



BASIC RESEARCH:

Biofilm and Enamel Changes after Interproximal Reduction and Polishing

Cambios en esmalte y biopelícula tras reducción interproximal y pulido

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ABSTRACT: This study examined the effects of three different interproximal enamel reduction (IER) techniques and subsequent polishing methods on enamel roughness and biofilm formation. Enamel samples were treated with diamond burs, abrasive strips, or diamond discs, followed by polishing using various techniques. Surface roughness was measured with Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and Profilometry (PM). Biofilm formation of *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* were evaluated *in vitro* after IER and polishing. The results shown SEM, AFM, and PM revealed increased roughness on enamel surfaces treated with all IER techniques, with PM showing higher Ra and Rz values for the diamond bur IER method. Polishing was performed after IER, reducing roughness across all methods, though not to control levels. Additionally, a significant increase in the levels of *S. mutans*, *L. acidophilus*, and *C. albicans* was observed on enamel surfaces treated with all IER techniques, with reductions seen after polishing. While



IER effectively addresses dental crowding, it inherently increases enamel roughness and consequently elevates the risk of biofilm accumulation and caries. This study emphasizes the importance of meticulous polishing procedures and highlights the need for long-term clinical studies to evaluate the enduring effects of IER on oral health.

KEYWORDS: Interproximal enamel reduction; Stripping; Enamel damage; Post-stripping polishing; Dental biofilm.

RESUMEN: Este estudio examinó los efectos de tres técnicas diferentes de reducción interproximal del esmalte (IER, por sus siglas en inglés) y los métodos de pulido posteriores sobre la rugosidad del esmalte y la formación de biopelícula. Las muestras de esmalte fueron tratadas con fresas diamantadas, tiras abrasivas o discos diamantados, seguidas de diferentes técnicas de pulido. La rugosidad superficial se midió mediante Microscopía Electrónica de Barrido (SEM), Microscopía de Fuerza Atómica (AFM) y Perfilometría (PM). La formación de biopelícula de *Streptococcus mutans*, *Lactobacillus acidophilus* y *Candida albicans* se evaluó *in vitro* después de los procedimientos de IER y pulido. Los resultados obtenidos mediante SEM, AFM y PM mostraron un aumento de la rugosidad en las superficies de esmalte tratadas con todas las técnicas de IER, siendo la fresa diamantada la que presentó los valores más altos de Ra y Rz según PM. El pulido realizado después de la IER redujo la rugosidad en todos los métodos, aunque sin alcanzar los niveles del grupo control. Además, se observó un incremento significativo en los niveles de *S. mutans*, *L. acidophilus* y *C. albicans* en las superficies tratadas con todas las técnicas de IER, con una reducción posterior al pulido. Aunque la IER es eficaz para corregir el apiñamiento dental, incrementa de forma inherente la rugosidad del esmalte y, en consecuencia, el riesgo de acumulación de biopelícula y caries. Este estudio enfatiza la importancia de realizar procedimientos de pulido meticulosos y destaca la necesidad de estudios clínicos a largo plazo para evaluar los efectos duraderos de la IER en la salud oral.

PALABRAS CLAVE: Reducción interproximal del esmalte; Stripping; Daño del esmalte; Pulido posterior al stripping; Biopelícula dental.

INTRODUCTION

In orthodontic treatments, one of the most common problems for achieving harmony between the arches is the lack of space caused by crowding and premature loss of deciduous teeth (1). This crowding, affecting approximately 50% of the population without orthodontic treatment (2), can be categorized in various degrees. Dental crowding, arises from a discrepancy between tooth size and arch dimensions (3). This imbalance often manifests in the anterior region of dental arches (3) and can be influenced by factors like large

tooth size, insufficient bony bases, and developmental patterns leading to reduced facial skeletal size without a corresponding decrease in tooth size (4). Addressing crowding is crucial as it not only impacts aesthetics but can also hinder proper oral hygiene, potentially increasing the risk of caries and periodontal issues. preventive approaches addressing crowding early in development are often more cost-effective and less complex (4). Nevertheless, when the problem is present, to address this issue, multiple procedures are employed, including extractions, maxillary expansion, distalization, and interproximal enamel reduction

(IER) (5). In recent years, the latter technique, considered less invasive, has been used more frequently (6).

IER is a minimally invasive technique that involves the selective and controlled removal of a small amount of enamel (no more than 0.5 mm of the teeth or restorations) from the interproximal surfaces of teeth (Figure 1), thereby creating space for alignment without tooth loss or expansion (3, 7, 8). There has been some concern regarding the potential adverse effects of IER on the enamel surface (9). The main tools for performing IER are diamond burs, abrasive strips, and diamond discs (10, 11). Diamond burs are the most commonly used due to its simplicity and accessibility in different areas of the oral cavity (12).

