

Evaluation of Remineralizing Capacity of P11-4, CPP-ACP, Silver Diamine Fluoride, and NovaMin: An *In Vitro* Study

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ABSTRACT

Aim and objective: To determine and compare remineralizing efficacy of NovaMin, CPP-ACP, silver diamine fluoride (SDF), and P11-4.

Materials and methods: Sixty permanent premolars were divided into four groups with 15 samples in each group; group I: self-assembling peptide (P11-4), group II: SDF, group III: Casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP), and group IV: NovaMin. Mineral content was assessed using a scanning electron microscope at 7, 14, and 21 days after remineralization with each agent.

Results: The mean remineralization in group I at 7 days was 1.73 ± 0.02 , at 14 days was 1.79 ± 0.01 , and at 21 days was 1.90 ± 0.03 . Mean remineralization in group II was 1.61 ± 0.01 , 1.64 ± 0.02 , and 1.73 ± 0.03 at 7, 14, and 21 days, respectively. Mean remineralization in group III was 1.62 ± 0.01 , 1.65 ± 0.02 , and 1.74 ± 0.05 at 7, 14, and 21 days, respectively. Mean remineralization in group IV was 1.59 ± 0.02 , 1.62 ± 0.07 , and 1.70 ± 0.09 at 7, 14, and 21 days, respectively. The maximum value was obtained on day 21. There was a significant difference in mean remineralization values between group I vs group II, group I vs group III, and group I vs group IV ($p < 0.05$).

Conclusion: Self-assembling peptides showed maximum remineralization in tested specimens followed by CPP-ACP, SDF, and NovaMin-containing toothpaste.

Clinical significance: CPP-ACP, SDF, and NovaMin-containing toothpaste can be indicated for remineralization of initial caries in clinical use.

Keywords: CPP-ACP, NovaMin, SDF, Self-assembling peptide.

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INTRODUCTION

Dental caries is highly prevalent in young adults. The change in eating habits, industrialization, and ineffective oral hygiene are key factors.¹ Dental caries is a dynamic process. There is continuous remineralization demineralization taking place. Now it has been proven that initial lesions or white spot lesions can be reversed back to normal with the use of remineralizing agents. Nowadays there is a growing interest in conservation teeth and remineralization of the initial lesion. But there is a lack of an effective remineralizing agent. Hence, various remineralizing agents are introduced, such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), NovaMin, P11-4, and silver diamine fluoride (SDF).²⁻⁶

NovaMin contains calcium sodium phosphosilicate having a size <20 microns. It also contains bioactive glass particulates. It is evident that NovaMin brings about remineralization by binding calcium, sodium, phosphorus, and silicate with the tooth surface, hence initiating the remineralization process.⁷ This is mediated through the release of calcium, phosphorous, silica, and sodium ions to the tooth structure. Another effective agent is casein phosphopeptide-amorphous calcium phosphate technology that is based on the stabilizing properties of milk and salivary proteins. The casein phosphopeptides allow high concentrations of calcium, phosphate, and fluoride ions to be stabilized in solution, in a form that is bioavailable for the promotion of remineralization.⁸ CPP-ACP is a milk product; hence it cannot be advised for patients with milk intolerance.⁹ Drawback of NovaMin is that it is slow acting and less effective compared to other remineralizing agents.¹⁰

Silver diamine fluoride (SDF) has been used as a cariostatic agent for many years. It is capable of initiating remineralization by liberating fluoride, thus ensuring the deposition of silver

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phosphate to restore the mineral content and favoring tooth surface rehardening.¹¹ Its disadvantages are black staining of caries lesion, gum swelling, tooth or gum pain, and gum bleaching.¹²

Recently self-assembling peptide (P11-4) which is a β -sheet-forming peptide that self-assembles into three-dimensional fibrillar scaffolds and mimics the action of the extracellular enamel matrix proteins found during tooth development has shown its

beneficial effect on the tooth surface.⁷ Its disadvantage is aesthetic appearance since treated enamel does not reoccurrence to full translucency.¹³

The present study compared remineralizing efficacy of NovaMin, CPP-ACP, SDF, and P11-4.

MATERIALS AND METHODS

This *in vitro* study was conducted in the department of conservative dentistry, involving recently extracted 60 permanent premolars due to orthodontic treatment purpose. Ethical clearance to conduct the study was obtained from the institutional ethical committee. The sample size was determined using the following parameters: significance level: 0.05, power: 80%, and many groups.

