



INCREASE IN ENAMEL VOLUME OF PREMOLARS BY REMINERALIZATION WITH S-PRG FILLER CONTAINING TOOTHPASTE FOLLOWING DEBONDING OF LINGUAL BUTTONS: AN IN-VITRO NANOMETRIC STUDY

Dental Science

Teresa Flores	Department of Orthodontics, Universitat Internacional de Catalunya, Sant Cugat del Vallés, Barcelona, Spain. - Corresponding Author
Juan R. Mayoral	Department of Restorative Dentistry, Universitat Internacional de Catalunya, Sant Cugat del Vallés, Barcelona, Spain.
Montserrat Artés	Associate Professor, Department of Orthodontics, School of Dentistry, Universitat Internacional de Catalunya, Sant Cugat del Vallés, Barcelona, Spain.
Jaume Llopis	Statistics department, University of Barcelona, Barcelona, Spain
Andreu Puigdollers	Associate Professor and Department Chair, Department of Orthodontics, School of Dentistry, Universitat Internacional de Catalunya, Sant Cugat del Vallés, Barcelona, Spain.

ABSTRACT

Objectives: To analyze changes in enamel and surface roughness after remineralization with S-PRG filler-containing toothpaste or artificial saliva, following the bonding/debonding of orthodontic buttons and removal of residual cement.

Methods: Orthodontic buttons were bonded to twenty premolars by Transbond XT (TX) or BeautyOrthoBond2 (BO2). Buttons were debonded and residual cement removed. Teeth were subdivided into four groups. They were remineralized with toothpaste containing S-PRG filler or with artificial saliva. Confocal microscopy was used to determine the enamel changes.

Results: Significant ($P < .05$) enamel changes were observed after resin removal in the TX and BO2 groups. Remineralization with S-PRG filler-containing toothpaste resulted in a significant increase in enamel volume. There was no change in enamel volume after remineralization with saliva.

Conclusions: Use of S-PRG filler-containing toothpaste increases enamel volume after the removal of residual cement following orthodontic buttons bonding/debonding. No changes were observed following artificial saliva.

KEYWORDS:

Confocal microscopy, S-PRG filler, self-etching, debonding

Introduction

Disruption of the demineralization /remineralization balance in teeth can lead to irreversible structural damage, as adult enamel is incapable of self-regeneration.¹ Removal of orthodontic brackets and residual cement causes inevitable enamel loss that is irreversible by biological mechanisms.²

Researchers have examined the potential use of surface (S)-prereacted glass-ionomer (PRG) filler in orthodontic bonding resins or toothpastes for inhibiting demineralization around orthodontic brackets or recovering enamel loss after bracket debonding.³

Nanometric techniques permit three-dimensional data to be obtained with minimum sample preparation. Reports of nanometric studies of healthy and affected enamel have described the enamel topography and surface roughness (SR), but have not provided data on enamel recovery.⁴ This in-vitro study analyzed the remineralization of premolars after the bonding/debonding of lingual buttons and polishing to remove remnant adhesive, with the goal of determining enamel loss and SR parameters before and after remineralization with an S-PRG filler-containing toothpaste or with artificial saliva.

Materials and methods

Twenty freshly premolars were stored in distilled water at 37°C. Teeth were attached with wax to a jig, and placed in a container.⁵ To define the measurement area during successive treatments, an area (1000×1000 μm) was marked on the flattest part of the buccal sides of teeth with a precision low-speed Isomet sectioning saw (Buehler, Lake Bluff, IL). Enamel was analyzed by confocal microscopy (CFM) to obtain baseline (pretreatment) data.

Samples were randomly divided into two equal groups ($n = 10$ each), according to whether TX (3M/Unitek, Moravia, CA) or BO2 (Shofu, Kyoto, Japan) was used as adhesive cement. Teeth in the TX group were etched with 35% phosphoric acid for 30 seconds, washed with an air/water spray for 20 seconds, and dried. Teeth in the BO2 group were conditioned with self-etching primer. Orthodontic buttons were bonded to teeth with TX or BO2, followed by light-curing for 30 seconds. Samples were stored in distilled water at 37°C /24 hours, followed by debonding. Cleaning of the residual adhesive was

performed by using a standardized polishing technique.

