Evaluation of a Novel Microencapsulated Orthodontic Cement to Prevent White Spot Lesions

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DECLARATION OF INTEREST

None of the authors have any interest, financial or otherwise, in any of the orthodontic cements utilized in this study.

The experimental cements were donated by the Premier Dental Products Company. Funding was provided by the company for materials and experimental testing of the cements only.

Central incisor brackets were donated by 3M Unitek, and premolar brackets were donated by American Orthodontics.

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Abstract

The esthetic result of orthodontic therapy can be compromised by white spot lesion (WSL) development. WSLs are associated with increased bacterial accumulation exacerbated by orthodontic appliances and poor patient oral hygiene. One strategy to reduce WSL formation is to utilize orthodontic cements capable of releasing ions to inhibit bacterial growth and remineralize enamel. The Premier Dental Supply Company's experimental cements incorporated with microcapsules containing a combination of Zn^{2+}, PO_4^{3-}, Ca^{2+}, and F^- were evaluated for their capability to minimize bacterial growth and the formation of WSLs and for their bond strength to enamel compared to a control. The experimental cements containing microencapsulated ions released significant levels of Zn^{2+}, PO_4^{3-}, Ca^{2+}, and F^- in an acidic aqueous solution (pH 5.15) over a 28-day period. The fluoride-containing formulation demonstrated a mild inhibition of bacterial growth. There were no significant differences in WSL formation on enamel for any of the cements. While there were no significant differences in the debonded adhesive remnants, the shear bond strengths were significantly lower for cements containing microcapsules, though they were still within the clinically-acceptable range. Future studies should focus on clinical capabilities of the cements and verify the adequacy of the bond strength.

Introduction

One of the most common goals of orthodontic therapy is to align a patient's teeth for an esthetically pleasing smile. Unfortunately, the increased bacterial accumulation associated with orthodontic appliances can impede this goal by developing white spot lesions (WSL). White spot lesions are visible, opaque spots on the tooth surface that appear chalky and white. Some are small blemishes on an otherwise healthy tooth; however, others can cover the majority of a tooth's surface and appear when smiling or speaking. White spot lesions ultimately compromise the esthetic benefit gained from orthodontic therapy.

White spot lesions occur by the same mechanism as dental caries – certain oral bacteria, such as *Streptococcus mutans*, form biofilms on teeth and ferment available sugars to create an acidic environment that demineralizes the enamel surface [1]. Generally, about 40-60% of orthodontic patients develop WSLs, but studies report a wide range depending on the detection method [2]. Lesions tend to regress through natural remineralization from ions present in saliva once orthodontic appliances are removed [3, 4]. Additionally, treatment strategies utilizing casein phosphopeptide-amorphous calcium phosphate pastes, microabrasion, and resin infiltration may provide some additional esthetic improvement [3, 5, 6].

There are many risk factors associated with WSL development, and the most significant is poor oral hygiene [7, 8]. Therefore, prevention is the most important and effective strategy to stop WSLs from forming. Unfortunately, all efforts to improve a patient's at-home care depend on an unpredictable level of cooperation, so approaches that do not rely on compliance are appealing. One example is through the utilization of a bonding agent capable of remineralizing demineralized enamel – such an approach may deter or eliminate the formation of WSLs without additional chair time or reliance on patient compliance. Bonding orthodontic appliances with

fluoride-releasing glass ionomer has been demonstrated to inhibit enamel demineralization; however, these materials exhibit lower bond strength than resin cements, and their fluoride reserves deplete rapidly [9-11].

The purpose of this study was to evaluate several formulations of orthodontic cements developed by the Premier Dental Products Company (henceforth, “Premier”) with microencapsulated ions for sustained release of zinc, phosphate, calcium, and/or fluoride (Premier Dental Products Company, Plymouth Meeting, PA). The primary aim was to evaluate the ability of the novel orthodontic cements to prevent white spot lesion development on enamel. Secondarily, the bond strengths of these cements to enamel were evaluated. The hypotheses tested were that, as compared to a popular commercial orthodontic resin cement as a control, the experimental cements would:

1. Release more ions,
2. More effectively inhibit bacterial growth,
3. Exhibit smaller white spot lesions,
4. Demonstrate similar shear bond strength, and
5. Exhibit similar adhesive remnants upon debonding.

Materials and Methods

Experimental Groups

The experimental cements were provided by Premier. These experimental cement formulations were developed based on the design of their microencapsulated pit and fissure sealant product, BioCoat (Premier Dental Products Company, Plymouth Meeting, PA). The method for producing the cements is proprietary, but these formulations were created specifically by Premier to test the hypotheses of this study and to determine the most optimal formulations for future testing. Briefly, spherical microcapsules (1.5 ± 0.75 μm) were prepared with bisGMA/TEGMA, 70% loading of a silicate glass (Schott), and fumed silica (Schott AG, Mainz, Germany). The following nomenclature will be used for the orthodontic cement and microencapsulated ion formulations (Premier's cements denoted with "P-"):

1. P-0 – no microcapsules
2. P-Z – 7% Zinc
3. P-CaP – 5% Ca^{2+} & 2% PO_4^{3-}
4. P-F – 4% F^-, 2% Ca^{2+}, & 1% PO_4^{3-}

Transbond XT, a commercial cement commonly used for orthodontic bracket bonding, was used as a control (denoted "TB"; 3M, St. Paul, MN).

Experimental Design

The cements were evaluated for their ability to resist WSL development in three ways:

1. Quantification of ion release in an acidic environment simulating a caries-inducing situation.

2. Quantification of *S. mutans* biofilm formation on the surface of the cement to evaluate antibacterial capabilities.
3. Image analysis of the presence and size of white spot lesions developed on orthodontically bonded teeth in the exposed enamel adjacent to the bonded brackets.

In addition, the physical properties of the cements were also evaluated:

4. Shear bond strength of brackets bonded to enamel.
5. Adhesive remnant index after debonding of brackets.

<u>Cement Disc Fabrication</u>

Cement discs were fabricated by placing uncured cement in a 1 mm thick rubber mold secured between two glass slides. The mold was 6 mm in diameter for the ion release studies and 4 mm in diameter for the biofilm studies. The discs were then cured with a Demi Plus curing light (Kerr, Orange, CA) with an 8-mm light-guide tip, and an output of 640 mW/cm^2 measured by a laser power meter (Power Max 5200, Coherent-Molectron Detector Inc, Santa Clara, CA). The light tip was placed directly against both sides of the glass slide and cured for 20 seconds each. The cured discs were then roughened on 600-grit silicon carbide paper on both the top and bottom surfaces to create a uniform surface for evaluating ion release and bacterial adhesion.

<u>Ion Release Studies</u>

An aqueous acidic solution of pH 5.15 (measured via a model 710A pH pH/ISE meter, Thermo Scientific Orion; Waltham, MA) was created with purified water (MilliporeSigma, Burlington, MA) and hydrochloric acid to promote ion release from the cements. Two milliliters of the acidic solution and one cement disc were placed in individual polypropylene vials. Five

cement discs for each formulation were immersed in the acidic solution and then transferred to another vial containing fresh acid after 6 hours, 1 day, 7 days, 14 days, and 28 days. A pilot test following this protocol demonstrated no ion saturation after 2 weeks. A 1 mL aliquot of the solution from P-F samples was reserved for fluoride release measurements. The remaining vials were analyzed for Ca^{2+}, Zn^{2+}, and P via Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Oregon Health & Science University's Elemental Analysis Core. Briefly, ICP-MS ionizes a sample utilizing inductively coupled plasma and then subsequently separates and quantifies the ions via mass spectrometry [12]. ICP-MS was chosen for sample analysis because of its capability to simultaneously measure multiple elements with very high sensitivity [13]. Its high accuracy and independence from elemental compounds lends ICP-MS well to quantifying trace elements [12].

ICP-MS Sample Preparation

First, the samples were vortexed to suspend any precipitates and equally distribute the contents. Then, 500 µl of each sample was added to 500 µl of 1% HNO_3 (trace metal grade, Fisher Scientific, Hampton, NH) in a 15-ml HNO_3-rinsed centrifuge tube. The final dilution for all samples was 2x. In a follow-up experiment, all samples for P-Z were re-measured at 100x dilution (10 µl sample + 990 µl 1% HNO_3) to ensure that the Zn^{2+} concentrations of these samples were within the standard calibration curve.

ICP-MS Controls and Standards

A National Institute of Standards and Technology Standard Reference Material number 1683f (NIST, Gaithersburg, MD) was prepared at 5x dilution (4 ml of 1% HNO_3 trace metal

grade, Fisher + 1 ml of NIST SRM 1683f) and measured to ensure accuracy of the standard calibration curve.

ICP-MS Measurements

Inductively coupled plasma mass spectroscopy analysis was performed using an Agilent 7700x equipped with an ASX 500 auto sampler (Agilent Technologies, Santa Clara, CA). The system was operated at a radio frequency power of 1550 W, an argon plasma gas flow rate of 15 L/min, and Ar carrier gas flow rate of 0.9 L/min. Elements were measured in kinetic energy discrimination (KED) mode using He gas (4.3 ml/min). Data were quantified using a 9-point (0, 0.5, 1, 2, 5, 10, 50, 100, 1000 ppb [ng/g] for P, Mn, Fe, Cu, and Zn and 0, 0.05, 0.1, 0.2, 0.5, 1, 5, 10, 50 ppm [µg/g] for Ca) calibration curve using external standards for P, Ca, Mn, Fe, Cu, Zn. For each sample, data were acquired in triplicate and averaged. A coefficient of variance (CoV) was determined from frequent measurements of a sample containing ~10 ppb of P, Mn, Fe, Cu, and Zn as well as ~1 ppm of Ca. An internal standard (Sc, Ge, Bi) continuously introduced with the sample was used to correct for detector fluctuations and to monitor plasma stability. Elemental recovery was evaluated by measuring the NIST reference material (water, SRM 1643f) and found to be within 90 - 110% for all determined elements.

One sample from each group was then evaluated for the presence of insoluble ion complex precipitants that may have adsorbed to the container walls during the soaking time. The samples were prepared by removing the remaining liquid and then gently rinsing the vials with water. One milliliter of 1% HNO_3 was added to each vial and an unused vial before being vortexed and measured as before. Phosphorous was not measured because the counter ion, Ca^{2+}, would already be detected if there were any precipitants.

Fluoride Release Quantification

Fluoride calibration curves were created using a fluoride-specific ion electrode (ATI Orion; Boston, MA) connected to a pH/ISE meter (model 710A, Thermo Scientific Orion; Waltham, MA) according to the manufacturer's instructions. Briefly, a 10 ppm F^- solution was created with low-level total ionic strength adjustment buffer (TISAB). The resulting solution was added incrementally to a solution of deionized water and low-level TISAB, and the millivolt reading was recorded. The calibration curve was created by plotting the log of the fluoride concentration versus the millivolt readings. The millivolt readings for P-F were then measured and compared to the calibration curves to determine fluoride concentration.

Biofilm Studies

Bioluminescent JM10 *Strepococcus mutans* bacteria were grown on BD Bacto™ Todd (BT) Hewitt Broth (Fisher scientific) agar plates inside a 5% CO_2 incubator at 37ºC for 2 days. The JM10 modification is a derivative of type UA159 specifically designed to enable luminescence of adenosine triphosphate to quantify viable bacterial cells [14, 15]. This assay is described as Luciferase assay because it utilizes the firefly luciferase reporter gene. A single colony was taken from this plate to inoculate 1 mL of sterile BT media and then incubated for another day under the same conditions. The bacteria were then added to 10 mL of media at a 500x dilution along with a 40% sucrose solution (1% sucrose final).

Nine discs of each cement were grouped into wells on a sterile 6-well plate. Five milliliters of the BT + sucrose solution was added to the wells to cover each sample. The plate was incubated at 5% CO_2 and 37ºC for one day. The media was removed and replenished with plain BT media for one hour prior to analyzing to provide nutrients to the cell and ensure that

they were in an active state (i.e. not starving). Each disc and their associated biofilm were placed into individual wells on a 96-well plate for Luciferase Assay analysis with fresh media. Ten microliters of a 0.75 mg/mL Co-Elenterazine solution (Prolume Ltd, Pinetop, AZ) was added to each well and vigorously agitated with the pipette to introduce oxygen and fuel the Luciferase reaction. The 96-well plate was then placed in a GloMax Discover NanoLuc Luciferase Ready device (GloMax® Discover System; Promega, Madison, WI) and analyzed.

Tooth Preparation

Fifty extracted human premolars with intact buccal surfaces free of white spot and carious lesions were stored in a 1% thymol solution until use. The teeth were divided into groups of 10 for each cement. Teeth were then soaked in isopropyl alcohol for one hour before use. The buccal surfaces were cleaned by a slow-speed rotary handpiece and rubber prophy cup with a fluoride-free pumice slurry for eight seconds. The buccal surface was etched with 37% phosphoric acid etch for 30 seconds before being rinsed for 10 seconds and air-dried with an air-water syringe (A-dec, Newberg, OR) for an additional 10 seconds. A metal maxillary premolar bracket (Mini Masters Series™, American Orthodontics Corporation, Sheboygan, WI) was bonded to each tooth with the specific cement and light-cured using a 7.5 mm, 1500 mW/cm^2 Satelec Mini LED ScanWave (A-dec, Newberg, OR) at 45º and 3 mm distance from the bracket pad for 10 seconds from both the mesial and distal aspects. These premolars were used in shear bond strength and adhesive remnant index assessments.

Thirty extracted human central incisors with intact facial surfaces were stored in a 1% thymol solution until use. These teeth were divided into groups of six for each cement. Metal central incisor brackets (Victory Series Twin, 3M Unitek, St. Paul, MN) were bonded as

previously described for the extracted premolars. Two coats of nail polish (Kleancolor Metallic Nail Polish, Santa Fe Springs, CA) were applied to the facial enamel leaving a 2-mm x 3.5-mm window of uncoated enamel gingival to the bracket. Teeth were photographed under a Nikon Binocular Stereo Microscope (SMZ-10, Nikon, Tokyo, Japan) with a mounted eyepiece camera (Edge Eyepiece Camera, Dino-Lite, Torrance, CA) for a baseline record. These central incisors were then utilized for white spot lesion induction and imaging analysis.

White Spot Lesion Development and Analysis

The central incisors were submerged in 5.25% bleach (Clorox, Oakland, CA) for two hours to sterilize them. They were then copiously rinsed with sterile water and then placed in sterile Falcon™ tubes (Fisher Scientific, Hampton, NH) by group with 8 mL of sterile BT media to cover all of the teeth. The tubes were placed in a 5% CO_2 chamber at 37ºC for 24 hours to verify sterility.

