup>25</sup>

The problem with most of the fluoride regimes is that they rely heavily on patient compliance. Geiger et al.<sup>19</sup> showed a clear association in which an increase in white spot incidence occurred with decreasing compliance and they reported that only 13% of patients were fully compliant with a fluoride regime. In light of these findings, efforts have been made to develop compliance-free fluoride delivery systems.<sup>26</sup> For example, manufacturers have incorporated fluoride into orthodontic adhesives to help prevent or reduce the formation of white

spot lesions. Glass ionomer cements have been shown to be effective in releasing the incorporated fluoride to the surroundings in-vitro.<sup>27,28</sup> However, previous studies have also shown that the amount of fluoride released is highest on the first day, sharply decreases on the second day, and gradually decreases to undetectable levels.<sup>29-31</sup> Even though the amount of released fluoride is sharply decreased by the end of the third day, the fluoride releasing ability can be recharged in-vivo to some degree with the use of a topical fluoride agent or fluoride containing toothpaste. In the literature, there is a controversy concerning the anticariogenic effects of these materials as the amount of recharge reported exhibits a wide range.<sup>32-34</sup>

Of the current fluoride products on the market, varnishes are easy to use and do not depend on patient cooperation. Fluoride varnishes adhere to the tooth surface and release fluoride for a long period of time. The longer contact time with enamel enables fluoride varnishes to incorporate significantly more fluoride into enamel when compared with acidulated phosphate fluoride gel and amine fluoride applications.<sup>35</sup> Brudevold et al.<sup>36</sup> observed that the efficiency of topical fluoride was directly related to the exposure period to enamel. A longer exposure period permanently increased the amount of fluoride retained in the enamel, enhanced formation of fluoridated hydroxyapatite, and reduced the acid solubility of enamel.<sup>26</sup> Studies of some current fluoride varnishes on the market have shown them to be effective in reducing enamel demineralization around orthodontic brackets, but they often need to be re-applied throughout treatment, resulting in extra clinical chair time and extra cost.<sup>37-39</sup>

The application of resin sealants to the enamel around orthodontic brackets to prevent demineralization has also been reported in the literature.<sup>15,37,40</sup> Placement of a sealant on the buccal surface of the tooth, after acid etching was thought to provide several benefits: increased bond strength, sealing of etched enamel, and protection against demineralization around the

bracket. It was suggested that the sealant could be effective in preventing demineralization during treatment.<sup>37</sup> Previous studies have proven that most of the chemically cured sealants do not effectively seal smooth enamel surfaces, because of oxygen inhibition of polymerization when the sealant is in contact with the air in a thin layer.<sup>37,41-43</sup> Alternatively, light-cured sealants have been proven to cure completely on smooth enamel surfaces, effectively preventing enamel demineralization in vitro.<sup>37,43,44</sup> However, subsequent clinical studies did not support the results of the laboratory research.<sup>45,46</sup> The unfilled or lightly filled light-cured sealant could not provide more protection than the chemically cured sealant. Mechanical and chemical wear of sealants in vivo could weaken the protection effect, resulting in decalcification under the sealant surface. Newer highly filled sealants have shown significant reduction of enamel demineralization in vitro and a resistance to toothbrush abrasion.<sup>37,38</sup>

Recently, a fluoride releasing bonding primer, Opal Seal (Ultradent, South Jordan, UT), has become commercially available. This product is 38% filled with glass ionomer and nano filler particles and is claimed by the manufacturer to exhibit a superior fluoride recharging ability. Furthermore, it is claimed that the primer is able to penetrate deeply into fissures of the teeth, resulting in a long lasting coverage and mechanical retention. Since a continuous fluoride supply to the tooth surface is anticipated to be available over a long period of time, it is thought that Opal Seal would have an increased potential to prevent demineralization adjacent to the brackets. To our knowledge, the effectiveness of Opal Seal in preventing the formation of WSLs around orthodontic brackets has not been established.

In the literature, visual inspection has historically been the principal method of examination to detect demineralization since the term “white spot lesion” was defined by Fejerskov et al.<sup>47</sup> as “the first sign of a caries lesion on enamel that can be detected with the

naked eye.<sup>47,48</sup> Lesions are identified after thoroughly drying the tooth and given a nominal rank depending on severity according to a visual white spot scale. Although this technique has been criticized for its high degree of subjectivity and variability between studies, the method produces results that can be more easily interpreted by the clinician when compared to minute differences in demineralization identified by more sensitive laboratory tests.<sup>49</sup>

Other methods to measure and compare enamel demineralization include quantitative light-induced fluorescence (QLF), polarized light microscopy, microradiographs, or microhardness testing. Since enamel hardness is thought to be affected by its mineral content, the microhardness test is widely used in laboratory studies to investigate enamel demineralization.<sup>50,51</sup> Previous studies have shown that microhardness is a reliable technique because it is simple, quantitative, and reproducible.<sup>50-53</sup> Featherstone et al.<sup>53</sup> reported a direct relationship between the hardness values and mineral content of the enamel. Kielbassa et al.<sup>54</sup> also determined a reliable correlation between microradiographic and microhardness data, strengthening the validity behind microhardness testing.

Therefore, the purpose of this in-vivo study was to investigate the effect of Opal Seal on enamel demineralization adjacent to orthodontic brackets using both qualitative and quantitative evaluation methods. The null hypothesis is that Opal Seal will show no difference in the amount of clinical white spot lesions or enamel demineralization, as determined by visual examination and microhardness, compared to Transbond XT.

