Introduction

Dental caries have a dynamic process. They initiate in the form of primary subclinical lesions and result in cavity formation following the primary clinical phase. In the management of tooth caries, detection of primary lesions and use of remineralizing agents are necessary to prevent caries progression and stop the demineralization process prior to cavitation. It has been confirmed that white spot lesions can be reversed by the use of mineralizing agents with high calcium concentration and that their progression towards cavitation may be prevented. CPP consist of a cluster of phosphoryl residues that stabilize ACP nanoclusters in metastable solution. It is a sticky protein that bonds to phosphate and calcium ions and stabilizes them in an amorphous form. CPP-ACP easily bonds to pellicle, soft tissue plaque and even enamel hydroxyapatite. It can also be incorporated into toothpastes and mouth rinses for more efficient caries prevention. CPP-ACP nano complex has shown cariostatic properties in in-vitro, in-situ, animal models and human studies. On the other hand, laser technology has become highly popular in dentistry and medicine in recent years.

Methods

Thirty sound maxillary extracted premolars were selected. The crowns were cut at the cervical line and were split into facial and palatal halves. Specimens were mounted in self-cure acrylic blocks in such way that the enamel surface was exposed to 4×4 mm. After a pH cycling of the specimens, they were randomly divided into 4 groups (n = 15), as follows: CG: Control group, LAS: CO2 laser, CP: CPP-ACP and LASCP: laser combined CPP-ACP treatment. The Vickers microhardness of the specimens was measured (500 g load, 5 seconds, 3 points). Data were analyzed using one-way ANOVA and post hoc Tukey tests (α = 0.05).

Results

The lowest mean Vickers microhardness value was observed in CG group (192.57±50.87 kg/mm²) and the highest in LASCP group (361.86±22.22 kg/mm²). There were significant differences between groups (P<0.001). The pairwise comparison of the groups revealed that there were significant differences between these groups: CG versus LAS, CP, LASCP (P<0.05) and LASCP versus LAS and CP (P<0.05). No significant difference between LAS group versus CP group (P>0.05) was observed.

Conclusion

The results of the current study revealed that CO2 laser and CPP-ACP were effective for improvement of enamel hardness value after demineralization. Incorporation of CO2 laser irradiation and CPP-ACP paste application provides additional remineralizing potential for demineralized enamel.

Keywords: CO2 laser; Casein phosphopeptide-amorphous calcium phosphate nano complex; Enamel microhardness.

Abstract

Introduction: It has been suggested that the application of casein phosphopeptide-amorphous calcium phosphate paste (CPP-ACP) and CO2 laser irradiation on enamel could increase the resistance of enamel to caries and acid attacks. The aim of the current study was to compare the influence of CPP-ACP paste application and irradiation of CO2 laser on microhardness of demineralized enamel.

Methods: Thirty sound maxillary extracted premolars were selected. The crowns were cut at the cervical line and were split into facial and palatal halves. Specimens were mounted in self-cure acrylic blocks in such way that the enamel surface was exposed to 4×4 mm. After a pH cycling of the specimens, they were randomly divided into 4 groups (n = 15), as follows: CG: Control group, LAS: CO2 laser, CP: CPP-ACP and LASCP: laser combined CPP-ACP treatment. The Vickers microhardness of the specimens was measured (500 g load, 5 seconds, 3 points). Data were analyzed using one-way ANOVA and post hoc Tukey tests (α = 0.05).

Results: The lowest mean Vickers microhardness value was observed in CG group (192.57±50.87 kg/mm²) and the highest in LASCP group (361.86±22.22 kg/mm²). There were significant differences between groups (P<0.001). The pairwise comparison of the groups revealed that there were significant differences between these groups: CG versus LAS, CP, LASCP (P<0.05) and LASCP versus LAS and CP (P<0.05). No significant difference between LAS group versus CP group (P>0.05) was observed.

Conclusion: The results of the current study revealed that CO2 laser and CPP-ACP were effective for improvement of enamel hardness value after demineralization. Incorporation of CO2 laser irradiation and CPP-ACP paste application provides additional remineralizing potential for demineralized enamel.