Enamel is a dense, highly mineralized tissue that serves as the first line of defense against dental caries. The surface of enamel is smooth and glossy, providing a low-friction environment that inhibits bacterial adhesion and biofilm formation. Enamel stripping is performed at the contact points, generating structural changes in the enamel surface and increasing roughness (13, 14). This surface alteration has been associated with a significant rise in bacterial adhesion and biofilm formation, which are factors linked to the development of caries and periodontal disease (15-17).

The primary microorganisms associated with dental caries are *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans* (18). After adhering to the enamel surface, these microorganisms metabolize carbohydrates from the daily diet through acid fermentation (19). Additionally, increased enamel roughness leads to a higher number of microorganisms, causing variation in the degree of saturation of the biofilm's fluid phase with respect to hydroxyapatite, ultimately resulting in acidic enamel dissolution (20).

One proposal to mitigate the initial roughness caused by stripping is the use of post-stripping dental polishing systems (21). Prior studies have shown that polishing techniques, such as rubber cups and prophylaxis pastes, can partially restore the smooth enamel surface and reduce the risk of caries and periodontal disease development (22). Thus, this study aimed to analyze the impact of different IER techniques and subsequent polishing methods on enamel surface roughness and biofilm formation of cariogenic microorganisms.

MATERIAL AND METHODS

SAMPLE OBTENTION

This *in vitro* study was conducted as an experimental, cross-sectional, and prospective investigation. Upper premolars specifically indicated for orthodontic extraction were obtained with informed consent. All procedures were performed in the University of the Valley of Guerrero Orthodontic Department. Premolars with caries, enamel fractures, or any other enamel alteration were excluded.

After extraction, any adhered tissue was removed, and the teeth were disinfected with a 5% sodium hypochlorite solution for 1 minute, washed with saline solution three times, and stored in saline solution at 4°C for no more than 15 days until processing.

INTERPROXIMAL ENAMEL REDUCTION

The samples were fixed on plaster supports and treated with three different enamel reduction techniques. The teeth were randomly divided into 4 groups (Figure 2), each consisting of 10 teeth. The control group was not intervened (CG), one group was treated with abrasive strips (Microdont® brand, 40 microns) (AS), other group was

treated using diamond disc (Komet® brand, 180 mm diameter) (DD), and the last was treated with diamond bur (fine grain TC-28 diamond bur, Azdent® brand) (DB). After IER processing, the teeth were washed and fixed and then stored at 4°C until analyses were done.

POLISHING TECHNIQUES

IER with DB (fine grain TC-28 diamond bur, Azdent® brand) was chosen to test the different polishing techniques. The teeth were randomly divided into 3 groups, each consisting of 10 teeth. The first group was treated with Sof-lex disc (3M Sof-lex disc medium-fine-ultrafine discs), the second group was intervened using polishing metal sandpaper (Microdont® extra fine metal band) and the last one was treated with polishing celluloid band (Microdont® extra-fine polyester band).

SURFACE ROUGHNESS MEASUREMENT

After performing interproximal enamel reduction (IER) on the teeth using each technique, the surface roughness was measured in the Ra (arithmetic mean of roughness) and Rz (average roughness amplitude) parameters. A calibrated roughness meter was used (Mitutoyo SJ-301, Mitutoyo American Corporation, EU), operating at a speed of 0.25 mm/s and a cut-off distance of 0.08 mm, with 5 repetitions. The tooth surfaces were aligned parallel to the roughness meter tip and secured using double-sided tape. Five teeth were used per technique, and the roughness was evaluated on both proximal surfaces in triplicate, for a total of 30 measurements per group.

SCANNING ELECTRON MICROSCOPY (SEM)

All samples were analyzed by SEM. The sample preparation was performed as follows: all the samples were washed with PBS for 10 minutes twice. After that, the samples were fixed using paraformaldehyde 4% and glutaraldehyde 2.5%

for 3 hours. Then the samples were dried, and gold coated. It was used a scanning electron microscope (JSM 5600 LV-SEM, JEOL, Japan) in low vacuum mode with secondary electrons at 5 KeV. Micrographs were taken at 100 X and 500 X.

ATOMIC FORCE MICROSCOPY

All samples were analyzed by atomic force microscopy (AFM). All the samples were washed with PBS for 10 minutes twice. After that, the samples were fixed using paraformaldehyde 4% and glutaraldehyde 2.5% for 3 hours. Then the samples were dried. Used an atomic force microscope (JSPM4210, JEOL, Japan), in tapping mode at 160 kHz and a force of 5 N/m using a cantilever probe NSC14 (MikroMasch, Tallinn, Estonia) with a height tip of 125 µm and an angled tip of 40°, performing a sweep over an area of 100 × 100 µm.

