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The Fluoride Recharging Capability of an Orthodontic Primer: an in vitro study

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THE FLUORIDE RECHARGING CAPABILITY OF AN ORTHODONTIC PRIMER: AN IN VITRO 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

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.
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Objective: The purpose of this study was to determine the fluoride recharging capability of Opal Seal, a fluoride releasing orthodontic primer, as compared to Transbond XT, the control.

Material and Methods: 1mm x 5mm disks of Opal Seal and Transbond were prepared according to the respective manufacturer's instructions. Initially, the samples were stored in deionized water (DI) for 8 weeks. The samples were then randomly divided into one of two groups: Over-the-counter (OTC) fluoride mouthwash and prescription strength (PS) fluoride mouthwash. The OTC group samples were immersed in 5mL of 0.0219% sodium fluoride containing mouthwash for one minute every day for seven days. The PS group samples were immersed in 5mL of 0.2% sodium fluoride containing mouthwash for one minute. All of the samples were suspended in 5mL fresh DI water and fluoride release measurements were taken at baseline (the end of initial 8 weeks of storage), 24 hours, 3 days, 5 days, 7 days, and 14 days.

Results: Opal Seal samples treated with the OTC fluoride mouthwash exhibited significant fluctuation in fluoride ion release across time ($p=0.0058$). However, there were no statistically significant differences in fluoride ion release between the individual timepoints and baseline.

Similarly, Opal Seal samples treated with the PS fluoride mouthwash exhibited significant variation in the fluoride ion concentration across time ($p< 0.001$), and a statistically significant increase over baseline was seen at 24 hours only ($p= 0.0006$). The control group samples treated either with the OTC or PS mouthwash did not exhibit any significant difference in fluoride ion release between any individual timepoint and baseline.

Conclusion: For Opal Seal and Transbond XT, there were no statistically significant differences of fluoride concentration at any timepoint compared to baseline measurements when using OTC mouthwash. When using PS mouthwash, there was a small, statistically significant increase of fluoride concentration of the Opal Seal samples after 24 hours but no differences were seen at any other timepoints. Opal Seal did not demonstrate a substantial amount of fluoride recharge when fluoride mouthwash is used as a fluoride delivery vehicle. Future well-designed randomized controlled trials are needed to evaluate the efficacy of Opal Seal primer when coupled with the use of fluoride mouthwashes.

Introduction

Enamel demineralization around brackets has long been a recognized problem in orthodontic patients with poor oral hygiene.[1-3] Enamel demineralization has been reported to occur in as much as 50% of orthodontic patients and can develop as soon as four weeks after the bonding appointment.[4,5] The presence of these incipient lesions often cause long term esthetic concerns for the patients, and in some severe cases, restorative intervention may be needed.[6,7]

The high incidence of demineralization can be attributed to the increased difficulty for orthodontic patients to perform effective oral hygiene measures in the presence of fixed appliances. Previous studies have shown an increase in plaque retention around brackets as opposed to patients without brackets.[8,9] Studies have also reported an increased level of *S. mutans* and *Lactobacilli* in the plaque of orthodontic patients. There is a decrease in oral pH due to acid production from bacterial fermentation of carbohydrates.[9,10] If the fermentation process continues, the pH in the oral cavity may reach critical levels within minutes. At the pH of 5.5, the demineralization process is initiated by the loss of calcium and phosphate ions from enamel to the oral environment. However, the buffering action of saliva may serve to increase the intraoral pH level which may reverse the flow of ions back into the enamel structure through a process called remineralization. When the continuous exchange between the demineralization and remineralization remains in balance, the dental tissues stay healthy. However, with the prolonged plaque retention around fixed appliances, the equilibrium can be skewed toward demineralization without allowing remineralization to occur.[11-13] As the progressive demineralization takes place, enamel crystal dissolution begins creating pores between the enamel rods. Due to mineral loss, the light refractive index of enamel is altered, giving the subsurface lesions an opaque and white appearance. The white spot lesions (WSLs) are therefore an early manifestation of caries.[4,8,14]

To prevent the formation of WSLs, certain preventive measures have been previously proposed. These include patient education, routine dental prophylaxis, and use of topical fluoride delivery systems.[8,15-18] In the literature, there is strong evidence that fluoride regimens can reduce demineralization during orthodontic treatment.[1,5,19-22] In the presence of fluoride ions, fluoroapatite is formed and it is this crystalline structure that makes the enamel resistant to caries.[23] In addition, numerous studies have shown that the use of fluorides can effectively decrease caries incidence by also promoting remineralization, especially when fluoride ions are readily available in the oral environment.[24]

