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ORIGINAL ARTICLE

An in Vitro Evaluation of Remineralizing Capacity of Self-Assembling Peptide (SAP) P11-4 and Casein Phosphopeptides-Amorphous Calcium Phosphate (CPP-ACP) on Artificial Enamel

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Abstract

Objective: To determine and compare the remineralizing capacity of self-assembling peptide (SAP) P11-4 and casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) on enamel. **Material and Methods:** Enamel samples were divided into 2 groups. Group I was treated with Self-assembling peptide (SAP) P11-4 and group II with casein phosphopeptides-amorphous calcium phosphate (CPP-ACP). In both groups, remineralizing capacity was assessed at baseline, 2 weeks, 6 weeks and 12 weeks. Student's t- test and ANOVA were applied, with the significance level set at 5%. **Results:** The mean calcium weight % was evaluated at baseline, 2 weeks, 6 weeks and 12 weeks. In Group I, there was increase in mean value (62.12 ± 1.24) from baseline to 12 weeks (67.36 ± 2.14). However, there was decrease in phosphate weight % from 37.16 ± 2.52 at baseline to 35.72 ± 2.11 at 12 weeks. In Group II, mean calcium weight % was 64.18 ± 1.52 at baseline, which ultimately increased to 66.01 ± 2.03 at 12 weeks. Phosphate weight % showed reduction from 37.34 ± 2.23 at baseline to 35.04 ± 2.02 at 12 weeks. Ca/P ratio showed significant improvement. There was significant difference in Ca/P ratio at 2 weeks, 6 weeks and 12 weeks in both groups ($p<0.05$). **Conclusion:** Self-assembling peptide (SAP) P11-4 found to be more effective and efficient as compared to casein phosphopeptides-amorphous calcium phosphate (CPP-ACP).

Keywords: Caseins; Phosphopeptides; Calcium Phosphates; Dental Enamel.

Introduction

Deciduous teeth are more prone to develop dental caries due to higher sugar intake among children. Dental caries is irreversible microbial disease of calcified tissue characterized by dimeralization of inorganic and destruction of organic portion of teeth. Outermost covering of tooth is made up of enamel, which is highly vulnerable to destruction. Once destruction occurs in enamel, the process cannot be reversed due to incapacity of ameloblasts [1].

The acidic environment of oral cavity produced by excessive sugar intake resulting from bacterial action on it, leads to significant reduction in pH. This fall in pH begins the dimeralization of enamel of teeth. The appearance of white spot on the enamel is the sign of dimeralization [2]. The process of dental caries starts with the appearance of white spot leading to loss of mineral from subsurface area even with intact superficial layer. With >30% dimeralization, the process becomes irreversible. These incipient lesions need to be detected at appropriate time so as to prevent this destruction [3].

Various remineralization agents are present in the market. An ideal agent should be toxic free, capable of initiating remineralization without any harm to tooth. There should be matrix-mediated mineralization equivalent to natural process. However, this property is lack by most of these agents [4]. The advent of self-assembling peptide (SAP) P11-4 has overcome this limitation. It has property of regenerating enamel. These agents initiate remineralization by forming three dimensional structures mimic extracellular matrix of teeth [5]. The present study was conducted to determine and compare the remineralizing capacity of self-assembling peptide (SAP) P11-4 and casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) on enamel.

Material and Methods

The present study was conducted in the Department of Oral and Maxillofacial Pathology. It consisted of 84 freshly extracted premolars in the children/adolescents age ranged from 14-20 years of age. Only those premolars were selected which required extraction due to orthodontic treatment. These enamel samples from premolars were prepared and stored in 10% formalin solution.

All teeth were cut into two pieces at cemento-enamel junction (CE junction) so that roots were separated from crowns. The coronal portion of teeth was dissected in mesio-distal plane dividing it into two halves with the help of diamond disks. The samples (N=168) were divided into 2 groups of 84 in each group: Group 1 samples were self-assembling peptide (SAP) P11-4 and Group 2 samples were casein phosphopeptides-amorphous calcium phosphate (CPP-ACP).

In all samples, artificial enamel lesions were prepared by placing wax sheet of size 3mm X 3mm on tooth surfaces and these samples were placed in remineralizing agents for 5 days. In Group I, samples were etched with 37% phosphoric acid for 5 minutes followed by washing with distilled water and dried. All samples were subjected to SAP P11-4 remineralizing agents for 6 minutes. In Group 2, CPP-ACP agents was applied to all samples and kept for 5 minutes. In both groups solution was changed after 2 weeks. In both groups, samples were assessed with scanning electron

microscopy (SEM) and energy-dispersive X-ray (EDX) at 2 weeks, 6 weeks and 12 weeks to detect remineralization. Calcium and phosphorous weight % was assessed with the help of EDX and thus Ca/P ratio was calculated.