Keywords: CO2 laser; Casein phosphopeptide-amorphous calcium phosphate nano complex; Enamel microhardness.
studies have evaluated the effect of lasers on the enamel and dentin.\textsuperscript{25,17} Among all of dental lasers, CO2 laser has the maximum absorption rate in hydroxyapatite and operates at a wavelength of 9-11 \( \mu \text{m}. \textsuperscript{18,19}\) This district of wavelength band coincides closely with some of the apatite absorption wavelengths.\textsuperscript{20} Therefore, CO2 laser irradiation causes chemical and morphological changes of enamel that subsequently alters the mineral and organic content of the tooth structure and increases enamel resistance to acid attacks.\textsuperscript{19,21} In addition, when considering dental caries CO2 laser irradiation improves the efficacy of fluorides.\textsuperscript{19,22,23} Irradiation of CO2 laser with fluoride application may produce fluoroapatite and calcium fluoride (CaF2) on the enamel surface. During episodes of enamel demineralization, these compositions serve as a depository of fluoride and are used up later for remineralization process.\textsuperscript{26} Nonetheless, the efficacy of lasers and remineralizing agent for promoting remineralization of enamel has not been investigated sufficiently.\textsuperscript{24,25}

The current study evaluated the effectiveness of CO2 laser, in association with CPP-ACP paste, on Vickers microhardness of demineralized enamel.

Methods
Thirty intact human maxillary premolars extracted during the previous 3 months were collected from dental centers and private office. The teeth were cleaned using a scalpel and toothbrush and immersed in 0.5% chloramine solution. They were examined under a stereomicroscope (Olympus, Shinjuku, Tokyo, Japan); to ensure the absence of structural defects, cracks or caries. The crown of each tooth was removed at the cervical line by using a diamond saw (Microslice 2, Metal Research, Cambridge, UK) with the water-coolant stream. Subsequently, the crowns of the teeth were sectioned into facial and palatal parts. Specimens were mounted in autopolymerizing acrylic resin blocks (Acropars, Kaveh, Tehran, Iran) in such way that a 4x4 mm facial or palatal enamel surface was exposed. In order to prevent dehydration of the teeth, they were stored in distilled water. The teeth were immersed in demineralization solution (pH = 4.6) for 8 hours and then removed and immersed in artificial saliva solution for a period of 1 hour. Afterwards, the teeth were immersed in a remineralizing solution (pH = 7) for a period of 15 hours. This cycling continued for 14 days to demineralize the enamel surface. The demineralizing and remineralizing solutions were refreshed every two days.\textsuperscript{26} The demineralizing solution comprised of 0.05 mM CaCl\(_2\), 2.2 mM Na\(_2\)HPO\(_4\) and 50 mM acetic acid. The solution containing 20 mM HEPES, 1.5 mM Ca\textsuperscript{2+} (CaCl\(_2\)), 0.9 mM phosphate (KH\(_2\)PO\(_4\)) and 1 ppm fluoride (NaF) was used as a remineralizing solution. Mounted specimens were randomly divided into four groups of fifteen (\( n = 15 \)), according to the treatments:

- **CG**: Specimens received no treatment (control group).
- **CP**: Specimens were treated with CPP-ACP
- **LAS**: Specimens were treated with CPP-ACP, then received CO2 laser irradiation.
- **CP**: Specimens were treated with CPP-ACP, then received CO2 laser irradiation.

Application of CPP-ACP
In CP and LASCP, according to the manufacturer’s instructions, CPP-ACP paste (MI Paste, Recaldent\textsuperscript{TM}, GC Co., USA) was applied directly on the demineralized enamel surface for five minutes and then washed.\textsuperscript{11,26}

CO2 Laser Irradiation
In LAS and LASCP, the surface of the specimens was lased with a CO2 laser at a wavelength of 10.6 \( \mu \text{m} \) (SmartXide2; DEKA Laser, Florence, Italy). Laser parameters were adjusted as follows: Power of 0.7 W, pulse frequency of 50 Hz, focal spot of 0.2 mm, pulse duration of 0.4 milliseconds, non-contact mode with 10 mm distance from the hollow tube tip to the tooth surface, spot size of 0.4 mm. Average power output was measured by using a power meter (Model 37-3002, Scientech Inc, Colorado, USA) and determined at 0.68 W. Thus, the power density was 5.41 W/mm\(^2\) and the laser fluency applied to enamel was 10.66 J/cm\(^2\). The demineralized enamel surface was scanned by the laser hand piece with a circular motion starting from the center and moving outward with a 2 mm/s speed for 5 seconds simultaneously with air spray (60%) application in a circle with 3 mm diameter\textsuperscript{27} and total energy of 3.5 J.

