Original Article

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Dentistry Section

Comparative Evaluation of Enamel Surface Roughness after Minimally Invasive Treatment of White Spot Lesions: An In-vitro Experimental Study

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ABSTRACT

Introduction: White Spot Lesions (WSLs) represent the initial demineralisation of enamel. In the past, invasive treatment methods required the removal of marginal tissue, which weakened the residual tooth structure. A further understanding of caries development, coupled with technological advancements, has led to a change in the paradigm, with non invasive or microinvasive treatments favoured over traditional restorative methods.

Aim: To evaluate the surface roughness of resin-infiltrated proximal WSLs with ICON® subjected to a pH cycling challenge and to compare its surface roughness with that of WSLs treated with Duraphat®.

Materials and Methods: An in-vitro experimental study was conducted at the Department of Restorative Dentistry, Faculty of Dentistry, Universiti Malaya, Kuala Lumpur, Malaysia, between September 2019 and July 2021. A total of 60 extracted sound premolars were included in the research and the teeth were randomly divided into four groups of 15 specimens each. The groups were assigned as Sound (negative control), Demineralised (positive control), ICON® and Duraphat®. All specimens, except for the sound group, were subjected to initial demineralisation in a standard acid buffer demineralisation solution without fluoride at pH 4.5 for seven days and enamel changes were confirmed using Optical Coherence Tomography (OCT). Resin infiltration (ICON®) and fluoride varnish (Duraphat®) were applied to their corresponding groups. A non contact profilometer (3D Alicona) was used to measure surface roughness (Ra) at baseline and after pH cycling; initial and post-cycling mean differences were recorded. Statistical data were analysed using one-way repeated measures Analysis of Variance (ANOVA). A p-value of <0.05 was considered statistically significant.

Results: The R_a at baseline revealed significant differences across the groups, except for ICON® (0.31 µm±0.01), compared to sound enamel (0.31 µm±0.03) with a p-value <0.05. After pH cycling, the enamel surfaces treated with ICON® (0.42 µm±0.01) were significantly smoother than those treated with Duraphat® (0.58 µm±0.01), with p-values <0.001. After seven days of acidic challenge, ICON® exhibited the least R_a change (0.11 µm).

Conclusion: The WSLs treated with ICON® showed approximately the same surface roughness as sound enamel, suggesting that the risk of developing caries around WSLs treated with ICON® is comparable to that around sound enamel.

Keywords: Dental resin, Fluoride varnishes, Optical coherence tomography

INTRODUCTION

The contemporary definition of dental caries is: "Caries is a dynamic, complex, multifactorial process involving the gradual loss of mineral compounds from hard dental tissue." The formation of initial caries in enamel occurs due to demineralisation, which is primarily caused by lactic acids produced by cariogenic bacteria such as *Streptococcus mutans* and Scardovia wiggsiae [1,2]. If the biofilm's pH level falls below the critical level of 5.5 and is not removed, minerals such as hydroxyapatite are lost, leading to an increase in the porosity between the enamel crystals. This softens the surface and allows the diffusion of acids, resulting in the demineralisation of the enamel subsurface. The increase in microporosities causes the development of WSLs on the enamel [2].

The WSLs represent the initial demineralisation of enamel. A rough, white-opaque appearance can be observed when caries is in an active state, but it exhibits a shiny and smooth surface when inactive [3]. Occasionally, WSLs can appear as a brown colouration caused by the absorption of extrinsic pigments by decalcified enamel [4]. According to the International Caries Detection and Assessment System (ICDAS), WSLs with no evidence of surface breakdown or underlying dentine shadowing can be scored as a 1 (initial caries) or 2 (distinct visual change in the enamel) [3,5].

Proximal zones have a high prevalence of caries due to the difficulty in cleaning these areas, coupled with poor dental hygiene compliance [6]. In the past, invasive treatment methods required the removal of marginal tissue, which weakened the residual tooth structure. A further understanding of caries development, coupled with technological advancements, has led to a change in the paradigm, where non invasive or microinvasive treatments are favoured over traditional restorative methods. One of the established non invasive treatments is fluoride varnish (Duraphat®), which is often referred to as the standard care for early carious lesions [7]. Fluoride enhances the hydroxy ions in hydroxyapatite crystals, forming fluor(hydroxy) apatite, which is more resistant to acid dissolution. It also promotes tooth remineralisation by adsorbing onto the surface of partially demineralised crystals and attracting calcium ions to help grow fluorapatite crystals [7]. Non invasive treatments manage carious lesions through mechanical removal of the biofilm, dietary control, or remineralisation [8,9].