Since fluoride is shown to have a protective effect, its use in mouthrinses and orthodontic cements have been proposed to help minimize the problem of WSLs. Geiger et al[1] have reported that as a patient's compliance with a fluoride regime increased the incidence of WSLs decreased. Since the preventive effect of fluoride mouthrinses heavily depends on compliance, various fluoride delivery systems have been developed to eliminate the need for patient cooperation. The use of fluoride releasing orthodontic cements was not found to be successful in preventing demineralization in areas adjacent to brackets as fluoride was released only to the area beneath the bracket.[25] This limitation led to the development of fluoride-releasing primers and sealants that can be used in the bonding process to protect the enamel surface not only under the bracket but also on the whole buccal surface.[26,27]

The application of resin sealants on the smooth surface enamel around the orthodontic brackets has been extensively studied during the last three decades.[17,26,28] The use of an acid etchant followed by the application of a sealant on the buccal surface of the tooth is believed to not only seal the etched enamel but also protect against demineralization around the bracket during orthodontic treatment. The orthodontic sealants can be chemically cured or light cured,

depending on the formula used. With the use of chemically cured resins, the potential to seal smooth enamel surfaces has been shown to be deficient due to oxygen inhibition causing a lack of complete surface polymerization.[26] Although light cured resins are reported to form a sealant layer, they are still susceptible to oxygen inhibition and incomplete surface polymerization. Therefore, the protective effect of light cured sealants remains questionable.[29]

The new generation glass ionomer sealants have been shown to provide a continuous layer as these materials polymerize without an oxygen inhibition layer.[30] Since the final sealant provides complete surface coverage, they are expected to protect the enamel from demineralization. Fluoride, in the form of a glass ionomer powder, provides an added benefit to sealants. However, the anticariogenic effects of sealants not only depend on the actual amount of fluoride released into the immediate environment but also on the sustainability of the ion release.[31] A continuous presence of fluoride within reach of the enamel is critical for the prevention of demineralization as well as for the remineralization potential of the enamel.[13] Even in low levels of concentration (0.03-0.1 ppm), fluorides have been shown to have a substantial effect in minimizing demineralization if the fluoride is available throughout the day.[32] Glass ionomer sealants are thought to provide the necessary sustained concentration of fluoride ions over a prolonged period of time due to their fluoride rechargeability. In essence, these materials may act as a fluoride pump as they uptake available exogenous fluoride ions from saliva and release the ions back to the saliva.[33]

Studies on fluoride releasing bonding agents have shown that while they are successful at releasing the incorporated fluoride into the surrounding oral environment, the amount of release is high only on the first day and sharply declines by the second day after application and continues to gradually decrease to undetectable levels.[34-36] The rapid decrease in fluoride ion

release indicates the need for the rechargeability of the fluoride releasing agents in order to provide the therapeutic level of fluoride concentration in saliva. Due to the wide range in the amount of fluoride reuptake observed, the anticariogenic property of fluoride releasing glass ionomer materials has been a subject of controversy.[37-39]

Recently, a new fluoride releasing glass ionomer product (Opal Seal, Ultradent, South Jordan, UT) has become commercially available for use in orthodontic patients with poor oral hygiene. Because of its high filler content (38%), Opal Seal is marketed as a primer with a superior fluoride recharging property.[40] However, there is not enough evidence in the literature to support this claim. In an earlier study by Ultradent, the Opal Seal disks were successfully recharged with the application of acidulated fluoride gel for four minutes. The amount of fluoride ions released at the end of a 24 hour period was shown to be at therapeutic levels.[41] The findings of a later independent study were in agreement with that of the Ultradent study showing substantial fluoride ion uptake 24 hours after the application of acidulated phosphate gel.[42] However, at the end of a 6-week period, a significant decrease was observed in the amount of fluoride released. On the other hand, the application of fluoride containing toothpaste showed limited fluoride recharge in the range of 0.0300-0.0489ppm at 24 hours.