Microhardness Testing
Vickers hardness test was performed for all treated specimens after storage in distilled water for 48 hours. Values were recorded with a microhardness tester microscope (Micrometer 1, Buehler, Lake Bluff, IL, USA) using 500-g load for 5 seconds in 3 points.\textsuperscript{28} The mean of the microhardness values for points was considered for the micro-hardness value of each sample. SPSS software (version 18) was used to categorize the data. Data were analyzed by one-way ANOVA and post hoc Tukey tests at the level of \( \alpha = 0.05 \) significance.

Results
Means and standard deviations are presented in Table 1. The lowest mean Vickers hardness value was observed in CG (192.57 \( \pm \) 50.87 kg/mm\(^2\)) and the highest in LASCP (361.86 \( \pm \) 22.22 kg/mm\(^2\)). One-way ANOVA indicated that there were significant mean differences between groups (\( P < 0.001 \)). The pairwise comparison of groups revealed that there were significant differences between these groups: CG versus LAS, CP, LASCP (\( P < 0.05 \)) and LASCP versus LAS and CP (\( P < 0.05 \)). No significant difference between LAS group versus CP group (\( P > 0.05 \)) was observed (Table 1).
Discussion

In contemporary dentistry, prevention of caries is superior to treatment. Conventional tooth treatment eliminates the carious tissue and restores cavity with restorative materials. Non-invasive approach for carious lesions is now considered by many researchers.

This study evaluated the effect of CO2 laser irradiation and CPP-ACP paste application on enamel remineralization of demineralized enamel. The results of the current study showed that CO2 laser with a 10.6 μm wavelength and controlled parameters significantly increased enamel surface microhardness (P < 0.05). Previous studies confirm these results. Some studies have reported that lasers with an emission wavelength of 10.6 μm are more efficient for caries prevention. Laser irradiation changes the ratio of calcium to phosphate and decreases the carbonate/phosphate ratio in tooth structure. The loss of carbonate results in more stable and less acid soluble products and increases enamel caries resistance. The decrease in carbonate content caused by CO2 laser irradiation was confirmed in previous studies. The enamel carbonate content can be reduced or eliminated depending on the amount of energy applied, and crystallinity can be increased. Laser causes recrystallization in the enamel structure and makes enamel more resistant to acid dissolution. Correa-Afonso et al reported that the presence of carbonate generates unstable apatite crystals. It was reported that CO2 laser could increase the crystalline stability making the enamel less vulnerable to acid, and increase fluoride uptake by the enamel.

A SEM assessment indicated CO2 irradiation on human enamel forms melted irregular masses of hydroxyapatite. These masses had coalescence appearance and it seems that recrystallization occurred. Chiang et al reported that change in enamel matrix permeability occurs after lasing. It produces a decrease in acid diffusion, and alterations in organic and inorganic components of the enamel lead to increased enamel microhardness and decreased enamel susceptibility to acid. Also, Daniel et al showed that CO2 laser beam was effective in the reduction of mineral loss around composite resin filling on root surfaces and CO2 laser could be a resource in the prevention of secondary caries lesions on elderly population.

In the current study, CPP-ACP was used alone and in conjunction with laser to treat demineralized enamel lesions. The results showed that CPP-ACP paste significantly increased the microhardness of demineralized enamel. It has been reported that a stable complex formed by casein phosphopeptide-amorphous calcium phosphate can be used for remineralization of primary enamel lesions. CPP-ACP maintains a saturated state of phosphate and calcium on the enamel surface, and by doing so, decreases demineralization and increases remineralization. CPP with the cluster sequence of Ser(p)-Ser(p)-Ser(p)-Glu-Glu can bond to ACP in the solution. This process retains a high level of phosphate and calcium ions on the surface of lesions, prevents demineralization, and improves remineralization of enamel.

CPP-ACP paste can bond to enamel, biofilm and soft tissues around the enamel and guides the free calcium and phosphate ions into enamel crystals, resulting in the reformation of apatite crystals. In other words, CPP-ACP enhances the remineralization of carious lesions via saturation of enamel minerals. Previous studies have also demonstrated the role of CPP-ACP in preventing the process of demineralization. Our study findings are in agreement with these studies.

