Effect of Caries Infiltration Technique and Fluoride Therapy on Microhardness of Enamel Carious Lesions

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Clinical Relevance

An increase in microhardness of demineralized enamel after resin infiltration and acid resistance after exposure to a second demineralizing challenge can show the efficacy of the technique in controlling white spot lesions.

SUMMARY

Enamel white spot subsurface lesions compromise esthetics and precede cavitation; therefore, they must be halted. The aim of this study was to evaluate the effect of a caries infiltration technique and fluoride therapy on the microhardness of enamel carious lesions. Subsurface carious lesions were produced in 60 bovine specimens with polished enamel surfaces. The specimens were divided into four groups (n=15), according to the treatment used: CON, control—immersion in artificial saliva; DF, daily 0.05% fluoride solution; WF, weekly 2% fluoride gel; and IC, resin infiltration (Icon). The specimens were kept in artificial saliva and evaluated for microhardness at five points: baseline, after caries production, after four and eight weeks of treatment, and a final evaluation after being submitted to a new acid challenge. The repeated-measures analysis of variance showed significant differences according to the type of treatment (TREAT; p=0.001) and time of evaluation (EV; p=0.001). The results of the Tukey test were TREAT: CON = 45.18 ($\pm$29.17)a, DF = 107.75 ($\pm$67.38)b, WF = 83.25 ($\pm$51.17)c, and IC = 160.83 ($\pm$91.11)d. Analysis of correlation between the TREAT and EV factors showed no significant differences for DF (138.63 $\pm$ 38.94) and IC (160.99 $\pm$ 46.13) after the new acid challenge. The microhardness results in decreasing order after eight weeks were IC > DF > WF > CON. It was concluded that the microhardness of carious lesions increased with the infiltration of resin, while the final microhardness after a new acid challenge was similar for DF and IC.

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INTRODUCTION

Dental caries is defined as the destruction of tooth tissues by acid that is generated as a by-product of bacterial metabolism in dental plaque.[1] The first clinical sign of enamel caries is a white spot lesion, which precedes cavitation. Therefore, the early diagnosis and treatment of white spot lesions is extremely important. White spot lesions are considered reversible if they are detected early.[2]

Great attention has been devoted to noninvasive treatment of early proximal carious lesions, with the maximum preservation of tooth structure,[3] with no need of restorations.

As a noninvasive treatment, the use of topical fluoride associated with plaque removal is indicated to promote lesion remineralization.[4] Remineralization is the natural repair process for noncavitated lesions and relies on calcium and phosphate ions, assisted by fluoride, to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization.[5] Fluoride ions incorporate into remineralizing enamel/dentin, changing carbonated apatite to a fluoroapatite-like form that is more acid tolerant and makes the hard tissues more acid resistant.[6]

Fluoride plays a key role in the prevention and control of dental caries.[7] However, this approach is not always successful, as it requires good compliance of the patient, with a change of harmful habits, with many of the patients abandoning the treatment before completion.[8]

Sealants have been used therapeutically on non-cavitated occlusal caries as an attempt to reduce lesion progression.[9,10] In addition, these materials have been applied to interproximal smooth surfaces after temporary tooth separation to act as diffusion barriers.[3,11]

Enamel carious lesions are characterized by mineral loss in the body of the lesion, whereas the surface remains comparably highly mineralized.[2,12] The pores within the body of enamel caries provide diffusion pathways for acids and dissolved minerals. Therefore, an alternative approach for superficial sealing might be to arrest carious lesions by infiltration of these pores with light-curing resins, creating a diffusion barrier within the lesion without establishing any material on the enamel surface.[13]

Based on the available laboratory and clinical studies, it seems convincing that resin infiltration of enamel lesions should reduce (or even stop) the progress of white spot lesions.[14] This technique is considered microinvasive and might bridge the gap between noninvasive and minimally invasive treatment of initial dental caries, postponing as long as possible the need for a restoration.[15]

Caries resin infiltration represents a new concept in dentistry and therefore needs to be better investigated. The aim of this current study was to evaluate the resin infiltration technique and remineralization of enamel caries with a fluoride solution or gel on the microhardness of enamel caries and to investigate the resistance of these treatments when subjected to a new acid challenge. The null hypotheses tested were 1) the treatments tested did not alter the microhardness of the initial enamel carious lesion and 2) demineralization subsequent to treatments did not influence the microhardness of treated carious lesions.

