The multifaceted roles of antimicrobial peptides in oral diseases

Abstract

Antimicrobial peptides are naturally occurring protein molecules with antibacterial, antiviral and/or antifungal activity. Some antimicrobial peptides kill microorganisms through direct binding with negatively charged microbial surfaces. This action disrupts the cytoplasmic membrane and leads to the leakage of the cytoplasm. In addition, they are involved in the innate immune response. Antimicrobial peptides play an important role in oral health, as natural antimicrobial peptides are the first line of host defence in response to microbial infection. The level of natural antimicrobial peptides increases during severe disease conditions and play a role in promoting the healing of oral tissues. However, they are insufficient for eliminating pathogenic micro-organisms. The variability of the oral environment can markedly reduce the effect of natural antimicrobial peptides. Thus, researchers are developing synthetic antimicrobial peptides with promising stability and biocompatibility. Synthetic antimicrobial peptides are a potential alternative to traditional antimicrobial therapy. Pertinent to oral diseases, the deregulation of antimicrobial peptides is involved in the pathogenesis of dental caries, periodontal disease, mucosal disease and oral cancer, where they can kill pathogenic microorganisms, promote tissue healing, serve as biomarkers and inhibit tumour cells. This narrative review provides an overview of the multifaceted roles of antimicrobial peptides in oral diseases.

1 Introduction

During the past two decades, the application of antimicrobial peptides in managing various diseases has drawn considerable attention from researchers (Fjell et al. 2011; van 't Hof et al. 2001). In general, multicellular organisms produce antimicrobial peptides as a defence mechanism against competing pathogenic microbes. Antimicrobial peptides are small molecules with a molecular mass between 1 and 5 kDa, and they have a tendency to form amphipathic structures: separate hydrophobic and hydrophilic domains in nonpolar solvents. Considerable research has led to the realisation that antimicrobial peptides do not merely act as direct antimicrobial agents but also represent effectors and regulators of the innate immune system through a range of actions (Fjell et al. 2011; Lai and Gallo 2009; Takeuchi et al. 2012).

Regarding the bactericidal mechanism of antimicrobial peptides, positively charged antimicrobial peptides likely associate with negatively charged microbial surfaces. They displace lipid and kill bacteria by inserting into the cytoplasmic membrane lipid bilayer's outer leaflet. (Fjell et al. 2011; van 't Hof et al. 2001). The mode of action by which antimicrobial peptides are attached and inserted into membrane bilayers is known as 'barrel-stave', 'carpet' or 'toroidal-pore' mechanisms (Tenovuo 2002). In addition, antimicrobial peptides inhibit cell wall formation, affect bacterial cell membranes' structure and inhibit protein folding or enzyme activity. Some antimicrobial peptides inhibit biofilm formation by competing with bacteria for adhesion (Ito et al. 2017). The mechanism of action also highlights antimicrobial peptides' selective toxicity; they kill bacterial pathogens without damaging host tissues. Because antimicrobial peptides usually work by attacking multiple hydrophobic and polyanionic bacterial targets, antimicrobial-peptide-resistant mutants infrequently arise

(Andersson et al. 2016). However, in cases where specific protein targets are involved, the possibility of bacterial resistance to antimicrobial peptides should not be ignored (Bechinger and Gorr 2017; Ho et al. 2016).

Natural antimicrobial peptides are naturally occurring molecules that all multicellular organisms produce as the first line of defence, and they display remarkable structural and functional diversity. They exist as antimicrobial substances on the epithelial lining and in the blood, leucocytes and the lymphatic tissue, where they defend host organisms against microbes. Natural antimicrobial peptides come in various forms, including defensins, histatins and cathelicidins, which have remarkably different structures (Table 1).

Table 1 Type, expression site and sequence of natural antimicrobial peptides

Peptide	Expression site				Sequence
	Salivary glands	Oral mucosa	Bone marrow	Dental pulp	
Cathelicidin					
LL37	+	+			LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES
Defensin					
hBD-1	+	+	+	+	GNFLTGLGHRSDHYNCISSGGQCLYSACPIFTKIQGTCYRGKAKCCK
hBD-2	+	+	+	+	GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP
hBD-3	+	+	+	+	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK
hBD-4	+				EFELDRICGYGTARCRKKCRSQEYRIGRCPNTYACCLRKWDESLLNRTKP
HNP1	+	+			ACYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP2	+	+	+		CYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP3	+	+			DCYCRIPACIAGERRYGTCIYQGRLWAFCC
Histatin					
Histatin-1	+		+		DSpHEKRHHGYRRKFHEKHHSHREFPFYGDYGSNYLYDN
Histatin-2	+				RKFHEKHHSHREFPFYGDYGSNYLYDN
Histatin-5	+				DSHAKRHHGYKRKFHEKHHSHRGY

Their bactericidal properties could be evaluated based on their *in vitro* effect on planktonic bacteria. However, many antimicrobial peptides have limited antibiotic activity due to the variability of the oral environment (Bechinger and Gorr 2017; Lei et

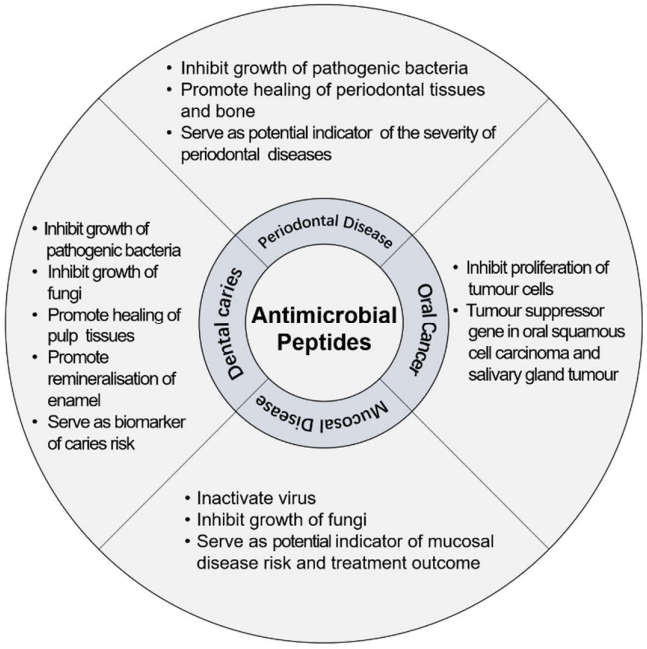
al. 2019). In addition, natural antimicrobial peptides generally have a short half-life, long sequence length and are unstable (Kumar et al. 2018; Shao et al. 2019).

To address the limitations of natural antimicrobial peptides, researchers designed and synthesised artificial antimicrobial peptides with structural modifications (sequence truncation or amino acid substitution) to achieve highly active, broad-spectrum activity and favourable pharmacokinetics with desirable stability (Bechinger and Gorr 2017; Shao et al. 2019). Researchers also modified the peptides, such as peptoids (peptide mimetics generated through backbone modification) and foldamers (unnatural oligomers of peptides). This was done to promote their antimicrobial activity and to prevent them from proteolysis (Luo et al. 2017; Olsen 2010). Furthermore, innovative drug delivery systems, such as nanoformulations, can further improve the stability, safety and efficacy of antimicrobial peptides (Mahlapuu et al. 2016). In addition, agents such as butyrate and vitamin D can enhance the expression of antimicrobial peptides and their function in some patients (Bayirli et al. 2020; Wang 2014).

Most oral diseases are inflammatory diseases resulting from the formation of mixed biofilms on the teeth or the periodontal tissues. Therefore, the control of dental microbial biofilms is critical to preventing or controlling dental diseases. Antimicrobial peptides do not produce bacteria resistance. They are safe because their degradation products are natural amino acids (Vlieghe et al. 2010). Antimicrobial peptides have the potential to be used in managing oral diseases, including dental caries, periodontal diseases, oral mucosal diseases and oral cancer (Figure 1). In addition, some databases were specifically developed for the properties and application of antimicrobial peptides (Niu et al. 2021). This narrative review is aimed at providing an overview of the role of antimicrobial peptides in the response to and treatment of oral diseases by collecting

information from different databases. Table 2 shows some of the natural and artificial antimicrobial peptides which were investigated.

