Anesthesia and Pain Medicine
Abbreviation: *Anesth Pain Med.*
Open Access
Volume: 20: Issue:01
Year: 2025

Remineralization Potential of Self-Assembling Peptide P11-4, 2% Arginine Enriched Sodium Fluoride and Functionalized Tri Calcium Phosphate Fluoride Varnishes in Depth of Artificial Carious Lesion: Polarized Light Microscopic Evaluation

Bardis Salah Abd Elaziz[1], Dina Hamdy [2], Mona Galal[3], Nagwa Mohammed Ali Khattab[2]
[1]Pediatric Dentistry & Dental Public Health, Faculty of Dentistry, Modern University for Technology and Information (MTI), Cairo, Egypt.
[2]Pediatric Dentistry & Dental Public Health, Faculty of Dentistr, Ain Shams University Cairo, Egypt.
[3]Operative Dentistry, Faculty of Dentistry, Ain Shams University, Cairo, Egypt.

Corresponding Author
Bardis Salah Abd Elaziz,
Pediatric Dentistry & Dental Public Health, Faculty of Dentistry, Modern University for Technology and Information

Article History:
Received: 28-03-2025
Accepted: 24-04-2025
Available Online: 06-05-2025

How to Cite the Article:
Bardis Salah Abd Elaziz, et al. Remineralization Potential of Self-Assembling Peptide P11-4, 2% Arginine Enriched Sodium Fluoride and Functionalized Tri Calcium Phosphate Fluoride Varnishes in Depth of Artificial Carious Lesion: Polarized Light Microscopic Evaluation, Anesthesia and Pain Medicine. 2025; 20(1): 51-59

ABSTRACT

Background: White spot lesions (WSLs) known as hidden caries. **Aim:** to evaluate the remineralizing potential of Self-assembling peptide P11-4, 2% Arginine Enriched Sodium Fluoride varnish and Functionalized Tri Calcium Phosphate Fluoride varnish regarding artificially produced carious lesions utilizing polarized light microscope (PLM). **Methods:** Thirty-two bovine enamel samples were prepared and artificially demineralized by submersion in an acidic solution (pH 4.4) for four days until a homogenous white spot lesion was formed and observed when the specimen dried. They were randomly located into four groups: (A): Self-assembling peptide P11-4 varnish; (B): 2% Arginine Enriched sodium fluoride varnish; (C): Tri-Calcium Phosphate Fluoride Varnish; and group (D): Untreated negative control. All samples underwent a standardized protocol of regulated pH cycling for 12 days. The extent of remineralization for each drug was evaluated using PLM. **Results:** All three examined materials demonstrated enhancement in the depth of artificial carious lesions. In comparison to the negative control group D (20.69±1.98), both groups B (80.22±9.18) and C (74.93±14.43) exhibited a statistically significant decrease in lesion depth relative to group A (34.45±12.73). **Conclusions:** All tested materials showed improvement in the lesion depth. Arginine containing fluoride and Tri Calcium Phosphate fluoride varnish showed better results than self-assembling peptide.

Keywords: Curodont Repair, Clinpro, Remineralization, Early carious lesions, Minimal invasive dentistry, Artificial caries.

BACKGROUND

A white spot lesion (WSL) is characterized as a white opacity resulting from subsurface enamel demineralization onsmooth surfaces. The white manifestation is owing to alterations in the light scattering of the demineralized enamel [1]. Thus, early intervention involving remineralization rather than restoration of white spot lesions is considered a crucial element of minimally invasive dentistry [2]. WSLs are triggered by pH changes caused by the microbes that continue to function metabolically within the biofilm. These oscillations induce an disparity in the processes of demineralization and remineralization. This results in the deterioration of dental hard tissues, leading to the development of a carious lesion [3,4].

Understanding the development of these lesions and their associated risk factors is crucial for implementing preventive strategies that mitigate the demineralization process prior to lesion progression[5].In recent decades, various treatments, in addition to the necessity for superior oral hygiene, have been implemented prevent the formation of WSLs, particularly the topical application of fluoride, which serves as the baseline against which other remineralization systems are evaluated[6,7].

Fluoride agents may reverse initial lesions but are only effective on the lesion's surface. This may result in the remineralization of the porous superficial layer, obstructing enamel pores, diminishing ionic exchange at the superficial surface and impeding the remineralization of the lesion, hence complicating the attainment of complete remineralization [8].

The optimal remineralising agent must deliver Ca^{2+} and PO_4^{3-} to the lesion. One of the recent advances in remineralization is self-assembling peptides, produced by Credentis AG, Windisch, Switzerland. Biocompatibility testing performed according to ISO 10993 standards has confirmed that peptide P11-4 demonstrates no evidence of cytotoxicity in biological systems [9] . Jablonski-Momeni et al, [10] Guided a trial to assess the effectiveness of 1,000 ppm self-assembling peptide in remineralising artificial early caries lesions, compared to 22,600 ppm fluoride varnish. P11-4 demonstrated a significantly enhanced remineralization capability. Another interesting technology is the amino acid arginine, which serves as the primary salivary component which elevates saliva pH, even in the presence of carbs. Arginine (L-arginine) can be sourced exogenously or endogenously, with the human body naturally producing and secreting it in saliva, either in free form or as salivary peptides[11].