Samples from the TX and BO2 groups were subdivided into four equal groups ($n = 5$ each), according to the remineralization technique. Teeth in the TX-Saliva and BO2-Saliva subgroups were stored in artificial saliva⁶ for 7 days at 37°C. Saliva was changed daily. Teeth in the TX-S-PRG and BO2-S-PRG subgroups were brushed with toothpaste containing 1 μm, 5 wt% S-PRG filler (Shofu) for 3 minutes daily for 7 days,⁷ rinsed with distilled water, and brushed for 5 minutes under copious water spray to remove toothpaste. Between tooth-brushing sessions, S-PRG samples were stored at 37°C in distilled water.

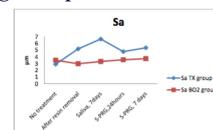
A Leica DCM 3D confocal microscope and Leica HCX PL Fluotar objectives at 10× magnification (Leica Microsystems, Wetzlar, Germany) were used to quantify the enamel volume and SR.⁸ Samples in the saliva subgroups were analyzed after 7 days of submersion. Samples in the S-PRG subgroups were analyzed 24 hours after the first tooth-brushing episode and 24 hours after the seventh day of brushing.

Statistical analysis

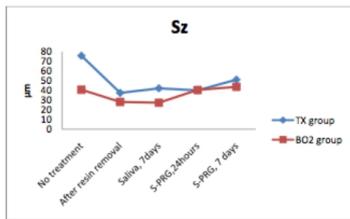
Analysis of variance (ANOVA) was used to determine the significant differences between the volume of enamel loss and roughness parameters among the groups. Normality was checked with the Shapiro-Wilk test and homogeneity of variance was checked with the Levene's test. The results were analyzed with a statistical software program (Statgraphics; Warrenton, VA, USA). The level of statistical significance was set at $p < 0.05$.

Results

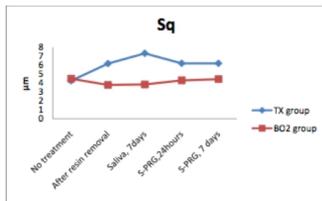
Graphic 1. Surface Roughness Parameters Sa, Sq and Sz of Enamel in Groups Before and After Remineralization with S-PRG Filler-Containing Toothpaste or with Saliva



Sa values of the TX, TX-Saliva, TX-S-PRG, and BO2 groups were significantly increased compared to baseline values and significantly greater Sa values were obtained in each TX group compared to the corresponding BO2 group.

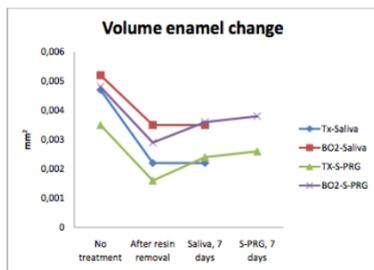


Saliva treatment significantly reduced Sz values with both cements. Sz values were higher in the TX vs BO2 group and in the TX-Saliva vs BO2-Saliva group.



Sq values of the TX and TX-Saliva groups were significantly increased compared to baseline values and Sq values were higher in the TX vs BO2 group and in the TX-Saliva vs BO2-Saliva group.

Graphic 2. Mean ± SD Volume of Enamel Loss (mm²) Before and After Remineralization With Saliva or With S-PRG Filler-Containing



Enamel volume was significantly lower after remineralization with saliva compared to untreated enamel, with no significant difference between cements. Remineralization with S-PRG filler-containing toothpaste for 24 hours or 7 days significantly increased the enamel volume compared to the volume after resin removal, but did not recover the volume to the level of untreated enamel. There was no difference in enamel volume between the 24-hour and 7-day remineralization times for either cement.

Toothpaste in Each Cement Group

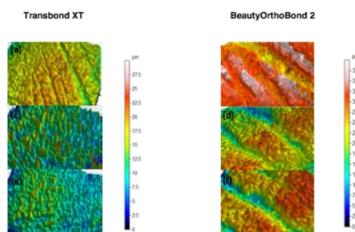


Figure 1. Representative confocal microscopy images illustrating surface roughness of premolar enamel for TX and BO2 groups without treatment (top), after resin removal (middle), and after submersion in artificial saliva for 7 days (bottom). Transition from blue to red indicates increasing enamel height.

