Antimicrobial peptides for the prevention and treatment of dental caries: A concise review

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Abstract

The objective of this study was to perform a comprehensive review of the use of antimicrobial peptides for the prevention and treatment of dental caries. The study included publications in the English language that addressed the use of antimicrobial peptides in the prevention and treatment of caries. These publications were also searchable on PubMed, Web of Science, Embase, Scopus, the Collection of Anti-Microbial Peptides and the Antimicrobial Peptide Database. A total of 3,436 publications were identified, and 67 publications were included. Eight publications reported seven natural human antimicrobial peptides as bactericidal to Streptococcus mutans. Fifty-nine publications reported 43 synthetic antimicrobial peptides developed to mimic natural antimicrobial peptides, fusing peptides with functional sequences and implementing new designs. The 43 synthetic antimicrobial peptides were effective against Streptococcus mutans, and nine peptides specifically targeted Streptococcus mutans. Ten antimicrobial peptides had an affinity for hydroxyapatite to prevent bacterial adhesion. Six antimicrobial peptides were also antifungal. Four antimicrobial peptides promoted remineralisation or prevented the demineralisation of teeth by binding calcium to hydroxyapatite. In conclusion, this study identified 67 works in the literature that reported seven natural and 43 synthetic antimicrobial peptides for the prevention and treatment of caries. Most of the antimicrobial peptides were bactericidal, and some prevented bacterial adhesion. A few antimicrobial peptides displayed remineralising properties with hydroxyapatite.

1. Introduction

Dental caries develop when plaque-associated bacteria produce acid that damages the tooth (Selwitz et al., 2007). Maintaining tooth minerals and controlling oral microbial biofilms are essential for preventing dental caries. The use of antibiotics in the management of dental caries is not acceptable because the excessive use could result in bacterial resistance and alter oral and intestinal flora. Moreover, antibiotics are often only briefly effective due to fluctuations in oral environment (Cheng et al., 2015), and antibiotics also have no remineralising properties to assist in the management of dental caries. Hence, researchers seek alternative antimicrobial agents for the control of biofilm in the management of caries. Pioneering research has shown that antimicrobial peptides can target pathogens and preserve healthy microflora in the mouth (Pepperney et al., 2011).

Antimicrobial peptides are naturally occurring protein molecules with antibacterial, antiviral or antifungal activity (Pepperney et al., 2011). In general, antimicrobial peptides are amphipathic and cationic. Most of these are oligopeptides consisting of short chains of 12 to 50 amino acids. The common secondary structures are α-helical, β-stranded, loop (β-hairpin) and extended structures (Dhople et al., 2006; Jenssen et al., 2006). Among them, α-helical peptides have membrane-disruptive properties designed to kill bacteria. In general, antimicrobial peptides are unstructured in free solution. They can partition into a membrane lipid bilayer and fold into their final configuration. Multicellular organisms produce antimicrobial peptides as an immune response to microbial infection. Such peptides also play modulatory roles and function as a bridge between innate and acquired immunity (Mai et al., 2017). They assist in defence against a broad range of microorganisms, including resistant species (Hancock, 2001; van ‘t Hof et al., 2001). Antimicrobial peptides can disrupt the cytoplasmic membrane, as well as penetrate cells and bind intracellular molecules. Furthermore, they can interfere with protein synthesis and prevent the synthesis of cell walls (Nguyen et al., 2011).

Antimicrobial peptides are produced as a host defence mechanism against infection