Prior studies indicated that including arginine into dentifrices improved the remineralization of early carious lesions relative to non-arginine toothpaste formulations. Furthermore, the incorporation of arginine to 5% sodium fluoride varnish produces a synergistic action, resulting in increased fluoride and arginine release[12,13]. The functionalized tricalcium phosphate (fTCP) fluoride system demonstrates enhanced remineralization efficacy through synergistic calcium-fluoride co-delivery. The stabilization mechanism involves mechanochemical processing of β-TCP with organic modifiers, which prevents premature calcium-fluoride complexation, maintains ionic bioavailability in aqueous formulations and enables simultaneous release of Ca^{2+}, PO_4^{3-}, and F^- upon application[14,15]. Previous research documented that (TCP) fluoride varnish has greater protection against a cariogenic challenge and showed a lower change in surface microhardness and depth of the lesion compared to 5% sodium fluoride varnish[16,17]. Consequently, this in vitro experiment aimed to assess the depth of carious lesion after application of self-assembling peptide P11-4, 2% Arginine enriched sodium fluoride, and tricalcium phosphate fluoride varnishes using PLM.

The null hypothesis assumed that: no anticipated difference in the remineralising ability of self-assembling peptide P11-4, 2% Arginine enriched sodium fluoride, and tricalcium phosphate fluoride varnishes in the treatment of white spot lesions.

METHODS

Sample size estimation

A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis. With an alpha level of (0.05), a beta of (0.2) i.e. power=80% and an effect size (f) of (0.790) calculated based on the results of previous study [18]; the calculated sample size (n) was a total of (28) samples. Sample size calculation was done using G*Power version 3.1.9.7. The sample size was augmented by 15% to account for potential damaged specimens (32 specimens, 8 in each group) [19].

Specimen collection:

A total of 32 enamel specimens were retrieved from bovine incisors (second dentition). Teeth with structural defects or cracks were excluded [20]. Blood and any debris were then detached using a hand scaler. The teeth were cleansed by toothbrush, rinsed, and preserved in a 0.1% (w/v) thymol in aqueous solution into a closed flask at 0°C till time of the experiment (maximum two months) [21].

Specimen Preparation:

The crowns were separated from their roots through precise sectioning at the cementoenamel junction utilizing a water-cooled diamond saw. The teeth were polished using silicon carbide paper grits of 180, 600, and 1200 microns in ascending order to attain a smooth enamel surface. Each specimen was encased in a mold of cold-cure acrylic resin material (Acrostone, England) with a 1 cm-high ring of polyvinyl chloride (PVC) that solidified around the tooth segment. Subsequently all specimens received double-layer application of acid-resistant varnish (Revlon ColorStay™), with a standardized 6×6 mm enamel window exposed on the labial surface. The window dimensions were precisely demarcated using calibrated graph paper (0.5 mm grid) and masking tape prior to varnish application[22].

Creation of artificial enamel lesions

Artificial enamel lesions were created in the 6mm×6mm exposed window of the ground enamel surface. Each specimen was individually submerged in a quiescent demineralizing solution. (2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05 mM acetic acid). The pH was modified to 4.4 with 1 mm potassium hydroxide (KOH). According to the equation: 2 ml/mm^2 of the exposed enamel area [23], each specimen was soaked in a volume of 72 mL (6x6= 36 mm^2 x 2 mL) for four days at room temperature [24,25].

Demineralizing solutions were not changed through the whole period of storage[26]. The samples were collected and cleansed with distilled water for one minute, then blot-dried using absorbent tissue paper[27]. The exposed 6×6 mm enamel

window was further divided by applying two coats of acid-resistant varnish (Revlon ColorStay™) to cover precisely 50% of the surface, creating a final test area measuring 6×3 mm on the labial aspect.

Grouping:

The enamel samples were equally allocated to four groups (n=8) based on their designated remineralization protocol: Group A: Self assembling peptide P11-4 varnish; Group B: 2% Arginine Enriched Sodium fluoride varnish (; Group C: Tri Calcium Phosphate fluoride varnish (positive control); and Group D: No treatment (negative control).

Treatment of artificial enamel lesions:

Group A: The lesion's surface was cleansed with a 3% hypochlorite solution for 20-second duration, cleansed with distilled water for 30 seconds, then etched using a 35% phosphoric acid gel (BISCO SELECT, HV ETCH high viscosity) for 5 seconds, rinsed again [28], and subsequently dried with absorbent paper. self-assembling peptide (Curodont™ Repair, Credentis AG) was reconstituted by adding 50 µL of sterile distilled water to the pre-measured vial. The solution was applied to lesions and allowed to remain undisturbed for 5 minutes to enable complete penetration and interaction with demineralized enamel structures [18].

Group B: The Arginine powder (L-arginine A5006, Sigma-Aldrich USA) was suspended at 2% *w/v* alongside to 5% sodium fluoride varnish (Citrine sodium fluoride varnish Dharma Research, Inc), where 200 mg of arginine powder was dispensed with 10 ml of 5 % sodium fluoride varnish and vigorously mixed for 60 sec on a glass slab utilizing a sterile micro brush. After dryness of the specimen with absorbent paper, the prepared varnish was applied evenly using a micro brush and left undisturbed for 5min [29,30,31].

