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Effect of Remineralizing Agent after Proximal Stripping of Enamel with Two Different Methods: A Comparative in-Vitro Atomic Force Microscopic Study

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Abstract

Introduction: For patients with mild-to-moderate crowding, enamel stripping is a viable alternative to tooth extractions in preserving teeth inclinations and transverse dimensions. Bioactive compounds such as CPP-ACP, which are derived from milk products, have been created to improve the remineralization of enamel by the use of active ions. The purpose of this study was to assess the remineralization potential of CPP-ACP on enamel surfaces after stripping by two different methods.

Methods: 34 extracted premolar teeth included in the study were divided into 2 groups. Stripping was carried out by two different techniques: Air-rotor and Strips and assessed under the microscope for surface roughness after which the surfaces were treated with CPP-ACP for 21 days and reassessed under the microscope for surface changes.

Results: The study showed significant reduction in surface roughness values in both the groups after application of CPP-ACP. Student t test (two tailed, paired and unpaired) was used to find the significance of study parameters on continuous scale within and between groups. Repeated measures ANOVA was used to find the significance of the study parameters. Bonferroni's post hoc analysis was carried out to test if the values of RM-ANOVA were significant.

Conclusions: Following stripping, the mean surface roughness values in Group 1 and Group 2 were 452.47 ± 19.98nm and 408.47 ± 14.81nm respectively, both significant. There was significant decrease in the surface roughness values of both the groups after application of CPP-ACP agent: 297.18 ± 60.35nm and 212.79 ± 36.30nm in Group 1 and Group 2 respectively.

Keywords: interproximal stripping, enamel surface roughness, remineralizing agent, CPP-ACP, atomic force microscope.

Introduction

Enamel stripping, also known as interproximal reduction is an alternative to extractions in patients with mild-moderate crowding as it allows transverse arch dimensions and anterior tooth inclinations to be maintained. The procedure entails a partial reduction of the enamel layer, resulting in the reduction of the mesiodistal tooth size so that a precise amount of space is created to allow the crowding to unravel [1]. The main indications for the technique include reshaping the proximal contacts, resolving the Bolton's discrepancy, treating mild or moderate crowding, and stabilizing the dental arch. Interproximal reduction is indicated in cases with mild-moderate crowding of 4mm or less [2]. Interproximal reduction (IPR) might be effective in improving alignment and enhance post-orthodontic stability, by reducing the width of enamel at interproximal spaces. [3]. As an alternative to tooth extraction and various non-extraction treatment modalities in patients with moderate crowding, Air-Rotor Stripping (ARS) was introduced by Sheridan, particularly on molars and premolars [4]. The potential increase in caries susceptibility of the stripped enamel surfaces is an area of concern [5]. White spot lesions (WSL), corresponding to the first visible stage of dental caries, have been defined as subsurface enamel porosity from carious demineralization which appears as white milky opacity on smooth surfaces [6]. The most common site for WSL formation is labio-gingival area around lateral incisors [7]. Tufekci et al., has reported in his clinical study that an increase the number of WSLs occurred during the first six months and followed by a slower rise in the next six-month period, thus emphasizing on the maintenance and evaluation of oral hygiene [8]. Recently, a new series of bioactive agents based on milk products have been developed that facilitate the remineralization of enamel by means of releasing active ions. These agents are based on a nano-complex combination of the milk protein casein-phosphopeptide (CPP) with amorphous calcium phosphate (ACP) [9]. CPP-ACP (Casein Phosphopeptide Amorphous Calcium Phosphate) agent serves as a medium which provides calcium and phosphate ions on the enamel surface, to depress demineralization, facilitate remineralization, resulting in improved microhardness of softened enamel. [10] Previous in-vitro studies have shown that enamel surfaces which have been abraded are more prone to demineralization than the intact surfaces [11]. Although smoother surfaces can be obtained by the stripping protocol followed by polishing, there is an increased risk for demineralization due to the removal of the aprismatic enamel layer, which protects the enamel surface, reducing enamel microhardness. The role of CPP-ACP has been described as localization of ACP at the tooth surface, which then provides free calcium and phosphate ions, maintaining a supersaturation state with respect to the enamel, promoting remineralization [12]. CPP-ACP is a milk product which helps in remineralization and prevents dental caries. Casein phosphopeptide can deliver amorphous calcium phosphate and also facilitates its binding with to the enamel [13].

This study aims to assess the efficacy of this agent on enamel surfaces after stripping using the most commonly used two methods: air-rotor and proximal strips.