Data Analysis

All statistical analysis was undertaken using SPSS v. 18.0 (IBM Corp., Armonk, NY, USA) with significance value $p<0.05$. Calcium and percentage in Group I and II was evaluated using ANOVA and Student t-test.

Ethical Aspects

The ethical approval was obtained from the Ethical Review Board of the College of Dental Sciences, Indore.

Results

Table 1 and Figure 1 shows that mean calcium weight% was 62.12 ± 1.24 at baseline, 63.85 ± 1.46 at 2 weeks, 65.28 ± 1.70 at 6 weeks which ultimately increased to 67.36 ± 2.14 at 12 weeks. The difference was significant ($p<0.05$). Phosphate weight % was 37.16 ± 2.52 at baseline, 37.24 ± 2.64 at 2 weeks, 36.15 ± 2.25 at 6 weeks and 35.72 ± 2.11 at 12 weeks. The difference was significant ($p<0.05$). Ca/P ratio was 1.75 ± 0.14 at baseline, which decreased to 1.72 ± 0.12 at 2 weeks and become 1.98 ± 0.16 at 12 weeks. The difference was significant ($p<0.05$).

Table 1. Calcium and Phosphate weight % and Ca/P ratio in Group 1.

Interval	Calcium Weight % Mean ± SD	Phosphate Weight % Mean ± SD	Ca/P ratio Mean ± SD
Baseline	62.12 ± 1.24	37.16 ± 2.52	1.75 ± 0.14
2 weeks	63.85 ± 1.46	37.24 ± 2.64	1.72 ± 0.12
6 weeks	65.28 ± 1.70	36.15 ± 2.25	1.77 ± 0.18
12 weeks	67.36 ± 2.14	35.72 ± 2.11	1.98 ± 0.16
p-value	0.001	0.001	0.001

ANOVA

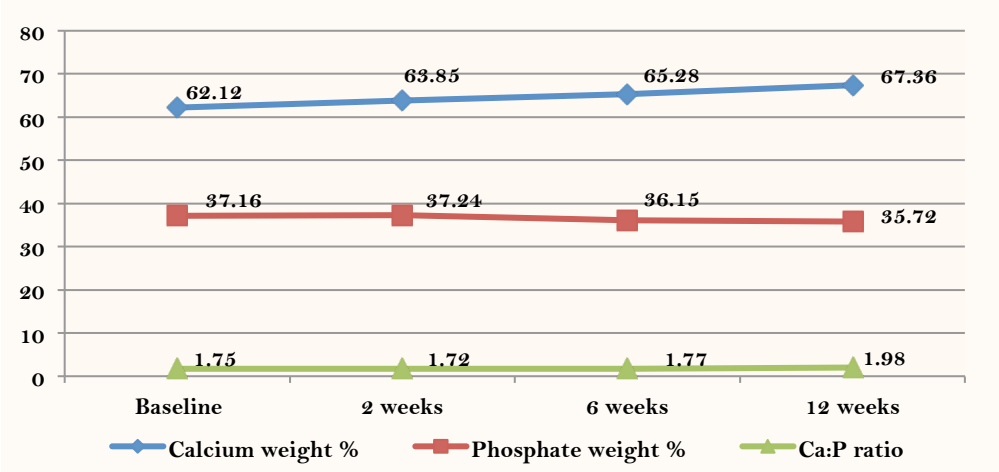

Figure 1. Calcium and Phosphate weight % and Ca/P ratio in Group 1.

Table 2 and Figure 2 shows that mean calcium weight % was 64.18 ± 1.52 at baseline, 67.80 ± 1.58 at 2 weeks, 65.40 ± 1.64 at 6 weeks which ultimately increased to 66.01 ± 2.03 at 12 weeks. Phosphate weight % was 37.34 ± 2.23 at baseline, 33.87 ± 2.28 at 2 weeks, 36.23 ± 2.12 at 6 weeks and 35.04 ± 2.02 at 12 weeks. Ca/P ratio was 1.77 ± 0.15 at baseline, 2.06 ± 0.14 at 2 weeks, 1.87 ± 0.17 at 6 weeks and 1.88 ± 0.14 at 12 weeks. The difference was significant (p<0.05).

