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Review

Human salivary proteins and their peptidomimetics: Values of function, early diagnosis, and therapeutic potential in combating dental caries

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ABSTRACT

Saliva contains a large number of proteins that play various crucial roles to maintain the oral health and tooth integrity. This oral fluid is proposed to be one of the most important host factors, serving as a special medium for monitoring aspects of microorganisms, diet and host susceptibility involved in the caries process. Extensive salivary proteomic and peptidomic studies have resulted in considerable advances in the field of biomarkers discovery for dental caries. These salivary biomarkers may be exploited for the prediction, diagnosis, prognosis and treatment of dental caries, many of which could also provide the potential templates for bioactive peptides used for the biomimetic management of dental caries, rather than repairing caries lesions with artificial materials. A comprehensive understanding of the biological function of salivary proteins as well as their derived biomimetic peptides with promising potential against dental caries has been long awaited. This review overviewed a collection of current literature and addressed the majority of different functions of salivary proteins and peptides with their potential as functional biomarkers for caries risk assessment and clinical prospects for the anti-caries application.

1. Introduction

Human saliva contains a large array of proteins, many of which possess a distinct biological function to maintain the homeostasis of the oral cavity system. Alternative splicing and post-translation modifications occurring in the course of gene expression lead to the formation of salivary proteins with various structures. Since Krasnow and Oblatt (Krasnow & Oblatt, 1933) firstly investigated the variation in salivary protein concentration in different individuals, there has been increasing interest in the application of salivary analyses to monitor general health. The National Institute of Dental and Craniofacial Research (NIDCR) started to fund three research groups comprising the Saliva Proteome Consortium in 2004, and they made an effort to identify and catalogue the human saliva proteome including saliva proteins as well as their structurally modified forms (Katsiougiannis & Wong, 2016). With the rapid development of proteomic technology and application of high resolution MS, in-depth proteomic analysis for the salivary protein polymorphisms has recently been achievable (Si, Ao, Wang, Chen, & Zheng, 2015; Sun et al., 2016; Wang, Wang, Wang et al., 2018). These studies revealed the salivary proteome as a sizeable collection of up to 1166 proteins, including 914 in parotid and 917 in submandibular/sublingual saliva (Denny et al., 2008). The majority of these proteins are synthesized and secreted into the oral cavity by the acinar cells of the salivary glands, which can be divided into a few families: proline-rich proteins (PRPs), salivary mucins, salivary α-amylase, salivary cystatins, histatins (small cationic histidine-rich peptides), and statherin and P−B peptide (Amado, Lobo, Domingues, Duarte, & Vitorino, 2010; Cabras et al., 2014; Gonzalez-Begne et al., 2009; Zhang, Sun, Wang, & Wang, 2013). The above findings have promoted the gaining of knowledge regarding the association between salivary protein composition and human health, and also provided a promising result in utilizing saliva to explore biomarkers for diagnosis purposes. The non-invasive and simple nature of saliva collection made it interesting to be used for the early diagnosis and risk assessment of a variety of oral diseases, such as Sjögren's syndrome, oral squamous cell carcinoma, periodontitis, and dental caries (Gallo et al., 2016; Hall et al., 2017; Nomura et al., 2012; Wang, Wang, Wang et al., 2018). With an expectedly increasing number of salivary protein species to be identified in the near future, now is the time to devote more attention to the comprehension of their function and to the application of their clinical practice.

Dental caries is one of the most common chronic diseases afflicting a large proportion of the world's population, involved in interactions between the tooth structure, the microbial biofilm formed on the tooth

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surface, as well as genetic influences and salivary function (Petersen, 2003; Pitts et al., 2017). Saliva contains a variety of proteins participating in maintaining the tooth integrity and preventing caries through several mechanisms: I) formation of acquired enamel pellicle to continuously protect against tooth wear (mucins and proline-rich glycoprotein); II) inhibition of demineralization of exposed tooth surfaces (mucins); III) promotion of enamel remineralization by attracting calcium ions (proline-rich proteins and statherin); IV) antimicrobial activities including prevention of cariogenic species adherence onto enamel surface (histatins and cystatins), microorganism aggregation and clearance from the oral cavity (agglutinin and immunoglobulins), and the secretion of antimicrobial peptides (AMPs) (Gao, Jiang, Koh, & Hsu, 2016; Guo & Shi, 2013; Van Nieuw Amerongen, Bolscher, & Veerman, 2004). Currently, the biological functions of salivary proteins have been extensively studied for their possible relevance to caries risk assessment. For example, low levels of statherin and truncated cystatin S in saliva are found to be associated with caries susceptibility (Rudney, Staikov, & Johnson, 2009). This enhances knowledge of the structure-function relationship of salivary proteins, making it possible to identify potential biomarkers for dental caries, and further to design small, biologically active peptides as instruments to fight against caries, as well as restore functionality in patients in whom the natural protection is compromised.

This review aims to summarize the following: (1) the protective function of major salivary proteins in cariology; (2) the identification of salivary proteins utilized as biomarkers for caries risk assessment; (3) bioactive peptides derived from salivary proteins for the therapeutic use against dental caries.

