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The Evaluation of Different Treatments of Incipient Caries Lesions: An in Situ Study of Progression Using Fluorescence-based Methods

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Clinical Relevance

Effective methods to control incipient caries lesions are needed. In this investigation, several methods provide encouraging results.

SUMMARY

This study aimed to evaluate *in situ* the inhibition of incipient caries lesion progression using different treatment protocols and to evaluate the effectiveness of fluorescence-based methods (DIAGNOdent, DIAGNOdent pen, and VistaProof fluorescence camera [FC]) in monitoring this process. The research was conducted in four phases: (1) at baseline, (2) after a first cariogenic challenge, (3) after treatment modalities, and (4) after a second cariogenic challenge. Sixteen volunteers used intraoral acrylic palatal appliances, each containing six enamel blocks (n=96). The cariogenic challenge was performed using a 20% sucrose solution over a 14-day period. The appliances were removed eight times a day and, upon removal, two drops

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of the solution were placed onto each enamel block. The enamel blocks were randomly assigned to three treatment groups: fluoride varnish ([FV] Duraphat; n=32), resin infiltrant ([RI] Icon; n=32), and adhesive system ([AS] Scotchbond; n=32). At the end of each phase, the surface microhardness (SMH) was measured, and two trained examiners evaluated the specimens using fluorescence-based methods. In addition, integrated mineral loss (ΔΔZ; vol%.min x μm) and lesion depth (ΔLD; μm) were evaluated using transverse microradiography. A two-way analysis of variance and a Tukey *post hoc* test were calculated (α=5%). Significant differences in SMH were observed according to the treatment, phases, and interaction of factors ($p<0.001$). Treatment with FV resulted in significantly higher SMH values in phases 3 and 4 compared to RI and AS, with the last two treatments resulting in similar values ($p>0.05$). The ΔΔZ value was similar for FV and AS but significantly higher for RI ($p=0.016$). ΔLD was not significantly different among the groups ($p=0.126$). Significant differences in the measurement of fluorescence for each fluorescence-based method were observed between each phase of the study ($p<0.05$). It can be concluded that all treatments were effective in inhibiting the *in situ* progression of incipient lesions, although to different degrees, with minor mineral loss changes observed for the AS and FV. Besides, all fluorescence-based methods tested, except for that using the FC device, were effective in monitoring caries lesion progression.

INTRODUCTION

The assessment of incipient caries lesion development and progression plays an important role in promoting adequate oral health care. It allows for the use of nonoperative preventive measures and minimally invasive treatments based on the control or arrest of caries lesion progression,[1] which is the current recommendation according to the Minimal Intervention Dentistry concept.[2]

In this context, it is possible to find several therapies, with different mechanisms of action, for controlling incipient caries lesions, such as fluoridated agents, adhesive sealants, and resin infiltration.[2] Fluoridated agents act by inhibiting enamel demineralization, increasing remineralization, and inhibiting acidic and aciduric bacterial enzyme production.[3,4] Adhesive sealants are used to arrest dental caries progression by preventing contact with the oral fluids through the use of a mechanical support to the tissue, thereby inhibiting further cariogenic challenge.[5-7] Similarly, the use of a resin infiltrant (RI) is an alternative treatment for arresting and inhibiting the progression of noncavitated caries lesions through the penetration of low-viscosity photopolymerizable resins into the enamel pores present in the body of the lesion, thereby preventing the diffusion of cariogenic acids and mineral dissolution.[8] The question remains unanswered as to whether fluoride varnish (FV), resin infiltration, or conventional adhesive might be able to inhibit dental caries progression in an oral cavity with constant cariogenic challenges.

The slow progression of caries lesions allows for early detection and monitoring, providing the opportunity for the correct management of the patient.[9] Thus, fluorescence-based methods have been suggested for the detection and quantification of caries lesions.[10] DIAGNOdent 2095 ([LF] KaVo, Biberach, Germany) and DIAGNOdent 2190 pen ([LFpen] KaVo) are fluorescence-based devices that emit a diode laser at a wavelength of 655 nm (within the rwed range of the visible spectrum) and capture the fluorescence emitted by the endogenous porphyrins (fluorophores) produced by the cariogenic bacteria. The detected fluorescence is transformed into numerical values ranging from 0 to 99.[11,12] The VistaProof intraoral fluorescence camera ([FC] Dürr Dental, Bietigheim-Bissingen, Germany) is another fluorescence-based device that illuminates the dental surface with a 405 nm wavelength light emitted by six LEDs (within the blue range of the visible spectrum). This system captures the image of the dental surface at the time of fluorescence emission and transforms the ratio of green (wavelength of approximately 510 nm) and red (wavelength of approximately 680 nm) fluorescence emitted by the dental tissues into numerical values. According to the manufacturer, these values are correlated with the extent of the caries lesion.[13]

