European Journal of Therapeutics

DOI: 10.5152/eurjther.2020.19110

Original Research

Is it Possible to Regain Lost Minerals to Initial Enamel Lesions Using Enamel Matrix Derivatives Combined with Casein Phosphopeptide Amorphous Calcium Phosphate: An *in Vitro* Study

Gül Keskin[1], Çiğdem Güler[2], Sibel Yıldırım[3]

[1]Department of Pediatric Dentistry, Gaziantep University Faculty of Dentistry, Gaziantep, Turkey
[2]Department of Pediatric Dentistry, Ordu University Faculty of Dentistry, Ordu, Turkey
[3]Department of Pediatric Dentistry, Selçuk University Faculty of Dentistry, Konya, Turkey

ABSTRACT

Objective: This study aimed to assess the efficacy of enamel matrix derivatives (EMD) used in combination with casein phosphopeptide amorphous calcium phosphate (CPP-ACP) to recover minerals to the enamel after artificial caries formation.

Methods: Fifty enamel samples were prepared from the buccal surfaces of extracted human third molars. The samples were divided into five groups: (G1) Untreated enamel samples (control), (G2) Application of 500 ppm sodium fluoride (NaF), (G3) CPP-ACP, (G4) EMD, and (G5) CPP-ACP + EMD. All of the samples were placed in an acidic buffer solution for 96 hours to simulate a carious lesion. Ca/P ratios were calculated using energy dispersive X-ray spectroscopy (EDX). After seven days of the remineralization procedure, the mineral contents of the samples were re-measured. All the data were analyzed statistically.

Results: There were no statistically significant differences in the mineral contents of the samples between the groups after demineralization ($p>0.05$). The Ca/P ratios of G2, G3, G4, and G5 increased significantly ($p<0.05$) after remineralization. The highest levels of the Ca/P ratio were obtained in G5.

Conclusion: Despite the limitations of this in vitro study, the combined use of CPP-ACP and EMD may increase the remineralization potential. Furthermore, this procedures may be an alternative for providing enamel remineralization in future clinical trials.

Keywords: Casein phosphopeptide amorphous calcium phosphate, enamel matrix derivatives, enamel remineralization, fluoride, SEM-EDX

INTRODUCTION

Dental caries is an infectious disease of the teeth that results in the dissolution and destruction of hard tissues such as the enamel and dentin (1). The first sign of dental caries is the initial caries lesion defined as "subsurface enamel porosity from carious demineralization" (2), and it can be reversed by the recovery of minerals to the lost structure (3). Some methods or materials like the application of topical fluoride or CPP-ACP provided aided remineralization (4). Fluoride is a classic anti-caries agent, although it is more effective for sound enamel than caries lesions (5) since its effectiveness is limited by the availability of calcium and phosphate ions (6). CPP, which is the protective factor in milk, was obtained by the digestion of casein with trypsin enzyme, using the selective precipitation method (7). CPP can stabilize calcium phosphate as a CPP-ACP complex (8), and these complexes promote remineralization by increasing calcium phosphate in the dental plaque (9).

More recently, biomimetic remineralization approaches are receiving increasing attention as a promising anti-caries therapy (10). The biomimetic synthesis of materials such as enamel can provide a noninvasive alternative treatment for early carious lesions. Amelogenin is the most common enamel matrix protein that plays a role in the formation and growth of the enamel crystal structure (11). Regarding the interaction of amelogenin and calcium phosphate, it is believed that amelogenin modulates the calcium phosphate nanocrystalline structure and plays an important role in enamel biomineralization (11). Amelogenin, calcium, and phosphate ions are important substances for the formation of organized hydroxyapatite crystals *in vitro* (12). Enamel matrix derivatives (EMD) are commercially available derivatives of enamel matrix proteins (EMP). EMD includes 90% amelogenin and 10% pig enamel matrix protein derivatives, which have been shown to promote periodontal ligament cell proliferation and collagen production, and also to enhance *in vitro* mineralization (13).