DETERMINATION OF BIOFILM FORMATION

Enamel samples measuring 4 x 4 mm were obtained from the coronal region of the treated tooth groups. Each tooth fragment underwent a disinfection process: first, it was immersed in 3% NaOCl for three minutes, followed by two rinses with sterile saline; then, it was placed in a 70% isopropyl alcohol solution for one minute, followed by two additional rinses with sterile saline. Subsequently, the fragments were placed into 6-well plates and exposed to the microorganisms separately. The bacterial concentrations were 1.5×10^8 CFU/ml for *S. mutans* and *L. acidophilus*, and 1.5×10^6 CFU/ml for *C. albicans*. The samples were incubated for 24 hours under anaerobic conditions for the bacteria and aerobic conditions for *C. albicans*. After incubation, the culture medium was removed, and the fragments were washed in a physiological solution to remove non-adherent cells. The tooth fragments were then stained with 5% crystal violet for 5 minutes. Finally, the fragments were washed once with saline, transferred to an Eppendorf tube, and 1 ml of methanol was added.

The solution was vortexed three times and then read in a spectrophotometer at 590 nm. The assay was performed in triplicate at different times.

STATISTICAL ANALYSIS

The data was analyzed using statistical software (Graphpad Prism V8). The normality of

the data was evaluated using the Shapiro-Wilk test ($p < 0.05$). The means and standard deviations were calculated for the different groups. The differences between the groups were analyzed using analysis of variance (ANOVA). Multiple comparison post-hoc Tukey test was used to determine which groups differed significantly. The level of significance was set at $p < 0.05$.

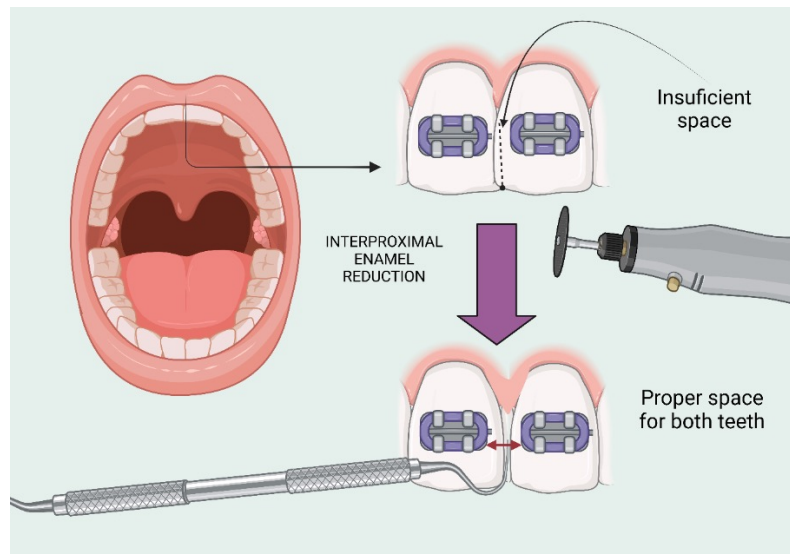


Figure 1. IER is a minimally invasive technique that involves the selective and controlled removal of no more than 0.5 mm of enamel from the interproximal surfaces of teeth or restorations. This process creates space for alignment without resulting in tooth loss or expansion.

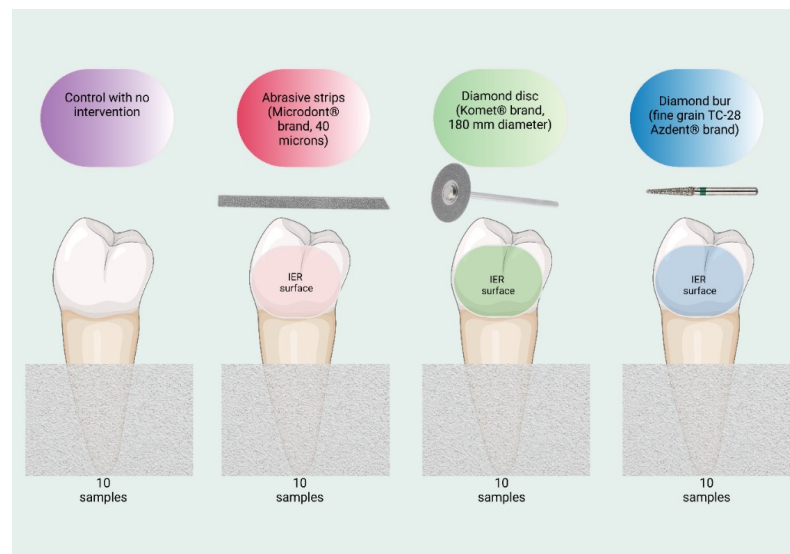


Figure 2. Three IER techniques were tested to determine which one produce the roughest enamel surface. The control group was not intervened, one group was treated with abrasive strips (Microdont® brand, 40 microns), other group was treated using diamond disc (Komet® brand, 180 mm diameter) and the last was treated with diamond bur (fine grain TC-28 diamond bur, Azdent® brand).