Figure 1 Antimicrobial peptides in oral diseases



2 Inhibition of cariogenic species and promotion of pulp tissue healing

Dental caries is one of the most common chronic diseases globally (Chu et al. 2013). It is localised tooth demineralisation resulting from the acidic by-products of bacterial fermentation (Selwitz et al. 2007). Pulp and periapical disease are secondary diseases of caries. Pulpal inflammation and infection usually occur when the pulp is exposed to bacteria (Nakashima and Akamine 2005). One of the most prevalent causes of endodontic treatment failure is the permanence of microorganisms within root canals. *Enterococcus faecalis* is one of the most frequently found bacteria in teeth with pulp necrosis. It forms a tenacious biofilm in the root canal and can survive in severe conditions, such as medical treatments or mechanical preparation, which causes apical periodontitis (Gomes et al. 2008). Moreover, pulp cells play a crucial role in pulp-

dentine complex regeneration, and cell migration is an essential phenomenon during the early stage of this process (Lin et al. 2014). Both natural and artificial antimicrobial peptides have been known to be promising candidates for the development of new oral antimicrobial therapeutics (Mor 2000).

Table 2 Oral disease–related types of antimicrobial peptides

Oral diseases	Peptide type (No)	Antimicrobial peptides				
Dental caries	Natural (9)	LL37	hBD-1	hBD-2	hBD-3	hBD-4
		Histatin-5	HNP1	HNP2	HNP3	
	Artificial (50)	AAP	AmyI-1-18	C10-KKWW	C11H	C16G2
		Cecropin-XJ	chrysophsin1	CLP-4	D-GL13K	D-Nal-Pac-525
		D1-23	dhvar5	DJK-5	DR9-RR14	DPS-PI
		gaegurin 6	GH12	G3	HBAMP	hBD3-C15
		hLF1–11	IMB-2	IDR-1002	Kappacin	KR12-KAEK
		KSL	KSL-W	L-K6	LFA-LFC	Lys-a1
		M8(KH)-20	MHAMP	mPE	MUC7-12mer	Nisin
		P113	Parasin I	pm11	Protamine	PsVP-10
		Pug-1	QP5	SHAMP	Ssp(A4K-A11K)	Sp−H5
		spB (390–T400K–402)		TVH19	VS2	VSL2
		ZXR-2				
Periodontal disease	Natural (9)	LL37	hBD-1	hBD-2	hBD-3	Histatin-1
		Histatin-2	HNP1	HNP2	HNP3	
	Artificial (19)	AMPCol	BAR	Bacitracin	Cateslytin	DSS-RKF
		GL13K	HBD3-C15	hLF1-11	LFchimera	LyeTx I
		Nal-P-113	oligopeptide	P-15	P15-CSP	SAP3-TA
		SMAP28	TiBPs	ZS37-37	ZS37-38	
Mucosal disease	Natural (8)	LL37	hBD-1	hBD-2	hBD-3	Histatin-5
		HNP1	HNP2	HNP3		
	Artificial (4)	dhvar4	dhvar5	KSL-W	ToAP2	
Oral cancer	Natural (7)	LL37	hBD-1	hBD-2	hBD-3	HNP1
		HNP2	HNP3			
	Artificial (1)	$hCAP_{109–135}$				

2.1 Natural antimicrobial peptides

Defensins were the first described antimicrobial peptides in mammals and consist of two subfamilies in humans: α-defensin (HNP) and β-defensin (hBD). hBDs (hBD-1, hBD-2 and hBD-3) are expressed in dental pulp and odontoblasts (Dommisch et al.

2005; Paris et al. 2009). Studies indicate that hBDs (hBD-1, hBD-2 and hBD-3) show activity against various oral pathogens, especially the principal causative organisms of *Streptococcus mutans* and *E. faecalis*. They also prevent *E. faecalis* from binding to the host cell (Lee et al. 2013; Lee and Baek 2012). hBD3 showed a promising antimicrobial effect among hBDs (Joly et al. 2004). Two other natural antimicrobial peptides, Histatin-5 (Krzysciak et al. 2015) and cathelicidin (LL37) (Guo et al. 2016) have also been proved to be effective against *S. mutans*. Most studies were *in vitro* and reported their findings base on planktonic bacteria. Several strains of *Actinomyces naeslundii, S. mutans* and *Lactobacillus spp* have low susceptibilities to LL37, hBD-2, HNP-1 and HNP-3, and the number of such strains in patients showed significant positive correlations with dental caries experience (Goeke et al. 2018).

Natural antimicrobial peptides are potential biomarkers because deregulation of antimicrobial peptides can affect the caries risk. (Jurczak et al. 2015; Munther 2020) or are genetic susceptibility markers of caries (Slebioda et al. 2020; Wu et al. 2020). It is suggested that a positive correlation exists between the salivary hBD-2 level and caries progression (Jurczak et al. 2015). The increase of HNP1-3 in saliva is associated with the reduction of *S mutans* and rate of caries progression (Wattanarat et al. 2020). However, the association between hBD-1 and caries experience is still inconclusive (Krasone et al. 2014). Nevertheless, prior studies indicated that natural antimicrobial peptides could be biomarkers for identifying individuals with high caries risk (Goeke et al. 2018).

In addition, endogenous antimicrobial peptides may play a role in the innate host defence of the human dental pulp and promote the healing of pulp tissues. In previous research, hBD-2 significantly increased the dentine sialophosphoprotein mRNA levels

in human pulp cells, which may have the ability to stimulate odontoblast differentiation (Shiba et al. 2003). hBD-4 may be useful in indirect pulp therapy because it can enhance the differentiation of stem cells into osteoblasts or odontoblasts in dental pulp (Zhai et al. 2020). LL37 also stimulates human pulp cell migration, odontoblastic differentiation, angiogenesis and reparative dentin formation (Horibe et al. 2018). Thus, LL37 might be useful for pulp regeneration and pulp capping. Moreover, HNP-1 and HNP-3 could be identified from acute apical abscesses, but their roles are still not clear (Alfenas et al. 2017).

2.2 Artificial antimicrobial peptides

To achieve the application of antimicrobial peptides to dental caries management, researchers have made many attempts to develop synthetic analogues or fragments of natural antimicrobial peptides from humans or other species. More than 40 artificial antimicrobial peptides have shown activity against planktonic cariogenic species (Table 3). In terms of their effects on cariogenic biofilms, HBD3-C15 (Ahn et al. 2017) and D1-23 (Kreling et al. 2016), both derived from hBD-3, demonstrated the inhibitory effect of *S. mutans* (Ahn et al. 2017; Kreling et al. 2016) and *Streptococcus gordonii* (Ahn et al. 2017) on biofilm formation. In another study, researchers attempted to load antimicrobial peptides to a bioadhesive liquid crystalline system to overcome the instability of natural antimicrobial peptides (Aida et al. 2018). This drug delivery system presented antibacterial activity against *S. mutans* biofilm with no cytotoxicity.

Table 3 The roles of natural and artificial antimicrobial peptides in oral diseases

