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A Comparison of the Enamel Remineralisation Potential of Self-Assembling Peptides

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ABSTRACT

Background: The aim of this research was to compare the efficacy of the remineralising potential of self-assembling peptides (SAPs): Curodont Repair (P11-4), P26, and leucine-rich amelogenin peptides (LRAP) with the standard 5% NaF varnish (Duraphat) on early enamel caries lesions (EECLs).

Methods: A demineralising solution (DS) was used to create artificial EECLs in human dental enamel specimens, which were randomly allocated to treatment groups: P11-4; P26 solution; LRAP solution; 5% NaF varnish; and deionised water (DIW). Each specimen was subjected to 8 days of pH cycling. Specimens from each test group were subjected to microcomputed tomography (micro-CT) and nanomechanical testing to assess mineral density (MD), hardness (H), and elastic modulus (EM) properties of sound, demineralised, and treated enamel.

Results: The mean MD percentage gain was highest in the P26 and P11-4 groups, followed by the LRAP, 5% NaF varnish, and DIW groups. There were statistically significant differences amongst groups. In the outer layer of EECLs, the EM and H were highest in P26 and P11-4 groups, followed by the LRAP and 5% NaF varnish. In the inner layer of EECLs, the EM and H were highest in P11-4 and P26 groups, indicative of enhanced penetration and remineralisation of the deeper parts of the artificial EECLs.

Conclusions: P26 and P11-4 SAPs are more effective than 5% NaF varnish in remineralising the depth of EECLs.

Introduction

Dental caries remains a major cause of tooth loss in the primary and permanent dentitions, despite being preventable.[1,2] It involves a cyclic de- and remineralisation process, resulting in an opaque chalky white appearance of the enamel where partial dissolution and loss of mineral content has occurred to form early enamel caries lesions (EECLs).[2]

Remineralisation of noncavitated EECLs aims to increase fluoride, calcium, and phosphate ion levels in the oral cavity. Various remineralising modalities include but are not limited to fluoride vehicles, amorphous calcium phosphate (ACP), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), calcium sodium phosphosilicate, 5000 ppm fluoride, and xylitol.[3,4] More recent modalities for managing EECLs include resin infiltration and biomimetic remineralisation.[5]

Biomimetic remineralisation aims to provide matrix-mediated remineralisation for the nucleation of calcium and phosphate ions gained from the saliva to regenerate hydroxyapatite (HAP) crystals in enamel. Biomimetic strategies include self-assembling peptides (SAPs) P11-4 (Curodont Repair, Credentis), amelogenin, amelogenin fragments (eg, leucine-rich amelogenin peptide [LRAP]), amelogenin in combination with chitosan, and amelogenin-inspired peptides P26 and P32.[6,7]

Numerous in vitro and a few in vivo studies have been conducted to assess the efficacy of SAPs in remineralising EECLs.[8] P11-4 is a short-chain peptide commercially synthesised for topical application on the EECLs which forms a 3D

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fibrillar structure resembling the extracellular matrix and promoting remineralisation and HAP formation. Le Norcy et al have shown that LRAPs are similar to the full-length and cleaved forms of amelogenin.[9] Mukherjee et al[7] explored the role of a smaller amelogenin peptide, providing evidence in support of enhanced remineralisation of enamel which contains highly oriented crystals with increased mechanical strength using a peptide-mediated approach. The enamel remineralisation potential of the SAPs is still unclear due to heterogeneity of study designs.

Micro-CT assessment in in vitro dental caries research is a conservative and widely used approach that provides 3D visualisations and allows mineral density (MD) assessment. It provides qualitative and quantitative data that are assessed using grayscale values representing the x-ray beam attenuation as it transmits through enamel.[10,11] Remineralisation of EECLs can also be assessed by mechanical testing such as nanoindentation. Whilst many studies have investigated the surface hardness, only a few have assessed mechanical properties in terms of hardness (H) and elastic modulus (EM) using nanoindentation techniques across the body of the EECLs.[12,13] The use of nanoindentation facilitates measurements at a nanoscale and the better distribution of minute changes with the use of a small and sharp indenter tip when compared with macro and micro indentation systems.[14]