These fluorescence-based methods (LF, LFpen, and FC) appear to aid the process of detecting caries lesions. However, their performance is still questionable when used on smooth surfaces. Studies have evaluated the performance of these devices for detecting and monitoring caries lesions on smooth surfaces[14-18] and for monitoring the remineralization process.[19-22] Recent studies have evaluated the use of fluorescence-based devices as complementary methods for monitoring incipient caries lesions treated with RI or dental sealants on smooth and occlusal surfaces, with controversial results.[23-26]

No previous study has been performed to evaluate the effectiveness of LF, LFpen, and FC devices in monitoring the inhibition of the progression of

noncavitated caries lesions on smooth surfaces *in situ* after different treatments based on a minimally invasive dentistry (MID) philosophy. Thus, the aims of this *in situ* study were to evaluate the effectiveness of (1) different treatments in the inhibition of incipient caries lesion progression, and (2) fluorescence-based methods in monitoring this process. The null hypotheses were that (1) there is no difference among treatments based on an MID approach for inhibiting the progression of incipient caries lesions, and (2) fluorescence-based methods are not able to monitor enamel lesion progression.

METHODS AND MATERIALS

Experimental Design

This prospective, Institutional Review Board–approved, double-blind *in situ* study involved four phases: baseline (phase 1); a first cariogenic challenge, involving demineralization induction for 14 days (phase 2); treatment modalities of specimens (phase 3); and a second cariogenic challenge, involving demineralization induction for an additional 14 days (phase 4).

Sample and Specimen Preparation (Phase 1)

The sample size was calculated based on the surface microhardness (SMH) remineralization data (primary outcome) from a previous study[22] and was calculated with a website power calculator (www.sealedenvelope.com). Considering a continuous outcome, a superiority trial was performed (α at 5% and β at 80%); the SMH of the control group after the remineralization phase was 43.9 ± 25.2 (mean ± standard deviation) and the limit of equivalence was at 18%, thus a total of 31 samples per group were required to detect possible differences.

A total of 200 enamel blocks (4×4×2 mm) were obtained from freshly extracted sound bovine incisors. Teeth were disinfected in a 2% formaldehyde solution for one month. The enamel blocks were obtained after two double sectionings (Isomet 1000; Buehler, Lake Bluff, Illinois, USA) of the widest portion of dental crowns.[27] The enamel specimens were then stored in a 0.1% thymol solution.

Each enamel block was prepared and successively polished with carbide paper of different grits (Ecomet 250; Buehler, Lake Bluff, IL, USA) and diamond abrasive paste (Teclago; Vargem Grande Paulista, São Paulo, Brazil).[22]

A microhardness tester with a Knoop diamond (HMV-2; Shimadzu Corporation, Tokyo, Japan) was used for SMH analysis, as described in previous studies.[20,22] Of the 200 enamel specimens, only the 96 that exhibited hardness of 368.3 ± 80.0 KHN were selected.

Measurements Using Fluorescence-based Methods

Each enamel block was fixed in an acrylic resin disk and analyzed by two experienced examiners (MBD and PHC) using LF, LFpen, and FC devices.[22] Each examiner individually assessed the enamel blocks using the fluorescence-based devices three times, and the mean values were recorded.[22]

The LF and LFpen fluorescence analyses were performed following the manufacturer's instructions.[22] Before measurement, each device with its specific tip was calibrated using a standard reference and swept across each enamel block.[21] The maximum fluorescence value was recorded. The FC analysis was conducted in a dark room. The images of the enamel blocks were analyzed using DBSWIN software (Dürr Dental, Bietigheim-Bissingen, Germany), which translates fluorescence into numbers.[13]

Participant Selection

A total of 16 healthy adult volunteers (3 males and 13 females, aged 20 to 40 years) who lived in a community with fluoridated water (0.7 ppm F) were selected and signed informed consent forms. They were in good general health (ie, no systemic illness, no drug use that affects salivary parameters, nonsmoking, not pregnant or breastfeeding, and no use of orthodontic appliances) and good oral health (ie, no active caries lesions or significant gingivitis/periodontitis).[22] All ethical and methodological aspects related to this *in situ* investigation were explained to the participants.

First Cariogenic Challenge (Phase 2)

The enamel blocks were immersed in a 70% alcohol solution for 30 minutes.[27] Then, the outer one-third of the enamel surface of each block was covered with nail varnish (sound control area), leaving two-thirds of the enamel for induction of artificial demineralization[27] under the *in situ* protocol. The enamel blocks (n=96) were randomly allocated according to the different treatment modalities (n=32 per group). Then, two enamel blocks per group were randomly assigned to each participant (n=6; www.sealedenvelope.com).

Each participant wore an intraoral palatal appliance containing six spaces. One enamel block was fixed with wax in each space, leaving a 1-mm gap for biofilm formation, and protected by a plastic mesh.[22]

The cariogenic challenge was performed through exposure to a 20% sucrose solution over 14 days. Participants were instructed to remove the intraoral appliance eight times per day, and 2 drops of the solution were placed onto each enamel block.[21] Then,

the participant was instructed to put the appliance back into the mouth 5 minutes after sucrose exposure. Participants were also instructed to wear the appliances except during meals, drinking, and oral care.[28] Volunteers were instructed to brush their natural teeth with the provided nonfluoride dentifrice (Cocoricó; Bitufo, Itupeva, São Paulo, Brazil).[21] They were also instructed to not use any fluoridated or antibacterial products.

