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The Effect of Zinc-Carbonate Hydroxyapatite versus Fluoride on Enamel Surfaces After Interproximal Reduction

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Summary: The aim of this study was to qualitatively investigate the effects of a zinc-carbonate hydroxyapatite (Zn-CHA) containing toothpaste on stripped enamel morphology in a pH cycling model *in vitro* and to compare the efficacy of this toothpaste versus fluoride one which still represent the gold standard to remineralize early enamel lesions. Twenty-one extracted lower incisors underwent to interproximal enamel reduction with metal strips (Horico 80 μm) on both mesial and distal surfaces. They were then sliced into mesial and distal halves and the 42 samples obtained were randomly assigned to 3 groups of 14 enamel specimens each. For 8 days, teeth were placed in lactic acid solution for 2 h three times a day with 2 h distilled water preservation in between. After each demineralization bath, samples of Group A were brushed with Zn-CHA containing toothpaste while samples of Group B were brushed with 1,400 ppm fluoride dentifrice for 5 min before immersion into water. Group C of untreated samples served as control. All the samples were then prepared for scanning electron microscopy (SEM) analysis. A score rating system was used to perform a non-parametric statistical analysis. No statistically significant differences were found between the samples brushed with fluoride toothpaste and those untreated (Groups B and C) where the highest grade of damage was found, while the lowest grade was recorded in the samples brushed with Zn-CHA (Group A) and there was a statistically significant difference between this group and the other two groups. SCANNING 36:356–361, 2014. © 2013 Wiley Periodicals, Inc.

Key words: orthodontic, SEM, fluoride, zinc-carbonate hydroxyapatite, surface analysis

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Introduction

In orthodontic therapy, interproximal enamel reduction (IER) or stripping is a frequent clinical procedure that involves reduction of mesiodistal tooth dimensions by grinding interproximal enamel surface (Rossouw and Tortorella, 2003; Zachrisson *et al*., 2007). Qualitative scanning electron microscopy (SEM) evaluations showed that all stripping methods produce a significantly rougher and irregular enamel surface (Danesh *et al*., 2007; Grippaudo *et al*., 2010) that facilitate plaque and bacteria retention (Jarjoura *et al*., 2006; Grippaudo *et al*., 2010) and so might translate into a caries disease.

Fluoride use (toothpaste, gel, varnishes, cements) is the most common and studied approach to reduce the incidence of demineralization, representing the gold standard in the prevention of enamel early lesions (Rossouw and Tortorella, 2003; Caldeira *et al*., 2012; Hamdan *et al*., 2012; Zanarini *et al*., 2012), although some lacks in its possibility of enhance an enamel remineralization (Late *et al*., 2010).

The interaction between fluoride and hydroxyapatite leads to the formation of fluorhydroxyapatite which is more resistant to further demineralization, but avoids at the same time the penetration of calcium and phosphate ions to rebuild the lesion in depth (Ten Cate, ’90).

For these reasons, research was redirected to develop novel preventive agents that can act similarly to fluoride as an adjunct or independent of it.

Recently it has been suggested that the compound casein phosphopeptide amorphous calcium phosphate (CPP-ACP) may reduce the incidence of demineralization working similarly to fluoride by maintaining the saturation of calcium and phosphate in plaque fluid, thereby discouraging the dissolution of these elements and also promoting remineralization if they are lost (Sudjalim *et al*., 2007; Zhang *et al*., 2011).

CPP–ACP complex showed to be effective in enhancing remineralization after stripping (Giulio *et al*., 2009) and it has been suggested for tooth demineralization prevention and caries prophylaxis before bracket bonding procedure (Sudjalim *et al*., 2007; Zhang *et al*., 2011).

Moreover carbonate-hydroxyapatite nanocrystals (CHA) form a persistent biomimetic mineral coating that covers and safeguards the enamel structure (Rimondini *et al.*, 2007; Roveri *et al.*, 2008a,b; Tschoppe *et al.*, 2011).

A dentifrice formulation with CHA and zinc ions added (zinc-carbonate hydroxyapatite, Zn-CHA) provides a cariostasis long-term effect, due to the ability of metal ions to be retained into the salivary pellicle and at the surface of bacterial biofilm for several hours after application (Saxton *et al.*, '86; Tschoppe *et al.*, 2011; Palmieri *et al.*, 2013).

The present study aimed to qualitative assess by SEM evaluation the morphological changes induced by exposure to a Zn-CHA versus fluoride toothpaste on abraded enamel surfaces after exposure to an acid based solution.