S. mutans was grown as before. Similarly, the bacteria were diluted with sterile media and mixed with sucrose to create a 1% sucrose solution. The samples were then incubated at 37ºC and 5% CO_2 for 14 days. The media was refreshed with 8 mL of sterilized media every 24 hours.

After the 14 days, the teeth were removed from the media, wiped with cotton gauze to remove any attached biofilm, and thoroughly air-dried under an air-water syringe for 10 seconds to reveal any demineralized area in the exposed enamel window. The teeth were photographed as before and the images imported into ImageJ (NIH - LOCI, Madison, WI). The demineralization photographs were coded by one evaluator (HD) to blind a separate evaluator measuring the demineralized area (JY). The image greyscale intensity levels were adjusted with ImageJ to

further improve white spot visualization, and all areas of demineralization were confirmed tactilely with an explorer tip. The demineralized area was then traced and measured by utilizing the baseline image as a comparison. A few samples were viewed under a scanning electron microscope to further calibrate the areas of demineralization observed in the photos.

Shear Bond Strength

The bonded premolars were subjected to multiple WSL development experiments as previously described to simulate a clinically-relevant situation. These samples were subjected to 80 total days of storage in aqueous media incorporating two separate periods totaling 12 days of exposure to the *S. mutans* biofilm grown in BT media at 37 ºC and 5% CO_2. This rigorous challenge ensured that the cements had taken up water during aging and depleted some of the ion microcapsules when subjected to the effects of an acidic biofilm for nearly two weeks. The premolars then prepared for shear bond strength (SBS) testing by being embedded perpendicularly into boxing wax to fully immerse the bracket and part of the facial enamel. Each tooth and its wax were then placed onto a cylindrical mold 2.5 cm in diameter by 2.5 cm thick. Epoxy resin was made by mixing EpoxiCure Resin 2 and Epoxy Hardener (Buehler, Lake Bluff, IL) at 5:1 by weight and poured into each mold to capture the un-waxed portion of each sample. The resin was left to set for 24 hours before removal. Each tooth was visually and tactilely inspected for resin flash; any flash on or around the bracket pad was then removed.

The debonding force was applied with the Universal Testing Machine hex bolt attachment (MTS Centurion, Eden Prairie, MN) with an occlusal-gingival load rate of 0.01 mm/sec applied to the bracket pad until it debonded from the enamel surface. Shear bond strength was calculated as the debonding force divided by the bracket base surface area provided

by American Orthodontics product specifications and verified by digital caliper measurements (0.180 in [4.572 mm] x 0.130 in [3.302 mm]).

Adhesive Remnant Index

After debonding, the teeth were examined under a Leica ES2 Stereomicroscope at 10x magnification (Leica Microsystems, Wetzlar, Germany). Adhesive Remnant Index (ARI) scores were assessed visually using the guidelines proposed by Årtun and Bergland [16]:

0 = No adhesive left on tooth

1 = Less than ½ of the adhesive left on tooth

2 = More than ½ of the adhesive left on tooth

3 = All adhesive left on the tooth, with distinct impression of the bracket mesh

Statistical Analysis

The quantities of viable bacteria measured via the luciferase assay, the WSL sizes, and the shear bond strengths were compared for the different cements using one-way Analysis of Variance (ANOVA) and Tukey's HSD test ($\alpha = 0.05$). The cumulative quantities of ions released were analyzed by one-way ANOVA and Newman-Keuls test ($\alpha = 0.05$). ARI values were compared with Fisher's Exact Test ($\alpha = 0.05$).

Results

Quantification and Characterization of Ions Released

The cumulative zinc, phosphorous, and calcium releases over the 28-day period are shown in Figures 1-3, respectively. The standard calibration curve for fluoride ion measurements is shown in Figure 4, followed by the cumulative fluoride ion release in Figure 5 and the fluoride release at each time-point in Figure 6. Fluoride Release of P-F at each Time The cumulative amount of zinc released by P-Z was significantly higher than for all other groups. Furthermore, both P-CaP and PF exhibited significantly more zinc release than P-0 and TB. For phosphorous release, P-CaP demonstrated significantly higher levels than all other groups. Both P-CaP and P-F released significantly more calcium than TB, P-0, and P-Z; furthermore, the calcium release of P-CaP was significantly higher than P-F. The release of fluoride was characterized but not compared to other groups. The cumulative release of phosphorous, calcium, and fluoride ions followed a logarithmic pattern whereas the release of zinc was linear over the 28-day period, and all goodness of fit (R^2) values were higher than 0.90.

There was no significant evidence of the formation of precipitants during the experiment (Table 1). There was a low level of copper detected in P-0 and a modest amount of zinc in P-Z. Measuring the empty vial revealed minimal background values for Cu and Zn that were normal and negligible, especially in light of the values from the actual dissolution experiment.

Biofilm Quantification

The number of viable bacteria grown on the cement discs as assessed by the luciferase assay is shown in Figure 7. Only P-F exhibited significantly less bacterial growth than the other four cements, including the control.

White Spot Lesion Development

The mean demineralized areas of the orthodontically bonded teeth are shown in Figure 8. There were no significant differences in demineralized area for any of the experimental groups ($p = 0.88$).

Mechanical Properties

The shear bond strengths of the cements when bonded to premolar brackets are shown in Figure 9. The control cement, Transbond, exhibited the highest SBS at 9.9 ± 2.9 MPa, but it was statistically equivalent to P-0 (8.3 ± 1.6 MPa) ($p = 0.3$). P-Z (6.8 ± 1.8 MPa), P-CaP (5.7 ± 1.2 MPa), and P-F (6.7 ± 1.1 MPa) all had significantly lower bond strength when compared to TB. P-CaP also demonstrated significantly lower SBS than P-0.

ARI scores are tabulated in Table 2. All groups had similar ARI scores when evaluated by Fisher's Exact Test ($p > 0.5$). None of the samples demonstrated debonding at the adhesive-enamel interface. Debonding the brackets most commonly left some partial amount of cement on the enamel surface (scores 1 and 2).

Discussion

A new cement for bonding orthodontic brackets that incorporates microencapsulated ions for the purpose of reducing white spot lesions was developed by Premier. The various formulations of the orthodontic cement were originally modeled after BioCoat, a pit and fissure sealant from Premier. BioCoat utilizes patented SmartCap resin microcapsules containing rechargeable solutions of phosphate, calcium, and fluoride. In recent reports, the SmartCap microcapsules in BioCoat have demonstrated sustained ion release and two-way permeability for remineralization in initial studies [17, 18]. The purpose of this study was to evaluate how the presence of these microcapsules in different formulations influenced white spot lesion formation and the bond strength of the orthodontic cement.

The experimental cements demonstrated significant amounts of zinc, phosphate, calcium, and fluoride release for their corresponding formulations over the 28-day period, thus demonstrating their efficacy as ion-releasing materials when exposed to mildly acidic conditions simulating a caries challenge. Each of these ions have potential benefits in oral health. Zn^{2+}, usually as ZnO or $ZnCl_2$, has demonstrated efficacy in inhibiting both Gram-positive and Gram-negative bacteria through the destabilization of microbial membranes [19-21]. A systematic review by Almoudi et al. concluded that even low concentrations of zinc can significantly inhibit the growth of *Strepococcus mutans* and would be appropriate for oral health applications [22]. Phosphate, calcium, and fluoride ions are all important in remineralizing enamel. PO_4^{3-} and Ca^{2+} are key constituents of the hydroxyapatite $[Ca_{10}(PO4)_6(OH)_2]$ crystal structure of enamel, and F^- is a common ion substitute that decreases the solubility of enamel [23].

The release of Zn^{2+} was linear throughout the trial period of 28 days. Clearly, the limit of release from the microcapsules had not been reached in one month, suggesting favorable

sustainability in clinical applications. In contrast, PO_4^{3-}, Ca^{2+}, and F^- release followed a logarithmic pattern, characteristic of diffusion controlled kinetics. As the ion reservoir depletes, the rate-limiting step is the diffusion of additional ions from microcapsules deeper within the cement. Additionally, the procedure of switching the cement discs to a fresh acidic environment at each time point was specifically used to avoid saturation of the ions in the solution. Therefore, the decrease in ion release with time is somewhat suggestive of a partial depletion of the microcapsule reservoir and not a saturation of the media. Another possibility is that the calcium and phosphate or calcium and fluoride released from the appropriate cements precipitate from the solution once the solution becomes saturated, thus causing the non-linear release kinetics. However, no evidence for such precipitation was found, and the ICP method used to quantify the ion release involved vigorous mixing and acid solubilization that should have dissolved any precipitates to recover all of the released ions.

Zinc may have been released linearly because of its high concentration relative to the other ions (7% versus the next highest at 5%). The linear release may also be because the microcapsule formation in P-Z only contained a single element. The multiple ions released from P-CaP and P-F could combine into insoluble ion complexes like $Ca_3(PO_4)_2$, CaF_2, or $Ca_5(PO_4)_3F$. However, as noted above, the subsequent investigation performed in this study revealed little evidence of precipitates, and, if precipitates were formed, they were not detected with the methodology used here. As diffusion of ions out of the microcapsules occurs within the solution absorbed by the cement, the multiple species of ions may be interfering with each other's diffusion. However, based on the evidence provided, it may be concluded that if the ions interfered with each other's release or diffusion due to complexing, this may have occurred within the resin structure, thus reducing the actual release of ions to the solution. A follow-up

study investigating the release of PO_4^{3-}, Ca^{2+}, and F^- as sole constituents of a formulation may provide more insight on the different patterns of ion release.

Given that orthodontic treatment often lasts longer than 18 months, sustained ion release for continued remineralization is imperative. This is why glass ionomer (GI) cements are appealing because of their capability to “recharge” by the continued uptake of fluoride from extraneous sources, usually from mouth rinse or toothpaste, and then re-releasing it. The deficiencies of GI materials include rapid depletion of the fluoride reservoir within 24 hours and lower bond strength [24, 25]. The experimental cement in this study showed substantial fluoride release for a longer duration, and perhaps the low levels released with increased duration may be sufficient for beneficial effects. In-vitro studies performed by O’Reilley et al. suggested 0.08 ppm of fluoride promotes enamel remineralization, which was exceeded within the first six hours in the present study [26]. While the microcapsules have previous evidence of rechargeability, it was not evaluated here [18]. Ultimately, studies of longer duration for all ion groups, the potential impact of microcapsule rechargeability, and a comparison group specifically for characterizing the fluoride release are desirable.

A modified *Streptococcus mutans* was grown on discs of each cement for one day to assess any antibacterial effect of the formulations. Only P-F demonstrated lower levels of bacterial growth relative to all other cements. In particular, P-CaP had no significant difference in bacterial growth which suggests that the presence of fluoride is a key factor. Fluoride as an antimicrobial for oral bacteria has been well-documented [27-29]. Fluoride acts as an enzyme inhibitor and negatively impacts bacterial metabolism; additionally, fluoride can disrupt the membranes of some bacteria and reduce their acid tolerance [27]. It is possible that one or more of these interactions occur with the release of fluoride from P-F. Marquis found that as little as

0.1 mM of fluoride can completely halt glycolysis of *S. mutans* [27]. P-F released 0.41 ppm (2.2 mM) of fluoride by six hours, and 1.26 ppm (66.3 mM) by twenty four hours.

Given the high levels of zinc release from P-Z, it is surprising that the formulation did not exhibit any significant difference in bacterial growth relative to the other cements. Much of the previous studies investigating Zn^{2+} in dental applications focus on $ZnCl_2$, while the contribution of ZnO as an antimicrobial is still disputed due to the complexity of the underlying mechanism [21]. He et al. showed $ZnCl_2$ levels as low as 0.2-0.3 mM inhibited glycolysis of mixed cultures of *Streptoccocus* species, but single organism biofilms were less sensitive and required 1 mM Zn [30]. Furthermore, initial zinc concentrations of 0.05 mM resulted in 72% less acid production of *S. mutans*. Another study by Gu et al. showed different effects of $ZnCl_2$ concentration based on plaque layer. Gu et al.'s work demonstrated that 2.5 mM of $ZnCl_2$ reduced the vitality of outermost plaque layers, 5 mM was required to reduce the vitality of bacteria in the middle layer of plaque, and levels as high as 20 mM were insufficient to reduce vitality in the inner plaque layer [20]. In this study, the first data at 6 hours showed over 8 ppm (120 mM) of Zn^{2+} released, and by 24 hours, 12 ppm (180 mM) had been released – and yet, there was no antimicrobial effect observed. It is possible that the bacteria in this study remained viable but with reduced acid production, though that would imply a decreased metabolism. The linear release of zinc may have been too slow for an antibacterial effect in just one day whereas a more logarithmic release would result in a more rapid initial release. It is possible that the benefits of the Zn^{2+} may not occur until a sufficient amount of ions have accumulated; however, this seems unlikely with the high levels seen in this study. Previous studies suggest that higher levels of Zn^{2+} are necessary to provide a lasting anti-glycolytic effect; 5 mM of Zn^{2+} was required for 30 min and 100 mM was

requried for 2 hours [30]. Interestingly, the cytotoxity of Zn^{2+} has been shown to be suppressed by the presence of Ca^{2+}; however, none of the current formulations combined Zn^{2+} and Ca^{2+} [31].

P-CaP showed no difference in bacterial growth suggesting that the potential beneficial effect of Ca^{2+} and PO_4^{3-} is limited to remineralizing the enamel when these ions are incorporated at these concentrations. Studies have demonstrated that oral bacteria have the capability to bind Ca^{2+} which is then released during a pH drop and can potentially lead to decreased enamel demineralization [32, 33]. Leitão et al.'s study suggested 1 mM Ca^{2+} resulted in an equilibrium of bound and unbound calcium ions to planktonic *S. mutans*. When the Ca^{2+} concentration was increased to 10 mM, the bacteria exhibited decreased affinity for calcium but still demonstrated an overall increase in the amount of bound calcium. Aranha et al.'s study found that calcium has a either a stimulatory or inhibitory effect on *S. mutans* depending on the dose [34]. 0.64 µM of calcium stimulated *S. mutans* growth but increasing calcium concentrations to 1.3 µM or 2.5 µM decreased steady-state *S. mutans* growth. During the present study, accumulated Ca^{2+} levels reached 0.99 mM and 0.22 mM after 24 hours P-CaP and P-F respectively, and thus lower *S. mutans* growth would be expected. However, it is unknown how the dose-dependent effect of calcium behaves past the 2.5 µM studied by Aranha et al. Data from Xie & Yang suggest that a minimum concentration of 10 mM Ca^{2+} is required to disrupt a model of *Staphylococcus aureus*'s membrane [35]. When tested against live cells of *S. aureus*, Ca^{2+} concentrations of 40 mM resulted in a maximum 60% loss in viability. While these data pertain to *S. aureus*, it may suggest that there was insufficient Ca^{2+} released after one day to exhibit an antibacterial effect in the present study. Furthermore, Salehi et al. found 100 ppm of Ca^{2+} was required for significant reductions in *S. sobrinus* viability, which was only achieved by P-CaP after one week [36].

