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REVIEW ARTICLE



# Self-assembling peptide $P_{11}$-4 in remineralization of enamel caries – a systematic review of *in-vitro* studies

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**ABSTRACT**

**Objective:** The present systematic review was conducted to investigate the effect of the self-assembling peptide (SAP) – $P_{11}$-4 in the remineralization of enamel caries.

**Material and methods:** The systematic search for studies was conducted through CINAHL, EMBASE, MEDLINE, Scopus, PsychINFO, and various key journals. This review was conducted in adherence to PRISMA standards and was registered in PROSPERO with registration number CRD42019110156. The methodological quality of the studies was graded through Cochrane's tool of risk of bias in non-randomized studies – of interventions (ROBINS-I).

**Results:** In total, 91 studies were identified for screening, and 12 studies were eligible. Ten studies showed effective enamel remineralization with $P_{11}$-4 compared to controls. One study showed a combination of $P_{11}$-4 with fluoride varnish or Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride (CPP-ACPF) leads to significantly higher remineralization compared to $P_{11}$-4 alone. Quality assessment of study showed 6 (50%) studies as medium risk of bias and 6 (50%) studies as low risk of bias.

**Conclusion:** To conclude, the present study results showed SAP- $P_{11}$-4 is effective in the remineralization of enamel caries.

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**KEYWORDS**
Self-assembling peptides; enamel caries; enamel remineralization; $P_{11}$-4; systematic review

## Introduction

Dental caries is a multifactorial disease initiated with cyclic demineralization and remineralization of enamel by cariogenic bacteria leading to the formation of the cavity [1]. The traditional modes of treatment for caries include drilling and replacement of damaged tissue by filling with dental biomaterials. These invasive treatments are associated with loss of healthy tooth structure, leading to repeated cycles of restoration, resulting in a major burden on individuals and public health costs [2]. To overcome the limitations associated with traditional invasive treatment, several non-invasive [3] and minimally invasive treatments [2] have been suggested for early caries, before the cavity formation. Authors [2–6] have suggested that the demineralization of enamel is reversed by the remineralization process that is based on the complex interaction between organic and inorganic components in an aqueous liquid, which transforms the protein-rich matrix substances into hard tissues. Different enamel matrix proteins are involved in the process of enamel remineralization like amelogenin, tufted in, and other serum proteins [4]. Along with the naturally available enamel proteins, several topical remineralizing agents have been used to inhibit the demineralization and enhance the remineralization of enamel and white spot lesions [6–10]. One of the recently tried remineralizing agents is a self-assembling peptide (SAP) $P_{11}$-4 [11,12]. Peptide-based materials represent an especially promising class of compounds due to their relative ease of synthesis and most importantly they have the same chemical structure of biological signals. Self-assembling peptide (SAP) $P_{11}$-4 undergo spontaneous assembling after being applied to or infiltrated into a carious lesion. They form a fibrillar network into which minerals are deposited leading to remineralization [11–14]. Many *in-vitro* and few in-vivo studies have been conducted previously to show the effect of SAP – $P_{11}$-4 in enamel remineralization [11,12,14–29] which report variable results based on their methodology. A systematic review to substantiate the results of *in-vitro* studies is lacking, thus, the present systematic review aimed to review *in-vitro* studies and evaluate the effect of SAP – $P_{11}$-4 in remineralization of enamel caries.

## Material and methods

This review was conducted in adherence to PRISMA standards of quality for reporting systematic reviews and meta-

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analyses [30]. The present review was registered in PROSPERO with registration number CRD42019110156.

### Questions

We sought to examine the quantitative effects of SAP $P_{11}$-4 in enamel remineralization. The research question was defined as follows:

The study included: *In-vitro* studies involving human or bovine teeth.
The intervention used: SAP $P_{11}$-4 in remineralization of enamel.
Comparison group: No treatment or treated with other remineralizing solutions.
Outcome analyzed: Changes in enamel mineral content (both demineralization and remineralization stage), expressed via surface micro-hardness test, surface fluorescence change.