Group C: Following surface drying with sterile absorbent paper, a uniform coating of tri-calcium phosphate fluoride varnish (Clinpro™ White Varnish, 3M ESPE) was applied using a microbrush applicator. The varnish was deposited in a continuous, single-direction brushstroke and allowed to set undisturbed for five minutes to ensure proper adhesion and ion release[32]. Group D: No treatment was done [33].

All samples underwent a standardized protocol of regulated pH cycling as per White [34]. Samples were alternately submerged for 21 hours in freshly prepared artificial saliva, formulated according to Ten Cate and Duijsters [35] (1.5mM $CaCl_2$, 0.9mM NaH_2 PO_4, 0.15mM KCl and distilled water) at pH 7.0, and for 3 hours in the demineralizing solution[23]. Blocks were cleansed with deionized water between solution alterations. The pH cycling was conducted over a duration of 12 days in sealed containers. The demineralizing solution and artificial saliva were refreshed daily [36].

Polarized light microscope analysis:

After pH cycling, acid-resistant varnish was completely eliminated using acetone [37]. PPLM (Leica DM 750P) was used. Sections around 300 µm in thickness were excised utilizing an air-cooled, diamond-coated band saw. All sections were refined to a thickness of approximately 200 µm. Each section acquired was examined for lesion depth [24].

Evaluation technique (measurement of lesion depth):

Sections were analyzed at 10x magnification using PLM to quantitatively evaluate the lesions, and photographs were captured. The lesion depth was assessed with an image analyzer (Software Image Pro Plus) at three different locations within each sample. Mean values were calculated and compared pre- and post-treatment by two calibrated blinded independent examiners, reliability assessments included calculation of Intraclass Correlation Coefficients (ICC) to determine both inter- and intra-examiner consistency. ICC for intra examiner reliability was 0.91 which indicated consistency of the examiner over time, while it was 0.86 for inter examiner reliability which indicated strong agreement between examiners [38] .

Statistical method:

Continuous data are expressed as mean ± standard deviation (SD). The Shapiro-Wilk test (significance level $\alpha = 0.05$) was used to assess normality, and Levene's test evaluated variance homogeneity. For lesion depth measurements that followed a normal distribution but exhibited unequal variances (Levene's test $p < 0.05$), Welch's ANOVA with Games-Howell post-hoc analysis was applied. Non-normally distributed reduction data were analyzed using the Kruskal-Wallis test, followed by Dunn's post-hoc comparisons with Bonferroni adjustment. A 95% confidence interval ($p < 0.05$ for significance) was applied for all statistical tests, which were conducted in R (version 4.4.0, R) [39].

RESULTS

Intergroup comparisons of lesion depth (µm):

Before the intervention, there was no significant difference between depths of lesions measured in different groups (p=0.464). Post-intervention, the difference was statistically significant, with group D (-ve control) exhibiting markedly greater depths than the other groups, and group A (Self assembling peptide) showed statistically higher lesion depths compared to groups B (2% Arginine Enriched Sodium fluoride varnish) and C (Tri Calcium Phosphate fluoride varnish) (p<0.001) **(Table 1)**.

Table (1): Intergroup comparison and summary statistics for lesion depth (µm).

Time	Lesion depth (Mean±SD) (µm)				est statistic	p-value
	Group (A)	Group (B)	Group (C)	Group (D)		
Before intervention	132.62±21.43^{A}	153.50±70.06^{A}	138.21±77.23^{A}	183.88±88.95^{A}	**0.91**	**0.464ns**
After intervention	85.20±10.13^{B}	26.29±9.08^{C}	27.14±7.75^{C}	175.62±56.37^{A}	**74.52**	**<0.001***

Within the same row, values marked with distinct superscript letters indicate statistically significant differences ($p < 0.05$), while "ns" denotes non-significant results.

Intergroup comparisons of lesion depth reduction (%):

There was a significant difference between reductions measured in different groups ($p<0.001$) **(Figure 1)**. Post hoc pairwise comparisons showed reductions measured in groups (B) and (C) to be significantly higher than those of other groups ($p<0.001$). In addition, the reduction measured in group (A) was significantly higher than that of group (D) ($p<0.001$) **(Figure 2)**.

