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Novel Peptide-based Remineralization of Dental Caries: A Systematic Review

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ABSTRACT

This systematic review aims to provide an understanding of the mechanisms of action, and to compare and evaluate the efficacy of novel peptide-based remineralisation strategies based on P11-4, QP5, DSS and Sp-H5 in the treatment of dental caries. Of the 46 studies included, P11-4 working via β-sheet fibrillar scaffold and de novo nucleation was found to be the most studied to have progressed to the clinical phase. Meanwhile, QP5 primarily stabilises amorphous calcium phosphate via domain-specific ion interactions, whereas DSS exhibits electrostatic adsorption and nucleation templating on demineralised surfaces. DSS has been studied in the form of varying numbers of amino acid repeats, with the most efficacy demonstrated in longer chains. Sp-H5 has been engineered to offer antimicrobial properties which enhance its remineralisation efficacy.

Keywords: dental caries, DSS, enamel remineralisation, novel peptides, P11-4, QP5, Sp-H5

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INTRODUCTION

Dental caries is an oral disease caused by acid-producing bacteria, which has led in demineralised lesions on the tooth surface [1]. These occur through plaque build-up. It is a global phenomenon, affecting over 2 billion adults with permanent teeth and over 530 million children with primary teeth [2]. Initial caries presents as white spot lesions (WSLs). These are areas of discolouration where enamel demineralisation has occurred [3]. Caries is only reversible in the early stages of demineralisation, where demineralised enamel is remineralised. If undetected and untreated, caries can progress to tooth decay, with permanent demineralisation of the enamel resulting in visible loss of dental tissues [4]. Untreated caries can have significant negative impact on the quality of life and even increase the risk of mortality [5]. Tooth decay requires restorative treatment, which is costly, invasive and not always locationally available, making them inaccessible to some [6, 7]. It is clear given the prevalence of this disease there is a need for more effective treatments than are currently available.

Fluoride is the current first-line approach, for example, water fluoridation, NaF varnish, and fluoride toothpastes. The presence of fluoride ions is thought to allow for the formation of fluorapatite (FA) within the enamel surface. FA is considered to be stronger than hydroxyapatite (HA), which is naturally present in enamel composition. FA has shown superior properties of density, crystallinity and structural stability [8, 9], as highlighted in another review [10]. NaF varnish rapidly gained clinical popularity, partly due to its high efficacy and ease of use among patients with special needs and young children [11]. Whilst it has demonstrated high efficacy in the reduction of caries, especially with large-scale, effective application in toothpastes [12], it is important to note, fluoride is only effective on early caries, with shallow lesions [13], and not for more advanced, deeper carious lesions. This clinical limitation highlights the need for biomimetic agents capable of infiltrating and regenerating subsurface lesions.

Calcium phosphate products are another line of treatment which include crystalline arrangements of HA [14] or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) commonly used in toothpastes. It reduces plaque and bacterial surface build-up and deposits [15]. It is worth noting that HA differs from ACP in its crystalline arrangement of calcium and phosphate ions, with ACP producing broad diffuse patterns on X-ray diffraction [16]. CPP-ACP has demonstrated remineralising abilities [17, 18]. However, it is considered to have lesser remineralisation effect, which is thought to be due to the lack of FA contributing to improved mechanical strengths. Combined with calcium fluoride treatment, CPP-ACFP is a promising development which has demonstrated higher efficacy than either CPP-ACP and fluoride varnish alone [17]. These current treatments still have limitations and therefore alternative or enhanced treatments are still required to manage this disease.

The new focus of research for treatment and prevention of caries is novel peptides. These can be targeted at early caries and have the capacity to self-assemble and therefore promote remineralisation. They may also have antimicrobial properties. Many are being investigated; however, this review will focus on four main specific novel peptides, namely P11-4, QP5, DSS and Sp-H5. They are formed of amino acid chains of varying lengths, as shown in **Error! Reference source not found.**.

Table 1: Synthesised novel peptide sequences

Peptide	Sequence	Study
P11-4	Ace-QQRFEWEFEQQ-NH2	(Kind et al., 2017)
QP5	QPY-QPV-QPH-QPM-QPQ-TKREEVD	(Ding et al., 2020)
DSS	$(Asp\text{-}Ser\text{-}Ser)_8$	(Zheng et al., 2019)
Sp-H5	Sp-DSHAKRHHGYKRKFHEKHHSHRGY	(Zhou et al., 2020; 2021)

The first peptide that became of interest is P11-4. It is specifically engineered to include 11 amino acids to provide its self-assembling abilities [19]. This is one of the most researched novel peptides, because of its ability to self-assemble and diffuse into the sub-surface lesion [20]. It is the only peptide in this review which has been tested to be safe and effective in clinical settings [21-23]. This peptide has been seen to visually reduce lesion size and appearance [24-26]. The mechanism of these effects is not completely understood, but it is thought to induce *de novo* nucleation, as first suggested by Kirkham et al. [27]. Since then, there has been a growing evidence base supporting and developing this theory [28, 29, 20].

Secondly, QP5 is derived from amelogenin, a naturally occurring enamel matrix protein (EMP). Amelogenin is the main EMP taking up over 90% of the proteins within the matrix [30, 31]. Amelogenin regulates the direction and orientation of crystal growth [32] therefore providing natural mineralising properties. It has been suggested the natural mechanism for amelogenin is the involvement of enamel ribbon synthesis to provide a framework for the formation of healthy, prismatic enamel [33]. QP5 may work via a similar mechanism, as the crystal regeneration in remineralisation is seen to be closer in structure to that of natural enamel [34]. Early evidence also supports remineralisation effects to be similar to NaF varnish [34-36].

Thirdly, DSS is derived from dentin phosphoprotein (DPP), which is largely composed of aspartate-serine-serine (DSS) repeats [37]. DPP is the most abundant non-collagenous protein in the dentin extracellular matrix [38]. It has demonstrated a high binding affinity to HA [39]. Various numbers of DSS repeats have been experimented with. It was shown that HA binding affinity and remineralisation effects increased with increasing number of repeats [40].

Lastly, Sp-H5, the most recently synthesised peptide, is derived from two naturally occurring domains combined. The aim was to produce a remineralising agent with antimicrobial properties. The two domains are phosphoserine (Sp) and histatin 5 (H5) [41]. Sp is a phosphoprotein, which are highly abundant in both enamel and dentin [42]. Calcium phosphate products containing phosphoserine sequences have demonstrated efficacy in remineralising the enamel [43]. H5 is a naturally occurring antimicrobial peptide found in saliva [44], which has been proven to offer bacterial protection against oral bacteria such as *S. mutans* [45]. The Sp domain was found to enhance the antimicrobial effects of H5 [41].