### Materials and Methods

Prior to the study, ethical approval was obtained from the Research Office of the Virginia Commonwealth University. Twenty-two orthodontic patients, 10-20 years of age, who were scheduled to have at least two of their premolars extracted for orthodontic purposes were recruited for the study. Informed consent was obtained from the patients 18 and older and from the parents of the younger patients. In addition, assent was obtained from patients 10-17 years old. Inclusion criteria was (1) overall good general health (2) age 10 years and older (3) premolars fully erupted and intact without visible defects on their buccal surfaces. A split mouth study design was utilized. In each patient, one premolar was randomly chosen as the experimental tooth and the contralateral tooth was assigned as the control.

On the day of the bonding appointment, tooth surfaces in both groups were cleaned with a rubber prophyl cup and a fluoride-free pumice, etched for 15 seconds with 37% phosphoric acid gel (3M Unitek, Monrovia, CA), rinsed with water for 5 seconds, and dried with an oil free air-water syringe. Opal Seal primer was applied to the teeth in the experimental group according to the manufacturer's instructions. Teeth in the control group received a non-fluoride conventional orthodontic primer (Transbond XT moisture insensitive primer, 3M Unitek). After positioning the pre-coated premolar brackets (APC II Mini Twin Bicuspoid, 3M Unitek) onto the enamel surfaces, the adhesive was light-cured three seconds from the mesial and three seconds from the distal aspects using a plasma arc visible-light curing unit (Ortholite, 3M Unitek). All of the bonding procedures were carried out by the same clinician. Subsequently, elastomeric spacers were placed around bracket wings to help with increased plaque accumulation so that the ideal environment for WSL development could be provided. Patients were not informed which tooth received the experimental orthodontic primer. All patients were instructed to brush twice a day

with an over the counter fluoride containing toothpaste. However, they were asked not to rinse with any antibacterial or fluoride containing mouthrinse.

Teeth were scheduled to be extracted approximately 6-8 weeks after the initial bracket placement. Patients were provided with bottles containing a 1% Chloramine T solution so that teeth would be placed into these containers immediately after extractions were performed. Upon collection from the patients, teeth were subsequently cleaned and placed into bottles filled with fresh 1% Chloramine T solution for further disinfection until experimental testing was performed.

## **Evaluation of WSLs on tooth surfaces**

### **1) Visual Examination**

Visual examination of the extracted teeth was carried out independently by two of the authors (D.P. and E.T) who were blinded to the experimental protocol conducted on each tooth. Each tooth was removed from its' individual storage bottle and air-dried for 5 seconds and the buccal surface was examined visually, with the naked eye, for enamel decalcification in the portion of the crown gingival to the bracket using the following scale (Figure 1):

Score 0 = No visible white spots or surface disruption (no decalcification)

Score 1 = Visible white spot without surface disruption (mild decalcification)

Score 2 = Visible white spot with a roughened surface but not requiring restoration  
(moderate decalcification)

Score 3 = Visible white spot lesion requiring restoration (severe decalcification).

In addition, a surface was considered as having a lesion when it had a white spot or was cavitated. Hypoplastic or fluorotic enamel was not scored as caries.