Role of antimicrobial in oral diseases	Antimicrobial peptides				
Dental caries					
● Inhibit growth of pathogenic bacteria	LL37	hBD-1	hBD-2	hBD-3	Histatin-5
	HNP1	HNP2	HNP3	AAP	AmyI-1-18
	C10-KKWW	C11H	C16G2	Cecropin-XJ	chrysophsin1
	CLP-4	D-GL13K	D-Nal-Pac-525	D1-23	dhvar5
	DJK-5	DR9-RR14	DPS-PI	gaegurin 6	GH12
	G3	HBAMP	hBD3-C15	hLF1–11	IMB-2
	IDR-1002	Kappacin	KR12-KAEK	KSL	KSL-W
	L-K6	LFA-LFC	Lys-a1	M8(KH)-20	MHAMP
	mPE	MUC7-12mer	Nisin	P113	Parasin I
	pm11	Protamine	PsVP-10	Pug-1	QP5
	SHAMP	Ssp(A4K-A11K)		Sp−H5	TVH19
	VSL2	SspB (390–T400K–402)		ZXR-2	VS2
● Inhibit growth of fungi	hBD-2	hBD-3	AmyI-1-18	DR9-RR14	
● Promote healing of pulp tissues	LL37	hBD-2	hBD-4	IDR-1002	
● Promote remineralisation of enamel	DR9-RR14	QP5	Sp−H5	TVH19	
● Serve as biomarker of caries risk	LL37	hBD-1	hBD-2	HNP1	HNP2
	HNP3				
Periodontal disease					
● Inhibit growth of pathogenic bacteria	LL37	hBD-1	hBD-2	hBD-3	Histatin-2
	HNP1	HNP2	HNP3	AMPCol	BAR
	Bacitracin	Cateslytin	DSS-RKF	GL13K	HBD3-C15
	hLF1-11	LFchimera	LyeTx I	Nal-P-113	oligopeptide
	P-15	P15-CSP	SAP3-TA	SMAP28	TiBPs
	ZS37-37	ZS37-38			
● Promote healing of periodontal tissues	LL37	hBD-1	hBD-2	hBD-3	Histatin-2
● Promote healing of bone	AMPCol	Bacitracin	GL13K	hBD-2	Histatin-1
	oligopeptide	P-15	P15-CSP	TiBPs	
● Serve as potential indicator of the severity of periodontal diseases	hBD-2	HNP1	HNP2	HNP3	
Mucosal disease					
● Inactivate virus	LL37	hBD-2	hBD-3		
● Inhibit growth of fungi	LL37	Lactoferricin	hBD-1	hBD-2	hBD-3
	Histatin-5	HNP1	HNP2	HNP3	ToAP2
	dhvar4	dhvar5	KSL-W	LL37	hBD-1
● Serve as potential indicator of mucosal disease risk and treatment outcome	hBD-2	hBD-3	Histatin-5	HNP1	HNP2
	HNP3				
Oral cancer					
● Inhibit proliferation of tumour cells	LL37	$hCAP_{109–135}$			
● Serve as tumour suppressor gene in oral squamous cell carcinoma	hBD-1	hBD-2	HNP1	HNP2	HNP3
● Serve as protooncogenes in oral squamous cell carcinoma	hBD-2	hBD-3			
● Serve as tumour suppressor gene in salivary gland tumour	hBD-1				

Some synthesised antimicrobial peptides, such as VSL2 (Winfred et al. 2014), D-GL13K (Hirt et al. 2018) as well as PR39 and its fragments (Veldhuizen et al. 2014) showed activity against *E. faecalis* at different levels. Other kinds of antimicrobial peptides such as DJK-5 (Wang et al. 2017a) also showed activity against *E. faecalis* and have been evaluated to determine whether they could be used as a root canal irrigation agent in the *in vitro* condition. IDR-1002 is a synthetic peptide which has anti-inflammatory and antibacterial properties against *E. faecalis*. It is also a potential synergistic agent of ciprofloxacin in regenerative therapies (Sousa et al. 2020).

Among the abovementioned artificial antimicrobial peptides, targeting antimicrobial peptides is currently of major interest in research. It creates a new opportunity for antimicrobial peptide development. Given the property of amino acids, some functional groups could be designed or grafted to antimicrobial peptides for selective targeting purposes. For instance, antimicrobial peptide C16G2 has been synthesised to target *S. mutans* (Eckert et al. 2006). C16G2 consists of two functionally independent moieties conjoined in a linear peptide sequence: a nonspecific antimicrobial peptide serves as the killing moiety. Meanwhile, a species-specific binding peptide comprises the targeting moiety that provides specific binding to *S. mutans*. It facilitates the targeted delivery of the attached antimicrobial peptide. Previous studies have shown that C16G2 is potent against *S. mutans* grown in liquid or biofilm states (Kaplan et al. 2011). Data have also shown that several bacterial species with metabolic dependency or physical interactions with *S. mutans* suffered a drastic reduction in their abundance. Meanwhile, *S. mutans*' natural competitors, including health-associated *Streptococci*, became dominant after C16G2 treatment (Guo et al. 2015). Other studies involved developing tooth-binding antimicrobial peptides by grafting a hydroxyapatite-binding antimicrobial peptide to a broad-spectrum antimicrobial peptide domain. This was done

to achieve the long-term retention of antimicrobial peptides in the oral cavity (Huang et al. 2016; Zhang et al. 2019).

3 Prevention of periodontal disease

Periodontal disease is an inflammatory disease stemming from biofilms on periodontal support tissue and is also closely related to systemic health. Periodontal infections are usually mixed, most often involving such anaerobes as *Porphyromonas gingivalis*, *Prevotella intermedia, Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum*. In addition, it has been reported that 90% of all types of implants show signs of inflammation, and 50% of all implants show signs of irreversible tissue destruction (Mombelli et al. 2012). The main reason for the failure of dental implants is peri-implantitis, which is generally the result of pathogenic biofilms. Antimicrobial peptide research regarding periodontal disease has mainly focused on two aspects: its antibacterial effect and its role in the regulation of immune responses. Moreover, antimicrobial peptides have recently been used to improve implant performance, as they possess broad-spectrum antimicrobial activity, promote osteogenic differentiation and reduce bone loss.

3.1 Natural antimicrobial peptides

Natural antimicrobial peptides have shown antibacterial activity against periodontal pathogens. hBD-2 showed an approximately equal level of antibacterial activity to that of minocycline at equimolar concentrations (Ouhara et al. 2005). Meanwhile, the antibacterial activity of natural β-defensin is reduced by the increase in concentration of metallic ions, such as sodium and calcium ions (Bowdish et al. 2006; Gursoy and Kononen 2012). Similarly, LL37 suppressed the *P. gingivalis*-induced proinflammatory

responses of human gingival fibroblasts in a paracrine manner (Into et al. 2010). However, *P. gingivalis* can down-regulate LL37 production (Tada et al. 2017) and directly degrade synthetic LL37 (McCrudden et al. 2013). All of the above limit the role of antimicrobial peptides as a potential therapeutic in the gingival crevice.

Levels of natural antimicrobial peptide differ in healthy people and patients with periodontitis (Gorr 2012). The LL37 level in saliva was positively correlated with severe periodontal destruction in patients with chronic periodontitis. This increase might occur as an innate immune response against the periodontopathic *Treponema denticol* (Takeuchi et al. 2012). Interestingly, edentulous patients had the lowest salivary antimicrobial peptide levels, suggesting that gingival tissues may contribute to the secretion of peptides in the oral environment (Davidopoulou et al. 2013). In addition, the level of the antimicrobial peptide in salivary and gingival crevicular fluid is associated with systemic status such as diabetes (Yilmaz et al. 2018) or vitamin D deficiency (Bayirli et al. 2020). These situations might lead to lower antimicrobial peptide levels in the oral cavity, as well as an increased risk of periodontitis (Bayirli et al. 2020; Turkoglu et al. 2016; Yilmaz et al. 2018). In addition, pregnancy could affect the salivary concentrations of hBD-1, hBD-2 and HNP-1 (Gursoy et al. 2016).

Antimicrobial peptides also carry a broad range of immunomodulatory activities and contribute to the bacterial clearance of the host. Antimicrobial peptides also boost specific innate immune responses and exert selective immunomodulatory effects on the host. Antimicrobial peptides scavenge the endotoxin lipopolysaccharides (LPSs) to prevent the LPSs from binding the Toll-like receptor 4 (TLR4) and triggering inflammation (Lai and Gallo 2009). For instance, LL37 has been reported to inhibit the TLR4-induced secretion of TNF-α and IL-6, and it is involved in neutrophil recruitment

into an inflammatory site within diseased periodontal tissues (Montreekachon et al. 2014). The expression levels of hBD-1, -2 and -3 are also associated with that of TNF-α (Saitoh et al. 2004). Researchers proposed three models to describe the mechanism of antimicrobial peptides' immunomodulatory actions in mammalian cells, namely alternate ligand model, trans-activation model and membrane disruption model (Lai and Gallo 2009). In the alternate ligand model, antimicrobial peptides bind to a specific receptor to activate signal. In the trans-activation model, antimicrobial peptides stimulate the release of a membrane-bound ligand, and the ligand then binds to its receptor to activate signal. In the membrane disruption model, antimicrobial peptides modify the membrane containing the receptor and alter the membrane activity to activate signal.