There is a growing literature base of exploratory research, investigating multiple novel peptides. The peptides in question have self-assembling and antimicrobial benefits. Therefore, there is potential for them to aid remineralisation, and for the prevention and treatment of caries. However, not enough is known about the mechanisms of action. In addition, it is not yet known how they can be brought into clinical settings, safely and

effectively on a large scale. Studies are underway in which novel peptides are combined with calcium and fluoride-based treatments [24, 29]. While findings suggest this combination to be the most effective treatment to date, there is not yet enough evidence to form a robust conclusion.

This systematic review sets out to provide an overview of current literature concerning novel peptides, P11-4, QP5, DSS and Sp-H5. The focus of this review is to comparatively evaluate the remineralisation effects of each peptide, and to summarise the known mechanisms by which these effects are produced. It looks at the effect of combining treatments, discusses the further implications of study findings and suggests areas to concentrate on and bridge the gaps in this field.

METHOD

Search strategy

The search was conducted across the three databases: Medline, Scopus and Embase, using 'Boolean Operators'. The papers were filtered by publication between 1st January 2003 – 17th April 2023, and search terms were constructed around the PICO framework (see Table 2), including; ("dental carie*" OR decay OR "carious lesion*" OR "white spot lesion*" OR "enamel carie*" OR cavit* OR erosion OR "dental tissue*") AND ("novel peptide*" OR antibiofouling OR "self-assembling peptide*" OR sap or "β-sheet forming peptide*" OR qp5* OR "amelogenin based peptide*" OR "enamel matrix derivative" OR "enamel matrix protein" OR dss* OR 8dss OR 3dss OR "aspartate-serine-serine" OR Sp-H5 OR H5 OR histatin) AND *(remineral** OR *"improv* enamel").* To note both Scopus and Embase required phrase-searching for the database to conduct the search without error messages. However, the search conducted on Medline did not use phrase searching, so the quotation marks were removed, due to incorrect exclusion of papers. The limits included abstract, title, (author) keywords and the paper was available in English. In Medline only, the expander of "apply equivalent subjects" and the "Boolean/Phrase" search model selected and the limit of 'Scholarly (Peer Reviewed) Journals' was manually selected, as the other two databases have this limit pre-built in. Additional methods of citation and hand-searching were conducted on review articles to provide a more thorough search. This was required particularly to locate papers on peptides other than P11-4, which have attracted less research attention.

PICO

The following PICO framework in Table 2 is being used for the systematic review.

Table 2: PICO framework

PICO	Criteria
Population	Patients/animals/models with dental caries
Intervention	Novel peptides (any of the four); P11-4, QP5, DSS or Sp-H5
(Comparison)	Fluoride or calcium-based products
Outcome	Improved enamel remineralisation

Inclusion criteria

Clinical studies were quality assessed further using the CASP critical appraisal scheme to consider areas susceptible to bias (CASP, 2018). The points of consideration included were: (1) a clear and focused question , (2) randomised assignment of treatment, (3) record of participant selection and followed up recorded, even if participant lost, (4) the level of blinding, if at all, (5) baseline data recorded, such as affecting factors of outcome or patient characteristics etc, (6) equal treatment for all groups, following of thorough protocol, (7) comprehensive reports, (8) calculation of statistics, such as confidence intervals, precision and effect size and lastly (9) consideration of all factors, e.g. benefits outweigh negative effects and/or cost. This method of appraisal does not include scoring each study by the number of criteria met (CASP, 2018), it is a more subjective approach.

Statistical analysis was not conducted in this study, as the type of data extracted was predominately qualitative and observational. Results between studies are largely heterogeneous, arising from a variety of approaches, therefore results are not numerically comparable.

The following criteria were used for inclusion in the review:

- Full access available
- Original studies (experimental/observational study design, not reviews or conference papers)
- Focus on one (or more) of the four peptides
- Relevant to PIO elements of the PICO framework in Table 2

Papers not meeting these requirements were excluded. The included studies were screened, and relevant data extracted, then displayed in tables in the results section accordingly.

Data extraction

The first author screened titles, abstracts and full texts using the criteria above. Calibration was conducted by the corresponding author, with any disagreements resolved by the second author. Data was extracted onto a spreadsheet, with a focus on remineralisation effect, potential mechanisms of action, cytotoxicity information and noted limitations.

Findings were stratified into (1) clinical evidence, (2) animal studies, and (3) *in vitro* investigations, as P11-4 has clinical and preclinical studies, both QP5 and DSS have animal and *in vitro* studies, while Sp-H5 have preliminary *in vitro* studies only. A narrative hierarchy was adopted (clinical > animal > *in vitro*) to contextualise outcomes.

Risk of bias assessment

To quality assess the data included in this study, a risk of bias assessment was conducted. This was adapted from the approach used in a previous peptide-focused systematic review [46]. The following nine criteria were interpreted: (1) control present, (2) sample size calculation, (3) synthesis via standard methods, (4) peptide characterisation, (5) peptide stability assessment, (6) biocompatibility, (7) amount and

concentration, (8) exposure time, (9) observer blinding scores. Studies were graded, dependent on how many criteria were met, high 0-3, medium 4-6 and low 7-9.