How to cite: Keskin G, Güler Ç, Yıldırım S. Is it Possible to Regain Lost Minerals to Initial Enamel Lesions Using Enamel Matrix Derivatives Combined with Casein Phosphopeptide Amorphous Calcium Phosphate: An in Vitro Study. Eur J Ther 2020; 26(3): 172–7.

ORCID iDs of the authors: G.K. 0000–0003–4569–7174; Ç.G. 0000–0002–2581–9050; S.Y. 0000–0001–8044–1874.

Corresponding Author: Gül Keskin **E–mail:** gulbeyret@hotmail.com

Received: 11.10.2019 • **Accepted:** 09.12.2019

In light of these knowledge,the null hypothesis of the present *in vitro* study was that CPP-ACP and EMD would have a positive synergistic effect on the recovery of the lost structure of the enamel surface. This *in vitro* study aimed to evaluate the remineralization potential of CPP-ACP and EMD in initial caries lesions by EDX and make comparisons between the groups.

METHODS

Preparation of the Enamel Samples

Fifty impacted human third molar teeth were collected for this study, and ethics committee approval was obtained from the İnönü University/Turkey Clinical Research Ethics Committee (2013/146). The teeth were stored in 4°C deionized water containing 0.2% thymol for at most 30 days. The samples were evaluated using a stereomicroscope (Nikon, Tokyo, Japan) at 2× magnification to choose thesound enamel surfaces. The teeth were then cut using the IsoMet ™ Low Speed Precision Cutter (Buehler, Germany). The enamel surface of the blocks was polished on a polishing machine (Ecomet 3, Bueller, IL, USA) and water-cooled to obtain a smooth enamel surface. Then, they were covered with two coats of acid-resistant nail varnish, except for a 3×3 mm^2 enamel window.

Caries Lesion Formation

Early artificial caries lesions were produced in 50 enamel samples. The samples were placed in a demineralization solution containing 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 50 mM acetic acid, and 1 M KOH. The pH of the solution was adjusted to 5.0, andthe demineralization was performed at 37°C for 96 hours (14).

pH-Cycling Model

The samples were divided into five groups: (G1) Untreated enamel samples (control), (G2) 500 ppm sodium fluoride (NaF, Oral-B Stages, Oral-B Laboratories, Netherlands), (G3) CPP-ACP (Tooth Mousse, containing 10% CPP-ACP; GC Int., Tokyo, Japan), (G4) EMD gel (Emdogain, lyophilized protein fractions dissolved in acetic acid, Straumann, Biora, Sweden), and (G5) CPP-ACP + EMD. pH-cycling was carried out using a remineralization solution (1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 150 mM KCl, 1 M KOH, and pH 7.0) and demineralization solution (2.2 mM CaCl2, 2.2 mM NaH_2PO_4, 50 mM acetic acid, and 1 M KOH, pH 5.0) (15). The experimental design included 2 demineralization/remineralization cycles a day for 10 days: immersion of the enamel samples in 10 mL of demineralizing solution, twice daily (at 9 AM and 9 PM) for 30 minutes; application of the solutions (described above) for 4 minutes(in group 5;CPP-ACP was applied for 4 minutes firstly, washing in distilled water for 10 seconds, then EMD gel was applied for 4 minutes); washing in distilled water for 10 seconds; immersion in 100 mL of the remineralizing solution (16).

Main Points:

- The combined use of CPP-ACP and EMD increased Ca/P% ratio at the enamel surface.
- CPP-ACP showed close efficacy to the fluoride in enamel remineralization.
- The use of EMD alone was not sufficient to reverse the mineral loss from the enamel.

SEM-EDX Analysis

SEM-EDX analysis was performed to determine the mineral content of the enamel samples after caries lesion formation and pH-cycling. Elemental analysis was performed on both halves of the exposed enamel surfaces in 20 Kv and fluoride (F) %, calcium (Ca) %, and phosphate (P) % values were recorded atomically. The Ca/P% ratio was then calculated. Image analysis was also performed using an SEM to determine the structure of the initial carious lesions and the variations after remineralization.

