ORIGINAL ARTICLE

# Prevention of demineralization around orthodontic brackets in vitro

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**Introduction:** The demineralization of enamel adjacent to orthodontic brackets is a significant clinical problem. The purpose of this in-vitro study was to investigate the effect of sodium fluoride (Colgate Neutrafluor 9000 ppm) (NaF) and 10% casein phosphopeptide-amorphous calcium phosphate (GC Tooth Mousse) (TM) on enamel demineralization adjacent to orthodontic brackets. **Methods:** Forty specimens were sectioned from the buccal or lingual surfaces of extracted sound third molars. Twenty specimens had molar tubes bonded with composite resin (Transbond XT, 3M, St Paul, Minn) (CR), and 20 were bonded with resin-modified glass ionomer cement (Fuji Ortho LC, GC America, Alsip, Ill) (RMGIC). A 2-mm window for enamel demineralization was prepared. The specimens were randomly divided into 4 treatment groups: control, TM, TM/NaF (50/50 w/w), and NaF. The treatment solutions were placed around the bracket margins, and the specimens were immersed inverted into a carbopol demineralization solution at 37°C. The enamel specimens were exposed for 96 hours, with the demineralization and topical solutions changed every 4 hours. Quantitative light-induced fluorescence (QLF) images were taken every 8 hours under controlled conditions. The difference in fluorescence (ΔF) and the proportional ΔF (%F) change between baseline and 96 hours was calculated. **Results:** RMGIC significantly reduced ΔF and %F when compared with CR (ANOVA, $P = .029$ and $P = .034$, respectively). Application of TM with CR, NaF with CR, and TM/NaF with CR significantly reduced ΔF and %F compared with the control CR (Tukey post-hoc test, $P < .001$). Application of TM/NaF with RMGIC significantly reduced ΔF and %F compared with the control RMGIC (Tukey post-hoc test, $P = .008$, $P = .019$, respectively). **Conclusions :** With the limitations of any in-vitro study, the following clinical conclusions can be drawn. The use of RMGIC alone can significantly decrease enamel demineralization compared with CR. The application of TM/NaF can provide significant additional prevention of enamel demineralization when RMGIC is used for bonding. The application of TM, NaF, or TM/NaF can significantly prevent enamel demineralization when CR is used for bonding. The use of both agents should be recommended for any at-risk orthodontic patient to provide preventive actions and potentially remineralize early (subclinical) enamel demineralization. (Am J Orthod Dentofacial Orthop 2007; 131:705,e1-705.e9)

The demineralization of enamel adjacent to orthodontic brackets is a considerable clinical problem, with reports of significant increase in the prevalence and severity of enamel demineralization after orthodontic treatment when compared with untreated control subjects. The prevalence of white spot lesions in orthodontic patients has been reported between 2% and 96%.[1-4] The increased prevalence of enamel decalcification during fixed appliance therapy is partly due to the irregular surfaces of brackets, bands, wires, and other attachments, which create stagnation areas for plaque, render tooth cleaning more difficult, and limit naturally occurring self-cleansing mechanisms, such as the movement of the oral musculature and saliva.[5] These in turn encourage lower plaque pH in the presence of fermentable carbohydrates, accelerate the rate of plaque accumulation and plaque maturation, and favor colonization of aciduric bacteria such as *Streptococcus mutans* and *lactobacilli*.[1-5] Early enamel caries appear as white spot lesions that develop as a result of a dietary carbohydrate and saliva-modified bacterial infection.[6-8] After active orthodontic treatment, the demineralization process normally decelerates due to alterations in these local environmental factors. Some white spot lesions might remineralize and return to normal or at least to a visually acceptable appearance. However, white spot lesions can also persist, resulting in an esthetically unaccept-

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able result. In severe cases, restorative treatment might be required.