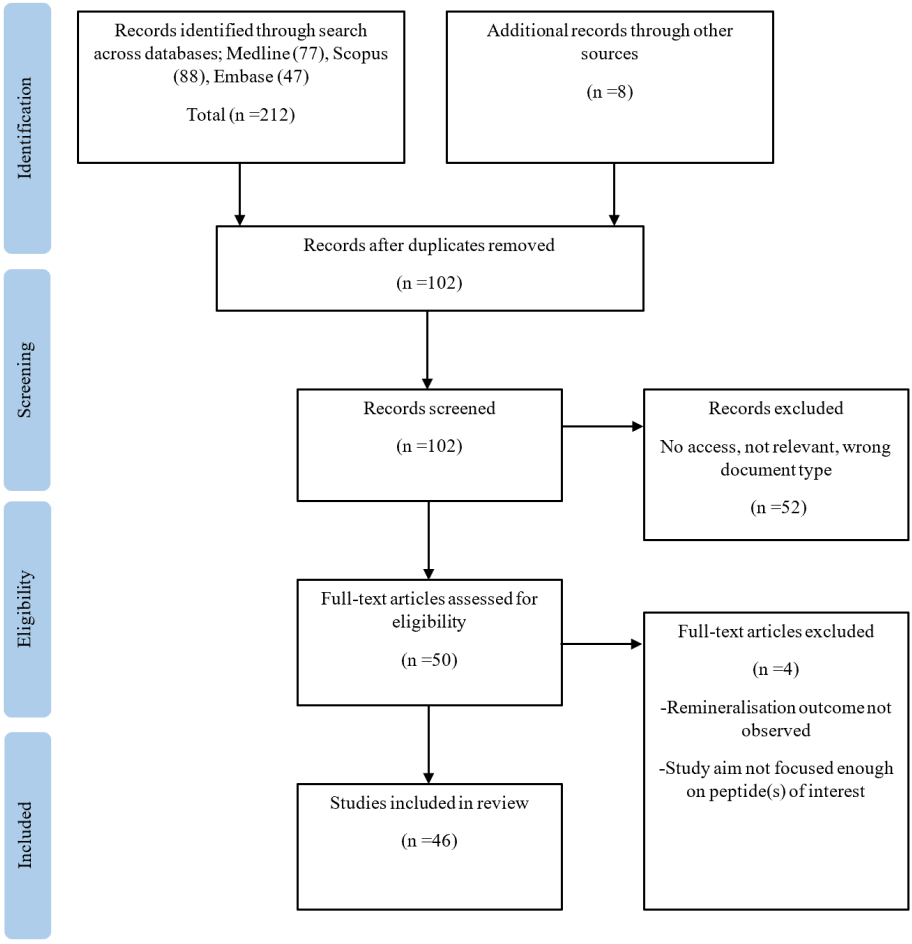

Figure 1: PRISMA diagram

RESULTS

Study selection

A total of 212 papers were identified across the three databases and through review citation searching. After the removal of duplicates, 102 papers were screened for relevance, where 50 were then excluded because they did not meet eligibility criteria. This resulted in 50 papers for full-text assessment. Four papers were excluded because remineralisation effect

was not demonstrated and the study aim lacks focus on the peptides of interest. This resulted in 46 papers being included in this review, as illustrated in the PRISMA diagram in Figure 1.

Risk of bias

According to the risk of bias assessment, risk was categorised to be low, medium or high risk, in 16, 29 and one papers respectively (see Table 3). 10 clinical studies were found and included in this review, with 8 at low and 2 at medium risk under these criteria. Of the 36 experimental studies the majority were found to have a medium risk of bias.

The only clinical studies were found on P11-4. 10 were critically appraised, using the CASP points of consideration as guidance. Due to small sample size and short follow-up period these studies were not robust enough to draw any final conclusions about the efficacy of P11-4. Two of the studies were case series, and therefore lacked a control, making the study less reliable. The study authors had accounted for patients lost to follow up and pointed out any limitations they noticed.

Table 3: Risk of bias assessment

Peptide	Study no.	Reference	Criteria									Score	Risk of Bias
			1	2	3	4	5	6	7	8	9		
Sp-H5	1	[47]	✓		✓	✓	✓	✓	✓	✓		7	Low
	2	[41]	✓		✓	✓	✓	✓	✓	✓		7	Low
DSS	3	[48]	✓		✓	✓		✓	✓	✓	✓	7	Low
	4	[49]	✓		✓	✓		✓	✓	✓		6	Medium
	5	[50]	✓		✓	✓	✓		✓	✓		6	Medium
	6	[51]	✓		✓	✓				✓		4	Medium
	7	[52]	✓		✓	✓				✓		4	Medium
	8	[40]	✓		✓	✓			✓	✓		5	Medium
	9	[53]	✓		✓	✓				✓		4	Medium
QP5	10	[36]	✓		✓	✓	✓	✓	✓	✓		7	Low
	11	[54]	✓		✓	✓	✓		✓	✓		6	Medium
	12	[34]	✓		✓	✓	✓		✓	✓		6	Medium
	13	[55]	✓		✓	✓	✓		✓	✓		6	Medium
	14	[56]	✓		✓	✓	✓		✓	✓		6	Medium
	15	[57]	✓		✓	✓	✓		✓	✓		6	Medium
	16	[35]	✓		✓	✓	✓	✓	✓	✓		7	Low
	17	[58]	✓		✓	✓	✓		✓	✓		6	Medium
P11-4	18	[25]*	✓	✓	**✓	-	✓	-	✓	✓	✓	7	Low
	19	[28]	✓		**✓	-	✓	✓	✓	✓		6	Medium
	20	[59]	✓		**✓	-	✓	-	✓	✓		6	Medium
	21	[60]	✓	✓	**✓	-	✓	-	✓	✓		6	Medium
	22	[61]	✓	✓	**✓	-	✓	-	✓	✓	✓	7	Low
	23	[62]	✓	✓	**	-	✓	-	✓	✓	✓	7	Low

				✓								
24	[63]*	✓	✓	** ✓	-	✓	-	✓	✓	✓	7	Low
25	[24]*	✓	✓	** ✓	-	✓	✓	✓	✓		7	Low
26	[64]	✓	✓	** ✓	-	✓	-	✓	✓	✓	7	Low
27	[29]	✓		** ✓	-	✓	-	✓	✓		5	Medium
28	[26]*	✓	✓	** ✓	-	✓	-	✓	✓		6	Medium
29	[65]*	✓	✓	** ✓	-	✓	-	✓	✓	✓	7	Low
30	[66]*	✓		** ✓	-	✓	-	✓	✓	✓	6	Medium
31	[67]	✓	✓	** ✓	-	✓	-	✓	✓	✓	7	Low
32	[68]	✓		** ✓	-		-		✓		3	High
33	[21]*	✓	✓	** ✓	-	✓	✓	✓	✓		7	Low
34	[69]	✓		** ✓	-	✓	-	✓	✓		5	Medium
35	[70]	✓	✓	** ✓	-	✓	-	✓	✓		6	Medium
36	[23]*	-		** ✓	-	✓	✓	✓	✓		5	Medium
37	[71]	✓	✓	** ✓	-	✓	-	✓	✓		6	Medium
38	[20]	✓		✓	✓	✓		✓	✓		6	Medium
39	[72]	✓		** ✓	-	✓	-	✓	✓	✓	6	Medium
40	[73]	✓		** ✓	-	✓	-	✓	✓		5	Medium
41	[74]	✓		** ✓	-	✓	-	✓	✓		5	Medium
42	[75]	✓		** ✓	-	✓	-	✓	✓		5	Medium
43	[76]	✓		** ✓	-		-	✓	✓		4	Medium
44	[77]	✓		** ✓	-		-		✓		3	Low
45	[22]*	-		✓	✓	✓	✓	✓	✓	✓	7	Low
46	[27]	✓		✓	✓			✓	✓		5	Medium

Note. Criteria: (1) control present, (2) sample size calculation, (3) synthesis via standard methods, (4) peptide characterisation, (5) peptide stability assessment, (6) cytocompatibility, (7) amount and concentration, (8) exposure time, (9) observer blinding. *Clinical study **Use of manufactured product named ‘Curodont

Repair/Protect' therefore assumed synthesised & characterised and cytotoxicity assessed prior, - criteria no longer required.