Statistical Analysis

After demineralization and application of the remineralization agents to the demineralized samples, a paired t-test was performed for each group to evaluate the mineral changes in the samples. The one-way ANOVA test was conducted to compare mineralization differences between the groups after demineralization and remineralization. In addition, the post-hoc Tukey test was used to compare the differences between the groups after remineralization.

RESULTS

The mean atomic values of the F%, Ca%, and P% values were recorded atomically using SEM-EDX, and the Ca/P% ratios of all the study groups were statistically compared (Table 1).

The SEM-EDX analysis method was used after applying the demineralization procedure, and it revealed that there was no statistically significant difference between the groups in terms of the mean atomic Ca/P% ($p<0.05$). After the remineralization, the mean atomic Ca/P% ratio in all groups, except for the control group, exhibited a statistically significant increase ($p<0.05$). According to the paired t-test, the highest Ca/P% ratio after treatment was obtained in group 5, whereas the lowest Ca/P% ratio was obtained in group 1 (Table 2).

Due to the intergroup comparison of the mean Ca/P% ratios after remineralization using the post-hoc Tukey test, the mean Ca/P% ratio of group 5 was found to be higher than that of group 2. However, the difference between these values was not found to be statistically significant ($p>0.05$). The mean Ca/P% ratio obtained from groups 2 and 5 was higher than the mean Ca/P% ratio obtained from group 3, and the mean Ca/P% ratio obtained from group 3 was higher than that obtained from groups 1 and 4 ($p<0.05$). The mean Ca/P% ratio obtained from group 4 was higher than that obtained from group 1. However, the difference between these values was not found to be statistically significant ($p>0.05$; Table 3).

By evaluating the SEM images according to the treatment groups, we observed the enamel surfaces of the groups (Figure 1). The small particles and cracks found in the control groups (a)could be due to the dissolved Ca/P ions from the enamel subsurface. Prism structure could be seen in NaF (b) and CPP-ACP+EMD (e) with smaller pores and showed a dense packed precipitation layer. CaF_2 deposition was also observed

Table 1. Comparison of the mean atomic values of the F%, Ca%, P%, and Ca/P% ratiosin the demineralization and remineralization periods

		Groups					
Elements %		**G1**	**G2**	**G3**	**G4**	**G5**	**OWA P**
F	Demineralization	13,44±1,29	13.44±0.84	13.57±1.05	13.45±1.06	13.59±0.84	0.054 0.99
	Remineralization	13,61±1,32	17.10±0.48	12.60±1.19	15.25±0.78	15.21±1.10	**37.862***
Ca	Demineralization	15,53±1,79	15.03±1.73	14.37±0.94	14.23±0.89	14.06±1.46	1.887 0.12
	Remineralization	15,43±0,83	17.81±0.29	20.81±1.37	15.59±1.11	18.29±1.26	**44.22***
P	Demineralization	8,74±0,81	8.56±0.85	8.34±0.30	8.38±0.73	8.09±0.56	1.305 0.28
	Remineralization	8,63±0,61	8.15±0.23	10.03±0.94	8.40±0.72	8.29±0.77	**11.923***
Ca/P	Demineralization	1,77±0,10	1.75±0.09	1.72±0.08	1.70±0.10	1.73±0.09	0.90 0.46
	Remineralization	1,79±0,08	2.18±0.06	2.07±0.12	1.86±0.09	2.21±0.11	**37.86***

Group 1: Control, Group 2: NaF, Group 3: CPP-ACP, Group 4: EMD, Group 5: CPP-ACP+EMD.
OWA: One-way Anova,
*: statistically significant difference (p<0.05)

Table 2. Comparison of the mean atomic values of the Ca/P% ratiosin the demineralization and remineralization periods