No previous studies have concurrently investigated the remineralisation efficacy of short-, medium-, and long-chain SAPs (P11-4, P26, LRAP) on EECLs compared with fluoride-based remineralising agents, which is of interest as it is a known effective strategy for arresting or reversing EECLs on facial/lingual surfaces of primary and permanent teeth.[15] The comparison of varying lengths of peptides advocates for the synthesis of a biocompatible, biodegradable, affordable cost of synthesis and easy handling of SAPs. At present, the long lengths, acidic nature, and cost of synthesis limit the practical design of SAP for enamel biomimetics. This research compared mineral density and nanomechanical properties for enamel specimens treated with these SAPs. The null hypotheses of this study are that (1) there is no difference in mineral density of the treated enamel amongst the test groups; (2) there is no difference in mechanical properties of the treated enamel amongst the test groups; and (3) there are no differences in remineralisation potential amongst different SAPs.

Materials and methods

Sample size calculation and statistical analyses

Statistical power analyses (G*Power, version 3.1.9.4)[16] were performed using analysis of variance (ANOVA). Repeated measures, within-between interaction, effect size of 0.25, statistical power of 0.8, and significance level of $\alpha = 0.05$ determined a minimum total sample size of 45 (n = 9, N = 45). As 2 specimens can be obtained per premolar tooth, a minimum 22.5 teeth (45/2) were required. To account for potential pre-test failures, this value was rounded up to 25 teeth and 10 teeth per experimental group. The research data were analysed using IBM SPSS Statistics Software (SPSS) version 27 (IBM Corporation). The normality of the data was verified using the Shapiro–Wilk test. The statistical significance amongst test groups was tested using 2-way ANOVA (micro-CT: remineralising agents and enamel experimental zones, nanoindentation: remineralising agents and enamel depth) and with post hoc Bonferroni test with confidence interval set at 95%.

Tooth preparation

Ethical approval was obtained for the collection of extracted sound premolar teeth from the Human Ethics Committee (Health) at the University of Otago, New Zealand (Ref. H19/106). Extracted sound premolar teeth were stored in thymol (0.1% w/v) solution at 4 °C. A diamond impregnated wheel (M0D13, Struers) in an automated precision cutting machine (Accutom 50, Struers) was used to vertically section the crown into buccal and palatal halves, followed by decoronation of teeth under water irrigation. Newly erupted teeth have an outer prismless enamel layer, which was removed with 1200-grit silicon carbide paper on an automated polisher under water irrigation (TegraPol-21, Struers) at 300 rpm. The grinding/polishing resulted in 3 distinct flat zones which were designated as sound enamel (SE) control, demineralised enamel (DE), and the demineralised treatment enamel (TE) zones. A 4 mm^2 window was marked and the boundaries outside this window were fully coated with a double layer of acid-resistant fast-drying nail varnish (Revlon). The nail varnish was applied to protect the SE and prevent the loss of minerals during artificial EECL formation and pH cycle.

Artificial EECL formation regime

The artificial EECL formation protocol included the immersion of each enamel specimen in 20 mL of lactic acid solution, with specimens being placed on an orbital shaker (Labnet and Biomaterials Lab, University of Otago) at 80 rpm for 96 hours.

The demineralising lactic acid buffer solution was prepared with analytical grade chemicals which included 0.1 M lactic acid and 0.16 mM of $Ca(H_2PO4)_2$ diluted to 1 litre deionised water and adjusted with 1M KOH to pH 4.4.[17] The demineralised control zone was covered with double layer of acid resistant fast-drying clear nail varnish (Revlon) in all enamel specimens.

Test groups

Fifty enamel specimens were randomly allocated into each of the following treatment groups (n = 10): Group 1: Curodont Repair (Credentis) containing SAP P11-4 (second generation; comprises the short-chain peptide sequence: Ace-QQRFEWEFEQQ-NH2); Group 2: P-26 (GL BioChem Ltd; comprises the medium-chain peptide sequence: MPLPSYEVLTPLKWPSTDKTKREEVD [phosphorylated serine]); Group 3: porcine leucine-rich amelogenin peptide (GL BioChem Ltd), 98% purity (comprises the long-chain peptide sequence: MPLPPHPGHPGYINFSYEVLTPLKWYQNMIRHPSLLPDLPLEAWP ATDKTKREEVD [serine, not phosphorylated]); Group 4: Duraphat (Colgate Palmolive) containing 5% NaF varnish; Group 5: deionised water (DIW) as the negative control.

