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Original

Micro-computed tomographic analysis of progression of artificial enamel lesions in primary and permanent teeth after resin infiltration

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Abstract: We investigated inhibition of lesion progression in artificial enamel lesions. Lesions were created on primary and permanent anterior teeth (*n* = 10 each) and were divided randomly into two groups with two windows: Group 1 (window A: resin infiltration; window B: negative control) and Group 2 (window A: resin infiltration + fluoride varnish; window B: fluoride varnish). After pH cycling, micro-computed tomography was used to analyze progression of lesion depth and changes in mineral density. Resin infiltration and resin infiltration + fluoride varnish significantly inhibited progression of lesion depth in primary teeth (*P* < 0.05). Inhibition of lesion depth progression in permanent teeth was significantly greater after treatment with resin infiltration + fluoride varnish than in the negative control (*P* < 0.05). Change in mineral density was smaller in the resin infiltration and resin infiltration + fluoride varnish groups; however, the difference was not significant for either group (*P* > 0.05). Resin infiltration is a promising method of inhibiting progression of caries lesions. (J Oral Sci 57, 177-183, 2015)

Keywords: fluoride; initial caries lesions; μCT; resin infiltration.

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Introduction

An ideal approach for treating non-cavitated enamel lesions—which are characterized by loss of minerals in the lesion body and comparatively high mineralization of the surface—is to maintain enamel integrity and minimize use of restorative treatment procedures (1). At an early stage, these lesions can be arrested or even remineralized by daily oral health procedures in patients with good oral hygiene (2,3). However, adherence to the required daily oral health procedures might be insufficient among children and adolescents.

For this reason, it has been suggested that infiltrating the existing lesion to prevent contact with oral fluids could arrest lesion progression (4,5). Recently, a new material, ICON (DMG, Hamburg, Germany), was developed to facilitate infiltration of low-viscosity resins into initial caries lesions. The effectiveness of this novel material has been tested *in vitro*. Several studies have investigated progression of artificial caries lesions using confocal microscopy and transversal microradiography (6,7). However, these methods require destructive sample preparation, and longitudinal analyses of the same lesion involve preparation of fragile single sections, which may be lost during the course of a study (8,9).

Micro-computed tomography (μCT) is an innovative, noninvasive, and nondestructive approach for investigating the processes that occur in dental hard tissues (10). μCT allows the use of the same specimens for several biological and mechanical tests (11,12). Additionally, μCT facilitates evaluation of the quantity and quality of dental tissues, depth of caries lesions (13-15), and

Table 1 Composition of materials used in the study

Materials	Composition
Resin infiltrant ICON	TEGDMA-based resin matrix, initiator, additives
Fluoride varnish Duraphat	50 mg sodium fluoride, equivalent to 22.6 mg fluoride, in an alcohol solution of natural resins
Negative control physiologic serum	0.85% sodium chloride solution

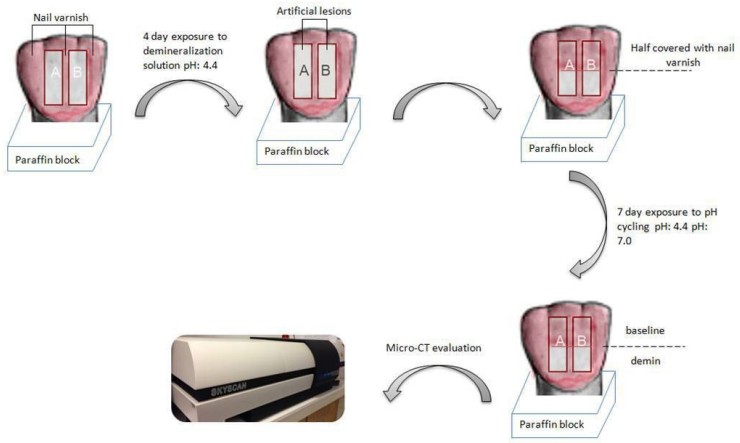


Fig. 1 Experimental set-up.

mineral loss in initial enamel caries lesions (11,16).

No study has used μCT to evaluate inhibition of lesion progression with the resin infiltrant ICON. Thus, this study used μCT to investigate inhibition of lesion progression in artificial primary and permanent enamel lesions after using resin infiltration, with and without fluoride varnish, and compares changes in lesion depth and mineral density associated with the use of fluoride varnish and physiologic serum.

