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Comparative evaluation of surface microhardness of enamel adjacent to orthodontic brackets when treated with CPP-ACP paste and Er, Cr: YSGG laser irradiation: An in vitro study

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ABSTRACT

Background: Fixed orthodontic treatment has a lot of benefits for the patients, but it is not without any potential risks. The most common side effect of orthodontic treatment with fixed appliances is in the form of incipient caries lesions around brackets and which is mainly due to limitation on self-cleansing mechanism.
Objectives: To evaluate the effect of Er, Cr: YSGG laser irradiation and CPP-ACP paste used alone or in combination on the surface microhardness of enamel adjacent to orthodontic brackets.
Materials and Methods: Initially starting with bonding brackets to each tooth and making acrylic resin blocks. Then LASER irradiation with appropriate settings was performed.
200 non-carious extracted human maxillary first premolars were bonded by metal brackets and were tested for microhardness on Vickers machine. They were randomly divided into 5 groups followed by treated with different caries prevention methods, then subjected to PH cycling model. All teeth were tested for surface microhardness in a 3mmx3mm area surrounding the orthodontic bracket.
Results: The mean VHN value of all the groups were found to be highly statistically significant. The highest mean VHN value was found in the group 5 followed by group 2, group 3 and group 4 and least mean VHN in group 1.
Conclusion: Combination of ER, CR:YSGG laser irradiation and CPP-ACP paste shows a highly effective preventive measure for white spot lesion. LASER irradiation with specific guidelines as followed m in this study is a more effective preventive measure as compared to CPP-ACP Paste application followed at regular intervals.

1. Introduction

The benefit of orthodontic treatment is not just limited to achieving a million-dollar smile, if treated successfully it improves overall function of stomatognathic system. Dentists highly recommend patients to opt orthodontic therapy owing to the benefits it offers. Alike all the other treatments, orthodontic treatment too has its potential risks.

One of the most common side effect of orthodontic treatment with fixed appliances is in the form of incipient carious lesions (white spot lesions) around brackets and bands. The greatest prevalence of white spot lesions are on the cervical and middle thirds of the crown of first molars, lateral incisors and canines.

Incipient carious lesions are characterized by opacity and mineral loss compared with healthy enamel. Initial caries development is in the form of white spot lesions (WSLs) on the labial surfaces is a serious side effect of orthodontic

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treatment with fixed appliances. Almost about 97 percent of the patients treated with fixed appliances show WSL after the completion of treatment.[1]

These lesions appear to be very resistant to complete remineralization. Thus, even after several years post orthodontic care, patients have a high number of these discolored lesions. Fixed orthodontic appliances create stagnation areas for plaque and limit the naturally occurring self-cleansing mechanism of the oral musculature and saliva. Overtime, the colonization of aciduric bacteria results in active carious lesions.[2] To prevent these unwanted side effects there are various chemical and physical methods available. Chemical methods include different forms of fluorides such as varnishes and fluoride-releasing adhesives, and CPP-ACP-containing paste.

Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) complexes act as a calcium and phosphate reservoir helping to maintain a state of supersaturation of these minerals, which can enhance enamel remineralization. CPP-ACP paste, when exposed to acid, releases calcium and phosphate ions. Subsequently, the concentration of calcium and phosphate ions increases in the oral cavity which gets deposited on the tooth surface, preventing caries.

Lasers are used I recent times as one of the preventive measures for white spot lesions. The theory of the creation of micro spaces within the irradiated enamel for mechanisms of increased enamel resistance has gained popularity. According to this theory, acidic components penetrate the enamel during demineralization leading to the release of calcium, phosphorus, and fluoride ions. With the formation of micro spaces into the lased enamel, the released ions are trapped, and minerals are deposited into the enamel surface. Various lasers were used to reduce the rate of enamel demineralization such as argon, diode (960 nm), Nd: YAG (1.06 μm), Er: YAG (2.9 μm), CO2 (9.6 μm) and Er, Cr: YSGG (2.8 μm). Both CO2 and Er, Cr: YSGG lasers are strongly absorbed by the hydroxyapatite of tooth structure.[3]

There are very few studies on remineralization surrounding the orthodontic brackets with the application of Er,Cr: YSGG laser, and CPP-ACP paste used alone or in combination. So this study aims to evaluate the effect of Er, Cr: YSGG laser irradiation and CPP-ACP paste used alone or in combination on surface microhardness of enamel adjacent to orthodontic brackets.