Since it is possible that patients would be more compliant with the use of a mouthwash as opposed to toothbrushing, it is of interest to evaluate the fluoride rechargeability of Opal Seal from fluoride containing mouthwashes. Therefore, the purpose of this in vitro study was to determine the fluoride recharge potential of Opal Seal samples after the application of an over-the-counter and a prescription strength mouthwash.

Materials and Methods

For this study, a commercially available fluoride releasing primer, Opal Seal, was used as the experimental product and a non-fluoride sealant, Transbond XT (3M Unitek, Monrovia, CA), served as the control. Disks, 5mm in diameter and 1mm in thickness, were prepared using polyethylene molds. Once placed on a 1mm thick glass slide, the molds were overfilled with either Opal Seal or Transbond and a second glass slide was placed on top and pressed down to form the disks. Each sample was then cured using a visible light-curing unit (Blue Ray 3, American Orthodontics, Sheboygan, WI) for 30 seconds on each surface. The disks were then inspected under 2.5X magnification for visible surface porosities and those with surface voids and defects were discarded. Subsequently, the remaining disks were hand polished on all sides using a standardized method with a 3000-grit fine abrasive paper (Buehler Ltd., Lake Bluff, IL) to ensure homogenous and smooth surfaces. After polishing, all the samples were again inspected at 2.5X magnification and any disks with surface imperfections were discarded. A total of 40 disks (n= 20, Opal Seal and n=20, Transbond XT) were prepared in this manner. A 0.4mm hole was drilled with a fine diamond bur in the center of each disk and a non-coated fishing line was threaded through the opening and tied to enable the teeth to be suspended in deionized (DI) water.

Prior to the study, 20mL high-density polyethylene vials were first immersed for 24 hours in a 5% solution of Decon 90 (Electron Microscopy Sciences, Hatfield, PA), a phosphate free detergent, according to the manufacturer's instructions. The bottles were then thoroughly scrubbed, rinsed with DI water, and completely dried using clean paper towels. The disks were individually inserted into the cleaned vials, each filled with 5mL DI water. The vials were kept at room temperature and the water was changed every other day for four weeks, and

subsequently twice a week for another four weeks. At the end of eight weeks, all of the samples were removed from their vials and the DI water solutions were stored for the baseline measurements (T0).

The Opal Seal (n = 20) and Transbond XT (n = 20) disks were then randomly distributed to one of two fluoride treatment groups, namely an over-the-counter (OTC) fluoride mouthwash group and a prescription strength (PS) fluoride mouthwash group as follows: Opal $Seal_{OTC}$ (n = 10), Opal $Seal_{PS}$ (n = 10), $Transbond_{OTC}$ (control, n = 10), $Transbond_{PS}$ (control, n = 10).