In the current study, no significant difference was noted between the impact of laser treatment and application of CPP-ACP paste on Vickers microhardness improvement of demineralized enamel. In other words, both methods equally enhanced the process of remineralization of lesions. Also, it was noted that CPP-ACP paste in conjunction with laser significantly increased the microhardness of enamel compared to the value in groups treated with laser or CPP-ACP paste alone (Table 1). CPP delivers small, stable hydrated calcium phosphate clusters into the intercellular matrix and localizes calcium and phosphate ions for easy access. These tiny clusters act as a reservoir and are refilled with calcium and phosphate ions. By doing so, they enhance the effect of laser on inhibiting demineralization and increasing remineralization.

This result confirms findings of Niazy’s study regarding the synergistic effect of CO2 laser in conjunction with CPP-ACP paste on the enhancement of remineralization. In fact, the change caused by laser enhances the penetration of CCpp-ACP nanocomplex into HA crystals in deeper layers. Thus, it has higher efficacy than the application of laser or CPP-ACP alone for remineralization.
Similar studies are required to compare other preventive measures, such as the use of fluoride and xylitol gums. This study had an in-vitro design and future studies are required to assess the efficacy of these techniques in a clinical setting.

**Conclusion**
Within the limitations of this study, our results demonstrated that CO2 laser irradiation, at 10.6 μm and application of CPP-ACP increased the microhardness of demineralized enamel. Incorporation of the CO2 laser to CPP-ACP formulation provides additional remineralizing potential.

**Conflict of Interests**
All of the named authors had no conflict of interest.

**Ethical Considerations**
The protocol of this investigation was approved by the Ethics Committee of Hamadan University of Medical Sciences.