(Dale et al., 2005). Natural antimicrobial peptides such as cathelicidin, defensins and histatin are found in oral cavities. They have antimicrobial properties against oral pathogenic bacteria (Mai et al., 2017). They may also have a role in protecting teeth and oral mucosa (Tao et al., 2005). Although antimicrobial peptides generally have a short half-life and show low stability in vivo (Bush, 2004), researchers are developing antimicrobial peptides with promising stability and cytotoxicity properties (Hancock et al., 2006). Because synthetic antimicrobial peptides may offer safe, effective and ecologically balanced promotion of oral and overall health, they provide a potential alternative to traditional antimicrobial therapy (Pepperney et al., 2011). In addition, because antimicrobial peptides can be readily applied to the oral cavity to kill both bacteria and fungi, they have great potential for the prevention and treatment of dental caries. Natural and artificial antimicrobial peptides have recently become promising candidates for the development of new oral antimicrobial therapeutics (Gordon et al., 2005). Authors have also reviewed the types and mechanisms of action for natural oral-cavity antimicrobial peptides and the application of artificial antimicrobial peptides in dentistry (Bechinger et al., 2017; Khurshid et al., 2018; Wang et al., 2017). However, no previous reviews have assessed the status and prospects of antimicrobial peptides relative to the management of dental caries. The objective of this study was to perform a comprehensive review of the use of antimicrobial peptides for the prevention of dental caries.

2. Materials and Methods

2.1 Eligibility Criteria

Original studies—not reviews—investigating antimicrobial peptides for the prevention and treatment of dental caries were included in this review. The included studies could investigate the antimicrobial and remineralising properties of antimicrobial peptides.

2.2 Search Strategy

Two independent investigators (NJY and YIX) conducted a literature search to identify English language publications, including advance online publications from inception to 2019. They chose six common databases: PubMed, Web of Science, Embase, Scopus, Collection of

Anti-Microbial Peptides and Antimicrobial Peptide Database (Figure 1). The last search was conducted on 31 January 2020. The keywords included (Antimicrobial peptide OR AMP OR Cathelicidin OR Defensin OR Histatin OR Statherin) AND (Caries OR Dental caries).

2.3 Study Selection and Data Extraction

The investigators removed duplicate publications, screened titles and abstracts; they excluded publications on antimicrobial peptides that did not relate to dental caries, publications on dental caries that did not relate to antimicrobial peptides, literature reviews, and other irrelevant publications. The two investigators retrieved the full texts of the remaining publications for review. They selected studies on the use of antimicrobial peptides for the prevention and treatment caries and performed a manual screening of the reference lists in the selected publications. They then discussed the inclusion of the selected publications with another investigator to achieve agreement on the list of publications included in this review.

2.4 Assessment of Risk of Bias

Two independent investigators (NJY and YIX) assessed the risk of bias from individual studies. The assessment was adapted from previous systematic reviews (AlShwaimi et al., 2016; Montagner et al., 2014). We used nine parameters to evaluate the quality of each study: (1) the presence of a control, (2) description of the calculation of the sample size, (3) synthesis of antimicrobial peptides using standard methods, (4) characterisation of antimicrobial peptides, (5) assessments of the stability of antimicrobial peptides, (6) assessments of the biocompatibility of antimicrobial peptides, (7) the amount of antimicrobial peptides used, (8) the exposure time to antimicrobial peptides, and (9) the blinding of observers. Publications reporting fewer than four items were classified as having a high risk of bias, whereas those reporting more than six were classified as low risk.

3. Results

The initial literature search revealed 4,392 publications, but 956 duplicate records were removed (Figure 1). After screening the titles and abstracts, 3,335 publications were excluded,

as they were unrelated to dental caries and antimicrobial peptides. Twenty-nine literature reviews were excluded. The reference lists of the selected publications were searched, and 33 publications that potentially met the inclusion criteria were added. After a full-text screening of the 105 publications, 38 were excluded because they examined the relationship between antimicrobial peptides and the risk of caries (n = 19) or oral disease not related to caries (n = 19). Sixty-seven publications met the eligibility criteria and were thus included in this review. Forty-nine studies had a medium risk of bias, 11 publications presented a high risk of bias, and only seven publications presented a low risk of bias (Table 1). Almost of all publications were in vitro laboratory studies. Only three were in vivo laboratory studies using rats (Han et al., 2017; Wang et al., 2018B) or rabbits (Zhang et al., 2019). In addition, one publication was an ex vivo study using tooth blocks embedded in a removable intra-oral appliance (Sullivan et al., 2011). Eight publications reported that seven human antimicrobial peptides were bactericidal to *Streptococcus mutans*. LL37, hBD-2 and hBD-3 were also lethal to *Candida albicans* (Joly et al., 2004; Lee et al., 2013; Maisetta et al., 2003; Nishimura et al., 2004; Ouhara et al., 2005).