Preparation of peptides

The second-generation P11-4 peptide was available as a commercial dental remineralising product (Curodont Repair). P11-4 is immobilised on a sponge and the water in the bottom part of the applicator. The P26 and porcine leucine-rich amelogenin peptide solutions were prepared at a concentration of 200 μg/mL and 120 μg/mL−1, respectively.[7,18] Peptide solutions were centrifuged (Sigma 2-5, DJB Labcare Ltd) at 11,000 g, 4 °C, for 20 minutes prior to the treatment of enamel specimens.

pH cycle

The 8-day pH cycling model used in this study was similar to the protocol outlined by Said et al.[19] The de- and remineralising solutions were prepared with reference to studies that considered the saturation of hydroxyapatite minerals present in human saliva under normal conditions and were renewed daily. The demineralising solution contained 2.2 mM of CaCl2, 2.2mM of KH2PO4, and 0.05 M of acetic acid and adjusted to a pH of 4.4 with 1 M KOH. The remineralising solution contained 1.5 mM of CaCl2, 0.9 mM of NaH2PO4, and 0.15 M of KCl, and the pH was adjusted to 7.0 with 5 M KOH.[20,21] A calibrated pH electrode and magnetic stirrer were used to prepare the de- and remineralising solutions.

Following treatment, each specimen was immersed in 10 mL of remineralising solution and placed on an orbital shaker (80 rpm) and maintained at ambient temperature. After 22 hours in remineralising solution, each specimen was thoroughly washed with deionised water, dried on fibreless napkins, and immersed in 20 mL demineralising solution and placed on an orbital shaker (80 rpm) at ambient temperature for 2 hours.

Micro-CT mineral density assessment

A micro-CT scanner (SkyScan 1172 X Ray Microtomography) was used to assess mineral density of enamel specimens.[17,22] A 3D printed platform was used to mount 3 reference phantoms and 4 enamel specimens perpendicular to the x-ray beam. Scans were performed at 80 kV, 100μA, Al+Cu filter, voxel size of 17.3μm camera resolution, 360° rotation angle, and 0.3° step size.[17]

The MD calibration used reference phantoms consisting of HAP discs with known concentration (0.145, 0.596, and 1.249 units) and known MD (1.165, 1.469, and 1.747g/cm^3).[22] The raw images were reconstructed with NRecon version 1.7.0.4 (Skyscan). The 3D image reconstruction settings were configured as smoothing 1, ring artefact correction 20, and beam hardening correction 30%.

Processed images were selected for MD assessment using CTAn version 1.16.1.0 (SkyScan) at 3 different zones per slice. The SE depicted baseline MD, the demineralised enamel depicted pretreatment MD of the artificial EECLs, and the treated demineralised enamel depicted posttreatment (after pH cycling) MD of the artificial EECLs.

MD was assessed with 3 round regions of interest (ROIs) with a diameter of 8 pixels used at 3 sites within each zone. This was repeated every 20 slices, obtaining 22 assessed slices per specimen. A total of 66 ROIs per zone were evaluated per specimen. The mineral gain and percentage of remineralisation were calculated formulas follows:

$$\text{Mineral Gain} = DZ_r - DZ_d$$
$$\text{Percent Remineralisation} := ((DZ_r - DZ_d)/DZ_d) * 100$$
$$\text{Where}: DZ_d \text{ is the MD difference between SE and DE and } DZ_r \text{ is the MD difference between TE and SE}$$

Mechanical characterisation with nanoindentation

Three specimens per test group (N = 15) were selected for mechanical characterisation of H and EM of enamel. A silicone mould was used to embed specimens in cold mounting EpoFix resin (Struers). A diamond impregnated wheel (M0D13 by Struers) in an automated precision cutting machine (Accutom 50 by Struers, Denmark) under water irrigation was used to horizontally section the specimens, exposing a cross-section of the artificial caries lesion.