2. Materials and Methods

The study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, initially starting with bonding brackets to each tooth and making acrylic resin blocks. Then LASER irradiation with appropriate settings was performed in private clinics in Pune.

Ethical clearance was obtained from the Scientific Advisory Committee and Institutional Ethics Committee.

2.1. Materials

1. Healthy human maxillary first premolars extracted for orthodontic purpose
2. Storage solution 1%thymol, nail varnish (leaving0 3mmX3mm exposed irradiated area
3. CPP-ACP paste (GC tooth mousse)
4. Acrylic resin blocks
5. Demineralization solution-20ml Calcium(2 0mMol/l, Phosphate(2 0mMol/l), Acetic acid (75 0 mMol/l buffered to pH 4 4),
6. Remineralization solution-30ml, Calcium (1 5mMol/l), Phosphate (0 9mMol/l), 0 15M KCl in 0 1m Tris buffer, pH 7
7. pH cycling model -for 28 days demineralization for 3hrs and remineralization for 17hrs. The remineralizing solution will be replaced every 48hrs, and demineralizing solution replaced every 5 days.
8. Conventional MBT metal brackets 0.022” slot Ormco, Glendora, USA (Bonded to extracted maxillary 1st premolars)
9. Light cure composite (Enlight light cure adhesive Ormco, Glendora, USA)
10. Bracket holding plier
11. Er,Cr:YSGG laser(Waterlase machine wavelength 2780nm, 0 75W,10s,8 5J/cm2,20hz frequency
12. Vicker’s micro-hardness tester
13. Etchant- Discover etchant gel
14. Bonding agent- Ortho solo universal bond enhancer
15. Probe, micro brush
16. Curing light gun

2.2. Methodology

245 Freshly extracted human maxillary 1st premolar teeth with sound buccal enamel without cracks, caries, fracture, and surface malformations were selected. After thorough examination 45 teeth were rejected due to cracks, fractures, carious lesions, with fillings, previously used for bonding. 200 non-carious extracted human maxillary first premolars with sound buccal enamel was taken, embedded in acrylic resin blocks. Each tooth was bonded by Conventional MBT metal brackets (0.022” slot) according to the manufacturer’s guidelines and standard practices. Nail varnish was applied to all the tooth surfaces, leaving 3mm x 3mm space surrounding the orthodontic bracket. All 200 samples were then tested for surface microhardness on the Vickers microhardness tester machine (Prima Germany, testing load 300 gm for 15 seconds) in a 3mmx3mm area surrounding the orthodontic bracket, after bonding.

All samples were then divided into 5 groups.

ER: CR, YSGG Laser irradiation was done by LASER machine (Waterlase USA) with accurate specifications, (wavelength 2780 nm, Power 0.75W, average exposure time

Group1 (40)	Control group which receives no surface treatment after demineralization
Group2 (40)	Application of CPP-ACP paste without demineralization
Group3 (40)	Er, Cr: YSGG laser irradiation before demineralization
Group4 (40)	Application of CPP-ACP paste before demineralization
Group5 (40)	Er, Cr: YSGG laser and CPP-ACP paste application before Demineralization

10s, Pulse energy 8.5J/cm2, frequency 20 Hz) scanning done once in each direction, horizontal and vertical, to promote homogeneous irradiation and to cover the entire testing area. The irradiation was performed by hand, screening was done on the enamel surface with a uniform motion by an experienced clinician and CPP-ACP paste was applied on the enamel surface by an applicator tip and left for 4 minutes. Then it was cleaned with cotton rolls and the remaining paste was allowed to remain for another 3 minutes. Finally, it was washed with normal saline. After 6 hours, the topical agent was re-applied to the tooth surface using the same method. This procedure was repeated every day for 5 days. Both LASER and Paste application in respective groups were done in 3mmx3mm space surrounding the orthodontic bracket. Samples were then subjected to pH cycling model, leaving samples of group 2 (CPP ACP paste without demineralization) which is the negative control group. All teeth were then tested again for surface microhardness in a 3mmx3mm area surrounding the orthodontic bracket.

After obtaining the results, inter and intragroup comparison was done.