Sixty tooth specimens were coated with acid-resistant nail varnish leaving a buccal surface of size 1 mm × 1 mm exposed, which was subjected to the demineralizing process. Sixty specimens were divided into four groups with 15 samples in each remineralizing agent; group I: self-assembling peptide/P11-4 (Curodont Repair, US), group II: silver diamine fluoride (SDF: 38% silver diamine fluoride, FAGamin®, Argentina), group III: casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP-GC Tooth Mousse®), group IV: NovaMin (Bioactive glass—calcium sodium phosphosilicate CSPS, GlaxoSmithKline, New York, US). Each specimen was treated with respective remineralizing agents two times daily for 3 minutes followed by incubation in artificial saliva at 37°C. Mineral content (% weight) was measured in all groups with scanning electron microscope-energy dispersive X-ray analysis (SEM-EDX) at time intervals of 7, 14, and 21 days after remineralization.

Statistical Analysis

Results of the study were clubbed and descriptive statistics were calculated using Statistical Package for the Social Sciences (SPSS version 19) software (IBM, USA). *p*-value < 0.05 was labeled significant.

RESULTS

Table 1 shows the distribution of teeth in various groups depending upon the type of remineralizing agent used.

Table 2 shows that mean remineralization in group I at 7 days was 1.73 ± 0.02 , at 14 days was 1.79 ± 0.01 , and at 21 days was 1.90 ± 0.03 . Mean remineralization in group II was 1.61 ± 0.01 , 1.64 ± 0.02 , and 1.73 ± 0.03 at 7, 14, and 21 days, respectively. Mean remineralization in group III was 1.62 ± 0.01 , 1.65 ± 0.02 , and 1.74 ± 0.05 at 7, 14, and 21 days, respectively. Mean remineralization in group IV was 1.59 ± 0.02 , 1.62 ± 0.07 , and 1.70 ± 0.09 at 7, 14, and 21 days, respectively. The maximum value was obtained on day 21. ANOVA test revealed a significant difference in values in each group. Higher remineralizing efficacy was found with group I followed by group III, II, and IV.

Table 3 shows that there was a significant difference in mean remineralization values between group I vs group II, group I vs group III, and group I vs group IV (*p* < 0.05). All groups were effective in remineralization whereas, the group I was much effective.

Table 1: Distribution of specimens

Groups	Group I	Group II	Group III	Group IV
Agent	P11-4	SDF	CPP-ACP	NovaMin
Number	15	15	15	15

Table 2: Assessment of remineralization at various intervals of time

Groups	Interval	Mean \pm SD	<i>p</i> -value
Group I: P11-4	7 days	1.73 ± 0.02	0.014
	14 days	1.79 ± 0.01	
	21 days	1.90 ± 0.03	
Group II: SDF	7 days	1.61 ± 0.01	0.032
	14 days	1.64 ± 0.02	
	21 days	1.73 ± 0.03	
Group III: CPP-ACP	7 days	1.62 ± 0.01	0.027
	14 days	1.65 ± 0.02	
	21 days	1.74 ± 0.05	
Group IV: NovaMin	7 days	1.59 ± 0.02	0.019
	14 days	1.62 ± 0.07	
	21 days	1.70 ± 0.09	

ANOVA, significance, *p* < 0.05

Table 3: Intergroup comparison for 21 days

Pairwise comparison	Mean 21 days remineralization
Group I vs group II	<0.05
Group I vs group III	<0.05
Group I vs group IV	<0.05
Group II vs group III	<0.05
Group II vs group IV	<0.05
Group III vs group IV	<0.05

Post hoc analysis, Tukey test, significance, *p* < 0.05

DISCUSSION

The affected dentin can be remineralized and the odontoblasts can form reparative dentin, thus avoiding pulp exposure.¹⁴ The method of physiologic remineralization demands collagen fibers and odontoblastic processes in the last layer of carious dentin.¹⁵ These fibers provide the site for the attachment of apatite crystals. There is a supply of calcium phosphate from the vital pulp by odontoblasts that promotes the process of remineralization.¹⁶

We found that self-assembling peptides had higher remineralization capacity followed by CPP-ACFP and SDF. Sinha et al. determined the remineralizing efficacy of SDF, glass ionomer Type VII (GC VII), and calcium hydroxide (Dycal). They demonstrated that there was an equivalent rise in the % of calcium level in GC VII and Ca(OH)₂ groups, followed by the SDF group. The maximum percentage of fluoride upsurge was observed in the GC VII group followed by the SDF group, and Ca(OH)₂ group.¹⁷

Manoharan et al. compared casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) and NovaMin in terms of remineralization potential on artificially induced enamel subsurface lesions with the help of SEM-EDX. They concluded that both groups had significant differences between Ca/P ratios of remineralized samples.¹⁸ These findings are in accordance with our results.