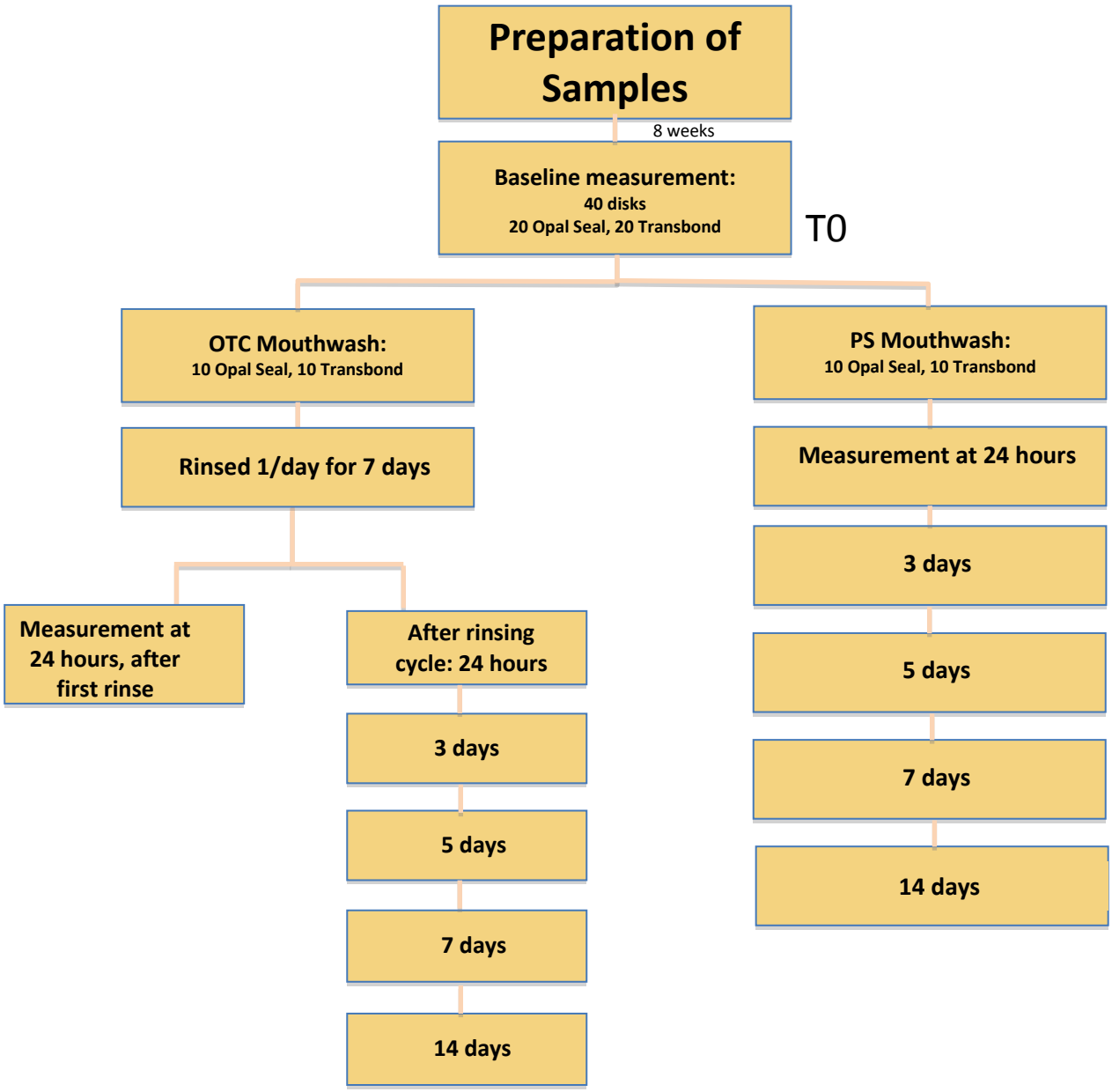
The samples in the OTC group were immersed in 5mL of 0.0219% sodium fluoride containing mouthwash (Crest Pro-Health Complete, Proctor & Gamble, Cincinnati, OH) for one minute and then rinsed for one minute using DI water. After this procedure, the disks were placed in a new vial filled with 5mL of fresh DI water. At 24 hours, the first measurement was taken. The samples were then immersed again in mouthwash and rinsed in DI water as described above every day for the following 6 days, making for 7 total days of charging with the application of OTC mouthwash each day. After the completion of the 7 days of fluoride charging, another measurement was performed at 24 hours, 3 days, 5 days, and 7 days with the DI water being changed at every interval. Measurements were also taken at 14 days with the DI water being changed every other day between measurements.

The samples in the PS group were immersed in 5mL of 0.2% sodium fluoride containing mouthwash (Colgate PreviDent Dental Rinse, Colgate-Palmolive, New York, NY) for one minute after which they were rinsed for one minute using DI water and each sample was placed in a new vial containing 5mL fresh DI water. Measurements were performed at 24 hours, 3 days, 5 days, and 7 days with the DI water being changed at every interval, and at 14 days with the DI water being changed every other day between measurements.

An ion analyzer (Fisher Scientific Accumet XL250, Thermo Fisher Scientific, Hampton, New Hampshire) was used to measure the fluoride ion concentration in the samples using a fluoride ion-specific combination electrode. The analyzer was calibrated using standards of known concentrations. The analyzer was re-calibrated after every eighth measurement to ensure precision. The samples of each timepoint were randomized and the operator was blinded to the treatment groups. Measurements were completed until there were six measurements of each sample at every timepoint. These six repeated measurements for each sample were averaged to create the value for the sample at each specific timepoint.

Statistical Methods

Comparisons for the fluoride ion release between the two groups were made using a repeated-measures mixed-model analysis of variance (ANOVA). Significant differences were identified using a Bonferroni-corrected alpha level with a significance level of 0.0011. All analyses were carried out using SAS software (SAS version 9.3, JMP Pro version 11.1, SAS Institute Inc., Cary NC).