Overall management of white spot lesions involves methods of both preventing demineralization and encouraging remineralization of existing lesions. Preventive measures take precedence, due to the challenges of treating patients who develop many white spot lesions. Successful preventive strategies include patient education, oral health promotion, regular professional oral hygiene visits, and appropriate patient compliance. For noncompliant patients, appropriate preventive medicaments, such as topical fluorides, can aid in reducing the demineralization of enamel surrounding orthodontic brackets.[9-11]

The scientific cornerstone for the use of fluoride in caries prevention has been based on the fact that fluoride can be incorporated into the crystalline lattice of the mineral of dental hard tissues, resulting in a mineral phase that is less soluble and more acid resistant.[12] Fluoride ions can be incorporated into the hydroxyapatite structure of tooth enamel by the replacement of hydroxy groups or by the redeposition of dissolved hydroxyapatite as less soluble fluoridated species.[13] Calcium fluoride is the major reaction product of topical fluoride treatment of enamel and has been found to be a major factor of the cariostatic mechanism of fluoride.[3] Any formed calcium fluoride can persist in dental plaque on the enamel surface for several weeks after a topical application, and it can be incorporated into the crystal lattice as fluorapatite. Studies have shown that fluorapatite formation from either a monthly high dose of fluoride (eg, with replacement of fluoride-containing elastomeric ligature ties) or continually available low doses of fluoride (eg, from fluoride-containing orthodontic adhesive cements or fluoride-containing elastomeric ligature ties) can be advantageous in reducing enamel decalcification during fixed appliance therapy.[14-16]

More recently, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been reported to have topical anticariogenic effects because of its ability to stabilize calcium and phosphate in an amorphous state, preventing the growth of calcium phosphate to the critical size required for precipitation.[17-20] The role of CPP-ACP in decreasing the incidence of dental caries in the community is expected to supplement the beneficial effects of topical fluoride.[17] The proposed anticariogenic mechanism of CPP-ACP involves the incorporation of nano-complexes into dental plaque and onto the tooth surface, thereby acting as a calcium and phosphate reservoir. A study showed that CPP-ACP incorporated into dental plaque can significantly increase the levels of plaque calcium and phosphate ions.[21] This mechanism is ideal for the prevention of enamel demineralization because there appears to be an inverse association between plaque calcium and phosphate levels and measured caries experience.[21] The localized CPP-ACP nano-complexes subsequently act to buffer free calcium and phosphate ions in the plaque fluid, to maintain a state of supersaturation of ACP with respect to enamel mineral, thereby limiting enamel demineralization and enhancing remineralization.[19,21] In addition, immunolocalization studies showed that CPP-ACP can be incorporated into supragingival dental plaque by binding to the surfaces of bacterial cells, to components of the intercellular plaque matrix, and to adsorbed macromolecules on tooth surfaces. These interactions can cause the formation of a less-cariogenic plaque.[22] Studies of the effects of CPP-ACP have so far shown promising dose-related increases in enamel remineralization in already demineralized enamel lesions.[19-21,23-25] The ability of CPP-ACP to prevent white spot lesion formation around orthodontic brackets has not, as yet, been reported.

Several methods of white spot quantification can be used in a clinical setting. The ideal method of assessment should be simple, noninvasive, reproducible, and precise. The great interest in optical methods of detection is driven by the need for early detection of carious lesions, to quantify changes in mineral loss in vivo for clinical practice and to allow more accurate, clinically based investigations that can be accomplished in shorter times.[26,27] Available optical methods include quantitative laser or light-induced fluorescence (QLF), electrical caries monitor, and digital imaging fiber-optic transillumination. The principle behind QLF is the observation of autofluorescence from a tooth.[28] When enamel demineralization takes place, minerals are replaced mainly by water from saliva, causing a decrease in the light path in the tooth substance. This results in less light absorption by enamel. Because fluorescence is a result of light absorption, the intensity of fluorescence decreases in demineralized regions of the enamel, which appear darker than sound tooth structures.[29-31]

The use of QLF allows for quantitative analysis ($\Delta F$) by comparative measurement of fluorescence radiance scattering from sound enamel with the fluorescence radiance scattered by demineralized enamel, which has been reported to be well correlated (0.73-0.83) with the degree of mineral loss from early enamel lesions in vitro when measured by longitudinal microradiography.[32-35] Light-induced fluorescence can be used for early detection and quantification of carious lesions. The device has been tested in clinical studies, with several studies validating it.[36-38] The use of QLF

has enabled the detection of demineralization before the affected areas are visible to a trained examiner.[39] Theoretically, these findings allow for early (subclinical) caries detection and subsequent preventive and remineralizing therapies to minimize esthetic damage and prevent the need for future restorative intervention.