There are a few limitations to this experimetal design. First, this approach only focused on the concentration of the bacteria in the biofilm but did not investigate any differences in bacterial adhesion. It is possible that the lower levels of bacterial growth associated with P-F were due to a bacteriocidal effect or due to a decrease in bacterial adherence – this protocol does not elucidate the specific mechanism. Another limitation is that these results primarily reflect the viability of the biofilm bacteria adhered to the cement disc. Removing the media and replenishing with fresh media one hour prior to the luciferase assay potentially removes the suspended bacteria that may otherwise be present. Therefore, it is difficult to discern if any of the materials have an antibacterial zone that extends to the surrounding media. A zone of inhibition to bacterial growth would be useful because demineralization can occur immediately adjacent and farther away from orthodontic appliances. Also, there is an underlying assumption that the environment used to grow this bacteria also facilitated ion release. Lastly, the study was only one day in duration in order to immediately elucidate any intial antibacterial effect. Longer-term studies combining antibacterial properties with prolonged ion release should be conducted.

Orthodontically bonded teeth were subjected to bacteria in an environment simulating dental caries to evaluate the influence of the various cement formulations on WSL formation. The extent of demineralization was not significantly different for any of the cements. However, this only evaluated the surface demineralization and not the lesion depth. Evaluating cross-sections of the teeth under scanning electron microscope or utilizing quantitative light-induced fluorescence may reveal differences in lesion depth. While the presence of demineralization was evident, the lesions were not consistent amongst the samples – this is apparent in the high standard deviation depicted in Figure 8. Area of Demineralization Some lesions were opaque and intense in color while others were more subtle. There is some evidence that the clinical color

intensity reflects the depth of the enamel demineralization [37]. The experiment conducted here was only for two weeks which cannot represent the full duration of orthodontic treatment. However, it does provide some revealing information during the most critical period; studies suggest that WSLs can form in as little as four weeks and that lesions develop most frequently during the first six months of treatment [38, 39]. The experimental formulations currently evaluatd were unable to significantly resist demineralization in this short timespan. The previous studies investigating the ion release and biofilm formation used cement disks which are not indicative of the surface area of cement exposed under an orthodontic bracket. Therefore, it is likely that the ion release is substantially less in the WSL studies. Further studies investigating the amount of ion release from the cement under a bracket would provide more clinically-relevant information. As stated before, the possibility of microcapsule recharge may alter the results clinically. However, because the microcapsules are contained in the cement matrix, the recharge may be limited. Overall, a clinical study, perhaps of a split-mouth design, for an extended duration would better elucidate any effect of the cements to resist WSL formation.

The shear bond strength to enamel of the experimental cements were also compared to the control. All of the formulations containing microcapsules (P-Z, P-CaP, and P-F) exhibited significantly lower SBS relative to the control. Furthermore, P-CaP demonstrated significantly lower bond strength compared to P-0. These results suggest that the presence of microcapsules lowers the shear bond strength. It is possible that the microcapsules interfere with the polymerization of the cement matrix or increases the risk of crack propagation. The microcapsules were added in a relatively small amount, but they did appear to weaken the cement matrix. They may also reduce the elastic modulus of the cement. Materials with lower elastic modulus generate lower interfacial stresses and result in lower shear bond strengths [40].

The experimental design was chosen in such a way that allowed for measuring the SBS of cements with some level of microcapsule depletion. This was intentionally performed to study a clinically-relevant situation where orthodontic appliances have been in place for some time. It is possible that the SBS changes as ions are released from the microcapsules. Nevertheless, all SBS values were within the minimum 5-10 MPa proposed by Reynolds to resist forces of mastication [41]. Follow-up studies should examine the adequacy of the bond strength clinically, compare the experimental composites to other commercial materials, and the effect of further microcapsule depletion on the SBS.

There were no significant differences in the ARI scores for the cements. Most of the brackets left some amount of cement regardless of formulation. Remnants of cement are unlikely to be removed from abrasive wear and may become discolored [42]. Therefore, the remaining cement necessitates cleanup which requires time and risks damaging the enamel. It would be more advantageous to have as little cement remnants as possible.

Conclusions & Future Research

A series of new cements containing a combination of microencapsulated zinc, phosphate, calcium, and fluoride ions were developed. The objectives of this study were to investigate the cement's capability to reduce or prevent white spot lesions and to evaluate the material's mechanical properties, specifically bond strength to enamel. Four different formulations were evaluated and compared to a control. All formulations containing microencapsulated ions demonstrated significant ion release for 28 days. The formulation containing fluoride exhibited significantly less bacterial growth than all other groups. There were no significant differences in the extent of enamel demineralization for orthodontically bonded teeth. The shear bond strength was significantly lower than the commercial control, but still within the minimum recommended for clinical use. There were no significant differences in the adhesive remnant indices. Therefore, the experimental cements successfully release potentially remineralizing and antibacterial ions without significant sacrifice of mechanical properties. Further studies must target the clinical efficacy of the cement, its longevity, and ultimately its ability to reduce or prevent white spot lesion development on enamel surfaces.

Literature Review

White Spot Lesions: Background, Prevalence, and Detection

White spot lesions (WSLs) result from subsurface enamel demineralization that creates light-scattering porosities [43]. WSLs are the decalcified precursors of carious lesions and have the same etiology. Bacteria, particularly *Streptococcus mutans* and *Lactobacillus* groups, ferment sugars and create an acidic environment that ultimately demineralizes the enamel surface [1]. WSLs develop in two stages: First, the surface softens as mineral content is lost from the enamel in an acidic environment at the critical pH of 5.5; second, dissolution occurs and a subsurface lesion forms that is subsequently surrounded by a layer of porous but richly mineralized tooth structure [44, 45]. This dissolution can potentially reduce the enamel's mineral content by 10-50% [46]. Studies have shown increased levels of the offending bacteria in the biofilm surrounding orthodontic appliances [47]. Ultimately, orthodontic appliances provide plaque-retentive areas that promote bacterial adhesion. Without a fluoride regimen, white spot lesions can develop within four weeks which is often the time-span between orthodontic visits, whereas carious lesions normally take at least six months to develop [44].

Studies report a wide range of white spot lesion prevalence from as low as 2% to as high as 97% though most settle on the average around 40-60% [2]. Some variation in reported WSL prevalence could be due to how clinicians define the lesions. Early on, Gorelick developed a scoring index for the severity of white spot lesions [39]:

1. No white spot
2. Slight white spot formation
3. Excessive white spot formation
4. White spot formation with cavitation

The large range of reported prevalence may also be attributed to the various methods of detecting lesions and the difficulties in standardizing the clinical examination [48]. For example, Boersma et al. used quantitative light-induced fluorescence to find 97% prevalence of at least one decalcification. Other sophisticated methods including microradiography, microscopy, and imaging analysis have been utilized to more accurately quantify WSLs [1]. Livas et al. proposed that quantification of WSLs through photographic imaging and computer analysis is preferred because other methods were deemed too subjective, inaccurate, or non-reproducible [49]. The typical method of detecting WSLs chairside is by visual inspection after air-drying the enamel surface to eliminate the moisture that can otherwise mask the chalky, white surface.

Generally, WSLs are more visible and noticeable after removal of orthodontic appliances [48, 50]. One reason is because the lack of appliances reveals more tooth surface; evaluators studying WSLs anecdotally remarked that diagnosing lesions adjacent to brackets was more difficult than without brackets present [51]. Boersma et al. also noted an increase in the total number of affected surfaces between the day of debond and the six-week recall visit. This increase was attributed to a decrease in gingival swelling that revealed additional tooth surface area [48].

Risk Factors

Many studies have investigated the risk factors associated with white spot lesion formation. Awareness of the pertinent risk factors enables clinicians to identify patients with potential for developing WSLs and adjust accordingly. Oral hygiene is the risk factor most commonly discussed and investigated. Several researchers have correlated poor oral hygiene with WSL development [52-54]. Zachrisson and Zachrisson demonstrated a fairly linear

correlation between plaque accumulation and caries development in orthodontic patients [7]. Khalaf found that poor oral hygiene was the greatest risk factor for developing WSLs with a risk ratio of 8.55 [8]. However, this finding is not universal; according to Hadler-Olsen et al.'s study, the trend between reduced WSLs incidence and compliance with oral hygiene was statistically insignificant [55]. Hadler-Olsen et al.'s patients had no severe WSLs with cavitations, suggesting that a good prophylactic regimen may reduce the severity but perhaps not the occurrence of WSLs. Patients that present with WSLs prior to orthodontic therapy likely have poor oral home care, and in fact, Julien et al. found that the presence of WSLs prior to treatment was significantly correlated to new WSLs during treatment [52]. Chapman et al. found that patients with poor to fair pretreatment oral hygiene had three times the incidence of WSLs compared to patients with good pretreatment oral hygiene [56]. Furthermore, despite an improvement in oral hygiene at the start of treatment, patients with a history of poor oral hygiene tended to still be at risk, likely due to a regression to previous inadequate home care [56]. Considering how crucial oral hygiene is to caries prevention, it seems logical that oral hygiene plays a role in WSL formation because the process is fundamentally identical.

The discussion about oral hygiene is often paired with investigating the effects of fluoride on white spot development. As with oral hygiene, fluoride use tends to reduce lesions but requires compliance [57]. One of the early studies by Øgaard et al. suggested that 50% of subjects with no preventive fluoride had an increased risk of WSLs during treatment with fixed orthodontic appliances [58]. In fact, Øgaard also suggested that low caries risk individuals with a fluoride regimen do not have any additional risk of caries development during orthodontic therapy compared to individuals not undergoing treatment [59]. According to Gavrilovic, multiple studies suggest that routine use of over-the-counter fluoridated toothpaste is inadequate

at inhibiting lesion development, but 0.05% or 0.2% sodium fluoride mouth rinses can reduce demineralization [60].

There are some biologic factors that may influence WSL formation. An individual's salivary flow rate and buffer capacity impact enamel demineralization and remineralization; salivary flow provides physical cleansing of the tooth surface, and the ions within saliva facilitate remineralization [47]. Low salivary clearance results in lower pH of the plaque on the upper incisors, increasing their susceptibility to WSLs [61]. Also, individual variation in salivary pH may influence the growth of acidic bacteria and ultimately demineralization of the enamel surface [60]. Unlike *S. mutans*, the presence of saliva and the salivary pellicle may promote adhesion of periodontal pathogens like *P. gingivalis*, providing potential competition for bacteria associated with WSLs [62]. There can be differences in tooth biology or structure as well; Julien's study on WSL prevalence revealed that patients with fluorosis had a significantly lower chance of developing WSLs [52].

Many studies have also investigated demographic factors associated with white spot lesions. Male patients are generally thought to be at higher risk than female patients due to poorer hygiene [8, 57]. Chapman et al. found WSLs with both higher incidence and severity in male patients [56]. However, these gender trends are not universal amongst studies [57]. Age is another commonly investigated risk factor. Younger patients in the preadolescent to adolescent age range have been found to be at a higher risk of WSLs occurrence and severity [56, 57]. However, again, this has not always been the case; O'Reilly et al.'s study observed that younger patients (age range 10-25) actually had a lower risk for WSLs [63]. Furthermore, they did not detect a strong association between age and oral hygiene. Therefore, it may be an oversimplification to equate younger age with poorer oral hygiene. One study found that the

white ethnic group was a risk factor for WSLs over other ethnicities [56]. Socioeconomic factors were not associated with caries prevalence according to Boersma [2]. Diet can also have an effect on WSL formation. Khalaf found that the use of fruit juices and/or carbonated soft drinks four or more times per week increase risk of WSLs [8]. In summary, patient demographics may provide some insight on the relative risk of WSLs but have too much variability to be reliable predictors.

The area of the dentition is also commonly investigated for WSL risk, though studies report various results. Many studies find WSLs most frequently in maxillary laterals [39, 53, 57]. The next most common tooth varies by study but generally include the maxillary or mandibular canine, the maxillary first premolar, or the maxillary central incisor [53, 54, 57]. According to Boersma's study, carious lesions during orthodontic treatment were more commonly found on molars and premolars [2]. Generally, larger lesions occur in the areas gingival to orthodontic brackets, possibly due to a lack of space between the gingival margin and the bracket for cleansing [5]. Interestingly, no lesions were found on teeth bonded to lingual retainers despite accumulations of stain and calculus [39]. This may reflect how important the accessibility of free-flowing saliva may be in the defense against decalcification. There were no differences in WSL incidence between the left and right sides of the mouth [53]. One study found that the maxillary arch developed more than twice as many lesions than the mandibular arch during orthodontic treatment [52]. Thus, the maxillary arch is both the primary esthetic zone and the most susceptible to decalcifications; therefore, special emphasis must be placed on reducing WSLs as much as possible.

Ironically, the presence of orthodontic appliances including brackets, wires, and bands can be a risk factor for WSL formation. Orthodontic appliances in general tend to promote

bacterial adhesion and challenge the patient's ability to sufficiently clean. Orthodontic brackets not only allow for the accumulation of plaque bacteria but also retain them long enough to mature. Maturation then allows the progression for more pathogenic microorganisms to prosper and cause enamel demineralization and gingival inflammation [62]. One study suggests that the removal of fixed appliances significantly reduced levels of *Lactobacillus* bacteria which were found to be correlated to decalcifications at the time of debond [2].

Between metallic and ceramic brackets, there was no significant difference in the number of *Streptococcus mutans* and *Lactobacillus acidophilus* found around either bracket type in a study performed by Anhoury et al. [64]. While the bacterial profile of several other species differed between the two materials, the authors found that there was no strong trend towards either material. Another study investigated the adhesion of *S. mutans* on various esthetic polymer bracket materials and found no significant difference in bacterial colonization [65]. In contrast, Van Gastel et al. performed a study comparing the bacterial adhesion in-vitro for various commonly used brackets. Their findings suggested that ceramic brackets had higher microbial adhesion than metallic brackets [66]. However, brackets with less bacterial adhesion had a higher ratio of aerobic, caries-inducing bacteria in their biofilm. Ultimately, like Anhoury et al., Van Gastel et al. concluded that different brackets can have significantly different bacterial profiles in their biofilm. Pellegrini et al. showed significantly reduced plaque retention for self-ligating brackets but only in the short term of 5 weeks; Buck et al.'s follow-up study to Pellegrini et al.'s at one-year showed no significant difference [67, 68]. Unfortunately, there is conflicting evidence on whether self-ligating brackets provide a significant reduction in plaque accumulation compared to traditional brackets ligated with elastomerics [47].

