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Evaluation of surface microhardness of teeth after remineralizing with Sodium fluoride with functionalized tricalcium phosphate in combination with Indium-Garnet-Arsenide Laser: an invitro study.

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ABSTRACT

Demineralization occurs due to dental caries, intrinsic and extrinsic erosive factors. Research has aimed at studying the cause, process and management of both de-mineralization and re-mineralization of tooth structure. In the field of remineralization therapies, much research has been devoted to developing systems of fluoride and calcium phosphate. Laser irradiation can effectively improve the acid resistance via changes in enamel. The present study is aimed at evaluating the microhardness of demineralized enamel following surface treatment using sodium fluoride with functionalised tri calcium phosphate, and in combination with diode laser. The teeth were divided into four groups of ten each: Intact tooth. (positive control - without any cyclic pH as all the groups), Demineralized tooth (negative control - with demineralising solution), Demineralized teeth surface treated using Sodium fluoride with functionalised tricalcium phosphate. (Clin-Pro White-3M), Demineralized teeth surface irradiated with Indium-Garnet-Arsenide laser (Diode laser: Kavo Gentle Ray 910nm) in conjunction with Sodium fluoride with functionalised tricalcium phosphate. (Clin-Pro White-3M). Enamel surface micro-hardness was measured using the micro-hardness tester with a Vickers diamond indenter in three dental areas (Vickers diamond, 100 g, 11 s, HMV; Shimadzu Corporation Tokyo, Tokyo, Japan), and the data obtained was sent for statistical analysis. Microhardness significantly differed between tested groups too i.e., there was statistical in microhardness of teeth surface treated with a combination of the Sodium fluoride with functionalized tricalcium phosphate (ClinPro White) and Indium-Garnet-Arsenide laser (Kavo GentleRay) from just application of Sodium fluoride with functionalized tricalcium phosphate (ClinPro White), implying the adjunctive and synergistic action of diode laser. Within the limitations of the study, combination Indium-Garnet-Arsenide laser in conjunction with sodium fluoride with functionalised tricalcium phosphate provides superior remineralization effect and brings about statistically significant increase in surface microhardness.

Keywords: Sodium fluoride with functionalized tricalcium phosphate, Indium-Garnet-Arsenide Laser, Diode Laser, ClinPro White.

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INTRODUCTION

Subsurface enamel lesions need to be detected and remineralized at the earliest to prevent the progression of disease (1). Demineralization occurs due to dental caries, intrinsic and extrinsic erosive factors such as acidic foods, beverages, medicines etc. In recent decades research has aimed at studying the cause, process and management of both de-mineralization and re-mineralization of tooth structure (2).

Teeth are comprised of the phosphate-based mineral Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ in the enamel, collagen in the dentine, and blood vessels and nerves in the pulp (3). Chemical demineralization of teeth is caused by acids through two primary means: dietary acid consumed through food or drinks and microbial attack from bacteria present in the mouth. During a typical demineralization regime, chemical dissolution of both the organic and inorganic matrix components takes place (4).

For net remineralization to occur, pH must be neutral and adequate levels of calcium and phosphate ions must be available. (2) In the field of remineralization therapies for the tooth, much research has been devoted to developing systems of fluoride and calcium phosphate. For such systems to work, it is essential that the calcium component is isolated from the fluoride component, less unfavourable reactions (i.e. calcium fluoride) occur; secondly, the topically applied fluoride and calcium interface constructively with the tooth (5).

Recently, it has been shown that laser irradiation can effectively improve the acid resistance via changes in enamel. Once the enamel surface is irradiated with laser it causes melting of the enamel crystals and then coalesce to form higher resistance crystals called pyrophosphate crystals. In case of fluoride application, the enamel crystals i.e. the hydroxyapatite crystals are replaced by fluorapatite crystals which are also resistant, but not as much as pyrophosphate crystal. Pyrophosphate crystals are completely impermeable barriers that are formed after laser irradiation, which prevent acids produced by micro-organisms to permeate into the enamel structure (6).

The present study is aimed at evaluating the microhardness of demineralized enamel following surface treatment using sodium fluoride with functionalised tri calcium phosphate, and in combination with diode laser.