### Study eligibility

The *in vitro* studies published in the English language only that investigated the changes in enamel mineralization following the application of SAP $P_{11}$-4 were included. Papers were excluded at this stage if they were *in vivo*, case report, editorial letter, case series, not investigating the changes in enamel mineralization following application of SAP $P_{11}$-4, SAP $P_{11}$-4 used for other purposes.

### Study identification

Various research databases were searched which include, Cochrane library (Cochrane review, Trials), Medline (PubMed, OVID Medline, and Ebsco), Embase (European studies, pharmacological literature, conference abstract), Web of Knowledge (Social science, conference abstract), SCOPUS (Conference abstracts, scientific web pages). For grey literature following databases were searched: Google scholar, Open Grey, National Library of Medicine, Social science research, For thesis (EthOS, DART-Europe), Institutional repositories (OpenDOAR, Bielefeld Base, Lenus, RIAN, e-publications@RCSI). No beginning date was used, and the last date of the search was March 4th, 2020.

### Search key-words

Key terms focussed on the specific search strategy which includes: peptides, self-assembling peptides, enamel, remineralization, demineralization, enamel caries, $P_{11}$-4, Curodont Repair (CDR), enamel lesions, surface treatment. The reference list of all included articles was searched for additional references.

### Study selection

All the titles and abstracts were screened independently and in duplicate for inclusion in the study. The inter-rater agreement for study inclusion, as assessed using an intra-class correlation coefficient, was 0.88. Conflicts were resolved by consensus discussion between the two reviewers.

*Risk of bias assessment:* Risk of bias was assessed using Cochrane's tool of risk of bias in non-randomized studies – of interventions (ROBINS-I) [31]. The following domains were assessed for risk of bias: bias due to confounding, the bias in selection, bias in classification of interventions, bias due to deviations from intended interventions, bias due to missing data, the bias in the measurement of the outcomes, bias in the selection of the reported result. Each domain is graded as low, moderate, serious risk of bias, critical risk of bias, or no information.

### Data extraction and data synthesis

The data were extracted independently by the two reviewers using a data extraction sheet, and any differences were resolved by discussion and consensus. The following data were extracted from each included study: first author, publication year, study type, study quality, sample size, inclusion criteria, treatment type, demineralization and remineralization cycles, method of analysis, changes (before, after and long term treatment), statistical analysis used, and the authors' conclusion.

## Results

Using our search strategy, we identified 87 articles with an additional 4 identified from our review of references and journal indices. Twelve *in-vitro* studies were included in the present systematic review (Figure 1). Quality assessment of the study showed 6 (50%) studies as medium risk of bias and 6 (50%) studies as low risk of bias (Table 1).

Studies used 691 enamel blocks. Eight of the included studies used extracted human teeth and four studies used bovine teeth. Ten studies showed effective enamel remineralization with SAP $P_{11}$-4 compared to controls. One study showed a combination of SAP with fluoride varnish or Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride (CPP-ACPF) leads to significantly higher remineralization compared to SAP- $P_{11}$-4 alone (Table 2).

Different demineralising agents were used. Seven *in-vitro* studies used acetic acid buffer, two studies used Buske's demineralization solution. Study outcomes were assessed using different techniques. Eight studies used artificial saliva to store the specimens post-treatment. Five studies assessed the qualitative changes in enamel remineralization using a scanning electron microscope (SEM) (Table 3).

The outcome of studies showed significant mineral gain after the application of SAP $P_{11}$-4 (Table 4).