The sectioned enamel specimens were polished with 1200-grit silicon carbide paper (Struers) on an automated polisher (TegraPol-21, Struers) under water irrigation. A polishing felt (ASFL Magnetic Cloth) and 1-μm diamond polishing paste (DP-Paste M, Struers) were used for the final polish. Specimens were cleaned in an ultrasonic cleaner (Unisonics) with ethanol to dislodge any dirt and sludge from polishing.[23]

Nanoindentation was conducted using the Hysitron TI 950 TriboIndenter (Bruker) with a 3-sided pyramid diamond Berkovich indenter tip to assess the H and EM of the lesion. Considering the dimension of the specimens, a consistent load of 10 mN was used to make 44 indents on the enamel along 2 rows per test zone. The first row of indents was approximately 30 μm from the surface of the lesion (outer layer) and the second row of indents was done with a spacing of approximately 30 μm, giving the inner indents at approximately 60 μm from the surface of the inner layer. The Oliver and Pharr analysis method was used for the calculation of H and EM from the unloading section of the load-displacement curves.[24–26] The EM was calculated from the reduced modulus and known dental enamel Poisson's ratio of v = 0.3.[27]

Results

Mineral density assessment

Micro-CT scans were used to assess MD in the 3 zones of enamel: SE, DE, and TE. The SE region provided a baseline MD, the DE region provided the MD after artificial EECL formation, and the TE region provided the MD following treatment with respective topical agents and 8-day pH cycle.

Table 1 provides a summary and comparison of the MD and mineral gain in the SE, DE, and the TE zones in each treatment group. The mean MD in the TE zone ranged from 1.94 to 2.30g/cm^3. The mineral gain in the TE zone was higher amongst the P26 group (38.6%) and P11-4 SAP group (28.5%), followed by the LRAP SAP and 5% NaF varnish groups. The mineral gain was lowest in the DIW group (8.3%). A 2-way ANOVA was conducted to assess the micro-CT data, considering 2 factors: the different groups of remineralising agents

Table 1 – Comparison of mineral density (g/cm^3) results of sound, demineralised, and treated enamel amongst groups.

	Group 1 SAP P11-4	Group 2 SAP P26	Group 3 SAP LRAP	Group 4 Fluoride varnish	Group 5 DIW
	Mean (SD) mineral gain density (g/cm^3)				
Sound enamel	3.02 (0.08)[a]	2.98 (0.10)[a]	2.92 (0.15)[a]	2.91 (0.09)[a]	2.95 (0.07)[a]
Demineralised enamel	1.89 (0.06)[b]	1.88 (0.12)[b]	1.83 (0.06)[b]	1.85 (0.07)[b]	1.85 (0.06)[b]
Treated enamel	2.22 (0.07)[A]	2.30 (0.08)[A]	2.08 (0.09)[B]	2.08(0.06)[B]	1.94 (0.08)[C]
Mineral gain	0.32 (0.06)	0.43 (0.05)	0.24 (0.04)	0.23 (0.04)	0.09 (0.03)
Mineral gain percentage	28.5 %	38.6 %	22.3 %	21.6 %	8.3 %

[a] There was no significant difference in the mean MD of sound enamel amongst all groups.

[b] There was no significant difference in the mean MD of demineralised enamel amongst all groups.

[A,B] There was no significant difference in the mean MD of treated enamel with similar superscripts ($P = .84$).

[C] Significantly different to other values in the same row ($P < .03$).MD, mineral density.

and the 3 experimental zones (SE, TE, and DE). The results of the analysis indicate a significant difference in their interactions ($P < .01$) amongst the various groups of remineralising agents and different zones, as shown in Table 1.

The Figure illustrates micro-CT images from the 5 test groups depicting the SE, TE, and DE zones. Varying degrees of remineralisation are evident in the TE zone.

Nanoindentation assessment

Mechanical properties assessment via nanoindentation included indentations in the 3 zones (SE, DE, and TE) and amongst the outer (30 μm) and inner (60 μm) layers. The EM and H of the enamel specimens were determined in all groups, and the data were analysed using a 2-way ANOVA. The analysis revealed a significant difference in their interactions ($P < .01$) amongst the various groups of remineralising agents and enamel depth, as shown in Table 2 and Table 3.

Table 2 and Table 3 show that the mean EM and H values were similar amongst groups in the SE and DE zones regardless of the depth of the indentations. The Bonferroni multiple comparison test revealed that the mean EM and H were significantly higher in P26, P11-4, LRAP SAPs, and fluoride varnish groups when compared to specimens in the DIW group.