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**References**
1. Featherstone JD. The continuum of dental caries--evidence for a dynamic disease process. *J Dent Res.* 2004;83 Spec No C:39-42.
2. Pitts NB. Clinical diagnosis of dental caries: a European perspective. *J Dent Educ.* 2001;65(10):972-978.
3. Abbas HM, Hamza HM, Ahmed HM. Minimal intervention approaches in remineralizing early carious lesions. *J Am Sci.* 2012;8(3):709-717.
4. Hicks MJ, Flaitz CM. Enamel caries formation and lesion progression with a fluoride dentifrice and a calcium-phosphate containing fluoride dentifrice: a polarized light microscopic study. *ASDC J Dent Child.* 2000;67(1):21-28.
5. Hicks J, Flaitz C. Role of remineralizing fluid in in vitro enamel caries formation and progression. *Quintessence Int.* 2007;38(4):313-319.
6. Xu HH, Weir MD, Sun L, Takagi S, Chow LC. Effects of calcium phosphate nanoparticles on CaPO4 composite. *J Dent Res.* 2007;86(4):378-383. doi:10.1177/154085910708600415
7. Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. *J Dent Res.* 1997;76(9):1587-1595. doi:10.1177/0022034597076091101
8. Chokshi K, Chokshi A, Konde S, et al. An in vitro Comparative Evaluation of Three Remineralizing Agents using Confocal Microscopy. *J Clin Diagn Res.* 2016;10(6):Zc39-42. doi:10.7860/jcdr/2016/18191.7984
9. Salehzadeh Esfahani K, Mazaheri R, Pishevar L. Effects of Treatment with Various Remineralizing Agents on the Microhardness of Demineralized Enamel Surface. *J Dent Res Dent Clin Dent Prospects.* 2015;9(4):239-245. doi:10.15171/joddd.2015.043
10. Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. *Spec Care Dentist.* 1998;18(1):8-16.
11. Theyyou R, Chanmitkul W, Thanatvarakorn O, et al. Casein phosphopeptide-amorphous calcium phosphate and glass ionomer show distinct effects in the remineralization of proximal artificial caries lesion in situ. *Dent Mater J.* 2013;32(4):648-653.
12. Sudjalim TR, Woods MG, Manton DJ, Reynolds EC. Prevention of demineralization around orthodontic brackets in vitro. *Am J Orthod Dentofacial Orthop.* 2007;131(6):705.e701-709. doi:10.1016/j.ajodo.2006.09.043
13. Grewal N, Kudupudi V, Grewal S. Surface remineralization potential of casein phosphopeptide-amorphous calcium phosphate on enamel eroded by cola-drinks: An in-situ model study. *Contemp Clin Dent.* 2013;4(3):331-337. doi:10.4103/0976-237x.118385
14. Castellan CS, Luiz AC, Bezinelli LM, et al. In vitro evaluation of enamel demineralization after Er:YAG and Nd:YAG laser irradiation on primary teeth. *Photomed Laser Surg.* 2007;25(2):85-90. doi:10.1089/pho.2006.2043
15. Chen CC, Huang ST. The effects of lasers and fluoride on the acid resistance of decalcified human enamel. *Photomed Laser Surg.* 2009;27(3):447-452. doi:10.1089/pho.2008.2312
16. Ana PA, Bachmann I, Zezell DM. Lasers effects on enamel for caries prevention. *Laser Phys.* 2006;16(5):865-875. doi:10.1134/s1054660x06050197
17. Kato IT, Kohara EK, Sarkis JE, Wetter NU. Effects of 960-nm diode laser irradiation on calcium solubility of dental enamel: an in vitro study. *Photomed Laser Surg.* 2006;24(6):689-693. doi:10.1089/pho.2006.24.689
18. Bahrololoomi Z, Fotuhi Ardakani F, Sorouri M. In Vitro Comparison of the Effects of Diode Laser and CO2 Laser on Topical Fluoride Uptake in Primary Teeth. *J Dent (Tehran).* 2015;12(8):585-591.
19. Kim JW, Chan KH, Fried D. Evaluation of enamel surface modification using PS-OCT after laser treatment to increase resistance to demineralization. *Proc SPIE Int Soc Opt Eng.* 2016;9692. doi:10.1117/12.2218662
20. Zancopo BR, Rodrigues LP, Parisotto TM, Steiner-Oliveira C, Rodrigues LK, Nobre-dos-Santos M. CO2 laser irradiation enhances CaF2 formation and inhibits lesion progression on demineralized dental enamel-in vitro study. *Lasers Med Sci.* 2016;31(3):539-547. doi:10.1007/s10103-016-1900-4
21. Souza-Gabriel AE, Colucci V, Turassi CP, Serra MC, Corona SA. Microhardness and SEM after CO2 laser irradiation of fluoride treatment in human and bovine enamel. *Microsc Res Tech.* 2010;73(11):1030-1035. doi:10.1002/jemt.20827
22. Hossain MM, Hossain M, Kimura Y, Kinoshita J, Yamada Y, Matsumoto K. Acquired acid resistance of enamel and dentine by CO2 laser irradiation enhances CaF2 formation and inhibits lesion progression on demineralized dental enamel-in vitro study. *Lasers Med Sci.* 2015;30(4):809-816. doi:10.1007/s10103-014-1837-6
23. Esteves-Oliveira M, Zexell DM, Ana PA, Yekta SS, Lampert F, Eduardo CP. Dentine caries inhibition through CO2 laser (10.6μm) irradiation and fluoride application, in vitro. *Arch Oral Biol.* 2011;56(6):533-539. doi:10.1016/j.aorb.2011.06.006
24. Niayz MA, Abdul-Hamid ES. Synergistic caries inhibitory effect of a rematerializing agent and CO2 laser on human enamel and root dentin. *Cairo Dent J*. 2009;25(3):415-424.

25. Heravi F, Ahrari F, Mahdavi M, Basafo S. Comparative evaluation of the effect of Er:YAG laser and low level laser irradiation combined with CPP-ACP cream on treatment of enamel caries. *J Clin Exp Dent*. 2014;6(2):e121-126. doi:10.4317/jced.51309

26. Somasundaram P, Vimala N, Mandke LG. Protective potential of casein phosphopeptide amorphous calcium phosphate containing paste on enamel surfaces. *J Conserv Dent*. 2013;16(2):152-156. doi:10.1016/j.jcd.2012.02.016

27. de Souza-e-Silva CM, Parisotto TM, Steiner-Oliveira C, Kamiya RU, Rodrigues LK, Nobre-dos-Santos M. Carbon dioxide laser and bonding materials reduce enamel demineralization around orthodontic brackets. *Lasers Med Sci*. 2013;28(1):111-118. doi:10.1007/s10103-012-0765-6

28. Khamverdi Z, Vahedi M, Abdollahzadeh S, Ghambari MH. Effect of a common diet and regular beverage on enamel erosion in various temperatures: an in-vitro study. *J Dent (Tehran)*. 2013;10(5):411-416.