Microinvasive treatments have been developed as alternatives that rely less on patient compliance and are generally more conservative than other treatment options. These therapies aim to halt the progression of initial carious lesions by infiltrating the microporosities within the enamel using a low-viscosity liquid resin. Caries infiltration is recognised as a microinvasive option for non cavitated enamel lesions that extend into the outer third of the dentine. This method preserves the integrity of the carious lesions. Infiltration and sealing techniques are commonly employed in these microinvasive

treatments. Clinically, infiltration technology has been used for non cavitated proximal caries [10].

Utilising capillary forces, the low-viscosity resin penetrates the pores of demineralised enamel. Once it sets, it forms a barrier that prevents acid diffusion, thereby protecting the tooth structure [11].

The Infiltration Concept (ICON®) (DMG Chemisch-Pharmazeutische Fabrik GmbH, Hamburg, Germany) is a commercially available resin infiltrant developed to arrest intermediary lesions in one visit with no mechanical preparation or anaesthesia. The approximal version of the product is specifically developed for hard tissues, preserving the treatment of incipient proximal caries. Not much is known about the long-term stability of the resin and the possible effects of the surface alterations. Resin degradation might result in surface destruction and the development of plaque on these sites due to an increase in surface roughness [11,12]. Therefore, resin degradation may be a risk factor for increased plaque accumulation and the development of secondary caries [13].

The current challenges in the in-vitro clinical validation of anti-caries treatments aimed at preventing tooth decay arise from a scarcity of studies that accurately replicate in-vivo delivery conditions [14,15]. Moreover, although much research on enamel surface roughness has focused on ground sections, there is a lack of data regarding natural enamel surfaces, particularly concerning intact proximal contacts that have successfully simulated the application of materials to teeth with these intact contacts. Therefore, the present study was conducted to determine whether treating WSLs with minimally invasive techniques could prevent further bacterial colonisation and retention by maintaining surface roughness within acceptable limits. The present study aimed to evaluate and compare the surface roughness of proximal WSLs treated with ICON® and Duraphat® after initial application and following a seven-day acidic challenge.

MATERIALS AND METHODS

An in-vitro study was conducted at the Department of Restorative Dentistry, Faculty of Dentistry, Universiti Malaya, Kuala Lumpur, Malaysia, between September 2019 and July 2021. A total of 60 extracted sound permanent human premolar teeth were obtained from private dental clinics and dental institutions in Klang Valley, Malaysia. Ethics approval was obtained from the Ethics Committee/IRB of the University of Malaya (Reference number: DF RD1927/0090 (P)) before conducting the study.

Study Procedure

The teeth were cleaned to remove debris and blood and they were disinfected in a 0.5% chloramine solution for one week. Leftover debris and residue were then removed using a prophylaxis brush and pumice powder and the teeth were stored in distilled water at 37°C.

Application jigs for maxillary and mandibular study models were fabricated using epoxy resin, featuring missing second premolars and first molars in each quadrant. The 'missing' teeth/empty slots in the models were replaced with artificial teeth in both the molar and premolar slots. Each tooth was embedded into a polyvinylsiloxane putty (Flexceed®, GC Dental Products Corp., Japan) before placement into the slot. The tooth orientation was adjusted to simulate intraoral contact between adjacent teeth. The dimensions of the contact points did not exceed 3 mm buccolingually and 2 mm occlusally-gingivally [16]. The consistency of the proximal contact was registered as a "snap" when dental floss was passed through the contact point. Upon setting, contact points and areas of demineralisation were determined and the putty was numbered accordingly [Table/Fig-1].

Sample preparation: Working window preparations were made on all specimens before baseline OCT scans and initial demineralisation.

[Table/Fig-1]: Lateral view of the jig with the specimen.