Antimicrobial peptides also promote tissue healing. Among natural antimicrobial peptides, hBDs are expressed in oral bone (Warnke et al. 2006) and mediate tissue remodelling by increasing levels of metalloproteinases in articular cartilage Further studies have demonstrated that hBDs could promote the proliferation of human mesenchymal stem cells, osteoblasts and keratinocytes in cultures (Warnke et al. 2013). In addition, hBDs and histatin-1 promoted bone regeneration and prevented infection (Lee et al. 2018; Peng et al. 2020; Sun et al. 2020).

Studies have shown that alterations in antimicrobial peptide–related genes may also be associated with periodontal inflammation. A low hBD-2 genomic (*DEFB4*) copy number increased the risk of severe chronic periodontitis (Jaradat et al. 2013). In addition, the mutation of the gene p.S34N in CAMP (LL37-encoding gene) associated with generalised aggressive periodontitis (Turkoglu et al. 2011).

Furthermore, activating natural antimicrobial peptides through therapeutic methods is

a useful strategy for exploring the application of antimicrobial peptides for management of periodontal disease. Laser irradiation has been used to induce the expression of antimicrobial peptides (LL37 and hBD-2), which promotes *P. gingivalis* elimination and tissue healing (Kim et al. 2015; Tang et al. 2017). Applying Cyclosporin A can extend inflammation, lead to neutrophil infiltration and increase the LL37 level in gingival crevicular fluid (Turkoglu et al. 2015).

3.2 Artificial antimicrobial peptides

Researchers have introduced new synthetic antimicrobial peptides for management of periodontal disease. A novel peptide, P-113, derived from Histatin5 was biocompatible but highly degradable in serum, plasma and saliva (Oudhoff et al. 2010). A novel antimicrobial peptide called Nal-P-113 was synthesised through replacing the histidine residues using the bulky amino acid of β-naphthylalanine. Nal-P-113 has demonstrated an inhibitory effect on a *P. gingivalis*–induced local inflammatory response and alveolar bone loss (Wang et al. 2017b). In addition, hBD3-C15, derived from hBD3, has shown the potential to be a therapeutic agent for the inhibition of bone destruction (Park et al. 2017). Several synthesised cathelicidins from cattle (BMAP28), sheep (SMAP28 and SMAP29) and pigs (PMAP23) have shown broad-spectrum antimicrobial activities (Brogden et al. 2007). Meanwhile, SMAP28 has shown the most antibacterial activity but also the most cytotoxicity (Weistroffer et al. 2008). Some researchers used SMAP28 as a template to develop a 'bacteria-specific' antimicrobial peptide targeting *P. gingivalis*, but the results showed the peptide lacked specificity (Bratt et al. 2010). Thus, further work should focus on developing new targeting domains that specifically involve selecting *P. gingivalis* (Bratt et al. 2010). Other poly acid nanoparticles that BAR (a peptide derived from *S. gordonii*) has modified might represent a potential

therapeutic approach to limiting *P. gingivalis* colonisation in the oral cavity (Kalia et al. 2017).

Researchers used the surface coating of antimicrobial peptides to prevent peri-implantitis or to promote the osseointegration of the implant. *In vitro* and *in vivo studies* showed that the surface coating of antimicrobial peptides was effective in inhibiting bacterial adhesion and biofilm formation (Coelho et al. 2014). Moreover, the coating of antimicrobial peptides on the implant surface reduces the vascular distribution and potential cytotoxicity of antimicrobial peptides (Yucesoy et al. 2015). Coating strategies include bonding covalently antimicrobial peptides to a titanium or hydroxyapatite surface; coupling the titanium-binding peptide domain with the antimicrobial peptides; employing the layer-by-layer technique; and using antimicrobial peptide biohydrogels.

The covalently immobilised coating process has been used to bond antimicrobial peptides to titanium surfaces using silane coupling agents to produce coatings. Chen et al. developed a GL13K-coated titanium implant that has demonstrated the inhibition of biofilm growth. The coated implant promoted the adhesion and proliferation of human gingival fibroblasts. It guided the growth of the fibroblasts along with the microgrooves (Chen et al. 2014). Furthermore, the GL13K-coated titanium implant had a similar bone growth rate as that of gold-standard dental implants during the six-week period after implantation in rabbit femurs (Chen et al. 2017). Bifunctional chimeric peptides were developed by coupling the titanium-binding peptide domain with the antimicrobial peptides (Wisdom et al. 2020). Bifunctional chimeric peptides had titanium-binding affinity and were antimicrobial. The surfaces that bifunctional chimeric peptides modified inhibited the adhesion of *S. mutans, Staphylococcus epidermidis* and *Escherichia coli* (Yazici et al. 2016). Proper selection of linkers could optimise the

performance of chimeric peptides (Wisdom et al. 2016). Another study coated a synthetic antimicrobial peptide, namely AMPCol, on smooth titanium surfaces using the layer-by-layer technique (Shi et al. 2015). The coating provided the sustained release of the antimicrobial peptides for the prevention of peri-implantitis. In addition, researchers adopted biohydrogels for the release of antimicrobial peptide (Yang et al. 2018). Most biohydrogels require an antibacterial agent to enhance the antimicrobial activity. A study reported that the gel containing antimicrobial peptides formed a strong mechanical barrier. The barrier inhibited *P. gingivalis* on gingiva and titanium alloys (Mateescu et al. 2015).

4 Antifungal/virus property and protection against oral mucosal diseases

Oral mucosal diseases are often associated with autoimmune inflammatory conditions or infections from fungi or viruses. Antimicrobial peptides play an essential role in innate immunity (Ganz 2003) and help to prevent infections and maintain oral health. Research has focused on fostering an understanding of the involvement of antimicrobial peptides in mucosal disorders (Dale and Fredericks 2005). (Table 3).

Oral candidiasis is a common oral mucosal disease. In the *in vitro* condition, several natural antimicrobial peptides (e.g., HNP-1, hBD-2, hBD-3, LL37, histatin 5 and Lactoferricin B) have shown antifungal activity (Bates et al. 2017). Recombinant human interleukin 17A induces the secretion of hBD-2 and LL37 (Jiang et al. 2020). Synthetic peptides, such as ToAP2, have also shown anti-fungal activity against *C. albicans* (do Nascimento Dias et al. 2020). Furthermore, a study reported a library of linear peptoids with significant fungicidal activity against *C albicans* biofilms (Luo et al. 2017). In another study, hBD-2 and hBD-3 in adult oral epithelial cells inactivated the human immunodeficiency virus (HIV) by forming oligomers with virions. This

caused a reduction of HIV transmission through adult oral epithelium (Herrera et al. 2016).

Some antimicrobial peptides are related to oral mucosal diseases. HBDs, HNP-1 and LL37 have been identified at high levels from saliva samples of patients with oral lichen planus, Behçet's disease and recurrent aphthous ulcer. The levels of these antimicrobial peptides considerably decreased after treatment (Davidopoulou et al. 2014; Kucukkolbasi et al. 2013). LL37 exhibits a noticeable antiviral activity against Kaposi's sarcoma-associated herpesvirus, which indicates that LL37 could play an essential role in the host defence against oral Kaposi's sarcoma-associated herpesvirus (Brice et al. 2018). In addition, low salivary levels of HNPs have been shown to be related to oral ulcer activity in Behçet's disease (Mumcu et al. 2013). Furthermore, the increased expression of the HNP (DEFA4) in oral leukoplakia might characterise its potency in malignant transformation (Wenghoefer et al. 2010). Further studies should focus on the application of specific antimicrobial peptides as an indicator of the disease risk or treatment outcome measurement.

5 Some antimicrobial peptides are tumour suppressors

Interest in the development of antimicrobial peptides as anticancer agents is growing. Some cationic peptides from the Antimicrobial Peptide Database have shown selectivity towards tumour cells (Kuroda et al. 2015; Niu et al. 2021). This is due to the negative membrane potential over the cytoplasmic membranes of many types of cancer cells, which attract cationic peptides (Utsugi et al. 1991). However, the relationship between antimicrobial peptides and oral cancer remains unclear.