RESULTS

THE IER TECHNIQUES PRODUCE DIFFERENT WEAR PATTERNS IN THE ENAMEL SURFACE

All the IER techniques evaluated generated a considerable increase in the surface roughness of human enamel when compared with the natural enamel (Table 1). Nevertheless, IER using a DD is the one that produces a higher Ra and Rz values of roughness (Figure 3). The surface roughness tester indicated that the IER using a DD produced a roughest enamel surface of 1.36 μm in Ra and 5.87 μm in Rz. IER using a DB generated 1.15 μm in Ra and 4.71 μm in Rz and the less aggressive IER technique was AS, producing a roughness of 0.54 μm in Ra, and 2.52 μm in Rz (Figure 3. A-B).

We then compared the IER techniques through SEM. The untreated enamel surface showed the heads of the enamel prisms at 500x and 1000X. Changes in the enamel surfaces were evident in the samples from teeth with IER treatments. Images of DB showed more irregular areas on the enamel, showing that although profilometry makes a quantitative evaluation of roughness, microscopy shows precise shape patterns, which has an impact on adhesion of microorganisms. AS generated roughness patterns with transverse fractures, and DD generated a more uniform wear pattern on the enamel according to SEM (Figure 4). Besides, in AFM, the microscale topography in AS and DD shows totally depressed areas (1.7 μm and 903nm respectively) consistent with the grooved wear pattern seen in SEM, demonstrating that the technique produces these

defects in the enamel, while in DB constant and slight depressions are produced (1.3 μm) (Figure 5).

THE THREE POLISHING TECHNIQUES EVALUATED GENERATED A SIMILAR DIMINUTION OF THE ROUGHNESS

We analyzed the effectiveness of three polishing techniques commonly used after IER using DB as model of IER as it is the most used technique. Polishers included Sof-lex disc (SD), metal sandpaper (MS), and polishing celluloid band (CB). All three techniques reduced the enamel roughness (Table 2) even with lower Ra and Rz values than natural enamel. SD, MS and CB showed not statistically significant differences between them but when compared to DB all polishing techniques showed a $p < 0.0001$ (Figure 3. C-D). Nevertheless, in both analysis, SEM and AFM, a lower presence of superficial streaks and lower roughness was observed after dental polishing (Figure 6). The results indicate that all polishing methods leave the enamel significantly less rough than the enamel intervened with DB IER. However, these polished surfaces do not appear as smooth as the natural enamel.

IER INCREASE THE RISK OF BIOFILM FORMATION

After processing the enamel surface with the IER and polishing techniques, a portion of the enamel from the contact area was obtained using a longitudinal cut, generating a 16mm square disk (4 x 4mm) by 1mm thick. After being disinfected, the enamel fragments were placed separately in a culture medium with *S. mutans*, *L. acido-*

philus, and *C. albicans*. The results showed that all enamel samples treated with IER techniques have a significant increase ($p < 0.05$) in biofilm formation of the three species when compared to natural enamel (Figure 7. A). These results suggest that IER techniques could produce wear patterns that increase the risk of developing caries due to increased biofilm formed on the enamel surface. Likewise, the three post-stripping polishing

techniques were evaluated, showing a significant decrease in biofilm formation for the three different techniques when compared to DB IER alone (Figure 7. B). Interestingly, the absorbance shown in the three polishing groups was like the absorbance seen in the natural enamel. These results suggest that the three polishing techniques significantly reduced the biofilm formation of the three cariogenic species to basal levels.

Table 1. Surface Enamel Roughness after IER techniques. Values express the number of micrometers of roughness, the standard deviation is shown in parentheses.