Objectives of the Study

To compare average roughness value, after proximal stripping using two different methods-

- Air-rotor

- Proximal Strips

2. To compare mean square roughness, after application of CPP-ACP remineralizing agent

- On air-rotor stripped surfaces
- On surfaces stripped with proximal strips

3. To analyze enamel surface changes after stripping and after application of remineralizing agent with the help of Atomic Force Microscope

Materials and Methods

This study aims to assess the following null hypothesis:

- There is no difference in the surface roughness of teeth stripped with air-rotor and teeth stripped with proximal strips
- There is no difference in the enamel surface roughness after application of remineralizing agent on both the groups

Part 1: Active stripping and surface roughness

34 extracted premolar teeth were included in the study by using the following criteria: (1) extraction of premolar teeth due to orthodontic reasons; (2) teeth with no caries; (3) no restorations on any surface; (4) teeth with intact crown and root; (5) teeth with no stains or calculus on any of the surfaces. No attempt was made to identify the gender, race or any previous fluoride exposure of the persons from which our sample was derived. Before stripping, all the samples were first thoroughly cleaned with pumice water and stored in normal saline. The teeth were randomly allocated into two groups of 17 samples each. There was no control group in this study as the initial surface roughness of teeth in control group would not have coincided with either of the groups, and comparison of the outcomes of the control group would have led to high variability and inaccuracies in statistical tests, in both the methods of stripping and application of remineralizing agent. Group 1 was subjected to stripping by air-rotor and Group 2 was subjected to stripping by PD proximal strips (Produits Dentaires, Vevey Switzerland). The enamel stripping was carried out based on the protocol proposed by Sheridan.[14] After that, stripping was carried out in Group 1 using Air-rotor handpiece using high speed air-rotor turbine handpiece with a light, wiping stroke and in Group 2 using proximal strips with a handle. The surfaces of all the teeth were stripped approximately 0.5 mm on one side, which was checked with the help of digital vernier calipers. (Figure 1) The samples were then assessed under the atomic force microscope on the side which underwent stripping for determining the surface roughness of each tooth and the average surface roughness values for each tooth was recorded for both the groups. No surface preparation was necessary for the samples before they would be assessed under the microscope (Figure 2) After the evaluation and recording the average roughness values, the teeth were stored in normal saline until the next step where the samples were then treated with CPP-ACP test agent (GC Tooth Mousse). (Figure 3)

PART 2: Application of remineralizing agent

For 21 days, the specimens in both groups received twice-daily treatments of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP paste). Using a tooth brush, the specimens were manually brushed for three minutes in a reciprocal back-and-forth motion with casein phosphopeptide-amorphous calcium phosphate paste. After that, they were placed in artificial saliva and allowed to remain at room temperature. Up until the point at which each sample received the agent treatment, artificial saliva was replenished every 24 hours. After a 21-day cycle, the specimens in groups I and II were once more examined using atomic force microscopy to

examine and re-evaluate the surface topographical changes brought about by remineralization and to check for alterations in the enamel surface roughness on each of the 34 tooth samples. (Figure 4) To evaluate the outcomes and compare any variations in the average surface roughness values, the average surface roughness values for all the teeth in both groups were once more recorded for each sample. The readings were then compared to the average roughness recorded before the administration of our test agent.

The artificial saliva had the following composition (grams/litre):

- Methyl p- hydroxybenzoate – 2 g/l
- $MgCl_2.2H_2O$ (Magnesium chloride dihydrate) -0.059 g/l
- $CaCl_2.2H_2O$ (Calcium chloride dehydrate)-0.166 g/l
- Sodium carboxy methyl cellulose- 10 g/l
- K_2HPO_4 (Dipotassium hydrogen phosphate)- 0.804 g/l
- KH_2PO_4 (Monopotassium hydrogen phosphate)- 0.326g/l
- KCl (Potassium chloride)-0.025 g/l
- KOH was present for adjusting the pH of the saliva to 6.75

Determination of Sample Size

As per the study conducted by Biavati et al. (Silvestrini Biavati F, Schiaffino V, Signore A, De Angelis N, Lanteri V, Ugolini A. Evaluation of Enamel Surfaces after Different Techniques of Interproximal Enamel Reduction. Journal of Functional Biomaterials. 2023 Feb 16;14(2):110.)[12], enamel roughness value for the groups using fine diamond bur and proximal strips were found to be [median (range)] 1.37 (2.2-0.25) and 1.79 (0.24-3.04) respectively. On converting these values to mean (SD), the men difference between the two groups was found to be 0.42 (0.61). So, assuming the same finding, the sample size was calculated at 95% confidence interval, 80% power, which was calculated to be around 33.9, i.e. 34 teeth samples.

Results

The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used for all statistical description.

Descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements were presented on Mean ± SD. Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant.

Student t tests (two tailed, paired and unpaired) were used to find the significance of study parameters on continuous scale within and between two groups. Repeated measures Analysis of variance (RM-ANOVA) was used to find the significance of study parameters within the group at different time intervals (Intra group analysis). Further Bonferroni's post hoc analysis was carried out if the values of RM-ANOVA test were significant. Keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%. Level of significance were as follows:

$p>0.05$: Not Significant

$p<0.05$: Significant

Table I shows the comparison of surface roughness of teeth before and after stripping in both the groups using an unpaired t-test. The average surface roughness of the tooth samples before stripping in the both the groups has no statistically significant difference and the mean values were 162.12 nm and 158.76 nm respectively. There was a statistically significant difference (p <

0.001) after stripping in both the groups, being 364.12 nm and 286.41 nm respectively, also suggesting that Group 1 (air-rotor) stripped surfaces had higher surface roughness as compared to Group 2.

Table II shows the comparison of mean surface roughness of teeth of Group 1 samples before and after application of CPP-ACP agent on the stripped surfaces using a paired t-test. There is a statistically significant ($p<0.005$) reduction in the mean surface roughness of the samples after application of CPP-ACP, suggesting a potential for remineralization and smoother enamel surfaces after application of the agent.

Table III shows the comparison of the mean surface roughness of teeth samples in Group 2 before and after CPP-ACP application on the stripped surfaces using a paired t-test and it also shows a statistically significant reduction in average surface roughness values similar to that of Group 1, suggesting that CPP-ACP application led to a smoother enamel surface.

Table IV and Table V summarise the findings and the significance of the results of both Group 1 and Group 2 respectively using repeated measures ANOVA test.

Bonferri's Post hoc analysis was done to represent the significance of the values obtained from repeated measures ANOVA tests which has been highlighted in Table VI.

Discussion

This study showed that both the group had statistically significant increased surface roughness values as compared to the unstripped enamel surfaces with mean surface roughness values in unstripped and stripped surfaces in group 1 being 162.12 nm (SD: 22.099) and 364.12 (SD: 47.474) respectively, and in group 2 being 158.76 nm (SD: 15.730) and 286.41 nm (SD: 29.572) respectively. According to the literature, Interproximal Enamel Reduction (IER) has become a consolidated technique used in orthodontic treatment in particular situations such as mild to moderate crowding, non-extraction therapy, Bolton's discrepancies, and prevention of relapse by enhancing stability [14,15]. Numerous studies have shown that stripping of the outer enamel leaves many grooves and furrows on the surfaces of the teeth, through IPR, which changes the enamel surface contour [15,16]. Using Scanning Electron Microscopy (SEM), the grooves and roughness on enamel surfaces have been observed on the interproximal enamel of both deciduous and permanent teeth. These grooves and furrows are regularly or irregularly distributed, over the entire treated area [16]. Biavati et. al reported in their study that all different stripping methods have left strikes and streaks on the enamel surface, contributing to its roughness [17]. Before performing IPR it is important to assess how much of enamel can be reduced. This can be made by projecting a line from the cervical line vertically to the occlusal plane because dentin is projected in a straight line from the cervical line [18]. Chudasama and Sheridan claim that only 0.5 mm should be removed from these contact points, as the interdental enamel is thin [19]. Sheridan and Ledoux claim that by interproximal enamel reduction of eight proximal surfaces of the premolars and molars, around 6.4 mm of space can be gained [20,21]. Fillion et al. suggests to never strip morethan 0.3 mm of the enamel from the upper incisors and 0.6 mm from the posterior teeth [22].

Furthermore, there is also a statistically significant difference between the enamel surface roughness between the Group 1 and Group 2 teeth samples after stripping. (Table I) This study shows statistically significant results ($p < 0.001$), which concludes that

there is greater surface roughness associated with stripping with air-rotor as compared to the proximal strips, which is in accordance to the study conducted by Gholamreza et. al. [23], that reported the same results with rougher enamel surface topography with air-rotor as compared to proximal strips. Another alternative for management of mild-to-moderate crowding is Air-rotor Stripping [4,24] Upon correct utilization of the technique, it is not evidenced that it can be harmful for the hard or soft tissues of the teeth [4,24]. Meredith et. al. [3] in their study of interproximal stripping with different methods under atomic force microscope concluded that there was a statistically significant difference in the enamel surface roughness in air-rotor (diamond coated burs) and proximal strips (diamond coated strips).

