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Original Article

Dentistry Section

In-vitro Comparative Evaluation of Enamel Microhardness Remineralised with Diode and Er,Cr:YSGG Lasers along with CPP-ACP Remineralising Agent

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ABSTRACT

Introduction: Enamel demineralisation is a common consequence of orthodontic treatment and has been treated with a variety of methods and recent use of lasers has been investigated.

Aim: To assess and compare the efficacy of diode laser, Erbium, Chromium-Doped: Yttrium, Scandium, Gallium and Garnet (Er,Cr:YSGG) with Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP) and CPP-ACP alone for remineralisation of the demineralised enamel surfaces.

Materials and Methods: This in-vitro experimental study was conducted at Blue Lab, SIMATS, Chennai, Tamil Nadu, India, from February 2021 to March 2021. A total of 40 extracted sound human teeth were obtained and subjected to demineralisation (T0) and divided into three experimental groups of 10 teeth each and one control group with 10 teeth. The study groups were as follows: Group 1- artificial saliva, group 2- CPP-ACP, group 3- Er,Cr:YSGG+CPP-ACP, group 4- diode laser+CPP-ACP. Vickers Microhardness test was done after demineralisation (T0) and after remineralisation (T1). Descriptive statistics, paired t-test and one-way Analysis of Variance (ANOVA) followed by Post-hoc Tukey's test were done to evaluate intragroup and intergroup differences.

Results: On intragroup comparison between T0 and T1 significant differences in microhardness were noted for all groups (p-value <0.05). At T1 the lowest mean microhardness value was noted in group 1 (210.13±41.20) and the highest mean value was noted in group 3 (255.86±41.53). Intergroup comparison revealed no significant difference in microhardness among the all four groups at T0 and T1 (p-value=1.0 and p-value=0.12 respectively).

Conclusion: It can be concluded that both diode laser and Er,Cr:YSGG when used along with CPP-ACP are equally effective in remineralising demineralised enamel surfaces.

Keywords: Casein phosphopeptide amorphous calcium phosphate, Demineralised enamel surface, Diode laser, Erbium laser

INTRODUCTION

Calcium and phosphate ions form the main structural component of the enamel [1]. During orthodontic treatment, enamel demineralisation occurs around the brackets as the teeth are subjected to acid challenge from cariogenic microbes which increase due to excess plaque accumulation. Fluoride plays a significant role in preventing demineralisation of enamel by altering the crystalline structure of hydroxyapatite to fluorapatite, which is less soluble and more resistant to acidic attack, as well as increasing remineralisation and having antibacterial properties, which is less soluble and more resistant to acidic attack, as well as increasing remineralisation and having antibacterial properties [2,3].

Since White Spot Lesions (WSLs) are reversible, early identification and treatment of WSLs during orthodontic treatment is critical. Casein Phosphopeptide- Amorphous Calcium Phosphate (CPP-ACP products have recently been developed for caries prevention and remineralisation of demineralised enamel [4,5]. When exposed to acid, CPP-ACP is added to the tooth surface and dental plaque, releasing calcium and phosphate ions. As a result, the concentration of calcium and phosphate ions in the oral cavity rises, causing them to deposit on the tooth surface, preventing caries and promoting remineralisation [6]. In-vivo and in-vitro studies have shown that CPP-ACP compounds can reduce demineralisation and increase remineralisation of enamel [7-9]. In a clinical trial, Iijima Y et al., found that adding 18.8 mg CPP-ACP to sugar free gums increased enamel resistance to demineralisation and improved remineralisation [10]. Some fluoride compounds contain CPP-ACP, which has been believed to have a higher propensity for enamel remineralisation due to fluoride release [11-13]. Wu G et al., used circulatory polarised imaging to evaluate the efficacy of tooth mousse (GC, Tokyo, Japan) containing CPP-ACP for enamel remineralisation and concluded that it can reduce the size of demineralised lesions and increase enamel remineralisation; its combination with the fluoride further enhances its remineralising ability and adequacy [14].