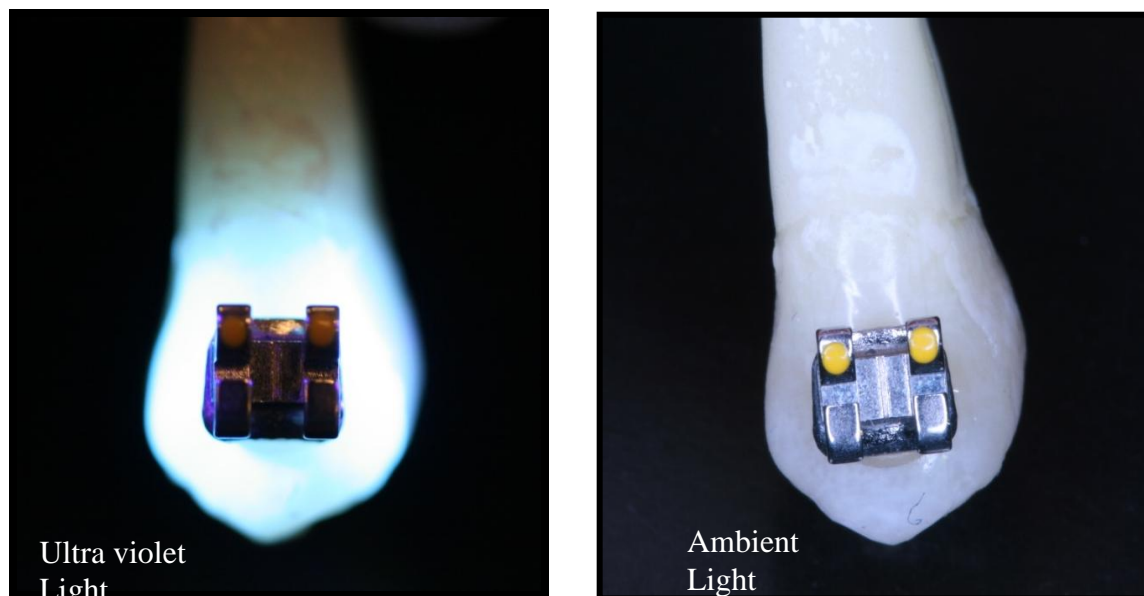




**Figure 1.** Example of white spot lesion visual examination scale.

## 2) Ultra Violet Light Examination

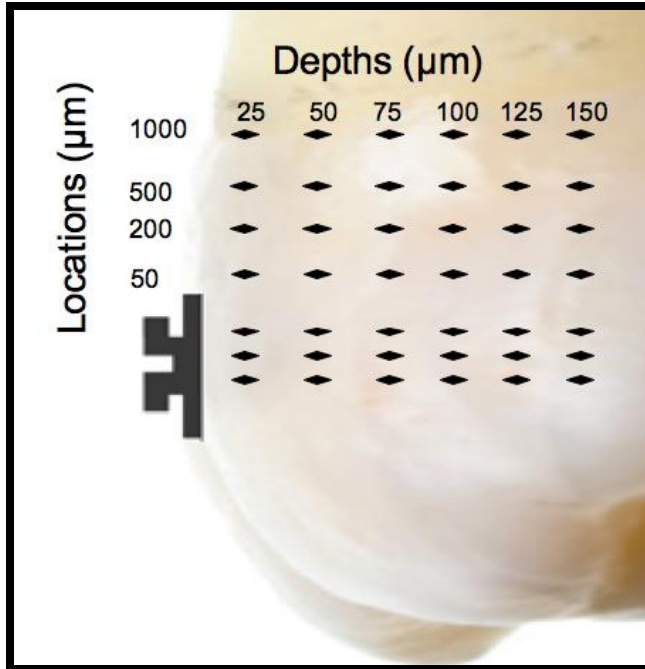
Opal Seal primer has the property of fluorescence under ultra violet light. Each tooth was air dried for 5 seconds and visualized under ultra violet light, a hand-held unit provided by the manufacturer, to determine the percentage of Opal Seal primer remaining on the tooth. Since an absolute percentage was difficult to determine, the percentage of primer remaining was recorded as 100%, 75%, 50%, 25% or 0%. The score of 100% corresponded to primer covering the entire buccal surface of the tooth and the score of 0% indicated no fluorescence of primer present (Figure 2).



**Figure 2.** 100% Opal Seal remaining viewed under ultra violet light and ambient light.

### 3) Hardness Test

For the cross-sectional microhardness testing, teeth were embedded in resin (EpoxiCure Resin, Buehler, Lake Bluff, IL) and allowed to cure overnight. They were then sectioned longitudinally through the bracket with a low speed saw (Accutom-5, Struers Inc., Westlake, OH) using a low concentration diamond blade. Samples were polished using 4000 grit laboratory grade silicon carbide wet grinding paper with non-adhesive back (Struers Inc.). The hardness of the enamel surrounding the brackets was evaluated by using a microhardness tester (Duramin-5 Hardness Tester, Struers Inc.). Indentations were made with the long axis of the diamond parallel to the outer enamel surface. The five distance locations were: underneath the bracket, 50  $\mu\text{m}$ , 200  $\mu\text{m}$ , 500  $\mu\text{m}$ , and 1000  $\mu\text{m}$  away from the bracket edge toward the gingival margin. For each depth location, the first indentation was made with a 25 gram force load, 10 second press time at 25  $\mu\text{m}$  deep from the enamel surface. The next mark was at 50  $\mu\text{m}$ , and subsequent indentations were done in 25  $\mu\text{m}$  steps toward the dentin up to 150  $\mu\text{m}$ , for a total of six depth indentations.



**Figure 3.** Depiction of locations and depths for hardness measurements.

The length of the indentation was used to determine the Knoop hardness number (KHN) for each sample. The Knoop hardness was calculated using the following formula:  $KHN = 14229L / d^2$ , where the load  $L$  is in grams force and the long diagonal  $d$  is in  $\mu\text{m}$ . The KHN of the six depth indentations was then reported in  $\text{kgf} / \mu\text{m}^2$  unit for each of the five distance sections. The mean of the three indentations underneath the bracket at depth  $150 \mu\text{m}$  was chosen as the KHN of the sound enamel for that specific tooth. The matrix of the remaining four section's site distance measurements ( $50 \mu\text{m}$ ,  $200 \mu\text{m}$ ,  $500 \mu\text{m}$ , and  $1000 \mu\text{m}$ ) away from the bracket edge toward the CEJ of the tooth, and 6 depth indentations ( $25 \mu\text{m}$ ,  $50 \mu\text{m}$ ,  $75 \mu\text{m}$ ,  $100 \mu\text{m}$ ,  $125 \mu\text{m}$ ,  $150 \mu\text{m}$ ) were then compared to the hardness value of the sound enamel as previously determined to reduce any discrepancies of enamel hardness amongst different samples.

### Statistical Analysis

A repeated-measures logistic regression was used to explore the differences between the percentage of visible white spot lesions observed in the Opal Seal group compared to the Transbond XT group (control). In addition, any potential interactions were further explored when appropriate. Regression analysis was also used to determine whether there was a correlation between the percentage of Opal Seal remaining on the teeth and the number of days in-vivo.