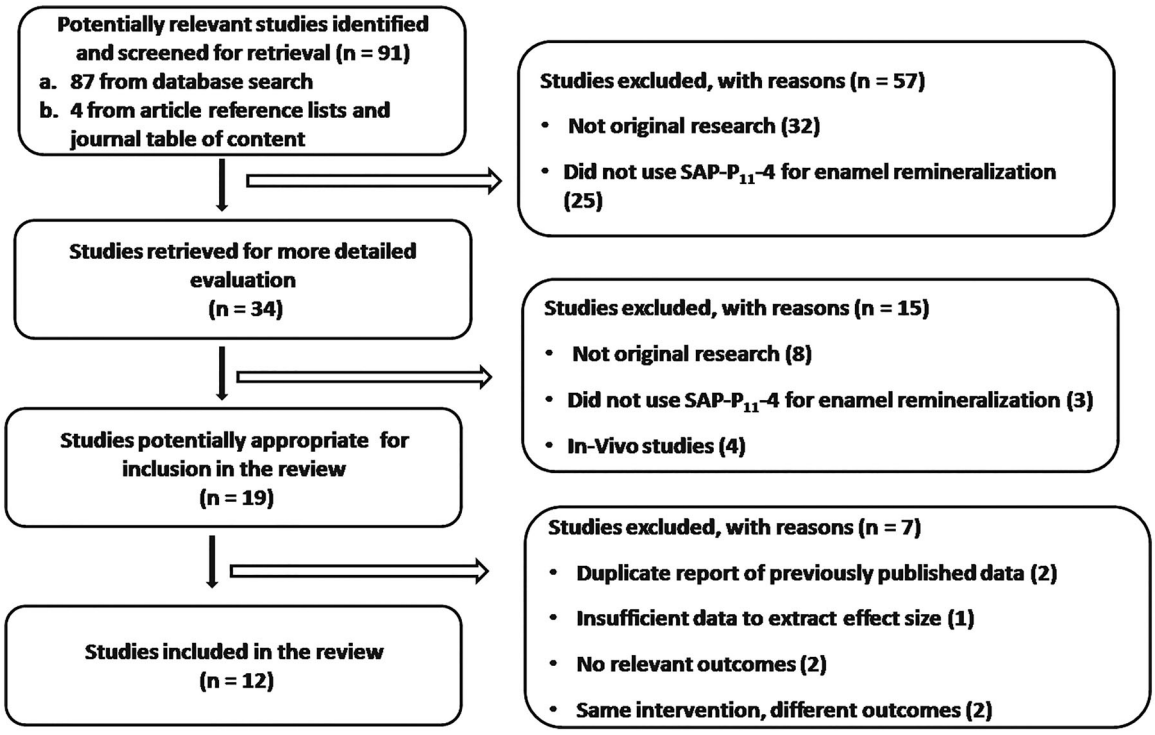


**Figure 1.** Study selection flow diagram for systematic review.

## Discussion

Self-assembling peptides received great attention from researchers in recent times because of their regenerative property. They are used in a variety of treatment purposes like bone and cartilage repair, repair of the central nervous, and cardiovascular system [32–34]. The present systematic review is conducted to evaluate the effectiveness of SAP – $P_{11}$-4 in enamel remineralization. Twelve *in-vitro* studies [14–22,25–27] were included in the present review. Quality assessment of study showed 6 (50%) studies [14,15,17,20,21,27] as medium risk of bias and 6 (50%) studies [16,18,19,22,25,26] as low risk of bias.

### *Role of self-assembling peptides in the regeneration of enamel*

Nine *in-vitro* [14–17,19,20,22,26,27] showed the application of $P_{11}$-4 is associated with significant enamel regeneration on the demineralized surface. The monomers of $P_{11}$-4 self-assembling peptide simulate the enamel matrix by congregating in a three-dimensional network. The hydroxyapatite crystals form on this enamel matrix with the aid of calcium phosphate from the saliva, thereby enhancing the regeneration of enamel. The study by Kamal et al. [25] showed a combination of $P_{11}$-4 with fluoride varnish or CPP-ACPF is associated with significantly higher remineralization compared to $P_{11}$-4 alone. This may be because the presence of high amounts of calcium, phosphate, and fluoride ions enhanced the regenerative potential of $P_{11}$-4 thereby increasing hydroxyapatite crystallization utilizing the high level of ions available [25].