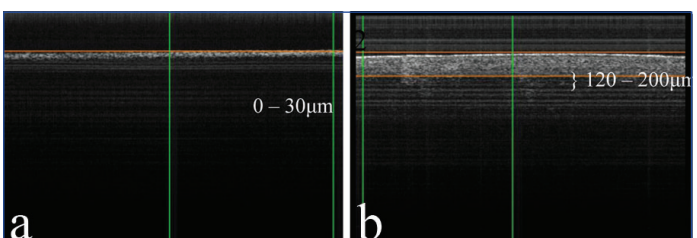
The teeth were cleaned in distilled water and custom-made three mm round stickers were applied to the designated demineralisation areas. After painting all surfaces with two layers of acid-resistant nail varnish (Essence, New York, USA), the stickers were removed with tweezers, leaving a circular window on the proximal surface, one mm below the contact area. These areas were then scanned using OCT for baseline data.

Optical Coherence Tomography (OCT) Scanning (Baseline): Customised individual Polyvinyl Siloxane (PVS) putty jigs were prepared and numbered for the 60 specimens to analyse enamel changes using OCT. Each specimen was placed in a jig for correct orientation and a baseline OCT B-scan was taken using the swept-source OCT (Thorlabs OCS1300SS Inc., New Jersey, USA) [Table/Fig-2a]. Before analysis, specimens were washed with distilled water and fixed on a 5° tilted micrometre metal stage, with B-scan images acquired using standard parameters for all specimens.

Experimental groups: The specimens were randomly divided into four groups, each containing an equal number of maxillary and mandibular teeth (n=15). The groups were assigned as follows: Group A received sound teeth, Group B received demineralised teeth, Group C was treated with ICON® and Group D received Duraphat®. The specimens were renumbered according to their assigned groups. To identify the groups, dental floss was tied at the root ends of the teeth and the floss ends were colour-coded with stickers. The specimens were stored in distilled water at 37°C when not manipulated.

Initial demineralisation: The first cycle of demineralisation aimed to create initial interproximal WSLs for Groups B, C and D. The demineralisation protocol was executed using a standard acid-buffer demineralisation solution that did not contain fluoride [17]. This solution consisted of 2.2 mM calcium chloride, 2.2 mM potassium meta-phosphate and a 50 mM acetate buffer. All the teeth were submerged in the acid buffer demineralising solution (pH 4.5) for seven days. After immersion, the specimens were visually inspected and the resulting WSLs were further analysed using OCT.

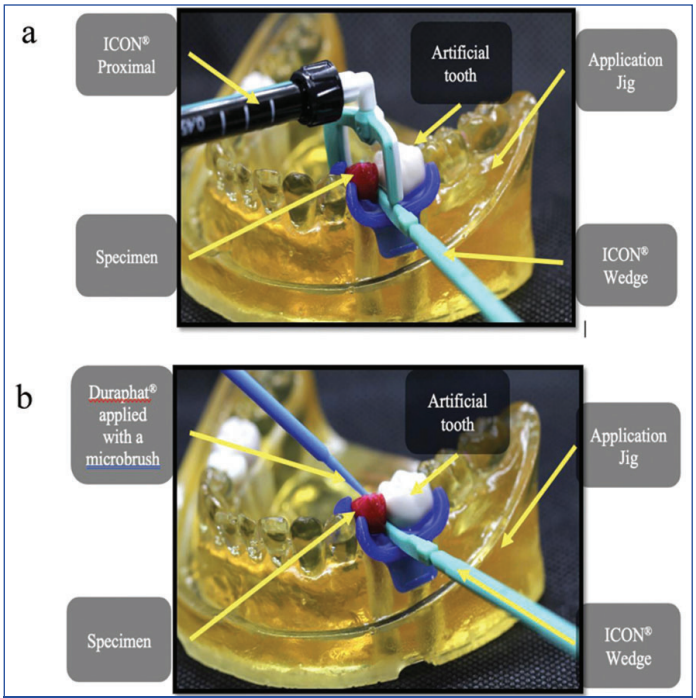
The scanning protocols for specimens in Groups B, C and D [Table/Fig-2b] were similar to the baseline scanning.



[Table/Fig-2]: (a) Baseline B-scans of proximal tooth surface of premolars with minimal (0-30 µm) surface demineralisation; (b) B-scans of artificially induced White Spot Lesion (WSL) with 120-200 µm of demineralised zone. All measurements of demineralised zones were made using the measuring ruler within the software.