There is some evidence that patients treated with lingual orthodontics develop fewer white spot lesions than patients treated with traditional labial brackets [69]. The WSLs that do develop on lingual surfaces have substantially less impact on smile esthetics than facial WSLs. In contrast, patients with full-coverage bonded acrylic palatal expanders tend to develop more white spot lesions than untreated patients even when cemented with glass ionomer [70].

No studies could be found comparing the incidence of WSLs with fixed appliances versus clear aligners. Azeem and Ul Hamid found 7 out of 25 clear aligner therapy patients developed at least one WSL during treatment [71]. However, it is unclear if their reported 28% incidence is significantly less than what is reported for fixed appliances. This is partially due to the aforementioned lack of standardization of evaluating WSLs between studies. Clear aligners and their associated attachments avoid the problem of having a wire interfere with contact points between teeth, and thus it seems logical that the WSL incidence would be lower. However, the full coverage of aligners can potentially trap sugar and reduce the cleansing provided by salivary flushing [72]. One study by Abbate et al. compared the periodontal health in teenagers treated with removable aligners and fixed appliances. After receiving standardized oral hygiene instructions, the patients in the aligner group exhibited significantly lower plaque index, probing depths, bleeding on probing scores, and higher oral hygiene compliance [73]. However, the study did not explain if it utilized aligners only or aligners with attachments. Bacterial accumulation has been shown on the clear aligner material itself even without attachments [74, 75]. Chhiber et al. performed a prospective randomized clinical trial and found significantly better oral health values for clear aligners compared to fixed appliances at nine months; however, there were no significant differences in oral hygiene levels at 18 months for aligners, self-ligated brackets, and conventional brackets [76].

In conjunction with the presence of orthodontic appliances, the treatment duration can also affect WSL formation. Gorelick's study showed no change in WSL incidence from months 12-16 to month 36 [39]. However, several other studies have found a positive correlation between WSL development and treatment duration [8, 57, 71]. In particular, treatment duration greater than 36 months was noted as a risk factor for developing demineralization [52]. Tufekci et al. investigated the development of WSLs at different time points during treatment and observed the highest rate of WSLs during the first six months of treatment with a notable decrease after twelve months [38]. Other studies have also noted that the initial six months of treatment have the highest risk of WSL formation and that longer treatment in general increases risk.

In summary, there are many potential risk factors for developing WSLs though few of them are definitive. The most consistent indicators tend to be poor oral hygiene and extended treatment duration. Thus, proper oral hygiene and home care should be emphasized to patients to reduce the risk of WSL.

WSL Treatment

Once a white spot lesion has developed, there are several treatment modalities to mitigate the esthetic damage. While WSLs are stable long-term and rarely progress to cavitation and decay, their unesthetic nature makes treatment appealing [39, 77]. Fortunately, there is clinical evidence that if the overlying enamel surface remains intact, the lesion can be remineralized [78]. Generally, there are two treatment strategies for white spot lesions: Remineralizing or masking the lesion [79].

The severity of lesions reduces naturally over time due to the remineralizing capabilities of saliva; simply removing appliances without any fluoride application can have a profound effect on lesion depth and mineral loss of established lesions according to Øgaard [77]. Lesions tend to revert most significantly during the first three months after orthodontic appliances are removed and then persist in a stable state [3]. Hamdan et al.'s survey revealed that many orthodontists and general dentists recommend immediate topical fluoride treatment to combat white spot lesions; however, multiple studies suggest that high fluoride concentrations arrest lesion development to the point of preventing complete repair and cause unesthetic yellow or brown staining [43]. Øgaard also suggested that ultimately lesions developed during orthodontic treatment should not be treated with concentrated fluoride because it will arrest the lesion, particularly a surface demineralization, and prevent complete repair [77]. However, Willmot recommended that lesions on non-labial, and thus non-esthetic, surfaces could benefit from highly concentrated fluoride to prevent lesion progression [5]. Some studies have demonstrated that sorbitol- or xylitol-based chewing gums provide significant remineralization when used five times a day over a three-week period; however, this could largely be attributed to salivary stimulation and not necessarily from the chewing gum itself [5].

CPP-ACP

Another proposed method to remineralize white spot lesions is through the use of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). However, the evidence supporting CPP-ACP is rather lacking. Oliveira et al. performed a study on the effects of CPP-ACP and fluoride for treating WSLs in vitro and found that highly-fluoridated dentifrices at 5000 ppm displayed more remineralization capabilities within the first 10 days but that the results were

equivalent to the non-treatment controls at 30 days [80]. Furthermore, CPP-ACP exhibited less remineralization with or without fluoride. Willmot also suggested that a CPP-ACP mousse delivered in a thermoplastic retainer worn nightly may aid WSL regression but may not be different than the natural reduction from saliva [5].

Bleaching

One potential strategy to mask white spot lesions is through bleaching the unaffected enamel to obtain a closer color match. Knosel et al. described a bleaching technique in which anterior teeth were bleached with an in-office gel for 60 minutes followed by a 2-week home bleaching protocol [81]. They demonstrated significant color change and patient satisfaction when evaluated with a questionnaire. This camouflage was achieved primarily by affecting and lightening the sound enamel structure. The area of the white spot lesion whitened similarly but only at the first follow-up. The authors and patients noted that the enamel surface looked more uniform but some opacity differences persisted. Lastly, the authors noted that bleaching has several detrimental side effects and should be reserved for cases with exceptional oral health and hygiene.

Microabrasion

Microabrasion is a technique first described in 1986 by Croll and Cavanaugh used to treat white spot lesions [82]. Briefly, microabrasion involves combining acid application, originally hydrochloric acid, and pumice abrasion to remove the superficial surface of enamel; the esthetic benefit occurs by compacting mineralized tissue into a prism-free region that changes light reflection, ultimately camouflaging the surface stains [83]. Murphy et al. demonstrated the

ability to provide an esthetic improvement with minimal loss of tooth structure via microabrasion followed by non-acidulated fluoride application. Their study revealed that tooth structure loss does not exceed 250 μm yet reduced WSLs by 83 ± 8.2% [6]. However, a simpler 37.5% phosphoric acid etch technique has been advocated and may also diminish WSLs. Some suggest delaying microabrasion for at least three months after appliance removal because of the spontaneous improvement of lesions due to salivary effects discussed previously [6]. Akin and Basciftci compared the capability of regular tooth brushing, sodium fluoride mouth rinse, CPP-ACP, and microabrasion on reducing WSL. WSLs decreased significantly for all treatment groups; however, microabrasion performed the most successfully with a 97% reduction in WSL surface area depending on the severity of the lesion. Brushing, fluoride mouth rinse, and CPP-ACP reduced WSLs 45%, 48%, and 58% respectively [84].

Resin Infiltration

Senestraro et al. described a minimally invasive resin infiltration technique to treat arrested WSLs [3]. The technique utilized a microabrasion technique and a light-cured resin infiltrate to improve the esthetics of existing lesions. The authors reported that the technique was more conservative on the enamel surface and only penetrated 80 μm compared to the 360 μm of other more invasive microabrasion techniques [3]. The authors had concerns about the resin staining in the long-term, as their follow-up was only eight weeks.

Despite these successes, Sonesson et al.'s systematic review ultimately concluded that microabrasion and resin infiltration are technique sensitive and have questionable long-term success [79]. Therefore, Sonesson et al. recommended that these invasive procedures be reserved for challenging esthetic cases. Sonesson et al. were unable to evaluate post-orthodontic

bleaching. Side effects of bleaching include increased tooth sensitivity and decreased enamel microhardness, suggesting that bleaching is a non-ideal treatment modality [3].

Overall, there are several methods to reduce WSLs once formed. However, treatment is costly; Ren et al. estimated that orthodontic complications that require professional care, including WSLs, develop in 15% of patients [85]; furthermore, they estimated that 60% of orthodontic patients suffer from at least one biofilm-related complication during treatment. Hamdan et al.'s survey revealed that slightly more than one-third of orthodontists have removed braces prematurely because of WSL issues [43]. Ultimately, Ren et al. estimated that it would take 1,000 full-time dentists and over $500,000,000 annually to address all of these problems [85]. With over four million adolescents and one million adults in North America currently undergoing orthodontic treatment, potential consequences like white spot lesions can be a serious public health concern [85]. Therefore, focusing on preventing WSLs from forming would be a more efficient approach.

Prevention

As previously discussed, adequate home care is one of the most significant factors in preventing white spot lesions. Unfortunately, not all orthodontic patients demonstrate satisfactory hygiene. Several strategies have been described to augment the prevention of white spot lesions in orthodontic patients.

Chairside Method

Some advocate that the method of bonding orthodontic appliances may impact WSL development. Specifically, excessive enamel etching during orthodontic bonding has been

thought to increase the risk of white spot formation, particularly when the etched enamel is not fully covered by adhesive [86, 87]. Additionally, Knosel suggests that etched enamel without the application of bonding sealers may increase plaque retention [87]. Tufekci et al. reported approximately a 9% decrease in enamel hardness in etched teeth during their study [88]. Therefore, they recommended applying etch only to the tooth surface where the bracket will be bonded.

Appliance Choice

As discussed previously, appliance choice and design may influence bacterial adhesion. Innovative antimicrobial coatings on brackets and wire, attempt to combat the inherent microbial accumulation around orthodontic appliances. Unfortunately, these coatings have not had enough clinical application to demonstrate efficacy; additionally, there are concerns that the reservoirs of the antibacterial agent on these appliances would have an insufficient duration or area of effect to offer any significant protection [85].

Another similar strategy utilizes fluoride-releasing elastomeric rings. One study using an in-situ caries model investigated mineral loss and lesion depth adjacent to fluoride-releasing elastomerics; the results suggested no statistically significant difference in anti-cariogenic effect but did not eliminate a potential local effect adjacent to the bracket [89]. In contrast, one initial prospective clinical trial demonstrated that fluoride-releasing elastomeric rings significantly reduced the occurrence of WSLs; only 16% of teeth with the fluoride-releasing elastomerics displayed WSLs compared to the 26% of the control teeth [90]. The high-risk lateral incisors exhibited the most benefit from the experimental elastomerics, and the overall severity of the lesions in the experimental group was decreased. Overall treatment times were similar between

the control and experimental groups despite the clinical impression of more rapid degradation of the fluoride-releasing elastomerics. To note, the study did report increased staining and swelling of the elastomerics as well as an increased difficulty of use for operators.

Fluoride Rinses, Gels, and Varnish

As discussed previously, fluoride can potentially supplement and improve oral hygiene to prevent white spot lesions. Many studies have investigated home care regimens utilizing various fluoridated mouth rinses and gels. One group of orthodontic patients utilizing a neutral 0.2% NaF oral rinse daily had a statistically significant reduction in lesion development: 39 ± 16 μm lesion depth with the rinse compared to 101 ± 26 μm without [77]. The study suggested a low-pH fluoride solution encourages calcium fluoride formation which reduces lesion development. One systematic review recommended a 0.05% NaF mouth rinse daily [29]. Other studies have shown that 0.5% stannous fluoride gel applied twice daily can also decrease enamel decalcification [91].

Fluoride varnish is another simple product commonly administered in the dental office that could combat WSLs. Srivastava suggested that topical fluorides, while not recommended for treating WSL, can be used to prevent formation [47]. In Restrepo's study comparing the capability of fluoride varnish and chlorhexidine gel to minimize white spot lesions, fluoride varnish demonstrated faster remineralization that was stable throughout 3 months [92]. Mehta et al. found only 3 of 63 teeth exhibited enamel demineralization during their 120-day study when utilizing the varnish Clinpro XT (3M, St. Paul, MN) during bonding [93]. The manufacturer has claimed that the varnish acts as an extended barrier and can remain for 6 months [94]. Ultimately, the option of fluoride varnish is so appealing because of its ease of application, contact duration, safety, and patient acceptance [92]. Azarpazhooh concluded that applying

fluoride varnish every six months for medium- and high-risks groups was the most cost-effective method for reducing decalcifications and caries [95].

There are some limitations with fluoride use. Lab studies performed by O'Reilly et al. determined that fluoride at 0.08 ppm was optimal to promote enamel remineralization [26]. Fluoride's protection is limited to approximately 30 μm of the outermost shell of enamel [96]; mean lesion depths can range from approximately 100-180 μm without fluoride use [77]. Despite the potential benefits, only slightly more than half of orthodontists prescribe fluoridated mouth rinse, and these regimens still rely on patient compliance [97].

Chlorhexidine

Studies have also investigated the use of other generic antimicrobial substances like chlorhexidine. Chlorhexidine has been cited as the most effective antimicrobial mouth rinse but at the long-term cost of staining teeth and affecting taste [85]. Restrepo et al. observed remineralization with two applications of chlorhexidine gel but attributed the improvement not because of the chlorhexidine's antibacterial property but rather its precipitation of phosphate salts [92].

Sealants

Although fluoride products and chlorhexidine can potentially protect teeth from WSLs, many of these options rely on the patients' compliance. Like fluoride varnish, sealants can be applied in the office throughout a patient's orthodontic treatment without relying on their compliance. Some studies have demonstrated success in preventing WSLs by applying sealant material to labial enamel, particularly gingival to brackets [47]. O'Reilly et al. found a slight but

significantly lower WSL incidence on sealed teeth (13.5%) compared to the unsealed teeth (17.7%) [54]. Interestingly, the WSLs that formed had no significant difference in severity [39]. Tufekci et al. evaluated the utilization of Opal Seal (Ultradent Products Inc., South Jordan, UT) as a protective sealant against WSLs. Opal Seal's higher filler content was marketed to have longer-lasting coverage than other sealant products. Tufekci's et al.'s study revealed no significant difference in incidence of WSLs between sealed and control teeth except within the first 90 days where sealed teeth had fewer WSLs. After 90 days, only an average of 50% of tooth surfaces had Opal Seal remaining [88]. Premaraj et al.'s study on Pro Seal (Reliance Orthodontic Products, Itasca, IL) and Opal Seal showed that surfaces coated with either sealant material showed less damage and higher mineral content after one week in an acidic environment [98]. Some researchers have shown that sealants suffer from effects of oxygen inhibition during polymerization and do not effectively seal the enamel surface [99]. Knosel et al. found more rapid sealant degradation in well-brushed posterior teeth but also suggested that plaque level was not correlated with deterioration of the sealant [100]. Therefore, the research suggests that sealants can be effective but need reapplication to provide protection throughout the full duration of treatment. Knosel et al.'s results indicate that reapplication would be necessary after 14 weeks of treatment; this equates to seven or more applications over a two-year treatment period.

Laser Therapy

A couple of studies have shown a reduction in enamel decalcification of orthodontically banded teeth with the prophylactic use of argon laser irradiation [101, 102]. Anderson et al. demonstrated that prophylactic laser therapy resulted in an over 90% reduction in lesion depth

and area after the five-week trial period [102]. However, the optimal energy emission, wavelength, and protocol for laser therapy have yet to be established.