### *Effect of pH on the activity of $P_{11}$-4*

The *in-vitro* studies by Golland et al. [21] and Wierichs et al. [18] showed the application of $P_{11}$-4 not associated with enamel regeneration. This may be attributed to the fact that at pH $<$ 8, SAP will be in a flocculated state which is unreactive compared to nematic state SAP at pH $>$ 8. Self-assembling peptides mask the initial caries lesions by reversing the demineralizing process under ideal remineralizing conditions. At pH $>$ 8 and low ionic strength, SAP will be in a nematic state, which is a Newtonian fluid of monomeric, random coil peptides and enhances the regeneration of enamel. Nematic peptides form the fibrillar gels within the demineralized lesions and induce remineralization by attracting calcium phosphate from the surrounding fluids. If the remineralizing condition is not ideal, SAP changed to a flocculated state from the nematic state and the flocculated peptide is relatively un-reactive [35]. Thus, regeneration of enamel is no longer promoted and flocculated peptides hamper the diffusion of calcium and phosphate ions into the enamel.

### *Effect of remineralizing solution concentration and length of exposure of the sample to a remineralizing solution*

For effective remineralization, the samples should be kept in the remineralization solution for a longer time, solution concentration and pH should remain unchanged, and the demineralization solution should have a higher pH [36]. In various studies [14–17,19,20,22,25,26] evaluating remineralization efficacy and time-dependent changes, following a one-time application of agents, the samples were stored in

remineralization solution until the time of assessment. The result showed, $P_{11}$-4 application resulted in a gradual increase in the mineralisation at 5–7 days [17,19,25,26], 14th day [14], 1-month [20,22] and 3-month intervals [16,27]. Sindhura et al. [27] showed, initially (at 1-week interval), $P_{11}$-4 showed a decrease in Ca: P ratio compared to the baseline value. This could be attributed to additional acid etching before the application of $P_{11}$-4 as recommended by the manufacturer which could have caused further loss of minerals from the tooth surface. A single application of $P_{11}$-4 to the lesion surface resulted in a significant net mineral gain, suggesting a sustained or incremental effect on tissue repair.

### Depth of remineralization after SAP application

The *in-vitro* study by Schmidlin et al. [15] showed enamel rehardening up to the depth of 200 μm after the application of $P_{11}$-4, indicating crystallisation of mineral, which are taken up by remineralizing surface.

### Method of assessment of remineralization

In the present review, two studies [16,26] used the DIAGNOdent method to evaluate remineralization along with VistaProof (VP) fluorescence and micro-computed tomography (μCT] analysis respectively. Various investigations have reported that although DIAGNOdent has low specificity, the ability to detect enamel lesions is successful [37,38]. Additionally, in some studies, this device had good reproducibility and was more reliable than bitewing radiographs [39,40]. Surface microhardness analysis (SMH) is a simple, fast, and reliable method to assess demineralisation and remineralization changes occurring in enamel [15,22,25]. SMH testing measures the material's resistance against plastic deformation from a standard source, allowing repeated measurements of the same specimen over some time, reducing the experimental variation, and reinforcing that SMH evaluations are a feasible choice to estimate mineral change.

### Quality of remineralization with SAP

Scanning electron microscope (SEM) is done for qualitative assessment of the remineralizing regimens [16,19,22,25,27], which helps in illustrating the surface ultra-morphological changes induced by different remineralizing agents. $P_{11}$-4 alone or in combination with other remineralizing agents demonstrated improved mineral content with surface morphology resembling natural enamel on quantitative and qualitative evaluation. Kind et al. [14] and Kirkham et al. [17] used Fourier transform infra-red spectroscopy (FTIR) and Transmission electron microscopy (TEM) measurements respectively. These methods assessed the mechanism of action of $P_{11}$-4 but it was not suitable to characterize the structural arrangement of the mineral within the carious lesion.