Surface analyzed	Ra μm	Rz μm
Natural Enamel (Control Group)	0.07 (0.048)	0.43 (0.213)
Interproximal Enamel Reduction Techniques:		
Stripping with Abrasive Strip (Microdont® brand, 40 microns)	0.54 (0.07)	2.51 (0.27)
Stripping with Diamond Disc (Komet® brand, 180 mm diameter)	1.36 (0.15)	5.83 (0.65)
Stripping with Diamond Bur (Fine grain TC-28 diamond bur, Azdent® brand)	1.15 (0.11)	4.71 (0.5)

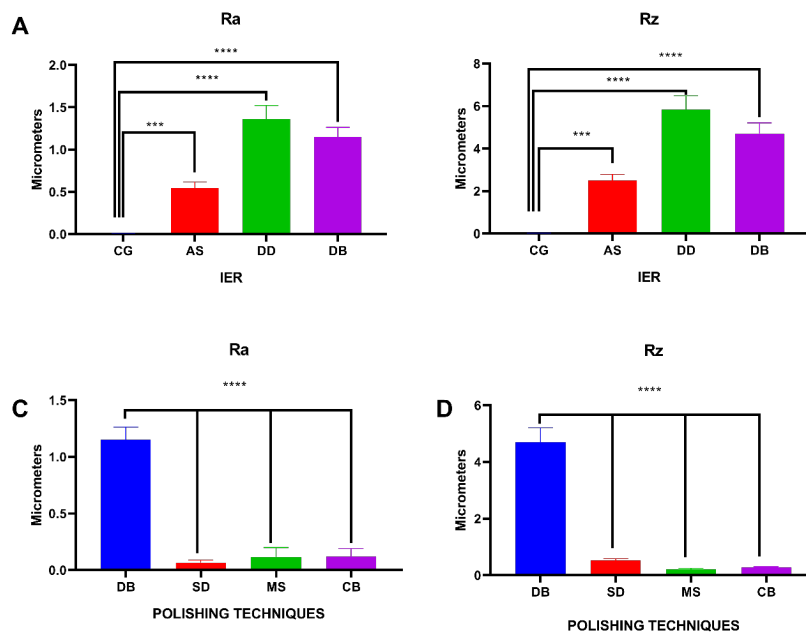


Figure 3. All IER techniques modified drastically the enamel surface showing statistically significant differences when compared to CG. A) Ra value: CG vs. AS $p=0.0006$ (***), CG vs. DD $p<0.0001$ (****), and CG vs. DB $p<0.0001$ (****). B) Rz value: CG vs. AS $p=0.0003$ (***), CG vs. DD $p<0.0001$ (****), and CG vs. DB $p<0.0001$ (****). C and D) All polishing techniques recover smoothness of the surface when compared to DB (**** $p<0.0001$).