The mean EM in the outer and inner enamel layers and the mean H in the outer layer were highest for the P26 SAP group. This was followed by P11-4 SAP, which had a similar mean EM but slightly higher mean H in the inner layer of the TE zone. LRAP SAP and fluoride varnish groups had similar mean EM and H values, and as expected, the mean EM and H were lowest in the DIW group.

Discussion

The null hypotheses of this study were rejected, as there is a difference in mineral density of the treated enamel amongst

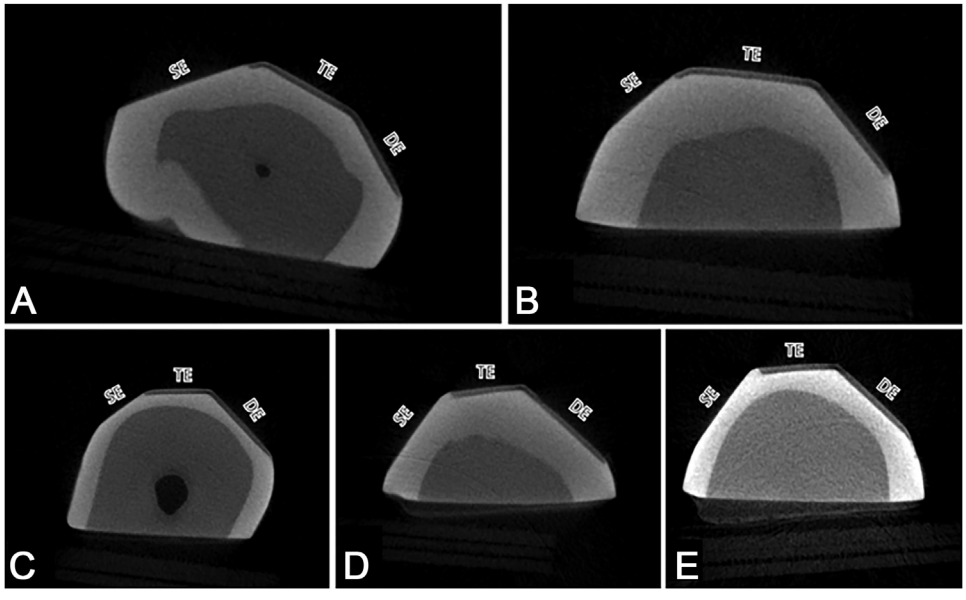

Fig – Post pH cycle microcomputed tomography images depicting 3 experiment zones: sound enamel (SE), treated enamel (TE), and demineralised enamel (DE). Varying levels of remineralisation (% mineral gain) are evident in the TE zones of the specimens in (A) P11-4, (B) P26, (C) LRAP; (D) 5% NaF; and (E) DIW.

Table 2 – Elastic modulus (GPa) in the outer and inner layers amongst the different treatment groups.

	Group 1 SAP P11-4	*Group 2 SAP P26*	*Group 3 SAP LRAP*	*Group 4 Fluoride Varnish*	*Group 5 DIW*
	Mean elastic modulus in GPa (SD)				
Sound enamel					
Outer layer 30 μm	71.00[a]	70.97[a]	72.43[a]	71.31[a]	64.31[a]
	(9.00)	(8.05)	(4.65)	(5.43)	(12.37)
Inner layer 60 μm	73.60[a]	75.67[a]	73.63[a]	71.69[a]	65.03[a]
	(6.42)	(5.86)	(2.98)	(6.14)	(12.18)
Demineralised enamel					
Outer layer 30 μm	5.24[b]	4.33[b]	5.18[b]	7.75[b]	14.63[b]
	(1.41)	(1.44)	(1.84)	(3.13)	(12.27)
Inner layer 60 μm	4.55[b]	5.13[b]	4.92[b]	7.93[b]	12.39[b]
	(2.01)	(2.23)	(1.19)	(2.12)	(11.14)
Treated enamel					
Outer layer 30 μm	22.79[A]	31.34[A]	21.56[A]	19.38[A]	15.86[C]
	(6.99)	(7.76)	(5.45)	(6.47)	(4.21)
Inner layer 60 μm	16.33[B]	17.18[B]	15.52[A]	14.34[A]	10.46[C]
	(2.83)	(2.62)	(1.83)	(2.67)	(2.53)

[a] There was no significant difference in the EM of sound enamel amongst all groups.

