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"Comparative evaluation of the Efficiency of Three Remineralising Agents on Enamel Surface after Reproximation- An Ex-Vivo study."

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ABSTRACT

Objective: Dental crowding represents the most prevalent issue among orthodontic patients. The reproximation technique involves the reduction, reshaping, and safeguarding of proximal enamel surfaces. Reproximation involves reducing, reshaping, and safeguarding proximal enamel surfaces. It is a crucial tool in cases where tooth extraction is on the borderline, aiming to create space and alleviate crowding. This study aimed to investigate, whether when an Oscillating saw with the diamond-coated metal strip is used for reproximation, the use of CPP-ACP, NovaMin and nano-hydroxyapatite application, post-reproximation procedure affects or enhances the remineralizing of the enamel surface.

Methods: Patients who report to the Dentofacial Orthopaedics and Orthodontics Department at NIMS Dental College and Hospital in Jaipur comprised the sample size. Individuals without cavities, cracks, fillings, fractures, or hypoplasia who had therapeutic extraction of their premolars as part of an orthodontic treatment plan were chosen. Before the commencement of the investigation, the patients provided their informed consent. The samples were divided into 5 groups. Patients were advised to apply the appropriate remineralizing paste to the stripped surfaces, based on their assigned groups and the exact paste provided. Photomicrographs were captured using SEM at various magnifications (500, 1000, 5000) for visual inspection, particularly focusing on surface roughness. A Micro Hardness Tester was utilized to measure Vickers hardness.

Results: This study's microhardness results showed that Group 1, which had intact enamel and had not had any stripping, had the greatest mean microhardness value, whereas Group 2, which had teeth extracted and polished, exhibited the lowest microhardness value among the stripped enamel samples. The findings indicate that CPP-ACP was more effective than Novamin and Nano-hydroxyapatite in enhancing approximated enamel's mineral content and microhardness.

Conclusion: The microhardness and surface roughness values of enamel surfaces dropped during

interproximal enamel peeling. On the stripped enamel surface, saliva and saliva mixed with remineralising agents (CPP-ACP, Novamin, Vantej) decreased surface roughness values elevated by reproximation.

Keywords: *Remineralising Agents, Enamel Surface and Reproximation*

INTRODUCTION

Dental crowding represents the most prevalent issue among orthodontic patients. The reproximation technique involves the reduction, reshaping, and safeguarding of proximal enamel surfaces. It is a crucial tool in cases where tooth extraction is on the borderline, aiming to create space and alleviate crowding. Additionally, it plays a role in addressing disparities identified by the Bolton index and preventing relapse. Reproximation is a long-standing clinical treatment used in conjunction with non-extraction orthodontic procedures. It involves removing dental enamel from between teeth to reduce the mesiodistal diameter of dental units[1].

The intricate structure of enamel causes it to vary over time in terms of both chemical composition and surface state. It consists of two thin inner and outer aprismatic layers at its extremities and a prismatic layer inside its centre. When enamel reaches maturity, it comprises over 95% mineral components, mostly in the form of hydroxyapatite or stacked calcium phosphate crystals. The outer aprismatic layer, which acts as a barrier to preserve the enamel and has the largest concentration of calcium and phosphate, must be removed to thin the enamel. This may increase the enamel's vulnerability to demineralization and raise the risk of dental hypersensitivity and carious infections. Applying a remineralizing agent is therefore essential for enhancing the integrity of the enamel surface[2].

Nevertheless, this process affects the enamel's surface quality and may make cavities and hypersensitivity more likely. It has been recommended to employ polishing discs and specific compounds, such as sealants and fluoride treatments, to combat the undesirable effects of reproximation. These interventions aim to create smoother enamel surfaces and bolster remineralization. Several substances are employed to enhance remineralization after tooth regrowth. One such drug is composed of ACP and milk protein CPP. They serve as a ca^{+} and P^{+} ion reservoir on the tooth's surface, which inhibits demineralization, encourages remineralization, and strengthens the softened enamel's microhardness[3].

Particles of nanohydroxyapatite (n-HAP) show promise in remineralizing early caries. The capacity of nano-HA to promote remineralization greatly increases at pH values below 7.0. When exposed to acidic solutions, Nano-HA effectively stops further mineral loss from the enamel surface and shows strong deposition onto the etched enamel surface. Considering this perspective, nano-HA could elicit varying remineralization effects depending on pH levels, potentially enhancing the process overall. Nano-HA holds promise as a potent repair material and anticaries agent. The restoration of demineralized enamel surfaces might be aided by the two mechanisms of particle- and ion-mediated remineralization that are unique to nano-HA. Another notable agent is an inorganic substance called NovaMin (calcium sodium phosphosilicate), which interacts in aqueous conditions to release phosphate, sodium, and calcium ions gradually[4].