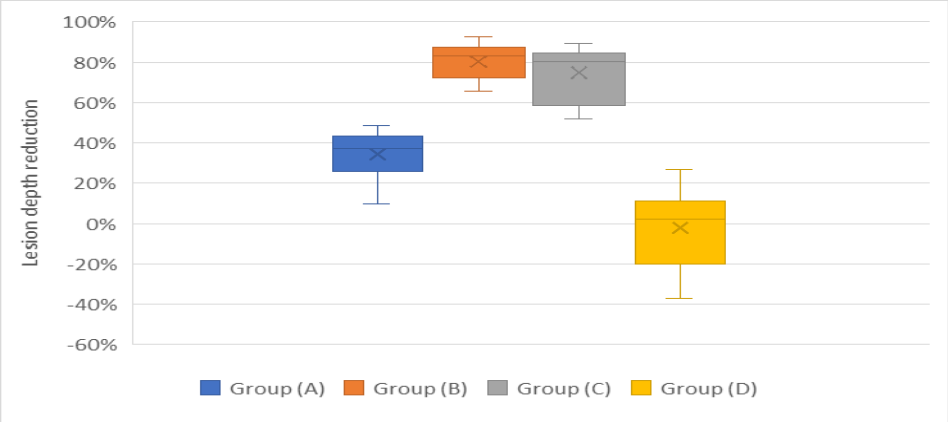

FIGURE 2: BOX PLOT FOR LESION DEPTH REDUCTION VALUES

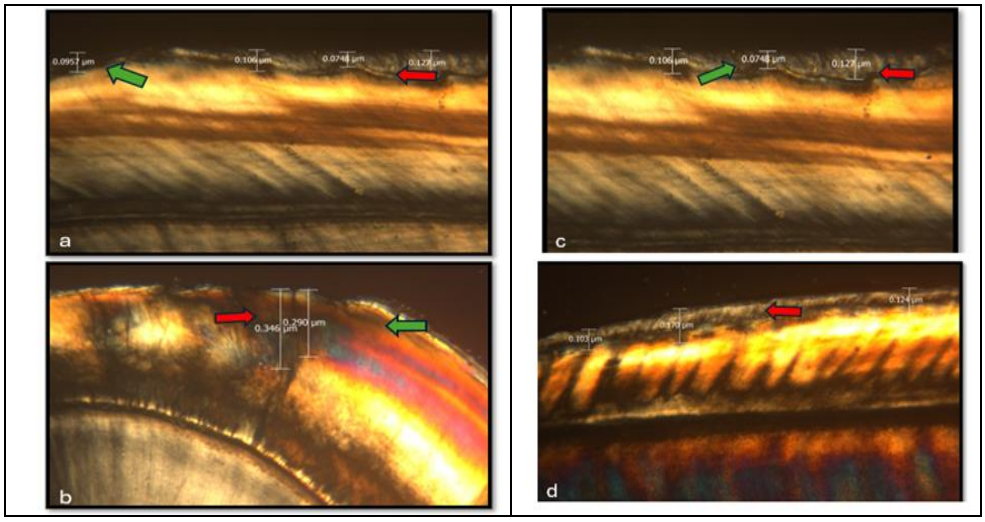

Figure 2: PLM images (×10) showed Demineralized area in enamel, green arrow revealed after treatment with self-assembling peptide (Curodont Repair). (b) demineralized area in enamel (red), green arrow: after treatment with 2 % arginine enriched NA fluoride varnish; (c) Demineralized area in enamel (red arrow), green arrow: After treatment with Tri Calcium Phosphate fluoride varnish; (d) Demineralized area in enamel in the control group (red arrow) in negative control group.

DISCUSSION

The management of incipient caries lesions has been documented since the beginning of the 20th century. Traditionally, caries diagnosis occurs only after cavitation, with treatment relying predominantly on surgical intervention. Decades of research have yielded advanced therapeutic approaches that promote enamel remineralization and inhibit the progression of WSLs [40].

The aim of our experiment was to assess the remineralizing ability of Self-assembling peptide P11-4, 2% Arginine Enriched Sodium Fluoride and Functionalized Tri Calcium Phosphate Fluoride varnish on the depth of artificially induced

carious lesions by polarized light microscope. All the previously mentioned materials are proven to be simple, secure, and efficacious noninvasive interventions for initial carious lesions [15, 29,41].

The novel material Self-assembling peptide was claimed to penetrate the subsurface of white spot lesions (WSLs) to establish a three-dimensional matrix that draws calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions from saliva, promoting the de novo synthesis of hydroxyapatite crystals [42- 44].

Arginine was utilized with 2% w/v, since it has been demonstrated that the inclusion of 2% L-arginine in 5% NaF varnish enhances its physical qualities and creates a stable matrix with sustained greater F/Arg release [31].

Tri-calcium phosphate fluoride varnish (Clinpro™ White Varnish) served as the positive control due to its demonstrated ability to enhance remineralization by supplying calcium, phosphate, and fluoride ions, with a high fluoride concentration of 22,600 ppm permitting a single application[45].

The negative control group was exposed to pH cycling following artificial caries production without any treatment, to eliminate the influence of artificial saliva on the remineralization of artificial caries observed in the other groups. Although the tested materials demonstrate potential for remineralization, there is currently insufficient reliable evidence to fully support their effectiveness. Further research is required to validate their efficacy [17, 46,47].

Artificial enamel lesions were created as they yield more consistently reproducible samples than natural lesions and thereby offer a dependable experimental model [24,27] . Bovine teeth were selected as the experimental substrate due to their greater accessibility and more homogeneous structural composition compared to human dentition. This enhanced uniformity reduces inter-specimen variability when assessing both caries formation and remineralization efficacy. Furthermore, the increased coronal dimensions of bovine teeth provide practical advantages for standardized sample preparation and treatment application [48].

PH cycling was conducted to replicate the kinetics of mineral depletion and accumulation in the caries process. Furthermore, the variables applicable within pH-cycling models exhibit greater sensitivity than those utilized in clinical settings. PH-cycling models may enhance the comprehension of the caries process. Moreover, they are time and cost-effective [48]. PLM was used for sample's evaluation as it is a delicate technique for evaluating de- and remineralization as well as being feasible and reliable method [49].