5.1 Natural antimicrobial peptides

The natural antimicrobial peptides such as hBDs may play different roles in oral cancer. The expression of hBD-1 showed a significant decrease in human oral squamous cell carcinoma cell line when compared with normal cells (Wenghoefer et al. 2008). In addition, hBD-1 deficiency promotes malignant transformation in pleomorphic adenomas. hBD-1 might be a potential tumour suppressor in oral squamous cell carcinoma and benign salivary gland tumours (Pantelis et al. 2009; Winter et al. 2011). In contrast, hBD-3 was considerably overexpressed in oral squamous cell carcinoma cells (Kesting et al. 2009) and might serve as proto-oncogenes (Winter et al. 2011). Interestingly, researchers have arrived at contrary findings on the effect of hBD-2 in stimulating oral squamous cell carcinoma cell lines (Kamino et al. 2014). The reason for this might be that hBD-2 on carcinoma cells is dependent on the concentration of the hBD-2 peptide and the types of cell lines (Kamino et al. 2014). HNPs' role in cancer is inconclusive. HNPs were overexpressed (greater than 12-fold) in the squamous cell carcinomas of the human tongue compared with nontumour tissue (Lundy et al. 2004). In the *in vitro* condition, HNP-1 simultaneously has an inhibitory effect on antitumorigenic matrix metallopeptidases-8 (MMP-8) and several protumorigenic matrix metallopeptidase (MMP-2 and -9) secretions of oral squamous cell carcinoma cells (Musrati et al. 2016). LL‐37 might act as a tumour suppressor in oral squamous cell carcinoma. The mechanisms may involve the induction of caspase-3 mediated apoptosis via the P53‐Bcl‐2/BAX signalling pathway (Chen et al. 2020). However, LL37 had no effect on the progression of squamous cell carcinoma on the tongue (Vierthaler et al. 2020).

5.2 Artificial antimicrobial peptides

hCAP109–135 derived from LL37-induced mitochondrial depolarisation and apoptosis in human oral squamous cell carcinoma cells. However, it did not induce apoptosis in healthy human cells in the *in vitro* condition (Okumura et al. 2004). No clear pattern currently exists in the relationship between antimicrobial peptides and oral cancer. However, evidence indicates that antimicrobial peptides might influence oral cancer via three mechanisms: as tumour suppressor genes, as an immune enhancer or as direct cytotoxic/anticancer agents. Antimicrobial peptides show potential for oral cancer treatment.

6 Conclusion

Antimicrobial peptides play an essential role in various oral diseases, such as dental caries, periodontal disease, mucosal disease and oral cancer. Antimicrobial peptides can kill pathogenic microorganisms, promote tissue healing, serve as biomarkers and inhibit tumour cells. Natural antimicrobial peptides are essential in immune resistance to oral diseases. Synthetic antimicrobial peptides are also a potential therapeutic strategy for managing oral diseases.

Ahn KB, Kim AR, Kum KY, Yun CH, Han SH. 2017. The synthetic human beta-defensin-3 c15 peptide exhibits antimicrobial activity against streptococcus mutans, both alone and in combination with dental disinfectants. J Microbiol. 55(10):830-836.

Aida KL, Kreling PF, Caiaffa KS, Calixto GMF, Chorilli M, Spolidorio DM, Santos-Filho NA, Cilli

EM, Duque C. 2018. Antimicrobial peptide-loaded liquid crystalline precursor bioadhesive system for the prevention of dental caries. Int J Nanomedicine. 13:3081-3091.

Alfenas CF, Mendes TAO, Ramos HJO, Bruckner FP, Antunes HS, Rocas IN, Siqueira JF, Jr., Provenzano JC. 2017. Human exoproteome in acute apical abscesses. J Endod. 43(9):1479-1485.

Andersson DI, Hughes D, Kubicek-Sutherland JZ. 2016. Mechanisms and consequences of bacterial resistance to antimicrobial peptides. Drug Resist Updat. 26:43-57.

Bates AM, Garaicoa JL, Fischer CL, Brogden KA. 2017. Diminished antimicrobial peptide and antifungal antibiotic activities against candida albicans in denture adhesive. Antibiotics (Basel). 6(1).

Bayirli BA, Ozturk A, Avci B. 2020. Serum vitamin d concentration is associated with antimicrobial peptide level in periodontal diseases. Arch Oral Biol. 117:104827.

Bechinger B, Gorr SU. 2017. Antimicrobial peptides: Mechanisms of action and resistance. J Dent Res. 96(3):254-260.

Bowdish DM, Davidson DJ, Hancock RE. 2006. Immunomodulatory properties of defensins and cathelicidins. Curr Top Microbiol Immunol. 306:27-66.

Bratt CL, Kohlgraf KG, Yohnke K, Kummet C, Dawson DV, Brogden KA. 2010. Communication: Antimicrobial activity of smap28 with a targeting domain for porphyromonas gingivalis. Probiotics Antimicrob Proteins. 2(1):21-25.

Brice DC, Toth Z, Diamond G. 2018. Ll-37 disrupts the kaposi's sarcoma-associated herpesvirus envelope and inhibits infection in oral epithelial cells. Antiviral Res.

158:25-33.

Brogden KA, Nordholm G, Ackermann M. 2007. Antimicrobial activity of cathelicidins bmap28, smap28, smap29, and pmap23 against pasteurella multocida is more broad-spectrum than host species specific. Vet Microbiol. 119(1):76-81.

Chen X, Hirt H, Li Y, Gorr SU, Aparicio C. 2014. Antimicrobial gl13k peptide coatings killed and ruptured the wall of streptococcus gordonii and prevented formation and growth of biofilms. PLoS One. 9(11):e111579.

Chen X, Ji S, Si J, Zhang X, Wang X, Guo Y, Zou X. 2020. Human cathelicidin antimicrobial peptide suppresses proliferation, migration and invasion of oral carcinoma hsc-3 cells via a novel mechanism involving caspase-3 mediated apoptosis. Mol Med Rep. 22(6):5243-5250.

Chen X, Zhou XC, Liu S, Wu RF, Aparicio C, Wu JY. 2017. In vivo osseointegration of dental implants with an antimicrobial peptide coating. J Mater Sci Mater Med. 28(5):76.

Chu CH, Wong SS, Suen RP, Lo EC. 2013. Oral health and dental care in hong kong. Surgeon. 11(3):153-157.

Coelho PG, Teixeira HS, Marin C, Witek L, Tovar N, Janal MN, Jimbo R. 2014. The in vivo effect of p-15 coating on early osseointegration. J Biomed Mater Res B Appl Biomater. 102(3):430-440.

Dale BA, Fredericks LP. 2005. Antimicrobial peptides in the oral environment: Expression and function in health and disease. Curr Issues Mol Biol. 7(2):119-133.

Davidopoulou S, Diza E, Sakellari D, Menexes G, Kalfas S. 2013. Salivary concentration of free ll-37 in edentulism, chronic periodontitis and healthy periodontium. Arch Oral Biol.

58(8):930-934.

Davidopoulou S, Theodoridis H, Nazer K, Kessopoulou E, Menexes G, Kalfas S. 2014. Salivary concentration of the antimicrobial peptide ll-37 in patients with oral lichen planus. J Oral Microbiol. 6:26156.

do Nascimento Dias J, de Souza Silva C, de Araujo AR, Souza JMT, de Holanda Veloso Junior PH, Cabral WF, da Gloria da Silva M, Eaton P, de Souza de Almeida Leite JR, Nicola AM et al. 2020. Mechanisms of action of antimicrobial peptides toap2 and ndbp-5.7 against candida albicans planktonic and biofilm cells. Sci Rep. 10(1):10327.

Dommisch H, Winter J, Acil Y, Dunsche A, Tiemann M, Jepsen S. 2005. Human beta-defensin (hbd-1, -2) expression in dental pulp. Oral Microbiol Immunol. 20(3):163-166.

Eckert R, He J, Yarbrough DK, Qi F, Anderson MH, Shi W. 2006. Targeted killing of streptococcus mutans by a pheromone-guided "smart" antimicrobial peptide. Antimicrob Agents Chemother. 50(11):3651-3657.

Fjell CD, Hiss JA, Hancock RE, Schneider G. 2011. Designing antimicrobial peptides: Form follows function. Nat Rev Drug Discov. 11(1):37-51.