Calcium sodium phosphosilicate, known as NovaMin, belongs to the category of highly biocompatible materials initially designed for bone regeneration purposes by Hench and Andersson in 1993. These materials exhibit reactivity upon exposure to bodily fluids, leading to the deposition of hydroxycarbonate apatite (HCA), a mineral closely resembling natural tooth mineral. NovaMin particles are included in dentifrices and are deposited onto dentin surfaces, obstructing dentinal tubules mechanically. Scanning electron microscopy (SEM) studies have highlighted challenges in eliminating coarse abrasions remaining after initial reproximation procedures. This SEM study set out to assess several polishing methods proposed in the literature and determine the relative merits of each remineralizing agent. An accurate experimental method for figuring out a material's mechanical properties in terms of microhardness is depth-sensing indentation. A computer procedure based on the Oliver-Pharr method was developed to increase the accuracy of predicting the elastic modulus. Using a Vickers indenter, a Fischerscope H100 depth measuring apparatus was utilized for this method.

Furthermore, the computer program attempted to determine Vickers's hardness to make up for the lack of software specifically designed for this purpose on the apparatus. Analysis of the indentation

data may reveal several characteristics of the materials under study, including Vickers hardness, contact depth, projected contact area, initial unloading stiffness, decreased modulus, and elastic modulus[5]. This study aimed to investigate, whether when an Oscillating saw with the diamond-coated metal strip is used for reproximation, the use of CPP-ACP, NovaMin and nano-hydroxyapatite application, post-reproximation procedure affects or enhances the remineralizing of the enamel surface.

METHODOLOGY

Patients who report to the Dentofacial Orthopaedics and Orthodontics Department at a private Dental College in Jaipur comprised the sample size. Individuals without cavities, cracks, fillings, fractures, or hypoplasia who had therapeutic extraction of their premolars as part of an orthodontic treatment plan were chosen. The patient's good periodontal health was confirmed. Before the commencement of the investigation, the patients provided their informed consent. The Committee for Institutional Ethics examined the procedure.

Premolars were reproximated on their mesial and distal surfaces using an oscillating saw equipped with a diamond-coated metal strip before therapeutic extraction planning was carried out. The premolars were then polished using 3M Sof-Lex discs. After re-proximation, patients were given the remineralizing agents (one patient one agent was provided) for 3 months. After exposure to the oral condition and the use of a remineralizing agent by the patient for 3 months, the teeth were considered for therapeutic extraction.

The samples were divided into 5 groups (10 samples in each group):

Group 1 comprised of the Control group in which No reproximation was done and no remineralizing agent was used.

Group 2, in which the proximal surface of premolars was reproximated with an oscillating saw with a metal strip covered with diamonds and polished with a Sof-Lex polishing disc.

Group 3, in which the Premolars' proximal surfaces were reproximated using an oscillating saw and a metal strip coated in diamonds. Then, a Sof-Lex polishing disc was used to polish the teeth, and CPP-ACP was used.

Group 4, in which the proximal surface of premolars was reproximated with an oscillating saw with a metal strip covered with diamonds and polished with a Sof-Lex polishing disc followed by the application of NovaMin.

Group 5, in which the proximal surface of premolars was reproximated with an oscillating saw with a metal strip covered with diamonds (Strauss, liberal traders) and polished with Sof-Lex polishing disc followed by the application of Nano-hydroxyapatite.

Before the commencement of the trial, informed permission was given by all adult patients and the parents of patients who were younger than eighteen. The patients also received comprehensive information regarding the study design. The study protocol received approval from the Scientific and Ethical Committee of NIMS University, Jaipur, Rajasthan.

Every tooth underwent ten oscillating saw strokes on its proximal surfaces and twenty Sof-Lex disc strokes. The remineralization agent application technique was started right away after the stripping process. After their regular at-home tooth brushing routine, patients were advised to apply the paste onto the approximated teeth for 3 minutes once a day for 3 months. They were also told to wait 30 minutes after applying the remineralizing paste before consuming any food or liquids.

After their first premolars were extracted, orthodontic therapy was administered to all research participants. Following extraction, each of the fifty teeth was collected and stored in deionized water until required. For ten minutes, the gathered samples were cleaned using an ultrasonic cleaner in deionized water. For SEM (Scanning Electron Microscopy) evaluation, samples from each group were prepared with gold coating to facilitate qualitative assessment of the enamel surfaces. Photomicrographs were captured using SEM at various magnifications (500, 1000, 5000) for visual inspection, particularly focusing on surface roughness.