MATERIALS AND METHODS

Forty freshly extracted premolar teeth free of cracks, hypo or hyper calcifications, free of caries and other defects were selected for the study. The teeth were divided into four groups of ten each as follows:

Group A: Intact tooth. (positive control - without any cyclic pH as all the groups).
Group B: Demineralized tooth (negative control - with demineralising solution).
Group C: Demineralized teeth surface treated using Sodium fluoride with functionalised tricalcium phosphate. (Clin-Pro White-3 M).
Group D: Demineralized teeth surface irradiated with Indium-Garnet-Arsenide laser (Diode laser: Kavo Gentle Ray 910nm) in conjunction with Sodium fluoride with functionalised tricalcium phosphate. (Clin-Pro White-3 M)

Specimens Preparation: Forty extracted premolar teeth with no abnormalities were processed according to OSHA regulation. The root was removed with a low-speed turbine under water cooling. The palatal area was immersed in acrylic circles (a circular base of acrylic where the sample was placed to facilitate the manipulation), and the buccal surface was mounted horizontally. They were polished using 800,1200 and 2400 grit silicon carbide paper. An acid-resistant nail varnish was applied around the exposed enamel surface, leaving an uncovered area of about 4×4 mm.

Demineralization: Following the proposal suggested by Prado et al., the white spot lesions were created by individually immersing acrylic-mounted enamel specimens in a demineralization solution that had 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid, and a pH adjusted to 4.6 with 1 M KOH over two days at 37°C; uniform demineralization was created on the surface of the enamel.

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Remineralization: The remineralizing solution contained Sodium fluoride with functionalised tricalcium phosphate. (Clin-Pro White-3 M); the sample containers were kept at a constant temperature (37 ◦C). Mineralizing agents were applied every day for 15 days with pH cycling: 3h demineralization and 21h remineralization. Both solutions were changed daily.

Laser irradiation: The laser was used in pulse mode. And pulse duration and pulse interval were 30ms:15Hz, optic fiber diameter: 600 µm and exposure time: 30 s. The irradiation distance was established at 3 mm from tooth and the enamel surface was exposed to the laser irradiation.

Surface Micro-Hardness (SMH): Enamel surface micro-hardness was measured after applying remineralizing agents using the micro-hardness tester with a Vickers diamond indenter in three dental areas (Vickers diamond, 100 g, 11 s, HMV; Shimadzu Corporation Tokyo, Tokyo, Japan). All readings were performed by the same examiner, using the same calibrated machine.

Data obtained was sent for statistical analysis.

RESULTS

There was significant difference ($p < 0.01$) between microhardness of remineralized teeth and the demineralized teeth, thus confirming the action of surface treatment protocols suggested in the present study. (Table 1, Chart 1)

Microhardness significantly differed between tested groups too i.e., there was statistical increase ($p = 0.03$ i.e. <0.05) in microhardness of teeth surface treated with a combination of the Sodium fluoride with functionalized tricalcium phosphate (ClinPro White) and Indium-Garnet-Arsenide laser (Kavo GentleRay) from just application of Sodium fluoride with functionalized tricalcium phosphate (ClinPro White), implying the adjunctive and synergistic action of diode laser. (Table 2)

Table 1: Comparison of microhardness between the study groups

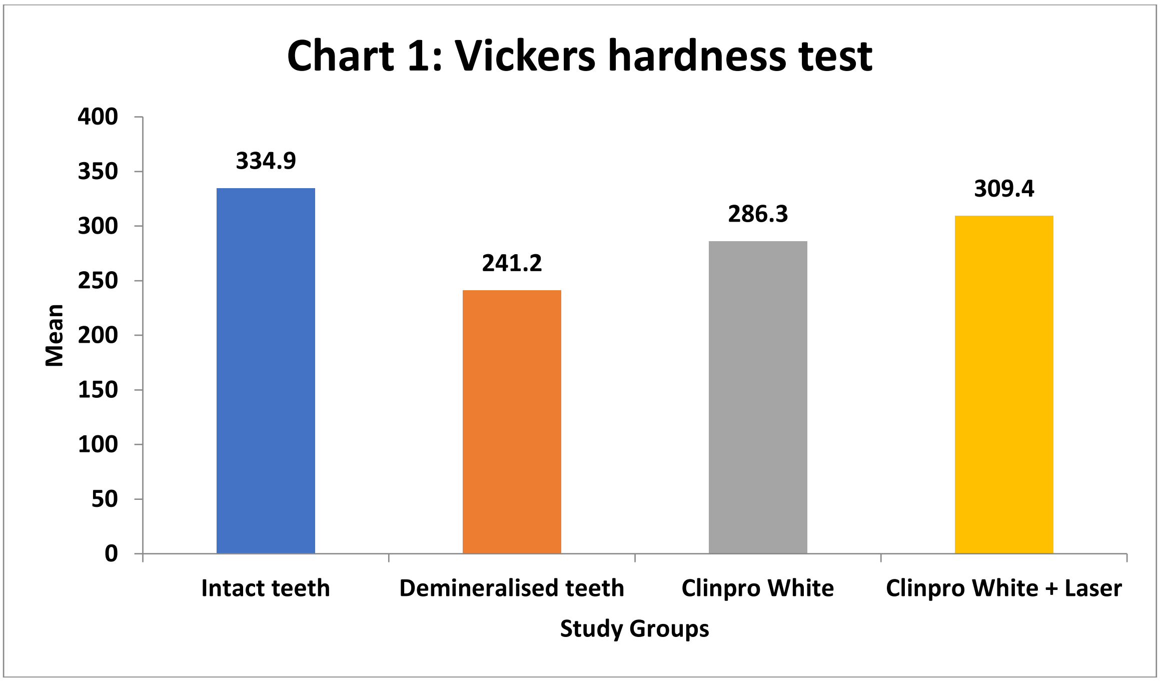
	N	Mean	SD	ANOVA	
				F	p-value
Intact teeth	10	334.90	15.44	87.81	<0.001*
Demineralized teeth	10	241.20	5.16		
Clinpro White	10	286.30	16.57		
Clinpro White + Laser	10	309.40	13.50		
Total	40	292.95	37.22		