Materials and Methods

The research protocol was approved by the Ethics Committee of the Ankara University Faculty of Dentistry in Ankara, Turkey (No: 19.10.2010-02/02). Oral and written informed consent was provided by the patients or parents/guardians of the patients.

Using data from previous μCT studies (14,15), we conducted a power analysis (Power and Precision software, Biostat, Englewood, NJ, USA). The findings indicated a minimum sample size of $n = 5$, based on an α of 5% and a power of 80%.

Lesion formation

Ten extracted or naturally exfoliated primary anterior teeth (canines and maxillary incisors) and 10 permanent anterior teeth extracted for periodontal reasons were cleaned of soft tissue debris and inspected for cracks, hyperplasia, and white spot lesions under a stereomicroscope (Leica MZ12, Leica Microsystems, Wetzlar, Germany). The 10 primary and permanent anterior teeth were then coated with an acid-resistant nail varnish (Maybelline, New York, NY, USA). Two narrow rectangular windows (approximately 4 × 2 mm) were left on the sound, intact buccal surface, and the teeth were embedded in paraffin blocks. The teeth were then immersed in demineralization solution (50 mL/tooth) for 96 h in a shaking incubator to produce lesions with a depth of 150-200 μm. The demineralization solution used for creation of the initial lesions and during pH cycling contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05 M acetic acid; pH was adjusted to 4.4 with 1 M KOH (17,18). After demineralization, each tooth had two initial enamel lesions (A, B), separated by nail varnish. The teeth were divided randomly into two equal groups ($n = 5$ each) and stored at 100% humidity until testing.

Test groups

For each tooth group, two windows were treated with different protocols. The compositions of the materials are shown in Table 1.

Group 1

Window A (resin infiltration): in accordance with the manufacturer's instructions, this window was etched with 15% HCl gel for 120 s, rinsed for 30 s, and dehydrated with 100% ethanol for 30 s. ICON (DMG, Hamburg, Germany) was applied with a microbrush for 3 min, excess material was gently removed with a small cotton pellet, and the resin was light-cured (Translux

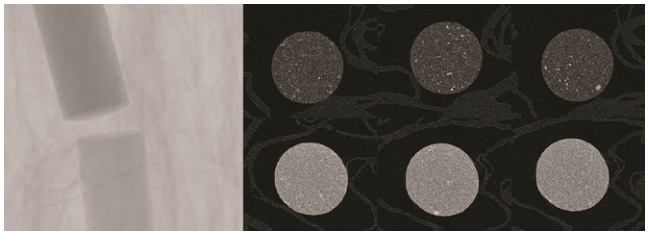


Fig. 2 Reconstructed images of conical phantoms at 0.25 and 0.75 gHAp cm^{-3}.

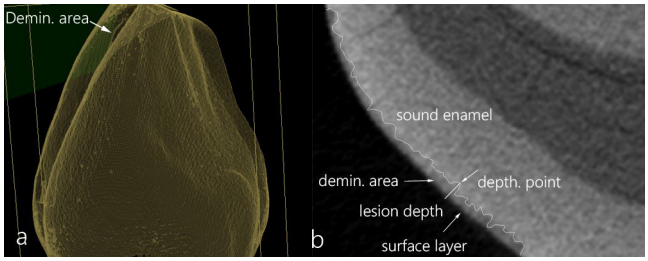


Fig. 3 (a) a 3D representation of the demineralized area, (b) a µCT image of a specimen, including the surface and inner area of the enamel.

CL; Heraeus Kulzer, Hanau, Germany) for 40 s at 400 mW/cm^2. ICON was then applied a second time for an additional 1 min.

Window B (negative control): physiologic serum was applied with a microbrush.

Group 2

Window A (resin infiltration + fluoride varnish): ICON was applied as described above. After ICON application, a small amount of Duraphat (Colgate, Palmolive, Waltrop, Germany) was applied with a microbrush.

Window B (fluoride varnish): a small amount of Duraphat was applied with a microbrush. To evaluate progression in the infiltrated lesions, half of the specimens were covered with a nail varnish (Fig. 1). One half of each specimen was used to measure lesion depths and mineral density directly after infiltration (baseline). The remaining halves were exposed to pH cycling for 7 days to simulate a cariogenic environment (Demin; Fig. 1).