A Micro Hardness Tester was utilized to measure Vickers hardness. The procedure involved applying a Vickers pyramid, a 136-degree angle pyramidal diamond indenter with a square base, to the tooth portions' exposed surfaces. The 200-gram load was applied and held for 10 seconds. Three indentations were done for each specimen, and the average hardness value was determined.

The data was entered into the computer (MS Word, Excel) and subjected to statistical analysis by using

SPSS (Statistical Package for Social Science) version 20.0
The Independent sample t-test and One-way ANOVA were done to compare the inter-group differences.

RESULTS

Table 1: Intergroup comparison based on Remineralising Efficiency.

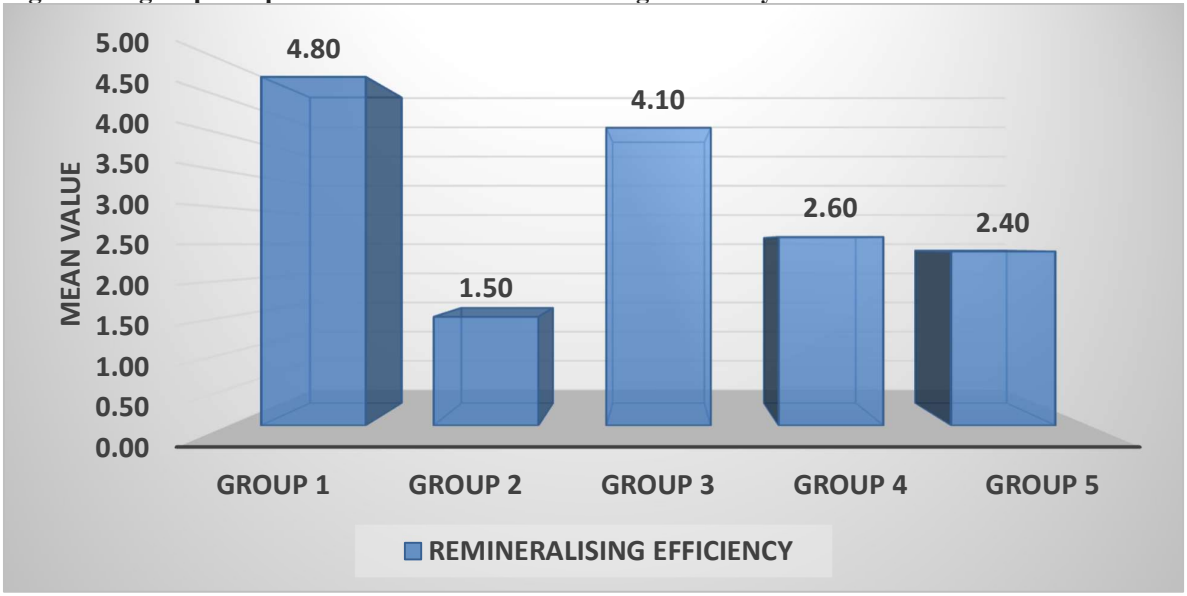
Group	N	Remineralising Efficiency		P value
		Mean	SD	
1	10	4.8	0.42	0.001*
2	10	1.5	0.52	
3	10	4.1	0.56	
4	10	2.6	0.51	
5	10	2.4	0.51	

ANOVA Test applied, SD- Standard Deviation, Level of Significance: ≤ 0.05, Statistically Significant *, Statistically Non-Significant **

ANOVA

Remineralising Efficiency	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	71.88	4	17.97	68.53	0.001*
Within Groups	11.8	45	0.262		
Total	83.68	49			

Fig 1: Intergroup comparison based on Remineralising Efficiency.



The mean Remineralising efficiency of Group 1, Group 2, Group 3, Group 4 and Group 5 was 4.8 ± 0.42, 1.5 ± 0.52, 4.1 ± 0.56, 2.6 ± 0.51 and 2.4 ± 0.51 respectively. There is a statistically, Significant difference between all 5 Groups. ($p \leq 0.05$*)

Table 2: Intergroup comparison based on Vicker's Hardness Test.