Laser irradiation has recently been proposed as a method of caries prevention. Caries can be prevented with CO_2, Erbium Doped Yttrium Aluminium Garnet (Er:YAG) lasers and Er,Cr:YSGG [15,16]. Water and hydroxyapatite crystals absorb these lasers very well, changing their crystalline structure and increasing their resistance to demineralisation [16]. While some recent studies have compared the efficacy of CPP-ACP with diode laser [17,18], CO_2 laser [19-21] and Er:YAG laser [16] in remineralising enamel surfaces, there are still only a few studies on Er,Cr:YSGG [22,23] and no studies have compared their efficacy in fluoride absorption for remineralisation. Thus, the aim of the study was to assess and compare the efficacy of diode laser, Er,Cr:YSGG and CPP-ACP for remineralisation of the demineralised enamel surface.

MATERIALS AND METHODS

This in-vitro experimental study was conducted at Blue lab, SIMATS, Chennai, Tamil Nadu, India, from February 2021 to March 2021. Ethical approval was obtained from the Institutional Review Board, (IHEC Ref NO: IHEC/SDC/ORTHO-2002/21/40). Permanent 1st premolar teeth extracted for orthodontic treatment were used. The teeth were selected using convenience sampling and a total sample of 40 teeth were included. The sample size was calculated using G power 3.1.9.7 which revealed a total sample size of 39 and it was increased upto 40 in this study with 10 samples in each group.

Inclusion criteria: Permanent premolars extracted for orthodontic purposes were included in the study. The teeth chosen were non carious, unrestored, and had an intact surface with no apparent fissures.

Exclusion criteria: Teeth having evident fissures, hypoplasia, enamel WSL and restored teeth were excluded from the study.

Study Procedure

A total of 40 extracted sound premolar teeth were disinfected in 0.1% chloramine T solution and stored in distilled water at 37°C. Enamel was ground using 1 μm, 2 μm and 3 μm aluminium oxide and 600, 800 and 2400 grit silicon carbide papers to obtain a smooth surface. It was then rinsed for 30 seconds with distilled water and dryed. The 40 teeth were divided into three experimental groups of 10 teeth and one control group with 10 teeth.

Demineralisation: All of the samples were subjected to demineralisation (T0). The demineralisation solution was prepared at Blue Lab of SIMATS using the reference described in the previous study [24]. At 37°C for 96 hours, each sample was stored in a separate container containing 50 mL of demineralising solution. The composition of demineralising solution included 3 mM calcium chloride 0.1 mM lactic acid, 0.2 guar gum and 3 mM potassium dihydrogen phosphate were used in this solution [25]. A 50% sodium hydroxide was used to change the pH to 4.5 [12]. The demineralised teeth were rinsed with deionised water for 20 seconds and air-dried after four days for the microhardness test. Before that, the roots were cut 2 mm below the cementoenamel junction. The teeth were then mounted in an epoxy resin mould such that their surface was at the level of the mould surface and they were located at the centre of the mould as shown in [Table/Fig-1]. A sticker measuring 2×2 mm was placed on the buccal surface of the teeth and a layer of nail varnish was applied on its surrounding surface. The sticker was then removed, and excess material was rinsed with distilled water. This was done to standardise the tooth surface subjected to remineralisation by control and intervention groups.

Vickers microhardness test was done at Chennai Mettex Lab Pvt. Ltd., Chennai, Tamil Nadu, India. The microhardness of the tooth surface was measured by applying a 50 gm load for 10 seconds to five points of each sample using a Vickers diamond indenter (Wopert, Darmstadt, Germany). After obtaining the Vickers Hardness Number (VHN), the mean microhardness of the five points was calculated and reported as the microhardness of enamel.

Remineralisation (T1)- Remineralisation was done for the experimental group

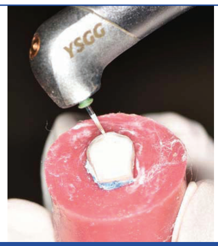
After demineralisation (T0) and microhardness testing the experimental groups were subjected to remineralisation (T1) with the following techniques.

Group 1- Demineralised teeth stored in artificial saliva stored for 1 week.

Group 2- CPP-ACP (GC Tooth Mousse®) paste was applied on the buccal surface of the teeth. This included 10% CPP-ACP, which was applied twice a day for 1 week.

Group 3- CPP-ACP (GC Tooth Mousse®) paste was applied on the buccal surface of the teeth once followed by one time 2780 nm Er,Cr:YSGG (WaterLase iPlus™) laser irradiated (0.25W power and 20Hz for 10 secs) to the respective site [Table/Fig-2].