METHODS AND MATERIALS

Sample Preparation

The methods described by Wiegand and others[16] were used to prepare the specimens. Thirty extracted, nondamaged, intact bovine incisors were stored in 0.1% thymol solution at room temperature until required. From each crown, two enamel-dentin specimens, 3 mm in diameter and 2.2-mm thick, were prepared from the labial surface using a trephine mill (Dentoflex, São Paulo, Brazil).

The specimens were positioned in a silicon mold with a cavity 6 mm in diameter and 2 mm deep. On the bottom of the mold, there was a second level cavity 3 mm in diameter and 0.1 mm deep. The specimens were positioned inside the internal cavity with the enamel surface facing the bottom of the mold. The mold was filled with acrylic resin (Extec Fast Cure Acrylic, Extec Corp, Enfield, CT, USA). The specimens were attached to a metal holder, and 0.1 mm of enamel was removed by polishing with sequential aluminum oxide abrasive papers (1200-, 2400-, and 4000-grit; FEPA-P, Struers, Ballerup, Denmark) in a polishing device (DP-10, Panambra Industrial e Técnica SA, São Paulo, SP, Brazil) for 20 seconds each. The dentin side of the specimens was abraded with 1200-grit abrasive paper, removing 0.1 mm of dentin and resulting in specimens with 1 mm of enamel and 1 mm of dentin. The prepared specimens were examined under a stereomicroscope to ensure the absence of cracks or other surface defects. After preparation, the specimens were stored in 0.1% thymol solution to avoid dehydration.

The microhardness determination was performed with a microhardness tester (FM-700, Future-Tech,

Tokyo, Japan) fitted with a 50-g load, which was used to make indentations on the enamel surface. The loaded diamond was allowed to sink and rest on the enamel surface for 10 seconds, and the Vickers hardness number (VHN) was determined. Three indentations, 100 μm apart, were performed at the center of each specimen and were averaged. The mean VHN value of each specimen was used for stratified allocation of all samples among the various experimental groups.

Sample Demineralization

Following the proposal by Queiroz and others,[17] artificial enamel subsurface lesions were created by individually immersing and storing the specimens in a buffer solution. The demineralizing solution was composed of 50 mM acetate buffer solution containing 1.28 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.74 mM $NaH_2PO_4 \cdot 2H_2O$, and 0.03 ppm F at a pH of 5.0 for 16 hours. The specimens were immersed separately in an unstirred solution at 37°C. The total volume of solution used was calculated using 2 mL/mm^2 of the enamel area.

Artificial saliva was prepared according to the formulation of Gohring and others[18] and consisted of hydrogen carbonate (22.1 mmol/L), potassium (16.1 mmol/L), sodium (14.5 mmol/L), hydrogen phosphate (2.6 mmol/L), boric acid (0.8 mmol/L), calcium (0.7 mmol/L), thiocyanate (0.2 mmol/L), and magnesium (0.2 mmol/L). The pH was between 7.4 and 7.8.

After this treatment, a new microhardness measurement was performed and baseline values were obtained. The specimens were then divided into four groups (n=15), according to the caries treatment employed:

CON (Control)—Specimens were stored in 5 mL of artificial saliva for eight weeks, which was changed every day.

DF (0.05% Fluoride Solution)—Specimens were immersed daily for 1 minute in 1 mL of 0.05% NaF solution for eight weeks. The fluoride solution was manipulated in the laboratory. After the daily fluoride immersion, the specimens were rinsed with deionized water and stored in artificial saliva.

WF (2% Neutral Fluoride Gel)—One milliliter of 2% NaF neutral gel (SS White Artigos Dentários Ltda, Rio de Janeiro, RJ, Brazil) was applied weekly for one minute on the surface of the specimens for eight weeks. After the gel application, the specimens were rinsed with deionized water and stored in artificial saliva.