Preparation of Samples
8 weeks
Baseline measurement:
40 disks
20 Opal Seal, 20 Transbond
T0
OTC Mouthwash:
10 Opal Seal, 10 Transbond
Rinsed 1/day for 7 days
Measurement at 24 hours, after first rinse
After rinsing cycle: 24 hours
3 days
5 days
7 days
14 days
PS Mouthwash:
10 Opal Seal, 10 Transbond
Measurement at 24 hours
3 days
5 days
7 days
14 days

Results

The results of the fluoride ion release measurements are summarized in Tables 1 and 2. The mean and standard error of the baseline measurements for the control (Transbond XT) and the experimental (Opal Seal) samples in the over-the-counter group were 0.218±0.012 and 0.177±0.017ppm, respectively. The values for the control and experimental samples in the prescription strength group were 0.212± 0.013 and 0.163±0.017ppm, respectively.

Fluoride release after exposure to the over-the-counter mouthwash

The statistical analyses indicated that the amount of fluoride ion released from the control samples in the OTC group varied across time (P = 0.0017). The fluoride ion release at 3 days and 14 days were significantly lower than the baseline with values (p=0.0002 and p=0.0007, respectively; Table 1, Figure 1). Similarly, the amount of fluoride ion released from the Opal Seal samples treated with the OTC mouthwash varied across time (P = 0.0058). The fluoride ion release at the subsequent measurement timepoints was not statistically significantly different than the release at baseline (Table 1, Figure 1). Overall, there were no statistically significant differences between the control and Opal Seal samples at any timepoint except at 14 days (P = 0.0010).

Fluoride release after exposure to the prescription strength mouthwash

The amount of fluoride ions released from the control samples in the PS group varied significantly across time as well (P = 0.0013). When compared to the baseline values, there were no statistically significant differences in the amount of fluoride ion release at any time point except at 3 days. (P = 0.0003; Table 2, Figure 2). Similarly, the fluoride ion release from the Opal Seal samples in the PS group varied significantly across time (P < .0001). At 24 hours, there was significantly more fluoride ion release as compared to the baseline (P = 0.0006; Table

2, Figure 2). Only the measurement at the 24 hour timepoint showed a significant difference in the fluoride ion release between the control and experimental groups ($P = 0.0002$). At the other timepoints, the experimental and control groups did not have statistically significant differences in the amount of fluoride ion released ($P > 0.02$).

Discussion

WSLs are a major concern for both the patient and orthodontist. Preventive measures include patient education, routine dental prophylaxis, and the use of various fluoride delivery systems.[8,15-18] In non-compliant patients, fluoride delivery systems that do not depend on cooperation may be used. These systems include fluoride releasing orthodontic resins and sealants. However, the efficacy of these materials is questionable because the amount of fluoride ion released decreases rapidly to undetectable levels within a few days.[34-36] Because of their fluoride recharge capability, combining a fluoride releasing orthodontic sealant with a topical fluoride application may exhibit synergistic anticariogenic properties.

A study by Marinho et al[43] demonstrated that the use of fluoride-containing toothpastes is as effective as the use of fluoride mouthwashes and varnishes in the prevention of enamel demineralization. However, as mentioned earlier, the preventive effect requires patient compliance so other fluoride delivery systems and materials such as fluoride releasing sealants, fluoride mouthwashes, and fluoride gels are usually needed to help prevent the formation of WSLs. Patient compliance with mouthwash could potentially be higher than toothbrushing, as it is easier for the patient to control plaque retention with a mouthwash in seconds as opposed to efficiently brushing with toothpaste.

In this study, fluoride mouthwash was chosen as the fluoride delivery vehicle. Specifically, fluoride recharge of Opal Seal was investigated after the application of an over-the-counter and a prescription strength mouthwash. Opal Seal is marketed not only as a fluoride releasing orthodontic primer, but also as a product with a superior fluoride recharging capability.[40] If this claim is true, Opal Seal could be used as an adjunct to orthodontic treatment of patients with poor oral hygiene to minimize the incidence and the severity of WSLs.

The findings of this study showed that the fluoride recharging capability of Opal Seal is not demonstrated when using OTC fluoridated mouthwash as a fluoride delivery vehicle. When using PS fluoridated mouthwash, the Opal Seal samples showed a statistically significant increase of 0.1 ppm fluoride ions over baseline levels of fluoride ion concentrations only at 24 hours. There were no statistically significant differences between the baseline fluoride ion concentration levels and the individual concentration levels measured at 3 days, 5 days, 7 days and 14 days after the application of the PS fluoride mouthwash.