Group 4- CPP-ACP (GC Tooth Mousse®) paste was applied on the buccal surface of the teeth once followed by one time 980 nm diode laser (EPIC X) irradiation (7W power for 15 secs) to the respective site [Table/Fig-3].

[Table/Fig-1]: Epoxy resin mould with the teeth mounted. **[Table/Fig-2]:** Erbium laser application for remineralisation (T1) in group 3. **[Table/Fig-3]:** Diode laser application for remineralisation (T1) in group 4. (Images from left to right)

Mounted samples of all the four experimental groups were immersed in artificial saliva for two weeks which was prepared and refreshed every three days. After two weeks, the teeth were rinsed with distilled water for 20 seconds and then they were sent for enamel microhardness testing.

The methodology of the present study was adopted from a similar investigation by Soltanimehr E et al., [24]. Demineralised mounted samples of all four groups were subjected to artificial saliva after subjecting them to the respective intervention except the control group. The artificial saliva used in this study included 0.381 g/L sodium chloride, 2.200 g/L gastric mucin, 0.738 g/L potassium hydrogen phosphate, 1.114 g/L potassium chloride and 0.213 g/L calcium hydrogen chloride [12].

STATISTICAL ANALYSIS

Data on microhardness values of all groups were analysed using IBM Statistical Package for the Social Sciences (SPSS) statistics software version 23.0. Significance of difference in enamel microhardness within the experimental groups before and after remineralisation was assessed using paired t-test and the significant difference among the experimental groups was assessed using one-way Analysis of Variance (ANOVA) followed by post-hoc. The normality test was performed with 95% confidence interval where (p-value <0.05) was considered significant.

RESULTS

The Kolmogorov-Smirnova tests for data normality revealed normal distribution of the data (p-value <0.05). [Table/Fig-4] gives the mean microhardness of each group before and after remineralisation. A paired t-test revealed significant differences in microhardness in all groups at T1 (p-value <0.05). After remineralisation with CPP-ACP and laser with CPP-ACP, there was a large difference in microhardness (p-value <0.05). Such that after remineralisation the lowest mean value was noted in the control group (210.13±41.20) and the highest mean value was noted in the erbium laser with CPP-ACP group (255.86±41.53). After demineralisation, one-way ANOVA revealed no significant difference in microhardness among the demineralised (T0) samples (p-value=1.00). After remineralisation (T1), there was no significant difference in microhardness (p-value=0.12) [Table/Fig-5]. [Table/Fig-6] presents intergroup comparison microhardness at T0 and T1. On intergroup comparison at T0 and T1 post-hoc tests results showed no significant difference (p-value >0.05).

Group	T0 Enamel microhardness Mean±SD	T1 Enamel microhardness Mean±SD	Sig. (2-tailed) p-value
Group 1	201.46±40.99	210.13±41.20	0.023*
Group 2	202.06±41.76	231.54±44.86	0.035*
Group 3	201.75±40.14	255.86±41.53	0.018*
Group 4	199.71±40.07	244.65±46.51	0.014*

[Table/Fig-4]: Means and SD of enamel microhardness at (T0) and (T1) paired t-test for significance of intragroup differences (p-value <0.05- significant).
SD: Standard deviation; p-value <0.05 was considered as statistically significant

Group	Groups	p-value
T0	Between four groups	1.00
T1	Between four groups	0.12

[Table/Fig-5]: One-way ANOVA for significance of inter group differences.
SD: Standard deviation; p-value <0.05 was considered as statistically significant

Dependent variables	(I) Groups	(J) Groups	Sig.
T0	Group 1	Group 2	1.00
		Group 3	1.00
		Group 4	1.00
	Group 2	Group 1	1.00
		Group 3	1.00
		Group 4	1.00
	Group 3	Group 1	1.00
		Group 2	1.00
		Group 4	0.99
	Group 4	Group 1	1.00
		Group 2	1.00
		Group 3	0.99
T1	Group 1	Group 2	0.69
		Group 3	0.10
		Group 4	0.30
	Group 2	Group 1	0.69
		Group 3	0.60
		Group 4	0.90
	Group 3	Group 1	0.10
		Group 2	0.60
		Group 4	0.93
	Group 4	Group 1	0.30
		Group 2	0.90
		Group 3	0.93