[b] There was no significant difference in the EM of demineralised enamel amongst all groups.

[A,B,C] Different uppercase superscripts indicate statistically significant difference ($P < .01$) in the EM of treated enamel in the same row.
EM, elastic modulus.

the test groups; there is a difference in mechanical properties of the treated enamel amongst the test groups; and there are differences in remineralisation potential amongst different SAPs. In the present study, SAP P26 and P11-4 groups showed statistically significant superior results in terms of mineral gain and re-hardening properties when compared with other test groups.

This finding is likely attributed to the permeation of SAPs into the body of the EECLs, forming 3D scaffolds mimicking the enamel matrix proteins for the enucleation of minerals.[28-30] SAPs in monomeric form tend to penetrate the EECLs owing to low viscosity which enables "self-assembly" in the presence of minerals and pH <7.4 conditions.[31] SAP LRAP has a large peptide sequence and acidic pH compared to SAP P26 and P11-4.[9,18] Fluoride, on the other hand, integrates into the surface of the EECLs, demonstrating a higher level of remineralisation on the surface when compared to the subsurface body of the EECLs.[32,33]

All treatment groups in this study had higher MD values when compared with the negative control DIW (1.94g/cm^3). The MD measured in our study is within the expected range to those observed by Hayashi-Sakai et al[34] for human teeth, who observed a range of 1.36 to 3.91 g/cm^3 for anterior teeth and 0.42 to 4.42 g/cm^3 for posterior teeth. In the present

Table 3 – Hardness (GPa) in the outer and inner layers amongst the different treatment groups.

	Group 1 SAPP11-4	*Group 2 SAP P26*	*Group 3 SAP LRAP*	*Group 4 Fluoride varnish*	*Group 5 DIW*
	Mean elastic modulus in GPa (SD)				
Sound enamel					
Outer layer 30 μm	3.85[a]	3.83[a]	3.81[a]	3.88[a]	3.62[a]
	(0.60)	(0.64)	(0.31)	(0.52)	(0.34)
Inner layer 60 μm	3.81[a]	3.79[a]	3.77[a]	3.87[a]	3.58[a]
	(0.43)	(0.50)	(0.27)	(0.49)	(0.36)
Demineralised enamel					
Outer layer 30 μm	0.28[b]	0.21[b]	0.24[b]	0.43[b]	0.30[b]
	(0.09)	(0.09)	(0.16)	(0.18)	(0.17)
Inner layer 60 μm	0.25[b]	0.23[b]	0.43[b]	0.43[b]	0.30[b]
	(0.14)	(0.10)	(0.18)	(0.17)	(0.17)
Treated enamel					
Outer layer 30 μm	1.01[A]	1.37[A]	0.89[A]	0.82[A]	0.60[C]
	(0.39)	(0.44)	(0.30)	(0.34)	(0.10)
Inner layer 60 μm	0.66[B]	0.63[B]	0.61[A]	0.58[A]	0.45[C]
	(0.10)	(0.09)	(0.08)	(0.13)	(0.08)

[a] There was no significant difference in the hardness of sound enamel amongst all groups.

[b] There was no significant difference in the hardness of demineralised enamel amongst all groups.

[A,B,C] Different uppercase superscripts indicate statically significant difference ($P < .01$) in the hardness of treated enamel in the same row.

study, there was an increase in net mineral gain when DE were treated with SAPs (P26 [38.6%], P11-4 [28.5%], LRAP [22.3%]) and with 5% NaF varnish (21.6%), when compared with the negative control (8.3%).

For EM and H assessment, no significant difference was observed in EM for the sound and demineralised groups amongst treatments, which is expected as EM values tend to be consistent for the same material unless there are major changes in structure. In the present study, the H of SE was highest at the surface and gradually stable until the dentine −enamel junction. He et al[35] assessed sound premolar teeth with a Berkovich indenter under 25 mN and reported the mean H as 3.98 ± 0.19 GPa on the outer enamel layer and 3.05 ± 0.41 GPa in the inner layer, whilst EM values were 82.67 ± 1.80 GPa on the outer layer and 56.80 ± 5.39 GPa in the inner layer. These values were comparable to the SE values in the present study. The mean hardness and elastic modulus values in the inner layer of TE treated with SAPs was statistically higher in the P11-4 (0.66 GPa and 16.33 GPa) and P26 groups (0.63 GPa and 17.18 GPa) when compared with the LRAP and 5% NaF varnish groups.

