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EFFECT OF REMINERALISATION AND RESIN INFILTRATION ON THE SURFACE HARDNESS OF THE DEMINERALISED DENTIN ; AN IN-VITRO STUDY

Endodontics

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ABSTRACT

Objective: Non-Carious Cervical Lesions (NCCL) are characterized by loss of hard dental tissue near the CEJ. NCCLs that undergo demineralization and long term remineralizing treatment alter the structure of dentin. The purpose for conducting this study was to assess the effect of surface micro hardness of the demineralised dentin after remineralising it with MI Paste Plus, Remin Pro and Icon Infiltrant **Method:** 25 extracted premolar teeth were sectioned into buccal & lingual halves exposing the dentin at the cervical aspect. All samples were demineralised using citric acid solution and were then divided into different groups according to the remineralising agents used. All samples from each group were subjected for analysis to check their surface hardness after 15 days using a Vickers hardness testing machine **Results:** The mean micro hardness in the Group I was 64.79 (SD=2.44), Group II was 40.82 (SD=2.98), Group III was 54.00 (SD=2.88), Group IV was 50.82 (SD=3.02) and Group V was 54.61 (SD=2.88). The micro hardness was highest in the Group I, followed by Group V and least in the Group II . The difference between the five groups was found to be statistically significant with f value of 90.879 and p value of 0.001 **Conclusion:** Within the limitations of this in vitro study, the sound dentin shows greatest microhardness, yet along with this, it has also been found that Remin Pro, MI Paste Plus and Icon Resin Infiltrant can efficiently increase the micro hardness of demineralised dentin.

KEYWORDS

Remineralization, Resin Infiltration, Remin Pro, Mi Paste Plus

INTRODUCTION

Loss of hard tissue at the cementoenamel junction (CEJ) is a condition commonly encountered in clinical practice. The prevalence rate of cervical lesions makes about 18 %. of the permanent teeth defects. Non Caries Cervical Lesions (NCCLs) are caused by tooth structure loss caused by a variety of etiologies such as chemical agents, occlusal stress, mechanical factors, or a combination of all of these. They are the result of increased speed and level of demineralization, which becomes dominant over time (1). Erosion, abrasion, and abfraction occur as a result of the interaction of several such factors . The loss of cervical tooth structure is compensated by restoring the defect to meet aesthetic and functional demands. Demineralization in NCCLs and long-term remineralizing treatment change the structure of dentin. Thus, it is a unique challenge for the clinician to successfully restore such demineralized and partially remineralized NCCLs.

Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into the crystal voids formed in the demineralized enamel to produce net mineral gain (2). The availability of calcium and phosphate ions is the limiting factor for net remineralization to occur (3). Many commercial products, such as fluoride compounds, have been introduced to date for remineralization of White Spot Lesions (WSLs). Despite the fact that the efficacy of fluoride for remineralization of WSLs has previously been established , recent studies have called into question the use of fluoride for treatment of these lesions . Fluoride uptake by the superficial layer of WSLs may reduce calcium and phosphate uptake, resulting in inadequate remineralization of deeper layers . Furthermore, applying fluoride alone does not improve the appearance of these lesions . As a result, several other compounds have been introduced for the remineralization of early caries.

Casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF), marketed as MI-Paste Plus, contains calcium, phosphate and fluoride stabilized by casein phosphopeptide. Dental remineralization technology has undergone advancements with combinations such as fluoride, hydroxyapatite (HA), and xylitol, ingredients used in Remin Pro (VOCO GmbH, Cuxhaven, Germany), which is believed to be associated with calcium in aqueous solution to inhibit the dissolution of calcium and/or phosphate ions from enamel and to act as a carrier of calcium required for enamel remineralization. HA is a major mineral component found in the dental matrix and is what gives teeth its rigidity.

The resin infiltration technique is a minimally invasive alternative treatment for non-cavitated carious lesions. The goal of low viscosity light-activated resin infiltration is to seal the carious lesion microporosities and form a diffusion barrier within the lesion, preventing acids from penetrating and depositing material on the enamel surface . Several studies have demonstrated the efficacy of this technique (4,5). The resin matrix has the ability to strengthen the enamel structure by increasing surface microhardness and preventing enamel surface breakdown (6).

Finding the best protocol for remineralization of incipient enamel lesions is a crucial step toward achieving preventive dentistry goals with minimal invasion. Thus the purpose of this study was to evaluate and compare the effects of MI-Paste Plus, Remin Pro, and ICON on the surface hardness of incipient enamel lesions. The null hypothesis for this study stated that the three materials would not differ significantly in their effect on the surface hardness on remineralised dentin.