Surface Roughness Measurement

- **Group-A and B:** The control groups (Group A - negative control and Group B - positive control) were embedded in epoxy resin with the area of demineralisation exposed and parallel to the scanning as closely as possible before surface roughness analysis. All 30 teeth were analysed in a similar manner to those in the treatment groups. Surface roughness measurements were taken using a non contact profilometer (3D Alicona, Infinite Focus G4 microscope, Alicona Imaging, Grambach, Austria) and the average surface roughness (R_a) was recorded for each specimen. A 3D image of the proximal enamel surfaces (80×80 µm in size) in the area of analysis was also obtained.
- **Group-C (ICON®)**: With the specimen seated in the application jig, the demineralised teeth were infiltrated with Infiltration Concept (ICON®). Each specimen required approximately 10 minutes to complete the etching and infiltration. A mini dam was used to isolate the tooth and a wedge provided in the kit was used to create interproximal space. Floss was used to gauge the space of around 50 µm, which was adequate for infiltration with ICON®. The resin infiltrant was placed following the manufacturer's instructions [Table/Fig-3a]. The specimens were then embedded in epoxy resin and surface roughness measurements were recorded using standardised scanning and analysis protocols.
- **Group-D:** Specimens in Group D were treated with fluoride varnish, Duraphat®. After initial WSL creation, the specimens were seated in the application jig and the fluoride varnish was applied following a standard protocol. The specimens were isolated and wedged in a manner similar to that used for the ICON® group. A precision electronic weighing balance (Model: AX224, Sartorius) was used to standardise the amount of varnish (0.2 mg) to be applied. A small 0.5 mm diameter microbrush was used to place the varnish inside the interproximal space and evenly spread it over the proximal surfaces using floss [Table/Fig-3b]. Any visible excessive varnish was removed using a new floss and a cotton swab pinched with tweezers. The varnish was left in situ to dry for 10 minutes, after which the surface was washed with running water. The specimens were embedded in epoxy resin and surface roughness was measured using the 3D Alicona.



[Table/Fig-3]: Application of (a) ICON® and (b) Duraphat® on the proximal surface in the application jig.

pH Cycling

A modified pH cycling model based on the White and Featherstone model was used for all specimens at 37°C for seven days [17]. Specimens were immersed in a demineralising solution (2.0 mmol/L Ca, 2.0 mmol/L PO_4, 0.075 mol/L acetate buffer, at a pH of 4.5) for six hours, alternating with immersion in a remineralising solution (1.5 mmol/L Ca, 0.9 mmol/L PO_4, 0.15 mol/L KCl, 0.02 mol/L cacodylate buffer, at a pH of 7.0) for 17 hours over the course of five days. The specimens were washed in deionised water for 30 seconds before immersion in each solution. They were further kept for two days in a fresh remineralising solution, washed in deionised water for 30 seconds and then stored in distilled water before surface roughness analysis.

Measurements of Surface Roughness (Ra): A 3D Alicona measurement was performed using the 40× objective at a vertical resolution of 20 nm on three areas (each 80×80 µm) on the designated proximal side. Three profile lines were analysed and the mean surface roughness (R_a) from three readings of each area was recorded for each specimen. The 3D Alicona was also used to capture 3D images of the proximal enamel surfaces (80×80 µm in size).

Scanning Electron Microscopy (SEM): Eight specimens representing each group for each time point were prepared using the identical step-by-step method and were subjected to SEM analysis. Two images were obtained from each sample at 500× and 3000× magnification from the region of interest using the Hitachi VP-SEM SU1510 (Hitachi High Technologies America, Inc.).

STATISTICAL ANALYSIS

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 23.0. Shapiro-Wilk tests were applied to assess the assumption of normality. Histogram plots, Shapiro-Wilk tests and inspection of skewness were used to determine normality. To compare the mean differences between groups at a single time point, one-way ANOVA and Dunnett's C3 post-hoc test were employed. In the comparison of evaluation periods (Δ_{Before}, Δ_{After}) for each group using repeated measures ANOVA, the assumption of sphericity was violated, as assessed by Mauchly's test of sphericity, $\chi^2(2)=0.00$, $p<0.05$. Therefore, a Greenhouse-Geisser correction was applied ($\varepsilon=0.648$), with $\alpha=0.05$.