	GROUPS					
	G1 n=10	**G2 n=10**	**G3 n=10**	**G4 n=10**	**G5 n=10**	**OWA P**
Demineralization	1.77±0.10	1.75±0.09	1.72±0.08	1.70±0.10	1.73±0.09	0.90 0.46
Remineralization	1.79±0.08	2.18±0.06	2.07±0.12	1,86±0,09	2.21±0.11	**37.86***
Difference	+0.01±0.14	+0.42±0.12	+0.35±0.16	+0,15±0,14	+0.47±0.14	
PTT (P)	0.801	**0.0001***	**0.0001***	**0.007***	**0.0001***	

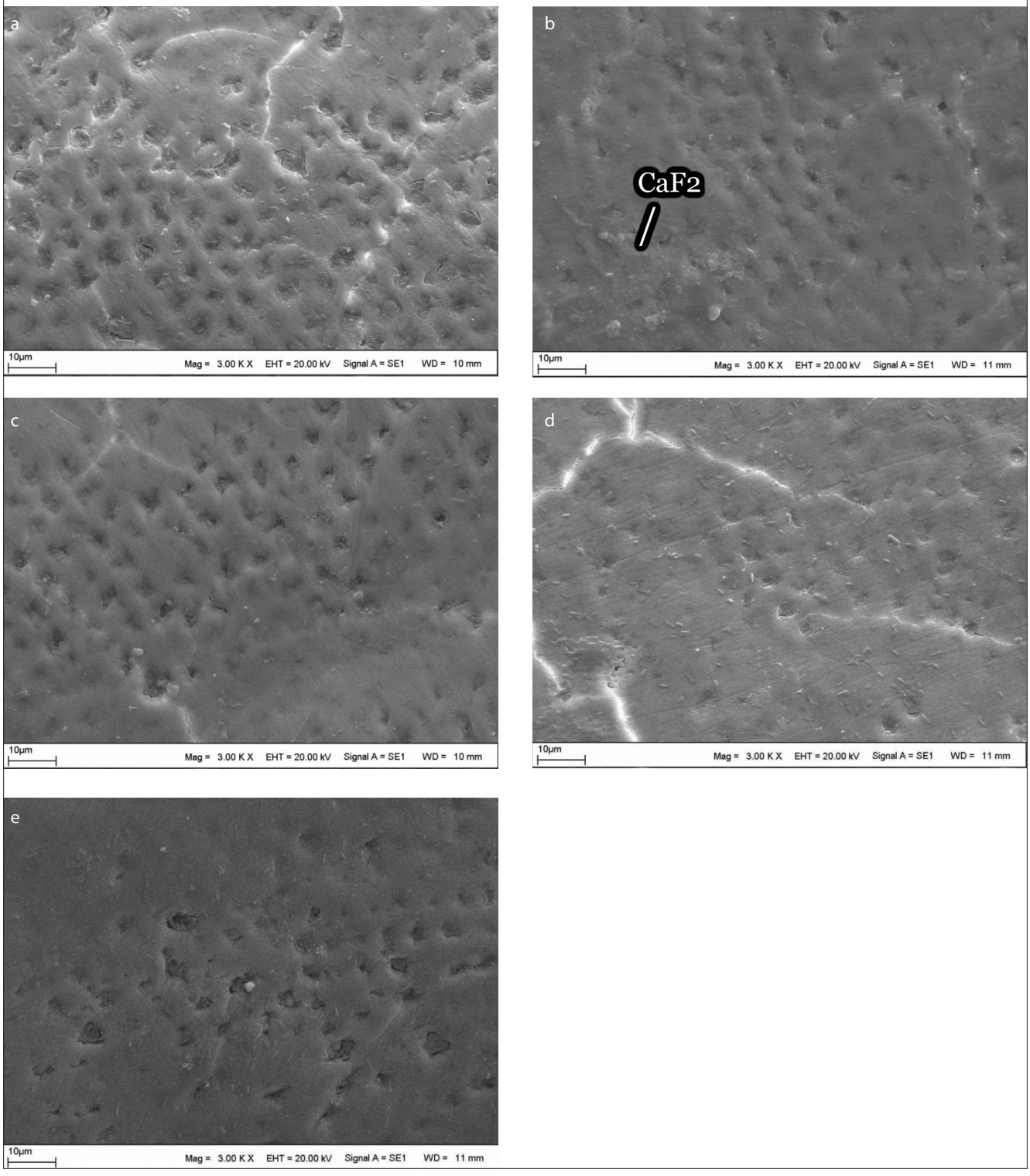
Group 1: Control, Group 2: NaF, Group 3: CPP-ACP, Group 4: EMD, Group 5: CPP-ACP+EMD.
OWA: One-way Anova, PTT: Paired T-Test,
*: statistically significant difference (p<0.05)