Interaction of novel peptides with caries

P11-4

P11-4 was found to be most researched peptide of the four. It had the largest evidence base, with 29 studies demonstrating remineralising effects (see Table 4). Of these, 10 were clinically based (eight RCTs and two case series) and 19 experimental studies.

Reported properties involved mineralising effects of P11-4 seen on tertiary dentin formation [28]. Additionally, the ability to inhibit progression of demineralisation [61, 74] suggests anti-erosive properties of P11-4 [71,74]. Combination treatments of P11-4 with fluoride or CPP-ACP enhanced effects and increased mineral deposition on eroded lesions [59], as described further in Table 4. Within study limits, P11-4 has shown greater effects to those of CPP-ACPF, with increased surface microhardness recovery (SMHR) of 62.06% and 48.41% respectively [73]. P11-4 demonstrated greater effects compared to fluoride in ability to remineralise deeper lesions within the study limits [60]. It had been suggested that this could be due to the ability of the fibre conformations of P11-4 [20].

It is noted that the crystal formations induced by P11-4 are structured more like the organic dentin scaffold, rather than the prismatic structure seen in enamel [72]. P11-4 may not be able to act on intact enamel, as the prismatic surface hinders remineralisation [61, 62]. Crystal structures formed by P11-4 are seen to have a "fan-like" appearance around matrix fibres [27, 63]. Furthermore, P11-4 is thought to act by *de novo* formation of HA as first suggested by Kirkham et al. in 2007. β-sheet ribbons of P11-4 act as a template for nucleation. After diffusing under the enamel surface, P11-4 is thought to self-assemble into a 3D scaffold. Its negative charge further assists the aggregation and alignment of HA ions, working in a "bottom-up" direction. The findings of numerous studies have since supported this pathway [21, 24- 26, 29, 62, 63, 65-67].

P11-4 has demonstrated biocompatibility in the available studies, although broader toxicological evaluation remains necessary. Three out of 10 clinical studies have explicitly stated no adverse effects [21-23]. Additionally, neither *in vitro* [74] nor *in vivo* studies have recorded cytotoxicity. P11-4 has shown 80% cell viability [28].

Table 4: Studies on P11-4

Study design		Study authors	Dental tissue, sample type	Combined treatment	Follow-up period	Results	
						Remineralisation effect	Suggested mechanism
Clinical	RCT	Gohar et al. [25]		×	3, 6 months	IDCAS and DIAGNOpen analysis both found P11-4 to have significant remineralisation capacity of WSLs at both follow ups, with superiority to fluoride varnish p <0.001	P11-4 remineralises bottom-up, with the ability to diffuse into subsurface of enamel lesion and imitate HA matrix formation, which then attracts Ca and P ions. Crystallisation ion theory.
		Bröseler et al. [63]		✓	90, 180 days	Significant reduction in size of early caries lesions, improved effect to fluoride varnish.	*De novo* HA formation, following diffusion of P11-4 into lesion, HA ions attracted.
		Doberdoli et al. [24]		✓	90, 180, 360 days	P11-4 found superior to fluoride varnish, both alone and combined with IDCAS-II scoring and fluorescence imaging.	*De novo* formation of HA.
		Kobeissi et al., [26]	Enamel, Human	×	1, 3, 6 months	IDCAS-II scoring found significant decrease of WSL caries ($p < 0.001$). DIAGNOpen found P11-4 to have significant effect over fluoride varnish at all timepoints.	*De novo* formation of HA.
		Sedlakova Kondelova et al. [65]		✓	30, 90, 180, 270 days	Regression of WSLs superior with P11-4 treatment. P11-4 prior to fluoride application did not negatively affect fluoride. Combination with delayed	*De novo* formation of HA deep within caries lesion.

				fluoride varnish treatment found superior to varnish alone.	
	Welk et al. [66]	×	45, 90, 180 days	Treatment with P11-4 found superior to control for lesions around orthodontic brackets.	*De novo* formation of HA.
	Jablonski-Momeni et al. [67]	×	4 weeks	Clinical results demonstrated beneficial effects in caries prevention, with fluorescence decrease seen. *In situ* (bovine) provided contradictory effects.	*De novo* formation of HA, monomeric P11-4 binds to surface.
	Alziky et al. [21]	✓	3, 6 months	Fluorescence imaging found combination treatment to be more effective in remineralisation than fluoride varnish alone.	Mechanism between fluoride and P11-4 thought to be complementary due to location of action, P11-4 is able to enter sub-surface whilst fluoride on its own cannot, prompting *de novo* formation of HA.
	Schlee et al. [23]	×	180, 360 days	No lesion deterioration detected and complete remineralisation observed in 4/28 lesions. Results suggest at least partial remineralisation effects of proximal initial caries.	P11-4 reliant on natural remineralisation mechanism from saliva, sensitive to mineral content, pH and flow rate.
Case series					
	Brunton et al. [22]	×	4, 8, 30, 180 days	Single application of P11-4 found effective and safe in promotion of mineralisation. Decreased lesion size and inhibited progression of lesion from day 30.	P11-4 thought to act as a nucleator, controlling nucleation (mineral deposition) and inducing formation of HA crystalline structure.

		Araújo et al. [28]	Dentin, Mouse MDPC-23	×	21 days	Mineral deposition demonstrated, 1 µg/ml had higher effect than 0.5 µg/ml and superior to calcium hydroxide. Cell migration observed.	Dentin mineralisation due to stimulation of odontoblast-like cells. Negatively charged domains of P11-4 act as nucleation template for HA.
		Memarpour et al. [59]	Enamel, Human	✓	28 days	%REMH found most improved combined with CPP-ACP, from 23.19% to 85.57%.	Crystal like structure formation observed. Mechanism observed varied with combinatory treatment.
		Özdemir et al. [60]	Enamel, Human	×	5 days	Statistically significant remineralisation effects between P11-4 and fluoride varnish ($p < 0.01$) with increased remineralisation depth of lesions.	-
Experimental	*In vitro*	Üstün et al. [61]	Enamel, Human	×	24hr	Remineralisation ability demonstrated, no statistical significance in effects between P11-4, NaF or CPP-ACP. Anti-erosive abilities similar to that of NaF, inferior to CPP-ACP.	Enamel required to have a flat surface to stop prismatic surface interfering with P11-4 aiding matrix formation.
		Lena Sezici et al. [62]	Enamel, Bovine	×	21 days	Significantly decreased fluorescence loss over time period, suggesting remineralisation effect.	*De novo* formation of HA crystals. Limited remineralisation ability due to prismatic structure of crystals.
		Jablonski-Momeni et al., [64]	Enamel, Human	✓	7, 30 days	Combined treatment with fluoride varnish alone found superior for enamel remineralisation of WSLs attached to orthodontic brackets.	-
		Kamal et al. [29]	Enamel, Human	✓	1, 4 weeks	Significant increase in SMH. Combined treatment with CPP-ACFP or fluoride	*De novo* mechanism.