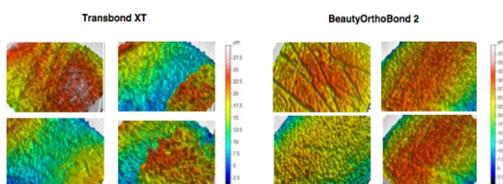


Figure 2. Representative confocal microscopy images illustrating surface roughness of premolar enamel for TX and BO2 groups without treatment (top left), after resin removal (bottom left), and after treatment with S-PRG filler-containing toothpaste for 24 hours (top right) or 7 days (bottom right). Transition from blue to red indicates increasing enamel height.

Discussion

In this in-vitro study, CFM was used to analyze changes in the enamel volume and SR of premolars after bonding/debonding of orthodontic buttons and polishing to remove residual cement. Remineralization abilities of artificial saliva and 5 wt% S-PRG filler-containing toothpaste were compared. CFM was used to analyze quantitative three-dimensional nanometric data.

SR affects the esthetic properties, bacterial adhesion, and plaque formation of enamel by altering the pathogenic environment.⁹ Researchers have analyzed the enamel SR by atomic force microscopy (AFM) and scanning electron microscopy (SEM) as nanometric techniques. In contrast to SEM, AFM does not dehydrate the surface enamel during sample preparation.¹⁰ Similar to AFM, CFM is a noninvasive nanometric technique that enables quantification of SR parameters with high measurement sensitivity and without altering the enamel surface quality.¹¹

Reported values of enamel loss after bracket debonding and cleaning are highly variable, ranging from 5–10 µm to 29.5–41.2 µm.² This high variability may be attributed to differences in the methods used for bracket bonding (ie, self-etching vs conventional etching cements)¹⁴ or analysis (eg, weight comparison, surfometry,¹⁴ profilometry,⁷ SEM¹³). Our CFM results revealed significant loss of enamel volume for premolars subjected to simulated orthodontic treatment compared to untreated enamel. Enamel loss was reduced, but not completely recovered, by remineralization with S-PRG filler-containing toothpaste. Similar changes in enamel volume were achieved after remineralization with toothpaste for 24 hours or 1 week. This finding may be attributable to the rapid decrease in fluoride release over time.¹⁵

In recent study, toothpastes containing 5 or 30 wt% S-PRG offered greater enamel remineralization abilities than NaF-containing toothpastes, as indicated by the improved surface hardness and elastic modulus values. Remineralization capability was primarily determined by the strontium- and fluorine-releasing capacities of the toothpastes, rather than the fraction of S-PRG filler.

Kaga et al⁷ reported that the buffering effect of S-PRG filler inhibits enamel demineralization. An aqueous solution containing S-PRG filler exhibited a rapid increase in pH at 1 day, a gradual increase over 6 days, and the lowest Ca ion concentration among remineralization solutions. Although human saliva is capable of hardening the enamel surface, calcifying solutions may have greater remineralizing potential due to their greater concentrations of calcium and phosphate.¹⁶ Calcium phosphate precipitates on the enamel surface as an amorphous precursor, which under goes rapid transformation to apatite crystals.¹⁷ Lippert et al¹⁸ observed no enamel hardening due to saliva. Consistent with results of Elkassas et al⁹, we observed no significant change in enamel volume after samples were submerged in artificial saliva for 1 week after resin removal.

Methods for measuring three-dimensional SR parameters have certain data collection and sensitivity limitations.¹⁹ For example, the topographic information needed to calculate SR parameters is difficult to obtain by SEM or profilometry.²⁰ Our CFM images of enamel provided clear definition of the enamel topography and allowed mean roughness data to be obtained. The decreased extents of peaks and valleys after treatment were increased after remineralization with S-PRG filler-containing toothpaste.

A previous study reported higher SR values after remineralization with 70wt% S-PRG compared to untreated enamel. The improvement could have been due to an increasing amount of filler particles on the enamel surface.¹⁵ We did not observe significant changes in SR between untreated enamel and samples remineralized with S-PRG toothpaste for 1 week (except for Sa in the TX group). However, the enamel surface was partially removed during polishing, which could decrease the SR after bonding and resin removal. Fluoride toothpastes are reportedly able to restore the surface of lesions,^{20,21} indicating their potential utility in cases of elevated caries risk, such as orthodontic patients.²¹

Conclusions

In-vitro treatment with toothpaste containing 1-µm, 5 wt% S-PRG filler for 7 days promoted an increase in enamel volume among teeth that had been bonded/debonded with orthodontic brackets and polished to remove residual cement.