Analysis of covariance (ANCOVA) was used to evaluate the effect of material (Opal Seal and Transbond XT), depth from the enamel surface (25, 50, 75, 100, 125, and 150  $\mu\text{m}$ ), position (under the bracket, and on the buccal surface in the cervical region at 0, 50, 200, 500, and 1000  $\mu\text{m}$  from the brackets), and their interactions. Specifically, a repeated-measures mixed-model ANCOVA, with the reference sound enamel hardness value (under the bracket at a depth of 150  $\mu\text{m}$ ) as a covariate, was used to model the mean hardness for each depth and distance. This method was used to account for the correlation within each subject and to correct for the fact that some individuals contributed two teeth (both experimental and control) and others only one (either experimental or control). SAS (version 9.1, SAS Institute Inc., Cary, NC) was used to perform all analyses. Statistical significance was set at  $p \leq 0.05$ .

## Results

### **Visual Examination**

A total of 72 teeth were evaluated for WSLs by two clinicians (Table I). In 14 individuals, four teeth (both maxillary and mandibular premolars) and in 8 individuals two teeth (either maxillary or mandibular premolars) were observed. In each individual, an equal number of control and experimental teeth were observed.

**Table I.** Teeth observed for WSLs visual examination.

Group	Tooth				All
	LL	LR	UL	UR	
Control	7	7	8	14	36
Experimental	7	7	14	8	36
All	14	14	22	22	72

The first rater recorded the presence of a WSL in 33% of the teeth (24 out of 72 teeth) and the second rater recorded WSL in 42% (30 out of 72 teeth). The two raters agreed in 62/72 cases. The chance-corrected kappa coefficient indicated an agreement of 0.71 (SE = 0.085). The absence or presence of WSL according to each of the groups of teeth is shown in Table II. Overall, a WSL was observed in 38% of the teeth, with 46% of control teeth and 29% of experimental teeth showing lesions.

A repeated-measures logistic regression was used to provide an answer to the primary aim of the study: Can we conclude that there is a difference in the presence of WSL in the experimental versus the control group after covarying out tooth location, bracket presence, rater difference, and days in-vivo? The multiple logistic regression that included all of these factors indicated that, over the course of the study, there was no significant difference in the amount of WSLs present between the experimental and control groups ( $p = 0.106$ ). However, a preliminary

analysis indicated a lack-of-fit, so the potential for interaction between pairs and days in-vivo was analyzed. The p-value for the interaction analysis indicated that the pairs effect (the control vs experimental difference) depended upon the days in-vivo ( $p = 0.009$ ). Table III provides the detailed information on percentage of white spots observed and the days in-vivo. As it can be seen from the table, the prevalence of WSLs was significantly lower in the experimental group compared to the control when observed earlier than 90 days ( $p = 0.009$ ).

**Table II.** WSLs visual examination results.

Groups	WSL		% yes	p-value
	no	yes		
Pairs				0.106
Control	39	33	46	
Experimental	51	21	29	
Tooth				0.222
LL	20	8	29	
LR	13	15	54	
UL	30	14	32	
UR	27	17	39	
Bracket present				
No	27	31	53	0.165
Yes	63	23	27	
Rater				0.083
1	48	24	33	
2	42	30	42	
Days in-vivo				0.100
less than 90 days	68	28	29	
90+ days	22	26	54	
Pairs * Days in-vivo interaction				0.009

**Table III:** Percentage of WSLs observed before and after 90 days.

Groups		WSL		% yes
		no	yes	
Control	Earlier than 90 days	27	21	44
	90+ days	12	12	50
Experimental	Earlier than 90 days	41	7	15
	90+ days	10	14	58

### Ultra Violet Light Results

Regression analysis indicated that there was no correlation between the percentage of Opal Seal remaining on the buccal surface of the tooth and the number of days the teeth were in the mouth ( $r = -0.06$ ,  $p > 0.6$ ).

### Hardness Testing Results

In order to account for the potential variability between maxillary and mandibular premolars, only maxillary premolars were tested for Knoop hardness. Therefore, a total of 44 maxillary premolars were available for hardness testing, but 20 of the teeth had brackets inadvertently debonded during extraction procedures. If the bracket was not present on a tooth, hardness testing could not be performed accurately due to difficulty in determining depth and distance without a reference point. Thus, a total of 24 premolars with brackets were tested for hardness as described in Table IV. Only these teeth were considered in the subsequent statistical analysis.

**Table IV.** Teeth for Knoop hardness testing.

Group	Tooth		
	UL	UR	All
Control	4	7	11
Experimental	8	5	13
All	12	12	24

The mean hardness number for every combination of depth and distance is reported in Table V (for descriptive purposes). The raw hardness mean values ranged from 274.7-317.7 kgf /  $\mu\text{m}^2$ .

**Table V.** Mean KHN hardness values.

E/C	Distance ( $\mu\text{m}$ )	Depth ( $\mu\text{m}$ )					
		25	50	75	100	125	150
C	0	301.5	301.4	303.6	305.9	310.1	310.2
C	50	286.4	283.8	299.5	303.5	297.5	307.1
C	200	279.1	279.2	288.8	296.0	306.3	300.7
C	500	281.3	298.6	294.2	307.5	307.3	309.0
C	1000	283.9	286.7	301.6	305.5	307.3	309.3
E	0	303.7	304.9	306.3	310.5	310.7	317.7
E	50	277.6	284.5	301.3	300.4	304.2	313.5
E	200	285.8	297.0	294.9	303.9	313.7	302.5
E	500	274.7	283.3	301.7	304.6	314.6	312.7
E	1000	285.3	289.3	298.9	307.6	313.7	307.3

Using a repeated-measures mixed-model ANCOVA with the reference hardness value for sound tooth enamel (under the brackets at a distance of 0 and depth a of 150  $\mu\text{m}$ ) as a covariate, the modeled hardness values were determined (Table VI).