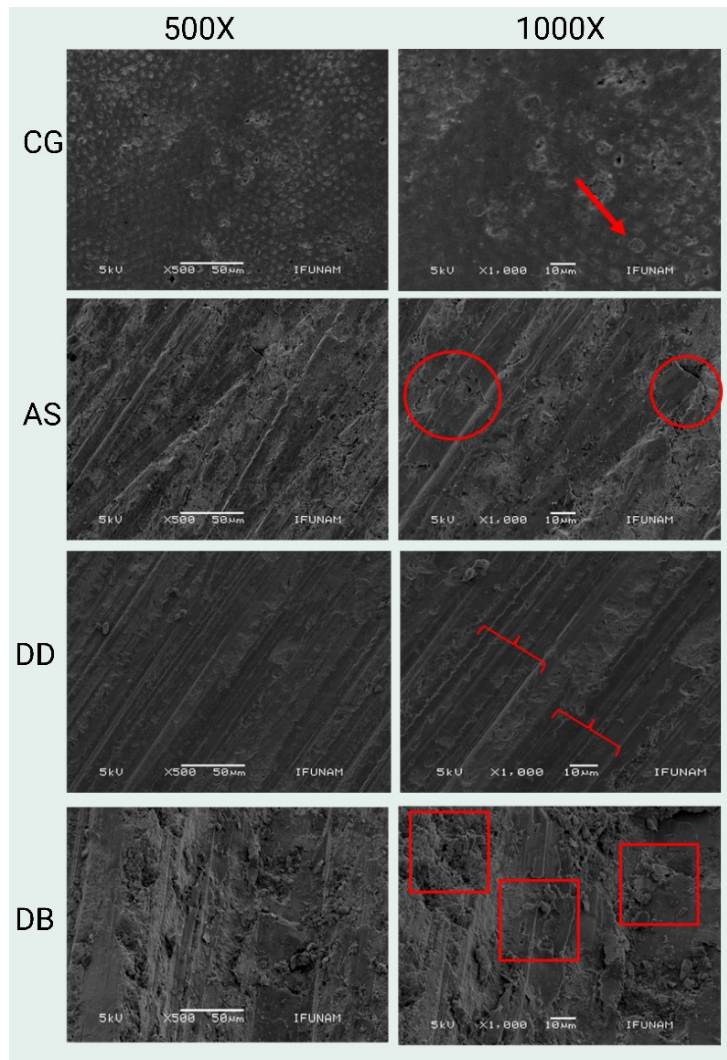


Figure 4. Examining enamel surface with SEM after IER. We analyzed the enamel surface untreated, where the heads of the hydroxyapatite prisms are observed (red arrow). The IER treatment using AS generated roughness patterns with transverse fractures (red circles). When the DD is used to IER, it generates uniform grooves in depth and continuity (red square brackets). Finally, we evaluated IER using a DB, which produced a more irregular surface, where striae, depressions and grooves were observed randomly (red squares).

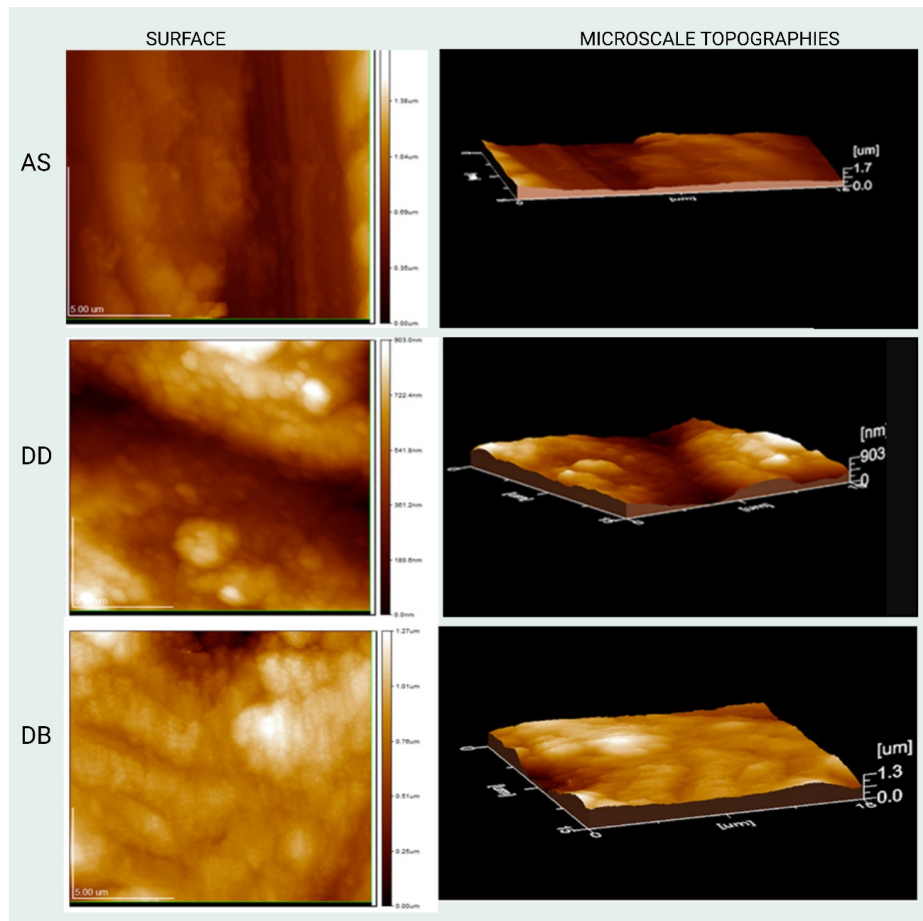


Figure 5. Examining enamel roughness after IER treatment using AFM. The use of AS generates roughness of 1.8 micrometers depth on average. DD produces grooves of 0.9 depth on average. Using DB depressions of 1.3 micrometers are generated, irregularly arranged, not forming grooves.

Table 2. Surface Enamel Roughness after polishing techniques. Values express the number of micrometers of roughness, the standard deviation is shown in parentheses.

Surface analyzed	Ra μm	Rz μm
Stripping with Diamond Bur	1.15 (0.11)	4.71 (0.53)
Polishing Techniques Evaluated:		
Polishing with Sof-lex disc (3M sof-lex disc medium-fine-ultrafine discs)	0.06 (0.021)	0.53 (0.06)
Polishing with Metal Sandpaper (Microdont® extra fine metal band)	0.11 (0.083)	0.21 (0.04)
Polishing with Celluloid band (Microdont® extra-fine polyester band)	0.12 (0.07)	0.29 (0.02)