During the experimental phases, the participants were questioned about the use and stability of the intraoral acrylic palatal appliances and any possible discomfort. The participants' understanding of and compliance with the clinical protocol was constantly monitored.

After the first cariogenic challenge (phase 2), all enamel blocks (n=96) were removed from each intraoral appliance and mildly brushed to remove biofilm; they were then immersed in an ultrasonic bath, in deionized water for 2 minutes. Then, the enamel blocks were fixed in acrylic disks and kept in a humid environment in a refrigerator until further analysis. Fluorescence-based SMH analyses were performed as previously described.[20,22] Afterwards, the other outer one-third of the enamel surface of each specimen was covered with nail varnish (demineralized control area), leaving a central area of the enamel that was previously demineralized[27] for the treatment of enamel blocks (phase 3) and the second *in situ* cariogenic challenge (phase 4).

A 14-day nontreatment period between the first and second cariogenic challenges allowed for examinations, and the samples were kept in a humid environment in a refrigerator during this period. A previous study has shown that fluorescence values decrease after this period of nontreatment due to the storage method.[29]

Treatment Modalities of Specimens (Phase 3)

The enamel blocks were treated according to the experimental groups (n=32): FV (5% NaF, Duraphat; Colgate-Palmolive, São Paulo, Brazil), RI (Icon; DMG, Hamburg, Germany), and AS (Adper Scotchbond Multi-Purpose; 3M ESPE Dental Products, St Paul, MN, USA).

Fluoride varnish was applied to the enamel surface using a standardized microbrush, and the samples were stored in artificial saliva[30] at 25°C for 6 hours (pH 6.8, 30 ml per sample) in order to promote the specimens' remineralization,[30] although this remineralization process continues with the effect of saliva during the use of the palatal appliances *in situ*. After that, the FV was removed using a blade and cotton swabs soaked in 50% acetone.[27] After this procedure, the nail varnish was applied to recover the control areas.[27]

Resin infiltrant was applied to the enamel blocks following the manufacturer's instructions. The demineralized enamel surfaces were etched for 2 minutes with 15% hydrochloric acid (HCl; Icon-Etch; DMG, Hamburg, Germany), water rinsed for 30 seconds, and air dried for 10 seconds. Then, ethanol (Icon-Dry; DMG, Hamburg, Germany) was applied for 30 seconds, followed by additional air drying for 10 seconds. The low-viscosity RI (Icon-Infiltrant; DMG, Hamburg, Germany) was applied on the surface for 3 minutes. After that, the resin was light cured for 40 seconds using a light-emitting diode device at 900 mW/cm^2 (Radii Cal; SDI Dental Products, Victoria, Australia). The infiltrant was additionally applied for 1 minute and light cured for 40 seconds. Then, the enamel blocks were polished using #4000 grit aluminum oxide abrasive papers for 10 seconds.

Adhesive was applied to the enamel surface after 37% phosphoric acid gel etching (step 1) (Super Etch; SDI Dental Products, Victoria, Australia) for 60 seconds before rinsing with water for 60 seconds. After gentle air drying, only the bond component (step 3—the hydrophobic component) of Adper Scotchbond Multi-Purpose was applied for 20 seconds using a microbrush, air dried for 2 seconds, and light-cured using a light-emitting diode device (Radii Cal; SDI Dental Products, Victoria, Australia) with output at 900 mW/cm^2 (measured with a radiometer) for 20 seconds. The enamel blocks were also polished using #4000 grit aluminum oxide abrasive papers for 10 seconds.

Then, the specimens were fixed in acrylic disks. Surface microhardness and fluorescence-based measurements were obtained as previously described. The time interval between this phase (phase 3) and phase 4 was one week,[22] as fluorescence values decrease only after one to two weeks of sample storage.[29]

Second Cariogenic Challenge (Phase 4)

In the second *in situ* cariogenic challenge, the six enamel blocks were washed with deionized water and fixed in each intraoral acrylic palatal appliance, with a new plastic mesh for biofilm accumulation.

The participants wore the appliances again for an additional 14 days and were instructed to follow the same protocol as described in phase 2. Then, the enamel blocks were removed from the intraoral appliances, cleaned, fixed in acrylic disks, and kept in a humid environment in a refrigerator until further analysis. Fluorescence-based and SMH analyses were performed as previously described.

Transverse Microradiography

The enamel blocks were sectioned perpendicularly to the central area, and one-half was analyzed using transverse microradiography (TMR). The preparation of the enamel blocks and methodology for acquiring microradiographs for each specimen obtained were performed as previously described by Cardoso and others.[27] The mineral content was calculated from one picture of each enamel specimen (at the initial and final lesion areas), and the step-wedge grey levels were obtained using the formula from Angmar and others.[31] Sound enamel mineral content was assumed to be 87 vol%. The lesion depth (LD) was obtained using a 95% threshold of the mineral content of sound enamel (82.7%). For the comparison between the initial and final lesion enamel areas (ΔZ, integrated mineral loss), the differences were calculated as follows: ΔΔZ = ΔZ initial lesion − ΔZ final lesion; ΔLD = LD initial lesion − LD final lesion.