29. Ten Cate JM. Fluorides in caries prevention and control: empiricism or science. *Caries Res*. 2004;38(3):254-257. doi:10.1159/000077763

30. Burke FJ. From extension for prevention to prevention of extension: (minimal intervention dentistry). *Dent Update*. 2003;30(9):492-498, 500, 502.

31. Esteves-Oliveira M, Zezell DM, Meister J, et al. CO2 Laser (10.6 microm) for caries prevention in dental enamel. *Caries Res*. 2009;43(4):261-268. doi:10.1159/00017858

32. Rechmann P, Fried D, Le CQ, et al. Caries inhibition in vital teeth using 9.6-mum CO2-laser irradiation. *J Biomed Opt*. 2011;16(7):071405. doi:10.1117/1.3564908

33. Daniel LC, Araujo FC, Zancope BR, et al. Effect of a CO2 Laser on the Inhibition of Root Surface Caries Adjacent to Restorations of Glass Ionomer Cement or Composite Resin: An In Vitro Study. *ScientificWorldJournal*. 2015;2015:298575. doi:10.1155/2015/298575

34. Zuerlein MJ, Fried D, Featherstone JD. Modeling the modification depth of carbon dioxide laser-treated dental enamel. *Lasers Surg Med*. 1999;25(4):335-347.

35. Steiner-Oliveira C, Rodrigues LK, Soares LE, Martin AA, Zezell DM, Nobre-dos-Santos M. Chemical, morphological and thermal effects of 10.6-microm CO2 laser on the inhibition of enamel demineralization. *Dent Mater J*. 2006;25(3):455-462.

36. Fried D, Zuerlein MJ, Le CQ, Featherstone JD. Thermal and chemical modification of dentin by 9-11-microm CO2 laser pulses of 5-100-micros duration. *Lasers Surg Med*. 2002;31(4):275-282. doi:10.1002/lsm.10100

37. Correa-Afonso AM, Bachmann L, de Almeida CG, Dibb RG, Borsatto MC. Loss of structural water and carbonate of Nd:YAG laser-irradiated human enamel. *Lasers Med Sci*. 2015;30(4):1183-1187. doi:10.1007/s10103-014-1532-5

38. Correa-Afonso AM, Bachmann L, Almeida CG, Corona SA, Borsatto MC. FTIR and SEM analysis of CO2 laser irradiated human enamel. *Arch Oral Biol*. 2012;57(9):1153-1158. doi:10.1016/j.archoralbio.2012.02.004

39. Mathew A, Reddy NV, Sugumaran DK, Peter J, Shameer M, Dauravu LM. Acquired acid resistance of human enamel treated with laser (Er:YAG laser and CO2 laser) and acidulated phosphate fluoride treatment: An in vitro atomic emission spectrometry analysis. *Contemp Clin Dent*. 2013;4(2):170-175. doi:10.4103/0976-237x.114864

40. Chiang YC, Lee BS, Wang YL, et al. Microstructural changes of enamel, dentin-enamel junction, and dentin induced by irradiating outer enamel surfaces with CO2 laser. *Lasers Med Sci*. 2008;23(1):41-48. doi:10.1007/s10103-007-0453-y

41. Munjal D, Garg S, Dhindsa A, Sidhu GK, Sethi HS. Assessment of white spot lesions and in-vivo evaluation of the effect of CPP-ACP on white spot lesions in permanent molars of children. *J Clin Diagn Res*. 2016;10(5):Zc149-154. doi:10.7860/jcdr/2016/19458.7896

42. Zero DT. Dentifrices, mouthwashes, and remineralization/ caries arrestment strategies. *BMC Oral Health*. 2006;6 Suppl 1:S9. doi:10.1186/1472-6831-6-s1-s9

43. Jayarajan J, Janardhanam P, Jayakumar P. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization - an in vitro study using scanning electron microscope and DIAGNOdent. *Indian J Dent Res*. 2011;22(1):77-82. doi:10.4103/0970-9290.80001

44. Walker G, Cai F, Shen P, et al. Increased remineralization of tooth enamel by milk containing added casein phosphopeptide-amorphous calcium phosphate. *J Dairy Res*. 2006;73(1):74-78. doi:10.1017/s0022029905001482

45. Asl-Aminabadi N, Najafpour E, Samiei M, et al. Laser-casein phosphopeptide effect on remineralization of early enamel lesions in primary teeth. *J Clin Exp Dent*. 2015;7(2):e261-267. doi:10.4317/jced.52165