2. Protective functions of salivary proteins and their potentials as biomarkers for caries risk assessment

Saliva contains a large number of secreted proteins, including major salivary glycoproteins (proline-rich proteins, mucins and immunoglobulins), minor salivary proteins (cystatins, lysozyme, lactoferrin, agglutinin, and amylase), AMPs (histatins, cathelicidin peptide LL-37, alpha-defensins, and beta-defensins), statherin and P-B peptide, which protect the tooth integrity through either against losing calcium and phosphate ions from the enamel surface or playing antimicrobial role directly or indirectly. The most common salivary proteins and their protective properties against dental caries are listed in Table 1. These molecules as well as their post-translational modifications with specific function may be better indicators of oral diseases, and could provide a promising repertoire in utilizing saliva to explore more sensitive and specific biomarkers for dental caries (Al-Tarawneh, Border, Dibble, & Bencharit, 2011). Initiation of salivary biomarker discovery and expanding the dataset of carious patients will help to establish a concise model for the early diagnosis and measurement of dental caries.

2.1. Major salivary glycoproteins

Major salivary glycoproteins account for approximately 50% of total salivary proteins, including proline-rich proteins (15–20%), mucous glycoproteins (20–30%), and immunoglobulins (5–15%).

2.1.1. Proline-rich proteins

The human salivary proline-rich proteins (PRPs) are a heterogeneous group of proteins produced from the parotid, submandibular, and sublingual salivary glands, which are abundant in saliva and constitute about 15–20% (w/w) of total proteins in whole human saliva and more than 50–60% (w/w) of proteins in parotid saliva (Kim et al., 1993; Manconi et al., 2016). PRPs are coded by a multi-family of six different genes (*PRH1*, *PRH2*, *PRB1*, *PRB2, PRB3*, and *PRB4*), strictly associated in a segment of ˜4.0 Kb in length on chromosome 12 at band 13.2, and a large number of PRPs have been discovered by differential RNA splicing and proteolytic cleavages after secretion (Kim et al., 1993; Maeda, Kim, Azen, & Smithies, 1985; Scherer et al., 2006). They are mainly classified into three groups: acidic PRPs (aPRPs), basic PRPs (bPRPs), and glycosylated PRPs (gPRPs) (Bennick, 1987; Schenkels, Veerman, & Amerongen, 1995). Accounting for 25–30% of all salivary proteins, the aPRPs possess a 30-amino acid N-terminal domain rich in aspartate and glutamate with a few serine phosphate residues, which are involved in the formation of the acquired enamel pellicle and act as salivary receptors for several plaque-forming bacteria (Amano et al., 1994). aPRPs binding to hydroxyapatite involves the acidic N-terminal domain and exposes the proline-rich C-terminal domain to oral bacteria binding (Manconi et al., 2016). Also, aPRPs (PRP-3 and its derived peptides) play a key role in the protection of tooth enamel through inhibiting calcium phosphate precipitation and thus promoting calcium homeostasis in oral cavity (Hay, Carlson, Schluckebier, Moreno, & Schlesinger, 1987; Vitorino et al., 2007). The family of bPRPs and gPRPs, encoded by the polymorphic *PRB1-PRB4* genes, includes 11 parent peptides/proteins and more than 6 parent glycosylated proteins, but a higher number of proteoforms with similar structures derived from polymorphisms and post-translational modifications (Padiglia et al., 2018). More recently, 55 new components of the family were extensively characterized by top-down liquid chromatography-mass spectrometry, bringing the total number of proteoforms to 109 (Padiglia et al., 2018). The significant structural differences presenting in the class of salivary PRPs suggest their crucial role in the oral protection. Some bPRP fragments are involved in enamel pellicle formation (PRB-1, IB-1, IB-8a, and IB-9), and others exhibit the ability to modulate the oral flora (Ruhl, Sandberg, & Cisar, 2004; Vitorino et al., 2007). Some other bPRPs (IB-7) could attach to a major adhesion antigen on the surface of *Streptococcus mutans* and other oral streptococci, whose arginine and lysine residues neutralize acids from carbohydrate metabolism within the biofilm (Levine, 2011; Nobbs, Jenkinson, & Jakubovics, 2011). In addition, the gPRPs is able to interact particularly with several types of microorganisms, such as *Fusobacterium nucleatum,* and is involved in the plaque formation (Kolenbrander & London, 1993; Schenkels et al., 1995).

In terms of the association between caries prevalence and PRPs, contradictory results were reported in previous studies. A cross-sectional study showed that subjects with high DMFT indices presented significant reduction of salivary aPRP (PRP-1), mucin 5B, and mucin 7 (Banderas-Tarabay, Zacarias-D'Oleire, Garduño-Estrada, Aceves-Luna, & González-Begné, 2002). Similarly, Vitorino et al. evaluated differential protein expression patterns in the whole saliva of caries-free and caries-susceptible individuals using 2-DE combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and found significantly higher quantities of aPRPs in caries-free samples (Vitorino et al., 2006). Based on iTRAQ and MRM analysis, our recent study found significant lower amounts of bPRPs (PRB2) in saliva samples from caries-susceptible children (Wang, Wang, Wang et al., 2018). On the contrary, Ribeiro et al. identified that the presence of proline-rich peptides IB-4 could significantly increase the risk of caries (Ribeiro et al., 2013). Another report by Bhalla et al. also observed a higher number of PRP bands in saliva of subjects with caries (Bhalla, Tandon, & Satyamoorthy, 2010). However, no obvious difference in the salivary levels of aPRPs was reported in other studies between the caries-free and caries-resistant subjects (Dodds, Johnson, Mobley, & Hattaway, 1997; Mandel & Bennick, 1983).