Fifty-nine publications reported 43 synthetic antimicrobial peptides. Twenty-five antimicrobial peptides were developed by mimicking natural antimicrobial peptides (Table 3). Six antimicrobial peptides were created with a new design (Table 4). Twelve antimicrobial peptides were developed through the fusion of peptides with antibacterial and functional sequences (Table 4 and Table 5 summarise their characteristics). The three methods of synthesising antimicrobial peptides involve mimicking natural antimicrobial peptides, fusing functional sequences, and implementing new designs. Mimicking their design means using natural antimicrobial peptides as templates to develop synthetic antimicrobial peptides.

Antimicrobial peptide mimesis includes full-length peptides, truncated peptides and modified peptides. For example, KR12-KAKE was derived from LL37 (da Silva et al., 2017). The segments hBD3-C15 (Ahn et al., 2017) and D1–23 (Aida et al., 2018) were derived from hBD-3. P-113 (Huo et al., 2011) and dhvar5 (Szynol et al., 2006) were derived from histatin-5. Each peptide maintained its activity against *Streptococcus mutans*.

Endogenous proteins could be used as templates for synthetic antimicrobial peptides. MUC7-12mer (Wei et al., 2006, 2007) was derived from saliva mucin. The peptide hLF1–11 (Huo et al., 2011) was derived from lactoferrin. Lv et al. (2015) developed the antimicrobial peptide QP5 based on the structure of amelogenin (Lv et al., 2015). In addition, the source for natural peptide templates was not limited to human beings. Developing novel antimicrobial peptides for prevention and treatment of caries has involved antimicrobial peptides from bovines (Dashper et al., 2005), frogs (Beckloff et al., 2007; da Silva et al., 2013; Kim et al., 2003; Shang et al., 2014; Wang et al., 2012), fish (Koo et al., 2008; Zhang et al., 2016), invertebrates (Chen et al., 2017; Hao et al., 2005; Li et al., 2010), bacteria (Ito et al., 2017; Lobos et al., 2009; Min et al., 2017; Okuda et al., 2010; Padilla et al., 2006; Su et al., 2018; Tong et al., 2010; Tong et al., 2011), and even plants (Taniguchi et al., 2015). The synthesis of antimicrobial peptides through the fusion of peptides with antibacterial and functional sequences could be described as a tuneable 'building-block' approach (He et al., 2010; Table 5). The antimicrobial peptide of C16G2 for inhibiting the growth of *Streptococcus mutans* has three main peptide segments: CSP_{C16}, GGG and G2 (Eckert et al., 2006). CSP_{C16} is a targeted domain of *Streptococcus mutans*, G2 was a broad-spectrum antimicrobial peptide, and GGG was the linker. In addition, peptide segments with affinity for hydroxyapatite and remineralising properties were used in the synthesis of antimicrobial peptides (Basiri et al., 2017; Huang et al., 2016; Yang et al., 2019; Zhang et al., 2019; Zhou et al., 2020). Researchers also developed synthetic antimicrobial peptides using a new design. Tu et al. (2016) and Wang et al. (2017) designed three amphipathic α-helical antimicrobial peptides based on an α-helical wheel projection.

In the included publications, the researchers used chlorhexidine or an antimicrobial peptide as a positive control and a BHI medium as a negative control. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and half-maximal inhibitory concentration (IC_{50}) were common methods used in the investigation of the antibacterial properties of the antimicrobial peptides. Analyses of biofilm biomass and evaluations of the minimum biofilm inhibition concentration ($MBIC_{50}$) were used to examine biofilm inhibition. Confocal laser-scanning microscopy and the counting of colony-forming units were also used to observe bacterial kinetics. Scanning electron microscopy and