This study showed that there was a statistically significant different between the enamel surface roughness in both the groups before and after the application of CPP-ACP agent. In group 1, the mean values for enamel surface roughness before and after agent application was found to be 452.47 ± 19.988 nm and 297.18 ± 60.352 nm respectively, which has been depicted in Table II. In group 2, the mean values for enamel surface roughness before and after agent application was found to be 408.47 ± 14.816 nm and 212.79 ± 36.306 nm respectively, which has been displayed in Table III.

Furthermore, there was also a statistically significant difference ($p < 0.001$) between enamel surface roughness between the two groups after the application of CPP-ACP agent, using an unpaired t test. These results are in agreement with E.C. Reynolds [24], who in his study also reported the increase in mineralization and decrease in average surface roughness (Ra) values of enamel surfaces after the application of CPP-ACP agent in which the enamel surface was subjected to different concentrations of CPP-ACP agents (0.1%, 0.5%, 1%) and reported increase in rate of mineralization with increasing concentration of CPP-ACP. As IPR inevitably alters the tooth enamel by changing the enamel surface contour, numerous qualitative studies have shown that removal of this outer enamel leaves many grooves and furrows on the surfaces of the teeth [15,16] In another study by Alessandri Guilio (2009) [25], it was concluded that topical applications of CPP-ACP can be expected to be effective in enhancing enamel remineralization, which was in accordance with this study.

Interproximal enamel reduction (IPR) is a clinical procedure involving the reduction and anatomic re-contouring of interproximal enamel surfaces of permanent teeth, which can be an alternative for patients with mild or moderate crowding.

Hudson et al., in their studies showed that thickness of enamel increases from the canines to the posterior teeth, whereas in anterior region the enamel thickness is much thinner with less than 1mm enamel [6]. Furthermore, these studies revealed that the enamel is slightly thinner in the distal than in the mesial surfaces, and there is no relationship between dental anatomy and enamel thickness. Regarding the advances in orthodontics, the application of extraction or expansion is difficult when treating patients who choose clear aligners like Invisalign as a treatment modality. [19,22]

The remineralization process through CPP-ACP involves diffusion of CaHPO40 and associated calcium and phosphate ions through the H20 (water) -filled pores of carious surface enamel into the body of the enamel lesion. Once inside the enamel lesion, the calcium and phosphate by dissociation, would increase the activities of Ca2+ and PO43-, thereby increasing the degree of

saturation with respect to Hydroxyapatite present on the enamel surface. The CPP, allows high concentrations of calcium and phosphate ions on the enamel surface, including CaHPO40, which diffuses into the enamel, helping the remineralization process. The high activities of the calcium and phosphate ions during remineralization occur through ACP which is bound to CPP. The bound ACP, by being in equilibrium with the ions of calcium and phosphate, will maintain the concentrations of the ions involved in diffusion into the lesion, providing further free ions [22].

Based on in-situ studies and randomized clinical trials [26,27]. CPP ACP paste was able to increase the remineralization of initial enamel caries lesions. There are different types of remineralizing agents available but one of the most commonly and commercially available is the GC Tooth Mousse (Recaldent) and hence this agent was chosen for our study.

This study highlights the effectiveness of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) as a potent agent for enamel remineralization, primarily by increasing the concentration of calcium and phosphate ions on the enamel surface. In the context of modern orthodontic treatment modalities, such as self-ligating brackets and clear aligner therapy, interproximal enamel reduction (stripping) has become a common adjunct to non-extraction protocols for gaining space. Given the associated risk of creating roughened enamel surfaces post-stripping, the application of CPP-ACP is recommended to enhance enamel recovery and reduce susceptibility to demineralization. Furthermore, the findings of this study may extend to the management of white spot lesions, a frequent and aesthetically concerning complication observed after the removal of fixed orthodontic appliances. The demonstrated remineralizing properties of CPP-ACP suggest its potential as a preventive and therapeutic agent in such cases. Despite promising results, the study presents certain limitations. The sample size was relatively small, which may affect the precision and generalizability of the findings. Future research with larger cohorts is necessary to validate and strengthen these clinical implications. Additionally, the use of atomic force microscopy for surface analysis carries inherent variability, as the exact evaluation site may differ among samples, potentially influencing the consistency of results. Another limitation is the financial constraint that restricted the study to testing only one commercially available remineralizing agent. Including a wider range of recent and advanced remineralizing products in future studies could offer a more comprehensive understanding of their effectiveness and clinical applicability.