2.3. Statistical analysis

The distribution of the dataset was assessed with a normality test (Kolmogorov– Smirnov test). Descriptive statistics were performed for all the findings. Mean and standard deviation (SD) were calculated. Mean of surface microhardness ie. Micro Vickers Hardness values for each group were compared statistically by one-way analysis of variance (ANOVA). Tukey post hoc comparison tests were performed to identify significance between-group differences. The results were considered significant at (P<0.05.)

3. Results

Table 1 shows Micro Vickers Hardness values of enamel adjacent to orthodontic brackets when treated with CPP-ACP paste and ER, CR: YSGG Laser irradiation using One-way ANOVA F test.

Pre-treatment MVHN for all groups was 101.3.

On overall comparative statistics of Micro Vickers Hardness values of enamel adjacent to orthodontic brackets when treated with CPP-ACP paste and ER, CR: YSGG Laser irradiation using One-way ANOVA F test, it was observed that highly statistically significant difference

($p<0.001$) was observed between all groups.

The highest MVHN value was found in Group 5 followed by Group 2, Group3, Group 4, and least ++MVHN in Group 1.

Table 2 shows Comparison of Micro Vickers Hardness value of enamel adjacent to orthodontic brackets when treated with various experimental groups.

Group 1 (Control) before and after demineralization

Pre-treatment MVHN score was 101.3 (3.15) which decreased to 91.16 (4.39), this difference was found to be of statistical significance on using paired t-test.

Group 2 (CPP-ACP paste without demineralization)

Pre-treatment MVHN score was 101.3 (3.15) which increased to a post-treatment value of 124.95 (2.49), this difference was found to be a high statistical significance on using paired t-test.

Group 3 (Er,Cr:YSGG) before demineralization

Pre-treatment MVHN score was 101.3 (3.15) which increased to a post-treatment value of 119.87 (2.24), this difference was found to be a high statistically significance on using paired t-test.

Group 4 (CPP-ACP paste) before demineralization

Pre-treatment MVHN score was 101.3 (3.15) which increased to a post-treatment value of 106.44 (2.45), but this difference was found to be a high statistical significance on using paired t-test.

Group 5 (Er,Cr:YSGG + CPP-ACP) before demineralization

Pre-treatment MVHN score was 101.3 (3.15) which increased to a post-treatment value of 127.4 (3.24), and this difference was found to be a high statistical significance on using paired t-test.

4. Discussion

White spot lesion is most common side effect of orthodontic treatment with fixed appliances. Once these lesions are established it is extremely difficult to achieve complete remineralization. Thus the gold standard method is to prevent them rather than curing once they appear. Ogaard B. et al[4] showed that even 5 years after treatment, orthodontic patients had a significantly higher incidence of white spot lesions than a control group of patients who didn't have orthodontic treatment.

Various preventive measures are fluorides, sorbitol-based chewing gums, varnishes, fluoride-releasing adhesives, and CPP-ACP (casein phosphopeptide amorphous calcium phosphate-containing paste), etc. which can decrease the risk of demineralization and remineralize previously demineralized enamel. Laser irradiation has also been shown to be effective for caries prevention.

Table 1: Comparative statistics of micro vickers hardness values of enamel adjacent to orthodontic brackets when treated with CPP-ACP paste and ER, CR: YSGG Laser irradiation using One-way ANOVA F test-

	Mean	SD	One way the ANOVA F test	P-value, Significance
Group 1 (Control) After demineralization	91.16	4.39		
Group 2 (CPP-ACP paste without demineralization)	124.95	2.49		
Group 3 (Er, Cr: YSGG) before demineralization	119.87	2.24	F=484.871	P<0.001**
Group 4 (CPP-ACP paste) before demineralization	106.44	2.45		
Group 5 (Er, Cr: YSGG + CPP-ACP) before demineralization	127.40	3.24		

Table 2: Pre and post comparison of micro vickers hardness value of enamel adjacent to orthodontic brackets when treated with various experimental groups

	Pre-treatment Mean (SD)	Post-treatment Mean (SD)	Paired t-test	p-value Significance
Group 1 (Control after demineralization	101.3(3.15)	91.16(4.39)	t=7.85	P=0.002*
Group 1 (CPP-ACP paste without demineralization)	101.3(3.15)	124.95 (2.94)	t=-18.76	P<0.001**
Group (Er, Cr: YSGG before demineralization)	101.3(3.15)	119.87(2.24)	t=-14.38	P<0.001**
Group 4(CPP-ACP paste before demineralization)	101.3(3.15)	106.44(2.45)	t=-3.46	=0.103(NS)
Group 5 (Er, Cr: YSGG+CPP-ACP) before demineralization	101.3(3.15)	127.4(3.24)	t=-21.68	P<0.001**