					had largest effects. Increased crystal densities and decreased pores.	P11-4 imitates HA matrix formation and attracts HA ion deposits and nucleates Ca ion.
	Stoleriu et al. [68]	Enamel, Human	×	8 weeks	Remineralisation effect seen through SMH analysis on both white and brown spot lesions, exception to depth 275 µm.	Involvement of natural saliva remineralisation, alongside *de novo* mechanism.
	Kamal et al. [69]	Enamel, Human	×	1, 4 weeks	Found mineralisation to be a time-dependant process, with higher SMH values at 4 weeks to 1 week.	Attraction of Ca and P ions to demineralised lesion surface to induce HA crystal regeneration.
	Suda et al. [71]	Enamel, Bovine	×	56 days	P11-4 may prevent acidic erosion and promote remineralisation	3D scaffold formation related to precipitation and availability of dissolved ions.
	Üstün et al. [70]	Enamel, Human	×	1, 7, 30 days	Remineralisation effects seen on early caries, significantly higher ($p < 0.001$) than that of NaF varnish, through fluorescence imaging and lesion depth. Remineralisation effects still remain lower than CPP-ACFP	-
	Kind et al. [20]	Enamel, Human	×	14 days	P11-4 demonstrated ability to enable subsurface regeneration of enamel lesion, through formation of higher aggregates throughout entire lesion and adsorption ability.	Support of *de novo* crystallisation with diffusion of P11-4 and small aggregates on subsurface lesion to then self-assemble around higher order aggregates or fibres within HA.
	Silvertown et al. [72]	Enamel, Human	×	7, 14, 30, 50 days	P11-4 showed to promote remineralisation with bulking effects and self-assembly ability.	P11-4 not seen to form alike enamel prismatic structure, rather a HA

						crystalline structure more similar to organic scaffold of dentin.
	Soares et al. [73]	Enamel, Human	×	30 days	Remineralisation seen highest in P11-4, followed by CPP-ACPF. SEM imaging showed formation of amorphous crystals scattered over surface lines, along the prismatic boarders.	-
	Ceci et al. [74]	Enamel, Human	×	8, 24, 36 hrs	Remineralisation property confirmed, and ability to prevent demineralisation after one application prior to demineralisation exposure.	3D scaffold formation, promoting HA crystal formation.
	Kucukyilmaz & Savas [75]	Enamel, Human	×	1, 4 weeks	QLF-D found P11-4 to be most effective agent in study to remineralise initial lesions. Higher fluorescence gain seen at 4 weeks.	Formation of fibrils at low pH and monomeric in high pH, slower remineralisation results thought to be due to accumulation of ions present in saliva, to aid remineralisation.
	Schmidlin et al. [76]	Enamel, Bovine	×	21 days	P11-4 shown ability to re-harden of enamel lesions, with access to deeper lesions. SMH	Mineral uptake ability to contribute to apatite formation.
	Jablonski-Momeni & Heinzel-Gutenbrunner [77]	Enamel, Human	×	1, 8, 12 weeks	P11-4 found effective in all samples, with remineralisation seen in 93% of samples through SEM imaging	Formation of crystal structures on surface, likely calcium phosphate precipitates from saliva.
In situ	Kirkham et al. [27]	Enamel, Human	×	5 days	Results suggest P11-4 aids modulation of mineral behaviour	HA nucleation *de novo*.

QP5

This search found eight experimental studies involving QP5 (see Table 5). Results were mixed but were largely considered similar to those of NaF varnish [34, 35, 58]. A variety of approaches were used to study QP5, where there was both the addition of further protein domains [36, 54, 56, 57] and the splitting into smaller chain sequences [55] where adsorption of remineralising ions increased with increasing concentration, due to higher saturation. QP5 in combination with additional agents such as fluoride showed increased efficacy to QP5 alone in all four studies. The two rat models found increased mineral gain and simultaneously with decreased mineral loss [35, 36]. The addition of CS, the antibacterial carrier material chitosan, offers prevention of *S. mutans* adhesion and growth, in turn inhibiting further demineralisation. CS-QP5 was most effective in lesion depths in a range between 20-100 μm. For lesions beyond 120 μm results were similar to that of other agents [56, 57].

A suggested mechanism involved the two domains, (QPX)5 and C-tail, working together to govern HA deposits onto enamel lesion surfaces, where electrostatic attraction causes binding between the C-tail and calcium ions present in the oral environment. The QPX repeats contain β-sheets which were suggested to provide a biomimetic scaffold for amorphous calcium phosphate (ACP) stabilisation [55, 58]. A lesser number of QPX repeats, (QPX)3 showed higher HA affinity to C-tail compared to (QPX)5 where hydrophilicity and hydrogen bonding were thought to be involved [55]. HA binding was significantly decreased when the domains were split apart, suggesting the overall structure of QP5 is important. QP5 demonstrated the highest concentration of calcium throughout nucleation, whereas the smaller domains did not show significant differences ($p > 0.05$). The author suggested the C-tail may also chelate calcium and phosphate ions, in turn stabilising ACP. The tail is thought to have a key role in influencing adsorption effects from (QPX)5 for effective remineralisation to occur.

Cytotoxicity effects were only mentioned in the two *in vivo* rat models, where no apparent adverse effects were found [35, 36]. Whilst 10% BQ hydrogel (bioactive glass and QP5) was deemed to be biosafe *in vivo* [36], further conformation required to progress to *in situ* studies.

DSS

DSS appeared in seven studies under this search, with varying lengths of DSS repeats tested. 8DSS at present is the most researched number of Asp-Ser-Ser repeats [40, 48, 49, 50, 52, 53]. It had comparable effects to NaF controls [48, 50]. Shorter repeats still demonstrated affinity for HA binding. It was seen to significantly increase with up to six repeats, and the differences were not considered significant when increased to eight repeats [40]. 3DSS still demonstrated efficacious remineralisation of enamel and induce the formation HA crystals on the surface [51] (see Table 6).

Remineralisation effects were seen to be significant on demineralised enamel, thought to be due to the rough surface. One study investigated the effects on native enamel and found no improvement of hardness or elastic modulus. This was thought to be due to the reduced binding affinity on a ‘smoother’ surface. The author noted DSS adversely lowered

the hardness and elastic modulus on untreated, healthy enamel, by destroying bonding [53]. It has been suggested that DSS had a preference of selective binding to less ordered surfaces [40]. Quantitative light-induced fluorescence digital (QLF-D) imaging techniques quantified mineral loss results, indicating 8DSS can inhibit demineralisation through inciting lesion regression and promoting remineralisation [48].