IC (Resin Infiltration)—Specimens were resin infiltrated (Icon, DMG, Hamburg, Germany) and stored in artificial saliva for eight weeks. The infiltration procedure was performed according to the manufacturer's instructions:

- Icon-Etch was applied for two minutes.
- Specimens were water rinsed and air dried for 30 seconds.
- Icon-Dry was applied for 30 seconds and air dried.
- Icon-Infiltrant was applied two times, the first time for three minutes and the second time for one minute. Both applications were light cured for 40 seconds;
- Specimens were polished with aluminum oxide abrasive papers (4000 grit; FEPA-P, Struers) for 20 seconds.

The specimens of all groups were reevaluated for microhardness at four and eight weeks after the beginning of the treatments. After these periods, the samples were immersed again in the previously described demineralizing solution to evaluate the acid resistance of the treated surfaces and were submitted to a microhardness final evaluation. The residuals from the statistical analyses were examined to check for departures from normality and variance heterogeneity. No violations of the assumptions were found. The data were statistically analyzed, using repeated-measures analysis of variance (RM ANOVA) and Tukey test. Analyses were performed with statistical software STATISTICA 10 (Stat Soft Inc, Tulsa, OK, USA).

RESULTS

Evaluation with RM ANOVA (time as the repeated variable) revealed significant differences for the factors of treatment, time, and interaction (p=0.001). The Tukey test was then applied for the treatment factor and showed that group IC exhibited the highest microhardness means, followed by groups DF, WF, and CON, respectively (Table 1).

Evaluation with the Tukey test for the time factor showed significant differences for all periods of time evaluated. Baseline values (after white spot lesion formation) presented the lowest VHN means, followed by values measured after the new acid challenge (Ac; when specimens were immersed again in the demineralizing solution). Means obtained after four weeks presented intermediate values, and after eight weeks of treatment, the specimens showed the highest microhardness means (Table 2).

Figure 1 shows the results of the Tukey test for the interaction between the treatment and time factors. The specimens stored in saliva for four weeks did not

Table 1: *Means and Standard Deviation (SD) Data for the Tested Groups and Results of Tukey's Test for Treatment Factor.*

Treatment	Mean	SD	Homogen Groups[a]			
IC	160.83	91.11	A			
DF	107.75	67.38		B		
WF	83.25	51.17			C	
CON	45.18	29.17				D

[a] *Different letters in the same column indicate significant difference.*

show significant differences in the microhardness mean when compared with the baseline groups (values obtained after white spot formation), but after 8 weeks, a significant increase in microhardness mean was observed.

Specimens exposed to daily 0.05% fluoride solution after eight weeks (WF-8W) exhibited significantly higher microhardness means than the specimens exposed to weekly 2% fluoride gel (DF-8W). The results of microhardness after the new acid challenge were significantly higher for the resin-infiltrated (IC-New Ac) and fluoride solution groups (DF-New Ac) when compared with the fluoride gel (WF-New Ac) and control groups (CON-New Ac). The highest microhardness means were obtained for the resin-infiltrated groups and exposed to artificial saliva for four and eight weeks (IC-4W and IC-8W).

Figure 2 presents the performance of all tested groups, at different periods of time, in which an increase of the microhardness means can be ob-

Table 2: *Means and Standard Deviation (SD) Data for the Tested Groups and Results of Tukey's Test for Time Factor.*

Time	Mean	SD	Homogen Groups[a]			
Baseline	19.67	8.43	A			
New acid challenge	108.86	58.28		B		
After four weeks	127.42	78.26			C	
After eight weeks	141.06	69.02				D

[a] *Different letters in the same column indicate significant difference.*

served for all treatments tested, especially for the resin-infiltrated groups after four and eight weeks. The illustration also presents the similar performance of the IC and DF groups after they were exposed to a new demineralizing solution.