2.1.2. Mucous glycoproteins

Human saliva contains two structurally and functionally distinct populations of mucous glycoproteins (mucins), the high-molecular-weight mucins (MG1, molecular mass > 1000 kDa), and low-molecular-weight mucins (MG2, molecular mass 150–200 kDa) (Amerongen & Veerman, 2002). Although secreted mucins are components of the non-immune oral host defense system, they are considered the first line of defense for the oral health (Tabak, 1995). Mucins are identified as acid-resistant proteins since it is still present in the acquired pellicle after

Table 1
Main properties of salivary proteins against dental caries.

Salivary proteins	Sources	Main functions	Reference
Proline-rich proteins	Parotid, submandibular, and sublingual glands	Remineralization of enamel, formation of AEP, neutralization of acids within the biofilm	Amano et al. (1994), Hay et al. (1987), Kim et al. (1993), Levine (2011), Manconi et al. (2016), Nobbs et al. (2011), Vitorino et al. (2007)
Mucins	Submandibular, sublingual, and labial glands	Inhibition of demineralization, agglutination of microorganisms, barrier protection, resistance of acid, decrease attachment of *S. mutans*	Buzalaf et al. (2012), Delecrode et al. (2015), Frenkel & Ribbeck (2015), Jordão et al. (2017), Levine et al. (1978)
Immunoglobulins	Major and minor salivary glands	Adaptive immune defense against bacterial, inhibition of bacterial adherence and colonization, microorganism aggregation and clearance, enhance the activity of lactoferrins and lysozymes	Law et al. (2007), Russell et al. (1999), Van Nieuw Amerongen et al. (2004)
Cystatins	Parotid, submandibular gland, and gingival crevicular	Protease inhibitor, formation of AEP, remineralization of enamel, bactericidal property	Baron et al. (1999), Dickinson (2002a), Dickinson (2002b), Lamkin et al. (1991), Vitorino et al. (2008)
Lysozyme	Major and minor salivary glands, neutrophil granulocytes, and gingival crevicular fluid	Antibacterial, cell wall lysis, inhibition of biofilm formation and *S. mutans* adhesion on HA	Hatti et al. (2007), Kho et al. (2005) Krzysciak et al. (2015), Moslemi et al. (2015)
Lactoferrin	Major and minor salivary glands, neutrophil granulocytes, and mucosal epithelial cells	Antimicrobial activity, immunomodulatory activity, cell growth modulation, iron homeostasis regulation, bacterial binding, agglutination of *S. mutans*	Baveye et al. (1999), Chierici (2001), Gorr & Abdolhosseini (2011), Lenander-Lumikari & Loimaranta (2000), Moslemi et al. (2015), Velusamy et al. (2016)
Agglutinin	Parotid, submandibular and sublingual glands	Microorganism aggregation and clearance	Bikker et al. (2002), Ligtenberg et al. (2010)
Amylase	Major and minor salivary glands	Formation of AEP, modulation of bacterial colonization, adhesion, and dental plaque formation	Scannapieco et al. (1993), Singh et al. (2015)
Histatins	Parotid and submandibular salivary duct cells	Formation of AEP, antibacterial and antifungal activities, regulation of oral hemostasis, adsorption on enamel surfaces, reduce *S. mutans* adhesion onto HA	Fernández-Presas et al. (2018), Gorr (2009), Oppenheim et al. (1986), Oudhoff et al. (2009), Richardson et al. (1993), Shimotoyodome et al. (2006)
LL-37	Salivary glands, epithelial cells, neutrophil leukocytes and gingival crevicular fluid	Antibacterial and antifungal activity	Isogai, Isogai et al. (2003, 2003b), Lo´ pez-Garcı´a et al. (2005), Zanetti et al. (2002)
Defensins	Neutrophil granulocytes, epithelial cells, gingival crevicular fluid	Microbicidal activity and antiviral activity	Ganz et al. (1985), Lehrer & Ganz (1999)
C-C motif chemokine 28	Salivary glands, epithelial cells	Antimicrobial activities, homing of certain types of lymphocytes, chemoattractant of IgA	Allen et al. (2007), Denny et al. (2008), Hieshima et al. (2003), Jiang et al. (2012)
Azurocidin	Azurophil granules of neutrophils	Antibacterial activity, strong affinity for LPS	Dhaifalah et al. (2014)
Statherin	Major and minor salivary glands	Formation of AEP, remineralization of enamel, inhibition of the spontaneous precipitation of calcium and phosphate salts, inhibition of *S. mutans* adhesion onto HA, antimicrobial activity	Shimotoyodome et al. (2006), Trindade & Amado (2015), Xiao et al. (2015)