Materials and Methods

Samples Preparation

Twenty-one lower permanent incisors extracted for orthodontic and periodontal reasons were collected. Every patient was informed about the study protocol and signed the informed consent before undergoing the extractions of the teeth and the subsequent investigations.

Samples were stored in 4°C distilled water for no longer than 30 days. Teeth with cracks visible under four magnifications, hypoplasia, white spots, caries, or reconstruction were not included in this study.

The incisors were placed on acrylic blocks to be aligned in an arch form and stripping was performed on mesial and distal surfaces under wet conditions to simulate intraoral environment. In order to standardize the procedure, IER was performed by the same operator (M.Z.), using a stripping diamond-coated metal hand-held strip (Horico 6 mm safe side 80 μm HOPF, RINGLEB & CO. GmbH & Cie., Berlin, Germany). Twenty strokes were made for each proximal surface using standard hand pressure and new strips have been used for each enamel surface to ensure ideal abrasion. The amount of enamel reduction was 0.5 mm per proximal surface, recorded by feeler gauges. After IER each incisor was removed from the block and sectioned by means of a diamond bur (Komet, Gebr Brasseler, Lemgo, Germany) along its major axis, thus separating the mesial and distal surfaces and obtaining 42 tooth sections.

They were randomly divided into the following groups:

Group A (14 tooth sections): enamel was brushed with an electric toothbrush and a Zn-CHA containing toothpaste (Blanx BioRepair Plus®; Coswell, Funo, Italy).

Group B (14 tooth sections): enamel was brushed with an electric toothbrush and a 1,400 ppm F^- containing toothpaste (Elmex®; GABA, Lörrach, Germany).

Group C (14 tooth sections): enamel surfaces remained untreated and served as control.

The assignment to the groups was carried out using a block randomization (block size = 3); to each tooth was assigned an envelope containing the number of the group it was assigned and a blind operator (E.P.) chose the envelope.

For 8 days, specimens, incubated at 37°C, were alternatively immersed in distilled water and demineralization solution as mentioned below.

An artificial demineralization was produced in order to amplify the effects of each treatment on enamel. Tooth sections were stored in a bath containing 75 ml of a demineralizing solution of 0.1 M lactic acid, buffered to pH 4.4 by ammonium hydroxide, for 2 h three times a day (from 9 a.m. to 9 p.m.) with a 2 h preservation in a 75 ml distilled water bath (pH 7.0) in between (Giulio *et al.*, 2009).

From 9 p.m. to 9 a.m. samples were incubated in distilled water (75 ml) at 37°C. After each demineralizing cycling, interproximal enamel surfaces of Groups A and B were brushed, respectively, with a Zn-CHA and a fluoride containing toothpastes. Every brushing session has been performed for 5 min by the same operator (E. P.) in a random fashion to avoid bias. The operator was trained and calibrated to maintain a constant pressure with an electric toothbrush with a timer and a pressure sensor (Trizone 5000, Oral-B, Procter & Gamble Co, Cincinnati, Ohio, USA). A "smear-size" toothpaste aliquot, weighing 0.10 g, controlled by a precision balance (KERN, NM 60-2, Kern & Sohn GmbH, Balingen, Germany) wetted with water was applied, closely resembling the *in vivo* usual tooth brushing procedure. Enamel surfaces of Group C were untreated and served as control.

After every treatment, each single sample was washed and gently brushed to remove residual toothpaste.

SEM Analysis

All teeth were carefully cleaned, fixed, dehydrated, and gold-palladium sputtered (Quorum-Emitech Sc 7620, Ashford, UK) for qualitative analysis by SEM (JSM-5200; JEOL, Tokyo, Japan).

A systematic method has been adopted for SEM images observation and interpretation. It provided four records on predetermined points on the surface of enamel sample, allowing repeated observations with 100% repeatability in finding the same observation fields (Marchionni *et al.*, 2010). Consequently, once identified, by 35×, the surface of enamel subjected to stripping on all the samples (Fig. 1), morphological analyses were made at 500× and 1,000× in the central area affected by IER. SEM images recorded at 1,000× were evaluated as regards the enamel damages by three experienced operators who randomly examined the samples twice in a blind manner. A modification of a scoring scale,