MATERIALS AND METHODS

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Praj Mettallurgical Laboratory, Pune. A total of 25 human permanent maxillary & mandibular premolar teeth which were extracted for Orthodontic or Periodontal reasons were collected.. Sound tooth structure free from cracks, restorations, stains, white spot lesions and caries evident by naked eye were taken for the study. Tooth with enamel hypoplasia or any other developmental anomalies and teeth with any visible or detectable caries, cracks & restorations were excluded from the study. Teeth were cleaned using ultrasonic scalers for removing any tissue remnants, plaque and calculus on the roots.

Crowns were separated from the roots by sectioning the teeth 1 mm apical to the cementoenamel junction using a diamond disc. The obtained crown portions were sectioned longitudinally into buccal and lingual halves (n = 50). The sectioned halves were placed in silicon rectangular blocks filled with self-cure acrylic resin, until the resin set. The dentin was exposed on the cervical one-third of the buccal and lingual surfaces of the teeth. Both surfaces were wet polished using 600-grit silicon carbide paper to obtain a flat surface. They were then inspected under a dental operating microscope to ensure that no remnants of enamel were present.

All the acrylic block samples were then allotted randomly into five groups (n = 10) according to the remineralizing agents used. Group 1 - Sound Dentin, Group 2 - Demineralised Dentin

Group 3 - Remin Pro, Group 4 - MI-Paste Plus, Group 5 - Icon Infiltrant.

Samples in group 1 (sound dentin) did not receive any further treatment while those in the rest of the groups (Group 2-5) were immersed in 1% citric acid solution (pH 2.5) for 3 days.

At the end of demineralization, the samples were washed with distilled water for 30 min.

Samples in group 2 (demineralized dentin) did not receive any further treatment. The samples in groups 3–5 were subjected to remineralization treatment using Mi-Paste Plus, Remin Pro and Icon resin Infiltrant according to the manufacturer's instructions

In the group 3, Remin Pro (VOCO GmbH, Cuxhaven, Germany) was applied on the surface of tooth samples. The Paste was made into slurry by dissolving dentifrice (5 g) in water (10 ml), which was then applied on the exposed dentin in 1mm thickness and brushed with the help of an toothbrush for 2 min. After 3 minutes, the samples were rinsed with deionized water for 30 seconds and dried with gentle airflow for 5 seconds. This was repeated twice a day for 15 days. All samples were stored in artificial saliva in between the experiment. In the group 4, Tooth Mousse Plus (MI Paste Plus; GC Corporation, Tokyo, Japan) was applied in the manner similar to group 3.

Finally, in the group 5, ICON (DMG, Hamburg, Germany) was also used according to the manufacturer's instructions. The ICON-Etch was applied on the surface of samples for 2 minutes. Then rinsed with water for 30 seconds. Surface was then dried with gentle air spray for 5 seconds. ICON-Dry was applied on the surface of samples for 30 seconds. Then again dried with gentle air flow for 5 seconds. One layer of ICON-Infiltrant was then applied on the surface of samples and remained for 3 minutes. Excess material were removed by a cotton roll and resin was light-cured for 40 seconds using a LED light-curing unit. After that, another layer of ICON- Infiltrant was applied on the surface of samples and remained for 1 minute. Afterwards, the resin was light-cured for 40 seconds.

Microhardness Evaluation

All the 10 samples from each group were subjected to analyse their surface hardness after 15 days using Vickers hardness testing machine. In each sample, three indentations were made along tooth surface. At each measurement, indentation was made using a 50 g load perpendicular to the indentation surface with a dwell time of 10 seconds. The indentations were made at a distance of 1000 micrometer from each other. The hardness values for each specimen was obtained as the average of the results for the three indentations.

Raw data was obtained and sent for statistical analysis

Statistical Analysis

Sample size estimation was done by using GPower software (version 3.0). Sample size was estimated for ANOVA. The significance was set at $p < 0.05$. Failure modes were analyzed using chi-square test

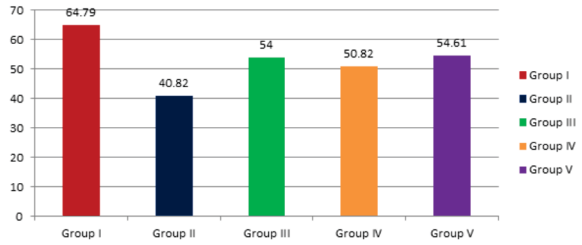
RESULT

The mean micro hardness in the Group I was 64.79 (SD=2.44), in the Group II was 40.82 (SD=2.98). in the Group III was 54.00 (SD=2.88), in the Group IV was 50.82 (SD=3.02) and in the Group V was 54.61 (SD=2.88). The micro hardness was highest in the Group I, followed by Group V and least in the Group II .