exposure to citric acid (Delecrode et al., 2015). The protective effect of mucins may be attributed to its high viscosity or chemical composition. Jordão et al. reported that artificial saliva with mucins could protect sound enamel against demineralization similarly to human saliva in situ (Jordão et al., 2017). MG1 is the primary mucin component of the dental pellicle coating the soft and hard tissues in the oral cavity, which provides an attachment site for bacteria and affects the adhesion of specific bacteria onto the tooth surface (Buzalaf, Hannas, & Kato, 2012). In spite that mucin 5B does not alter *S. mutans* growth, it could decrease surface attachment and biofilm formation by maintaining *S. mutans* in the planktonic form (Frenkel & Ribbeck, 2015). As an important antimicrobial salivary agent, MG2 can agglutinate *S. mutans* and *S. sanguis* via promoting the clearance of them from the oral cavity (Levine et al., 1978). However, mucins have also been shown to provide nutrients for a variety of bacterial species that inhabit mucosal surfaces (Derrien et al., 2010). Some previous studies found that *S. mutans* could scavenge nutrients from mucins to enhance its growth and survival (Mothey, Buttaro, & Piggot, 2014; Renye, Piggot, Daneo-Moore, & Buttaro, 2004).

The importance of mucin 5B and mucin 7 has been the focus of much research in the past two decades (Humphrey & Williamson, 2001). The presence or absence of mucin 5B and mucin 7 in the oral cavity could alter individuals' susceptibility to dental cavity formation, which could then be an easily accessible and highly predictable clinical diagnostic biomarker of dental caries. The importance of mucin 7 in caries prevention has been demonstrated in elderly populations, in whom the diminished levels of mucin 7 were found to be significantly associated with elevated *S. mutans* titers in saliva (Baughan, Robertello, Sarrett, Denny, & Denny, 2000). In addition, salivary mucins expression in relation to dental caries was also found to be different between preschool and school children (Angwaravong, Pitiphat, Bolscher, & Chaiyarit, 2015). Both mucin 5B and mucin 7 showed up-regulated levels in caries-susceptible children in response to dental caries (Wang, Wang, Wang et al., 2018). For adolescents, the salivary mucin 1 and mucin 5B levels were significant higher in subjects with high intensity of dental caries (DMF > 11) compared to those with low DMF (Gabryel-Porowska et al., 2014).

2.1.3. Immunoglobulins

The salivary immunoglobulins constitute 5–15% of whole salivary proteins, belonging primarily to the IgA subclass and to a lesser extent to the IgG and IgM subclass (Van Nieuw Amerongen et al., 2004). The secretory IgA immune response represents the first line of adaptive immune defense against mutans streptococci. SIgA composes 60% of the immunoglobulin in saliva, which plays antimicrobial roles through neutralizing bacterial toxins and enzymes, inhibiting bacterial adherence and colonization, reducing the hydrophobicity of bacteria and aggregating the bacteria together (Russell, Hajishengallis, Childers, & Michalek, 1999; Van Nieuw Amerongen et al., 2004). Salivary IgA has also been shown to enhance the activity of several enzymes, such as lactoferrins and lysozymes (Law, Seow, & Townsend, 2007).

Previous investigations have reported contradictory results in the field regarding the association of salivary levels of sIgA and dental caries. A systematic review and meta-analysis presented evidence that

supported the presence of increased sIgA levels in caries-active subjects based on 314 abstracts (Fidalgo et al., 2014). Likewise, a recent study collected saliva samples from 70 children and 43 elderly adults with and without dental caries, and demonstrated a significantly higher level of sIgA in both children with severe early childhood caries (ECC) and the elderly subjects with root caries (RC) using qPCR and ELISA (Yang et al., 2015). On the contrary, Colombo et al. in a more recent study explored positive correlations between salivary IgA antibody response against *S. mutans* GbpB and *S. mutans* counts, and found children with severe ECC had significantly lower IgA antibody levels to *S. mutans* GbpB (Colombo, Pereira et al., 2016). However, no significant correlation between IgA concentration and caries activity was observed in other reports (Koga-Ito, Martins, Balducci, & Jorge, 2004; Shifa, Muthu, Amarlal, & Rathna Prabhu, 2008). Salivary IgG functions in the protection of oral cavity by inhibiting the growth, adherence and acid production of *S. mutans* (Gregory, Kindle, Hobbs, Filler, & Malmstrom, 1990). Controversy still remains in research regarding IgG and IgM as biomarkers for caries. A cross-sectional study exhibited a higher salivary IgG level among children with ECC (Bagherian, Jafarzadeh, Rezaeian, Ahmadi, & Rezaity, 2008). Another study supported the correlation between higher levels of total salivary IgA and IgG and children with ECC, but no significant difference in total IgM level was found between children with and without ECC (de Farias & Bezerra, 2003). Based on the evidence currently available, the diagnostic value of salivary immunoglobulins as biomarker for caries risk has not yet been ascertained.

2.2. Minor salivary proteins

Besides the major proteins, saliva is abundant in lots of proteins with relatively lower concentrations, many of which are enzymes exerting significant biological activity even in low levels.