The high affinity for HA, through local surface charge distributions that vary with pH, and increased ability to attract calcium ions via electrostatic forces [40, 48, 49, 52] suggests DSS can function as a nucleation template in dentin and enamel remineralisation, inducing HA precipitation, after binding to the positively charged surfaces on the dentin collagen structure [49]. There have been conflicting results where DSS has failed to act as a nucleation template. The author noted that DSS depends on its phosphorylation state and its environment which affect the affinity for calcium phosphate aggregation and binding [40]. 8DSS can prevent further loss of calcium and phosphate ions, whilst also attracting them from the surrounding oral environment [48].

Cytotoxicity effects were recorded for in one study [49] where 8DSS was deemed biocompatible with a cell viability of over 87%. The only *in vivo* rat model for this peptide recorded no adverse events [48]. There was no other mention of cytotoxicity assessments.

Sp-H5

Sp-H5 was only found in two *in vitro* studies under this search, having been first synthesised in 2020. The peptide was found to have fast and significant remineralisation effects on demineralised enamel. Findings included reduced mineral loss and crystal regeneration in both studies [41, 47] (see **Error! Reference source not found.**). The newly formed structure was described as needle-like projections with a crystalline microstructure [47]. Demineralisation inhibition was also demonstrated in both studies. This is likely to be due to its additional antibacterial properties, which arise from the presence of the H5 domain. The author suggested Sp-H5 may work against bacterial adhesion by preferentially adsorbing onto the enamel surface which could inhibit the adhesion of *S. mutans* [41].

Sp-H5 was seen to have high levels of electrostatic interaction with PO43- in HA, due to its positive charge [41, 47]. It was seen to be adsorbed vertically due to Sp domain changing its molecular conformations onto the HA surface (H5 alone adsorbed horizontally). There was the suggestion of steric hindrance and electrostatic repulsion because negatively charged molecules present in both H5 and Sp have different levels of attraction to Ca2+. The size of Sp-H5 and presence of Sp is thought to increase adsorption [41].

The second study focused on the isolation of the effective domains in Sp-H5, to create a peptide with greater efficacy. Sp-H5 is now the less favourable remineralisation agent as the newly found derivative P-113-DPS had improved abilities. P-113 was extracted from H5 as it is thought to be most involved in antimicrobial activity. The antimicrobial activity of P-113 is due to two cationic lysine residues, Lys2 and Lys10. Both residues play a role in increasing cell membrane permeability and interacting with intracellular structures.

Additionally, the Sp domain was doubled to SpSp (referred to as DPS) to see if there could be increased interaction with calcium ions.

Table 7 displays the sequences of both peptides, where their similarities lie.

Table 5: Studies on QP5

Study authors	Study design	Dental tissue, sample type	Treatment	Results		
				Remineralisation Effect (on demineralised enamel), SMHR%	Adsorption and Binding	Suggested Mechanism
Liu et al. [36]	*In vitro / in vivo*	Enamel, bovine / enamel, rat	BQ hydrogel	Improved remineralisation both *in vitro* and *in vivo*. 7 days of remineralisation SMRH% of BQ and QP5 found 61.36%, 43.12% respectively. Loss of Ca and P significantly lower on BQ and QP5 treated samples compared to untreated controls (similar to NaF).	Both BQ and QP5 found to increase binding to HA.	Fluorescence results of BQ suggest QP5 induces deposits Ca and P *in vitro.*
Ding et al. [54]	*In vitro*	Enamel, human	QP5-LF QP5-HF	Improved remineralisation effect on demineralised enamel and enhanced formation of HA crystals to be larger. After 12 days pH-cycling significantly higher %SMHR in QP5-HF compared to NaF and QP5 alone.	QP5 saw advantageous adsorption capacity for HA binding. QP5 mostly remained on enamel surface after rinsing.	QPX determines QP5 adsorption to enamel surface for HA interaction. QP5 forms complex with ACP through Ca interaction, added F could form QP5-ACFP complex with ability to get deeper within demineralised enamel.

Li et al. [34]	*In situ*	Enamel, bovine	QP5	Demonstrated strongest inhibitory effect and caused crystal growth. 7 days of remineralisation SMHR% most increased in QP5 compared to other amino acids tested and second to NaF (not significantly $p < 0.05$).	Surface adsorption seen on precipitates.	Induction of crystal aggregation, involving "coordination polyhedron growth unit theory". QP5 has more adsorption sites, has ability to assist aggregation.
Wang et al., [55]	*In vitro*	Enamel, bovine	QP5 (and split into domains*)	(QPX)5 within QP5 demonstrated to have greatest binding affinity. QP5 found to have highest Ca conc. throughout nucleation and found to inhibit nucleation most, stabilising ACP for over 2 h.	Adsorption increased with increased conc. up to 200 μM. Hydrophilicity could affect HAP binding.	Adsorption reliant on β-sheet secondary structure. Stabilisation from C-tail domain may be offered through chelation of Ca and P ions.
Ren et al. [56]	*In vitro*	Enamel, bovine	CS-QP5	Promoted mineralising effect demonstrated, potential reversal of lesion progression. 12-day pH cycling, CS found to enhance existing remineralisation ability of QP5, shown by SMHR%, from 45.78% to 50.06%.	Reduced mineral loss from surface adsorption through creation of surface barrier. Significant inhibition of bacterial adhesion (95.43%) compared to CS and QP5 alone, 94.91 and 27.97% respectively.	CaP clusters stabilised by gel.

Ren et al. [57]	*In vitro*	Enamel, bovine	CS-QP5	Improved remineralisation in early lesions. Significantly higher mineral content and increase SMHR% compared to other agents.	Inhibition of biofilm formation. Both (QPX)5 and C-tail domains contribute to adsorption ability.	QP5 involved in orienting HA crystal growth on enamel surface. Isoelectric point suggests presence of multiple anionic surfaces.
Han et al. [35]	*In vivo*	Enamel, rat	QP5	Significantly increased remineralisation effects and reduced mineral loss compared to control (similar to NaF). Subsurface remineralisation demonstrated and reduced lesion depth through increased thickness of remineralisation area.	Adsorption thought to be promoted by both QPX and C-tail domains.	Self-assembly occurs spontaneously producing fibrillar scaffolds. Multiple anionic surfaces allow for Ca ion interaction.
Lv et al. [58]	*In vitro*	Enamel, bovine	QP5	12-day pH cycling showed improved SMHR% (similar to NaF). Reduced lesion depth and mineral loss.	C-tail thought to promote Ca binding.	Structural change thought to occur to β-sheet/turn to then form HA *de novo*.