Demineralizing and remineralizing solutions

The demineralization solution used for creation of the initial lesions and during pH cycling contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05 M acetic acid; pH was adjusted to 4.4 using 1 M KOH. The remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl; pH was adjusted to 7.0. This solution simulated supersaturation of apatite minerals found in saliva and was similar to that used by Cate and Duijsters (17,19).

Model of pH cycling

The teeth underwent pH cycling in a shaking incubator for 7 days. Each cycle involved 3 h of demineralization twice per day with 2 h of remineralization in between. Sections were then placed in the remineralizing solution overnight. Fresh demineralizing and remineralizing solutions were prepared for each cycle, and separate containers were used for each group throughout the experimental period. Each demineralization and remineralizing session was followed by thorough rinsing with clean water.

µCT evaluation

A high-resolution desktop µCT system (Skyscan 1172, Skyscan, Kontich, Belgium) was used to scan the specimens. The scanning conditions were 1,000 kVp, 100-mA beam current, 0.5-mm Al/Cu filter, 13.74-µm pixel size, rotation in 0.5º steps, and 40% beam hardening. To minimize ring artifacts, the detector was air-calibrated before each scan. Each sample was rotated 360° within an integration time of 5 min. The mean scanning time was about 2 h. Other settings included beam-hardening correction and input of optimal contrast limits (0-0.0005), based on data from prior scanning and tooth reconstruction.

µCT image analysis

Nrecon software (ver. 1.6.7.2; Skyscan) and CtAn (ver. 1.12.9, Skyscan) were used for visualization and quantitative measurement of samples. A modified version of the algorithm described by Feldkamp et al. (20) was used to obtain axial 2-dimensional (2D) 1,000 × 1,000-pixel images. For the reconstruction parameters, ring artefact correction and smoothing were fixed at zero, and beam artefact correction was set at 40%. The contrast limits were those specified in the SkyScan instructions. A similar procedure was used to measure the gray values of two bone mineral density (BMD) phantom rods. To aid mineral density (MD) calculations, grayscale values were converted into MD values (gHAp cm^{-3}) with a linear calibration curve based on grayscale values obtained from two mineral concentration conical phantoms of 0.25 and 0.75 gHAp cm^{-3} (Fig. 2). Calculations of difference in mineral loss (ΔZ; gHAp cm^{-3}) for each specimen were made by calculating MD values from the entire demineralized area as the mean loss of surface and inner part of the demineralized area (Fig. 3). Mineral loss was calculated by subtracting demin MD values from baseline phantom rod MD values, to correct alignment inaccuracies from the specimen.

Lesion depth (LD) was calculated by subtracting the area of demineralization from the estimated area of sound

enamel before demineralization and pH cycling. For each tooth area, 3 calculations from 3 enamel levels were performed, and the mean value was used as the final LD. LD difference (ΔLD; μm) was calculated by subtracting the demin LD values in each group from baseline LD values (Fig. 4).

Statistical comparisons

Differences in ΔLD and ΔZ between groups were examined with the Mann-Whitney *U* test. The Kruskal-Wallis H test with Bonferroni correction was used to determine the statistical significance of observed differences between groups. All analyses were performed using SPSS software (ver. 20; Chicago, IL, USA) at a significance level of $\alpha = 0.05$.

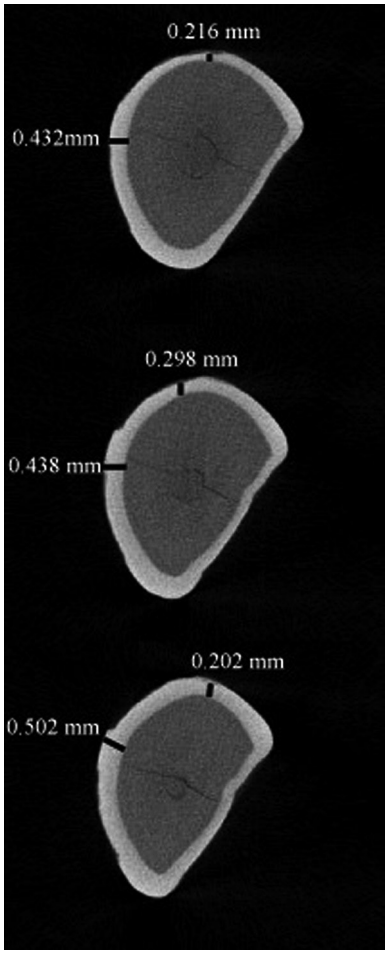


Fig. 4 Measurement of lesion depth.