The purpose of this study was to investigate the effect of sodium fluoride (Colgate Neutrafluor 9000 ppm, Colgate Palmolive Pty Ltd, Sydney, Australia) (NaF) and 10% casein phosphopeptide-amorphous calcium phosphate (GC Tooth Mousse, GC Corp, Tokyo, Japan) (TM) on enamel demineralization adjacent to orthodontic brackets in vitro.

## MATERIAL AND METHODS

Ethics approval (HREC Project No 050376) was obtained from the Human Research Ethics Committee of the University of Melbourne before this research project.

Twenty-five previously collected third molars from a fluoridated community were stored in 10% neutral buffered formalin solution for at least 2 weeks. All teeth were rinsed and stored moist in double deionized water (MilliQ water, Millipore Corp, Billerica, Mass) before experimental use. The outer enamel surface was polished wet to a mirror finish by using Soflex discs (3M, St Paul, Minn) on a slow-speed contra-angle dental handpiece. The roots were removed with a water-cooled diamond blade saw (Minitom, Streurs, Copenhagen, Denmark). The coronal pulp chambers were extirpated with a slow speed bur in a contra-angle handpiece. Buccal and lingual enamel specimens were sectioned from the coronal portion of the tooth with the water-cooled diamond blade saw. From these specimens, 40 enamel specimens, free of caries and fluorotic or hypomineralized lesions and other visible enamel defects or extraction damage, were selected (Fig 1).

The cut surfaces of the 40 specimens were painted with transparent, acid-resistant nail varnish (Revlon, New York, NY) and allowed to set overnight. The specimens were then mounted on sticky wax sticks and secured to the inside of plastic lids of 15-mL specimen jars. Molar tubes (Victory Series, 3M Unitek, Monrovia, Calif) were bonded to the enamel specimens according to the manufacturer's instructions. The specimens were allocated randomly into 2 groups. Half of the specimens (20) were treated with GC Ortho conditioner (GC America, Alsip, Ill) before adhesion of the molar tubes with resin-modified glass ionomer cement (RMGIC) (Fuji Ortho LC, GC America). The remaining 20 specimens were treated with Transbond Plus Self Etching Primer (3M Unitek) before adhesion of the molar tubes with a light-cured resin composite (RC) cement (Transbond XT, 3M Unitek). Any visible excess cement was removed before light curing with an Ortholux LED curing light (3M Unitek, Monrovia, Calif), which emits light of 430 to 480 nm at an intensity of 1000 mW/cm$^2$. Acid-resistant clear nail varnish was applied to the buccal surface of each specimen, leaving exposed enamel of approximately 2 mm surrounding the orthodontic molar tube. The specimens were randomly assigned to 1 of 8 groups (5 specimens per group) (Table I).

**Fig 1.** Prepared enamel specimen mounted on sticky wax stick and bonded with molar tube.

Subsurface lesions were created in the enamel windows according to the method of White.[40] The demineralization solution contained 20 g per liter of carbopol 907 (carboxypolymethylene, BF Goodrich, Cleveland, Ohio), 500 mg per liter of hydroxyapatite (Bio-Gel, HTP, Bio-Rad Laboratories, Richmond, Calif), and 0.1 mol per liter of lactic acid (Ajax Chemicals, Auburn, New South Wales, Australia). The solution was adjusted to a pH of 4.8. All specimens were immersed in 10 mL of the solution for 96 hours at 37°C, with the solution changed every 4 hours. The immersion of untreated enamel specimens in the demineralization solution for 96 hours was expected to result in the creation of 100-μm deep enamel lesions and represents approximately 3 months of real time. The 4-hourly application of topical medicaments therefore corresponds to approximately 2 weekly applications. Enamel slabs from groups 1 and 2 (control groups)