The intergroup comparison between the five groups was done using the One Way ANOVA and difference between the five groups was found to be statistically significant with f value of 90.879 and p value of 0.001. The intergroup comparison between the group III & V was done using the independent t test and difference between the five groups was found to be statistically nonsignificant with p value of 0.635

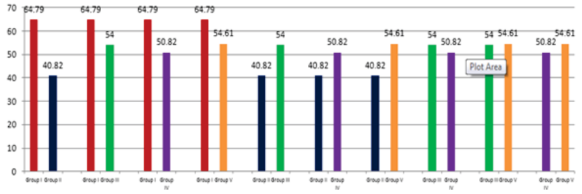
Mean Micro Hardness In The Five Groups

	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Group I (Sound Dentin)	64.79	2.44	0.772	60.60	68.40
Group II (Demineralised Dentin)	40.82	2.98	0.942	36.30	45.60
Group III (Remin Pro)	54.00	2.88	0.912	49.40	58.40
Group IV (MI-Paste Plus)	50.82	3.02	0.957	46.80	55.60
Group V (Icon Infiltrant)	54.61	2.88	0.912	50.90	60.20



Intergroup Comparison Of Mean Microhardness Between The Groups

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2957.007	4	739.252	90.879	.000
Within Groups	366.050	45	8.134		
Total	3323.057	49			



DISCUSSION

The main objective of this study was to assess the effect of surface micro hardness of demineralised dentin after remineralising with MI Paste Plus, Remin Pro and Icon Infiltrant. The results showed that MI paste plus, Remin Pro and Icon Infiltrant increased the surface microhardness of demineralised dentin but was found to be lower than the sound dentin.

Studies have shown that dentin remineralized at a much faster rate than enamel . Similar results were reported by Laheij et al. 2010 (7). who evaluated in situ remineralization of enamel and dentin. According to ten Cate (8) in 2008 , proteins such as dentin phosphoprotein may play a role in enhanced mineralization of dentin.. Similarly van Strijp et al. (9) observed full remineralization of dentinal lesions after using fluoride toothpastes and observed that remineralization conditions for dentin lesions may be favorable compared to enamel lesions. They suggested that the demineralized organic matrix of dentin may constitute a scaffold to enhance remineralization .

In this study, all the remineralising agents has shown a significant increase in the surface hardness of demineralised dentin.The increasing demand for non-invasive therapeutic procedures for dental caries has encouraged researchers to assess and compare the efficacy of remineralizing agents available in the market . CPP-ACP is among the commonly used remineralizing agents and its efficacy has been widely investigated (10). MI Paste Plus is a type of CPP-ACPF available in the market that contains amorphous calcium and phosphate as well as 900-ppm fluoride, stabilized by casein phosphopeptide (11).

It has been found that remineralizing agent Remin Pro contains fluoride (1450 ppm), HA, and xylitol (antibacterial agent) which can mitigate dental hypersensitivity, prevent demineralization, and enhance remineralization of subsurface lesions. HA provides remineralizing agents with the potential to repair microcracks in enamel and dentin, thereby improving the hardness of enamel and dentin by HA present in preparations like Remin Pro (12).

Incipient decay can now also be managed with other conservative methods (13). Resin infiltration (RI) which was developed in Germany to treat early enamel proximal caries. It is now commercially available in the name ICON. It's low viscosity that penetrates the porosities of the lesions, making it superior to the former technique that caused

sealing of the occlusal lesions (14).

The prevalence rate of cervical lesions makes about 18% of the permanent teeth defects.

Premolars are the most commonly affected teeth. Thus, the current study was conducted on Maxillary & Mandibular Premolar teeth. In the current study, dentin was exposed on the cervical aspect of the buccal and lingual surfaces of the teeth so that it closely relates to the condition of Non Caries Cervical Lesion's. (NCCL's) The demineralization-remineralization cycle was adopted from a previous study (15). This closely mimics an erosive intraoral environment, in which cycles of demineralization and remineralization occur.

The results of our study showed that Remin Pro to be more effective than MI Paste Plus in remineralization of demineralised dentin. The phosphopeptide stabilizes the calcium and phosphate ions and creates a super-saturated state of these ions adjacent to tooth structure.

Remineralization of tooth structure occurs in presence of high levels of these ions and increased pH (16). In fact, the density of hydroxyapatite crystals increases by penetration of calcium, phosphate and fluoride ions into these crystals (17). Leila et al. (18) in their study showed MI Paste Plus to be more effective than Remin Pro in remineralising the tooth structure, which was in contrast to our findings. Although the efficacy of high concentration of calcium for dental remineralization has been previously confirmed, high concentration of calcium can cause its quick absorption by the superficial layers and thus, less remineralization occurs in deeper layers of the lesion. Casein phosphopeptide present in MI Paste Plus prevents its fast deposition and stabilizes the calcium and phosphate compounds (17). According to Vyavhare et al. (19) MI Paste can be used as an adjunct to fluoride but due to lower remineralization level, it cannot be used as an alternative to fluoride. It should be noted that natural saliva and dental biofilm provide a suitable environment to preserve the afore-mentioned super-saturated state of ions in the oral cavity during treatment with CPP-ACP compounds (20). Considering the absence of these factors in vitro, the effect of these compounds can only be attributed to their application time on dental samples; this explains the difference in the results of in vivo and in vitro studies.