Through the TMR analysis, it is possible to compare the percentage of mineral loss and LD of the first and second cariogenic challenges, as the enamel was protected with nail varnish in phase 1 (healthy control area) and after phase 2 (demineralized control area), leaving a central band of the demineralized enamel (for the treatment of specimens [phase 3] and the second *in situ* cariogenic challenge [phase 4]).[27] So, it is possible to compare, through the TMR software, the differences between the areas of demineralization of each phase.

Statistical Analysis

Data analysis was performed using MedCalc for Windows (version 12.3.0; MedCalc Software, Mariakerke, Belgium) and Statistica for Windows (version 8.0; Stat Soft, Inc., Tulsa, OK, USA). The significance level was set at 5%. Outcome variables were the mean values of LF, LFpen, FC, SMH, ΔΔZ, and ΔLD, and the phases (1, 2, 3, and 4) and experimental groups (FV, RI, and AS) were the variation factors.

The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check the data for normal distribution. All requirements for the analysis of variance (ANOVA) were met. Two-way ANOVA and the Tukey *post hoc* test were performed for statistical comparisons.

Quantitative data were represented as means and standard deviations for all phases of the study (phase 1 [baseline], phase 2 [after the first cariogenic challenge], phase 3 [after treatment modalities], and phase 4 [after the second cariogenic challenge]).

Inter-examiner reproducibility for fluorescence-based methods (LF, LFpen, and FC devices) was assessed by calculating the intraclass correlation coefficient (ICC), which ranges from poor (<0.40) to excellent (>0.75).

A receiver operating characteristic (ROC) analysis was carried out to assess the performance of LF, LFpen and FC devices in monitoring the inhibition of incipient caries lesion progression following different treatment modalities. The area under the ROC curve (Az) was calculated to indicate the overall accuracy of each device.[9] Moreover, with the ROC analysis, the optimal cut-off points between sound and demineralized surfaces were calculated. With these cut offs, sensitivity and specificity values were also calculated for each method in phases 2 and 4.

RESULTS

In total, 16 subjects were able to finish the experimental periods. No participants reported adverse events or side effects.

Table 1 represents the SMH analysis of the enamel blocks with different treatments in all phases of the study. The two-way repeated measures ANOVA indicated differences for treatments (p<0.001), phases (p<0.001), and interactions between the two factors (treatments and phases) (p<0.001). With respect to the phases of the

Table 1: *Surface Microhardness in KHN (mean ± standard deviation) of the Enamel Blocks with Different Treatments (Experimental Groups) in All Phases of the Study*[a]

Experimental Groups	Phase 1	Phase 2	Phase 3	Phase 4
FV	376.3 ± 21.7 A,a	143.5 ± 47.9 B,a	110.4 ± 64.2 C,a	62.7 ± 54.5 D,a
RI	371.3 ± 21.5 A,a	143.9 ± 56.1 B,a	34.2 ± 17.8 C,b	36.8 ± 19.1 C,b
AS	366.2 ± 27.3 A,a	149.4 ± 60.4 B,a	36.8 ± 19.1 C,b	25.4 ± 15.5 C,b

Abbreviations: ANOVA, analysis of variance; AS, adhesive system; FV, fluoride varnish; KHN, Knoop hardness number; RI, resin infiltrant.
[a] Significant differences are represented by different uppercase letters within the same row and different lowercase letters within the same column (two-way repeated measures ANOVA and Tukey post hoc test; p<0.05).

study, no statistically significant difference was noted for SMH values among groups (p>0.05) within phases 1 and 2, demonstrating homogeneity for all groups at baseline and after the first *in situ* cariogenic challenge. However, FV SMH values were greater than RI and AS in both phase 3 (after treatment) and phase 4 (after the second *in situ* cariogenic challenge) (p<0.05). With respect to treatment modalities and their respective phases, SMH values reduced in each assessed phase, except for RI and AS, in which no statistically significant difference was noted for SMH values in phases 3 and 4 (p>0.05).

Table 2 shows the results of the TMR analysis. Fluoride varnish and the AS were able to inhibit lesion progression in a similar pattern and differed significantly from RI, which led to statistically significant higher demineralization according to the integrated mineral loss (p=0.016). However, when LD was considered, none of the treatments resulted in significant changes when submitted to the second demineralization challenge, and they did not differ significantly from each other (p=0.126).