All three tested materials demonstrated a decrease in the lesion depth under polarized light microscopy; nevertheless, a significant difference was observed among the reductions assessed in the various groups. Reductions seen in groups B (2% arginine enriched NaF varnish, 80.22±9.18) and C (TCP fluoride varnish, 74.93±14.43) were significantly greater compared to those in groups A (Self-assembling peptide P11-4, 34.45±12.73) and D (negative control, 1.98±20.69). The decrease observed in group (A) was significantly higher than that in group (D).

Group A (self-assembling peptide) demonstrated a reduction in artificial caries, but to a lesser extent than groups B and C. This finding aligns with Golland et al[50]., who indicated that the majority of crystals formed by the self-assembling peptide were irregular, thereby diminishing its remineralization capacity. Additionally, Kamal et al [18] observed that self-assembling peptides exhibited enhanced efficacy only when paired with fluoride, as this synergy facilitates enamel regeneration by attracting and nucleating Ca^{2+}, hence generating de novo hydroxyapatite precipitation. These findings came in agreement with Alahdal et al.[51] who assessed the impact of the self-assembling peptide on the micro-Vickers hardness (VH) of eroded enamel, indicating that it did not exhibit a significant difference compared to control regarding VH and μSBS.

Furthermore, Memarpour et al. [52]demonstrated that primary teeth treated with the self-assembling peptide had the lowest ratio of surface enamel microhardness compared to fluoride toothpaste, (CPP-ACP), and fluoride bioactive glass toothpaste. A recent investigation by Wahba et al[53]., indicated that the P11-4 is ineffective in remineralizing caries in deciduous teeth.

On the other hand, this result came in contrast with Alkilzy et al. [54] where superior outcomes were achieved with self-assembling peptide in treating WSL. This difference may be explained by the combination of fluoride and the self-assembling peptide in the mentioned study.

Nevertheless, 2% arginine-enriched sodium fluoride varnish (group B) demonstrated efficacy in halting caries progression and reducing lesion depth, as arginine-fluoride complexes can be retained within enamel, serving as a reservoir that releases fluoride during acid exposure, thereby facilitating the remineralization process. Additionally, it elevates pH, establishing favorable conditions for remineralization and augmenting the synergistic effect of fluoride [30].

Consistent with previous findings, Bijle et al. [55] demonstrated that supplementing sodium fluoride toothpaste with 2% arginine significantly improved remineralization of artificial enamel lesions compared to conventional fluoride toothpaste ($p<0.01$). The outcomes are also in line with Cheng et al[56] and Oliveira et al. [57] who established that the combined application of fluoride and arginine enhances surface microhardness in both intact and demineralized bovine enamel specimens.

The functionalized tri-calcium phosphate (fTCP) fluoride varnish (Group C) demonstrated significant caries lesion depth reduction ($p<0.05$) by serving as a dual mineral reservoir. The fTCP-fluoride synergy promotes formation of fluorapatite crystals with 30% greater acid resistance compared to conventional fluoride-formed hydroxyapatite [15]. These findings align with existing literature on TCP-fluoride synergism, Alamoudi et al.[17] demonstrated through controlled in vitro experiments that TCP incorporation significantly enhances fluoride varnish's protective efficacy. Similarly,

Rirattanapong et al.[58]showed in their study that fluoride varnish containing tricalcium phosphate has a high remineralizing ability and can inhibit progression of WSLs.

The negative control group demonstrated minimal remineralization capacity, as salivary ions alone produced only superficial mineral deposition. Without supplemental calcium and phosphate sources, the natural ion gradient proved insufficient to drive meaningful mineral uptake into the lesion body, compared to the other remineralizing agents [47].

Consequently, the outcomes of this study rejected the null hypothesis, indicating a significant variation in lesion depth among the various testing materials. This study adhered to the checklist for reporting in vitro study [59] and complied with standardization protocols, with all procedures conducted by the same operator consistently. Blinding was verified for both examiners and statisticians.

The pH-cycling model, while widely employed in dental research to evaluate the cariostatic efficacy of agents, has inherent limitations. It does not fully replicate the oral environment, including variations in dietary habits, oral hygiene practices, fluoride exposure, and salivary composition. Nevertheless, it remains the most frequently utilized method in such studies[60]. Furthermore, bovine teeth present higher porosity, which allows a faster diffusion of ions to the demineralized area when compared with natural teeth [48].

CONCLUSIONS

Although, all the three tested materials showed improvement in the lesion depth. Yet, 2% Arginine Enriched Sodium Fluoride and Functionalized Tri Calcium Phosphate Fluoride varnish showed better results than Self-assembling peptide.

Clinical relevance: These biomimetic approaches are particularly valuable for high-risk patients like orthodontic cases, with arginine and TCP-fluoride showing superior remineralization maintaining optimal pH conditions.

List of Abbreviations

WSLs**:** White Spot Lesions
f TCP: Functionalized Tri Calcium Phosphate.
PLM: Polarized Light Microscope.

Acknowledgements

The authors would like to express their sincere gratitude to Dr. Bassam.A for his expert assistance in the statistical analysis of this study.

Authors' contributions

All authors participated in the study of conception and design. Teeth collection, preparation, and treatment were performed by BS. Data analysis was done by BS, NK, DH and MG. BS wrote the first draft of the manuscript. NK and DH revised and edited the manuscript. All authors read and approved the final manuscript.

Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB). Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Ethics approval and consent to participate

The research was reviewed and approved by the Research Ethics Committee of the Faculty of Dentistry, Ain Shams University with reference number (FDASU-Rec ID 032107). All methods were performed according to the ethical principles of Declaration of Helsinki. The study followed CRIS (Checklist for reporting In-vitro Studies).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the first author "Bardis Salah" upon request.

Competing interests

The authors declare no competing interests.

REFERENCES

1. Øgaard B. White Spot Lesions During Orthodontic Treatment: Mechanisms and Fluoride Preventive Aspects. Semin Orthod 2008;14:183–93. https://doi.org/10.1053/j.sodo.2008.03.003.
2. Bakry AS, Abbassy MA. Increasing the efficiency of CPP-ACP to remineralize enamel white spot lesions. J Dent 2018;76:52–7. https://doi.org/https://doi.org/10.1016/j.jdent.2018.06.006.
3. Guo L, Shi W. Salivary biomarkers for caries risk assessment. J Calif Dent Assoc 2013;41. https://doi.org/10.1080/19424396.2013.12222284.

4. Yap J, Walsh LJ, Naser-Ud Din S, Ngo H, Manton DJ. Evaluation of a novel approach in the prevention of white spot lesions around orthodontic brackets. Aust Dent J 2014;59:70–80. https://doi.org/10.1111/adj.12142.
5. Chapman JA, Roberts WE, Eckert GJ, Kula KS, González-Cabezas C. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances. American Journal of Orthodontics and Dentofacial Orthopedics 2010;138:188–94. https://doi.org/10.1016/j.ajodo.2008.10.019.
6. Du M, Cheng N, Tai B, Jiang H, Li J, Bian Z. Randomized controlled trial on fluoride varnish application for treatment of white spot lesion after fixed orthodontic treatment. Clin Oral Investig 2012;16:463–8. https://doi.org/10.1007/s00784-011-0520-4.
7. Zero DT. Dentifrices, mouthwashes, and remineralization/caries arrestment strategies. BMC Oral Health, vol. 6, BioMed Central Ltd.; 2006. https://doi.org/10.1186/1472-6831-6-S1-S9.
8. Deyhle H, Dziadowiec I, Kind L, Thalmann P, Schulz G, Müller B. Mineralization of early stage carious lesions in vitro-a quantitative approach. Dent J (Basel) 2015;3:111–22. https://doi.org/10.3390/dj3040111.
9. Davies RPW, Aggeli A, Beevers AJ, Boden N, Carrick LM, Fishwick CWG, et al. Self-assembling β-sheet tape forming peptides. Supramol Chem 2006;18:435–43. https://doi.org/10.1080/10610270600665855.
10. Jablonski-Momeni A, Korbmacher-Steiner H, Heinzel-Gutenbrunner M, Jablonski B, Jaquet W, Bottenberg P. Randomised in situ clinical trial investigating self-assembling peptide matrix P11-4 in the prevention of artificial caries lesions. Sci Rep 2019;9. https://doi.org/10.1038/s41598-018-36536-4.
11. Morris SM. Arginine: beyond protein23. Am J Clin Nutr 2006;83:508S-512S. https://doi.org/https://doi.org/10.1093/ajcn/83.2.508S.
12. Cheng X, Xu P, Zhou X, Deng M, Cheng L, Li M, et al. Arginine promotes fluoride uptake into artificial carious lesions in vitro. Aust Dent J 2015;60:104–11. https://doi.org/https://doi.org/10.1111/adj.12278.
13. Agnello M, Cen L, Tran NC, Shi W, McLean JS, He X. Arginine Improves pH Homeostasis via Metabolism and Microbiome Modulation. J Dent Res 2017;96:924–30. https://doi.org/10.1177/0022034517707512.
14. Bennett T Amaechi Ramalingam Karthikeyan PKMKNACM, Karlinsey RL. Remineralization of eroded enamel by a NaF rinse containing a novel calcium phosphate agent in an in situ model: a pilot study. Clin Cosmet Investig Dent 2010;2:93–100. https://doi.org/10.2147/CCIDEN.S11844.
15. Karlinsey RL, Pfarrer AM. Fluoride Plus Functionalized β-TCP: A Promising Combination for Robust Remineralization. Adv Dent Res 2012;24:48–52. https://doi.org/10.1177/0022034512449463.
16. Tuloglu N, Bayrak S, Tunc E Sen, Ozer F. Effect of fluoride varnish with added casein phosphopeptide-amorphous calcium phosphate on the acid resistance of the primary enamel. BMC Oral Health 2016;16. https://doi.org/10.1186/s12903-016-0299-4.
17. AlAmoudi SA, Pani SC, AlOmari M. The Effect of the Addition of Tricalcium Phosphate to 5% Sodium Fluoride Varnishes on the Microhardness of Enamel of Primary Teeth. Int J Dent 2013;2013:486358. https://doi.org/10.1155/2013/486358.
18. Kamal D, Hassanein H, Elkassas D, Hamza H. Complementary remineralizing effect of self-assembling peptide (P11-4) with CPP-ACPF or fluoride: An in vitro study. J Clin Exp Dent 2020;12:e161–8. https://doi.org/10.4317/JCED.56295.
19. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175–91. https://doi.org/10.3758/BF03193146.
20. El-zankalouny SM, Abd El Fattah WM, El-Shabrawy SM. Penetration Depth and Microhardness in Incipient Carious Lesions. vol. 41. 2016.
21. Min JH, Inaba D, Kwon HK, Chung JH, Kim BI. Evaluation of penetration effect of resin infiltrant using optical coherence tomography. J Dent 2015;43:720–5.
22. Min JH, Inaba D, Kwon HK, Chung JH, Kim BI. Evaluation of penetration effect of resin infiltrant using optical coherence tomography. J Dent 2015;43:720–5. https://doi.org/https://doi.org/10.1016/j.jdent.2015.03.006.
23. Torres CRG, Rosa PCF, Ferreira NS, Borges AB. Effect of caries infiltration technique and fluoride therapy on microhardness of enamel carious lesions. Oper Dent 2012;37:363–9. https://doi.org/10.2341/11-070-L.
24. Shah SP, Birur PN. Polarized light microscopic evaluation of remineralization by casein phosphopeptide-amorphous calcium phosphate paste of artificial caries-like lesion: An: in vitro: study. Journal of Indian Academy of Oral Medicine and Radiology 2015;27.
25. Pintanon P, Sattabanasuk V, Banomyong D. Effectiveness of Caries Infiltration and CPP-ACP Containing Paste on Color Change and Surface Hardness of Artificial White Spot Enamel Lesions Correspondence to 2016:133. https://doi.org/10.14456/jdat.2016.12.
26. Alkattan R, Lippert F, Tang Q, Eckert GJ, Ando M. The influence of hardness and chemical composition on enamel demineralization and subsequent remineralization. J Dent 2018;75:34–40. https://doi.org/https://doi.org/10.1016/j.jdent.2018.05.002.
27. Buckshey S, Anthonappa RP, King NM, Itthagarun A. Remineralizing potential of ClinPro ® and tooth mousse plus ® on artificial carious lesions. Journal of Clinical Pediatric Dentistry 2019;43:103–8. https://doi.org/10.17796/1053-4625-43.2.6.