RESULTS

Surface Roughness Comparisons

Surface roughness results (mean, standard deviation) for each material at each period of evaluation (before pH cycling and after pH cycling) are shown in [Table/Fig-4]. The highest R_a value before pH cycling was observed in the demineralised group (0.51 µm, SD=0.04). Before pH cycling, the sound group (0.31 µm, SD=0.03) and the ICON® group (0.31 µm, SD=0.01) demonstrated the lowest R_a values compared to the other groups. The one-way ANOVA test showed that there was a statistically significant difference in Ra between the groups ($p<0.001$). The post-hoc test indicated that there were no statistically significant differences between the sound and ICON® groups ($p=1.000$). All other post-hoc results were statistically significant.

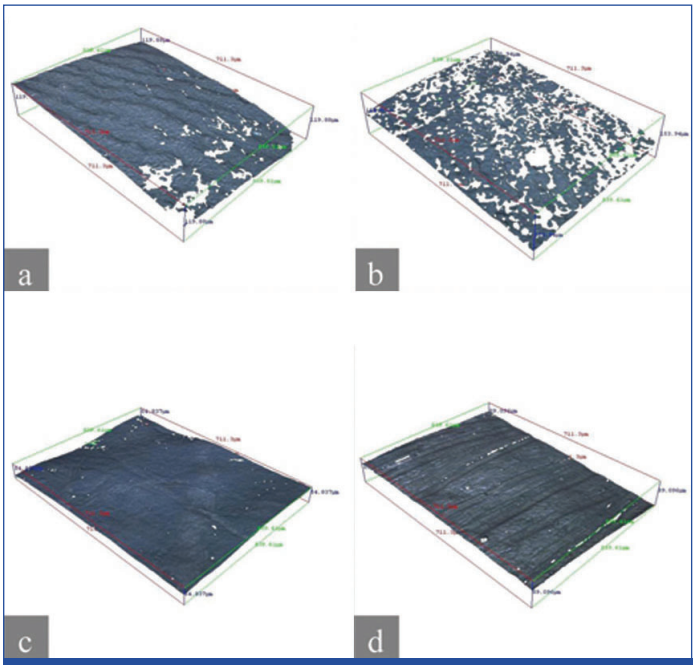
Groups	Δ_{Before} pH cycling Mean±SD		Δ_{After} pH cycling Mean±SD		Mean difference	**p-value
Sound	0.31	0.03	0.51	0.02	0.20	<0.001
Demineralised	0.51	0.04	0.75	0.02	0.24	<0.001
ICON®	0.31	0.01	0.42	0.01	0.11	<0.001
Duraphat®	0.41	0.01	0.58	0.01	0.17	<0.001
*p-value	<0.001		<0.001			

[Table/Fig-4]: Surface roughness value (Ra) for each group at each period of evaluation (n=60) (p>0.05).
*p-values comparing the groups at each time point (one-way ANOVA)
**p-values comparing time points for each group (repeated measure ANOVA)

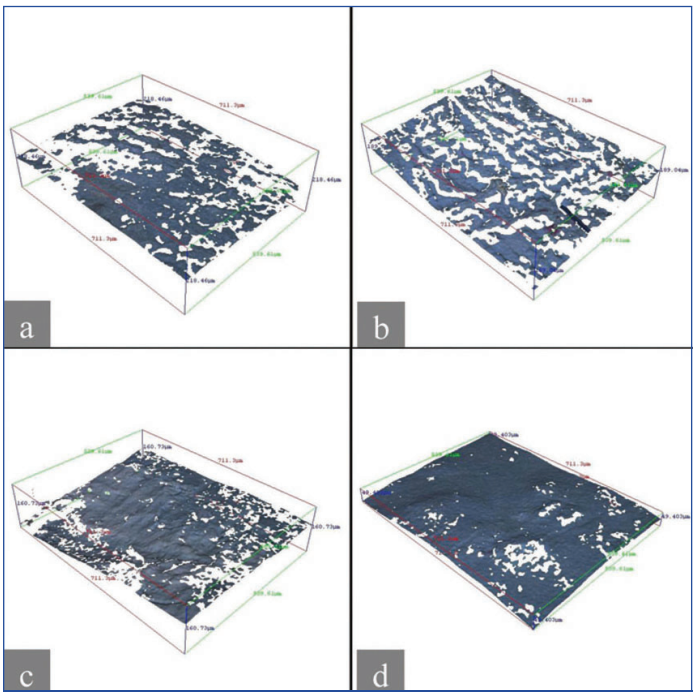
After pH cycling, the highest R_a value was observed in the demineralised group (0.75 µm, SD=0.02), while the ICON® group (0.42 µm, SD=0.01) recorded the lowest R_a value when compared to the other groups. The one-way ANOVA showed that there was a statistically significant difference between the R_a values ($p<0.001$). In the Dunnett T3 post-hoc results, all groups showed statistically significant differences ($p<0.001$).