**Table VI.** Mean KHN hardness values modeled by repeated–measures mixed-model ANCOVA.

E/C	Modeled Distance ( $\mu\text{m}$ )	Depth ( $\mu\text{m}$ )					
		25	50	75	100	125	150
C	0	302.3	302.3	304.4	306.7	310.9	311.0
C	50	287.1	284.5	300.2	304.2	298.2	307.9
C	200	279.8	279.9	289.5	296.7	307.0	301.4
C	500	282.0	299.3	294.9	308.2	308.0	309.7
C	1000	284.6	287.4	302.3	306.2	308.0	310.0
E	0	302.3	303.5	304.9	309.0	309.3	316.3
E	50	276.0	283.0	299.8	298.9	302.6	312.0
E	200	284.2	295.5	293.4	302.3	312.2	301.0
E	500	273.2	281.7	300.2	303.1	313.1	311.2
E	1000	283.8	287.8	297.3	306.0	312.1	305.8

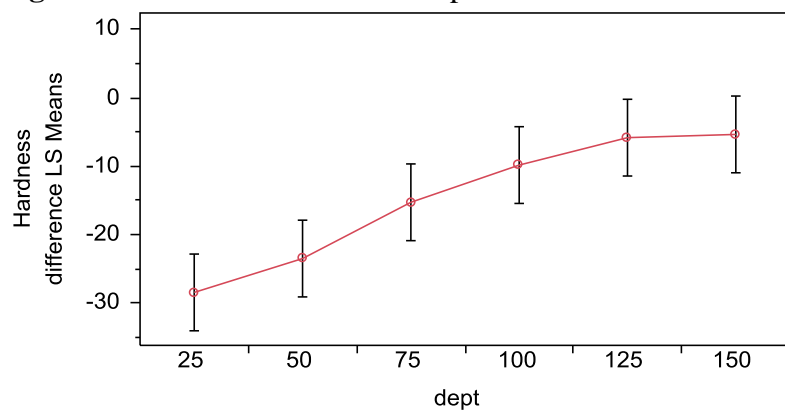
In order to compare the hardness values, the model used as a response variable the difference between the site-specific hardness and the hardness of that tooth at distance = 0  $\mu\text{m}$ , depth = 150  $\mu\text{m}$ . When these hardness-difference values were modeled using a repeated-measures mixed-model ANCOVA with the reference hardness as a covariate, the results indicated that teeth in the control group had significantly higher hardness values than teeth in the



experimental group ( $p = 0.0004$ ). Even though ANCOVA indicated a statistically significant difference between the hardness of the groups, the average difference of hardness values between the experimental and control groups was so small ( $6.9 \text{ kgf} / \mu\text{m}^2$ , 95% CI = 3.12 to 10.72) that solid conclusions could not be drawn.

The results of ANCOVA also indicated that there was a significant difference in hardness values across the 6 depths from the enamel surface ( $p < 0.0001$ ). The hardness increased as the depth got deeper indicating that the superficial layer of enamel was most affected by demineralization (Figure 4).

**Figure 4.** Hardness trend across depths.



ANCOVA also showed that there was a significant difference in the hardness among the 5 distances away from the edge of the bracket ( $p < 0.0001$ ). The hardness under the bracket was greater than the hardness values at any distance away from the edge of the bracket. This indicated that enamel directly under the bracket is less affected by demineralization compared to the area adjacent to the edge of the bracket in a cervical direction.

## Discussion

Demineralization around orthodontic appliances, in the form of visible white spot lesions, has been commonly reported in patients with inadequate oral hygiene.<sup>2</sup> White spot lesions develop due to prolonged bacterial plaque accumulation, leading to acidic dissolution of enamel. The incidence of white spot lesions in orthodontic populations has been reported in a range of 50-96%, and lesions can be clinically visible in as little as 4 weeks after placement of fixed appliances.<sup>1-5,17</sup> The high incidence and rapid onset of lesions makes white spot prevention a matter of great importance to both patients and orthodontists. In the present split-mouth study, a highly filled, fluoride releasing orthodontic primer, Opal Seal, was evaluated for its potential to prevent enamel demineralization.

### **Visual Examination**

Direct visual examination was performed to determine the incidence of white spot lesions in the sample population. Although visual exam has been criticized for its high degree of subjectivity and variability between studies, the method produces results that can be more easily interpreted by the clinician when compared to minute differences in demineralization identified by more sensitive laboratory tests.<sup>49</sup> The overall incidence of visual WSLs in this study was found to be 38% of all teeth examined. The control teeth had an incidence of 46% whereas the teeth that received Opal Seal application had an incidence of only 29%.

The duration that teeth were subjected to the oral environment was relatively short ( $67 \pm 30$  days) compared to the average 2 year duration of comprehensive orthodontic treatment, but there was a substantial amount of demineralization seen in both experimental and control teeth. In fact, the percentage of white spot lesions found in this study was comparable to the results of

visual examination reported in the literature. A recent prevalence study published by Tüfekçi et al.<sup>55</sup> reported that 38% of orthodontic patients examined visually at 6 month into fixed appliance treatment had at least one white spot lesion. The prevalence was found to be 46% when patients were examined one year into orthodontic treatment. Chapman et al.<sup>56</sup> found an incidence of at least one tooth having a WSL to be 36% upon examining intraoral photos post orthodontic treatment. Gorelick et al.<sup>2</sup> reported at least one WSL in 50% of patients examined after removal of fixed appliances.