DISCUSSION

The first null hypothesis of this current study was rejected, as all of the treatments tested increased the microhardness of the initial enamel carious lesion. Remineralization of noncavitated lesions has been reported for more than one century, when demineralized enamel was observed to harden in the presence of saliva.[19] In fact, patients with diminished salivary flow show an increased caries incidence.[20] Saliva can act on the acids themselves (via buffering or neutralization), on the bacteria (via inhibition of the metabolic process involved in acid production), and on the enamel (by maintaining chemical supersaturation in the adjacent plaque fluid).[14] In this present study, the remineralization potential of saliva was observed, as demineralized specimens immersed in artificial saliva showed increased microhardness values. Nevertheless, this remineralizing action was too small when compared with the remineralizing potential obtained when fluoride and resin-infiltration treatments for caries were used.

In the presence of fluoride, the remineralizing effect of saliva has been shown to be enhanced.[21] Fluoride would make demineralization more difficult and remineralization would be favored. This is essentially the basis for the effect of fluorides on dental tissues in reducing dental caries.[1]

The remineralization action of highly concentrated fluorides, such as those found in oral rinses, was observed in previous studies that showed the prevention of incipient caries progression.[22] In the present study, the remineralization action of fluoride in white spot lesions was also observed, but the 0.05% fluoride solution daily applied was considered more effective than the 2% fluoride gel applied weekly (ie, the frequency of application was more important than its concentration). The mainstay in caries prevention and remineralization is frequent exposure to low levels of fluoride.[23] Higher fluoride concentrations can cause rapid mineral precipitation on the enamel surface and obturation of the surface enamel pores that communicate with the underlying demineralized lesion. This process can further limit remineralization of the subsurface demineralized enamel.[24]
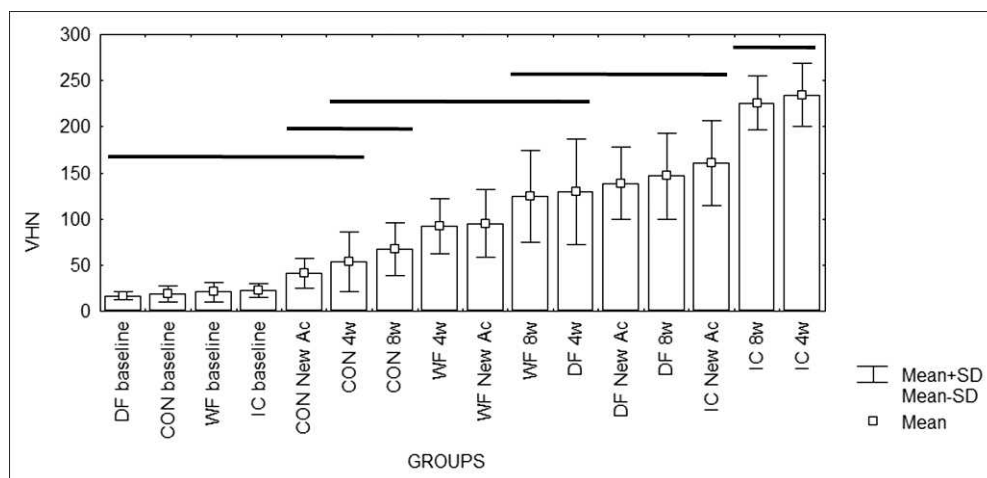
Figure 1. *Graph of microhardness means for the tested groups in different times and results of the Tukey test (horizontal bars) for the interaction between treatment and time factors.*

After the new acid challenge, the groups treated with fluoride exhibited no significant reduction in microhardness values compared with the groups treated for eight weeks (Figure 1). The remineralized enamel surface is different from the original in its composition and structure and is more resistant to demineralization than sound enamel.[25] The applied fluoride incorporates into enamel crystals, thereby forming a fluoroapatite-like mineral that improves the ability of the enamel to resist further acid challenge.[23,26]

The caries infiltration technique is an innovative approach investigated in previous studies, showing good results in hampering the progression of enamel caries.[12,27-30]