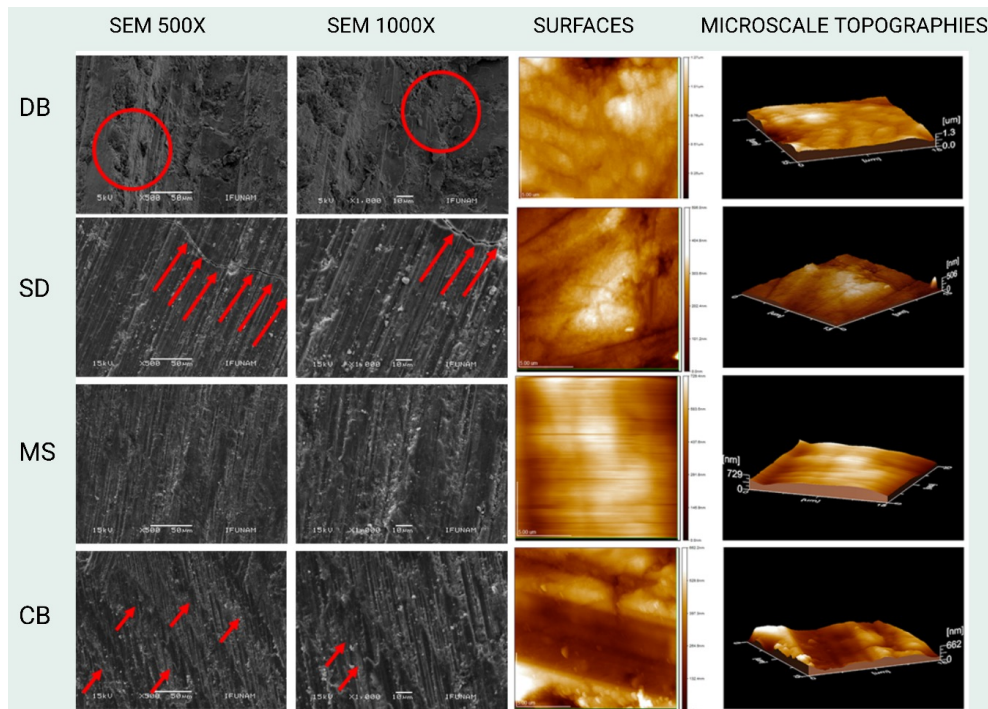


Figure 6. To evaluate three different polishing techniques, the enamel samples previously treated with IER using a DB which generates a roughness characterized by an irregular surface, where striae, depressions, and grooves are observed randomly (red circles). The analyzed the enamel after polishing with a SD found fractures in the samples (red arrows). In the analysis of the samples polished with MS found a roughness pattern more homogeneous and regular than the other experimental groups. In the group of samples polished with CB found repetitive deep grooves.

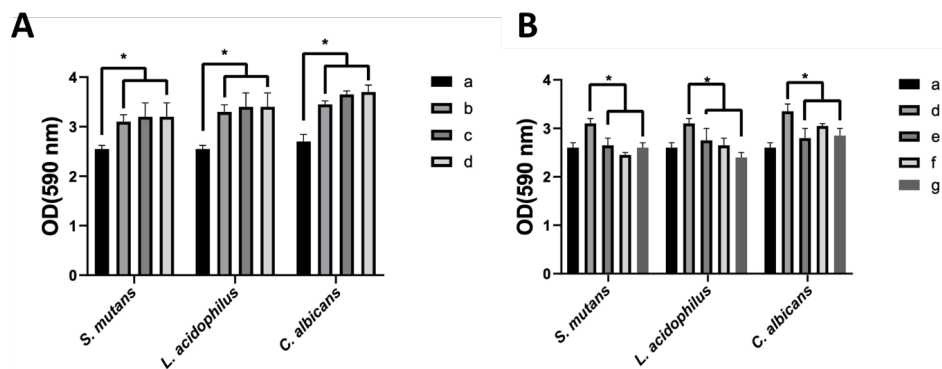


Figure 7. Evaluation of the biofilm by crystal violet assay of *S. mutans*, *L. acidophilus*, and *C. albicans* in the enamel of different groups (a): Control group without any procedure, (b) Stripping with abrasive strips (Microdont® brand, 40 microns), (c) Stripping with diamond disc (Komet® brand, 180 mm diameter), (d) Stripping with diamond bur (fine grain TC-28 diamond bur, Azdent® brand), (e) Stripping with bur (fine grain TC-28 diamond bur, Azdent® brand) and polishing Sof-lex disc (3M Sof-lex disc medium-fine-ultrafine discs), (f) Stripping with bur (fine grain TC-28 diamond bur, Azdent® brand) and polishing metal sandpaper (Microdont® extra fine metal band), (g) Stripping with bur (fine grain TC-28 diamond bur, Azdent® brand) and polishing celluloid band (Microdont® extra-fine polyester band). The average results of three replicates are shown, and error bars represent the standard error of the mean (SEM). *Statistically different compared in panel A (a vs b, c, d) and the panel B (d vs e, f, g) $p < 0.05$, Kruskal–Walli's test.