The results of this study indicated an incidence rate that was somewhat higher than those reported in the literature. Unlike many other visualization methods that rely on clinical photos or intraoral examination of teeth, the visual exam in this study was performed by viewing the air-dried facial surface of the extracted tooth. It is likely that any areas of decalcification would be more identifiable with this technique, because there was no gingiva or saliva present to interfere with direct visualization of the cervical area of the tooth. In addition, a large elastomeric spacer was placed around the wings of the bracket to help facilitate retention of plaque and create a favorable environment for WSL development. The presence of the spacer might have served as a physical barrier to plaque removal and likely altered the local environment around the periphery of the bonded bracket, leading to more rapid white spot lesion development. A similar technique was employed by Farhadian et al.<sup>39</sup> using T-loops to increase plaque retention, and although WSL incidence rates were not reported, significant demineralization in both the experimental and control groups during a comparable time period was reported in that study.

Although the inter-rater reliability correlation for this study showed a good agreement, with a Cohen Kappa value of 0.71, both raters noted that identifying WSLs was more challenging in teeth with brackets still attached compared to those with brackets lost during

extraction procedures. The difficulty to accurately diagnose WSLs on teeth with brackets bonded could lead to underestimation of the incidence rate of lesions while the brackets are still present on the teeth. Therefore, during orthodontic treatment it is likely that more WSLs would be seen after the braces are removed as it is possible for the clinician to not notice WSLs until the removal of appliances.

In this study, a repeated-measures logistical regression was used to determine if there was a significant difference in the amount of WSLs present in the experimental versus the control group. The results indicated that Opal Seal showed no difference in the amount of clinical white spot lesions compared to Transbond XT, over the course of the study ( $p = 0.106$ ). However, when the interaction between Opal Seal versus Transbond XT and the number of days in-vivo was investigated, there was a significant reduction in WSLs seen in the Opal Seal group compared to control when the teeth were observed prior to 90 days in-vivo ( $p = 0.009$ ). The time-dependent nature of these results may be explained by the possible loss of Opal Seal primer over time and the fluoride release and recharging ability of the product.

### **Sealant Retention**

Since average orthodontic treatment time is approximately 24 months, the ability for a sealant to stay on the tooth surface throughout treatment is important in order to be effective in preventing demineralization.<sup>57</sup> Previous in-vivo studies where sealants were used to protect enamel surfaces showed a lack of sealant retention, likely due to the inability of the sealant to resist mechanical abrasion from tooth brushing and mastication.<sup>49,58</sup> In-vitro studies have found that filled sealants can withstand simulated wear and decrease the number and intensity of white spot lesions.<sup>37,38</sup> These findings have been further supported by an in-vivo study by Benham et

al.<sup>59</sup> that showed a highly filled resin sealant significantly decreased the occurrence of WSL in patients, resisted mechanical abrasion, and remained intact on teeth over more than 15 months of treatment. Although these studies suggest that greater filler content leads to greater sealant retention, a recent study by Van Bebber et al.<sup>60</sup> found that adding more filler particles does not necessarily make the sealant more durable or wear resistant. Opal Seal is reported to have 38% filler particles, which is thought to impart greater wear resistance compared to unfilled resins. In addition, it is claimed that this product is able to “penetrate deeply into fissures of teeth, resulting in a long lasting coverage and mechanical retention.”<sup>61</sup> In the present study, Opal Seal was noted to stay on over 50% of applied tooth surfaces at 90 days. However, there was no correlation between amount of sealant remaining of the teeth and the number of days in-vivo ( $r = -0.06, p > 0.6$ ). The duration of sealant retention ranged from 40-112 days, but the investigation of longitudinal sealant retention was not an objective of this study. Therefore, further study of Opal Seal retention is warranted to determine if and when reapplication of sealant is necessary.

### **Fluoride Release and Recharge**

The protective effect of Opal Seal was most apparent in teeth observed before 90 days in the oral cavity. A possible explanation for this could be related to the fluoride release of the product. Basdra et al.<sup>29</sup> examined fluoride-releasing bonding agents and found that the highest concentration of fluoride was within the first 24hrs of placement. The fluoride release dramatically declined in a few days and after 90 days no fluoride was detectible. According to Ultradent, Opal Seal releases fluoride and recharges fluoride uptake.<sup>61</sup> The ability of Opal Seal to recharge fluoride has been shown in an in-house laboratory study where disks with an initial fluoride release concentration of 1.20ppm were recharged to a fluoride concentration level of

7.79ppm when exposed to 1.23% acidulated fluoride. The results of that study also showed that the level of fluoride release decreased from over 1ppm to less than 0.2ppm after one week of observation.<sup>62</sup> Despite a drastic reduction in the amount of fluoride released, it has been suggested that even sub-ppm fluoride release, in the range of 0.02ppm-0.06ppm, can have a significant effect on the demineralization and remineralization process.<sup>63</sup> There are currently no published studies that have analyzed the fluoride release and recharging effect of this product, but an in-vitro study by Soliman et al.<sup>64</sup> analyzed fluoride release from Pro Seal, a similar fluoride containing product. It was shown that the amount of fluoride release significantly decreased from 0.07ppm in the first week to 0.01ppm by week 17. It was also noted that Pro Seal had the ability to be recharged with 1.23% acidulated phosphate fluoride, but not with over the counter fluoride containing toothpaste. Even though the experimental protocols differed, the study by Soliman et al. reported less fluoride release and recharging ability compared to the Opal Seal study by Ultradent Corp. In the current study, subjects were instructed to carry out routine oral hygiene measures (brushing and flossing) but to refrain from using any supplementary fluoride applications. If the fluoride reuptake for Opal Seal is similar to that of Pro Seal when using over the counter fluoride toothpaste, then it is unlikely that a fluoride recharging effect would be seen during the study period. Although fluoride release was not investigated in this study, it could be speculated that Opal Seal lost its protective effect against visible WSLs after 90 days due to the reduced fluoride release and limited fluoride reuptake from the over the counter toothpaste used by the patients during the study.