Group	N	Vicker's Hardness Test		P value
		Mean	SD	
1	10	380	52	0.071**
2	10	308.17	40	
3	10	331.33	48	

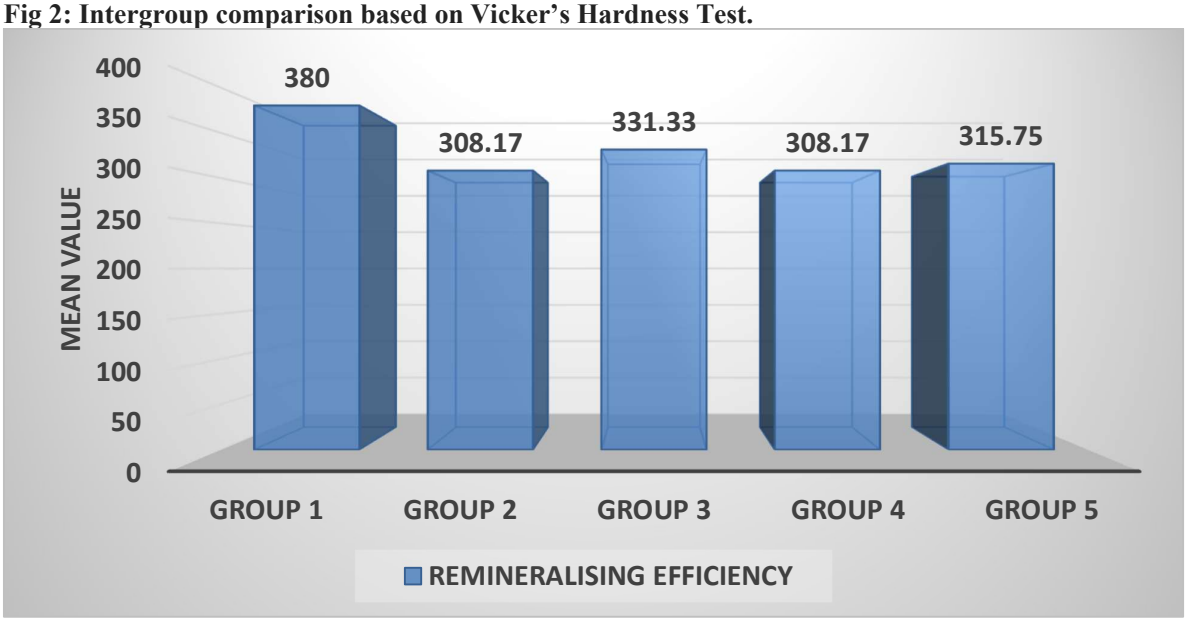
4	10	308.17	39	
5	10	315.75	42	

ANOVA Test applied, SD- Standard Deviation, Level of Significance: ≤ 0.05, Statistically Significant *, Statistically Non-Significant **

ANOVA

Vicker's Hardness Test	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	628.95	21	1791.97	128.53	
Within Groups	120	165	0.262		0.071**
Total	748.95	186			

Fig 2: Intergroup comparison based on Vicker's Hardness Test.



The mean Remineralising efficiency of Group 1, Group 2, Group 3, Group 4 and Group 5 was 380 ± 52, 308.17 ± 40, 331.33 ± 48, 308.17 ± 39 and 315.75 ± 42 respectively. Statistically, No, Significant difference exists between all 5 Groups. ($p > 0.05$**)

DISCUSSION

Enamel proximation is a common orthodontic treatment used to treat black triangles caused by gingival recession between neighbouring teeth, tooth crowding, and Bolton tooth-size disparities. While some dental specialists remain sceptical about its association with caries and periodontal disease, no direct link has been identified between the stripping procedure and elevated susceptibility to these conditions. Nonetheless, certain preventive measures have been suggested following enamel stripping to mitigate potential adverse effects[6,7].

Until now, the majority of research examining the surface properties of enamel ground by

stripping techniques has mostly depended on qualitative assessments made using SEM. Visualization of surface morphology, such as ground enamel, is possible through microscopic analysis. To quantitatively evaluate the degree of surface alterations, especially surface roughness, they need further techniques. Hence, in this study, microhardness tests were also employed to gather quantitative data, providing a more comprehensive understanding of the enamel's response to stripping procedures[8].

Arman et al. asserted that reproximation methods resulted in rougher surfaces compared to intact enamel. It's important to note, nevertheless, that before the surface evaluation, they altered the structure of the sound enamel by refining it with silicon-carbon discs rated at 1200 grit. There is a clear correlation between the mineral content of enamel and its microhardness. Mineral content is important because it prevents erosion and dissolution of enamel. Furthermore, evaluation of microhardness can provide oblique proof of mineral increase or loss in tooth-hard tissues[9].

According to our research, there were no appreciable variations in the permanent tooth sample's mean enamel microhardness values across the groups. This suggests that the tested stripping techniques were able to maintain the hardness of unharmed enamel surfaces even after polishing with Sof-Lex discs. Thus, it can be said that using Sof-Lex discs to polish stripped enamel does not result in additional enamel structure grinding, making this polishing method safe to use[10].