Table 3. Comparison of the mean atomic values of the Ca/P% ratios between the groups after remineralization

Group	**Group**	**PHT(P)**	**Group**	**Group**	**PHT(P)**	**Group**	**Group**	**PHT(P)**
G1	G3	**0.0001***	G2	G3	**0.014***	G3	G4	**0.0001***
	G4	0.118		G4	0.0001*		G5	**0.003***
	G5	**0.0001***		G5	0.556		G2	**0.014***
	G2	**0.0001***		G1	0,0001*		G1	**0.0001***
Group	**Group**	**PHT(P)**	**Group**	**Group**	**PHT(P)**			
G4	G3	**0.0001***	G5	G3	**0.003***			
	G5	**0.0001***		G4	**0.0001***			
	G2	**0.0001***		G2	0.556			
	G1	0.118		G1	**0.0001***			

Group 1: Control, Group 2: NaF, Group 3: CPP-ACP, Group 4: EMD, Group 5: CPP-ACP+EMD
PHT: Post Hoc Tukey,
*: statistically significant difference (p<0.05)

Figure 1. a-e. Scanning Electron Microscope (SEM) images (× 3.00 k). a) G1: Control b) G2: NaF c) G3: CPP-ACP d) G4: EMD, and e) G5: CPP-ACP+EMD



in the NaF (b) group, but CPP-ACP+EMD (e) seemed to have a less porous structure than the NaF (b) group. In the CPP-ACP groups (c) and EMD (d), patch-like structures were clearly seen while some enlarged pores were also observed (Figure 1a-e).

DISCUSSION

This study evaluated the effectiveness of using EMD combined with CPP-ACP on the remineralization of initial caries lesions, and it was found that using the two materials in combination may

have a synergistic effect on biomimetic remineralization. These materials may be proposed as a new treatment alternative forinitial caries lesions. However, there is need for further research to clarify this.

The progression of caries or enabling of remineralization depends on the balance between pathological (demineralization) and protective factors (improving remineralization and decreasing bacteria formation) (17). Among the protective factors, fluoride is the most commonly known agent that supports remineralization. Sodium fluoride (NaF), which has a very high water solubility, is the simplest fluoride compound that can be used (18). When toothpaste containing fluoride with an ionic bond (NaF) is used, a CaF_2 layer accumulates on the dental hard tissue while teeth are being brushed. This reserve is used over time, and fluoride concentrations rise in the enamel and saliva (19). A toothpaste containing 500 ppm NaF was used with the positive control group in this study. Controlling the decay process with the fluoride treatment may not be sufficient in individuals who are at a higher risk of caries. Therefore, new anti-caries agents and delivery systems have recently been developed.

EMD is a purified acidic extract of EMP that is secreted from Hertwing's epithelial root sheath in the development of the swine teeth at the embryonic stage (20). EMP that mainly consists of amelogenin, has a hydrophobic structure (21). EMD consist of 90% amelogenin and 10% proline-rich non-amelogenin, tuftelin, tuft protein, serum, ameloblastin, amelin, and saliva proteins (22). EMD regulates enamel biomineralization by inducing and guiding crystal formation, supporting crystal growth, protecting the mineral phase, combining mineral ions, and regulating the growth rate (23).