Conclusion

There was statistically significant increase in the surface roughness values of enamel after stripping with both the procedures, as highlighted by the graphs in both the groups. Application of GC Tooth Mousse (CPP-ACP) shows a statistically significant decrease in the mean surface roughness values of stripped enamel surfaces, for both of the groups, with greater reduction in mean surface roughness in Group 2, which was stripped with the help of proximal strips

CPP-ACP (GC Tooth Mousse) can be used and an adjunct to proximal stripping to enhance remineralization of roughened enamel surfaces. Its application can be extended for management of white spot lesions, which can be appreciated post debonding, as localized demineralized lesions on enamel surfaces, though further long term studies are suggested.

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Legend Tables and Figures

Table 1: Comparison of roughness of teeth surface in terms of {Mean (SD)} before and after stripping among both the groups using unpaired t test

Variables	Group	N	Mean (in nm)	Std. Deviation (± SD)	t value	p value
Before stripping	Group 1	17	162.12	22.099	0.510	0.614
	Group 2	17	158.76	15.730		
After stripping	Group 1	17	452.47	19.988	5.728	<0.001**

	Group 2	17	408.47	14.816		

Table 2: Comparison of roughness of teeth surface in terms of {Mean (SD)} before and after agent application in group 1 using paired t test

Time interval	N	Mean	Std. Deviation (± SD)	t value	p value
Before agent application	17	452.47	19.988	9.356	<0.001**
After agent application	17	297.18	60.352		

Table 3: Comparison of roughness of teeth surface in terms of {Mean (SD)} before and after agent application in group 2 using paired t test

Time interval	N	Mean	Std. Deviation (± SD)	t value	p value
Before agent application	17	408.47	14.816	20.452	<0.001**
After agent application	17	212.79	36.306		

Table 4: Comparison of roughness of teeth surface in terms of {Mean (SD)} at different time intervals in group 1 using repeated measures ANOVA test

Time interval	N	Mean	Std. Deviation (± SD)	Wilk's Lambda value	P value
Initial roughness of the teeth	17	162.12	22.099	141.390	<0.001**
After air-rotor stripping	17	452.47	19.988		
After application of remineralizing agent	17	297.18	60.352		

Table 5: Comparison of roughness of teeth surface in terms of {Mean (SD)} at different time intervals in group 2 using repeated measures ANOVA test

Time interval	N	Mean	Std. Deviation (± SD)	Wilk's Lambda value	P value
Initial roughness of the teeth	17	158.76	15.730	145.439	<0.001**
After stripping with PD proximal strips	17	408.47	14.816		
After application of remineralizing agent	17	212.79	36.306		

Table 6: Bonferroni's post hoc analysis

	Initial roughness of the teeth	After proximal stripping using proximal strips	After application of remineralizing agent
Initial roughness of the teeth (Group II)	-	<0.001**	<0.001**
	Initial roughness of the teeth	After proximal stripping using air-rotor	After application of remineralizing agent
Initial roughness of teeth (Group I)	-	<0.001**	<0.001**

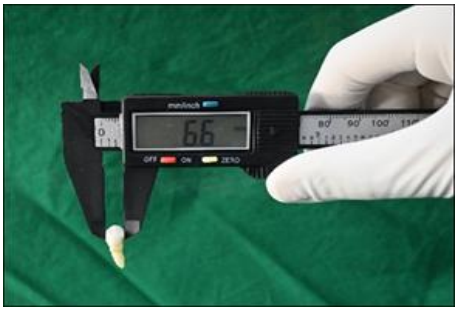

Figure 1: Mesio-distal measurement of tooth using digital vernier calipers after proximal stripping

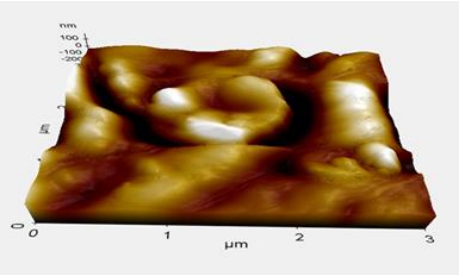

Figure 2: 3D image of surface roughness appearance of enamel of after stripping under the atomic force microscope

Figure 3: GC Tooth Mousse (CPP-ACP) Remineralizing agent

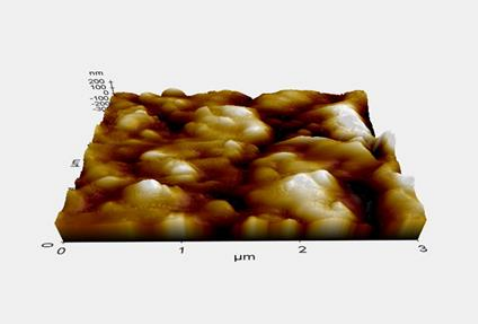

Figure 4: 3D image of surface roughness appearance of enamel of tooth sample after application of remineralizing agent