Table 3 represents the fluorescence values of the enamel blocks with different treatments in all phases of the study. For the LF device, the two-way repeated measures analysis of variance indicated statistically significant differences only for phases (p<0.001); no statistically significant difference was noted for treatments (p=0.3918) and interaction between the factors (treatments and phases) (p=0.5631). For the LFpen device, significant differences were indicated for phases (p<0.001) and the interaction between the factors (p=0.0418); no statistically significant difference was noted for the treatment (p=0.1662). For the FC device, significant differences were indicated for phases (p<0.001); no statistically significant difference was noted for treatments (p=0.1598) and interaction between the factors (p=0.5788). The Tukey *post hoc* test analysis showed that LF values were ranked from lower to greater values as phase 1 < phase 3 < phase 4 < phase 2; LFpen values were ranked as phase 1 < phase 3 = phase 4 < phase 2; and FC values were ranked as phase 1 = phase 3 = phase 4 < phase 2. LFpen fluorescence values were lower for RI when compared to FV in phase 3 (after treatment). It was interpreted that there was interaction between the factors the LFpen device because all treatment modalities were different in phase 3, which was not found for the other phases.

Fluorescence-based methods detected significant differences after the first *in situ* cariogenic challenge (phase 2). However, after treatments (phases 3 and 4), fluorescence values were significantly lower compared to those observed in phase 2. These results are confirmed in Table 4, which presents sensitivity, specificity, and area under the ROC curve values in phases 2 and 4 for all fluorescence-based methods (LF, LFpen, and FC devices).

Table 5 represents the inter-examiner reproducibility calculated using ICC for the fluorescence measurements for the experimental groups in all phases of the study. Intraclass correlation coefficient values varied from 0.3653 (FC device, group 1, phase 3) to 0.8857 (FC device, group 1, phase 4). The reproducibility values indicated fair to good agreement for the fluorescence-based methods in phases 1 and 2, and fair to excellent agreement in phases 3 and 4 for all experimental groups.

DISCUSSION

Different treatment modalities for the inhibition of incipient caries lesion progression on smooth surfaces have been discussed in the literature. Some studies have evaluated the synergistic effect of resin infiltration

Table 2: *Mean and Standard Deviation of the Lesion-integrated Mineral Loss (ΔΔZ, %vol.min x μm; ΔZ Initial Lesion – ΔZ Final Lesion) and Depth (μm; ΔLD = LD Initial Lesion – LD Final Lesion) for Enamel Specimens Treated With Different Materials (Experimental Groups) After Initial Demineralization*[a]

Experimental Groups (n)	ΔΔZ (%vol.min x μm) (ΔZ initial – ΔZ final lesion)	ΔLD (μm) (LD initial – LD final lesion)
FV (n=25)	-373.2 ± 113.8 A	-6.7 ± 4.3 A
RI (n=28)	-485.7 ± 187.9 B	-8.9 ± 6.1 A
AS (n=22)	-387.7 ± 132.4 A	-9.5 ± 4.6 A
p-value	0.016 [b]	0.126

Abbreviations: AS, adhesive system; FV, fluoride varnish; LD, lesion depth; RI, resin infiltrant; ΔΔZ, delta integrated mineral loss; ΔZ, integrated mineral loss; ΔLD, delta lesion depth; μm, micrometer; %vol.min, volume percentage of minerals.
[a]Significant differences are represented by different uppercase letters within the same column (ANOVA and Tukey post hoc test; [b] p<0.05).

Table 3: *Fluorescence Values (Mean ± Standard Deviation) of the Enamel Blocks with Different Treatment Modalities (Experimental Groups) in all Phases of the Study*[a]

Fluorescence-based Method	Experimental Groups	Phase 1	Phase 2	Phase 3	Phase 4
LF	FV	2.8 ± 2.1 A,a	17.1 ± 4.8 B,a	13.9 ± 4.1 C,a	14.1 ± 5.6 D,a
	RI	2.7 ± 2.3 A,a	17.0 ± 5.2 B,a	13.1 ± 3.6 C,a	14.6 ± 4.3 D,a
	AS	2.8 ± 2.1 A,a	16.6 ± 5.1 B,a	12.1 ± 4.0 C,a	14.3 ± 5.1 D,a
LFpen	FV	3.1 ± 2.4 A,a	20.7 ± 5.9 B,a	15.8 ± 5.4 C,a	15.2 ± 5.9 C,a
	RI	3.3 ± 2.4 A,a	20.4 ± 6.1 B,a	12.7 ± 3.1 C,b	15.1 ± 5.5 C,a
	AS	3.1 ± 2.0 A,a	19.9 ± 6.1 B,a	13.5 ± 3.3 C,a	14.9 ± 5.4 C,a
FC	FV	0.9 ± 0.1 A,a	1.1 ± 0.1 B,a	0.8 ± 0.1 A,a	0.9 ± 0.1 A,a
	RI	0.9 ± 0.1 A,a	1.1 ± 0.1 B,a	0.8 ± 0.1 A,a	0.8 ± 0.1 A,a
	AS	0.9 ± 0.1 A,a	1.1 ± 0.1 B,a	0.8 ± 0.1 A,a	0.8 ± 0.1 A,a

Abbreviations: ANOVA, analysis of variance; AS, adhesive system; FC, intraoral fluorescence camera; FV, fluoride varnish; LF, DIAGNOdent; LFpen, DIAGNOdent pen; RI, resin infiltrant.