**Table 1.** Risk of bias assessment of included studies using Cochrane's risk of bias in non-randomized studies – of interventions (ROBINS-I).

| | Author | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ROBINS –I criteria | Kirkham/2007 | Jablonski-Momeni/2014 | Takahashi/2016 | Schmidlin/2016 | Silvertown/2017 | Golland/2017 | Soares/2017 | Wierichs/2017 | Kind/2017 | Sindhura/2018 | Ustun/2019 | Kamal/2020 |
| BC | L | L | L | L | L | L | L | L | L | L | L | L |
| BSP | L | L | L | M | L | L | L | L | M | L | L | M |
| BCI | S | M | L | L | S | L | M | L | L | M | L | L |
| BDI | M | L | M | M | M | M | L | M | M | M | L | L |
| BMD | M | L | L | L | M | M | L | L | L | M | L | L |
| BMO | M | L | L | M | M | L | L | L | M | M | L | L |
| BSR | L | L | L | L | L | L | L | L | L | L | M | L |
| Overall score | M | L | L | M | M | M | L | L | M | M | L | L |

BC: Bias due to confounding; BSP: Bias in selection; BCI: Bias in classification of interventions; BDI: Bias due to deviations from intended interventions; BMD: Bias due to missing data; BMO: Bias in measurement of the outcomes; BSR: Bias in selection of the reported result; L: Low risk of bias; M: Moderate risk of bias; S: Serious risk of bias; C: Critical risk of bias; NI: No information.

**Table 2.** Descriptive details of *in vitro* studies included in systematic review.

| Author/year | Study groups/ inclusion criteria of in-vivo studies | Total sample | Sample type | Tooth type | Statistical analysis | Authors conclusion |
|---|---|---|---|---|---|---|
| Kirkham / 2007 | G1-$P_{11}$-4<br>G2-control | 8 window of enamel 0.75cm$^2$ | Human teeth | Premolar | NA | Application of $P_{11}$-4 remineralizing agent is associated with significant enamel regeneration |
| Jablonski-Momeni / 2014 | G1-$P_{11}$-4, stored in remineralizing agent<br>G2-control 1<br>G3-control 2 | 40 samples | Human teeth, smooth surfaces | molars and premolars | Chi-square test and ROC | Application of $P_{11}$-4 remineralizing agent is associated with significant enamel regeneration |
| Takahashi / 2016 | G1-$P_{11}$-4<br>G2-control without $P_{11}$-4 | 24 bovine teeth, enamel blocks (4x4x 1 mm) | Bovine teeth | Incisors | ANOVA | Application of $P_{11}$-4 is associated with significant enamel remineralization. |
| Schmidlin / 2016 | G1-$P_{11}$-4<br>G2-EMD<br>G3-F<br>G4-negative control | 24 bovine teeth, 48 enamel blocks, $5 \times 8$ mm | Bovine teeth | Mandibular incisors | Two way ANOVA | Application of $P_{11}$-4 and EMD is associated with significant enamel remineralization |
| Silvertown / 2017 | G1-$P_{11}$-4<br>G2-placebo control | 62 smooth surfaces ($\leq 6 \times 6$mm) | Human teeth | Molars and premolars | *t*-test | Application of $P_{11}$-4 is associated with significant enamel remineralization |
| Golland / 2017 | G1-$P_{11}$-4<br>G2-F<br>G3-control | 33 Bovine enamel discs, (diameter 15 mm, height 9 mm) | Bovine teeth | Mandibular incisors | ANOVA | No significant remineralization after application of $P_{11}$-4 |
| Soares / 2017 | G1-$P_{11}$-4<br>G2-CPP ACPF<br>G3-BAG<br>G4-FHA | 60 human teeth, $5 \times 5$ mmwindow of exposed enamel | Human teeth | Premolars | One-Way ANOVA | Application of $P_{11}$-4 is associated with significant enamel remineralization |
| Wierichs / 2017 | G1-$P_{11}$-4<br>G2-LVR<br>G3-F a<br>G4-F b<br>G5-no intervention | 20 bovine teeth, 300 enamel blocks ($5 \times 3.5 \times 3$ mm) | Bovine teeth | Incisors | One Way-ANOVA | Application of a low-viscosity resin could mask caries lesions significantly, whereas $P_{11}$-4 could neither inhibit lesion progression nor mask the lesions considerably |
| Kind/ 2017 | G1-$P_{11}$-4<br>G2- Placebo control | Enamel blocks of 4x4x6mm | Human teeth | Premolars | NA | Application of $P_{11}$-4 is associated with significant enamel remineralization |
| Sindhura/2018 | G1-$P_{11}$-4<br>G2 –CPP-ACP | 12 teeth, 24 Enamel surface of 4x4mm | Human teeth | Premolars | One-Way ANOVA and *t*-test | Application of $P_{11}$-4 is associated with significant enamel remineralization |
| Ustun/2019 | G1-$P_{11}$-4<br>G2-CPP ACPF<br>G3-NaF<br>G4-control | 16 teeth, 32 surfaces | Human teeth | Mandibular third molars | One-Way ANOVA | $P_{11}$-4 showed superior remineralization with higher Ca: P ratio and uniform mineral deposition on incipient lesions compared to other groups as per DIAGNOdent and μCT analysis |
| Kamal/2020 | G1- control<br>G2- FV<br>G3 –CPP-ACPF<br>G4 –SAP<br>G5 –SAP + FV<br>G6 –SAP + CPP-ACPF | 60 teeth, 60 buccal surfaces 12x2mm | Human teeth | Permanent molars | One-Way ANOVA | Combination of SAP with FV or SAP with CPP-ACPF significantly higher remineralization compared to SAP alone |