28. Wierichs RJ, Kogel J, Lausch J, Esteves-Oliveira M, Meyer-Lueckel H. Effects of Self-Assembling Peptide P11-4, Fluorides, and Caries Infiltration on Artificial Enamel Caries Lesions in vitro. Caries Res 2017;51:451–9. https://doi.org/10.1159/000477215.
29. Yip K, Abdalla MM, Bijle MN, Yiu C. Effect of arginine-fluoride varnish on preventing enamel erosion by paediatric liquid medicaments. BMC Oral Health 2023;23. https://doi.org/10.1186/s12903-023-03621-8.
30. ALBLOOSHI NA, KRITHIKADATTA J, YIU C, BIJLE MN. Fluoride release potential of arginine-incorporated fluoride varnishes. Dent Mater J 2024. https://doi.org/10.4012/dmj.2023-238.
31. Bijle MN, Ekambaram M, Lo E, Yiu C. Physicochemical characteristics of arginine enriched naf varnish: An in vitro study. Polymers (Basel) 2020;12:1–14. https://doi.org/10.3390/polym12122998.
32. Karlinsey RL, Pfarrer AM. Fluoride plus functionalized β-TCP: a promising combination for robust remineralization. Adv Dent Res 2012;24:48–52. https://doi.org/10.1177/0022034512449463.
33. Farhana F, Harish K, Shetty S. Evaluation of the Effect of Self Assembling Peptide-Curodont on Microhardness of Bleached Enamel Surface: An In Vitro Study. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN 2020;19:51–4. https://doi.org/10.9790/0853-1901155154.
34. White DJ. The application of in vitro models to research on demineralization and remineralization of the teeth. Adv Dent Res 1995;9:175–93.
35. Ten Cate JM, Duijsters PPE. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res 1982;16:201–10.
36. Amaechi BT. Protocols to study dental caries in vitro: pH cycling models. Methods in Molecular Biology, vol. 1922, Humana Press Inc.; 2019, p. 379–92. https://doi.org/10.1007/978-1-4939-9012-2_34.
37. Singhal RK, Rai B. Remineralization potential of three tooth pastes on enamel caries. Open Access Maced J Med Sci 2017;5:664–6. https://doi.org/10.3889/oamjms.2017.090.
38. Bansal K, Gauba K, Tewari A, Chawla HS, Sahni A. In vivo remineralization of artificial enamel carious lesions using a mineral-enriched mouthrinse and a fluoride dentifrice: A polarized light microscopic comparative evaluation. Journal of Indian Society of Pedodontics and Preventive Dentistry 2010;28:264–70. https://doi.org/10.4103/0970-4388.76156.
39. Team RC. RA language and environment for statistical computing, R Foundation for Statistical. Computing 2020.
40. Kobeissi R, Badr SBY, Osman E. Effectiveness of self-assembling peptide P11-4 compared to tricalcium phosphate fluoride varnish in remineralization of white spot lesions: A clinical randomized trial. Int J Clin Pediatr Dent 2020;13:451–6. https://doi.org/10.5005/jp-journals-10005-1804.
41. Alkilzy M, Qadri G, Splieth CH, Santamaría RM. Biomimetic Enamel Regeneration Using Self-Assembling Peptide P11-4. Biomimetics 2023;8. https://doi.org/10.3390/biomimetics8030290.
42. Bröseler F, Tietmann C, Schleich R, Drechsel T, Bommer C. Effect of Curodont™ Repair in Patients with Buccal Carious Lesions n.d.
43. Kamal D, Hassanein H, Elkassas D, Hamza H. Comparative evaluation of remineralizing efficacy of biomimetic self-assembling peptide on artificially induced enamel lesions: An: in vitro: study. Journal of Conservative Dentistry 2018;21:536–41.
44. Elsayed H. Evaluation of the state and shade of white spot lesions after treatment with different remineralizing agents (An in-vivo comparative study). Al-Azhar Journal of Dental Science 2021;24:239–45. https://doi.org/10.21608/ajdsm.2020.46840.1123.
45. Manchanda S, Liu P, Sardana D, Peng S, Lo ECM, Yiu CKY. Randomized clinical trial to compare three fluoride varnishes in preventing early childhood caries. J Dent 2024;147:105141. https://doi.org/https://doi.org/10.1016/j.jdent.2024.105141.
46. Elewa S, Elzainy M, Hussein S. The Effect of Self Assembling Peptides Versus Sodium Fluoride Varnish on Artificially Induced Enamel Lesions in Human Premolars, In Vitro Study. (Scanning Electron Microscope and Energy Dispersive X-ray Analysis). Egypt Dent J 2023;69:2793–805. https://doi.org/10.21608/edj.2023.228076.2674.
47. Abdslam A, Farag M, Mahfouz Omer S. " Evaluation of the Effect of Two Different Concentration of Arginine on Fluoride Uptake by Demineralized Enamel Surfaces" in vitro study. Dental Science Updates 2022;3:199–208. https://doi.org/10.21608/dsu.2022.117363.1098.
48. Buzalaf MAR, Hannas AR, Magalhães AC, Rios D, Honório HM, Delbem ACB. pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. Journal of Applied Oral Science 2010;18:316–34.
49. Bansal K, Gauba K, Tewari A, Chawla HS, Sahni A. In vivo remineralization of artificial enamel carious lesions using a mineral-enriched mouthrinse and a fluoride dentifrice: A polarized light microscopic comparative evaluation. Journal of Indian Society of Pedodontics and Preventive Dentistry 2010;28:264–70. https://doi.org/10.4103/0970-4388.76156.