DISCUSSION

This *in vitro* study analyzed and compared the enamel roughness produced by three different IER techniques and their corresponding polishing protocols. Topological analysis revealed an increase in enamel irregularity after IER, regardless of the method used when compared to untreated natural enamel. Even after polishing the enamel, the surfaces did not return to baseline levels observed in the natural enamel. Microbiological analysis showed an increase in biofilm formation for the three cariogenic species examined. However, after polishing the IER areas, the values of biofilm formation for these three species decreased to levels similar to the untreated control group. The results of this study indicate that IER procedures significantly increase enamel roughness, regardless of the specific technique used as have been shown before (23-26).

During IER, 1 mm of enamel wear is performed, mainly at the contact points. However, this wear changes the topography and can induce caries formation in the long term (17). Likewise, previous studies have shown that the topography changes after IER, and the retention of food and bacteria is essential for developing diseases in the oral cavity (18). In this regard, the results show that the irregular patterns observed by SEM and the roughness evaluated by AFM increased significantly when compared to the control group. These data suggest that the enamel removal technique by IER generates a topographical change in the surface of the treated enamel, which could be a determinant in the accumulation of dental biofilm.

One of the purposes of IER is to gain space in the arches to solve dental crowding (19). Various protocols have been described, and materials have been created to perform enamel polishing after Stripping to eliminate the generated roughness (19). Some authors mention that the roughness

decreases to values similar to the enamel surface of a tooth without any procedure (20), however, polishing the enamel at the contact points due to its location limits its effectiveness (21). Our results showed that the surfaces receiving polishing reduced the roughness generated by IER. Nevertheless, it does not reach the levels of the untreated natural enamel.

The primary organisms involved in the development of caries are *S. mutans*, *L. acidophilus*, and *C. albicans* (22). In this regard, recent research has described the microbiota that colonizes the surface of the enamel (23) being one of the factors of colonization the retentive areas (24). Our data showed that the IER procedure on the enamel favors biofilm formation for the three species of cariogenic microorganisms suggesting that IER is a risk factor for developing caries in the treated areas. However, performing post-stripping polishing significantly reduces biofilm formation for the three species. In conclusion, while IER can effectively address dental crowding, it inherently increases enamel roughness and, consequently, the risk of biofilm accumulation and caries. This study underscores the importance of meticulous polishing protocols and highlights the need for long-term clinical studies to assess the lasting impact of IER on oral health. It would be also valuable to consider other aspects inherent to patients such as saliva composition, dietary habits, or oral hygiene practices and how they interact with IER outcomes

Finally, because this study was conducted *in vitro*, the full conditions of a dynamic oral environment including variations in saliva composition, microbiological diversity, biofilm development, dietary habits, mechanical forces, and daily oral hygiene practices were not fully replicated. Therefore, although changes in the enamel surface that favored colonization by the three microorganisms were observed, the nature and inherent limitations of the study must be considered.

CONCLUSION

IER effectively creates space to address dental crowding but inevitably increases enamel roughness and predisposes surfaces to greater biofilm accumulation. Polishing plays a crucial role in reducing these undesirable effects, though it does not fully restore enamel to its natural condition. Future long-term *in vivo* studies are necessary to evaluate how IER-induced surface changes interact with patient-specific factors and to determine their real clinical significance over time.

AUTHOR CONTRIBUTION STATEMENT: Conceptualization: G.G.O.M. and J.A.A.C.; Data curation: J.A.A.C., S.A.S.D. and G.G.O.M.; Formal analysis: M.F.R.G., Y.P.E. and G.P.R.; Funding acquisition: N.S.A.S., V.M.M.A. and G.G.O.M.; Investigation: M.F.R.G., Y.P.E., G.P.R., J.A.A. and A.G.A.; Methodology: M.F.R.G., Y.P.E. and G.P.R.; Project administration: C.E.V.M., A.F.L. and G.G.O.M.; Resources: C.E.V.M., A.F.L. and G.G.O.M.; Software: C.E.V.M., A.F.L. and G.G.O.M.; Supervision: G.G.O.M. and J.A.A.C.; Validation: M.B., J.A.A.C. and G.G.O.M.; Visualization: M.B., J.A.A.C. and G.G.O.M.; Writing-original draft: M.B., J.A.A.C. and G.G.O.M.; Writing-review & editing: M.B., J.A.A.C. and G.G.O.M.

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