**Table I.** Allocation of 40 enamel specimens into 8 treatment groups

| *Group* | *n* | *Cement type* | *Topical medicament* |
|---|---|---|---|
| 1 | 5 | CR* | Control |
| 2 | 5 | RMGIC† | Control |
| 3 | 5 | CR | CPP-ACP (TM with Recaldent) |
| 4 | 5 | RMGIC | CPP-ACP (TM with Recaldent) |
| 5 | 5 | CR | NaF 9000 ppm gel |
| 6 | 5 | RMGIC | NaF 9000 ppm gel |
| 7 | 5 | CR | CPP-ACP (50% TM with Recaldent) and 9000 ppm NaF gel |
| 8 | 5 | RMGIC | CPP-ACP (50% TM with Recaldent) and 9000 ppm NaF gel |

*Composite resin (Transbond XT, 3M Unitek, Monrovia, Calif).
†Resin-modified glass ionomer cement (Fuji Ortho LC, GC America, Alsip, Ill).

were immersed in the demineralization solution without exposure to any topical solution. Enamel slabs from groups 3 to 8 were treated with a 0.5-mL smear of the predetermined topical solution before immersion in the demineralization solution to simulate the application of these topical solutions in a clinical setting. At 4-hourly intervals, all enamel specimens were rinsed with Milli-Q water, with topical solutions reapplied to the specimens in groups 3 to 8 and the demineralization solution changed.

At 8-hourly intervals, all enamel specimens were rinsed with Milli-Q water, blotted with paper tissues, and air dried for approximately 2 minutes before image collection with QLF. The QLF device used in this study consisted of a camera connected to a computer with image-capturing software (Inspektor Research System BV, Amsterdam, The Netherlands), with noncoherent light from a xenon arc lamp with a blue filter ($\lambda = 488$ nm, 10-20 mW $cm^{-2}$). A QLF camera platform and video-repositioning software (Inspektor Research System BV) were used to ensure that images of tooth surfaces were captured with the same camera position and from the same angle (Fig 2). The video-repositioning technique displayed baseline and live images simultaneously and computed their correlation based on similar geometry of the enamel lesion and the edge of the bracket base. The imaging correlation software was set to automatically capture images when the correlation was higher than 0.90 (Fig 3). The ambient light was controlled to less than 88 lux.

The images were stored in a computer, with 2 copies of the data copied onto DVDs and an external hard drive. The data were analyzed by using custom-made software (QLF 2.0.36, Inspektor Research System BV). Mean fluorescence loss ($\Delta F$) of the lesions was calculated by using the QLF software, excluding the orthodontic bracket from the analysis window. The $\Delta F$ values were exported to an Excel (Microsoft, Redmond, Wash) worksheet for statistical analysis and checked for accurate transfer.

**Fig 2.** QLF apparatus.

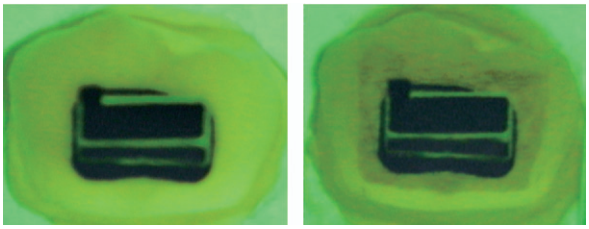

**Fig 3.** QLF images: **A**, at baseline (0 hours) and **B,** at end of experiment (96 hours).

### Statistical analysis

Descriptive statistics including mean, standard deviation, and minimum and maximum values were calculated for each test group with software (version 13.0, SPSS, Chicago, Ill). Significance for all statistical tests was predetermined at $P < .05$. For QLF analysis,