Orthodontic Cement

Improving the bracket bonding agent or cement is another potential way to reduce WSLs without relying on patient compliance. Ultimately, a bonding agent needs adequate bond strength to withstand the forces of mastication. Reynolds proposed that bond strengths of 6-8 MPa are sufficient [41]. Additionally, an ideal cement would be easy to apply in little time and leave less residual resin when debonding [86]. Most strategies to improve cements involve introducing components to strengthen the material or provide remineralization capabilities.

Self-Repairing Cements

Generally, dental composites can potentially be improved by utilizing nanotechnology, incorporating antimicrobial substances, or developing self-repairing materials capable of regenerating [103]. For orthodontic cements, the esthetic benefits of nanotechnology and nanoparticle fillers is subdued by the presence of a metal bracket. However, self-repairing materials could potentially be beneficial and are fabricated by incorporating reactive species or encapsulated healing substances into the matrix [104]. Huyang et al. developed a biocompatible, self-healing composite containing a healing liquid and healing powder in the composite matrix [105]. Cracks in the material trigger release of the healing liquid which subsequently reacts with the healing powder distributed throughout the matrix. This results in the formation of a reparative glass ionomer cement. The self-healing composite had similar fracture toughness and elastic modulus compared to the control but also demonstrated a 25% healing capacity of

fracture toughness after insult [105]. A self-repairing orthodontic cement could potentially reduce material fatigue and ultimately the number of debonded brackets. More relevantly, an ideal self-repairing cement would have reduced material creep and marginal degradation, resulting in less areas for bacterial accumulation. However, it is unclear if this would provide a clinically significant benefit because orthodontic brackets still provide many plaque retentive surfaces.

Antibacterial Cements

Another strategy to improve orthodontic bonding materials is to incorporate antibacterial components. Adding antimicrobial properties via zinc, silver, or antibiotics could beneficially reduce bacterial accumulation, demineralization, and caries [103]. Zhang et al. developed a resin-modified glass ionomer orthodontic cement modified with silver nanoparticles. Their initial results demonstrated higher protein-repellant capabilities compared to the control without sacrificing bond strength [106]. One of their separate but related studies suggested that the silver nanoparticles also maintained release for at least a year. Zhang et al. have also shown a potent antibacterial effect when incorporating dimethylaminododecyl methacrylate in dental adhesives [107]. Similarly, Melo et al. integrated an ammonium-based monomer capable of covalently copolymerization into a commercial orthodontic cement without sacrificing bond strength [108]. The design utilized an antibacterial strategy that does not require the release of components and was shown to sustain its effect even after six months. These strategies are so appealing because it directly targets the original etiology, i.e. bacteria, of white spot lesion formation.

Remineralization Materials

The last and perhaps most common material strategy is to utilize bonding agents capable of remineralizing enamel. Often, these materials release calcium, phosphate, and/or fluoride ions to remineralize adjacent enamel. In particular, fluoride ion release is so appealing because of its ability to repel bacteria and remineralize enamel.

Fluoride-Releasing

Glass ionomer cements capable of releasing fluoride are perhaps one of the most commonly researched orthodontic cements. Eissaa et al. studied the fluoride-releasing Transbond Plus Color Change (3M, St. Paul, MN) adhesive and revealed the formation of calcium-fluoride globules on the enamel adjacent to the occlusal and proximal surfaces of brackets [97]. While these compounds reduced the progression of WSLs, they were unable to prevent lesions gingival to the brackets. Yap et al. showed potential for various glass ionomer materials to inhibit enamel demineralization after seven days [9]. Glass ionomer materials are also appealing because they're relatively moisture-tolerant; Coups-Smith et al. demonstrated that orthodontic brackets bonded with glass ionomer cements in a wet environment still exhibit clinically acceptable bond strengths [109].

Though several studies have demonstrated the remineralization potential of glass ionomer, the material has several weaknesses. Typically, studies show that glass ionomer bonding materials have low strength and low sustainability of fluoride release [106, 108, 109]. Shammaa et al. demonstrated that some glass ionomer cements have significantly lower bonding strength while others like Fuji Ortho LC (GC Corporation, Tokyo, Japan) may have enough bond strength to withstand forces of mastication [110]. Gaworski et al. found significantly higher bond

failures with brackets bonded with glass ionomer cements in vivo compared to conventional resins [111]. Gaworski also found similar levels of enamel decalcification between the glass ionomer and conventional resin bonding groups. Melo et al. attempted to augment the material's low strength by incorporating micro- and nano-particle fillers; however, they found that the resin-modified glass ionomers (RMGI) had substantially less fluoride release [10]. Another weakness of glass ionomers is their inadequate duration of fluoride release. Like many other studies, Chadwick and Gordon reported that 70% of the fluoride reservoir in orthodontic materials is expended within the first month [11]. Many glass ionomers are capable of fluoride re-uptake and re-release; however, Damen et al. demonstrated that the fluoride reabsorbed by the glass ionomers is almost completely released within the first day, limiting its usefulness [24]. Furthermore, the beneficial fluoride releasing capability of RMGI is somewhat diminished by the increased bacteria accumulation around the material's relatively rough surfaces [106]. According to Eissaa, glass ionomer's effective zone is limited and is only able to protect the enamel one millimeter around the bracket [97].

Some manufacturers have combined the concepts of sealants with fluoride-releasing capabilities. Premaraj et al. compared Pro Seal and Opal Seal in their ability to prevent WSLs [98]. Pro Seal exhibited significantly more fluoride release but also higher levels of *S. mutans* adherence. The fluoride release in both Pro Seal and Opal Seal was reduced to low levels after 21 days. Compared to glass ionomers, these sealants may have a longer-lasting effect of fluoride release. However, neither 24 hours nor 21 days of fluoride release are nearly sufficient enough for orthodontic treatment, and sealant degradation is still a significant concern as discussed previously.

Ca^{2+} and PO_4^{3-} Releasing

There are some concerns with Ca^{2+} & PO_4^{3-} releasing composites that echo those of glass ionomer, primarily the limited duration of ion release and inadequate bond strength. Previous Ca-PO_4 composites for restorative applications had about half of the flexural strength compared to unfilled composites [112]. Xu et al.'s study demonstrated that silanization of Ca-PO_4 fillers was unable to increase the composite's strength [112]. However, they used nanoparticle fillers to create a Ca^{2+} and PO_4^{3-} releasing resin that had flexural strength and elastic modulus similar to a control.

Miscellaneous

Several researchers designed a novel self-assembling peptide that could form a scaffold to mimic the characteristics of an enamel matrix [96]. Their peptide P_{11}-4 forms into a network in the presence of a carious lesion. The peptide network then triggers nucleation of new hydroxyapatite crystals and results in remineralization of the affected tooth structure. This potentially could have an application with white spot lesions as well. Like other antibacterial strategies, reducing the amount of bacterial accumulation would reduce the acidic assault that ultimately causes demineralization.

Bioactive Glass

Another potential remineralization additive for dental composites is bioactive glass (BAG). The biomimetic properties of bioactive glass enable it to form tooth-like hydroxyapatite when immersed in body fluids [113]. Brown et al.'s results demonstrated that the BAG orthodontic resins released significant amounts of calcium and phosphate. Furthermore, the BAG composites raised the pH in an acidic environment to a neutral pH which persisted for 100 hours.

Davis et al. found BAG composites to exhibit capabilities of calcium and fluoride recharge and re-release [114]. However, the release rapidly decreased within 22 hours.

Unfortunately, the overall quality and quantity of reliable evidence investigating remineralizing agents are lacking [115]. More and higher quality studies with comparable protocols using defined detection methods must be performed to fully evaluate options for white spot lesion resistance. There is a lot of potential for materials with remineralizing capabilities – the right formulation with sustainable ion release and adequate mechanical properties could significantly reduce white spot lesions.

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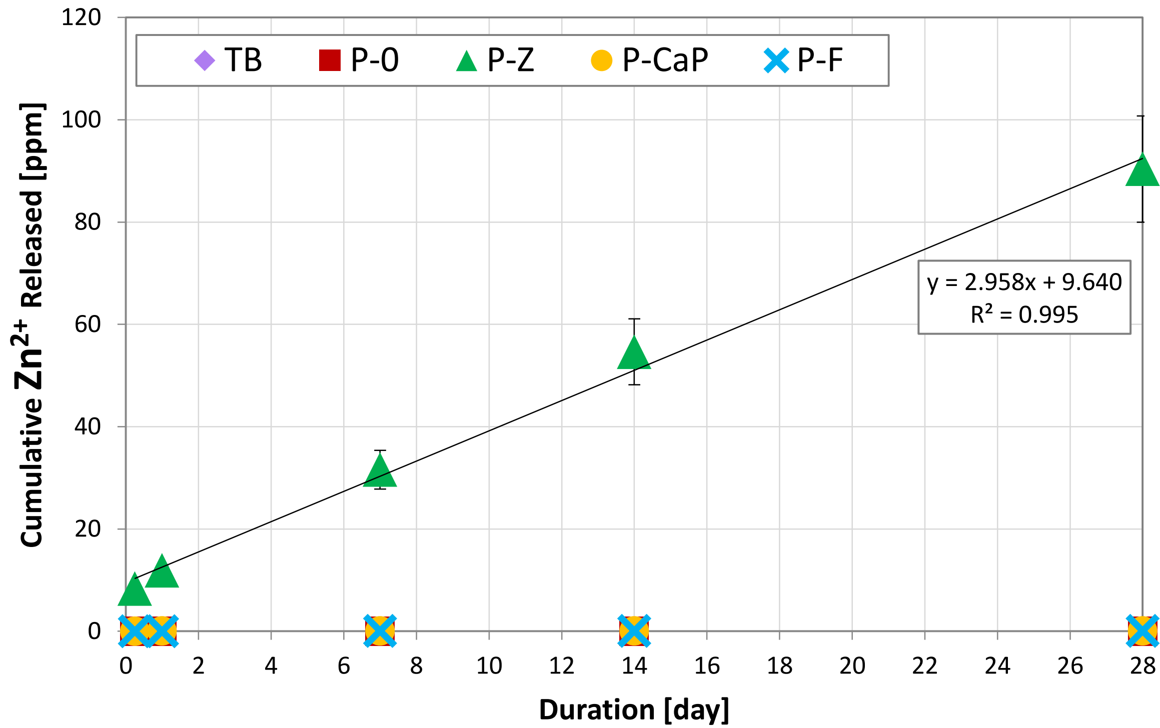

Figure 1. Cumulative Zinc Release

Cumulative zinc released from the experimental cements and commercial control over 28 days in a pH 5.15 aqueous solution. Each value is mean ± SD; n = 5. The P-Z formulation exhibited significant zinc release that was sustained linearly throughout the duration ($p < 0.05$). The remaining composites exhibited negligible zinc release as expected.

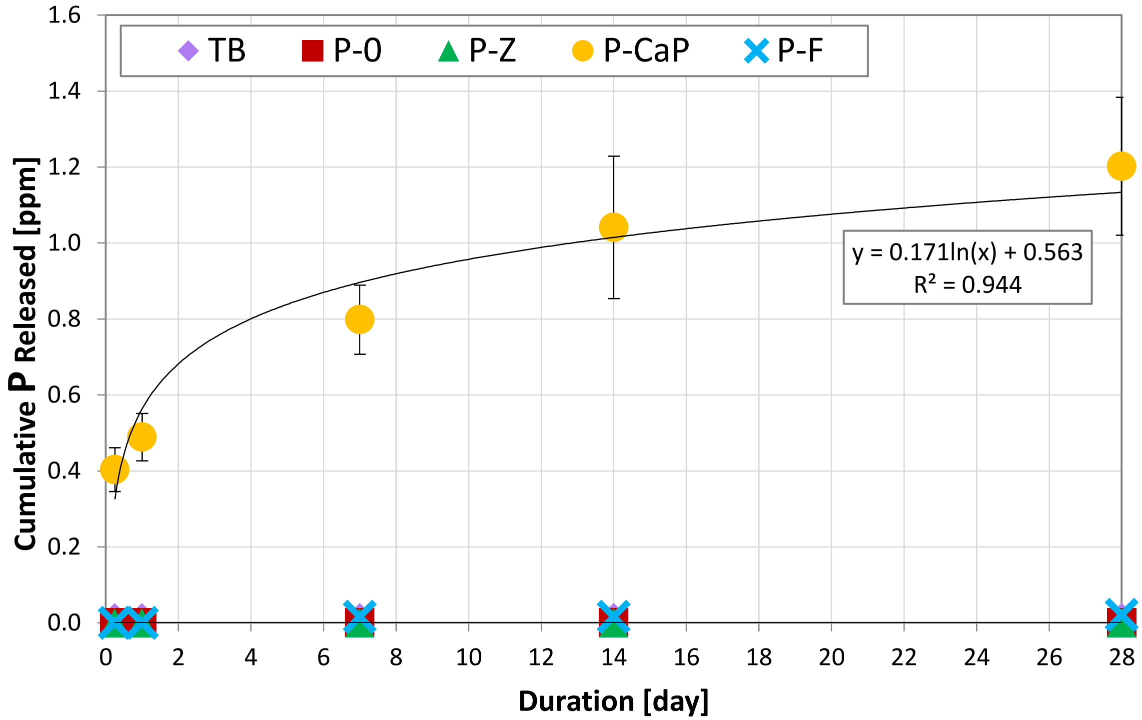

Figure 2. Cumulative Phosphorous Release

Cumulative phosphorous released from the experimental cements and commercial control over 28 days in a pH 5.15 aqueous solution. Each value is mean ± SD; n = 5. Only the P-CaP at 2% PO_4^{3-} released significant levels of phosphorous compared to the control and the other experimental cements ($p < 0.05$).