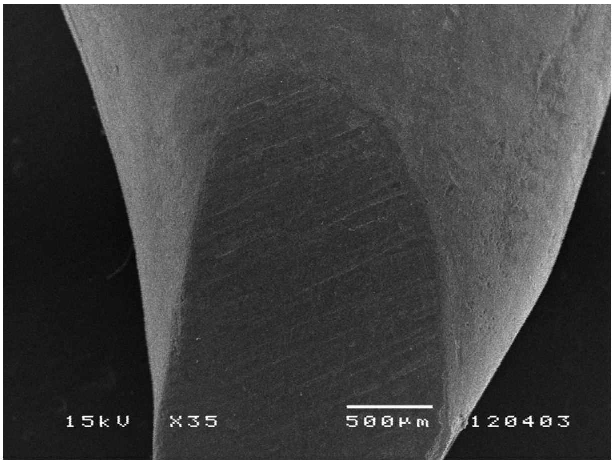

Fig 1. SEM micrograph (35×) showing the enamel surface affected by IER.

previously used for enamel surface after an acid attack (Nucci *et al.*, 2004), was adopted to describe the amount of enamel damage after IER (Table I).

Statistical Analysis

A pilot study was carried out on a sample of five enamel surfaces that were scored after observation on SEM; by setting a within group standard deviation (assuming those from different groups are homogenous) equal to 0.6 and a minimal difference of interest equal to 1 at a $\alpha = 0.0167$ (as per the Bonferroni correction) and $\beta = 0.8$ a total sample size of 13 surfaces in each of the three groups would give more than 80% power.

Kruskal–Wallis test for nonparametric data was performed to SEM scoring and multiple comparisons were assessed by Mann–Whitney test. Because of multiple testing, the significance level of a single test was set to 0.01.

To evaluate the method error, intra- and interobserver reliability checks were carried out using the Intraclass Correlation Coefficient (ICC > 0.9 in a 95% CI).

Results

The morphological analysis of all the images made at different magnifications on predetermined points, according to the systematic observation method, showed the earliest pattern of demineralization in the samples brushed with Zn-CHA containing toothpaste (Group A). A representative SEM image of all the observed specimens (Fig. 2A) showed the presence of irregularities on enamel surface and dissolution at the junction area between the rod and the interrod (interprismatic tissue): preferential enamel damage occurred at the peripheral areas of the prisms while the enamel rod cores were not affected and their margins were still evident.

When enamel was brushed with a fluoride containing toothpaste (Group B) the enamel prism pattern showed a predominant dissolution of enamel rods exposing delicate interprismatic enamel (Fig. 2B).

In the untreated samples of Group C a diffuse demineralization involved the rod core, with decomposition of morphology of prisms: they were severely affected and a greater prism-core dissolution compared with that in the interprismatic areas gave the enamel a "keyhole pattern" or "honeycomb pattern" of demineralization (Fig. 2C).

Figure 3 displays means and standard deviations in the scoring scale deriving from the observations made by the three evaluators on the 1,000× images for each group.

Mann–Whitney test for multiple comparisons showed no statistically significant differences between untreated samples and those brushed with fluoride containing toothpaste (Groups B and C) where the highest grade of damage was found. A statistically significant difference was recorded between the Group A, where the lowest score was observed, and the other two groups.

Discussion

Enamel demineralization is a widespread problem during orthodontic treatment.

Nowadays, it remains the most common dental disease facing mankind and prevention of initiation and interruption in progression of early lesions are the desirable modes of caries management (Hamdan *et al.*, 2012).

IER, a frequent clinical procedure, associated with an orthodontic therapy, affects enamel morphology (Danesh *et al.*, 2007; Zachrisson *et al.*, 2007; Grippaudo *et al.*, 2010) producing a rougher and irregular surface

Table I Scoring criteria used for the evaluation of SEM images

Grade	Status
0	Enamel surface remained perfectly intact with no grooves, pits, and porosity
1	Presence of surface irregularities on enamel surface, without demineralization of prismatic and/or interprismatic enamel
2	Presence of wrinkles and demineralization of prismatic/interprismatic enamel
3	Diffuse demineralization involved the rod core, with decomposition of morphology of prism