transmission electron microscopy were used to observe the morphology of microbe cells and the biofilm. Different methods have been used to investigate the affinity of hydroxyapatite for antimicrobial peptides. For instance, Huang et al. (2016) used confocal laser-scanning microscopy to observe peptides conjugated with fluorescein on hydroxyapatite. Zhou et al. (2020) used the micro-BCA method to measure the change in peptide concentrations before and after exposure to hydroxyapatite powder. Zhang et al. (2019) used rabbit models to evaluate the inhibition of tooth-binding antimicrobial peptides against biofilm. For testing the remineralisation or prevention of the demineralisation of teeth against cariogenic challenges, a majority of the studies (5/7) used a chemical pH-cycling model. In contrast, one study used animal models (Han et al., 2017), while the other used an in vivo model (Sullivan et al., 2011). Table 6 shows the properties of synthetic antimicrobial peptides for the prevention of caries. All 43 antimicrobial peptides were effective against *Streptococcus mutans* through various mechanisms. GH12 inhibited *Streptococcus mutans* by killing the bacteria directly and inhibiting their virulence factors (Wang et al., 2018B). C16G2 and 2_1G2 are selectively targeted antimicrobial peptides (STAMPs) for *Streptococcus mutans* (Eckert et al., 2006; Guo et al., 2015; He et al., 2010; Kaplan et al., 2011; Li et al., 2010B; Sullivan et al., 2011). STAMPs can generate biofilms that are free of *Streptococcus mutans* in vitro by directly killing *Streptococcus mutans* in multispecies biofilm. Unlike most studies on antimicrobial peptides, C16G2 was evaluated in Phase II clinical trials (Guo et al., 2017). IMB-2 showed a synergistic effect with 10 ppm of sodium fluoride in killing *Streptococcus mutans* (Mai et al., 2011). C11H can also downregulate or suppress the expression of 21 types of *Streptococcus mutans* proteins (Huo et al., 2018; Huo et al., 2011). SspB(390–T400K–402) is a competitive peptide that prevents the adhesion of *Streptococcus mutans* to the salivary pellicle on hydroxyapatite (Okuda et al., 2010).

The functional domains of HBAMP (Huang et al., 2016), SHABP (Yang et al., 2019), MHABP (Yang et al., 2019), *Sp*−H5 (Zhou et al., 2020) and DPS-PI (Zhang et al., 2019) exhibit hydroxyapatite affinity. QP5, DR9-RR14, *Sp*−H5 and TVH19 can promote remineralisation or prevent the demineralisation of teeth in studies (Basiri et al., 2017; Han et al., 2017; Lv et al., 2015; Ren et al., 2019; Wang et al., 2019; Zhou et al., 2020).

4. Discussion

In this review, apart from the commonly used databases, we searched the *Collection of Anti-Microbial Peptides* and the *Antimicrobial Peptide Database* to create a comprehensive list of antimicrobial peptides used in the prevention and treatment of caries. These two databases were specifically developed for antimicrobial peptides. They supplied information related to the sequence, activity, source organism, target organisms, protein family and application, all of which is essential for discovering and designing novel antimicrobial peptides.

4.1 The role of natural antimicrobial peptides in preventing and treating caries

Studies have indicated that defensins hBD-2 and hBD-3 show activity against various oral pathogens, especially the main cariogenic organism *Streptococcus mutans* (Joly et al., 2004; Lee et al., 2013; Maisetta et al., 2003; Nishimura et al., 2004; Ouhara et al., 2005). Among the β-defensins, hBD3 showed the best antimicrobial effect. Other natural antimicrobial peptides—LL37 (Ouhara et al., 2005), α-defensins (HNP1-3) (Wattanarat et al., 2015) and histatin-5 (Krzysciak et al., 2015; Zhou et al., 2020)—were effective against *Streptococcus mutans*. LL37, hBD-2 and hBD-3 were also effective against *Candida albicans* (Joly et al., 2004; Lee et al., 2013; Maisetta et al., 2003; Nishimura et al., 2004; Ouhara et al., 2005). These findings demonstrate that natural antimicrobial peptides play a role in the host's defence system against cariogenic pathogens.