[a] *Significant differences are represented by different lowercase letters within the same column and different uppercase letters with the same row (two-way repeated measures ANOVA and Tukey post hoc test;* $p<0.05$*).*

and fluoride application.[32,33] However, there is lack of comparison among them as separate treatments, as they have different mechanisms of action.

It is important to highlight that this is the first study that has evaluated caries lesion progression after different treatment modalities in a highly cariogenic environment, without the effect of residual fluoride from the dentifrice using an *in situ* model. To the best our knowledge, there is one *in situ* study in the literature that has evaluated the inhibition of caries progression through the use of resin infiltration and sealing. In this study, volunteers used the appliances for approximately 3 months, with two 30-minute exposures to 10% sucrose daily.[34] In the present investigation, the null hypothesis that there was no difference among the treatments based on an MID approach to inhibit the progression of incipient caries lesions was partially accepted. Fluoride varnish, RI and the AS were effective in inhibiting caries lesion progression, as indicated by the TMR analysis when considering LD (ΔLD). Considering the differences between the LD values at baseline and after treatment and the second cariogenic challenge, it can be assumed that they are not clinically relevant, as they represent a difference of less than 10 μm (as indicated by ΔLD). Concerning the integrated mineral loss (ΔΔZ) values, FV and the AS led to a statistically significant lower subsurface demineralization compared with the RI Icon. This fact might be explained by the differences

Table 4: *Sensitivity, Specificity, Area Under the ROC Curve (Az) Values, and Cut-off Points for LF, LFpen, and FC Devices for Phases 2 and 4*

Phase	Fluorescence-based Method	Sensitivity	Specificity	Az	Cut-off Points
2	LF	0.835	0.933	0.868	>8
	LFpen	0.990	1.000	1.000	>9
	FC	0.948	0.807	0.932	>0.9
4	LF	0.469	0.667	0.556	>14
	LFpen	0.401	0.745	0.545	>16
	FC	0.349	0.781	0.557	>0.8

Abbreviations: Az, area under the ROC (receiver operating characteristic) curve; FC, intraoral fluorescence camera; LF, DIAGNOdent; LFpen, DIAGNOdent pen.

Table 5: *Inter-examiner Reproducibility Represented by the Intraclass Correlation Coefficient and 95% Confidence Interval for LF, LFpen, and FC in the Experimental Groups for All Phases of the Study*

Fluorescence-based Method	Experimental Groups	Phase 1	Phase 2	Phase 3	Phase 4
LF	FV	0.6019 (0.3845-0.8057)	0.6575 (0.3983-0.8328)	0.4561 (0.3141-0.7345)	0.5400 (0.3570-0.6290)
	RI	0.6074 (0.4055-0.9060)	0.5136 (0.3123-0.6355)	0.6693 (0.3225-0.8386)	0.6268 (0.2354-0.8178)
	AS	0.5301 (0.3744-0.7706)	0.6244 (0.3305-0.8166)	0.6050 (0.3109-0.8072)	0.4529 (0.3207-0.7330)
LFpen	FV	0.6919 (0.4416-0.8982)	0.5106 (0.3075-0.7123)	0.7525 (0.4929-0.8792)	0.4913 (0.3214-0.7517)
	RI	0.6127 (0.3038-0.8128)	0.5504 (0.3260-0.7317)	0.5385 (0.4463-0.7747)	0.7981 (0.5865-0.9015)
	AS	0.6626 (0.4562-0.8226)	0.6629 (0.3094-0.8354)	0.7576 (0.5035-0.8817)	0.4996 (0.3502-0.7558)
FC	FV	0.6884 (0.5080-0.8405)	0.7210 (0.4285-0.8638)	0.3653 (0.3001-0.6902)	0.8857 (0.7659-0.9442)
	RI	0.6750 (0.5546-0.9193)	0.7446 (0.4767-0.8753)	0.7187 (0.4238-0.8627)	0.5003 (0.2285-0.7073)
	AS	0.6937 (0.3721-0.8078)	0.6000 (0.3806-0.8047)	0.4510 (0.3633-0.5898)	0.6832 (0.6832-0.8454)

Abbreviations: AS, adhesive system; FC, intraoral fluorescence camera; FV, fluoride varnish; LF, DIAGNOdent; LFpen, DIAGNOdent pen; RI, resin infiltrant.

in the mechanism of action and application protocol of the products (per the manufacturer's instructions). The fact that RI demonstrated higher mineral loss can be attributed to the rather harsh etching procedure (the use of 15% hydrochloric acid for 2 minutes) that may have led to the removal of the surface layer of the lesion, increasing the subsurface demineralization, as shown by Freitas and others[35] in an *in vitro* model. In contrast, the study by Paris and Meyer-Lueckel[34] replaced the 15% hydrochloric acid with 37% phosphoric acid for 5 seconds for both RI and sealing treatments, for better permeability and to diminish the surface layer removal. Moreover, our results corroborated the study by Gelani and others[32] (with respect to $\Delta\Delta Z$ values), who used an *in vitro* model for evaluating lesion progression. In their study, fluoride gel was able to inhibit lesion progression in the same way as resin infiltration combined with fluoride gel application, which was significantly better than RI alone. According to Meyer-Lueckel and Paris[36]—and also employed in the present study—RI should be applied after 2 minutes of etching with HCl to penetrate more deeply and occlude the enamel pores generated by demineralization, to inhibit lesion progression. Thus, considering the mechanism of action of resin infiltration, greater mineral loss detected in this group might not be an indicator of lack of effectiveness.