**Table II**. Mean fluorescence loss (ΔF) and proportional fluorescence (%F) loss at baseline (T0) and 96 hours (T96) for individual enamel specimens and treatment groups

| *Specimen* | *ΔF* | | *ΔF subtraction** | *Mean ΔF difference per group ± SD* | *Proportional change in ΔF*[†] | *%F ± SD*[‡] |
|---|---|---|---|---|---|---|
| | *T0* | *T96* | | | | |
| Control CR | | | | | | |
| 11 | −9.13 | −14.5 | −5.39 | −6.69 | −0.59 | 67.8 |
| 12 | −9.91 | −17.6 | −7.69 | ±0.89 | −0.78 | ±11.1 |
| 13 | −11.9 | −18.2 | −6.3 | | −0.53 | |
| 14 | −9.15 | −16.1 | −6.95 | | −0.76 | |
| 15 | −9.68 | −16.8 | −7.12 | | −0.74 | |
| Control RMGIC | | | | | | |
| 51 | −10.4 | −12 | −1.6 | −2.96 | −0.15 | 29.7 |
| 52 | −11.2 | −15.7 | −4.5 | ±1.27 | −0.40 | ±12.4 |
| 53 | −9.81 | −13.7 | −3.89 | | −0.40 | |
| 54 | −8.38 | −11.4 | −3.02 | | −0.36 | |
| 55 | −10.5 | −12.3 | −1.8 | | −0.17 | |
| TM CR | | | | | | |
| 21 | −9.13 | −12.3 | −3.17 | −2.64 | −0.35 | 30.4 |
| 22 | −9 | −10.7 | −1.7 | ±0.99 | −0.19 | ±11.9 |
| 23 | −7.76 | −10.1 | −2.34 | | −0.30 | |
| 24 | −9.39 | −11.3 | −1.91 | | −0.20 | |
| 25 | −8.51 | −12.6 | −4.09 | | −0.48 | |
| TM RMGIC | | | | | | |
| 61 | −8.68 | −10.9 | −2.22 | −2.05 | −0.26 | 23.6 |
| 62 | −7.02 | −9.4 | −2.38 | ±1.12 | −0.34 | ±13.2 |
| 63 | −9.55 | −13.2 | −3.65 | | −0.38 | |
| 64 | −9.51 | −10.7 | −1.19 | | −0.13 | |
| 65 | −10.3 | −11.1 | −0.8 | | −0.08 | |
| NaF CR | | | | | | |
| 31 | −10.6 | −11.5 | −0.9 | −1.96 | −0.08 | 17.7 |
| 32 | −12.2 | −13.4 | −1.2 | ±0.89 | −0.10 | ±9.1 |
| 33 | −13.4 | −16.1 | −2.7 | | −0.20 | |
| 34 | −11.2 | −13.3 | −2.1 | | −0.19 | |
| 35 | −9.38 | −12.3 | −2.92 | | −0.31 | |
| NaF RMGIC | | | | | | |
| 71 | −11.7 | −13.9 | −2.2 | −2.16 | −0.19 | 20.7 |
| 72 | −8.49 | −11.5 | −3.01 | ±0.56 | −0.35 | ±8.4 |
| 73 | −10.8 | −12.4 | −1.6 | | −0.15 | |
| 74 | −12.2 | −14.5 | −2.3 | | −0.19 | |
| 75 | −10.9 | −12.6 | −1.7 | | −0.16 | |
| TM/NaF CR | | | | | | |
| 41 | −8.34 | −9.29 | −0.95 | −1.9 | −0.11 | 18.9 |
| 42 | −10.2 | −15 | −4.8 | ±1.77 | −0.47 | ±17.1 |
| 43 | −10.4 | −12.8 | −2.4 | | −0.23 | |
| 44 | −14.4 | −15 | −0.6 | | −0.04 | |
| 45 | −8.52 | −9.29 | −0.77 | | −0.09 | |
| TM/NaF RMGIC | | | | | | |
| 81 | −7.35 | −7.98 | −0.63 | −0.81 | −0.09 | 8.3 |
| 82 | −10.6 | −11.9 | −1.3 | ±0.31 | −0.12 | ±2.7 |
| 83 | −10.1 | −10.8 | −0.7 | | −0.07 | |
| 84 | −10.3 | −11.2 | −0.9 | | −0.09 | |
| 85 | −10.3 | −10.8 | −0.5 | | −0.05 | |

*T0-T96.
[†](T0-T96)/T0.
[‡]([T0-T96]/T0) ×100%.