P=0.05-no significant (NS) *p<0.05-significant **p<0.001-highly significant

In this study, we used GC Tooth Mousse containing Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) complexes that act as a calcium and phosphate reservoir which help to maintain a state of supersaturation of these minerals, which can enhance enamel remineralization. CPP-ACP paste when exposed to acid, releases calcium and phosphate ions. Subsequently, the concentration of calcium and phosphate ions increases in the oral cavity and thus they deposit on the tooth surface, preventing caries and enhancing remineralization.

Various studies were conducted by Schmidlin P. et al,[3] Kwon Y. et al,[5] Chin- Ying S. et al,[6] which showed similar results.

On LASER irradiation and combination with fluoride for caries prevention,there are very few studies that compared Er, Cr: YSGG LASER, and CPP-ACP paste application for caries prevention but no studies on their combined effect. So we conducted this study to evaluate and assess the effect of Er, Cr: YSGG LASER irradiation and CPP-ACP paste when used alone or in combination on surface microhardness of enamel adjacent to orthodontic brackets.

After obtaining surface microhardness of all groups ie. Group1- Control group which receives no surface treatment after demineralization, Group 2 -Application of CPP-ACP paste without demineralization, Group3 -Er, Cr:YSGG laser irradiation before demineralization, Group 4 - Application of CPP-ACP paste before demineralization, Group 5 Er, Cr: YSGG laser and CPP-ACP paste application before demineralization; their results were compared and it was observed that a highly statistically significant difference was observed between all groups. This suggests that ER, CR: YSGG laser irradiation done at proper specifications and CPP-ACP paste application done at specific intervals acts as a preventive measure for WSLs.

The highest mean VHN (Vickers hardness number)value was found in group 5 that combined Er, Cr: YSGG Laser irradiation + CPP-ACP paste application, stated that LASER irradiation plus CPP-ACP paste application shows a synergistic effect and can be used as the highest preventive measure for white spot lesions. This is in accordance with the study conducted by Asl-Aminabadi et al[7] reported the synergistic remineralizing effect of CPP-ACP paste and Nd: YAG laser on enamel. Subramaniam et al[8] also reported increased surface microhardness of teeth after laser irradiation, followed by CPP-ACP application which is in concordance with our present study.

Synergistic effect was due to incorporation of calcium nano-complexes onto the tooth surfaces. These numerous nano-clusters of calcium deposits on the tooth surface could act as a reservoir to replenish the soluble calcium and phosphate ions that have diffused into the subsurface enamel. In this manner application of CPP-ACP enhances the action of lasers to inhibit demineralization.[9]

Our study findings are similar to a study done by Ana P. et al,[10] they found that lasers can induce crystallographic changes on enamel while effectively increasing its acid

resistance and significantly inhibiting caries development and progression. Moreover, a combination of a laser and fluoride seems to be the most promising treatment for caries prevention.

Contrary to our study, previously done studies by Heravi et al[11] did not find synergistic effects of CPP- ACP paste and Er: YAG laser and low-level laser on remineralization.

Our Results demonstrated that groups 3, 4, 5 values were highly significant when compared to each other. The present findings were consistent with the results reported by Poosti et al, Souza-e-Silva, and Miresmaeili et al that showed, after irradiation with laser, chemical and structural alterations in enamel such as decreased carbonates, fusion, and re-crystallization of hydroxyapatite crystals make enamel more resistant to acid attacks.[12]

In this study, it was found that LASER irradiation followed by CPP-ACP Paste application showed a remarkable result, which proved to be the most effective measure of prevention against WSLs. These findings were similar to studies done by Reichmann P et al, Tagomori and Morioka et al, they found that laser-modified enamel has an enhanced uptake of acidulated phosphate fluoride and that this fluoride uptake was greater when laser treatment was performed before the fluoride treatment, but some studies found no statistical difference in lesion depth regardless of whether fluoride treatment occurred before or after laser irradiation.[13]

The laser parameters in the present study were selected according to the study done by Reza Fekrazad et al,[14] who showed caries inhibition without destruction of the enamel structure, and increased acid resistance was seen by ER, CR: YSGG laser irradiation.