The infiltration technique, in contrast to the application of sealants, in which the diffusion barrier remains on the enamel surface, creates a diffusion barrier inside the enamel lesion and possibly strengthens the demineralized enamel structure with the resin matrix, preventing cavitation.[13] In the present study, the infiltration technique showed significantly higher microhardness means than all other tested groups (Table 1). This reflects the ability of the low-viscosity resin to fill the spaces between the remaining crystals of the porous lesion and
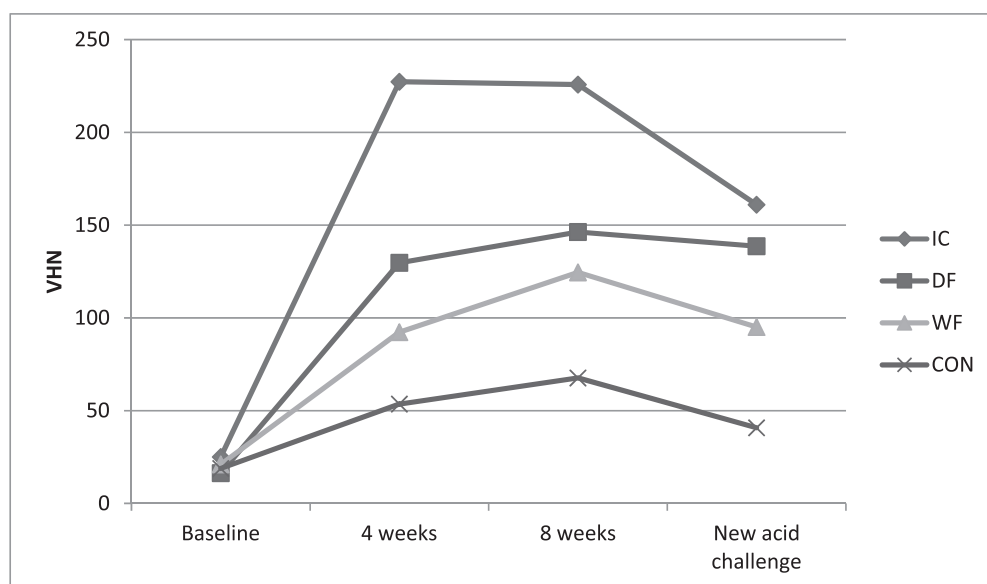

Figure 2. *Graph of microhardness values for the tested groups in different times.*

reharden the demineralized tissue, improving its mechanical strength.

Previous studies showed that although the resin-infiltration technique results in a significant reduction in lesion progression under demineralizing conditions, some demineralization can still occur after Icon treatment.[12,15,28] This result was also observed in the present study, in which the artificial lesions infiltrated with Icon presented lower micro-hardness means after the new acid challenge. This could be due to the partial dissolution of the remaining mineral in the lesion body that was not completely embedded within the resin matrix or by the resin shrinkage during light curing, which results in leakage and consequently reduction of acid resistance.[13] Nevertheless, despite this reduction in microhardness after the demineralization process, the resin-infiltrated group exhibited micro-hardness means similar to those obtained by the daily fluoride-treated group after the new acid challenge (Figure 2).

This result shows that resin infiltration is a promising technique to treat enamel caries, but the oral hygiene and diet education methods should be instituted for high-risk caries patients, and perhaps a new infiltration of resin should be conducted. It was shown that a repeated application of resin can reduce the leakage of acids in the lesion body.[31]

Although all tested treatments were capable of enhancing the microhardness of demineralized enamel, it should be emphasized that any strategy to reduce the progression of carious lesions should be based on the control of caries as a biofilm-dependent disease, and it should be controlled with tooth brushing by means of oral hygiene education and dietary control.[32]

It has to be considered that an artificial bovine enamel lesion model was used, but this limits the external validity of the study because, under clinical situations, the lesions to be resin infiltrated are deeper.[15] More studies are needed to confirm the efficacy of resin infiltration technique in clinical conditions.

CONCLUSION

It was concluded that the microhardness of initial enamel carious lesions increased significantly with the resin-infiltration technique and the final micro-hardness after a new acid challenge was similar in the specimens infiltrated with resin and treated with 0.05% daily fluoride solution.

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