The efficacy of Remin Pro for remineralization of incipient enamel lesions has beenpreviously confirmed. Kamath et al. (21) indicated that Remin Pro in-creases the hardness of bleached enamel. Shetty et al. (22) have also confirmed the efficacy of this material for increasing the micro hardness of incipient carious le-sions, which is in agreement with our findings. Remin Pro is a water-based paste made of hydroxyapatite, fluo-ride and xylitol . It seems that hydroxyapatite can fill the porosities of the incipient carious lesions. The fluoride in the composition of Remin Pro seals the tu-bules while xylitol exerts antibacterial effects. Thus, this compound can stop demineralization and induce remineralization of incipient enamel lesions. Our results are also in accordance with Thakur Sandeep et al. (23) and Jaya Shankara et al. (24) . These studies were in accordance as the studies were similar to the present study on account of remineralization and the remineralizing agent Remin Pro had shown the better remineralization potential compared with other agents used in their studies.

In the present study, micro hardness values of deminerlised dentin following the use of ICON were higher than the other remineralising agents used in the study but the hardness value were lesser than the sound dentin. Resin infiltration of lesions is performed aiming to fill the enamel lesion porosities and increase enamel resistance to acid attacks. This can aid in stopping caries progression and increase the strength of lesions . Arslan et al. (25) in their study showed that infiltration of incipient enamel lesions with ICON resin increased the micro hardness and decreased bacterial accumulation while Prasada et al. (26) in their study indicated that use of ICON resin also improved the appearance of WSLs.

ICON's 15% HCl gel erodes the surface layer more effectively than 37% phosphoric acid (H 3PO4) gel, and using the HCl conditioning for two minutes with ICON could have led to deeper resin penetration than using phosphoric acid gel for the etching process. The amount of tooth structure removed following the use of this etchant is around 40 μ; (45) while in micro-abrasion treatment of WSLs; the tooth structure is removed to 250-μ depth (27). Also, the use of ICON dry which contains 99% ethanol for 30 s before application of the infiltrant increases the penetration coefficient by reducing the viscosity and contact angle and improves infiltration depth. (28). In addition, higher ethanol content can inhibit polymerization of resin and thus reduce infiltration success. (29). ICON-Infiltrant is a methacry-late-based resin containing BISGMA and TEGDMA. Increased concentration of TEGDMA, HEMA and ethanol increases the resin penetration coefficient. Application of Resin Infiltrant was done twice in our study, which caused increase in microhardness of the lesions, compensated to polymerization shrinkage and filled porosities and crevices in the infiltrated lesion body, thereby increasing hardness and decreasing mineral loss when exposed to demineralization again (30).

In this in vitro study, extracted human premolars were used and the procedure and time needed for the formation of lesions, the application of the treatment material and how the material works on the lesions may be different from what happens in vivo. The lesions in each tooth may also be different, i.e. the amount of demineralization in each tooth may vary depending on the amount of fluoride to which they were exposed before extraction. Other limitations unique to our research are that the pH cycling cannot fully replicate the oral environment. In about 3 days of exposure to demineralization acids, the lesions were formed, while oral cavity lesions may develop over a period of months or years.

Hence from this study, it can be concluded that ICON material performs well in increasing the microhardness. The ICON material is used as an infiltrant and prevent further demineralization by formation of resin tags where the material penetrates deep into the tooth surface.

CONCLUSION

Within the limitations of this in vitro study, the sound dentin shows greatest microhardness, yet along with this, it has also been found that Remin Pro, MI Paste Plus and Icon Resin Infiltrant can efficiently increase the micro hardness of demineralised dentin. On compairing the remineralising agents, it can be stated although Icon resin infiltrant showed the highest microhardness value among the tested groups followed by Remin Pro and then MI paste plus, there was no significant difference between them.

Icon material performed well in increasing the micro hardness. Hence it can be used as an infiltrant. The Icon material prevents further demineralization by formation of resin tags where the material penetrates into the enamel and dentin. Future long-term clinical trials should be conducted to determine the superiority of these agents in vital teeth

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Research Committee of Babu Banarasi Das University on 11/10/2021

Conflict of Interest: The authors have no conflicts of interest to declare.

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