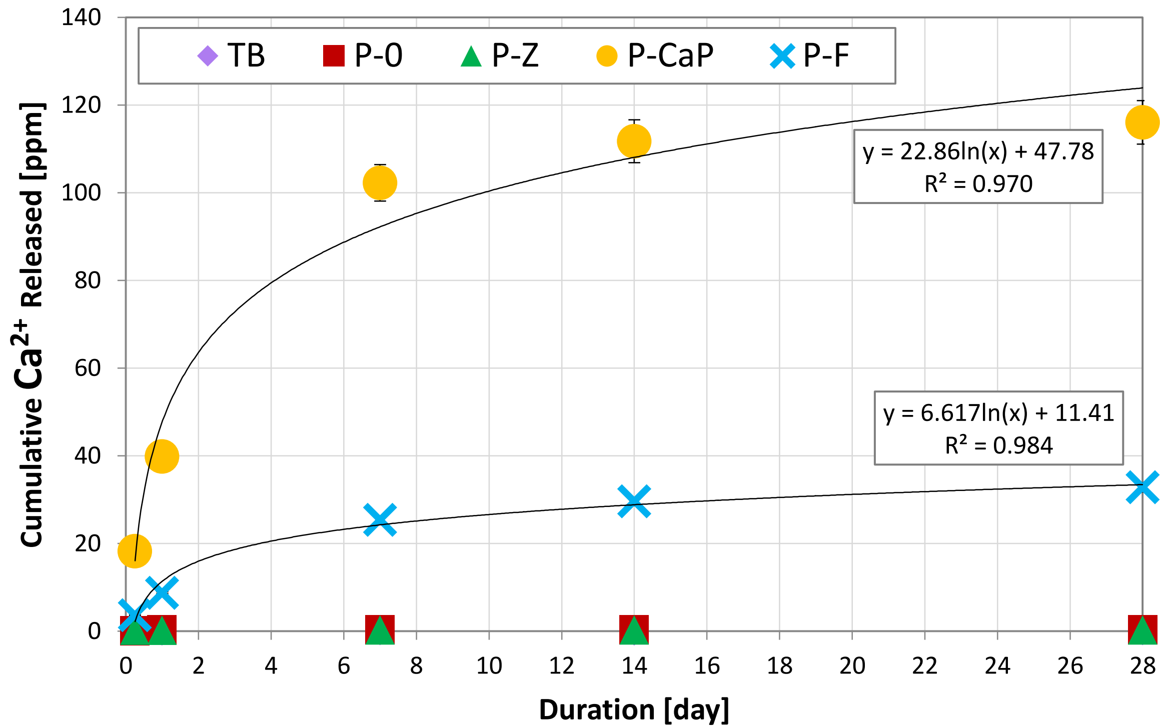

Figure 3. Cumulative Calcium Release

Cumulative calcium released from the experimental cements and commercial control over 28 days in a pH 5.15 aqueous solution. Each value is mean ± SD; n = 5. Both P-CaP (5% Ca^{2+}) and P-F (2% Ca^{2+}) exhibited significant levels of calcium release compared to the control, P-0) and P-Z ($p < 0.05$). P-CaP released significantly more calcium than P-F ($p < 0.05$).

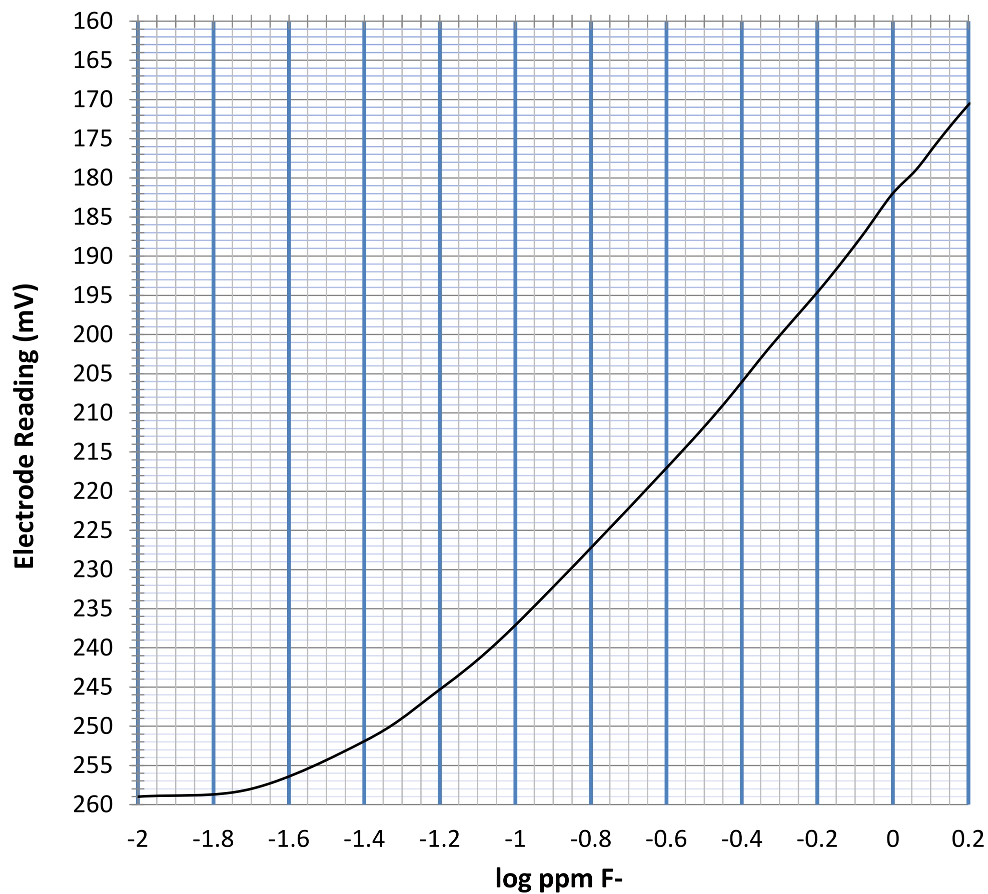

Figure 4. Fluoride Calibration Curve

Fluoride calibration curve created with a fluoride-specific electrode and incremental mixtures of TISAB and 10 ppm F^-.

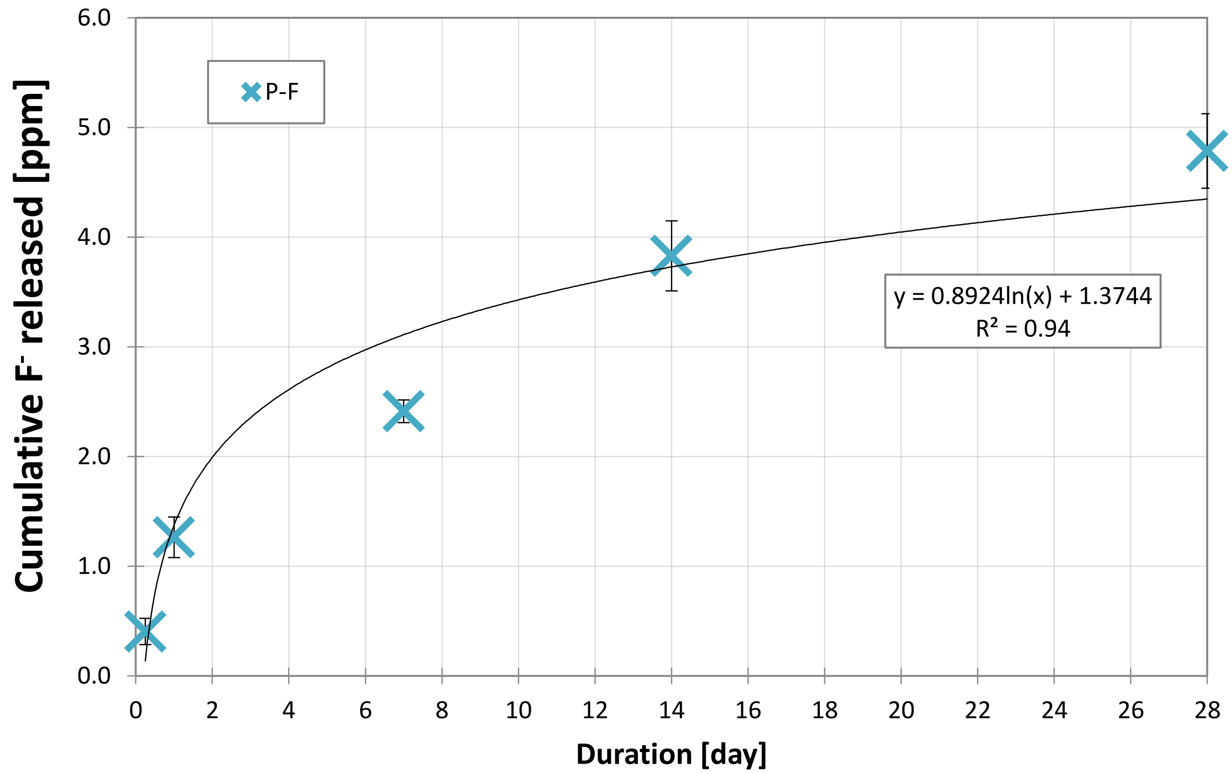

Figure 5. Cumulative Fluoride Release

Cumulative fluoride released from P-F over 28 days in a pH 5.15 aqueous solution. Each value is mean ± SD; n = 5. Fluoride release was sustained in a logarithmic fashion over 28 days.

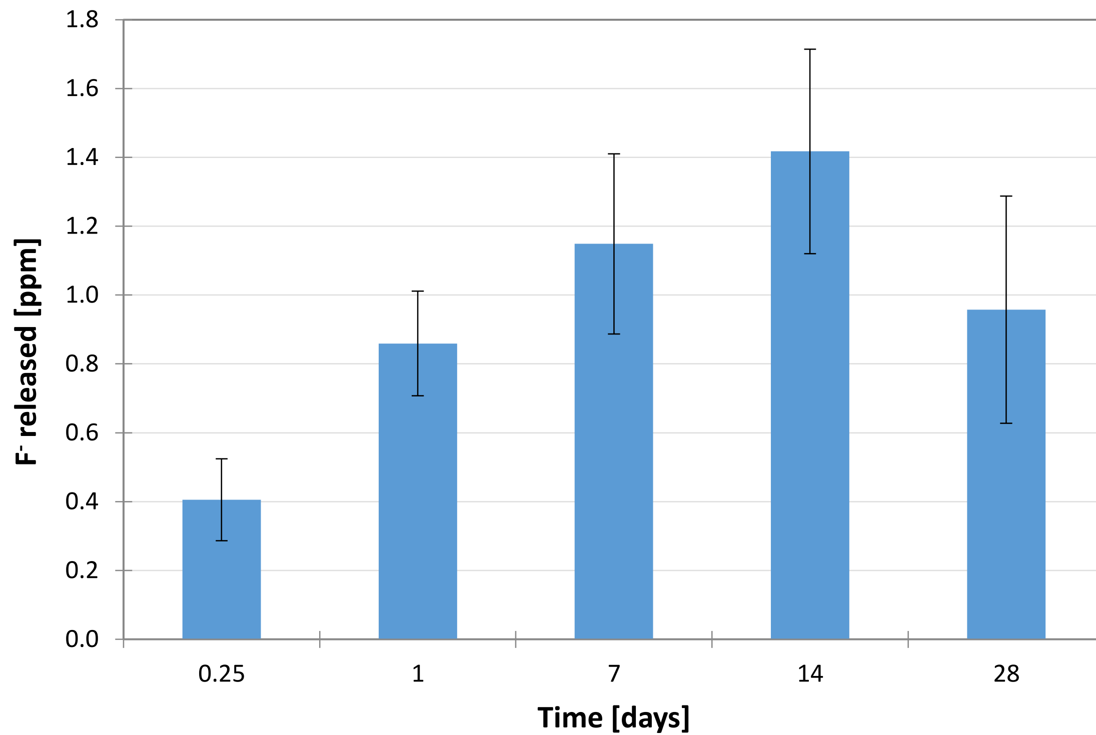

Figure 6. Fluoride Release of P-F at each Time

Fluoride released from P-F at each time point in a pH 5.15 solution. Each value is mean ± SD; n = 5.

Table 1. Ion Precipitate Investigation

Evaluation of the vials containing each cement formulation for ion precipitants. All of the values were within reason and attributed as background data when compared to the empty vial control. While there was some slight amount of Zn in the P-Z container, it was negligible compared to the detected levels (< 1%).

Sample Name	Ca [mg/l]	Mn [ug/l]	Fe [ug/l]	Cu [ug/l]	Zn [ug/l]
Control	<0.024	<0.100	<0.311	1.930	1.903
P-0	<0.024	0.163	0.492	5.293	2.439
P-Z	<0.024	<0.100	1.261	0.379	23.157
P-CaP	<0.024	<0.100	<0.311	0.075	1.934
P-F	<0.024	<0.100	<0.311	0.079	1.852

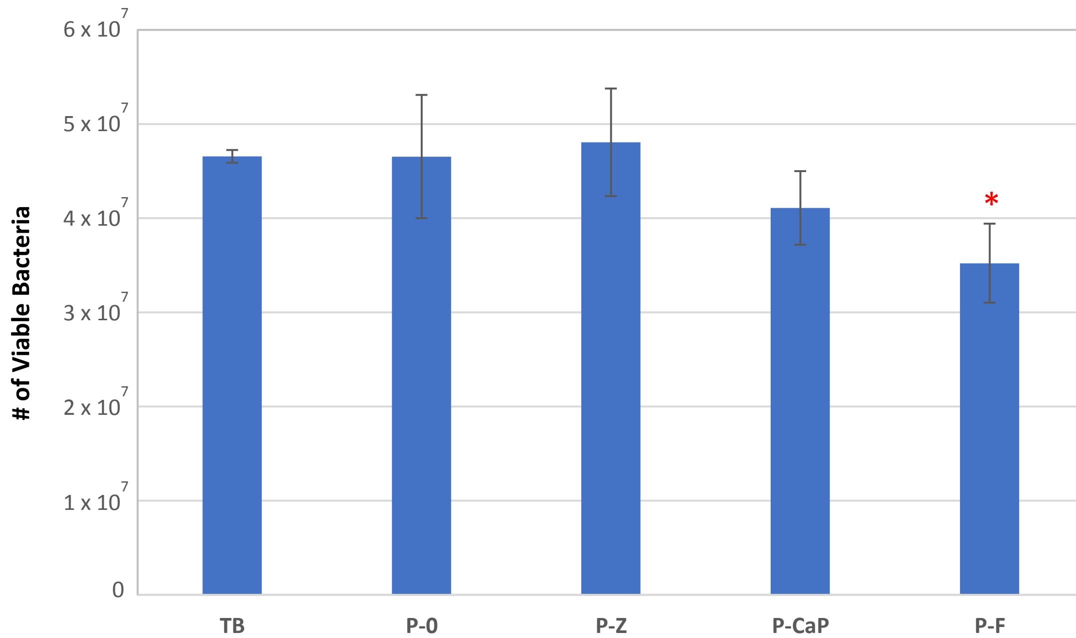

Figure 7. Biofilm Quantification

Number of viable bacteria quantified by the luciferase assay after one day of growth on a composite disc. Each value is mean ± SD; n = 3-7. The asterisk denotes significant difference between P-F and the other four cements ($p < 0.05$).