Table 2. Calcium and Phosphate weight % and Ca/P ratio in Group 2.

Interval	Calcium Weight % Mean ± SD	Phosphate Weight % Mean ± SD	Ca/P ratio Mean± SD
Baseline	64.18 ± 1.52	37.34 ± 2.23	1.77 ± 0.15
2 weeks	67.80 ± 1.58	33.87 ± 2.28	2.06 ± 0.14
6 weeks	65.40 ± 1.64	36.23 ± 2.12	1.87 ± 0.17
12 weeks	66.01 ± 2.03	35.04 ± 2.02	1.88 ± 0.14
p-value	0.001	0.001	0.001

ANOVA

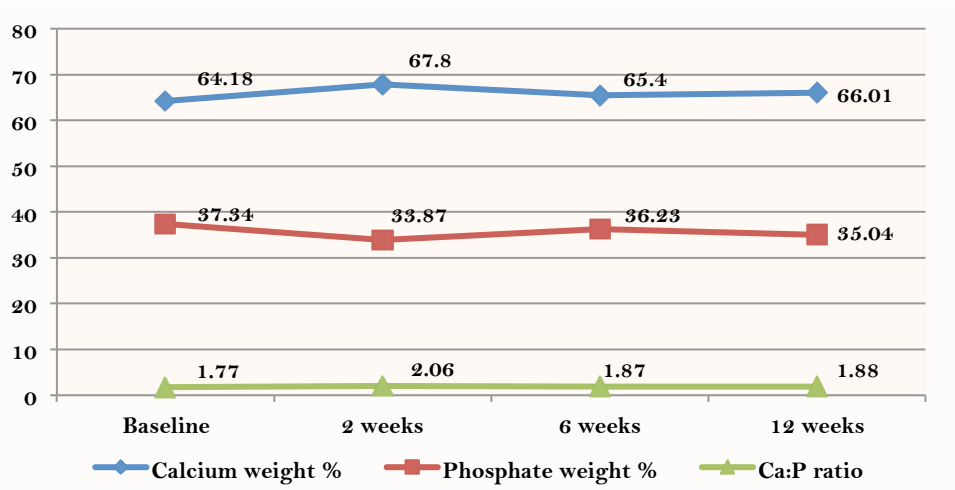

Figure 2. Calcium and Phosphate weight % and Ca/P ratio in Group 2.

Figure 3 showed significant difference in Ca/P ratio at 2 weeks, 6 weeks and 12 weeks. The difference was significant (p<0.05).

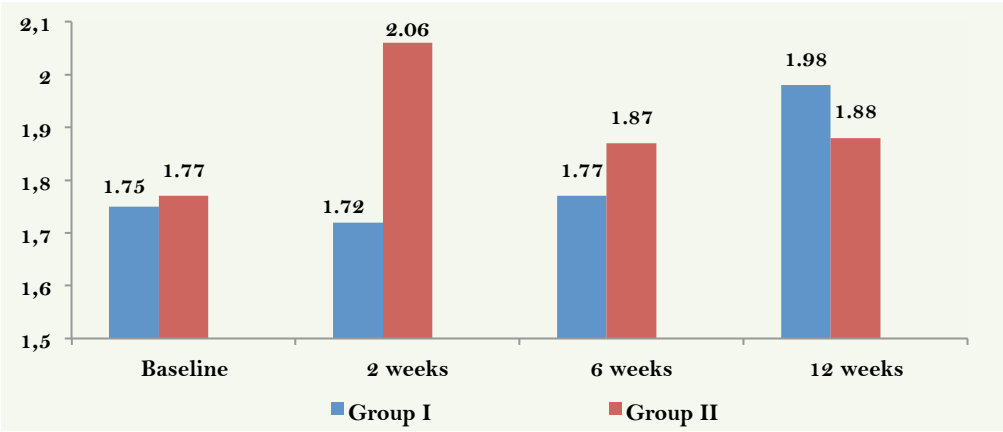

Figure 3. Comparison of Ca/P ratio in both groups.

Discussion

The process of dental caries starts with the loss of inorganic and organic portion of teeth. The appearance of white spots on enamel of teeth indicates the disease process and hence there should be some agent, which can reverse this process. However, this property is deficient in most of the agents, which could initiate remineralization. In pediatric dentistry, the prevalence of dental caries is quite high. In process of dimeralization, Ca10 (PO4)6(OH) 2 + 14 H+ → 10 Ca+ + 6 H2PO4+ H2O reaction occurs [6].