In this study, Transbond XT disks served as the control group. Since Transbond XT does not have fluoride in its composition, it is not expected to recharge and release fluoride. One interesting finding was that the baseline measurements of the deionized water solutions in which the Transbond XT were kept indicated low levels of fluoride. This was somewhat surprising as the high purity deionized water that was used as the storage medium should not have any fluoride in its composition since the samples had not yet been exposed to topical fluoride application at this timepoint (T0, baseline). Therefore, it would be ideal to carry out the baseline measurements prior to the application of topical fluorides to determine the purity of the water solution. However, due to time constraints, it was not possible to determine the baseline measurements prior to the start of the experimental procedures. Instead, at the end of 8 weeks of initial storage period, once the samples were removed from their vials, the water solutions were kept aside until the fluoride ion measurements were performed for all timepoints.

In previous studies, Transbond XT samples were shown to exhibit low levels of fluoride ion uptake and release after the application of topical fluorides. The limited fluoride recharge was attributed to diffusion of fluoride ions into pores and cracks within the disk rather than actual fluoride uptake into its composition.[44] Similarly, in the present study the control samples

have exhibited low levels of fluoride ions at all timepoints. Since the fluoride levels did not increase with a level of statistical significance over the baseline measurements, it can be concluded that no detectible fluoride ion uptake and release of Transbond XT took place this study.

As previously stated, Opal Seal is marketed as a primer with fluoride releasing and recharging ability. According to a previous in vitro study conducted by the manufacturer, Opal Seal has exhibited an initial fluoride release of 0.68 ppm upon depletion over a span of 14 days, with 65% (0.44 ppm) of that being released within the first 24 hours. Upon fluoride recharge, Opal Seal demonstrated a release of approximately 0.3 ppm at 24 hours following an exposure to 1.23% acidulated phosphoric fluoride (APF) gel for four minutes. However, the fluoride ion release following the application of APF gel dropped significantly after the 24 hour timepoint.[41] Another independent study that compared fluoride rechargeability of Opal Seal to Pro Seal demonstrated a 1.0 ppm fluoride release at 24 hours and 0.04 ppm at 6 weeks after the application of APF gel. It was also shown that Opal Seal disks exhibited a fluoride recharge of 0.04 ppm at the 24 hour timepoint after the application of the over-the-counter fluoridated toothpaste.[42]

In the current study, when using OTC fluoride mouthwash, the fluoride ion concentration in the Opal Seal samples fluctuated across time with statistical significance ($p=0.0058$), but when each timepoint was compared individually with the baseline measurements, no statistically significant changes were noted. In the Opal Seal samples treated with PS fluoride mouthwash, the fluoride ion concentration fluctuated across time as was seen with the OTC group. However, there was also a statistically significant increase noted at the 24 hour measurement when

compared to baseline. While this change was statistically significant, the clinical significance of an increase of only 0.10ppm is not known at this time.

Previous studies have shown that the amount of fluoride ions needed to initiate remineralization is lower than the concentration needed to inhibit demineralization. While a fluoride concentration of 0.15ppm in the saliva is needed to remineralize enamel, at least 1ppm of fluoride concentration is needed to be continuously present in saliva to prevent the initial demineralization process.[45,46] According to this information, Opal Seal has the potential to uptake and release acceptable levels of fluoride to inhibit demineralization only at 24 hours post-APF gel treatment, but there is no evidence to support long lasting fluoride release beyond this time as the fluoride ion release was below therapeutic levels after 6 weeks.

In the literature, the application of resin sealants to the enamel surfaces around orthodontic brackets following acid etching has been shown to reduce demineralization by creating a protective film.[26,28] A clinical study that investigated the efficacy of Opal Seal on demineralization around orthodontic brackets showed a decrease in the number of WSLs for a period up to 90 days.[47] However, Opal Seal demonstrated diminishing efficacy over time as there was no difference in the WSL incidence between the experimental and control teeth after 90 days. The ability of Opal Seal to prevent demineralization only up to 90 days may be attributed to the decrease in the amount of fluoride ion released over time. Since toothbrushing habits of the patients were not recorded in the study, it is not possible to postulate on the fluoride recharge capability of Opal Seal with the use of over-the-counter toothpaste. It is possible that even though fluoride recharge had taken place, the concentrations were too low to have a protective effect after 90 days. Therefore, well-designed controlled clinical studies are further needed to

determine the long-term effect of Opal Seal when combined with topical fluoride delivery systems including toothpastes, mouthwashes, and varnishes.