One of the topics of *in situ* and *in vitro* investigations is the degree of demineralization and LD (<50 μm) of artificial caries-like enamel lesions, which is not consistent with what is observed *in vivo,* as discussed previously.[35,37,38] In the present study, the mean LD was approximately 20 ± 6.5 μm, which can influence the results of the different treatment modalities.

Regarding the SMH analysis, a similar pattern of inhibition of lesion progression was observed after the second cariogenic challenge. It should be noted that the surfaces treated with resin infiltration and an AS exhibited lower SMH values after phases 3 and 4. Previous studies have shown increased caries lesion microhardness after resin infiltration,[35,39,40] since the low viscosity resin fills the lesion and creates a barrier to the lesion and the lesion body. However, the studies by Torres and others[41] and Neres and others[42] demonstrated a significant reduction in SMH of the group treated with resin infiltration after a new cariogenic challenge, which can be attributed to the incomplete dissolution of the remaining mineral content of the lesion body

that was not fully embedded in the resin matrix or polymerization contraction. Neres and others[42] also demonstrated higher surface roughness (ie, grooves and cracks) after enamel conditioning with HCl.

Adhesive systems do not have high penetration power, and, when present, penetration occurs only on the surface of natural enamel caries lesions.[8] However, in this study, the AS acted as a barrier to the new cariogenic challenge in the same pattern as the RI,[43] which might be attributed to the shallow artificial lesion produced in this study model. This is consistent with the literature, which shows better effectiveness in protecting enamel dissolution in early enamel lesions.[44] Previous studies using ASs to penetrate into artificial caries lesions showed surface sealing instead of penetration and occlusion of pore space.[37,45] On the other hand, RI leads to a full, but partially inhomogeneous, penetration of artificial caries lesions.[44] In addition, it is a treatment option for active white spot lesions because it promotes an aesthetic masking of these lesions,[39,46-48] with some color change after a new acid challenge.[46,49] If phase 2 had been completely skipped, the results for both the resin infiltration and AS treatments would probably be different for microhardness analysis, since it would be performed on a sound surface with no incipient lesion. Moreover, it is important to mention that applying HCl also promotes a rough surface and could influence SMH values.

With respect to the group treated with FV, a reduction in surface microhardness was also observed after phases 3 and 4. However, these SMH values were significantly higher than the values observed for RI and ASs. Fluoride precipitates calcium from saliva and promotes the formation of calcium fluoride (CaF_2) reserves when highly concentrated agents are used. In the present study, the higher cariogenic environment and the absence of daily fluoride dentifrice use by the participants may have led to an increase in the dissolution rate of CaF_2, thereby increasing the demineralization rate of the lesion. However, when using fluoride therapies, the lesion body does not remineralize to the same level of the previous surface zone.[50] The remineralization of the outer surface of the enamel does not improve the aesthetics and structural properties of the deeper lesion.[51] It should be emphasized that FV was applied only once, simulating a professional clinical condition.[40] Thus, the short contact time between the varnish and the surface may have influenced the results. It is already known that regular applications of FV may increase anticaries properties.[40] In this context, RI can have a better aesthetic resolution, as it can penetrate the deeper layers of the lesion. However, it can also present color alteration over time after staining processes.[49]

The second hypothesis of this investigation was rejected, as the fluorescence-based methods were able to monitor the enamel lesion progression. In general, the fluorescence values showed substantial differences between phases 1, 2, and 3 for all treatment modalities, proving to be effective for monitoring the progression of *in situ* enamel caries lesions. These results corroborate the findings of Spiguel and others,[21] Moriyama and others,[22] Diniz and others,[17] and Rodrigues and others,[18] who also used artificial caries-like lesions on smooth surfaces. The fluorescence values of LF and LFpen were significantly higher after *in situ* cariogenic challenge when compared with baseline values. According to Mendes and Nicolau,[14] an increase in LF values after artificial demineralization could be explained by an increase in porosity and light scattering on the enamel surface. However, LF's effectiveness is uncertain in artificial lesions created with no oral bacterial metabolites, as the LF device identifies changes in the organic content of the tooth structure (fluorophores and other chromophores produced by cariogenic bacteria) rather than inorganic content.[10,11] Previous studies reported that an increase in fluorescence values can be related to the penetration of bacteria, which produce substantial amounts of endogenous porphyrins and organic compounds, into the enamel lesions.[17,21]

It should be noted that LF and LFpen measurements were different at all phases of this investigation. The LFpen fluorescence values were higher than LF values, as shown in previous studies.[13,17,52,53] These differences between LF and LFpen fluorescence values can be associated with the type of probe tips in both devices (diameters and materials), which may impact the amount of light excitation and the level of fluorescence emitted by the dental tissues.