2.2.1. Cystatins

Cystatins are a superfamily of cysteine-peptidase inhibitors with various proteoforms, including type 1 cystatins (cystatins A and B), type 2 cystatins (C, D, E, F, S, SN, SA), type 3 cystatins, or kininogens (Manconi et al., 2017). The major “S-type” cystatins belong to type 2 comprising cystatin S, SN and SA with a molecular mass ranging from 13.5 to 14.5 kDa, and they participate in multi-functions in the oral cavity (Dickinson, 2002b). Cystatin S may be either monophosphorylated on Ser_3 (cystatin S1, accounting for 60–70% of total cystatin S forms) or diphosphorylated on Ser_1 and Ser_3 (cystatin S2, accounting for 20–30%) (Messana et al., 2008; Vitorino et al., 2004). Besides of modified forms identified in cystatin S, cystatin B in saliva is major present as S-modified derivatives, namely S-glutathionylated, S-cysteinylated, and S-S 2-mer (Cabras et al., 2012). “S-type” cystatins are major components of the acquired enamel pellicle and their phosphorylated forms have capacity of binding to hydroxyapatite (HA), which is reduced but not eliminated by dephophorylation (Dickinson, 2002a; Lamkin, Jensen, Setayesh, Troxler, & Oppenheim, 1991; Vitorino, Calheiros-Lobo, Duarte, Domingues, & Amado, 2008). They also inhibit calcium phosphate precipitation and control the process of enamel remineralization (Dickinson, 2002a). Besides, type-2 cystatins also exhibit strong bactericidal and virucidal properties (Baron, Gansky, Ryder, & Featherstone, 1999; Dickinson, 2002a). Cystatin S, a putative indicator of caries, was present at a higher level in elderly with RC in a previous study (Preza, Thiede, Olsen, & Grinde, 2009). In contrast, a strong correlation between cystatin S, cystatin SN2, cystatin SAIII and the lowest values of DMFT was reported in another work (Vitorino et al., 2006). Consistent with this result, our previous study also found down-regulated levels of cystatin S and cystatin SN in high caries-susceptible children using MRM validation, indicating their importance in the maintenance of teeth integrity (Wang, Wang, Wang et al., 2018). Using a modified proteomic approach, Rudney et al. suggested that levels of a truncated cystatin S missing the first eight N-terminal amino acids might be potential risk indicators for caries development (Rudney et al., 2009).

2.2.2. Lysozyme

In the oral cavity, lysozyme is an antibacterial enzyme, especially against gram-positive bacteria, which is secreted from major and minor salivary glands, gingival crevicular fluid, and salivary leukocytes (Moslemi et al., 2015). It also has the ability to bind to gram-negative bacteria lipopolysaccharides (LPS), thus contributing to the reduced risk of development of an inflammatory response when high amounts of exotoxin occur in the organism (Krzysciak et al., 2015). Due to the biological activity of lysozyme, the ability of cariogenic microorganisms toward the formation of biofilm is inhibited, and also the adhesion of *S. mutans* to HA is reduced (Hatti, Ravindra, Satpathy, Kulkarni, & Parande, 2007; Kho, Vacca Smith, Koo, Scott-Anne, & Bowen, 2005). Apart from the antibacterial effect, lysozyme also exhibits antiviral and antifungal properties (Krzysciak et al., 2015).

Various studies in the past have attempted to relate salivary lysozyme and caries activity. Mass et al. found an association between low levels of lysozyme and decreased amounts of *S. mutans* and lactobacilli (Mass, Gadoth, Harell, & Wolff, 2002). A recent study demonstrated significantly increased lysozyme level in caries-free children compared with ECC children, and suggested reduced amounts of lysozyme may be risk factor for childhood caries (Moslemi et al., 2015). Supporting this claim, lower levels of salivary lysozyme were correlated with low caries increment over 4 years of 28 adults by cluster analysis (Jentsch, Beetke, & Gocke, 2004). However, equivocal conclusions of the resemblance of salivary lysozyme between caries-active samples and caries-free controls were reported in some other studies. Stuchell and Mandel found no significant difference in lysozyme concentrations between caries-free and caries-active groups (Stuchell & Mandel, 1983). Also, a two-year cohort study based on longitudinal analysis indicated that single antimicrobial agents including lysozyme had not sufficiently strong power to have diagnostic significance in vivo with respect to future caries (Kirstilä, Häkkinen, Jentsch, Vilja, & Tenovuo, 1998).

2.2.3. Lactoferrin

As a ubiquitous biodiverse molecule, lactoferrin is typically found in saliva at concentrations of 1 to 7ug/ml (Scannapieco, 1994). Lactoferrin has been shown a diverse range of physiological functions, such as antimicrobial/antiviral activities, immunomodulatory activity, cell growth modulation, and iron homeostasis regulation (Baveye, Elass, Mazurier, Spik, & Legrand, 1999; Chierici, 2001). Its unique capability of binding iron leads to its characterization as a “metal iron chelator” (Gorr & Abdolhosseini, 2011). The iron-free state known as apo-lactoferrin, is capable of directly binding to bacteria via interactions through the tail end of the N-terminal region consisting of 47 amino acids, and agglutinating *S. mutans*, thus helping to remove the agglutinated bacteria from the oral cavity through mechanical action of saliva (Moslemi et al., 2015). In an in vivo study, the lactoferrin-knockout infected mice had significantly higher colonization with *S. mutans* and more carious lesions, indicating that endogenous lactoferrin could exert a protective effect against caries development (Velusamy, Markowitz, Fine, & Velliyagounder, 2016). Lactoferrin does not stand alone in its multifunctionality, which can also bind to salivary agglutinin acting together to bind microbes (Lenander-Lumikari & Loimaranta, 2000). Besides, it has also been proposed that lactoferrin’s effect can be correlated with lysozyme, lactoperoxidase, and IgA (Rudney, Hickey, & Ji, 1999). Due to its antimicrobial activity, salivary lactoferrin has been thought to play an important role in the prediction of caries risk. Moslemi et al. collected unstimulated saliva from 42 children, and reported a higher concentration of lactoferrin in caries-free individuals than that in ECC individuals (Moslemi et al., 2015). This study suggested reduced amounts of lactoferrin as a risk factor for childhood dental caries (Moslemi et al., 2015). Similarly in adults, a 4-year study among 28 young adults also linked low caries increment with low level of

lactoferrin in unstimulated saliva (Jentsch et al., 2004). However, the relationship between salivary lactoferrin and caries susceptibility has not yet shown consistent validity.