Group 5 had higher MVHN as compared to Group 2 but the difference was not found to be statistically significant. This result showed that even though LASER and CPP-ACP paste combination is a highly effective preventive measure for WSLS, only Daily application of CPP-ACP paste can also be used as an effective preventive measure.

Our study findings favor CPP-ACP (GC Tooth Mousse), which was similar to a study conducted by Brochner A. et al.[15] Where it was found that when treated with CPP-ACP after debonding of orthodontic appliances, resulted in a significant reduction in the area of the lesion. A systematic review was conducted which compared conventional fluoride supplements to CPP-ACP, which showed that the use of CPP-ACP can be more beneficial of fluoride agents and aid in the reduction of demineralization spots.

Our study findings favor results of a systematic review and meta-analysis done by Ma X. et al[16] which stated that casein phosphopeptide amorphous calcium phosphate (CPP-ACP) provided a remineralizing benefit superior to that of non-intervention or placebo, they concluded that CPP-ACP exhibited excellent remineralization of WSLs compared to the other groups, with greater percentages of WSL regression, lower enamel surface roughness, and the highest surface microhardness recovery.

Mettu et al.[17] reported that CPP-ACP increases enamel surface microhardness. The same was found in our study. Furthermore, in a study conducted by Oliveira PR. et al.[18] who evaluated the effect of MI paste (Recaldent) containing CPP-ACP with and without fluoride on enamel demineralization and concluded that remineralizing effect of MI paste on early enamel lesions was not found, although,slightly less demineralization was observed when compared with artificial saliva.

Next group which showed more surface microhardness is group 3 (ER, CR: YSGG LASER irradiation after demineralization), which showed highly significant MVHN as compared to groups 1 and 4. This suggests that LASER irradiation done following proper specifications is a more effective preventive measure as compared to CPP-ACP Paste application followed at regular intervals. In our present study, we used Er, Cr: YSGG (2.8 μm, Waterlase, Biolase, USA), which did not ablate the enamel but changed its morphological or chemical composition. We used energy densities below the ablation threshold (P= 0.75 W). High energy laser irradiation of enamel alone, at a specific wavelength, has been shown to cause remineralization.[8]

The next group that showed an increase in surface microhardness is group no. 4 (CPP-ACP paste group before demineralization), and the least surface microhardness is shown by group no. 1 (Control group). This suggests that CPP-ACP paste application alone can be used as an effective remineralizing agent for the treatment of WSLs. The anti-cariogenic mechanism of CPP-ACP is achieved by the incorporation of the nano-complexes of the ACP into plaque and onto the tooth surface.[19]

Since detection of carious lesions is possible at an early stage, instead of waiting till cavitation, efforts should be made to remineralize the lesion in earlier stages using appropriate remineralizing agents. Although the result of the present study indicates that Er, Cr: Y SGG laser irradiation has significantly increased the microhardness of enamel surrounding orthodontic brackets, a combination of both Er, Cr: YSGG laser irradiation and CPP-ACP paste application had shown a synergistic effect on increasing the surface microhardness of enamel and thus can be used as one of the preventive measures for white spot lesions.

We hope that the results of this study substantially contribute to the clinicians in managing WSLs in orthodontics and dentistry in general.

5. Limitation

This study was conducted in laboratory under controlled settings (in-vitro). The findings and outcome of this study may be distinct in clinical settings, hence further studies are required in the clinical setup to establish standard protocols

for using LASER and CPP-ACP paste and other methods for reducing WSL during orthodontic treatment.

6. Conclusion

Based on the results from this in-vitro study, we derived the following conclusions:

1. Combination of ER,CR:YSGG laser irradiation and CPP-ACP paste shows a highly effective method in preventing white spot lesions during orthodontic therapy.
2. LASER irradiation done following proper specifications mentioned in this study is a more effective preventive measure as compared to CPP-ACP Paste application followed at regular intervals.
3. Laser irradiation should be done before CPP-ACP paste application at the beginning of the treatment on critical areas like around orthodontic brackets, which enhances protection of enamel against demineralization.
4. Daily application of CPP-ACP paste alone can also be used as an effective method.

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8. Conflicts of Interest

None to declare.

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