G: Groups; 8DSS: aspartate-serine- serine; LVR: Low viscosity resin, Fa-fluoride solution of 10,000 ppm F; Fb: fluoride solution of 43,350 ppm F; F: fluoride solution; EMD: Enamel matrix derivative; $P_{11}$-4: Self assembling peptide (SAP- $P_{11}$-4); FV: Fluoride varnish; CPP -ACPF: Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride; BAG: Bioactive glass; NaF: Sodium Fluoride; FHA: fluoride enhanced Hydroxyapatite gel; control 1: after application of $P_{11}$-4, samples stored in water; control 2: samples stored in the remineralizing agent only; ROC: Receiver operating characteristic; ANOVA: Analysis of variance; RE: Remineralization of enamel.

**Table 3.** Demineralisation and remineralization protocol, storage type, outcome analysis used.

| Author/year | Demineralisation protocol | Application of P11-4 and Remineralization protocol | Storage – pretreatment | Tooth cutting | Depth of lesion | Storage –post treatment | Outcome analysis | Follow-up |
|---|---|---|---|---|---|---|---|---|
| Kirkham / 2007 | Acidified (acetic acid) gelatine gel, pH 4.8, for 6 weeks, 8 separate pH-cycling | 10 μL of either(a) a solution of 5 mg/mL $P_{11}$-4, pH-8, for 30 min | NA | NA | 100 μm | NA | Electron diffraction and elemental analysis of electron dense deposits, TEM | 5 days |
| Jablonski-Momeni / 2014 | 21 days using Acetate buffer, pH 4.9 (MHDP) | $P_{11}$-4 applied to the sample surface and allowed to work for about 5 min, pH-7 | Chloramine solution | MD | NA | Artificial saliva | DIAGNOdent (DD) and VistaProof (VP) fluorescence and SEM | 3 months |
| Takahashi / 2016 | Lactic acid buffer solution ($pH = 4.75$) for 10 min twice a day and stored in artificial saliva | $P_{11}$-4 was applied on the etched enamel surface, followed by treatment with under-saturated 0.1-M lactic acid buffer solution for 10 min. | Physiological saline | BL | NA | Artificial saliva | Ultrasonic velocity was measured using a pulser/ receiver, field-emission SEM | 28 days |
| Schmidlin / 2016 | 20 mL agitated Buskes demineralization acidic buffer solution at 37 °C for 18 days | 50 ul solution for 5 min | Water | Corono-incisal direction | 25 μm to 300 μm | Artificial saliva | Subsurface microhardness (KHN) | 18 days |
| Silvertown / 2017 | 20 sec exposure to NaClO, 20 sec rinsing with water, 20 sec etching with 37% phosphoric acid | 20 sec rinsing with water, 5 min contact with $P_{11}$-4 | Deionized distilled water | NA | 272 ± 136 μm | Artificial saliva | Canary System assessment and Histological validation | 50 days |