4.2 Synthesis of antimicrobial peptides

The majority of the antimicrobial peptides reported in the literature were synthetic. In this study we divide these peptides into three categories according to the method of synthesis: (1) mimesis, (2) new design, and (3) fusion of peptides with antibacterial and functional sequences. The development of antimicrobial peptide mimesis uses natural antimicrobial peptides as templates. QP5 was designed based on the structure of human amelogenin, which takes part in enamel biomimetic remineralisation. QP5 consists of five sequences of Q-P-X and a seven-residue hydrophilic tail. With the addition of a hydrophilic tail, the peptide becomes water-

soluble and calcium-binding (Lv et al., 2015). SspB(390–T400K–402) is a truncated fragment from the SspB of *Streptococcus gordonii*, but the threonine (T) at position 400 is substituted with lysine (K) (Okuda et al., 2010). This substitution caused SspB(390–T400K–402) to have a higher binding activity to the salivary pellicle and inhibited the growth of *Streptococcus mutans* biofilm on hydroxyapatite.

New designs and the fusion of peptides with antibacterial and functional sequences are two methods of synthesis used in the function-based design. Based on their intended purposes, a combination of template-assisted, minimalist and sequence-modification approaches is commonly used in the design of novel antimicrobial peptides (Wang et al., 2017A; Wang et al., 2017B). Tu et al. (2016) developed GH12 using an α-helical wheel projection based on the principles of α-helix folding (Tu et al., 2016). They used cationic histidine (H) and hydrophobic residue leucine (L) to create an amphipathic α-helix. In addition, they put a glycine (G) in the first position and included tryptophan (W) to help anchor the peptides to the lipid bilayer surface. Xiang et al. (2018) used computer-aided design to develop antimicrobial peptides (Cardoso et al., 2019). They designed C10-KKWW via software modelling and used SYBYL X-2.0 to simulate the docking of PtxA (an enzyme that is essential for *Streptococcus mutans*' energy metabolism) and candidate peptides. C10-KKWW is a selectively targeted antimicrobial peptide against *Streptococcus mutans* using computer-aided technology (Xiang et al., 2018). The truncation or modification of a peptide sequence is common in synthesis by fusing antibacterial and functional sequences. C16G2 (Eckert et al., 2006), IMB-2 (Mai et al., 2011) and C11H (Huo et al., 2018), which are specific target sequences for *Streptococcus mutans*, are truncated fragments from the competence-stimulating peptide of *Streptococcus mutans* (Qi et al., 2005). M8(KH)-20 is a dual-targeted antimicrobial peptide that targets *Pseudomonas aeruginosa* via KH and *Streptococcus mutans* via M8 (He et al., 2009). It can eliminate *Streptococcus mutans* and *Pseudomonas aeruginosa* selectively in a multispecies biofilm.

4.3 Secondary structure of antimicrobial peptides

Antimicrobial peptides have limited sequence homologies and a wide range of secondary structures. The common secondary structures are amphipathic α-helices,

amphiphilic β-strands, β-hairpins (loop) and extended structures (Dhople et al., 2006; Jenssen et al., 2006). In this review, the secondary structure was reported for 15 of the 43 antimicrobial peptides, most of which are α-helices. Three methods were used to analyse the secondary structures of antimicrobial peptides: (1) circular dichroism spectroscopy (Chen et al., 2017; da Silva et al., 2017; Koo et al., 2008; Li et al., 2010A; Lv et al., 2015; Shang et al., 2014; Tu et al., 2016; Zhang et al., 2016), (2) nuclear magnetic resonance spectroscopy (Kaplan et al., 2011; Mai et al., 2011), and (3) computer simulation (Okuda et al., 2010; Zhou et al., 2020). Not all antimicrobial peptides fit into the four types of secondary structures (da Silva et al., 2017; Min et al., 2017). Moreover, the secondary structures of antimicrobial peptides can change in different solutions or conditions (Jenssen et al., 2006; Tu et al., 2016; Zhou et al., 2020). GH12 displayed an α-helical structure in a buffer mimicking the amphiphilic structure of the natural bilayer membrane, but it showed more of a β-sheet structure in the sodium phosphate buffer (Tu et al., 2016). *Sp*-H5 changed from a helical to an unwinding loop structure during adsorption to a hydroxyapatite surface (Zhou et al., 2020).