*p<0.05 Statistically significant p>0.05 Non-significant, NS

Table 2: Pairwise comparison of microhardness between the study groups (Tukey Post Hoc test)

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Intact teeth	Demineralized teeth	93.70	6.01	<0.001*	77.52	109.88
	Clinpro White	48.60	6.01	<0.001*	32.42	64.78
	Clinpro White + Laser	25.50	6.01	0.001*	9.32	41.68
Demineralized teeth	Clinpro White	-45.10	6.01	<0.001*	-61.28	-28.92
	Clinpro White + Laser	-68.20	6.01	<0.001*	-84.38	-52.02
Clinpro White	Clinpro White + Laser	-23.10	6.01	0.003*	-39.28	-6.92

*p<0.05 Statistically significant p>0.05 Non-Significant, NS



DISCUSSION

It has been generally accepted that the combination of a fluoride regimen, oral hygiene instructions, and dietary control can contribute greatly to the inhibition of demineralization. Since the 1990s, people have been interested in the anti-carious effect of milk in which casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) plays a crucial role in suppressing demineralization and promoting remineralization. CPP-ACP has been shown to slow the progression of caries significantly and to promote the regression of early lesions in randomized, controlled clinical trials (7). However, systematic review with meta-analysis studies indicated that CPP-ACP has a short-term remineralization effect (8).

Several studies have reported the effect of laser irradiation on tooth enamel, either alone or in combination with fluoride (9, 10). It has been demonstrated that combined application of laser and fluoride has a synergistic effect. Laser enhances the effect of fluoride on enamel structure both superficially by forming calcium fluoride (CaF_2) and in its crystalline structure (11). Several theories have been suggested regarding the mechanisms by which laser irradiation increases enamel resistance to caries, such as surface melting and re-crystallization of enamel hydroxyapatite crystals (12), reduction in permeability and solubility of enamel (13,14), and deposition of calcium fluoride (15). Many studies conducted using different types of lasers have shown significant enamel and dentin resistance to dental caries. The application of CO_2 laser combined with fluoride has been proven to be effective for caries prevention in several studies (16, 17).

Diode laser is now widely used in dental practice. It has been shown that diode laser irradiation combined with fluoride application increases fluoride uptake in permanent teeth and thus is an effective method for preventing dental caries (18, 19). Souza et al. reported that diode laser irradiation induces melting and resolidification of the enamel surface of primary teeth and through this mechanism, it increases the resistance of enamel to acids. Thus, it probably plays an important role in caries prevention (20).

Functionalised tricalcium phosphate (fTCP) is produced through beta tricalcium phosphate (βTCP) complexes being milled with sodium lauryl sulfate (SLS) (21). The βTCP crystal structure exhibits several reactive sites including calcium-oxygen clusters (CaO_3, CaO_7, and CaO_8) and lattice defects (22). These reactive sites are available to undergo chemical interaction with anions such as fluoride ions. To reduce the reactivity of these sites within b-TCP complexes, the anionic surfactant SLS is added for incorporation within these reactive sites. The incorporation of SLS within the βTCP structure therefore impedes fluoride ions from combining with calcium ions in the dentifrice, so in turn potentially increasing the concentration of both calcium and fluoride to tooth surfaces (21, 23).

Additionally, Karlinsey et al. (24, 25) have reported a synergistic relationship between fTCP and fluoride with regard to enamel remineralisation; enamel that is remineralised through a fluoride/fTCP combination demonstrates significantly greater surface and subsurface rehardening following pH cycling compared to that achieved by fluoride application alone.

CONCLUSION

"Proactive Intervention Dentistry" - a concept aiming at delivering precision dental care through minimizing progression of disease, includes a critical procedure of remineralization of dental hard tissues. Within the limitations of the study, combination Indium-Garnet-Arsenide laser in conjunction with sodium fluoride with functionalised tricalcium phosphate provides superior remineralization effect and brings about statistically significant increase in surface microhardness.

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