Note. (BG) bioactive glass, (BQ) BG-QP5 hydrogel, (LF) 500ppm NaF, (HF) 1000ppm NaF, (CS) chitosan ACP (amorphous calcium phosphate). *QP5 comprised of (QPX)5 and C-tail.

Table 6: Studies on DSS

Study authors	Study design	Dental tissue, sample type	No. of DSS repeats	Remineralisation effect *Suggested mechanism
Zheng et al. [48]	*In vivo*	Enamel/slight dentinal, rat	8	Enhanced remineralisation effect on demineralised enamel. Decreased mineral loss (similar effects to NaF)
Liang et al. [49]	*In vitro*	Dentin, human	8	Effective induction of mineral regeneration on demineralised dentin. Increased surface hardness and elastic modulus. Reduced surface roughness. *Remineralisation induced via electrostatic attraction between 8DSS (-ve) on collagen fibrils (+ve). 8DSS acted as nucleation template during submersion in saliva (artificial), which led to aggregation of calcium and phosphate ions around collagen fibrils
Yang et al. [50]	*In vitro*	Enamel, bovine	8	Increased remineralisation on demineralised enamel. Increased mineral deposition at both surface layer and lesion body. Reduced lesion depth and mineral loss (similar effects to NaF).
Chung et al., 2012	*In vitro*	Enamel, human	3	Increased surface nano-hardness and elastic modulus of demineralised enamel. Promotion of HA crystalline formation. Reduced surface roughness.
Hsu et al. [52]	*In vitro*	Enamel, human	8	Increased surface nano-hardness and elastic modulus of demineralised enamel. Increased mineral deposition and reduced surface roughness.
Yarborough et al. [40]	*In vitro*	Dentin / enamel, human	2, 4, 6, 8	Significantly increased HA binding affinity with increased with no. of repeats (up to 6DSS). 8DSS increased calcium phosphate precipitate.
Hsu et al. [53]	*In vitro*	Enamel, human	8	No effect on native enamel, due to smooth surface. Increased remineralisation on demineralised enamel. Improved hardness and elastic modulus

Note. (-ve) negatively charged, (+ve) positively charged.

Table 7: Sp-H5 shared sequence

Peptide	Sequence
Sp-H5	**Sp**-DSH**AKRHHGYKRKFH**EKHHSHRGY
P-113-DPS	**AKRHHGYKRKFH-Sp**Sp

In regard to cytotoxicity, Sp-H5 and P-113-DPS were both classed to be biocompatible with human samples, with 8 µM/mL appropriate for clinical safety. Sp-H5 showed 100% stability in human saliva, whilst P-113-DPS showed 65.4%. Both were classified as desirable [47]. These are also shown in Table 8.

Table 8: Studies on Sp-H5 and derivatives

	Study authors	Zhou et al. [41]	Zhou et al. [47]
	Dental tissue, sample type	Enamel, human	Enamel, human
	Study design	*In vitro*	*In vitro*
Results	Adsorption	Adsorption capacity of Sp-H5 superior to control (H5) Δconc. 5.39 µg/mL compared to 1.90 µg/mL, most of the reaction occurred within first 5 mins	Adsorption to HA surface seen with Δconc., most of the reaction occurred within first 20 mins. P-113-DPS and Sp-H5 Δconc. was 4.13nM/mL and 3.27nM/mL respectively
	Antibiofouling, antibacterial	32 µmol/mL to kill 99.9% of *S. mutans*, antibacterial ability to inhibit adhesion of bacteria to coated surfaces at 4 µmol/mL	128 µM/mL to kill majority of *S. mutans* bacteria, same ability for both P-113-DPS and Sp-H5
	Remineralisation effect (on demineralised enamel)	Significant reduced mineral loss compared to control (H5) $p < 0.05$ Ability to inhibit demineralisation Generation of 2.5 µm crystal layer (both Sp-H5 and H5)	Significant ability to reduce mineral loss compared to other agents, $p < 0.001$ Strong inhibitory ability for demineralisation Generation of 6 µm and 8 µm crystal layer for P-113-DPS and Sp-H5 respectively.
	Cytocompatibility	Not found to be damaging to healthy cells	8 µM/mL suggested appropriate for clinical safety

Note. Studies used varying volumes of different concentrations so results not completely comparable.

As a summary, P11-4 enables scaffold-based β-sheet assembly and *de novo* HA formation; QP5 stabilises ACP; DSS promotes electrostatic templating; Sp-H5 integrates remineralisation with antimicrobial activity. Only Sp-H5 and derivatives demonstrate strong antibacterial behaviour. In terms of clinical validation and translational readiness, P11-4 is the only peptide with clinical trials; QP5 and DSS remain preclinical; Sp-H5 is early stage *in vitro*.

DISCUSSION

The 46 included studies collectively provide varying levels of evidence supporting remineralising potential for P11-4, QP5, DSS and Sp-H5, though effect sizes and study quality were heterogeneous. It is not clearly established how they compare in efficacy to existing fluoride and calcium-based products. For example, whilst P11-4 alone was found to produce remineralisation effect, it was still less than that of CPP-ACFP [70], whereas a clinical study found P11-4 greater in remineralising early occlusal caries to fluoride varnish within study limits [24].

There is evidence P11-4 can remineralise more deeply within a lesion than fluoride (Soares et al., 2017) which are in a more advanced state [25]. There is the largest, most developed evidence base for P11-4. It is the only peptide which has been tested in a clinical setting. It has been researched for longer, and no cytotoxic effects have been reported [21, 22].

From a clinical positioning perspective, P11-4 currently functions as an adjunctive regenerative agent for early lesions. QP5 and DSS are best considered preventive or supportive remineralisation systems pending further validation. Sp-H5 remains investigational with potential dual-use of antimicrobial and remineralisation benefits.

There are similarities in the peptide binding mechanisms as we understand them. They all have a high affinity for HA and calcium. P11-4 is known to act as a template for *de novo* nucleation [20, 27]. DSS has also been hypothesised to act similarly [40]. The QPX domain within QP5 is also thought to be involved in nucleation [55]. The surface of enamel has been seen to interfere with peptide binding mechanisms. The remineralisation ability of 8DSS is only seen on acid-etched enamel, however, it was shown to have a detrimental effect on the hardness and elastic modulus of native healthy enamel [53].