Wang et al. (24) obtained many new hydroxyapatite crystals using EMP and showed that it plays an important role in hydroxyapatite crystals formation and growth. It was therefore demonstrated that EMP induces enamel remineralization. Chen et al. (25) used EMP to obtain hydroxyapatite nanorods, and thus simulated the enamel mineralization process. These rods were similar to mature enamel not only in terms of their chemical composition, but also in terms of their size. Xiang et al. (26) investigated the remineralization effect of EMDs on initial enamel carious lesions. According to the results of this study, it was found that EMD plays an essential role in promoting the remineralization of initial carious lesions. However, the complete remineralization of such lesions could not be achieved. Considering the findings in these studies, our study aimed to enhance the understanding of the remineralization activity of EMD. Therefore, we used casein phosphopeptide amorphous calcium phosphate, which is a remineralization agent containing Ca and P.

We also used an SEM in this study to determine the structure of the initial carious lesion on the enamel and the changes that occurred after the treatment. Moreover, the changes created by the administered treatment agents at the mineral level in the enamel samples were evaluated using a microanalytical technique with EDX. SEM is a well-defined technique being used in almost every field of modern medicine, and it is generally used as a supportive technique in various studies. Energy dispersive X-ray spectroscopy (EDS, EDX, or XEDS) is an analytical technique that can be used in combination with SEM for the chemical characterization or elemental analysis of samples (27).

It has been reported in the literature that CPP-ACP remineralization pastes increase the Ca/P% ratio on demineralized enamel surfaces, and thus have the potential ofrecovering minerals to dental hard tissues (28, 29). Recently, biomineralization of the enamel with a regrown enamel-like mineral layer has been considered in the treatment of initial caries lesions. Self-assembling peptide (30), amelogenin (31), or EMD (26, 32) can be used for this purpose. In our study, SEM-EDX measurements showed that all the groups except the control group exhibited remineralization. It was found that EMD could play an essential role in promoting the remineralization of the initial carious lesions, andprevious studies also support this finding (23, 26, 32). However, when used alone, it could not provide complete remineralization of such lesions. CPP-ACP administration provided an effective remineralization of the enamel and therefore, CPP-ACP agent can be an alternative to fluoride in enamel remineralization. The fact that the Ca/P% ratio was higher in the group that received the combination of Emdogain and CPP-ACP compared to the groups that received these materials separately supports the opinion that EMD is an important promoter of enamel bio-remineralization and a modulator of calcium phosphate nanocrystal structures (11). Furthermore, the highest Ca/P% ratio was obtained from the group that received the combination of Emdogain and CPP-ACP. SEM images also supported the data obtained in our study using the EDX microanalytical method.

CONCLUSION

In this study, we found that using a combination of CPP-ACP and EMD could enhance the remineralization activity of each other. However, further studies are required to evaluate the profit-loss relationship and the remineralization activity in terms of the cost, in order to create a standard procedure with regard to the frequency of application, and to evaluate the possible effects on oral-dental and general health in the case of combined CPP-ACP and EMD use.

Ethics Committee Approval: Ethics committee approval was received for this study from the Clinical Research Ethics Committee of İnönü University (2013/146).

Informed Consent: Written informed consent was obtained from participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - G.K.; Design - G.K., Ç.G., S.Y.; Supervision - G.K.; Resources - G.K., Ç.G.; Materials - G.K.; Data Collection and/or Processing - G.K.; Analysis and/or Interpretation - G.K., Ç.G., S.Y; Literature Search - G.K.; Writing Manuscript - G.K.; Critical Review - G.K., Ç.G., S.Y.; Other - G.K., Ç.G., S.Y.

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

Financial Disclosure: The work was supported by the Scientific Research Project Coordination Unit of İnönü University (2013/91).