Furthermore, α-helical peptides display membrane-disruptive properties for killing bacteria. However, the relationship between the secondary structure and the antibacterial properties of antimicrobial peptides is unclear. Some peptides share similar secondary structures with minimal differences in sequence yet exhibit different antibacterial activities (Friedrich et al., 2000). In addition, the disruption of the secondary structure of some antimicrobial peptides completely abolishes membrane translocation and reduces their antimicrobial activity (Powers et al., 2004). The secondary structure of antimicrobial peptides should be investigated further to explain their antibacterial mechanisms.

4.4 Stability of antimicrobial peptides in human saliva

Antimicrobial peptides are generally unstable in saliva. Thus, researchers have aimed to develop antimicrobial peptides with improved stability. Assessing stability and cytotoxicity is essential for studies on novel antimicrobial peptides. However, this review revealed that stability in saliva was assessed on only six of 43 antimicrobial peptides. The researchers who did so used reversed-phase high-performance liquid chromatography (HPLC) to quantify the

concentrations of antimicrobial peptides in saliva at 37 °C over time (Huang et al., 2016; Na et al., 2007; Sullivan et al., 2011; Tu et al., 2016; Wang et al., 2019). The stability of GH12, HBAMP and TVH19 in saliva was maintained at 99.8% after 0.5 hr, 80.5% after 1 hr and 86.7% after 12 hr, respectively (Huang et al., 2016; Na et al., 2007; Sullivan et al., 2011; Tu et al., 2016; Wang et al., 2019). Half (50.0%) of the C16G2 was maintained after 19 min in saliva. Because C16G2 can reduce the growth of *Streptococcus mutans* significantly within 1 min, it was used in a mouth rinse (Sullivan et al., 2011). The stability of antimicrobial peptides in saliva increased after substitution of residues (Na et al., 2007). Although they did not assess the stability of IMB-2 in saliva, Mai et al. (2011) reported that IMB-2 maintained its bactericidal properties in physiological salt conditions, acidic pH conditions and human saliva.

4.5 Biocompatibility of antimicrobial peptides

The biocompatibility of antimicrobial peptides can be assessed with cytotoxicity tests. In 59 studies, human gingival fibroblasts and erythrocytes were the most common cells used for cytotoxicity testing. However, some researchers used monocytic cells (Shang et al., 2014), human mesenchyme cells (Min et al., 2017) and bone mesenchymal stem cells (Zhang et al., 2019), which are not common for cytotoxicity testing in dentistry. In a clinical or in-vivo study, the side effects of antimicrobial peptides on oral tissue can be evaluated by assessing the mucosal tissues (Sullivan et al., 2011; Wang et al., 2018B).

4.6 Antibacterial properties of antimicrobial peptides

The mechanism of antimicrobial peptides against bacteria is generally explained as a positively charged peptide being associated with negatively charged microbial membranes. The peptides are inserted into the outer leaflet of the cytoplasmic membrane lipid bilayer, leading to the leakage of cytoplasm (Fjell et al., 2011). The models for how antimicrobial peptides are inserted into membrane bilayers were described as barrel-stave, carpet or toroidal-pore mechanisms (Jenssen et al., 2006). C16G2 selectively killed *Streptococcus mutans* by forming pores in cell membranes (Kaplan et al., 2011). Apart from cell membranes, antimicrobial peptides can act on other targets of microorganisms (Bechinger et al., 2017). C11H could down-regulate or suppress the expression of 21 types of proteins of *Streptococcus*

mutans by acting on bacterial genes (Huo et al., 2018; Huo et al., 2011).