The study's microhardness results showed that the average remineralizing efficiency for Groups 1, 2, 3, 4, and 5 was 315.75 ± 42, 331.33 ± 48, 308.17 ± 39, and 380 ± 52. This study's microhardness results showed that Group 1, which had intact enamel and had not had any stripping, had the greatest mean microhardness value. In contrast, Group 2, which had teeth extracted and polished, exhibited the lowest microhardness value among the stripped enamel samples.

The mean microhardness values in Groups 3 (CPP-ACP), 4 (Novamin), and 5 (Nano-hydroxyapatite) were greater than those in Group 2. Between Groups 4 and 5, no statistically significant changes were discovered. The findings indicate that CPP-ACP was more effective than Novamin and Nano-hydroxyapatite in enhancing approximated enamel's mineral content and microhardness. Saliva and remineralizing agents worked together to cause the alterations in Groups 4 and 5. Notably, undamaged enamel had the highest microhardness findings.

All enamel reduction techniques studied in this research notably altered the enamel surface, creating furrows and grooves as observed through SEM examination. Results indicate that achieving similar morphological properties to intact enamel was not achieved solely through enamel polishing following reproximation. Nevertheless, using Sof-Lex polishing discs after reproximation resulted in smoother enamel surfaces. Similar recommendations were made by Piacentini et al., who proposed utilising diamond-coated discs and tungsten-carbide burs to reduce enamel before applying Sof-Lex discs. Additionally, in Groups 3, 4, and 5, the application of remineralizing agents led to a reduction in surface roughness[11].

The SEM findings showed that the mean remineralizing efficiency for Groups 1, 2, 3, 4 and 5 was, respectively, 4.8 ± 0.42, 1.5 ± 0.52, 4.1 ± 0.56, 2.6 ± 0.51, and 2.4 ± 0.51. All five groups differ statistically significantly from one another. The surface roughness values increased following stripping when remineralizing chemicals were applied or when the area was exposed to saliva[12].

Swarup JS and Rao A, found that when compared to 2% sodium fluoride, nano-hydroxyapatite (n-HAP) particles have more potential for remineralizing early caries[13]. Following bleaching therapy, Burwell et al. discovered that the use of remineralization agents, such as nano-hydroxyapatite (n-HAP), reduced hypersensitivity. This study also shows that nano-hydroxyapatite aids in the remineralization of reproximated enamel surfaces, however, it does so with less effectiveness than CPP-ACP[14].

Mohanty P et al inferred that novamin enhanced remineralization[15]. A study conducted by Livas C et al demonstrated that CPP-ACP exhibited superior remineralizing potential compared to tricalcium phosphate (TCP) and bioactive glass (BAG) Novamin. This study is also by CPP – ACP which has more remeneralising efficiency than Novamin[16].

Using SEM, Gumustas B et al. conducted an in vitro investigation to ascertain the impact of CPP-ACP therapy on the morphology of the approximated enamel surfaces following a lactic acid solution exposure. They concluded that, after interdental stripping, topical administrations of CPP-ACP might successfully encourage enamel remineralization. The results of this investigation support those of the present study. CPP-ACP stabilizes calcium and phosphate ions at the tooth surface by binding to pellicle and plaque. Phosphorus and calcium are held in reserve in this complex and stick to the surfaces

of the teeth. The bonded CPP-ACP releases phosphate and calcium ions in response to acid challenge, preserving the supersaturated mineral environment. Enamel's demineralization is lessened and its remineralization is encouraged by this process[17].

CONCLUSION

The microhardness and surface roughness values of enamel surfaces dropped during interproximal enamel peeling[18]. On the stripped enamel surface, saliva and saliva mixed with remineralising agents (CPP-ACP, Novamin, Vantej) decreased surface roughness values that had been elevated by reproximation. On saliva and peeling enamel, there was no statistically significant change in microhardness between the applications of CPP-ACP, Novamin, and Vantej. Saliva and saliva combined with remineralizing agents (CPP-ACP and fluoride) were found to decrease surface roughness values on stripped enamel surfaces[19]. In terms of SEM analysis and surface roughness testing, undamaged enamel has a smoother surface followed by CPP-ACP, Novamin and Vantej groups than the reproximated enamel surface without any use of remineralising paste[20]. The surface roughness values indicated that CPP-ACP paste has better remeneralising properties than Novamn and Vantej. According to Microhardness, there is no discernible difference between any of the five groups in the Vickers hardness testing.

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