Results

Primary teeth

The differences in ΔZ among the four groups were not statistically significant ($P > 0.05$). ΔZ was 0.31 g/cm^3 for the negative control, 0.27 g/cm^3 for fluoride varnish, 0.14 g/cm^3 for resin infiltration, and 0.13 g/cm^3 for resin infiltration + fluoride varnish (Table 2).

There were statistically significant differences in ΔLD between groups. Progression of caries depth was significantly greater in the negative control and fluoride varnish groups than in the resin infiltration and resin infiltration + fluoride varnish groups ($P < 0.05$). Progression of caries depth was 64.4 μm in the negative control, 57.2 μm for fluoride varnish, 21 μm for resin infiltration, and 5.78 μm for resin infiltration + fluoride varnish (Table 2).

Permanent teeth

Differences in ΔZ between groups were not statistically significant ($P > 0.05$). ΔZ was 0.23 g/cm^3 for the negative control and fluoride varnish, 0.17 g/cm^3 for resin infiltration, and 0.14 g/cm^3 for resin infiltration + fluoride varnish (Table 2).

Progression of caries depth was 56.98 μm for the negative control, 54.8 μm for the fluoride varnish, 26.34 μm for resin infiltration, and 13.74 μm for resin infiltration + fluoride varnish. As compared with the negative control, caries inhibition was significantly greater for resin infiltration + fluoride varnish ($P < 0.05$).

In analysis comparing primary and permanent teeth, there were no statistically significant differences between groups ($P > 0.05$). There was no significant difference in ΔZ or ΔLD between primary and permanent teeth for any of the applied materials.

Table 2 Mean ΔZ and ΔLD values in each group

	Negative control	ICON	Duraphat	ICON + Duraphat
Primary teeth				
ΔZ	0.31 ± 0.12	0.14 ± 0.05	0.27 ± 0.25	0.13 ± 0.06
ΔLD	64.4 ± 0.04^a	21 ± 0.06^b	57.2 ± 0.03^a	5.78 ± 0.03^b
Permanent teeth				
ΔZ	0.23 ± 0.09	0.17 ± 0.09	0.23 ± 0.12	0.14 ± 0.08
ΔLD	56.98 ± 0.09^a	26.34 ± 0.14^a	54.8 ± 0.20^a	13.74 ± 0.09^b

Within rows, different superscript letters indicate significant differences ($P < 0.05$).

Discussion

Resin infiltration is a relatively new method for treating initial caries lesions. To our knowledge, this is the first report to use μCT to evaluate progression of artificially induced initial caries lesions. There are several methods of measuring mineral concentrations and densities of hard dental tissues, including chemical analyses of microsamples, contact microradiography, and transversal microradiography (12). However, these methods require time and are destructive. In contrast, μCT measures the mineral density of hard tissues such as bones and teeth, has a resolution of 5-30 μm, and is more accurate than other methods (12,14). Because the thickness of the sample used for μCT depends on the size of the X-ray beam, slices of the sample examined can be thinner and more regular than samples prepared by physical cutting (10).

Studies using μCT to assess bone morphology and architecture have shown that the results correlate well with 2D histologic sections and that μCT evaluation is thus an alternative method to nondestructive bone biopsies in analyzing 3D bone architecture (21,22).

In a comparison of the reliability of transversal microradiography and μCT in detecting lesion depth and mineral density of caries lesions, Hamba et al. (14) showed that, when using an Al/Cu filter μCT system, LD and ΔZ measurements correlated with those of transversal microradiography. They concluded that μCT is preferable for studies of demineralization and remineralization, because of its nondestructive nature.

Lautensack et al. (23) reported that synchrotron X-ray μCT yielded high-resolution images and was able to show variations in density inside the enamel structure. They concluded that quantitative image analysis methods allow for visual examination and quantification of the degree of demineralization in different samples. With μCT, the 3D morphology of demineralization areas can be examined, which is not possible with classical 2D imaging techniques.

Oral health procedures such as brushing and flossing are recommended for patients as the first step in preventing caries lesions (24). However, Duraphat or other high-concentration fluoride methods are considered the treatment of choice for such lesions (25).