Conclusions

1. There was no statistically significant recharge and release of fluoride demonstrated at any timepoint by Opal Seal when using over-the-counter mouthwash or Transbond XT when using either the over-the-counter or prescription strength mouthwash.
2. With the use of prescription strength mouthwash, Opal Seal demonstrated a statistically significant recharge and release of fluoride at 24 hours but not at any other timepoints.
3. Opal Seal did not demonstrate a substantial amount of fluoride recharge when using fluoride mouthwash as a fluoride delivery vehicle.
4. Well-designed randomized controlled studies are further needed to determine the clinical efficacy and rechargeability of Opal Seal.

Figure 1. Fluoride concentrations (ppm) in the Over the Counter Group (95% CIs)

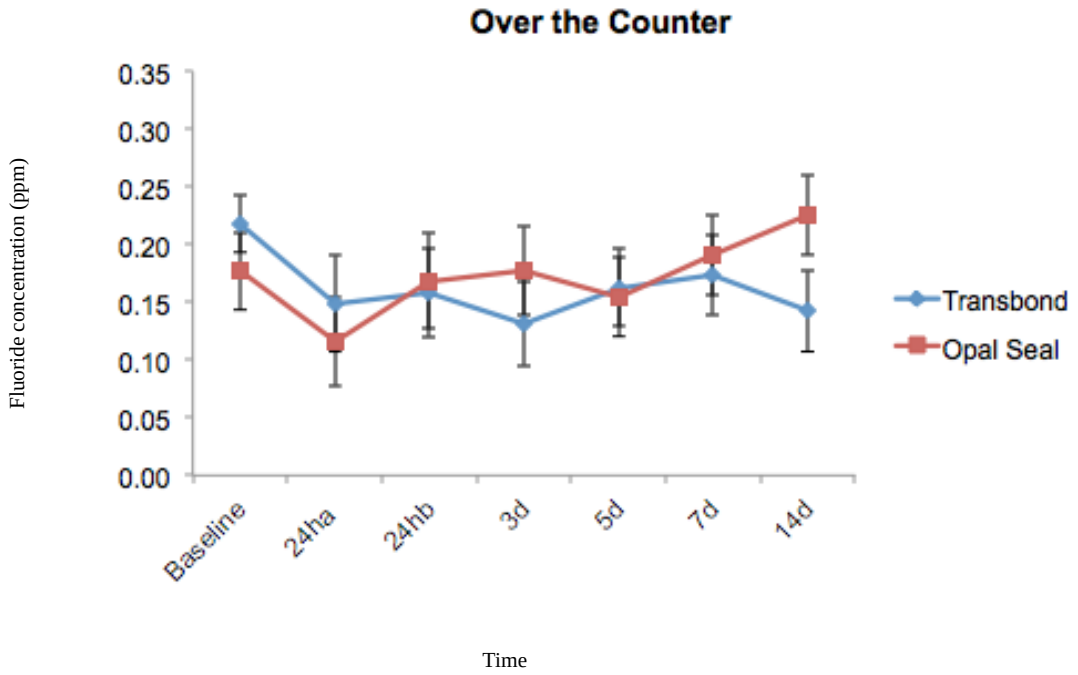


Figure 2. Fluoride concentrations (ppm) in the Prescription Strength Group (95% CIs)