[Table/Fig-6]: Post-hoc test for significance of difference in microhardness among groups after demineralisation (T0) and after remineralisation (T1)
p-value <0.05 was considered as statistically significant

DISCUSSION

The study aimed to evaluate and compare the efficacy of diode laser 980 nm wavelength and erbium laser 2790 nm wavelength when combined with CPP-ACP and compared with only CPP-ACP for remineralisation of demineralised enamel surfaces using Vickers microhardness evaluation. Mean microhardness values at T0 and T1 when subjected to intragroup comparison showed a significant difference (p-value <0.05) for all groups as was shown in [Table/Fig-4]. In control group, due to spontaneous remineralisation of the demineralised enamel in presence of calcium ions from artificial saliva there was improvement in VHN values [12] and it was also noted that on remineralisation the microhardness values improved in all groups. When subjected to intergroup comparison at T1 no significant difference in VHN values were observed but the mean VHN values were higher for both laser groups. At T1 the erbium laser group had higher remineralisation (VHN was 255.86±41.53) followed by diode laser (VHN was 244.65±46.51) and least in CPP-ACP (VHN was 231.54±44.86) but the differences were not statistically significant.

This was the first study to report on microhardness assessment comparison between remineralising agents alone and remineralising agents combined with either diode laser or erbium laser. Previous studies have reported comparisons of remineralising potential of remineralising creams like CPP-ACP or a fluoride varnish when combined with CO_2 lasers and diode lasers [24-26]. The ability of different wavelengths of erbium laser (Er:YAG) in increasing the enamel resistance to acids has been previously evaluated [27]. Liu JF et al., de Andrade LEH et al., and Apel C et al., investigated the effect of the Er:YAG laser on enamel resistance to caries and found that sub ablative laser irradiation increased enamel resistance to acids [27-29]. In the present study, authors also noted a similar effect by using lasers for remineralisation. Apel C et al., suggested that preventing unwanted enamel ablation is difficult and impossible to achieve with currently available lasers and wavelengths [29]. They assessed demineralisation using spectrometry, while in present study we combined CPP-ACP with diode laser in one group and erbium laser in another group and assessed the microhardness, which is a reproducible technique with an error rate of less than 5% [30]. The microhardness values measured in our study were similar to the values reported in a previous study [24,26].

Fluorides and remineralising agents like CPP-ACP are gold standard methods for remineralising demineralised enamel surfaces and have been studied extensively. CPP-ACP paste applied in subjects with demineralised enamel surfaces binds to enamel, plaque biofilm, and soft tissues around the enamel, guiding free calcium and phosphate ions into enamel crystals and causing apatite crystal reformation [13]. Also, a study by Chokshi K et al., evaluating the remineralisation potential of CPP ACP using confocal microscopy reported a good remineralisation potential of CPP-ACP containing agents [31].

When a laser light is absorbed by enamel, the energy is converted to heat, causing chemical and microstructural changes that in turn reduce its relative solubility and the depth of lesions in demineralisation experiments. Hypotheses have been proposed by Stern to explain why acidic dissolution of enamel is reduced after heating [32]. Furthermore, after laser treatment, the amount of hydroxide and pyrophosphate increases. A study by Ana PA et al., on the effect of Er,CR:YSGG lasers along with fluorides concluded that thermal effect and increased roughness of lasers was responsible for promoting enamel uptake [33]. A study by Khamverdi Z et al., reported synergistic effects of CO_2 lasers when used along with CPP-ACP [34]. It has been reported that laser-induction improves the penetration of the CCP-ACP nano complex into deeper layers of HA crystals [35,36]. Hence, aligning with the available literature, we evaluated the combined effect of CPP-ACP with the two different lasers and studied their effects on microhardness of enamel.

A 960 nm diode laser was used in the study by Kato IT et al., alone without a remineralising agent and they reported that it was not effective in reduction of calcium solubility [37]. In the present, we used a 980 nm laser with 7W power and we noted a good remineralising potential when diode lasers were combined with CPP-ACP. The laser parameters decide the depth of penetration and temperature rise, which determine chemical (low temperature) or morphological changes in the dental substrate, as well as adverse effects on the pulp. The current study's laser parameters were adapted from a previous study [38]. Diode laser irradiation increases the absorption of ions, especially fluoride, by the enamel structure [18,39].