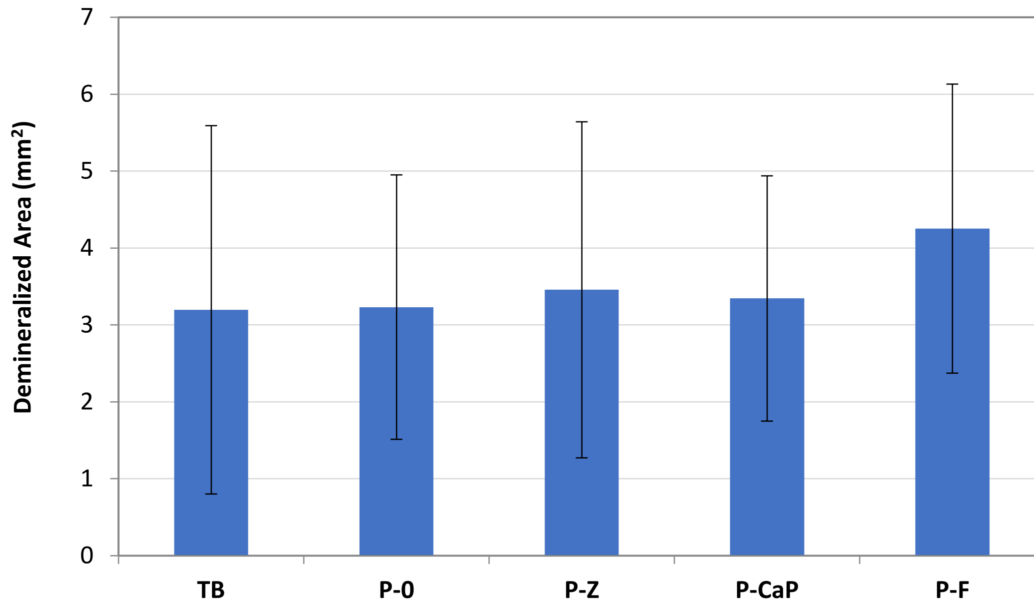

Figure 8. Area of Demineralization

Induced area of demineralization (mm^2) in the exposed enamel section gingival to orthodontic brackets bonded with an experimental cement. Each value is mean ± SD (n = 6). There were no stastitically significant differences noted in the size of demineralization ($p > 0.89$).

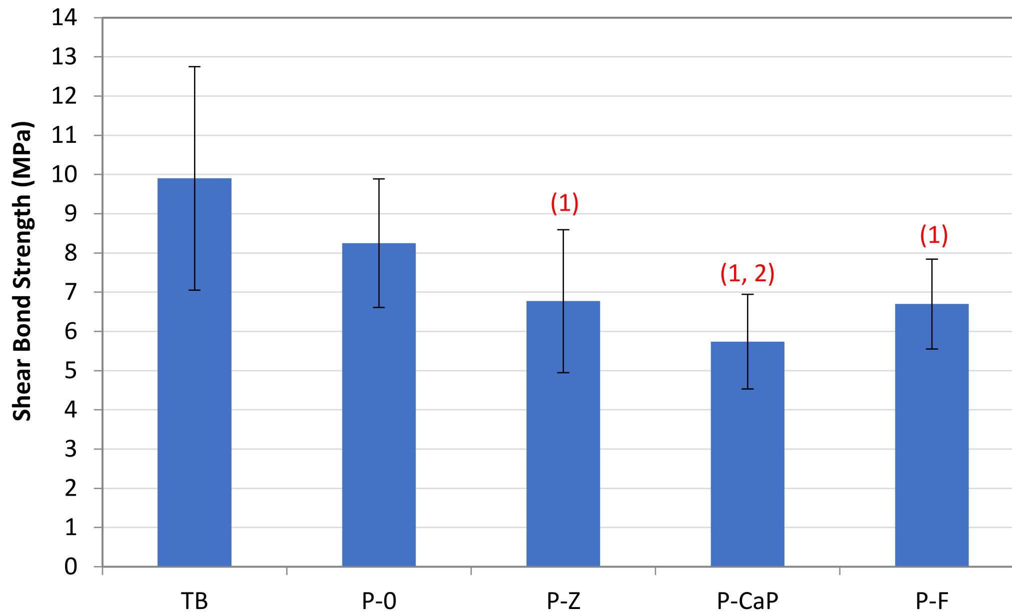

Figure 9. Shear Bond Strength

Shear bond strength of each cement. Each value is mean ± SD (n = 8-10). Numbers denote significantce compared to TB (1) and P0 (2). The microencapsulated formulations, P-Z, P-CaP, and P-F had significantly lower bond strength compared to TB. P-CaP had significantly lower bond strength than P-0.

Table 2. ARI Scores

ARI scores for each cement; n = 8-10.

Cement	ARI Score			
	0	**1**	**2**	**3**
TB	0	2	6	0
P-0	0	6	3	1
P-Z	0	4	5	1
P-CaP	0	4	5	0
P-F	0	3	7	0

Appendix

Table 3. Quantity of Zinc Released by each Composite as Measured by ICP-MS

Zn^{2+} Sample [ppb]	Duration [days]				
	0.25	1	7	14	28
TB 1	6.13	3.76	3.82	2.11	3.33
TB 2	5.11	2.53	2.08	1.67	2.3
TB 3	4.84	2.76	4.09	1.36	1.53
TB 4	5.27	2.23	2.15	5.09	2.5
TB 5	8.02	1.55	1.36	1.66	6.03
Average	5.87	2.57	2.70	2.38	3.14
SD	1.29	0.81	1.19	1.54	1.74
P-0 1	1.57	1.87	2.35	1.25	1.33
P-0 2	2.84	2.04	1.20	1.23	1.53
P-0 3	3.13	5.49	2.24	1.36	5.53
P-0 4	16.4	12.9	7.69	5.15	4.59
P-0 5	4.85	3.82	1.00	1.43	1.21
Average	5.76	5.22	2.90	2.08	2.84
SD	6.06	4.54	2.75	1.72	2.06
P-Z 1	7,477	3,312	18,712	23,609	31,109
P-Z 2	8,437	3,640	21,970	28,150	36,726
P-Z 3	8,528	3,253	19,118	22,425	49,488
P-Z 4	9,021	4,270	23,326	22,509	29,225
P-Z 5	8,150	3,217	15,621	18,474	32,152
Average	8323	3538	19749	23033	35740
SD	567	442	3010	3462	8166
P-CaP 1	33.3	11.6	47.9	6.55	6.65
P-CaP 2	23.6	8.98	8.86	6.25	4.54
P-CaP 3	33.3	7.13	7.39	2.71	4.96
P-CaP 4	12.3	20.3	2.24	1.75	1.34
P-CaP 5	30.6	6.58	8.02	4.38	4.64
Average	26.62	10.92	14.88	4.33	4.43
SD	8.93	5.60	18.64	2.12	1.92
P-F 1	11.9	4.54	8.62	3.79	3.89
P-F 2	9.8	8.53	10.33	4.69	6.76
P-F 3	10.5	7.97	6.8	8.29	5.48
P-F 4	13.1	29.8	6.32	6.41	8.98
P-F 5	9.59	10.06	5.02	4.47	5.39
Average	10.98	12.18	7.42	5.53	6.10
SD	1.49	10.05	2.08	1.82	1.90

ΣZn^{2+} Sample [ppb]	Duration [days]				
	0.25	1	7	14	28
TB 1	6.13	9.89	13.71	15.82	19.15
TB 2	5.11	7.64	9.72	11.39	13.69
TB 3	4.84	7.6	11.69	13.05	14.58
TB 4	5.27	7.5	9.65	14.74	17.24
TB 5	8.02	9.57	10.93	12.59	18.62
Average	5.87	8.44	11.14	13.52	16.66
SD	1.29	1.18	1.67	1.76	2.43
P-0 1	1.57	3.44	5.79	7.04	8.37
P-0 2	2.84	4.88	6.08	7.31	8.84
P-0 3	3.13	8.62	10.86	12.22	17.75
P-0 4	16.4	29.3	36.99	42.14	46.73
P-0 5	4.85	8.67	8.67	10.1	11.31
Average	5.76	10.98	13.68	15.76	18.60
SD	6.06	10.50	13.19	14.90	16.16
P-Z 1	7477	10789	29501	53110	84219
P-Z 2	8437	12077	34047	62197	98923
P-Z 3	8528	11781	30899	53324	102812
P-Z 4	9021	13291	36617	59126	88351
P-Z 5	8150	11367	26988	45462	77614
Average	8323	11861	31610	54644	90384
SD	567	934	3786	6433	10399
P-CaP 1	33.3	44.9	92.8	99.4	106
P-CaP 2	23.6	32.6	41.4	47.7	52.2
P-CaP 3	33.3	40.4	47.8	50.5	55.5
P-CaP 4	12.3	32.6	34.8	36.6	37.9
P-CaP 5	30.6	37.2	45.2	49.6	54.2
Average	26.62	37.54	52.42	56.75	61.17
SD	8.93	5.28	23.09	24.46	26.03
P-F 1	11.9	16.44	25.06	28.9	32.74
P-F 2	9.8	18.3	28.7	33.4	40.1
P-F 3	10.5	18.5	25.3	33.6	39.0
P-F 4	13.1	42.9	49.2	55.6	64.6
P-F 5	9.59	19.7	24.7	29.1	34.5
Average	10.98	23.16	30.58	36.11	42.21
SD	1.49	11.10	10.54	11.14	12.89

Note: Values of 1.00 were detected as <1.00 and considered 0

Table 4. Quantity of Phosphorous Released by each Composite as Measured by ICP-MS

P Sample [ppb]	Duration [days]				
	0.25	1	7	14	28
TB 1	39.0	24	24	24	24
TB 2	25.8	24	24	24	24
TB 3	24	24	24	24	24
TB 4	24	24	24	24	24
TB 5	24	24	24	24	24
Average	27.4	24.0	24.0	24.0	24.0
SD	6.6	0.0	0.0	0.0	0.0
P-0 1	24	24	24	24	24
P-0 2	24	24	24	24	24
P-0 3	24	24	24	24	24
P-0 4	24	24	24	24	24
P-0 5	24	24	24	24	24
Average	24	24	24	24	24
SD	0	0	0	0	0
P-Z 1	24	25	26	24	24
P-Z 2	24	24	24	24	24
P-Z 3	24	24	24	24	24
P-Z 4	24	24	24	24	24
P-Z 5	24	24	24	24	24
Average	24	24	24	24	24
SD	0	0	1	0	0
P-CaP 1	381	99	348	213	92
P-CaP 2	342	93	270	291	141
P-CaP 3	409	46	282	53	180
P-CaP 4	497	98	343	376	105
P-CaP 5	387	93	304	281	285
Average	403.2	85.8	309.4	242.8	160.6
SD	57.7	22.4	35.2	120.9	77.5
P-F 1	24	24	24	24	25.2
P-F 2	24	24	78	24	24
P-F 3	24	24	24	24	24
P-F 4	24	24	24	24	24
P-F 5	24	24	24	24	24
Average	24.0	24.0	34.8	24.0	24.2
SD	0.0	0.0	24.1	0.0	0.5

Σ P Sample [ppb]	Duration [days]				
	0.25	1	7	14	28
TB 1	39.0	39.0	39.0	39.0	39.0
TB 2	25.8	25.8	25.8	25.8	25.8
TB 3	0	0	0	0	0
TB 4	0	0	0	0	0
TB 5	0	0	0	0	0
Average	13.0	13.0	13.0	13.0	13.0
SD	18.3	18.3	18.3	18.3	18.3
P-0 1	0	0	0	0	0
P-0 2	0	0	0	0	0
P-0 3	0	0	0	0	0
P-0 4	0	0	0	0	0
P-0 5	0	0	0	0	0
Average	0	0	0	0	0
SD	0	0	0	0	0
P-Z 1	0	0	0	0	0
P-Z 2	0	0	0	0	0
P-Z 3	0	0	0	0	0
P-Z 4	0	0	0	0	0
P-Z 5	0	0	0	0	0
Average	0	0	0	0	0
SD	0	0	0	0	0
P-CaP 1	381	480	828	1041	1133
P-CaP 2	342	435	705	996	1137
P-CaP 3	409	455	737	790	970
P-CaP 4	497	595	938	1314	1419
P-CaP 5	387	480	784	1065	1350
Average	403.2	489.0	798.4	1041.2	1201.8
SD	57.7	62.2	90.9	187.2	181.5
P-F 1	0	0	0	0	25.2
P-F 2	0	0	78	78	78
P-F 3	0	0	0	0	0
P-F 4	0	0	0	0	0
P-F 5	0	0	0	0	0
Average	3.7	8.8	25.4	29.6	32.8
SD	0.0	0.0	34.9	34.9	33.9

Note: Values of 24 were detected as <24 and considered 0

Table 5. Quantity of Calcium Released by each Composite as Measured by ICP-MS

Ca^{2+} Sample [ppm]	Duration [days]				
	0.25	1	7	14	28
TB 1	0.175	0.113	0.113	0.113	0.113
TB 2	0.113	0.113	0.137	0.113	0.113
TB 3	0.148	0.113	0.113	0.113	0.113
TB 4	0.113	0.113	0.113	0.113	0.113
TB 5	0.199	0.113	0.113	0.113	0.174
Average	0.150	0.113	0.118	0.113	0.125
SD	0.038	0.000	0.011	0.000	0.027
P-0 1	0.113	0.113	0.113	0.113	0.270
P-0 2	0.113	0.113	0.113	0.113	0.113
P-0 3	0.485	0.119	0.113	0.113	0.113
P-0 4	0.113	0.133	0.113	0.113	0.113
P-0 5	0.200	0.318	0.113	0.160	0.113
Average	0.205	0.159	0.113	0.122	0.144
SD	0.161	0.089	0.000	0.021	0.070
P-Z 1	0.425	0.113	0.113	0.113	0.132
P-Z 2	0.240	0.113	0.139	0.113	0.113
P-Z 3	0.158	0.113	0.156	0.113	0.113
P-Z 4	0.132	0.218	0.113	0.113	0.113
P-Z 5	0.113	0.113	0.113	0.113	0.113
Average	0.214	0.134	0.127	0.113	0.117
SD	0.128	0.047	0.020	0.000	0.008
P-CaP 1	18.8	23.0	58.8	8.7	4.0
P-CaP 2	15.3	22.1	67.6	10.9	4.6
P-CaP 3	19.2	21.5	62.1	8.9	4.2
P-CaP 4	20.9	21.0	65.0	10.0	4.4
P-CaP 5	16.9	20.5	58.7	8.9	4.4
Average	18.2	21.6	62.4	9.5	4.3
SD	2.2	1.0	3.9	0.9	0.2
P-F 1	3.7	4.9	15.4	4.0	3.2
P-F 2	3.6	5.1	17.2	4.3	3.6
P-F 3	3.5	5.1	17.3	4.0	2.7
P-F 4	3.8	5.3	16.9	4.4	3.3
P-F 5	3.7	5.2	16.1	4.6	3.2
Average	3.7	5.1	16.6	4.3	3.2
SD	0.1	0.1	0.8	0.3	0.3