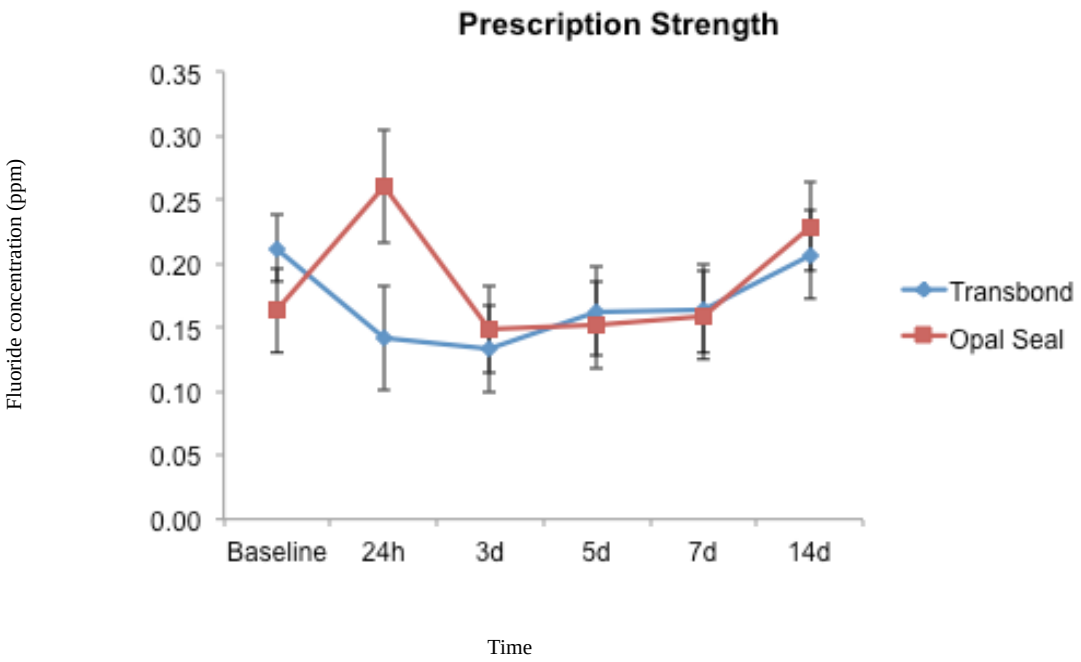


Table 1. Fluoride ion release measurements for the control and experimental groups after exposure to the over-the-counter fluoride mouthwash

Group	*Time*	*LS Mean*	*SE*	*95%*	*P-value*
Transbond XT					0.0017 *
	T0	0.218	0.012	(0.193 to 0.242)	
	24 h[a]	0.149	0.021	(0.107 to 0.191)	0.0062
	24 h[b]	0.158	0.020	(0.119 to 0.197)	0.0123
	3d	0.131	0.019	(0.094 to 0.168)	0.0002 *
	5d	0.163	0.017	(0.130 to 0.196)	0.0100
	7d	0.173	0.018	(0.139 to 0.208)	0.0425
	14d	0.142	0.018	(0.107 to 0.177)	0.0007 *
Opal Seal					0.0058 *
	T0	0.177	0.017	(0.144 to 0.210)	
	24 h[a]	0.116	0.020	(0.077 to 0.155)	0.0209
	24 h[b]	0.168	0.021	(0.127 to 0.210)	0.7594
	3d	0.178	0.020	(0.139 to 0.217)	0.9711
	5d	0.155	0.018	(0.120 to 0.190)	0.3826
	7d	0.191	0.018	(0.156 to 0.225)	0.5703
	14d	0.226	0.018	(0.191 to 0.261)	0.0467

* T0 values are compared to subsequent time point using a Bonferroni-corrected alpha =0.0011.

24 hours[a]– Fluoride release 24 hours after one day of charging with OTC mouthwash

24 hours[b] – Fluoride release 24 hours after 7 days of charging with OTC mouthwash

Table 2. Fluoride ion release measurements for the control and experimental groups after exposure to the prescription strength fluoride mouthwash

Group	*Time*	*LS Mean*	*SE*	*95%*	*P-value*
Transbond XT					0.0013 *
	T0	0.212	0.013	(0.186 to 0.238)	
	24 h	0.142	0.021	(0.101 to 0.183)	0.0044
	3d	0.133	0.017	(0.099 to 0.167)	0.0003 *
	5d	0.163	0.018	(0.128 to 0.198)	0.0225
	7d	0.165	0.017	(0.130 to 0.199)	0.0265
	2wk	0.207	0.017	(0.173 to 0.241)	0.8010
Opal Seal					<.0001 *
	T0	0.163	0.017	(0.130 to 0.196)	
	24 h	0.260	0.022	(0.216 to 0.305)	0.0006 *
	3d	0.149	0.017	(0.115 to 0.183)	0.5381
	5d	0.152	0.017	(0.118 to 0.187)	0.6416
	7d	0.160	0.017	(0.125 to 0.194)	0.8720
	2wk	0.229	0.017	(0.195 to 0.263)	0.0061

* T0 values are compared to subsequent time point using a Bonferroni-corrected alpha =0.0011.

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