In enamel, the surface fluorapatite exhibits higher H and flexibility when compared to hydroxyapatite crystals.[36] The penetration of fluoride ions into the deeper layers of the enamel subsurface lesion may have been reduced because of the strong attraction of fluoride ions to hydroxyapatite crystals. SAPs P26 and P11-4 can be more effective in the remineralisation of EECLs as they are able to remineralise the surface (30 μm) and the body (60 μm) of the lesion. The SAPs P26 and P11-4 were more effective in remineralising DE possibly because of stabilisation of minerals due to the presence of chains of amino acids. There were no statistically significant differences in H and EM values in the inner enamel layer amongst specimens treated with SAPs P26 and P11-4; however, there was a statistically significant difference when comparing SAPs P26 and P11-4 with the LRAP, 5% NaF varnish, and DIW groups at the inner layer.[37] This finding is consistent with Schmidlin et al,[13] who had found that SAP P11-4 had higher micromechanical properties at a depth of 125μm (258 KHN) and similarly at 200μm (326 KHN) when compared to other fluoride-based remineralising agents. In the present study, H values in the TE zone were higher than the DE zone; however, a decreasing gradient was observed as the depth of the TE zone increased. The H and EM values of the treated EECLs were associated with the mineral gain across the depth of the lesion.

Despite the promising evidence in caries prevention in biomimetic remineralisation through organic and inorganic interactions, there are not many products suitable for clinical applications. Curodont Repair (P11-4) is a bioactive peptide synthesised from a 11 amino acid residue (Ac-QQRFEWEFEQQ-NH_2) with its N-terminally acetylated, C-terminal amidated peptide.[38] SAP P11-4 has a slightly alkaline pH to maintain its monomeric form; as it permeates into the acidic EECLs environment (pH 4.5 to 6), it starts to self-assemble and form a scaffold which has a high affinity for calcium ions, promoting natural repair.[28,31] Saha et al[39] demonstrated that the calcium binding site is made up of 2 SAP P11-4 strands and the 2 central “GLU” or “E” of each strand make up the binding sites. The results of the present study are consistent with previous in vitro[6,13,28,29] and in vivo[30,31,40-43] studies, indicating a stronger efficacy in remineralising EECLs with SAP P11-4 when compared with 5% NaF varnish.

The P26 has 12 amino acid residues of the C-terminus and 14 amino acid residues from the N-terminus (MPLPSYEVLTPLKWPSTDKTKREEVD: Serine - S[16] is phosphorylated).[44] Mukherjee et al[45] demonstrated that P26-mediated nucleation of HAP crystals on demineralised dentin significantly facilitates the recovery of mineral density. Furthermore, SAP LRAP is a 56-59 residue amelogenin (MPLPPHPGHPGYINFSY EVLTPLKWYQNMIRHPSLLPDLPLEAWPATDKTKREEVD) which is similar to the full-length and cleaved forms of amelogenin.[9] The use of LRAP in in vitro remineralisation of EECLs has confirmed its potential to initiate remineralisation[12,46]; however, it has a large peptide sequence and acidic pH and is not cost-effective for clinical application. The N- and C-terminal amelogenin domains and nonphosphorylated LRAP are sufficient in forming amorphous calcium phosphate in HAP crystals, promoting remineralisation.[9,18,47]

There is wider acceptance of noninvasive dental treatments amongst patients, and biomimetic remineralisation and the synergistic effect of fluoride may be effective for the management of EECLs. Future research should investigate the remineralisation potential of SAPs and its synergistic effects with other fluoride and nonfluoride agents in in vivo studies.[40]

Conclusions

Self-assembling peptides (SAPs) promote remineralisation of demineralised enamel. SAPs P26 and P11-4 (Curodont Repair) were more effective in remineralising deeper (60 μm) parts of demineralised enamel when compared with SAP LRAP and 5% NaF varnish. SAPs could be a promising treatment strategy in preventive and minimally invasive dentistry.

Conflict of interest

None disclosed.

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Author contributions

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