Surface Topography (Non Contact Profilometry)

All surfaces showed an increase in the depth of pores and valleys after exposure to pH cycling. The ICON® group exhibited minimal changes in surface topography after being subjected to pH cycling for 168 hours compared to the baseline 3D model [Table/Fig-5a-d, 6a-d].



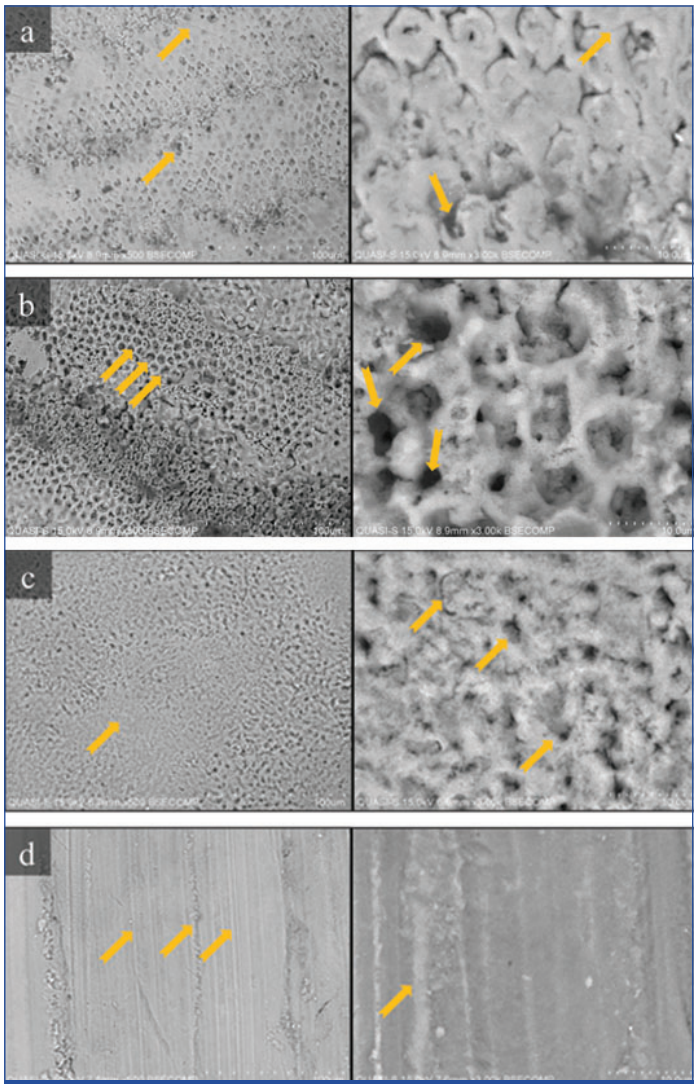
[Table/Fig-5]: Representative 3-Dimensional (3D) interpretation and surface profile of: a) Sound; b) Demineralised; c) ICON®; and d) Duraphat® groups before pH cycling.
*The 3-D measurements were taken at three sites of 80×80 µm each. The 3-D images were represented from one site (80×80 µm) and may not be a true representative of the entire surface of the sample



[Table/Fig-6]: Representative 3-Dimensional (3D) interpretation and surface profile of: a) Sound; b) Demineralised; c) ICON®; and d) Duraphat® groups after pH cycling.
*The 3-D measurements were taken at three sites of 80×80 µm each. The 3-D images were represented from ONE site (80×80 µm) and may not be a true representative of the entire surface of the sample

Surface Topography (Scanning Electron Microscope)

Sound enamel [Table/Fig-7a] showed some pits and scratches but, in general, had a smooth and nearly homogeneous surface. The enamel surface after demineralisation displayed craters of variable depths, which appeared as irregular hexagonal pitted surfaces [Table/Fig-7b]. The proximal enamel surface treated with ICON® showed blockage of the enamel rods, resulting in a smooth surface but uneven topography [Table/Fig-7c]. The surface treated with Duraphat® revealed complete blockage of enamel rods by a continuous mineralised outer layer of fluoride [Table/Fig-7d]. This mineralised layer appeared smooth but had marked vertical lines that coincided with the orientation of flossing.