Ganz T. 2003. Defensins: Antimicrobial peptides of innate immunity. Nat Rev Immunol. 3(9):710-720.

Goeke JE, Kist S, Schubert S, Hickel R, Huth KC, Kollmuss M. 2018. Sensitivity of caries pathogens to antimicrobial peptides related to caries risk. Clin Oral Investig. 22(7):2519-2525.

Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. 2008. Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain

reaction. J Endod. 34(5):537-540.

Gorr SU. 2012. Antimicrobial peptides in periodontal innate defense. Front Oral Biol. 15:84-98.

Guo L, McLean JS, Yang Y, Eckert R, Kaplan CW, Kyme P, Sheikh O, Varnum B, Lux R, Shi W et al. 2015. Precision-guided antimicrobial peptide as a targeted modulator of human microbial ecology. Proc Natl Acad Sci U S A. 112(24):7569-7574.

Guo YJ, Zhang B, Feng XS, Ren HX, Xu JR. 2016. Human cathelicidin ll-37 enhance the antibiofilm effect of egcg on streptococcus mutans. BMC Oral Health. 16(1):101.

Gursoy M, Gursoy UK, Liukkonen A, Kauko T, Penkkala S, Kononen E. 2016. Salivary antimicrobial defensins in pregnancy. J Clin Periodontol. 43(10):807-815.

Gursoy UK, Kononen E. 2012. Understanding the roles of gingival beta-defensins. J Oral Microbiol. 4.

Herrera R, Morris M, Rosbe K, Feng Z, Weinberg A, Tugizov S. 2016. Human beta-defensins 2 and -3 cointernalize with human immunodeficiency virus via heparan sulfate proteoglycans and reduce infectivity of intracellular virions in tonsil epithelial cells. Virology. 487:172-187.

Hirt H, Hall JW, Larson E, Gorr SU. 2018. A d-enantiomer of the antimicrobial peptide gl13k evades antimicrobial resistance in the gram positive bacteria enterococcus faecalis and streptococcus gordonii. PLoS One. 13(3):e0194900.

Ho YH, Shah P, Chen YW, Chen CS. 2016. Systematic analysis of intracellular-targeting antimicrobial peptides, bactenecin 7, hybrid of pleurocidin and dermaseptin, proline-arginine-rich peptide, and lactoferricin b, by using escherichia coli proteome

microarrays. Mol Cell Proteomics. 15(6):1837-1847.

Horibe K, Hosoya A, Hiraga T, Nakamura H. 2018. Expression and localization of cramp in rat tooth germ and during reparative dentin formation. Clin Oral Investig. 22(7):2559-2566.

Huang ZB, Shi X, Mao J, Gong SQ. 2016. Design of a hydroxyapatite-binding antimicrobial peptide with improved retention and antibacterial efficacy for oral pathogen control. Sci Rep. 6:38410.

Into T, Inomata M, Shibata K, Murakami Y. 2010. Effect of the antimicrobial peptide ll-37 on toll-like receptors 2-, 3- and 4-triggered expression of il-6, il-8 and cxcl10 in human gingival fibroblasts. Cell Immunol. 264(1):104-109.

Ito T, Ichinosawa T, Shimizu T. 2017. Streptococcal adhesin sspa/b analogue peptide inhibits adherence and impacts biofilm formation of streptococcus mutans. PLoS One. 12(4):e0175483.

Jaradat SW, Hoder-Przyrembel C, Cubillos S, Krieg N, Lehmann K, Piehler S, Sigusch BW, Norgauer J. 2013. Beta-defensin-2 genomic copy number variation and chronic periodontitis. J Dent Res. 92(11):1035-1040.

Jiang L, Fang M, Tao R, Yong X, Wu T. 2020. Recombinant human interleukin 17a enhances the anti-candida effect of human oral mucosal epithelial cells by inhibiting candida albicans growth and inducing antimicrobial peptides secretion. J Oral Pathol Med. 49(4):320-327.

Joly S, Maze C, McCray PB, Jr., Guthmiller JM. 2004. Human beta-defensins 2 and 3 demonstrate strain-selective activity against oral microorganisms. J Clin Microbiol.

42(3):1024-1029.

Jurczak A, Koscielniak D, Papiez M, Vyhouskaya P, Krzysciak W. 2015. A study on beta-defensin-2 and histatin-5 as a diagnostic marker of early childhood caries progression. Biol Res. 48:61.

Kalia P, Jain A, Radha Krishnan R, Demuth DR, Steinbach-Rankins JM. 2017. Peptide-modified nanoparticles inhibit formation of porphyromonas gingivalis biofilms with streptococcus gordonii. Int J Nanomedicine. 12:4553-4562.

Kamino Y, Kurashige Y, Uehara O, Sato J, Nishimura M, Yoshida K, Arakawa T, Nagayasu H, Saitoh M, Abiko Y. 2014. Hbd-2 is downregulated in oral carcinoma cells by DNA hypermethylation, and increased expression of hbd-2 by DNA demethylation and gene transfection inhibits cell proliferation and invasion. Oncol Rep. 32(2):462-468.

Kaplan CW, Sim JH, Shah KR, Kolesnikova-Kaplan A, Shi W, Eckert R. 2011. Selective membrane disruption: Mode of action of c16g2, a specifically targeted antimicrobial peptide. Antimicrob Agents Chemother. 55(7):3446-3452.

Kesting MR, Loeffelbein DJ, Hasler RJ, Wolff KD, Rittig A, Schulte M, Hirsch T, Wagenpfeil S, Jacobsen F, Steinstraesser L. 2009. Expression profile of human beta-defensin 3 in oral squamous cell carcinoma. Cancer Invest. 27(5):575-581.

Kim J, Kim S, Lim W, Choi H, Kim O. 2015. Effects of the antimicrobial peptide cathelicidin (ll-37) on immortalized gingival fibroblasts infected with porphyromonas gingivalis and irradiated with 625-nm led light. Lasers Med Sci. 30(8):2049-2057.

Krasone K, Lace B, Akota I, Care R, Deeley K, Kuchler EC, Vieira AR. 2014. Genetic variation in the promoter region of beta-defensin 1 (defb 1) is associated with high caries

experience in children born with cleft lip and palate. Acta Odontol Scand. 72(3):235-240.

Kreling PF, Aida KL, Massunari L, Caiaffa KS, Percinoto C, Bedran TB, Spolidorio DM, Abuna GF, Cilli EM, Duque C. 2016. Cytotoxicity and the effect of cationic peptide fragments against cariogenic bacteria under planktonic and biofilm conditions. Biofouling. 32(9):995-1006.

Krzysciak W, Jurczak A, Piatkowski J, Koscielniak D, Gregorczyk-Maga I, Kolodziej I, Papiez MA, Olczak-Kowalczyk D. 2015. Effect of histatin-5 and lysozyme on the ability of streptococcus mutans to form biofilms in in vitro conditions. Postepy Hig Med Dosw (Online). 69:1056-1066.

Kucukkolbasi H, Kucukkolbasi S, Ayyildiz HF, Dursun R, Kara H. 2013. Evaluation of hbetad-1 and hbetad-2 levels in saliva of patients with oral mucosal diseases. West Indian Med J. 62(3):230-238.

Kumar P, Kizhakkedathu JN, Straus SK. 2018. Antimicrobial peptides: Diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. Biomolecules. 8(1).

Kuroda K, Okumura K, Isogai H, Isogai E. 2015. The human cathelicidin antimicrobial peptide ll-37 and mimics are potential anticancer drugs. Front Oncol. 5:144.

Lai Y, Gallo RL. 2009. Amped up immunity: How antimicrobial peptides have multiple roles in immune defense. Trends Immunol. 30(3):131-141.

Lee JK, Park YJ, Kum KY, Han SH, Chang SW, Kaufman B, Jiang J, Zhu Q, Safavi K, Spangberg L. 2013. Antimicrobial efficacy of a human beta-defensin-3 peptide using an

enterococcus faecalis dentine infection model. Int Endod J. 46(5):406-412.