## Microhardness

In this study, Knoop microindentation was used as a quantitative measurement technique to determine the amount of enamel demineralization. The KHN test was selected over other microindentation methods because it has been shown to be appropriate for testing specimens of etched surface enamel with less sensitivity to elastic recovery and measurement errors.<sup>65</sup> In addition, published studies have demonstrated a linear relationship between KHN and mineral content suggesting that changes in microhardness are well correlated to changes in tooth mineralization.<sup>53,54</sup> Nonetheless, there is inherent variability during hardness testing because enamel is a heterogeneous material with more tightly packed hydroxyapatite crystals towards the outer enamel layers resulting in Knoop hardness values and hydroxyapatite density that varies significantly from the outer to the innermost layers of enamel.<sup>66</sup> Also, fluoride content is known to play a role in the hardness of tooth enamel such that microhardness would show variation because of the inhomogeneous fluoride incorporation into the enamel structure. The KHN of sound human enamel has been reported to be 355-431 with significant variability between studies.<sup>67,68</sup> The wide range in the KHN values may be attributed to both different locations tested on individual teeth and differences in the hydroxyapatite and fluorapatite ratios within the enamel structure of those teeth. In order to take into account the differences in the KHN within the same tooth because of the location and how much fluoride has been incorporated to the enamel, demineralization was calculated by comparing the value of KHN at a specific measurement point to that of the sound enamel of the same tooth, specifically underneath the bracket at a depth of 150  $\mu\text{m}$ .

The original intent of the hardness testing in this split-mouth study was to compare the hardness values of 22 patients' experimental teeth with the corresponding matched control teeth

(44 teeth total), but 20 out of 44 teeth had brackets inadvertently debonded during extraction procedures (45%). It was necessary to have a bracket present to provide a reference point for determining hardness of the sound tooth underneath the bracket at a depth of 150  $\mu\text{m}$ . Therefore, teeth without brackets could not be used for hardness testing, as it was not possible to determine the exact location of the bracket. After exclusion of teeth without brackets and those without a matched set of experimental and control teeth from the same individual, the number of matched pairs with brackets was found to be too low for meaningful statistical comparison. Therefore, instead of using matched pairs, KHN values of the 24 maxillary teeth with brackets (Table IV) were analyzed using a repeated-measures mixed-model analysis of covariance (ANCOVA) with the reference hardness (underneath the bracket at a depth of 150  $\mu\text{m}$ ) as a covariate to model the data. This statistical model accounted for the correlation within each subject, the effect of repeated measures, and it corrected for the fact that some subjects contributed two teeth and others only one due to bracket loss.

The raw mean hardness values expressed in Table V suggest that there was little variation in hardness at any combination of depth or distance. The KHN values for all teeth tested ranged from 274.7-317.7  $\text{kgf} / \mu\text{m}^2$  showing only 43  $\text{kgf} / \mu\text{m}^2$  unit difference between the highest and lowest hardness values. This difference is smaller than the variations reported in the normal range of KHN for sound tooth enamel (355 - 431  $\text{kgf} / \mu\text{m}^2$ ).<sup>67</sup> The average KHN values of sound tooth enamel found in this study were 310-317  $\text{kgf} / \mu\text{m}^2$ , which was lower than the reported range, but similar to that of a recent study by Poole et al.<sup>69</sup> The results of the repeated-measures mixed-model ANCOVA found that the average difference of hardness values between the experimental and control groups was only 6.9  $\text{kgf} / \mu\text{m}^2$  (95% CI = 3.12 to 10.72). When the small difference in KHN found in this study is compared to the expected difference of the KHN



of softened tooth enamel (150-255 kgf /  $\mu\text{m}^2$ ) reported in the literature, it can be seen that the values are vastly different.<sup>67</sup> Therefore, solid conclusions cannot be drawn from the statistical analysis even though the p-value indicated a statistically significant difference ( $p = 0.0004$ ). In light of these findings, it cannot be concluded that there is a meaningful difference in hardness between Opal Seal and Transbond XT.

The results of ANCOVA showed a statistically significant change in hardness difference across the 6 depths from the enamel surface ( $p < 0.001$ ). The hardness increased as the depth got deeper (Figure 4). It was found that enamel at a depth of 25  $\mu\text{m}$  from the tooth surface was approximately 9% less hard compared to sound enamel (at a depth of 150  $\mu\text{m}$ ). This trend was true for both experimental and control teeth, and is likely explained by the application of 37% hydrochloric acid to etch enamel during the bonding procedure. This is supported by studies in the literature reporting that initial acid etch significantly reduces the hardness of tooth enamel.<sup>17,30,69</sup> The results also showed that the hardness across the five distances was not constant ( $p < 0.001$ ). When compared to the hardness values directly underneath the bracket, the hardness at distances 50, 200, 500, and 1000  $\mu\text{m}$  away from the edge of the bracket towards the gingiva showed less hardness overall. There was little difference between the hardness at the distances 50, 200, 500 and 1000  $\mu\text{m}$ , as these values were uniformly lower than that of the hardness underneath the bracket. This finding supports the notion that the hardness underneath the bracket is a valid control, as its value was significantly greater than the hardness measured at the 4 distances from the edge of the bracket.