| Golland / 2017 | Five days in 30 mL of Buske'sdemineralisation solution | G1- 25 μL of a 10 mg/mL monomeric $P_{11}$-4 solution for 5 min<br>G2- 10,000 ppm amine fluoride for 5 min<br>G3- no treatment | Artificial saliva | NA | | Artificial saliva | QLF | 5 days |
| Soares / 2017 | Acetic acid with pH 4.4 l for 96 h, | Application of $P_{11}$-4 for 2 min, 20 mL remineralising solution for 17 h with a pH of 7.0. | 10% formalin solution | NA | NA | Remineralizing solution | Surface Microhardness using Vicker'sMicrohardness Testing machine and SEM | 1 month |
| Wierichs / 2017 | Acetic acid with a pH of 4.8, 28 days, 6 × 60 min demineralization/ day | Remineralizing (120 min)<br>G1- $P_{11}$-4 contact of solution for about 5 min.<br>G2 – LVR infiltration for 3 min followed by light-cured for 40 s.<br>G3 and G4 – F solution was applied, allowed to set for about 5 min. | 0.08% thymol | NA | 130μm | NA | Transversal Microradiographic and Colorimetric Analysis | 28 days |
| Kind/ 2017 | Acetic acid at pH 4.4 for 72 hr | 10 μL of $P_{11}$-4, Remineralization at a pH of 7.4 | NA | MD | 70 ± 30 μm | NA | MALDI-TOF, FTIR | 14 days |
| Sindhura/2018 | Lactic acid at pH 4.5 for 96 hr | One-time application of P11-4 and CPP-ACP | 10% formalin | MD | NA | Artificial saliva | EDX and SEM | 3 months |
| Ustun/2019 | Distilled water, 2 mM Ca (Ca[NO3]2), 2 mM PO4 (KH2PO4), and 75 mM of acetate at 4.3 pH for 72 hr. | One-time application of P11-4, CPP-ACP and and Duraphat varnish | 0.1% thymol | BL | NA | Artificial saliva | DIAGNOdent and μCT analysis | 30 days |
| Kamal/2020 | Acetic acid at pH 4.4 for 96 hr | One-time application of solutions | 0.1% thymol | Buccal surface | NA | Artificial saliva | SEM and VMT | 1 m |

ICDAS: International caries detection and assessment system; LF: laser florescence; m: month; SEM: scanning electron microscope; EDX: Energy Dispersive X-ray; QLF: quantitative light-induced fluorescence; TEM: Transmission electron microscopy; MD: mesio-distal; BL: bucco-lingually; MHDP: methyl hydroxyl di-phosphonate; NA: Not available or not applicable; MALDI-TOF: Matrix-assisted laser desorption/ionization with time-of-flight analysis; FTIR: Fourier transform infra-red spectroscopy; KHN: Knoop hardness-measuring device; VMT: Vickers Microhardness Tester; CPP-ACPF: Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride.