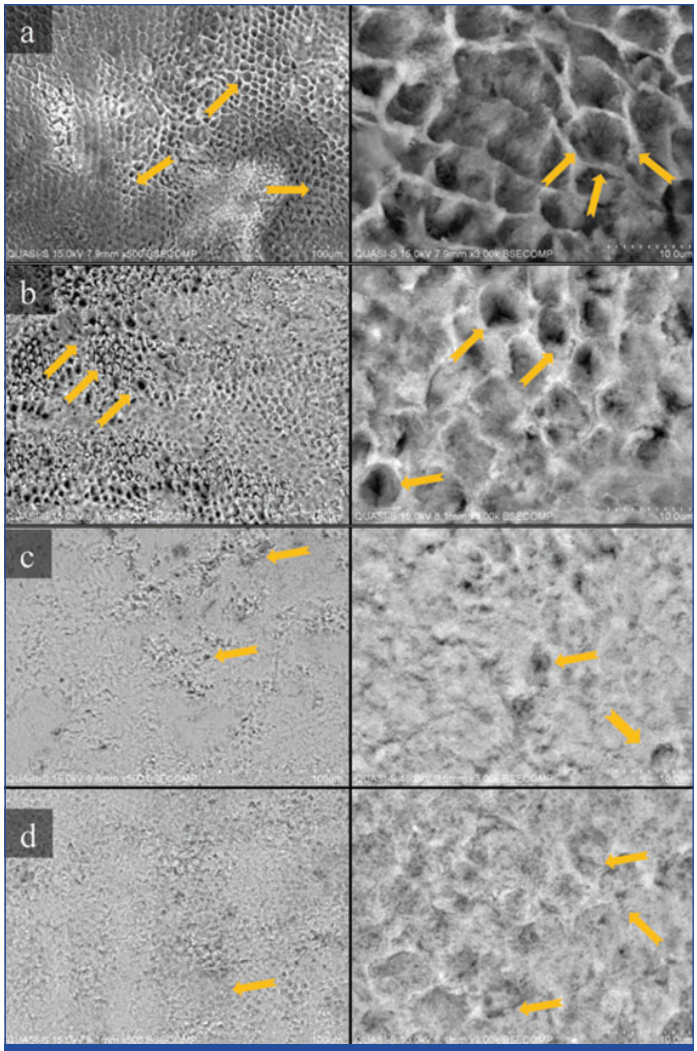


[Table/Fig-7]: Representative SEM images of enamel surfaces for all the groups (500x, 3000x magnifications): a) Sound; b) Demineralised; c) ICON®; d) Duraphat® before pH cycling.

After pH cycling, an evident increase in surface roughness was observed in both the sound [Table/Fig-8a] and demineralised [Table/Fig-8b] groups, as more hexagonal pitted surfaces emerged due to enamel rods losing their core while retaining their outer peripheral structure. Both the ICON® [Table/Fig-8c] and Duraphat® [Table/Fig-8d] groups revealed a near-homogeneous surface, with only slight changes in their morphological features. Both showed localised peeling of the surface, revealing honeycomb-shaped enamel rods, wherein the prismatic orifices resulting from pH cycling became more visible. The core of the enamel remained intact.

DISCUSSION

Previous studies on WSLs utilised various imaging techniques, including light microscopy, stereomicroscopy, polarised light microscopy, confocal microscopy, Transverse Microradiography (TM), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM)



[Table/Fig-8]: Representative SEM images of enamel surfaces for all the groups (500x, 3000x magnifications): a) Sound; b) Demineralised; c) ICON®; d) Duraphat® after pH cycling.

[18,19]. However, these methods often require samples to be sectioned and processed, which can lead to alterations or loss of critical structures. In the present study, OCT was employed to analyse the proximal surface and subsurface of WSLs while preserving the intact surface layer. OCT allows for both quantitative interpretation of the backscattering signal intensity and qualitative analysis through B-scans, which reveal changes in enamel structure between carious and control samples. To eliminate depth as a confounding factor, we standardised the depth of the artificially induced WSLs to 120-200 µm using B-scans [20].