Kamal et al. determined the efficacy of biomimetic self-assembling peptide (P11-4), CPP-ACPF, and fluoride-based delivery systems on enamel remineralization on 40 extracted human molars and found that P11-4 had maximum statistically substantial average SMH followed by CPP-ACPF and fluoride.¹⁹ These findings are similar to our result.

Kumar et al.²⁰ compared monofluorophosphate, casein phosphopeptide-amorphous calcium phosphate, and calcium sodium phosphosilicate on demineralized enamel lesions.

Results showed that calcium sodium phosphosilicate had 3874.1 μm^2 mean lesion area and 107282.6 value for total fluorescence and casein phosphopeptide-amorphous calcium phosphate had 7371.2 μm^2 and 233765.9 of mean lesion area and total fluorescence, respectively. No treatment group showed a mean area of 16449.2 μm^2 and 759743.1 of total fluorescence having a significant difference between groups.²⁰ Elsayad et al. in their study demonstrated that fluoride aggregation to CPP-ACP may be helpful to provide a synergistic effect on enamel remineralization of early carious lesion.²¹ de Oliveira et al. evaluated remineralizing efficacy of enamel lesions with fluoride dentifrice over casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP). They found remineralization with all tested groups but greater efficacy with the fluoride dentifrice group.²²

Narayana et al. evaluated the remineralization capacity of bioactive glass, fluoride toothpaste, CPP-ACP (tooth mousse), CPP-ACPF, and found greater efficacy with bioactive glass.²³ Akyildiz and Sonmez assessed the remineralization outcome of SDF with nanosilver fluoride using Vickers microhardness values and scanning electron microscope evaluation and found that SDF was effective compared to NSF.²⁴ Wierichs et al. evaluated remineralizing efficacy of NaF, NaF plus TCP, NaF plus CPP-ACP, and SDF varnishes and found remineralization of artificial caries with only NaF plus CPP-ACP and SDF.²⁵ Similarly we found effectiveness with CPP-ACP and SDF.

We found higher effectiveness of P11-4 followed by CPP-ACP, SDF, and NovaMin in remineralization. Remineralizing efficacy with casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP) could be because it discharges greater quantities of phosphate and calcium ions at all pH levels, CPP enhances fluoride integration into the biofilm and noticeably upsurges remineralization of enamel, presence of phosphate, which may either compete with fluoride for calcium bonds or precipitous, whereas the remineralization effect of fluoride depends on the availability of calcium and phosphate.²² Fluoride from SDF can help in the admission of silver phosphate to re-establish the mineral content, resulting in rehardening of the tooth structure. The fluoride released from SDF can penetrate deeper into dentin than into enamel and increases calcium, phosphate, and fluoride ions in caries affected dentin. The negative effect of SDF is blackish discoloration beneath the site of application due to the deposition of silver phosphate with an increase in phosphate ions.¹⁷ The better remineralization effect of P11-4 observed in the present study could be due to matrix-mediated mineralization and diffusion of the bioactive peptide into the porosities and assembled within the subsurface lesion into a three-dimensional fibrillar scaffold; gradual raise in the Ca:P ratio; assemble of peptides into fibers, containing clusters of negative charges made up of four Glu residues that provide a potential Ca^{2+} -binding site.²⁶

The present study compared remineralizing efficacy of NovaMin, CPP-ACP, SDF, and P11-4. There is very limited study on this comparison. This study helps to identify and use a better remineralizing agent for remineralization of the initial carious lesion to reduce the burden on the community and to improve oral health.

We observed that self-assembling peptides followed by CPP-ACP and SDF had higher remineralization capacity. Hence, we can suggest that self-assembling peptides can be used as effective remineralizing agents.

The weakness of the study was the lesser sample size with *in vitro* analysis. We assessed samples up to 21 days which was not sufficient for complete remineralization. Further *in vivo* studies

are required with a larger sample size with scanning electron microscopic evaluation of remineralization.

CONCLUSION

It was found that self-assembling peptides showed maximum remineralization in tested specimens, whereas those treated with NovaMin-containing toothpaste, SDF, and CPP-ACP had comparable remineralization potential.

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