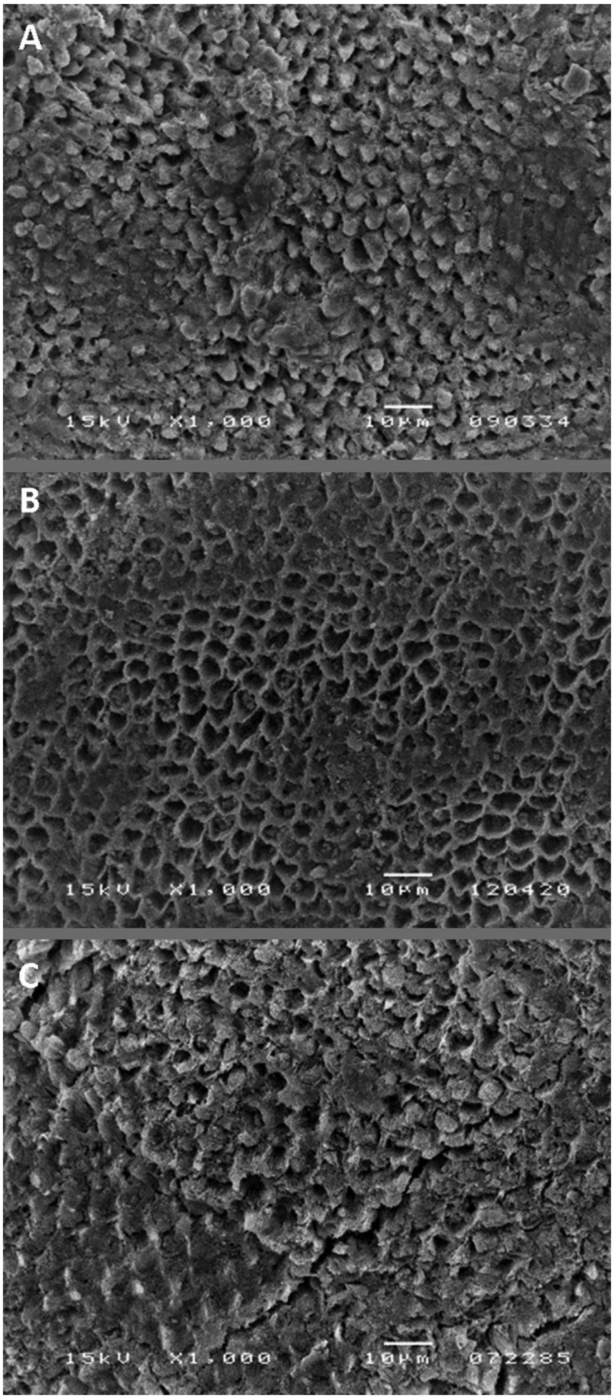

Fig 2. **A**: SEM micrograph (1,000×) from Group A. Severe dissolution at the junction area between the rod and the interrod was recorded while the rod cores were still evident. **B**: SEM micrograph (1,000×) from Group B. The enamel prism pattern showed a predominant dissolution of enamel rods exposing delicate interprismatic enamel, looking like an etched enamel. **C**: SEM micrograph (1,000×) from Group C. Diffuse demineralization involved the rod core, resulting in a lesion forming the "keyhole" like structure.

(Rossouw and Tortorella, 2003; Danesh *et al.*, 2007; Grippaudo *et al.*, 2010) that facilitate plaque and bacteria retention (Jarjoura *et al.*, 2006; Danesh *et al.*, 2007) and so might translate into a caries disease. It is initiated via the demineralization of tooth hard tissue by organic acids produced from fermentable carbohydrate by dental plaque cariogenic bacteria (Caldeira *et al.*, 2012; Hamdan *et al.*, 2012).

The use of fluoride products has been recommended by several authors to prevent unwanted side effects, such as demineralization, during and after an orthodontic therapy (Rossouw and Tortorella, 2003; Zero, 2006; Danesh *et al.*, 2007; Grippaudo *et al.*, 2010; Hamdan *et al.*, 2012).

Fluoride ions, in the presence of calcium and phosphate ions, can help replace the lost mineral of early caries lesions by remineralization (Zero, 2006; Hamdan *et al.*, 2012). The noninvasive treatment of early caries lesions by remineralization has the potential to be a major advance in the clinical management of the disease but there is still a little evidence about stripping and it is questionable whether fluoride treatment results in clinically significant benefits (Jarjoura *et al.*, 2006; Zero, 2006; Cochrane *et al.*, 2010; Late *et al.*, 2010).

Recently new biomimetic CHAs have been introduced and suggested as mineralizing product. This material mimic for composition, structure, nanodimension, and morphology bone apatite crystals, its chemical–physical properties resemble closely those exhibited by enamel natural apatite (Rimondini *et al.*, 2007; Roveri *et al.*, 2008a,b) and caused a progressive deposition of carbonate-hydroxyapatite into the eroded enamel surface scratches and pits forming a persistent biomimetic mineral coating, which covers and safeguards the enamel structure (Roveri *et al.*, 2008a; Tschoppe *et al.*, 2011).

The present study evaluate, by SEM analysis, the healing efficacy of Zn-CHA and fluoride products on enamel after IER procedures.

Since in all histomorphological studies the observations are based on merely qualitative criteria, a classification scale was used in order to help quantifying and to describe the damage grade on enamel. Scoring criteria modification of demineralization evaluation (Nucci *et al.*, 2004) was followed, as reported in Table I: a score of zero was assigned to enamel surface perfectly intact with no grooves, pits, and porosity, while a score of three to those where diffuse demineralization involved the rod core, resulting in a lesion forming the "keyhole" like structure.