Resin infiltration is currently recommended for arresting lesion progression in non-cavitated initial caries lesions. Resin infiltration acts through capillary movement to form a barrier in the caries lesion and enhances the enamel structure, thereby preventing enamel breakdown and subsequent cavity formation. The resin used for infiltration has a high penetration coefficient, and the enamel acts as a sponge that absorbs the infiltrant. The infiltrant completely fills the porous structure, preventing diffusion of organic acids into the lesion and inhibiting caries progression (4,5).

In our study, infiltrated lesions in primary teeth were significantly shallower than those in the negative control and fluoride groups. These findings are consistent with those of previous studies of commercially available adhesives used for sealing incipient caries lesions (6,7,26). The results of those earlier studies led to the development of the ICON material used here.

The results of an *in vitro* study of the color-masking effects of ICON showed that it was effective in masking white spot lesions and returning teeth to their natural color. However, after a new acid challenge, the resin infiltration exhibited some color changes, which suggests that some demineralization can still occur after resin infiltration treatment. Such demineralization may be caused by partial dissolution of the remaining minerals in the lesion body, which were not completely embedded within the resin matrix, or by resin shrinkage during light-curing (27).

Fluoride can remineralize incipient caries lesions at a superficial level. However, deeper segments of the enamel are more susceptible to demineralization, because the outer enamel is less soluble than the inner enamel (28-30). In the present study, we used a combination of resin infiltration with fluoride varnish to assess the effects of fluoride on remineralization. After resin infiltration, fluoride varnish was used for its remineralizing effect on the outer enamel, to provide better protection against continuous acid attacks if the resin matrix failed to infiltrate the entire lesion because of resin shrinkage.

In the present study, we compared the use of resin infiltration alone and in combination with fluoride varnish to determine whether fluoride varnish further inhibited lesion progression. Resin infiltration + fluoride varnish was better in arresting the initial lesions, as indicated by lesion depths in primary and permanent teeth. Although the difference between resin infiltration and resin infiltration + fluoride varnish was not statistically significant, the difference may still be clinically important. The absence of a statistically significant difference may have been due to the small number of samples.

Although ΔZ did not significantly differ between groups, the ranking of ΔZ values was resin infiltration + fluoride varnish < resin infiltration < fluoride varnish < negative control in primary teeth, and resin infiltration + fluoride varnish < resin infiltration < fluoride varnish = negative control in permanent teeth.

When permanent and primary teeth were compared,

there were no statistically significant differences. However, inhibition in permanent teeth was significantly greater for resin infiltration + fluoride varnish than for the negative control. In primary teeth, both resin infiltration and resin infiltration + fluoride varnish resulted in significantly shallower lesions as compared with the negative control and fluoride varnish groups. However, for resin infiltration and resin infiltration + fluoride varnish, progression of lesion depth did not significantly differ between primary and permanent teeth.

This study has some limitations. Difficulties in collecting caries-free primary teeth during exfoliation, economic constraints, and limits in access to μCT limited the number of teeth included in the study groups and the number of scans. Although the power analysis indicated that at least five teeth should be used in each study group, a large sample of both primary and permanent teeth is needed, and further studies with larger samples are necessary.

Another limitation of the study is the degree of initial lesion depth. In this study, μCT was performed after application of the materials. However, to standardize the procedure, μCT scans should be obtained before and after application, to select the same degree of mineral loss and lesion depth for each sample. Future studies of ΔZ and ΔLD should be done in this manner.

In the present study, the results for ΔZ and ΔLD differed. Although change in mineral density did not significantly differ between groups, this may have been due to the above-mentioned limitations, and the numerical differences may still be clinically important. It is possible that in a study with a larger number of samples, change in mineral density would differ significantly and correlate with change in lesion depth. However, this is the first study to investigate the effects of resin infiltration, alone and in combination with a fluoride varnish, and compare the findings with those from fluoride varnish alone and a control group. Within the limitations of our study, the resin infiltration technique combined with fluoride varnish shows promise in arresting progression of initial caries lesions in primary and permanent teeth. However, the synergistic effects of the materials should be further evaluated, *in vitro* and *in vivo*, in studies with larger samples.

In conclusion, resin infiltration is a promising method for inhibiting progression of caries lesions in primary and permanent teeth, and its use in combination with fluoride products results in further inhibition. To confirm these promising findings, future studies will require larger samples and, possibly, natural lesions.

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