Lee PH, Chen MY, Lai YL, Lee SY, Chen HL. 2018. Human beta-defensin-2 and -3 mitigate the negative effects of bacterial contamination on bone healing in rat calvarial defect. Tissue Eng Part A. 24(7-8):653-661.

Lee SH, Baek DH. 2012. Antibacterial and neutralizing effect of human beta-defensins on enterococcus faecalis and enterococcus faecalis lipoteichoic acid. J Endod. 38(3):351-356.

Lei J, Sun L, Huang S, Zhu C, Li P, He J, Mackey V, Coy DH, He Q. 2019. The antimicrobial peptides and their potential clinical applications. Am J Transl Res. 11(7):3919-3931.

Lin LM, Ricucci D, Huang GT. 2014. Regeneration of the dentine-pulp complex with revitalization/revascularization therapy: Challenges and hopes. Int Endod J. 47(8):713-724.

Lundy FT, Orr DF, Gallagher JR, Maxwell P, Shaw C, Napier SS, Gerald Cowan C, Lamey PJ, Marley JJ. 2004. Identification and overexpression of human neutrophil alpha-defensins (human neutrophil peptides 1, 2 and 3) in squamous cell carcinomas of the human tongue. Oral Oncol. 40(2):139-144.

Luo Y, Bolt HL, Eggimann GA, McAuley DF, McMullan R, Curran T, Zhou M, Jahoda PC, Cobb SL, Lundy FT. 2017. Peptoid efficacy against polymicrobial biofilms determined by using propidium monoazide-modified quantitative pcr. Chembiochem. 18(1):111-118.

Mahlapuu M, Hakansson J, Ringstad L, Bjorn C. 2016. Antimicrobial peptides: An emerging category of therapeutic agents. Front Cell Infect Microbiol. 6:194.

Mateescu M, Baixe S, Garnier T, Jierry L, Ball V, Haikel Y, Metz-Boutigue MH, Nardin M, Schaaf

P, Etienne O et al. 2015. Antibacterial peptide-based gel for prevention of medical implanted-device infection. PLoS One. 10(12):e0145143.

McCrudden MT, Orr DF, Yu Y, Coulter WA, Manning G, Irwin CR, Lundy FT. 2013. LI-37 in periodontal health and disease and its susceptibility to degradation by proteinases present in gingival crevicular fluid. J Clin Periodontol. 40(10):933-941.

Mombelli A, Muller N, Cionca N. 2012. The epidemiology of peri-implantitis. Clin Oral Implants Res. 23 Suppl 6:67-76.

Montreekachon P, Nongparn S, Sastraruji T, Khongkhunthian S, Chruewkamlow N, Kasinrerk W, Krisanaprakornkit S. 2014. Favorable interleukin-8 induction in human gingival epithelial cells by the antimicrobial peptide ll-37. Asian Pac J Allergy Immunol. 32(3):251-260.

Mor A. 2000. Peptide-based antibiotics: A potential answer to raging antimicrobial resistance. DRUG DEVELOPMENT RESEARCH. 50:440-447.

Mumcu G, Cimilli H, Karacayli U, Inanc N, Ture-Ozdemir F, Eksioglu-Demiralp E, Ergun T, Direskeneli H. 2013. Salivary levels of hnp 1-3 are related to oral ulcer activity in behcet's disease. Int J Dermatol. 52(10):1198-1201.

Munther S. 2020. The impact of salivary lactoperoxidase and histatin-5 on early childhood caries severity in relation to nutritional status. Saudi Dent J. 32(8):410-416.

Musrati AA, Tervahartiala T, Gursoy M, Kononen E, Fteita D, Sorsa T, Uitto VJ, Gursoy UK. 2016. Human neutrophil peptide-1 affects matrix metalloproteinase-2, -8 and -9 secretions of oral squamous cell carcinoma cell lines in vitro. Arch Oral Biol. 66:1-7.

Nakashima M, Akamine A. 2005. The application of tissue engineering to regeneration of

pulp and dentin in endodontics. J Endod. 31(10):711-718.

Niu JY, Yin IX, Wu WKK, Li QL, Mei ML, Chu CH. 2021. Antimicrobial peptides for the prevention and treatment of dental caries: A concise review. Arch Oral Biol. 122:105022.

Okumura K, Itoh A, Isogai E, Hirose K, Hosokawa Y, Abiko Y, Shibata T, Hirata M, Isogai H. 2004. C-terminal domain of human cap18 antimicrobial peptide induces apoptosis in oral squamous cell carcinoma sas-h1 cells. Cancer Lett. 212(2):185-194.

Olsen CA. 2010. Peptoid-peptide hybrid backbone architectures. Chembiochem. 11(2):152-160.

Oudhoff MJ, Blaauboer ME, Nazmi K, Scheres N, Bolscher JG, Veerman EC. 2010. The role of salivary histatin and the human cathelicidin ll-37 in wound healing and innate immunity. Biol Chem. 391(5):541-548.

Ouhara K, Komatsuzawa H, Yamada S, Shiba H, Fujiwara T, Ohara M, Sayama K, Hashimoto K, Kurihara H, Sugai M. 2005. Susceptibilities of periodontopathogenic and cariogenic bacteria to antibacterial peptides, {beta}-defensins and ll37, produced by human epithelial cells. J Antimicrob Chemother. 55(6):888-896.

Pantelis A, Wenghoefer M, Haas S, Merkelbach-Bruse S, Pantelis D, Jepsen S, Bootz F, Winter J. 2009. Down regulation and nuclear localization of human beta-defensin-1 in pleomorphic adenomas of salivary glands. Oral Oncol. 45(6):526-530.

Paris S, Wolgin M, Kielbassa AM, Pries A, Zakrzewicz A. 2009. Gene expression of human beta-defensins in healthy and inflamed human dental pulps. J Endod. 35(4):520-523.

Park OJ, Kim J, Ahn KB, Lee JY, Park YJ, Kum KY, Yun CH, Han SH. 2017. A 15-amino acid c-

terminal peptide of beta-defensin-3 inhibits bone resorption by inhibiting the osteoclast differentiation and disrupting podosome belt formation. J Mol Med (Berl). 95(12):1315-1325.

Peng Y, Li L, Yuan Q, Gu P, You Z, Zhuang A, Bi X. 2020. Effect of bifunctional beta defensin 2-modified scaffold on bone defect reconstruction. ACS Omega. 5(8):4302-4312.

Saitoh M, Abiko Y, Shimabukuro S, Kusano K, Nishimura M, Arakawa T, Nakashima K, Takuma T, Kaku T, Igarashi S. 2004. Correlated expression of human beta defensin-1, -2 and -3 mrnas in gingival tissues of young children. Arch Oral Biol. 49(10):799-803.

Selwitz RH, Ismail AI, Pitts NB. 2007. Dental caries. Lancet. 369(9555):51-59.

Shao C, Zhu Y, Lai Z, Tan P, Shan A. 2019. Antimicrobial peptides with protease stability: Progress and perspective. Future Med Chem. 11(16):2047-2050.

Shi J, Liu Y, Wang Y, Zhang J, Zhao S, Yang G. 2015. Biological and immunotoxicity evaluation of antimicrobial peptide-loaded coatings using a layer-by-layer process on titanium. Sci Rep. 5:16336.

Shiba H, Mouri Y, Komatsuzawa H, Ouhara K, Takeda K, Sugai M, Kinane DF, Kurihara H. 2003. Macrophage inflammatory protein-3alpha and beta-defensin-2 stimulate dentin sialophosphoprotein gene expression in human pulp cells. Biochem Biophys Res Commun. 306(4):867-871.

Slebioda Z, Wozniak T, Dorocka-Bobkowska B, Wozniewicz M, Kowalska A. 2020. Beta-defensin 1 gene polymorphisms in the pathologies of the oral cavity-data from meta-analysis: Association only with rs1047031 not with rs1800972, rs1799946, and rs11362. J Oral Pathol Med.

Sousa MGC, Xavier PD, Cantuaria APC, Porcino RA, Almeida JA, Franco OL, Rezende TMB. 2020. Host defense peptide idr-1002 associated with ciprofloxacin as a new antimicrobial and immunomodulatory strategy for dental pulp revascularization therapy. Microb Pathog.104634.