**Table III**. Mean fluorescence (ΔF) change and mean proportional fluorescence (%F) change for treatment groups

| *Treatment group* | *Mean ΔF change per group* | *SD* | P *value** | *Mean %F* | *SD* | P *value** |
|---|---|---|---|---|---|---|
| Control CR | −6.69 | 0.89 | | 67.8 | 11.1 | |
| Control RMGIC | −2.96 | 1.27 | | 29.7 | 12.4 | |
| TM CR | −2.64 | 0.99 | .000† | 30.4 | 11.9 | .001† |
| TM RMGIC | −2.05 | 1.12 | 0.407 | 23.6 | 13.2 | 0.776 |
| NaF CR | −1.96 | 0.89 | .000† | 17.7 | 9.1 | .000† |
| NaF RMGIC | −2.16 | 0.56 | 0.518 | 20.7 | 8.4 | 0.512 |
| TM/NaF CR | −1.9 | 1.77 | .000† | 18.9 | 17.1 | .000† |
| TM/NaF RMGIC | −0.81 | 0.31 | .008† | 8.3 | 2.7 | .019† |

*Tukey post-hoc statistical analysis comparing with control test samples.
†Statistically different to control test samples ($P < .05$).

the difference in fluorescence (ΔF) between baseline and 96 hours was calculated. The proportional change in fluorescence (%F) was calculated according to the formula $\%F = (\Delta F_{baseline} - \Delta F_{96hr})/(\Delta F_{baseline} \times 100\%)$ and analyzed with analysis of variance (ANOVA). The post-hoc Tukey multiple comparison test was performed to determine statistically significant changes in fluorescence ($P < .05$) between groups.

## RESULTS

The control CR test group showed the greatest amount of enamel demineralization, whereas the TM and NaF RMGIC groups had the least amount of demineralization (Table II). One-way ANOVA showed statistically significant ($P < .05$) differences between all test groups, but not within each test group (Table III).

The CR specimens showed significantly greater demineralization than the RMGIC specimens at 96 hours (CR %F = 33.71 ± 23.85; RMGIC %F = 20.56 ± 12.23; *t* test, $P = .034$). The use of RMGIC alone (control RMGIC) significantly reduced ΔF and %F when compared with control CR (Tukey post hoc test, $P = .029$ and $P = .034$, respectively). Control CR ΔF and %F were significantly greater when compared with all test materials (Tukey post hoc test; $P < .001$). Statistically significant differences were observed in all CR test groups, with $P < .000$ for all 3 tested medicaments when compared with CR control. For the RMGIC test groups, the topical application of TM or NaF gel reduced the ΔF when compared with the control RMGIC samples, but not significantly ($P = .407$, $P = .518$, respectively). The combination of TM and NaF reduced the ΔF and %F significantly when compared with the control RMGIC samples (Tukey post hoc test; $P = .008$, $P = .019$, respectively) (Table III).

## DISCUSSION

The use of topical medicaments that can successfully prevent white spot lesion formation during orthodontic treatment would be beneficial for patients at risk of developing such lesions. The use of topical fluoride in its various forms (toothpaste, mouthrinse, gels, varnishes, fluoride-releasing cements, and elastomeric auxilliaries) has, to date, been the most commonly used caries preventive protocol during orthodontic treatment for at-risk patients, in addition to patient education and regular hygiene visits.[36] The regular exposure of dental enamel to the various forms of topical fluoride has been found to have a greater effect in the prevention of enamel demineralization rather than the remineralization of existing lesions because of its considerable ability to decrease the solubility of minerals in the enamel crystal lattice in the presence of acid, with fluoride ions acting as a catalyst for mineral formation.[41,42] The substitution of hydroxide ions by fluoride ions in hydroxyapatite further decreases the critical pH for the dissolution of tooth mineral containing fluorapatite.[43] For fluoride ions to remineralize a preexisting white spot lesion, 10 calcium ions and 6 phosphate ions are required with every 2 fluoride ions to form 1 unit cell of fluorapatite. Remineralization of white spot lesions by fluorides, particularly lesions on the labial aspect of the maxillary anterior teeth, is often calcium-limited and therefore ineffective.