Although remineralization did not completely restore enamel volume, values were significantly greater after treatment with S-PRG than with saliva alone.

Remineralized enamel showed similar SR values to intact enamel, except for Sa in the TX group.

References

1. Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, Moore BK. Effect of CPP-ACP paste on mechanical properties of bovine enamel as determined by an ultrasonic device. *J Dent.* 2006;34:230-236.
2. Pus MD, Way DC. Enamel loss due to orthodontic bonding with filled and unfilled resins using clean-up techniques. *Am J Orthod.* 1980;77:269-283.
3. Ijima M, Ito S, Nakagaki S, et al. Effects of immersion in solution of an experimental toothpaste containing S-PRG filler on like-remineralizing ability of etched enamel. *Dent Mater J.* 2014;33:430-436.
4. Poggio C, Lombardini M, Colombo M, Chiesa M, Bianchi S. Impact of two toothpastes on repairing enamel erosion produced by a soft drink: an AFM in vitro study. *J Dent.* 2010;38:868-874.
5. Flores T, Mayoral JR, Giner L, Puigdollers A. Comparison of enamel-bracket bond strength using direct-and indirect-bonding techniques with a self-etching ion releasing S-PRG filler. *Dent Mater J.* 2015;34:41-47.
6. Torres CR, Rosa PC, Ferreira NS, Borges AB. Effect of caries infiltration technique and fluoride therapy on microhardness of enamel carious lesions. *Operative Dent.* 2012;37:363-369.
7. Kaga M, Kakuda S, Ida Y, et al. Inhibition of enamel demineralization by buffering effect of S-PRG filler-containing dental sealant. *Eur J Oral Sci.* 2014;122:78-83.
8. Webb RH. Confocal optical microscopy. *Rep Prog Phys.* 1996;59:427-471.
9. Elkassas D, Arafa A. Remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. *J Dent.* 2014;42:466-474.
10. Hegedus C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent.* 1999;27:509-515.
11. Poggio C, Dagna A, Chiesa M, Colombo M, Saibante A. Surface roughness of flowable resin composites eroded by acidic and alcoholic drinks. *J Conservative Dent.* 2012;15:137-140.
12. Horiuchi S, Kaneko K, Mori H, et al. Enamel bonding of self-etching and phosphoric acid-etching orthodontic adhesives in simulated clinical conditions: debonding force and enamel surface. *Dent Mater.* 2009;28:419-425.
13. Fjeld M, Øgaard B. Scanning electron microscopic evaluation of enamel surface exposed to 3 orthodontic bonding systems. *Am J Orthod Dentofacial Orthop.* 2006;130:575-581.
14. Hosein I, Sherriff M, Ireland AJ. Enamel loss during bonding, debonding, and cleanup with use of a self-etching primer. *Am J Orthod Dentofacial Orthop.* 2004;126:717-724.
15. Hahnel S, Wastl DS, Schneider-Feyrer S, et al. Streptococcus mutans biofilm formation and release of fluoride from experimental resin-based composites depending on surface treatment and S-PRG filler particle fraction. *J Adhes Dent.* 2014;16:313-321.
16. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. *J Den Res.* 1997;76:1587-1595.
17. Shen P, Cai F, Nowicki A, Vicente J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. *J Den Res.* 2001;80:2066-2070.
18. Lippert F, Parker DM, Jandt KD. In vitro demineralization/remineralization cycles at human tooth enamel surfaces investigated by AFM and nanoindentation. *J Colloid Interface Sci.* 2004;280:442-448.
19. Karan S, Kircelli BH, Tasdelen B. Enamel surface roughness after debonding. *Angle Orthod.* 2010;80:1081-1088.
20. Kakaboura A, Fragouli M, Rahiotis C, Silikas N. Evaluation of surface characteristics of dental composites using profilometry, scanning electron, atomic force microscopy and gloss-meter. *J Mater Sci Mater Med.* 2007;18:155-163.
21. Gjorgievska ES, Nicholson JW, Slipper IJ, Stevanovic MM. Remineralization of demineralized enamel by toothpastes: a scanning electron microscopy, energy dispersive X-ray analysis, and three-dimensional stereo-micrographic study. *Microsc Microanal.* 2013;19:587-595.