ΣCa^{2+} Sample [ppb]	Duration [days]				
	0.25	1	7	14	28
TB 1	0.175	0.175	0.175	0.175	0.175
TB 2	0.000	0.000	0.137	0.000	0.000
TB 3	0.148	0.148	0.148	0.148	0.148
TB 4	0.000	0.000	0.000	0.000	0.000
TB 5	0.199	0.199	0.199	0.199	0.373
Average	0.104	0.104	0.132	0.104	0.139
SD	0.097	0.097	0.078	0.097	0.154
P-0 1	0.000	0.000	0.000	0.000	0.270
P-0 2	0.000	0.000	0.000	0.000	0.000
P-0 3	0.485	0.604	0.604	0.604	0.604
P-0 4	0.000	0.133	0.133	0.133	0.133
P-0 5	0.200	0.518	0.518	0.678	0.678
Average	0.137	0.251	0.251	0.283	0.337
SD	0.213	0.290	0.290	0.332	0.295
P-Z 1	0.425	0.425	0.425	0.425	0.425
P-Z 2	0.240	0.240	0.240	0.240	0.240
P-Z 3	0.158	0.158	0.158	0.158	0.158
P-Z 4	0.132	0.736	0.736	0.736	0.736
P-Z 5	0.000	0.000	0.000	0.000	0.000
Average	0.191	0.312	0.312	0.312	0.312
SD	0.157	0.282	0.282	0.282	0.282
P-CaP 1	18.8	41.8	100.6	109.3	113.3
P-CaP 2	15.3	37.4	105.0	115.9	120.5
P-CaP 3	19.2	40.7	102.8	111.7	115.9
P-CaP 4	20.9	41.9	106.9	116.9	121.3
P-CaP 5	16.9	37.4	96.1	105.0	109.4
Average	18.2	39.8	102.3	111.8	116.1
SD	2.2	2.3	4.2	4.9	5.0
P-F 1	3.7	8.6	24.0	28.0	31.2
P-F 2	3.6	8.7	25.9	30.2	33.8
P-F 3	3.5	8.6	25.9	29.9	32.6
P-F 4	3.8	9.1	26.0	30.4	33.7
P-F 5	3.7	8.9	25.0	29.6	32.8
Average	3.7	8.8	25.4	29.6	32.8
SD	0.11	0.22	0.86	0.95	1.05

Note: Values of 0.113 were detected as <0.113 and considered 0

Table 6. Quantity of Fluoride Released by P-F as Measured by Fluoride-Specific Electrode

F^- Sample [ppm]	Duration [days]				
	0.25	1	7	14	28
TB 1	0.23	1.01	1.29	1.32	0.74
TB 2	0.56	0.98	0.71	1.41	0.68
TB 3	0.43	0.91	1.11	1.91	0.74
TB 4	0.41	0.73	1.29	1.35	1.37
TB 5	0.40	0.67	1.35	1.10	1.26
Average	0.41	0.86	1.15	1.42	0.96
SD	0.12	0.15	0.26	0.30	0.33

ΣF^- Sample [ppb]	Duration [days]				
	0.25	1	7	14	28
TB 1	0.23	1.24	2.53	3.85	4.58
TB 2	0.56	1.54	2.25	3.66	4.34
TB 3	0.43	1.34	2.45	4.35	5.09
TB 4	0.41	1.14	2.43	3.78	5.15
TB 5	0.40	1.06	2.41	3.51	4.77
Average	0.41	1.26	2.41	3.83	4.79
SD	0.12	0.19	0.10	0.32	0.34

Table 7. Luciferase Values for each Composite

Sample	Luciferase Value				
	TB	**P-0**	**P-Z**	**P-CaP**	**P-F**
blank	*4.25E+03*	*1.76E+03*	*3.98E+03*	*8.60E+03*	*6.23E+03*
1	4.68E+07	5.39E+07	4.22E+07	3.85E+07	4.13E+07
2	4.58E+07	4.15E+07	5.22E+07	4.16E+07	3.47E+07
3	4.71E+07	5.02E+07	4.47E+07	3.77E+07	3.20E+07
4		4.06E+07	5.78E+07	4.41E+07	3.29E+07
5			5.05E+07	3.75E+07	
6			4.65E+07	4.71E+07	
7			4.26E+07		
Average	4.66E+07	4.66E+07	4.81E+07	4.11E+07	3.52E+07
SD	6.81E+05	6.54E+06	5.71E+06	3.91E+06	4.20E+06

Table 8. Demineralized Area of Teeth Orthodontically Bonded with the Cements

Sample	Demineralized Area (mm^2)				
	TB	P-0	P-Z	P-CaP	P-F
1	1.374	5.042	7.675	6.308	1.808
2	6.479	5.053	2.790	2.739	4.102
3	5.602	3.929	1.658	3.812	3.565
4	0.412	2.766	3.648	1.743	6.909
5	3.120	1.266	2.008	2.611	3.157
6	2.197	1.332	2.965	2.856	5.968
Average	3.197	3.231	3.544	3.588	4.252
SD	2.393	1.719	2.125	1.595	1.880

Table 9. Shear Bond Strengths for each Composite

Sample	Shear Bond Strength [MPa]				
	TB	P-0	P-Z	P-CaP	P-F
0	14.1	8.8	11.2	5.6	6.7
1	8.9	10.2	5.1	7.0	5.9
2	12.1	6.5	8.1	5.6	5.6
3	6.8	10.0	5.9	3.4	6.8
4	6.9	8.4	6.0	5.1	7.5
5	10.0	9.8	5.6	7.1	6.8
6	12.9	9.3	6.6	6.6	6.4
7	7.5	6.6	5.4	5.5	4.7
8		5.7	6.2		8.5
9		7.2	7.6		8.1
Average	9.9	8.3	6.8	5.7	6.7
SD	2.9	1.6	1.8	1.2	1.1

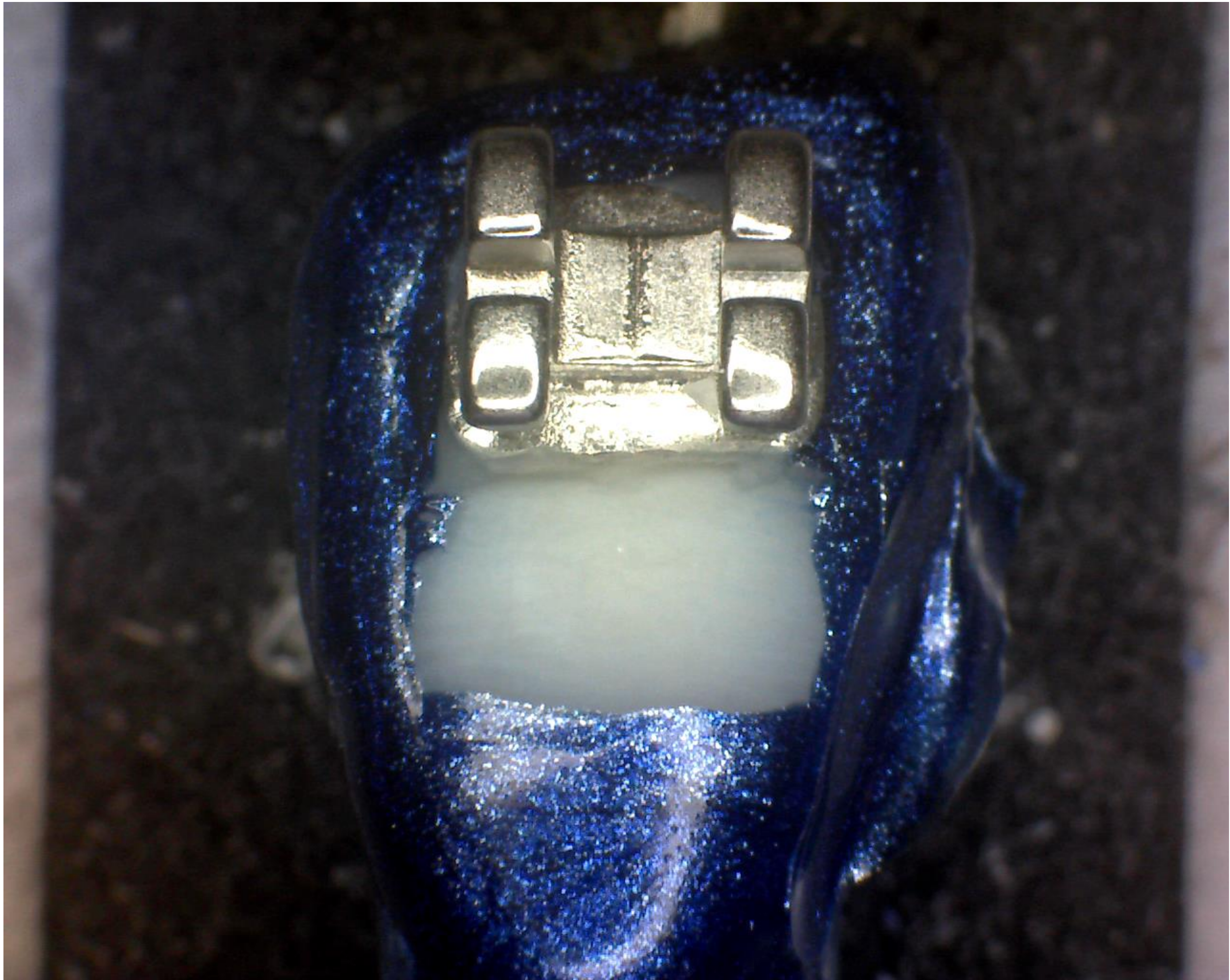

Figure 10. Example Tooth Pre-Demineralization

Figure 11. Example Tooth Post-Demineralization

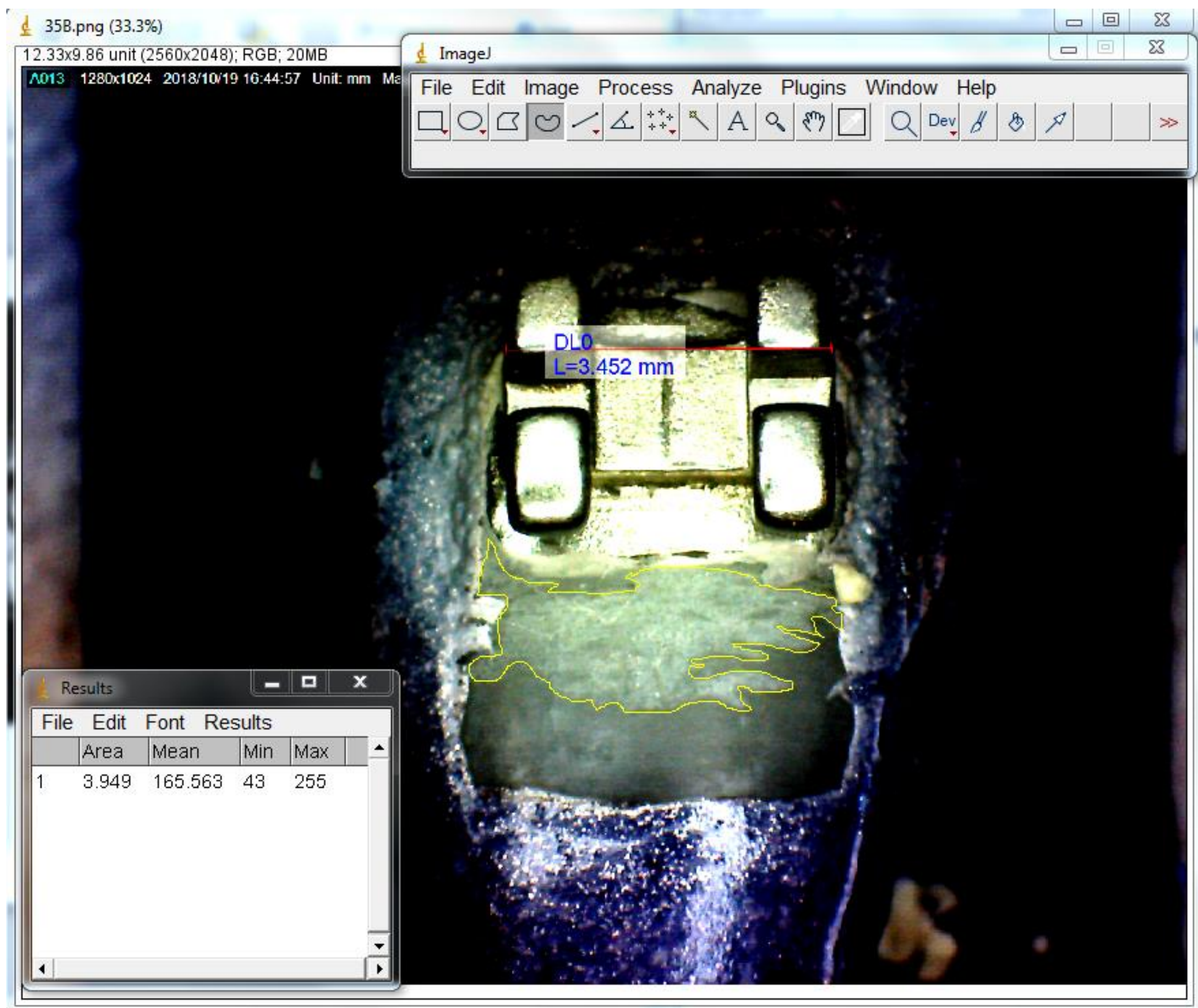

Figure 12. Example Demineralization Measurement

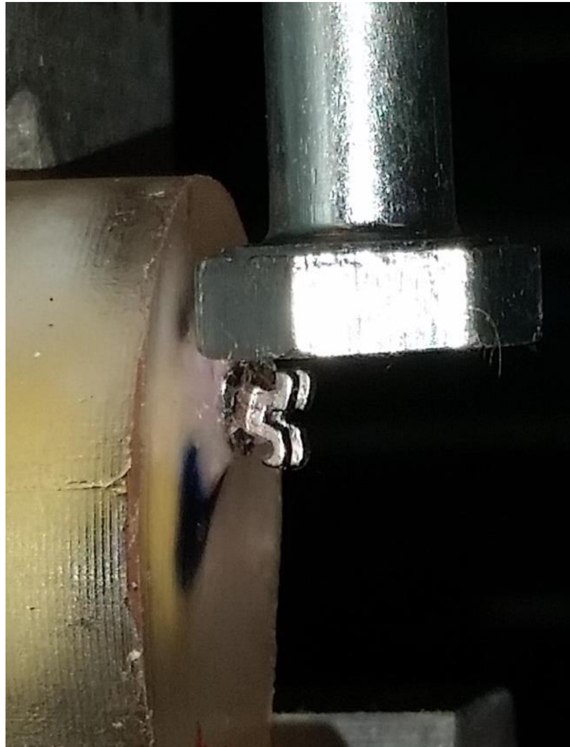

Figure 13. Hex Attachment for Debonding

The hex attachment was arranged to apply a shear force to the bracket pad.

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