REFERENCES

1. Somasundaram P, Vimala N, Mandke LG. Protective potential of casein phosphopeptide amorphous calcium phosphate containing paste on enamel surfaces. J Conserv Dent 2013; 16: 152-6. [Crossref]
2. Gocmen GB, Yanikoglu F, Tagtekin D, Stookey GK, Schemehorn BR, Hayran O. Effectiveness of some herbals on initial enamel caries lesion. Asian Pac J Trop Biomed 2016; 6: 846-50. [Crossref]
3. Wang X, Wang Y, Wang K, Ren Q, Li H, Zheng S, et al. Bifunctional anticaries peptides with antibacterial and remineralizing effects. Oral Dis 2019; 25: 488-96. [Crossref]
4. Nakata T, Kitasako Y, Sadr A, Nakashima S, Tagami J. Effect of a calcium phosphate and fluoride paste on prevention of enamel demineralization. Dent Mater J 2018; 30; 37: 65-70. [Crossref]
5. Alkilzy M, Tarabaih A, Santamaria RM, Splieth CH. Self-assembling Peptide P11-4 and Fluoride for Regenerating Enamel. J Dent Res 2018; 97: 148-54. [Crossref]
6. Pinto de Souza SCT, Araújo KC, Barbosa JR, Cancio V, Rocha AA, Tostes MA. Effect of dentifrice containing fTCP, CPP-ACP and fluoride in the prevention of enamel demineralization. Acta Odontol Scand 2018; 76: 188-94. [Crossref]
7. Buckshey S, Anthonappa RP, King NM, Itthagarun A. Remineralizing Potential of Clinpro® and Tooth Mousse Plus® on Artificial Carious Lesions. J Clin Pediatr Dent 2019; 43: 103-8. [Crossref]
8. Attiguppe P, Malik N, Ballal S, Naik SV. CPP-ACP and Fluoride: A Synergism to Combat Caries. Int J Clin Pediatr Dent 2019; 12: 120-5. [Crossref]
9. Fatemeh M, Marjan S, Homa N, Mahsa S. CPP-ACP: Effect on Dental Plaque Acidity after Water Rinsing Following Topical Fluoride Therapy. J Clin Pediatr Dent 2017; 41: 22-6. [Crossref]
10. Lv X, Yang Y, Han S, Li D, Tu H, Li W, et al. Potential of an amelogenin based peptide in promoting reminerlization of initial enamel caries. Arch Oral Biol 2015; 60: 1482-7. [Crossref]
11. Fan Y, Wen ZT, Liao S, Lallier T, Hagan JL, Twomley JT, et al. Novel amelogenin-releasing hydrogel for remineralization of enamel artificial caries. J Bioact Compat Polym 2012; 27: 585-603. [Crossref]
12. Tian K, Peng M, Ren X, Liao C, Fei W. Regeneration of tooth-like hydroxyapatite depended on amelogenin functional section monolayer: a new approach for tooth repair. Med Hypotheses 2012; 79: 143-6. [Crossref]
13. Kubasad G, Ghivari S, Garg K. Replantation of an avulsed tooth with an extended extra oral period. Indian J Dent Res 2012; 23: 822-5. [Crossref]
14. Mohd Said SN, Ekambaram M, Yiu CK. Effect of different fluoride varnishes on remineralization of artificial enamel carious lesions. Int J Paediatr Dent 2017; 27: 163-73. [Crossref]
15. Oliveira GM, Ritter AV, Heymann HO, Swift E Jr, Donovan T, Brock G, et al. Remineralization effect of CPP-ACP and fluoride for white spot lesions in vitro. J Dent 2014; 42: 1592-602. [Crossref]
16. Peric TO, Markovic DL, Radojevic VJ, Heinemann RM, Petrovic BB, Lamovec JS. Influence of pastes containing casein phosphopeptide-amorphous calcium phosphate on surface of demineralized enamel. J Appl Biomater Funct Mater 2014; 12: 234-9. [Crossref]