2.2.4. Agglutinin

Salivary agglutinin (encoded by the deleted in malignant brain tumors 1 (*DMBT1*) gene) is an innate immune receptor glycoprotein comprising ˜10% of total salivary protein in children and 5% in adults (Sonesson, Ericson, Kinnby, & Wickström, 2011), which was originated as an *S. mutans*-agglutinating glycoprotein (Bikker et al., 2002). Agglutinin is known to bind a variety of oral bacteria and viruses, and mediate the attachment of *S. mutans* to HA on the surface of the tooth (Ligtenberg, Karlsson, & Veerman, 2010). Some studies revealed a correlation between high levels of salivary agglutinin and increased amounts of *S. mutans* in dental plaque and caries susceptibility (Carlén, Olsson, & Ramberg, 1996). In contrast, a previous study found a two-fold enhancement of salivary agglutinin in caries-resistant group compared with caries-susceptible individuals (Rosan, Appelbaum, Golub, Malamud, & Mandel, 1982). More recently, protein variants of agglutinin have been suggested to affect caries susceptibility (Jonasson et al., 2007).

2.2.5. Amylase

Salivary α-amylase is the most plentiful and physiologically active enzyme in the oral cavity, and is also found in abundance in the acquired enamel pellicle, which has ability to modulate bacterial colonization and to provide additional glucose for the biofilm formation (Scannapieco, Torres, & Levine, 1993; Singh et al., 2015). In addition, it also could bind to the membrane of *S. mutans* and *Lactobacillus*, promoting their removal from the oral cavity and lowering the risk of dental caries (Scannapieco et al., 1993). On the other hand, all these activities depend on an intact enzyme configuration. Amylase binds to bacteria in plaque while free amylase in saliva may facilitate dietary starch hydrolysis to provide additional low-molecular weight carbohydrates for metabolism by plaque microorganisms. Then the resulting acid production may be added to the pool of acid in plaque to accelerate tooth demineralization and further progression of dental caries (Arya & Taneja, 2015; Scannapieco et al., 1993). Until now, there is, however, no sufficient evidence of a relationship between α-amylase and dental caries susceptibility. Recently, a longitudinal study found that α-amylase activity was significantly higher in saliva of caries-free children (aging 24–48 months) than children with ECC (Borghi et al., 2017). They also established a negative correlation between caries and α-amylase activity and suggested low salivary activity of this enzyme may be considered risk predictors for ECC (Borghi et al., 2017). In contrast, another group demonstrated a positive correlation between the number of DMF and amylase level in whole saliva among 32 male adults (Vitorino et al., 2006).

2.3. Antimicrobial peptides

In recent years, a continuous growth of interest has been observed in antimicrobial peptides (AMPs) in the light of an alarming increase in the incidence of pathogens resistant to conventional drugs, which is regarded as an important public health problem around the world. The three main AMP families are defined by biochemical and structural characteristics: α-helical peptides without cysteine (the cathelicidins); peptides with an unusually high proportion of specific amino acids, for example, the histatins; and peptides with three disulfide bonds (α- and β-defensins).

2.3.1. Histatins

The histatins comprise a family of low molecular weight cationic proteins, including three main members (histatin 1, histatin 3 and histatin 5) with other members generated from the proteolytic cleavage of them, generally exhibiting apoptotic activities on various microbial strains, as well as strong antifungal activity (Edgerton & Koshlukova, 2000). Histatin 1 and histatin 3 are derived from the available genes *HTN1* and *HTN3* present in humans (Helmerhorst, Van't Hof, Veerman, Simoons-Smit, & Nieuw Amerongen, 1997). Histatin 5, a peptide composed of 24 amino acids derived from histatin 3, has potent inhibitory effect against *Candida species (Candida albicans, Candida glabrata, Candida krusei, and Cryptococcus neoformans)* growth and bacterial co-aggregation (Gorr, 2009; Oppenheim et al., 1986). Recently, Fernández-Presas et al. found histatin 5 could induce ultrastructural damage in *S. mutans* through an apoptosis-like death mechanism (Fernández-Presas et al., 2018). Besides that, histatins are also involved in the regulation of oral hemostasis and bonding of metal ions in saliva (Oudhoff et al., 2009). Histatins, especially histatin 1, also possess high affinity for enamel surfaces and participate in the formation of acquired enamel pellicle (Richardson, Johnsson, Raj, Levine, & Nancollas, 1993). Shimotoyodome et al. elucidated that the N-terminal domain of histatin 1 could competitively reduce the adhesion of *S. mutans* onto HA surfaces by inhibiting the adsorption of salivary high-molecular weight glycoproteins (Shimotoyodome, Kobayashi, Tokimitsu, Matsukubo, & Takaesu, 2006).