In this review on the use of antimicrobial peptides for preventing and treating dental caries, all 67 studies reported that their antimicrobial peptides showed activity against *Streptococcus mutans.* Apart from *Streptococcus mutans,* common cariogenic bacteria include, but are not limited to, *Streptococcus subrings, Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Actinomyces naeslundii* (Mei et al., 2013). Therefore, it is necessary to assess the activity of antimicrobial peptides against other cariogenic microorganisms as well. However, the included studies did not provide evidence for novel antimicrobial peptides against cariogenic microorganisms besides *Streptococcus mutans.* Besides bacteria, fungi such as *Candida albicans* may contribute to the virulence of dental caries (Xiao et al. 2018). Only six antimicrobial peptides showed activity against *Candida albicans* (Basiri et al., 2017; Beckloff et al., 2007; Koo et al., 2008; Shang et al., 2014; Taniguchi et al., 2015; Wei et al., 2006, 2007).

4.7 Remineralising properties of antimicrobial peptides

Hydroxyapatite affinity is a desirable property of antimicrobial peptides for its applications in preventing and treating caries. Hydroxyapatite affinity increases the substantivity of antimicrobial peptides with hydroxyapatite and hence increases their activity against cariogenic biofilm. Some hydroxyapatite-binding antimicrobial peptides also show activity that promotes remineralisation or prevents the demineralisation of enamel and dentine (Han et al., 2017; Lv et al., 2015; Ren et al., 2019; Wang et al., 2019). In the review, all of the studies that showed remineralising properties used laboratory or animal models. Further studies are required before clinicians can use antimicrobial peptides for preventing and treating caries in clinical practice.

4.8 Future directions of study

This review indicated that several natural antimicrobial peptides play a role in the host's defence system against cariogenic pathogens. However, the mechanism is still unclear and requires more studies to gain support (Davidopoulou et al., 2012). This review also summarised three methods for developing novel antimicrobial peptides for the management of dental caries. In future

studies, researchers should continue to expand the library of novel antimicrobial peptides which are potentially useful in the management of dental caries. According to the assessment of the risk of bias, most of the included studies showed a medium risk of bias. All studies had negative or positive controls. Moreover, most of the studies used a synthetic method (56/67) to create antimicrobial peptides and reported the amount (64/67) and treatment time (56/67) of the antimicrobial peptides. However, fewer studies assessed structure (36/67), stability (6/67) and biocompatibility (14/67). It should be noted that these assessments are essential for the development of novel antimicrobial peptides (Hancock et al., 2006). In addition, only one study out of the 67 reported a calculation of sample size, and no studies included a blind design. Calculation of sample size is essential for the scientific validity of any research design (Charan et al., 2013), and non-blinded studies tend to report larger effect sizes and more significant *p*-values in the life sciences (Holman et al., 2015). Overall, it is necessary to develop a standard research process for the study of antimicrobial peptides used in the treatment and prevention of caries. On the other hand, further pre-clinical and clinical research should be conducted immediately after the development of novel antimicrobial peptides for preventing and treating caries.

As a limitation, we should mention that this review involved a search for literature published in the English language. We did not search unpublished registries; nor did we search for studies reported in, for example, proceedings of scientific conferences or prospective trial registries.

5. Conclusion

In this review, we found that several studies reported the bactericidal properties of natural antimicrobial peptides against *Streptococcus mutans*. In addition, several studies reported on the use of synthetic antimicrobial peptides for preventing and treating caries. The synthetic antimicrobial peptides were bactericidal, particularly for *Streptococcus mutans*, and some prevented bacterial adhesion. A few antimicrobial peptides possessed remineralising properties for hydroxyapatite. Because most of the publications are laboratory studies, further research is essential for warranting the clinical use of antimicrobial peptides for preventing and treating caries.

Declarations of interest

None.

CRediT authorship contribution statement

John Yun Niu: writing - original draft, data curation, formal analysis; **Iris Xiaoxue Yin**: data curation, formal analysis, writing—review and editing; **William Ka Kei Wu**: writing—review and editing; **Quan-Li Li**: writing—review and editing; **May Lei Mei**: conceptualisation, supervision, writing—review and editing, funding acquisition; **Chun Hung Chu**: conceptualisation, supervision, writing—review and editing, funding acquisition.

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