The isolation of the smaller active domains within the peptides facilitated the advancements in mechanism understanding, as seen with QP5 and Sp-H5 [47, 55]. The isolation and manipulation of smaller domains of P-113-DPS within Sp-H5 has already allowed for the development of a more effective peptide, where Sp-H5 is already being superseded [47]. Additionally, (QPX)3 was found to have greater binding properties to HA within study limits, compared to the longer sequence (QPX)5 [55]. This suggests the possibility of shorter chains with an increased or similar scale effect. This could be more economically viable to offer as a treatment. Shorter chains may mean lower costs. There were similar findings for shorter chains with 3DSS [51]. However, earlier researchers found reducing length of DSS chains decreased remineralising abilities with lowered affinity for HA [40]. There may be a compromise in length of chains which provides satisfactory efficacy whilst taking into account economic factors. So far only P11-4 and QP5 have been combined with existing fluoride or calcium-based treatments. For both peptides they were more effective in combination than alone [24, 54, 65]. Sp-H5 initially

evolved from H5. Its derivative P-113-DPS has since shown greater efficacy and potential. It is likely that this will be the subject of further study rather than the original Sp-H5 [47]. The isolation of P-113 in this study provided development for the understating of the antimicrobial domain within the mechanism. The suggested mechanism of action is thought to be Sp-H5 inducing cell death by binding to the cell wall of bacteria, increasing cell membrane permeability, and directly interacting with the intracellular DNA.

Limitations

Three types of limitations are be discussed here, namely (1) limitations within this study, (2) limitations found in the papers, and (3) general limitations within the field.

Firstly, with regards to limitations within this review, the inconsistent terminology between the papers made it hard to gather all potentially relevant papers. In addition, the existing literature base was already rather limited. This can be explained as research in this field has only been taking place for approximately 20 years. The variability in study design made it hard to conduct uniform quality assessment. A statistical analysis was not possible, and data extraction was largely qualitative. This is likely to have introduced an element of bias with subjective quality assessment, including CASP. The chosen peptides had considerably different evidence bases available. Again, this may have made unbiased comparison less possible. For these reasons, it is not possible to draw firm conclusions about the merits of the different peptides from this review.

Secondly, there were many limitations noted in the studies. Common ones include the use of bovine samples. Although they are easier to obtain their composition is not the same as human tissue [71, 77, 78]. 10 studies in this review experimented using bovine tissues. It is important to note only one of the eight studies on QP5 tested human tissue [54]. But there were two rat models used which were *in vivo*. The validity of these results should be viewed with this in mind. Three papers were excluded during screening, as they failed to produce expected remineralisation or inhibition of lesion progression with P11-4 [79-81]. The authors considered that the use of bovine samples contributed to this finding. They also considered the artificial conditions to have an effect. A common limitation across the included papers was that they were conducted *in vitro* rather than a real oral environment. Artificial saliva could not simulate these conditions and presented associated issues with storage [61, 62, 69]. It was suggested by Kamal et al. [69] that remineralisation *in vitro* took longer because of lower concentrations of HA minerals compared to natural saliva.

As an aside, a review [82] highlighted that where the manufacturer of commercial elements used in experimentation also funds the experiment, there is the potential for introduction of bias.

Another main issue found in the papers is linked to small sample sizes. For example, one of the clinical studies only included 15 patients [22]. Also, only short-term effects were evaluated *in vitro*, with only a short period of sample demineralisation prior to testing [61, 76]. The longest clinical follow up was a year. This study also found clinical radiographs to be a low quality and an inaccurate visualisation technique for evaluating results. Not only is this technique poor quality, but it also unnecessarily exposes the patient to harmful radiation so it cannot be used often [23]. Even new and improved imaging techniques come with limitations. An example is laser fluorescence which has the advantage of being non-destructive and highly sensitive, but this can make it produce false positives [25]. Micro-

CT analysis was also found to be promising but expensive, so imaging could only be afforded to be conducted once at the end of the study [60].

Thirdly, this review revealed the limitations of our knowledge of the subject area. For example, the biocompatibility of these peptides other than P11-4 are not understood. As discussed above, the mechanisms of how these peptide work need to be fully understood. Furthermore, primary teeth are different from adult teeth, for example in their sensitivity to fluoride. Whilst a few studies have included primary teeth, there has not been a large enough focus on them.

Despite the above limitations, all mechanistic interpretations have been reviewed to ensure alignment with the cited primary studies, without extrapolation beyond reported findings.

Implications for next steps

More work needs to be done to address the limitations mentioned previously. More studies are required, of larger sample sizes, over longer time spans, that use consistent analysis techniques and terminology to allow for comparisons between findings. There needs to be further focus on the other three peptides so that there is the same level of knowledge as there is of P11-4. This will allow the merits to be compared and the best ones to be further developed. Valid comparisons among teeth models (human, animal or synthetic substrates) will also be required. There is the need of further *in vivo* studies to progress to clinical trial, both small and large across populations. More accurate imaging techniques that are both safe for the patients and cost-effective will be required as a part of these future studies. Techniques such as QLF-D or DIAGNOdent analysis may be part of the solution [26, 48]. Future areas of study should include biocompatibility, and continuous effort to unravel the mechanisms of action of these peptides.

The overall aim in dental caries management is effective preventative and non-invasive management strategies, as this will reduce the need for long-term invasive and costly treatments [21]. In more detail, P11-4 and the other novel peptides may have a role to play in regenerative dentistry. They may reduce WSLs around orthodontic bonding, whilst also increasing bond strength and enamel [67]. The technology currently available on the market remain cost prohibitive for general dental use. CURODONT Repair contains P11-4 and is sold online for 270EUR for up to 10 clinical treatments, treating 1-3 teeth, depending on the size of the lesions. The use of shorter length peptides might be able to reduce the price if similar efficacy is seen or considered enough [51, 55]. On the other hand, the specific isolation of smaller domains could be an expensive area to research. It has been suggested P11-4 could be put in toothpaste for daily use, which would increase the speed of roll out and be a cost-effective method of delivery [77]. This would also remove the issue of current P11-4 treatments requiring high clinical precision [25, 26].

CONCLUSION

Although the current treatments for dental caries, fluoride and calcium phosphate-based products promote remineralisation, they come with limitations. Novel peptides show potential for future clinical treatments. All four peptides, P11-4, QP5, DSS and Sp-H5 have all demonstrated remineralisation, but their translational maturity differs significantly.

P11-4 is the most clinically advanced, with multiple human trials supporting safety and efficacy. QP5 and DSS show promising preclinical results but require *in vivo* validation. Sp-H5 remains early-stage. Studies have varied in their approaches and therefore results are not comparable. If combined with NaF varnish or CPP-ACP, there is greater effect to peptides alone. CS and Sp domains also enhance efficacy. There are gaps in the current knowledge, and all these peptides are at different stages of research with varying evidence bases.

In future all the mechanism pathways must be the focus of research, in all potential contexts of use and application, including on prismatic enamel and in differential oral environments. Clinical cytotoxicity needs to be established, and the use of non-destructive analysis techniques shall be encouraged to aid the development of novel peptides in dentistry for them to become widely available clinically.

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