**Table 4.** Outcome of *in-vitro* studies included in the systematic review.

| Author/Year | Changes at demineralization | | Changes at remineralization | |
|---|---|---|---|---|
| | Test[a] | Control | Test[a] | Control |
| Kirkham/2007 | NA | NA | Mineral gain- 9 μg/mm$^2$ | Mineral loss – 2μg/mm$^2$ |
| Jablonski-Momeni/2014 | DD = 9<br>VP = 10 | DD = 9<br>VP = 6 | DD = 2*<br>VP = 0 | DD = 8<br>VP = 3 |
| Takahashi/2016 | UV<br>4,987 ± 67 m/s, | UV<br>4,927 ± 56 m/s | UV<br>5,111 ± 95 m/s* | UV<br>4,832 ± 80 m/s, |
| Schmidlin/2016 | KHN- at 25 μm depth<br>43 ± 18 | KHN- at 25 μm depth<br>55 ± 28 | KHN- at 25 μm depth<br>52 ± 26* | KHN- at 25 μm depth<br>73 ± 41 |
| Silvertown/2017 | CN<br>44 ± 3.8 | CN<br>40 ± 2.3 | CN<br>24 ± 4.9* | CN<br>39 ± 2.6 |
| Golland/2017 | QLF<br>−49.7 ± 12.8 | QLF<br>44.4 ± 18.6 | QLF<br>0.4 ± 3.1 | QLF<br>−1.1 ± 2.1 |
| Soares/2017 | SMH<br>283.7 ± 18.4 | SMH<br>229.2 ± 23.6 | SMH recovery<br>62.06%* | SMH recovery<br>15.3 % |
| Wierichs/2017 | CIEL<br>13.1 | CIEL<br>13.9 | CIEL<br>11.7 | CIEL<br>7.9* |
| Kind/2017 | MALDI-TOF- 1,100 m/z | NA | MALDI-TOF − 1,596 m/z,*<br>Positive staining with Congo Red | Negative staining with Congo Red |
| Ustun/2018 | DD = 11.0 ± 2.0 | DD = 11.6 ± 3.9 | DD = 4.1 ± 0.4* | DD = 10.3 ± 3.0 |
| Sindhura/2019 | Ca-Ph<br>1.73 ± 0.16 | Ca-Ph 1.78 ± 0.14 | Ca-Ph* 1.95 ± 0.10 | Ca-Ph<br>1.87 ± 0.11 |
| Kamal/2020 | SAP<br>SMH<br>228.6 ± 8.8<br>SAP + FV*<br>SMH<br>239.2 ± 10.7<br>SAP + CPP-ACPF*<br>SMH<br>240.5 ± 11.6 | SMH<br>224.8 ± 4.9 | SAP<br>SMH<br>334.1 ± 8.9<br>SAP + FV*<br>SMH<br>350.5 ± 10.9<br>SAP + CPP-ACPF*<br>SMH<br>352.4 ± 13.9 | SMH<br>237.9 ± 2.6 |

DD: DIAGNOdent values; VP: VistaProof; MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization with Time-of-Flight Analysis; Test: $P_{11}$-4 group; Control: Other treatment techniques; TMA: Transversal Microradiographic Analysis; CIEL: Colorimetric Analysis (Mean value); SMH: Surface micro hardness; UV: Ultrasonic velocities; CN: Canary number; QLF: quantitative light-induced fluorescence; Ca-Ph: Calcium phosphate ratio; SAP: Self assembling peptide; FV: Fluoride varnish; CPP-ACPF: Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride. *$p$ value $< .05$.

### *Study limitations*

Due to heterogeneity across the studies that resulted from variations in pH cycling, lesion localization, depth of the lesion, method of application of SAP, method of outcome assessment, and controls used, performing meta-analysis with funnel plots and forest plots construction was not possible. In the future, various lesions of different depth should be analyzed to get more information about the tested agent. Randomized controlled trials need to be conducted to accurately substantiate the evidence in this field of research.

## Conclusion

This is the first systematic review done to assess the effectiveness of SAP – $P_{11}$-4 in enamel remineralization. The results of the present systematic review showed $P_{11}$-4 is effective in the remineralization of early enamel caries. Combination of $P_{11}$-4 with other mineralizing agents has synergizing effect.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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