A previous study verified a strong correlation between large amounts of histatin 1 and the absence of dental caries using HPLC-MS (Maeda et al., 1985). In a more recent work, a salivary peptidome profiling analysis was conducted using magnetic bead-based MALDI-TOF MS separately at the different time point of before, 1 and 4 weeks after dental treatment. Histatin 1 showed higher levels in children with severe ECC after 4 weeks treatment compared with before treatment (Sun et al., 2016). In agreement with their results, our recent study found significant lower amounts of histatin 1 in saliva samples from high caries-susceptible children based on iTRAQ and MRM analysis (Wang, Wang, Wang et al., 2018). More recently, histatin-1 was also validated to exhibit decreased salivary abundance in both caries-susceptible adults and elderly subjects using a discovery-through-verification pipeline (Wang, Wang, Zheng et al., 2018). On the other hand, another study revealed a significant increase in the concentration of histatin 5 in children with severe ECC compared to children without caries lesions and correlated with the progression of dental caries (Jurczak, Kościelniak, Papież, Vyhouskaya, & Krzyściak, 2015). Gornowicz et al. found that adolescents with high severity of caries (DMF > 11) had statistically increased level of salivary histatin 5 compared to those with low caries susceptibility (Gornowicz et al., 2014). Consistently, Colombo et al. later reported a positive correlation between histatin-5 and *S. mutans* levels in saliva samples from children with and without ECC (Colombo, Ribas et al., 2016).

2.3.2. Cathelicidin peptide LL-37

Cathelicidins are AMPs from the family of α-helical peptides with N-terminal regions carrying highly conserved cathelin domains and a C-terminal region that is less well conserved and carries antimicrobial properties (da Silva et al., 2012). LL-37, a long cationic α-helical peptide from human cathelicidin CAP18, is expressed in neutrophils and epithelial cells and thus presents in saliva and gingival crevicular fluid. LL-37 has the function of stimulation of neutrophils, monocytes, mast cells and T cells (Khurshid et al., 2016). Results from numerous studies demonstrated potent antimicrobial activity of LL-37 against gram-positive and negative bacteria, fungi, viruses and parasites (Isogai, Isogai et al., 2003; Lo´ pez-Garcı´a, Lee, Yamasaki, & Gallo, 2005). LL-37 could neutralize bacteria through forming ionic channels in the cell membranes of the microorganisms and by ability to bind LPS of bacterial membranes (Zanetti, Gennaro, Scocchi, & Skerlavaj, 2002). Another group revealed a stronger killing action of LL-37 derived synthetic peptides against *S. sanguis* (Isogai, Hirata et al., 2003). High salivary levels of LL-37 seem to increase caries resistance. However, limited evidence exists about the association between LL-37 concentration and caries risk. Davidopoulou et al. collected unstimulated whole saliva from 49 children aged 2–18 years old and found significantly lower

concentrations of LL-37 in children with high caries activity compared to caries-free children (Davidopoulou, Diza, Menexes, & Kalfas, 2012). Their finding indicated that LL-37 is an important molecule of immunity in the oral environment and play a protective role against caries. On the contrary, a longitudinal study showed a positive correlation between elevated concentrations of salivary LL-37 and higher numbers of *S. mutans* (Malcolm et al., 2014). This result contradicts another report in which there was no correlation between LL-37 and *S. mutans* levels, and a week positive association was detected between dmfs and LL-37 (Colombo, Ribas et al., 2016). Unlike the above-mentioned results, levels of LL-37 were not correlated with caries experience through immunoassay of saliva samples from 149 middle school children in a previous study (Tao et al., 2005). Therefore, the association between salivary LL-37 levels and caries susceptibility is not yet clear.

2.3.3. Defensins

Defensins, small cationic antimicrobial peptides with non-specific antimicrobial activity against gram-positive, gram-negative bacteria and *C. albicans*, are grouped into two subfamilies referred to as α- and β-defensins (Lehrer & Ganz, 1999). The neutrophil α-defensins, (human neutrophil peptides 1–3 (HNP1–3)), have been detected in whole saliva and mainly participate in non-oxidative microbial killing (Ganz et al., 1985). The human β-defensins (HBD1–3) are widely expressed in oral epithelial cells, gingival crevicular fluid, and saliva. Variable expression of α- and β-defensins within the oral cavity could render an individual at risk for dental caries (Sahasrabudhe, Kimball, Morton, Weinberg, & Dale, 2000). Tao et al. previously reported a higher salivary level of HNP1–3 in caries-free children than children with caries, which were not correlated with levels of salivary *S. mutans*, suggesting low levels of salivary HNP1–3 as useful measurement of caries risk for children (Tao et al., 2005). More recently, a randomized double-blinded clinical trial found that probiotic supplementation (*Lactobacillus paracasei* SD1) could enhance salivary HNP1–3 levels and reduce mutans streptococci numbers (Wattanarat et al., 2015). Also, an in vivo study found a relationship between an increasing level of HBD-2 with the decreasing amount of *S. mutans* after probiotic (*Lactobacillus reuteri*) induction in the oral cavity of the Wistar rats (Kusumaningsih, Subijanto, Indrawati, & Devijanti, 2016). Jurczak et al. showed a significant higher concentration of HBD-2 in children with ECC compared to the healthy controls and correlated with the caries progression (Jurczak et al., 2015). In addition, previous work suggested the *beta defensin 1 gene* (*DEFB1*) was associated with higher caries experience, and variation in the expression of HBD-1 within the oral cavity could be related to individual susceptibility for caries (Ozturk, Famili, & Vieira, 2010). However, other studies demonstrated no statistical differences between caries-free children and children with ECC considering the HNP1–3 salivary levels, and yet no correlation between HNP1-2, HBD2-3 levels and host caries experience (Colombo, Ribas et al., 2016; Phattarataratip et al., 2011; Toomarian, Sattari, Hashemi, Tadayon, & Akbarzadeh Baghban, 2011). The salivary defensins levels of preschool children could not yet been determined as a predictor for severe ECC in a recent study (Toomarian et al., 2011).