Even though microhardness is widely used to determine enamel demineralization, there are some potential concerns with this technique, including the need for a high number of teeth for testing and an adequate length of time teeth are in-vivo. In a study by Pascotto et al.<sup>30</sup> the

effect of resin modified glass ionomer cement on enamel demineralization around brackets was investigated using Knoop hardness test on teeth that were subjected to similar in-vivo conditions. It was concluded that there were significant differences in microhardness when a total of 36 teeth (24 maxillary and 12 mandibular) were examined at the end of 30 days in-vivo. In the current study, teeth were kept longer in the mouth, however the sample size was relatively smaller (a total of 24 teeth, all maxillary premolars) than that of the study by Pascotto et al.<sup>30</sup> Therefore, it is possible that a larger sample size would be needed to show any differences between the groups, if present. In addition, in the study by Pascotto et al.<sup>30</sup>, depths were recorded in increments of 10  $\mu\text{m}$ , starting at a depth of 10  $\mu\text{m}$  and proceeding to 90  $\mu\text{m}$ . Significant differences were reported between the experimental and control at depths of 10  $\mu\text{m}$  and 20  $\mu\text{m}$ , but there were no differences reported from 30-90  $\mu\text{m}$ . In this study, there were no significant differences between the experimental and control teeth for any depth, but the first depth was measured at 25  $\mu\text{m}$ . Therefore, if a shallow lesion is present at a depth of less than 25  $\mu\text{m}$ , it would not have been detected with the current study's protocol.

For this study, the initial indentation and increment depth of 25  $\mu\text{m}$  was chosen to avoid sample surface cracks that can obscure accurate measurement, which often occur as indentations are made close to each other and close to the enamel surface.<sup>53,69</sup> In addition, it was thought that the effect of acid etch could confound the results of hardness testing if indentations were made close to the enamel surface, because it has been shown that a mineral loss of 3% to 8% to a depth of 25  $\mu\text{m}$  is associated with initial acid etch.<sup>17</sup> Therefore, it may be preferable that in future microhardness studies, a smaller indentation increment should be used with a variable load protocol as described by Featherstone<sup>53</sup> to help avoid surface cracking.

The duration of the current study is thought to be adequate to produce significant demineralization. It is well supported in the literature that enamel demineralization can occur within 3 weeks of fixed appliance placement and this difference can be detected by microhardness, even if it is not present clinically.<sup>5,17,30</sup> A longer observation period would improve the quality of the study, by increasing the depth of the lesions; however, it would be unethical to further prolong treatment time by delaying premolar extractions.

In summary, the results from the visual examination support the null hypothesis that there is no difference in the amount of clinical white spot lesions observed in teeth treated with Opal Seal compared to those treated with Transbond XT, over the course of the study.

The findings of the hardness tests indicated significant differences ( $p=0.0004$ ) between the hardness value of the experimental and control groups with the teeth in the control group having a higher hardness value. However, despite the statistical significance, the differences between the values of the experimental and control were so small that solid conclusions could not be drawn from the hardness test.

Currently, there are no published studies that have investigated the effectiveness of Opal Seal in preventing enamel demineralization. Therefore, it is not possible to make direct comparisons with other studies. Future well-designed randomized controlled studies are needed to determine the retention of the primer on the tooth surface, the fluoride release from the primer into saliva, and the efficacy of Opal Seal in preventing WSLs.

### Conclusions

The results of the visual examination in this study indicated that there was no significant difference in the number of WSLs observed in teeth treated with Opal Seal compared to teeth treated with Transbond XT ( $p = 0.106$ ) over the duration of the study. However, there was a significant reduction in the number of WSLs in teeth treated with Opal Seal if they were observed prior to 90 days in-vivo, but after 90 days there were no differences between the experimental and control teeth. This finding suggests that Opal Seal exhibits some efficacy in preventing demineralization but this protective effect likely diminishes with time.

Differences in hardness values between teeth treated with Opal Seal and Transbond XT were observed in this study. However, despite the p-value of 0.004, the differences were so small that solid conclusions could not be drawn from the hardness test. Therefore, in light of these findings, it could not be concluded whether Opal Seal is effective or not in preventing white spot lesions in an orthodontic patient population.

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### Vita

Dr. Daniel R. Pennella was born in Rochester, New York on April 21, 1982. After several relocations during his childhood, Dan graduated valedictorian of Southridge High School in Kennewick, WA in 2000. He attended Wake Forest University where he graduated Summa Cum Laude with a Bachelor of Arts degree in Chemistry and a Minor in Political Science in 2004. Dan graduated from the College of Dental Medicine at the Medical University of South Carolina as valedictorian of his class in May 2009. He is currently a postgraduate resident in the Orthodontic program at Virginia Commonwealth University and will receive a certificate in Orthodontics along with a Master of Science degree in Dentistry in June 2011. Dr. Daniel Pennella will enter the private practice of orthodontics in Lexington, South Carolina.