According to Subramaniam P and Pandey A the microhardness of primary enamel irradiated with Er,Cr: YSGG laser had increased [40]. Previous studies evaluating enamel microhardness after remineralisation with lasers are presented in [Table/Fig-7] [24,34,41, 42]. The higher water content of primary enamel results in more laser absorption. As a result, depending on the substitute used, it may be necessary to change the laser parameters. In the present study the laser parameters of 0.25 W and 20 Hz were used by Serdar-Eymirli P et al., in the study [23].

Author's name and year	Place of study	Number of samples	Materials compared	Parameters compared	Conclusion
Soltanimehr E et al., (2019) [24]	Kermanshah	117 extracted primary anterior teeth	Group 1: Ftcp varnish Group 2: Ftcp+diode laser Group 3: Ftcp CO_2 laser Group 4: CPP-ACP Group 5: CPP-ACP+diode Group 6: CPP-ACP+CO_2 Group 7: Diode laser Group 8: CO_2 Group 9: Control group	Microhardness Enamel Porosity	Highest microhardness was achieved with CPP-ACP The efficacy of the diode and CO_2 lasers were the same.
Khamverdi Z et al., (2018) [34]	Iran	30 sound maxillary extracted premolars	CG: No treatment (control group) LAS: CO_2 laser irradiation CP: Treated with CPP-ACP LASCP: Treated with CPP-ACP, then received CO_2 laser irradiation	Microhardness testing	Incorporation of the CO_2 laser to CPP-ACP formulation provides additional remineralising potential.
Rafiei E et al., (2020) [41]	Iran	78 premolar teeth with intact crowns	Group 1- CO_2 laser irradiation Group 2- CO_2 laser irradiation+Remin Pro Group 3- Remin Pro+CO_2 laser irradiation Group 4- Remin Pro Group 5- negative control group- (C–) Group 6- positive control group- (C+)	Vickers micro hardness SEM analysis	The use of CO_2 laser and Remin Pro significantly increased the microhardness of WSLs.
Ghelejkhani A et al., (2021) [42]	Tehran, Iran	35 extracted sound third molars	Group 1: CPP-ACP Group 2: MI Paste Plus Group 3: Fluoride varnish Group 4: Er, Cr: YSGG laser Group 5: Er, Cr: YSGG laser+CPP-ACP Group 6: Er, Cr: YSGG laser+MI Paste Plus Group 7: Er, Cr: YSGG laser+fluoride varnish	Enamel microhardness	Laser therapy before the application of remineralising agents did not significantly enhance enamel resistance to demineralisation.
Present study (2021)	Chennai, India	40 extracted Premolar	Group 1: Artifical saliva- control group Group 2: CPP-ACP Group 3: Er, Cr: YSGG laser+CPP-ACP Group 4: Diode+CPP-ACP	Enamel microhardness	No significant difference in the micro hardness levels between the two types of lasers studied.

[Table/Fig-7]: Table depicting studies evaluating enamel microhardness after remineralisation with lasers [24,31,41,42].

Limitation(s)

The limitations of this study included a limited sample size, microhardness tests not performed before subjecting the teeth to demineralisation and the study design. The results of in-vitro evaluations cannot be completely applied clinically since they are performed in a controlled environment.

CONCLUSION(S)

The following conclusions can be proposed in the present study, use of lasers along with remineralising agents improves enamel microhardness more than when using remineralising agents alone but the effect was not significant. There was no significant difference in the micro hardness levels between the two types of lasers studied hence both are equally good at remineralising demineralised enamel surfaces when used along with remineralising agents. Future studies should be planned with remineralising agents other than fluoride varnish and CPP-ACP along with lasers and their efficacy can be evaluated. Lasers with different exposure parameters can be studied and enamel porosity with AFM can also be evaluated.

Authors contribution: Data collection, tabulation, result formulation, manuscript writing was done by the principal investigator (PV). Manuscript verification, proofreading and result verification was done by a second investigator (RKJ).

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