An ideal remineralization agent should be capable to providing calcium and phosphate, prevent calculus formation, active in acidic pH and accelerates the remineralizing power of saliva. In this regard, fluorides have been used in dentistry since long. In the presence of fluorides, the acid produced by bacteria by acting on carbohydrates gets cleared by saliva [7]. With this, there is rise in pH of saliva above 5. The biofilm fluid is supersaturated with respect to HA and FA [8]. If the biofilm still contains F, then the lost Ca and P by enamel can be recovered more efficiently. In attempt to assess the remineralizing capacity, two agents such as SAP P11-4 and CPP-ACP agents were compared. In both groups, calcium weight%, phosphate weight% and calcium/phosphate ratio was evaluated.

The casein phosphopeptides (CPPs) are developed from the tryptic digest of casein. It is aggregated with calcium phosphate and purified through ultra filtration. It has been observed that it aggravates stabilization of calcium and phosphate ions by liberating small sequences of peptides (CPPs) through partial enzymatic digestion. This whole process leads to the development of a remineralization based on the formation of casein phosphopeptide-stabilized amorphous calcium fluoride phosphate complexes (CPP-ACFP) and casein phosphopeptide-stabilized amorphous calcium phosphate complexes (CPP-ACP) [9].

Anionic P114 is a rationally-designed self-assembling peptide which undergoes well characterized hierarchial self-assembly as three-dimensional fibrillar scaffolds in reaction to specific environmental triggers [10]. It was previously confirmed that on application of P11-4 on to the tooth the peptide diffuses into the subsurface microproes leading to formation of 3 D scaffold. These are made up of small fibers, which are similar to proteins found in teeth development and supports hydroxyl apatite.

We observed that mean calcium weight % was evaluated at baseline, 2 weeks, 6 weeks and 12 weeks. In Group 1, there was increase in mean value (62.12 ± 1.24) from baseline to 12 weeks (67.36 ± 2.14). However, there was decrease in phosphate weight % from 37.16± 2.52 at baseline to 35.72 ± 2.11 at 12 weeks. We found that there was increase in Ca/P ratio in Group 1. A previou study evaluated the remineralization pattern of CPPs and fluorides on enamel surfaces and found that there was no increase in remineralization after 5 days [12]. In present study, we evaluated remineralization at several intervals such as baseline, 2 weeks, 6 weeks and 12 weeks with the help of SEM and EDX.

In Group 2, mean calcium weight % was 64.18 ± 1.52 at baseline, which ultimately increased to 66.01 ± 2.03 at 12 weeks. Phosphate weight % showed reduction from 37.34 ± 2.23 at baseline to 35.04 ± 2.02 at 12 weeks. Ca/P ratio showed significant improvement. There was 93% of remineralization of enamel samples evaluated after 3 months intervals with the help of SEM [13]. The enamel demineralization capacity of self-assembling peptide scaffolds with ultrasonic method. Authors confirmed the formation of needle shaped crystals [14].

We observed that significant difference in Ca/P ratio at 2 weeks, 6 weeks and 12 weeks when we compared both groups. It has been suggested that self-assembling peptide P11-4 diffusion into the subsurface lesion body and assembles there in into higher order fibrils, thus facilitating mineralization by of the subsurface volume by mimicking the natural biomineralization of the tooth enamel, and it remains within the lesion body as a scaffold built-in by the newly formed hydroxyapatite [15].

Previous study evaluated the remineralization capacity of CPP-ACP paste on enamel lesions [16]. Enamel surfaces of 6 years old children were prepared and divided into two groups as native enamel, 500ppm F as positive control and water as negative control. Ultrastructural and roughness was evaluated by atomic force microscopy. Authors found CPP-ACP paste repaired the microstructure of enamel. There was significantly increased hydroxyapatite crystal size and Ca/P molar ratios with CPP-ACP paste as compared to NaF. Both CPP-ACP and NaF decrease roughness, and increase the nanohardness and elastic modulus. Other researchers also found SAP P11-4 better than CPP-ACP [17].

The limitation of the study was small sample size. The selection of only two remineralizing agents was done. However, involvement of multiple agents could have resulted better results.

Conclusion

Self-assembling peptide (SAP) P11-4 found to be more effective and efficient as compared to casein phosphopeptides- amorphous calcium phosphate (CPP-ACP).

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

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