50. Golland L, Patrick /, Schmidlin R, Schätzle M. The Potential of Self-assembling Peptides for Enhancement of In Vitro Remineralisation of White Spot Lesions as Measured by Quantitative Laser Fluorescence. Oral Health Prev Dent 2017;15:147–52. https://doi.org/10.3290/j.ohpd.a37931.
51. Alahdal K, Almohareb T, Aldeeb L, Maawadh A, Alshamrani AS, Alrahlah A. Use of Reparative Agents Topical Fluoride Activated by CO2 Laser and Curodont™ Repair and NR-5™ on Vickers Hardness and Micro-Shear Bond Strength of Eroded Enamel to Composite Restoration. Applied Sciences (Switzerland) 2023;13. https://doi.org/10.3390/app132312717.
52. Memarpour M, Razmjouei F, Rafiee A, Vossoughi M. Remineralization effects of self-assembling peptide P11-4 associated with three materials on early enamel carious lesions: An in vitro study. Microsc Res Tech 2022;85:630–40. https://doi.org/https://doi.org/10.1002/jemt.23937.
53. Wahba N, Schwendicke F, Kamel MA, Allam G, Kabil N, Elhennawy K. Preventing and Arresting Primary Tooth Enamel Lesions Using Self- Assembling Peptide P11-4 In Vitro. J Int Soc Prev Community Dent 2022;12.
54. 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. https://doi.org/10.1177/0022034517730531.
55. Bijle MNA, Ekambaram M, Lo ECM, Yiu CKY. The combined enamel remineralization potential of arginine and fluoride toothpaste. J Dent 2018;76:75–82. https://doi.org/https://doi.org/10.1016/j.jdent.2018.06.009.
56. Cheng X, Xu P, Zhou X, Deng M, Cheng L, Li M, et al. Arginine promotes fluoride uptake into artificial carious lesions in vitro. Aust Dent J 2015;60:104–11. https://doi.org/https://doi.org/10.1111/adj.12278.
57. Oliveira PHC, Oliveira MRC, Oliveira LHC, Sfalcin RA, Pinto MM, Rosa EP, et al. Evaluation of Different Dentifrice Compositions for Increasing the Hardness of Demineralized Enamel: An in Vitro Study. Dent J (Basel) 2019;7. https://doi.org/10.3390/dj7010014.
58. Rirattanapong P, Vongsavan K, Saengsirinavin C, Pornmahala T. EFFECT OF FLUORIDE VARNISHES CONTAINING TRI-CALCIUM PHOSPHATE SOURCES ON REMINERALIZATION OF INITIAL PRIMARY ENAMEL LESIONS. vol. 45. 2014.
59. Krithikadatta J, Gopikrishna V, Datta M. CRIS Guidelines (Checklist for Reporting In-vitro Studies): A concept note on the need for standardized guidelines for improving quality and transparency in reporting: in-vitro: studies in experimental dental research. Journal of Conservative Dentistry 2014;17:301–4.
60. Singhal RK, Rai B. Remineralization potential of three tooth pastes on enamel caries. Open Access Maced J Med Sci 2017;5:664–6. https://doi.org/10.3889/oamjms.2017.090.