Ulrich I et al., reported no significant difference in surface roughness between resin-infiltrated WSLs and sound enamel [21]. Using AFM, Taher NM showed no significant difference in surface roughness among different treatment options; however, these studies were carried out on sound enamel [19]. Based on the findings of the present study, it may be speculated that the degradation of the resin over time resulted in a rougher surface. The infiltrant used in this study was Triethylene Glycol Dimethacrylate (TEGDMA), which has a relatively high solubility that influences water absorption and degradation of the polymer [22].

The surface roughness of the resin influences the adhesion and growth of cariogenic biofilms on their surface. In the present study, the R_a of the untreated initial carious lesion was 0.51 µm, indicating a higher risk for plaque accumulation and further caries progression. The R_a value was 0.31 µm immediately after infiltration. After pH cycling, the R_a value of the ICON® was 0.42 µm, which was still above the threshold for plaque retention. All the samples revealed higher values when compared to the 0.20 µm threshold value. Intraorally, patients can interpret surface changes when the R_a value is more than 0.50 µm [23]. Increased surface roughness may result in further plaque accumulation on the surface of the initial carious lesion, promoting surface demineralisation and further caries progression.

The present study demonstrated that both infiltrated enamel surfaces and fluoride varnish-treated surfaces became rougher after pH cycling. To the authors knowledge, the effect of fluoride varnish on the surface roughness of enamel has received little attention. The present findings are best explained by the micromorphological changes that occur in the enamel during pH cycling, which lead to an increase in porosity and degradation of the enamel and restorative materials [24]. However, the true reason for increased surface roughness remains a topic of speculation. These results are consistent with findings from a study where infiltrated lesions were subjected to thermocycling in combination with acidic challenges [21].

In the present study, there was a significant increase in surface roughness in the ICON® group compared to the control group. Although this suggests that the ICON® material tends to increase plaque accumulation on proximal surfaces, some studies have conversely shown an increase in surface hardness and caries resistance [22,25]. Taher NM et al., demonstrated that in human premolars with healthy enamel, there was no significant difference in surface roughness before and after the application of ICON® material [22], which is in agreement with our study. In a similar study a year later, Taher reported a non homogeneous layer with groups of small enamel grains scattered on the surface when analysed under AFM [19], which might explain the higher yet insignificant increase in surface roughness.

The present study found that the fluoride varnish group had lower R_a values compared to the positive control group. Other research [26,27] has indicated that highly concentrated fluoride can protect enamel from further demineralisation. This protection occurs because calcium fluoride leaches slowly and easily when exposed to acid, effectively preventing the dissolution of minerals from the enamel by providing a hypermineralised physical barrier on the enamel surface. The present findings align with those of two additional studies that also reported the protective effects of fluoride on enamel. Specifically, enamel treated with two different formulations of fluoride varnish showed a significant reduction in surface profile when compared to a placebo varnish and control [28]. In their study, Soares LES and De Carvalho Filho ACB noted that enamel protected with fluoride varnish and subjected to an acidic challenge exhibited significantly lower R_a values compared to unprotected enamel samples exposed to the same challenge [29].

Limitation(s)

Laboratory studies are conducted in artificial and controlled settings, which limits the ability to generalise the findings to real-world situations (external validity). The lesions in each tooth may vary, as the degree of demineralisation can differ based on the amount of fluoride exposure prior to extraction. Research has demonstrated that resin infiltration behaves differently in artificially induced WSLs compared to naturally occurring WSLs. Additionally, laboratory experiments cannot fully replicate the complexities of the oral environment. While artificially induced WSLs are created after just one week of exposure to demineralising acids, in the oral cavity, these lesions can take months or even years to develop.

CONCLUSION(S)

The study concluded that WSLs infiltrated with ICON® exhibited surface roughness similar to that of sound enamel and remained relatively unchanged after exposure to acidic conditions. In contrast, the application of fluoride varnish (Duraphat®) on proximal WSLs significantly increased surface roughness compared to sound enamel. However, when compared to untreated lesions, the roughness values (R_a) remained significantly lower both before

and after pH cycling. Additionally, all samples in the present study showed roughness values above the 0.20 µm threshold, indicating potential plaque retention.

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