Sun P, Shi A, Shen C, Liu Y, Wu G, Feng J. 2020. Human salivary histatin-1 (hst1) promotes bone morphogenetic protein 2 (bmp2)-induced osteogenesis and angiogenesis. FEBS Open Bio. 10(8):1503-1515.

Tada H, Shimizu T, Matsushita K, Takada H. 2017. Porphyromonas gingivalis-induced il-33 down-regulates hcap-18/ll-37 production in human gingival epithelial cells. Biomed Res. 38(3):167-173.

Takeuchi Y, Nagasawa T, Katagiri S, Kitagawara S, Kobayashi H, Koyanagi T, Izumi Y. 2012. Salivary levels of antibacterial peptide (ll-37/hcap-18) and cotinine in patients with chronic periodontitis. J Periodontol. 83(6):766-772.

Tang E, Khan I, Andreana S, Arany PR. 2017. Laser-activated transforming growth factor-beta1 induces human beta-defensin 2: Implications for laser therapies for periodontitis and peri-implantitis. J Periodontal Res. 52(3):360-367.

Tenovuo J. 2002. Clinical applications of antimicrobial host proteins lactoperoxidase, lysozyme and lactoferrin in xerostomia: Efficacy and safety. Oral Dis. 8(1):23-29.

Turkoglu O, Berdeli A, Emingil G, Atilla G. 2011. A novel p.S34n mutation of camp gene in patients with periodontal disease. Arch Oral Biol. 56(6):573-579.

Turkoglu O, Eren G, Emingil G, Azarsiz E, Kutukculer N, Atilla G. 2016. Does smoking affect gingival crevicular fluid ll-37 levels following non-surgical periodontal treatment in

chronic periodontitis? Arch Oral Biol. 61:98-105.

Turkoglu O, Gurkan A, Emingil G, Afacan B, Toz H, Kutukculer N, Atilla G. 2015. Are antimicrobial peptides related to cyclosporine a-induced gingival overgrowth? Arch Oral Biol. 60(3):508-515.

Utsugi T, Schroit AJ, Connor J, Bucana CD, Fidler IJ. 1991. Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. Cancer Res. 51(11):3062-3066.

van 't Hof W, Veerman EC, Helmerhorst EJ, Amerongen AV. 2001. Antimicrobial peptides: Properties and applicability. Biol Chem. 382(4):597-619.

Veldhuizen EJ, Schneider VA, Agustiandari H, van Dijk A, Tjeerdsma-van Bokhoven JL, Bikker FJ, Haagsman HP. 2014. Antimicrobial and immunomodulatory activities of pr-39 derived peptides. PLoS One. 9(4):e95939.

Vierthaler M, Rodrigues PC, Sundquist E, Siponen M, Salo T, Risteli M. 2020. Fluctuating role of antimicrobial peptide hcap18/ll37 in oral tongue dysplasia and carcinoma. Oncol Rep. 44(1):325-338.

Vlieghe P, Lisowski V, Martinez J, Khrestchatisky M. 2010. Synthetic therapeutic peptides: Science and market. Drug Discov Today. 15(1-2):40-56.

Wang D, Shen Y, Ma J, Hancock REW, Haapasalo M. 2017a. Antibiofilm effect of d-enantiomeric peptide alone and combined with edta in vitro. J Endod. 43(11):1862-1867.

Wang G. 2014. Human antimicrobial peptides and proteins. Pharmaceuticals (Basel). 7(5):545-594.

Wang HY, Lin L, Fu W, Yu HY, Yu N, Tan LS, Cheng JW, Pan YP. 2017b. Preventive effects of the novel antimicrobial peptide nal-p-113 in a rat periodontitis model by limiting the growth of porphyromonas gingivalis and modulating il-1beta and tnf-alpha production. BMC Complement Altern Med. 17(1):426.

Warnke PH, Springer IN, Russo PA, Wiltfang J, Essig H, Kosmahl M, Sherry E, Acil Y. 2006. Innate immunity in human bone. Bone. 38(3):400-408.

Warnke PH, Voss E, Russo PA, Stephens S, Kleine M, Terheyden H, Liu Q. 2013. Antimicrobial peptide coating of dental implants: Biocompatibility assessment of recombinant human beta defensin-2 for human cells. Int J Oral Maxillofac Implants. 28(4):982-988.

Wattanarat O, Nirunsittirat A, Piwat S, Manmontri C, Teanpaisan R, Pahumunto N, Makeudom A, Sastraruji T, Krisanaprakornkit S. 2020. Significant elevation of salivary human neutrophil peptides 1-3 levels by probiotic milk in preschool children with severe early childhood caries: A randomized controlled trial. Clin Oral Investig.

Weistroffer PL, Joly S, Srikantha R, Tack BF, Brogden KA, Guthmiller JM. 2008. Smap29 congeners demonstrate activity against oral bacteria and reduced toxicity against oral keratinocytes. Oral Microbiol Immunol. 23(2):89-95.

Wenghoefer M, Pantelis A, Dommisch H, Reich R, Martini M, Allam JP, Novak N, Berge S, Jepsen S, Winter J. 2008. Decreased gene expression of human beta-defensin-1 in the development of squamous cell carcinoma of the oral cavity. Int J Oral Maxillofac Surg. 37(7):660-663.

Wenghoefer M, Pantelis A, Najafi T, Deschner J, Allam JP, Novak N, Reich R, Martini M, Berge S, Fischer HP et al. 2010. Gene expression of oncogenes, antimicrobial peptides, and

cytokines in the development of oral leukoplakia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 110(3):351-356.

Winfred SB, Meiyazagan G, Panda JJ, Nagendrababu V, Deivanayagam K, Chauhan VS, Venkatraman G. 2014. Antimicrobial activity of cationic peptides in endodontic procedures. Eur J Dent. 8(2):254-260.

Winter J, Pantelis A, Reich R, Martini M, Kraus D, Jepsen S, Allam JP, Novak N, Wenghoefer M. 2011. Human beta-defensin-1, -2, and -3 exhibit opposite effects on oral squamous cell carcinoma cell proliferation. Cancer Invest. 29(3):196-201.

Wisdom C, VanOosten SK, Boone KW, Khvostenko D, Arnold PM, Snead ML, Tamerler C. 2016. Controlling the biomimetic implant interface: Modulating antimicrobial activity by spacer design. J Mol Eng Mater. 4(1).

Wisdom EC, Zhou Y, Chen C, Tamerler C, Snead ML. 2020. Mitigation of peri-implantitis by rational design of bifunctional peptides with antimicrobial properties. ACS Biomater Sci Eng. 6(5):2682-2695.

Wu L, Li Z, Zhou J, Ma B, Yu F, Zheng X, Hu X, Ma Z, Su X. 2020. An association analysis for genetic factors for dental caries susceptibility in a cohort of chinese children. Oral Dis.

Yang K, Han Q, Chen B, Zheng Y, Zhang K, Li Q, Wang J. 2018. Antimicrobial hydrogels: Promising materials for medical application. Int J Nanomedicine. 13:2217-2263.

Yazici H, O'Neill MB, Kacar T, Wilson BR, Oren EE, Sarikaya M, Tamerler C. 2016. Engineered chimeric peptides as antimicrobial surface coating agents toward infection-free implants. ACS Appl Mater Interfaces. 8(8):5070-5081.

Yilmaz D, Caglayan F, Buber E, Kononen E, Aksoy Y, Gursoy UK, Guncu GN. 2018. Gingival crevicular fluid levels of human beta-defensin-1 in type 2 diabetes mellitus and periodontitis. Clin Oral Investig. 22(5):2135-2140.

Yucesoy DT, Hnilova M, Boone K, Arnold PM, Snead ML, Tamerler C. 2015. Chimeric peptides as implant functionalization agents for titanium alloy implants with antimicrobial properties. JOM (1989). 67(4):754-766.

Zhai Y, Yuan X, Zhao Y, Ge L, Wang Y. 2020. Potential application of human beta-defensin 4 in dental pulp repair. Front Physiol. 11:1077.

Zhang LY, Fang ZH, Li QL, Cao CY. 2019. A tooth-binding antimicrobial peptide to prevent the formation of dental biofilm. J Mater Sci Mater Med. 30(4):45.