Fluoride-releasing bonding agents were developed to allow for compliance-free, constant exposure to topical fluoride. In the late 1980s, glass ionomer cements were proposed as an alternative to the more commonly used composite material for bracket bonding.[44] The proposed benefits of glass ionomer cements include no need for pretreating the enamel with acid to create conditions for mechanical bonding, the release of fluoride over several months, and the possible development of a modified, less cariogenic microflora.[45-47] Fluoride-releasing glass ionomer cements and RMGIC have been shown to exert some cariostatic effects in both prospective and longitudinal clinical trials.[48,49] The results of our study are consistent with previous studies, with the use of RMGIC alone showing significantly reduced enamel demineralization when compared with control CR samples. Nevertheless, there are significant concerns regarding the reduction in both shear and tensile bond strengths of glass ionomer cements and RMGIC compared with CR cements, as shown in both in-vivo and in-vitro studies.[44,50,51] How-

ever, an in-vitro study by Choo et al[52] reported similar shear bond testing to failure of brackets bonded with RMGIC and CR cements. The clinician must therefore consider the benefits of fluoride release from a cement vs reduction in bond strength and the possibility of increased bond and bracket failures.

In this study, the use of NaF gel or TM alone reduced enamel demineralization for specimens bonded with both CR and RMGIC. However, the amount of demineralization was significantly reduced only for specimens bonded with CR, suggesting that the application of either topical solution would only provide an additional preventive benefit when CR is used as the bonding material of choice. The use of NaF gel combined with TM (50/50 w/w) demonstrated significant reductions in enamel demineralization for specimens bonded with either cement. This implies that the use of both solutions together might provide preventive benefits when used with CR or RMGIC. In this study, the NaF gel and the TM were combined immediately before application to the tooth surfaces. For clinical purposes, it can be presumed that the use of a fluoride mouthrinse followed by TM before retiring would provide similar effects and benefits.

Our findings lead to the question of which topical agent or combination of agents provides the greatest benefit for patients undergoing orthodontic treatment. Studies of the effects of CPP-ACP have so far shown promising dose-related increases in enamel remineralization in already demineralized enamel lesions.[19-21,23-25] With the limitations of any in-vitro study, it can be inferred that the use of TM would be beneficial in patients with enamel demineralization, because it might remineralize existing enamel lesions and also prevent the development of further white spot lesions. The use of topical fluoride would be ideal in poorly compliant patients who have not yet developed enamel lesions. In this in-vitro study, the combination treatment was shown to provide the greatest prevention of white spot development, probably due to the synergistic effect of both the NaF gel and the TM. Thus, the use of both agents perhaps can be recommended for an at-risk orthodontic patient to provide preventive actions and potentially remineralize early (subclinical) enamel demineralization. This combination treatment might minimize esthetic damage and prevent the need for future restorative intervention; however, studies to investigate the clinical effectiveness of topical agents during orthodontic treatment would be required to confirm these recommendations.

## CONCLUSIONS

With the limitations of any in-vitro study, the following clinical conclusions can be drawn.

1. The use of RMGIC for orthodontic bonding would appear to significantly prevent the development of enamel demineralization, even without the additional application of a topical agent.
2. If composite resin has been used for bonding, it is likely that the separate topical application of either TM or NaF gel (9000 ppmF) will prevent enamel demineralization.
3. If RMGIC has been used for bonding, the application of the combination of TM and NaF gel (9000 ppmF) would appear to be required to obtain preventive effects in addition to those of the glass ionomer cement itself.
4. The use of both agents should be recommended for any at-risk orthodontic patient to provide preventive action and potentially remineralize early (subclinical) enamel demineralization.

Clinical studies to investigate the clinical effectiveness of topical agents during orthodontic treatment would be required to confirm these in-vitro results.

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