The highest grade of damage was found in the samples untreated (Group C) and in those treated with fluoride toothpaste (Group B), as the enamel prism pattern showed a predominant dissolution of rods exposing interprismatic enamel (Fig. 2B and C), and no statistically significant differences were recorded between the two groups.

The lowest score of damage was recorded in the samples brushed with Zn-CHA containing toothpaste where the dissolution was observed at the junction area between the rod and the interrod but the rod cores were still evident.

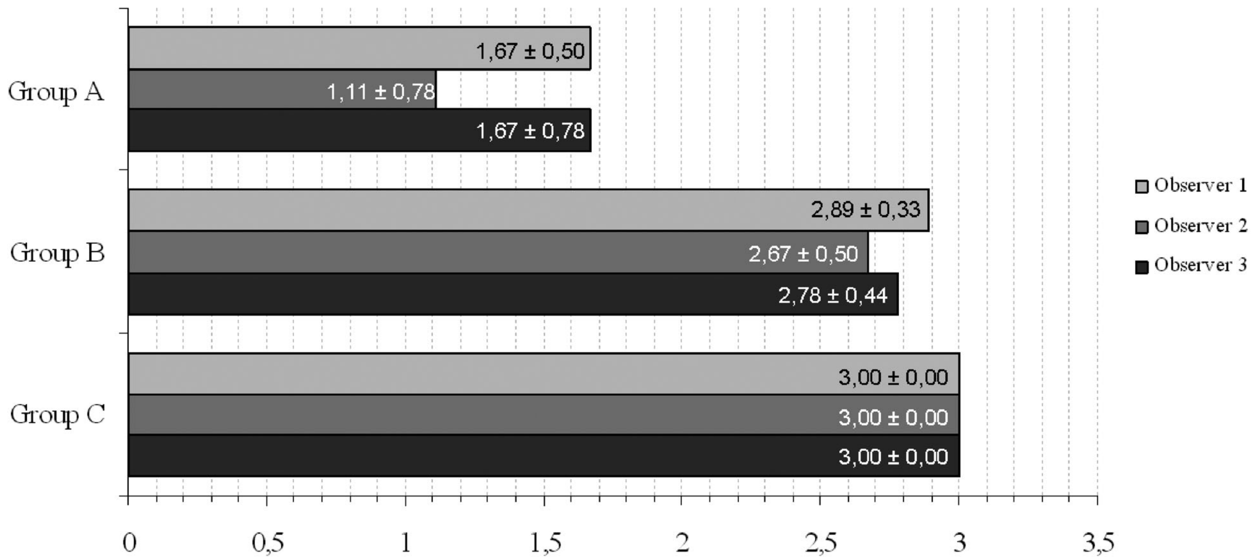

Fig 3. Means and standard deviations of SEM scores of the three observers.

These results showed a low reparative potential of fluoride dentifrice, as already demonstrated by several previous *in vitro* studies (Jarjoura *et al*., 2006; Zero, 2006; Cochrane *et al*., 2010; Late *et al*., 2010) and confirm that acid attack starts at the rod sheath space, which is the junction area between the rod and the interrod (Xue *et al*., 2009) once the lesion became established, demineralization proceed inward until the core was completely dissolved, resulting in the lesion forming a "keyhole" like structure (Darling, '67; Nucci *et al*., 2004; Xue *et al*., 2009).

The grade of damage observed in enamel surfaces after brushing sessions with the Zn-CHA containing dentifrice (Group A) highlighted the persistence of rod integrity resembling a less advanced demineralization level if compared with samples brushed with fluoride containing toothpaste (Fig. 2A). Similar results were recorded by Tschoppe *et al*. (2011) who showed that nanohydroxyapatite toothpastes exert similar capacities to mineralize enamel and dentine subsurface lesions unlike the fluoride toothpastes displaying the lowest mineralizing effects on both the tissues.

Zn-CHA reduced enamel damage on abraded samples after IER more than fluoride one did and proved to be an effective and viable alternative.

Conclusions

Within the limitations of this study and considering the abovementioned observations, it can be concluded that the use of a Zn-CHA containing toothpaste seemed to be an efficient way to protect stripped enamel surfaces from demineralization *in vitro*. Further quantitative and *in vivo* clinical studies would be required to clarify the effects of remineralizing products during orthodontic IER procedure and the role of saliva and biofilm in a natural mineralization in the oral environment.

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