17. Featherstone JD, Domejean S. The role of remineralizing and anticaries agents in caries management. Adv Dent Res 2012; 24: 28-31. [Crossref]
18. Rugg-Gunn A, Banoczy J. Fluoride toothpastes and fluoride mouthrinses for home use. Acta Med Acad 2013; 42: 168-78. [Crossref]
19. Altenburger MJ, Bernhard J, Schicha TD, Wrbas KT, Hellwig E. Comparison of in vitro fluoride uptake from whitening toothpastes and a conventional toothpaste in demineralised enamel. Schweiz Monatsschr Zahnmed 2010; 120: 104-13.
20. Hammarstrom L. Enamel matrix, cementum development and regeneration. J Clin Periodontol1997; 24: 658-68. [Crossref]
21. Kobayashi E, Fujioka-Kobayashi M, Saulacic N, Schaller B, Sculean A, Miron RJ. Effect of enamel matrix derivative liquid in combination with a natural bone mineral on new bone formation in a rabbit GBR model. Clin Oral Implants Res 2019; 30: 542-9. [Crossref]
22. Youssef AR, Emara R, Taher MM, Al-Allaf FA, Almalki M, Almasri MA, Siddiqui SS. Effects of mineral trioxide aggregate, calcium hydroxide, biodentine and Emdogain on osteogenesis, Odontogenesis, angiogenesis and cell viability of dental pulp stem cells. BMC Oral Health 2019; 19: 133. [Crossref]
23. Ieong CC, Zhou XD, Li JY, Li W, Zhang LL. Possibilities and potential roles of the functional peptides based on enamel matrix proteins in promoting the remineralization of initial enamel caries. Med Hypotheses 2011; 76: 391-4. [Crossref]
24. Wang ZW, Zhao YP, Zhou CR, Li H. The study on the enamel remineralization by enamel matrix proteins' inducing. Sichuan Da Xue Xue Bao Yi Xue Ban 2008; 39: 579-82.
25. Chen H, Clarkson BH, Sun K, Mansfield JF. Self-assembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure. J Colloid Interface Sci 2005; 288: 97-103. [Crossref]
26. Xiang C, Ran J, Yang Q, Li W, Zhou X, Zhang L. Effects of enamel matrix derivative on remineralisation of initial enamel carious lesions in vitro. Arch Oral Biol 2013; 58: 362-9. [Crossref]
27. Ellingham STD, Thompson TJU, Islam M. Scanning Electron Microscopy-Energy-Dispersive X-Ray (SEM/EDX): A Rapid Diagnostic Tool to Aid the Identification of Burnt Bone and Contested Cremains. J Forensic Sci 2018;63: 504-10. [Crossref]
28. Zhou C, Zhang D, Bai Y, Li S. Casein phosphopeptide-amorphous calcium phosphate remineralization of primary teeth early enamel lesions. J Dent 2014; 42: 21-9. [Crossref]
29. Hegde MN, Moany A. Remineralization of enamel subsurface lesions with casein phosphopeptide-amorphous calcium phosphate: A quantitative energy dispersive X-ray analysis using scanning electron microscopy: An in vitro study. J Conserv Dent 2012; 15: 61-7. [Crossref]
30. Alkilzy M, Santamaria RM, Schmoeckel J, Splieth CH. Treatment of Carious Lesions Using Self-Assembling Peptides. Adv Dent Res 2018; 29: 42-7. [Crossref]
31. Ruan Q, Zhang Y, Yang X, Nutt S, Moradian-Oldak J. An amelogenin-chitosan matrix promotes assembly of an enamel-like layer with a dense interface. Acta Biomater 2013; 9: 7289-97. [Crossref]
32. Schmidlin P, Zobrist K, Attin T, Wegehaupt F. In vitro re-hardening of artificial enamel caries lesions using enamel matrix proteins or self-assembling peptides. J Appl Oral Sci 2016; 24: 31-6. [Crossref]