A significant difference could be observed between phases 3 and 4 for the LF device, with higher fluorescence values in phase 4, but the difference was not clinically relevant. In contrast, LFpen and FC devices demonstrated similar fluorescence values between phases 3 and 4. This was expected, as the different treatment modalities were able to hamper the lesion progression *in situ* in the same way. In recent clinical studies,[25,26] LFpen was able to detect significantly lower fluorescence values immediately after resin infiltration application and after six months of follow-up on white spot lesions on buccal surfaces when compared with baseline. The main idea of using fluorescence-based methods to monitor the inhibition of incipient caries lesion progression would be to detect a decrease in

light scattering on the enamel surface with the use of resin infiltration and, consequently, the change in fluorescence values. In an *in vitro* study, Markowitz and Carey[24] evaluated the LF device in assessing the ability of resin infiltration to improve the optical properties of artificial white spot lesions. The authors found no differences in the fluorescence values when analyzing the effect of demineralization and RI treatment on the appearance of the tooth structure. The fluorescence values remained at the low end of the instrument's range (close to zero). This fact could be attributed to the caries induction with pH 4.5 lactic acid gel and the absence of bacterial biofilm.

When evaluating the performance of fluorescence-based methods for caries detection and monitoring, there is no scientific evidence on the optimal cut-off points that should be used to determine the extent of caries lesions on smooth surfaces. In the present study, the baseline (sound surface) fluorescence values are consistent with the scale recommendation proposed by the manufacturers and by Lussi and Hellwig.[12] However, the range of fluorescence values for LF and LFpen devices in phases 2, 3, and 4 indicates enamel caries lesions. For the FC device, the range of fluorescence values in phases 3 and 4 are in the range of a sound surface, according to the manufacturer's interpretation (0.0-1.0), which could be a limitation of the device in detecting the progression of treated initial lesions.

The optimal cut-off points obtained by the ROC analysis for the detection of enamel lesions were >8 (LF), >9 (LFpen), and >0.9 (FC). The cut-offs for the detection of enamel lesion progression after treatment modalities were >14 (LF), >16 (LFpen), and >0.8 (FC). In phase 2, higher sensitivity, specificity, and Az values were observed for the fluorescence-based methods, showing their ability to identify initial caries lesion development, similar to the findings of Diniz and others.[17] However, in phase 4, the sensitivity and Az values were lower for all fluorescence methods, showing a moderate capacity to monitor the caries progression after different treatments. Another important aspect is related to the cut-off point for the FC device in this phase, which is not consistent with fluorescence values of enamel lesion progression and instead indicates a sound enamel surface. Thus, care must be taken when interpreting the FC values in monitoring incipient lesions.

A reliable caries detection method should present consistent data between diverse examiners and evaluations in order to be useful for monitoring carious development.[12] In general, the inter-examiner reproducibility varied from fair to excellent values for all fluorescence devices and phases of the study, with different results according to the treatment modality. For the FC device, the wide range of agreement between the examiners was observed for the group treated with FV in phases 3 and 4. Previous studies have demonstrated good to excellent results for fluorescence-based devices in detecting and monitoring caries progression.[13,16,17,22]

Some limitations of the present investigation should be indicated, such as the depth of the enamel lesion that was expected to be deeper, the *in situ* study design that did not include a crossover model, and the high cariogenic challenge model simulating a patient with high caries risk. It should be mentioned that SMH analysis was performed after each phase of this *in situ* study once the surface test was not destructive and consider further surface assessments.[17,20-22,27] To confirm the results, TMR was done to provide a quantitative measure of the mineral content and LD, since it is an invasive technique that allows for the comparison of differences between the areas of demineralization of each phase.[27]

Despite the promising results of this *in situ* study, which is a close representation of the oral environment, new investigations should be performed in clinical conditions to confirm the capacity of different treatment modalities in order to inhibit enamel lesion progression and to confirm the performance of fluorescence-based methods in monitoring this process. It is important to highlight that fluorescence-based methods are adjunct methods and must be used in combination with visual examination in clinical practice.

CONCLUSION

It can be concluded that FV, RI and AS were effective in inhibiting the *in situ* progression of incipient caries lesions, although at different levels, with minor mineral loss changes for AS and FV. In addition, the fluorescence-based methods were effective in monitoring caries lesion progression for all treatment modalities, except for the FC device.

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Regulatory Statement

This study was approved by the local institutional review board of Cruzeiro do Sul University (protocol #013/2015, Brazil) and by the local animal research committee of Cruzeiro do Sul University (protocol #001/2015; Brazil) for the use of bovine teeth.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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