2.3.4. C-C motif chemokine 28

Chemokines are a superfamily of small proteins (8–16 kDa) that play pivotal roles in innate and acquired immunity (Allen, Crown, & Handel, 2007). The C-C motif chemokine 28 is a 128-amino acid peptide, which is principally expressed in plenty of epithelial cells, including salivary glands (Zhang et al., 2013). The C-C motif chemokine 28 participates in broad-spectral antimicrobial activities as well as homing of certain types of lymphocytes (Hieshima et al., 2003). As it is the chemoattractant of IgA antibody-secreting cells (IgA^+ ASCs), a previous study found that the delivery of a recombinant eukaryotic plasmid expressing C-C motif chemokine 28 to the rat parotid glands was able to induce high levels of C-C motif chemokine 28 and sIgA in saliva, which inhibited *S. mutans* in a biofilm (Jiang, Lan, Hu, Li, & Jiang, 2012). Besides, a positive correlation between the salivary content of C-C motif chemokine 28 and sIgA and the degree of dental caries in children was established in another study (Liu et al., 2015).

2.3.5. Azurocidin

Azurocidin is a 37 kDa cationic antimicrobial protein expressed in azurophil granules of neutrophils, which has strong antibacterial properties against gram-negative bacteria due to its strong affinity for LPS (Dhaifalah et al., 2014). A differential expression level of azurocidin in saliva was found between caries-active and caries-free children using electrospray ionizati

on ion-trap tandem mass spectrometry, which may lay some foundation for biomarker research of caries susceptibility (Yan, Huang, Xue, Jia, & Yang, 2014).

2.4. Other salivary peptides

2.4.1. Statherin

Statherin is a tyrosine-rich acidic salivary phospho-peptide composed of 43 amino acids and an unusually potent inhibitor of calcium phosphate precipitation, which is encoded by a single gene, *STATH* (Inzitari et al., 2006; Schlesinger & Hay, 1977). Statherin has been known as a potential precursor of the acquired pellicle due to its strong affinity to HA. Like salivary phosphoproteins, statherin binds calcium and maintains the supersaturated saliva through inhibiting the spontaneous precipitation of calcium and phosphate salts, which enhances enamel remineralization and protects the integrity of teeth (Humphrey & Williamson, 2001). Furthermore, statherin peptide phosphorylated on residues 2 and 3 indicated a strong affinity and fast adsorption on HA, and a significant inhibitory effect when compared with unphosphorylated statherin-derived peptides (Xiao et al., 2015). Shimotoyodome et al. found that the negative charges in the N-terminal domain of statherin could inhibit *S. mutans* adhesion onto HA surfaces (Shimotoyodome et al., 2006). Moreover, a peptide (R^{12}FGYGYGPYQPVPEQPLYPQ^{32}) within statherin exhibits antimicrobial activity against *Staphylococcus aureus* (Trindade et al., 2015). In a past report by Vitorino et al., a strong correlation between elevated level of statherin and the absence of dental caries was established (Vitorino et al., 2005). Similarly, statherin was found to be significantly up-regulated in caries-free children with the highest fold change compared with high caries-susceptible children through iTRAQ analysis (Wang, Wang, Wang et al., 2018). These findings imply a protective role of statherin against dental caries. Further studies should be conducted for supporting the hypothesis that statherin can be used as biomarker for caries risk prediction.

2.4.2. P-B peptide

Salivary P-B peptide is usually included in the bPRPs family but it shows some similarities with statherin. Differently from classical bPRPs, P-B peptide is secreted both from parotid and submandibular/sublingual glands and displays three Tyr residues in the sequence (Messana et al., 2008). In a previous study, P-B peptide appeared to be more abundant in saliva obtained from caries free adults than caries-susceptible adults (Ayad et al., 2000). However, none specific association between salivary P-B peptide and caries susceptibility has been proposed to date.

3. Therapeutic anti-caries effects of bioactive peptides derived from salivary proteins

A goal of modern dentistry is the non-invasive management of non-cavitated caries lesions involving antimicrobial and remineralization systems in an attempt to prevent disease progress. The study of peptides descended from salivary proteins as a new class of therapeutic agents against dental caries has attracted considerable interest in many

Table 2
Bioactive peptides derived from salivary proteins for therapeutic use against dental caries.

Peptide analogues	Native salivary proteins	Main functions	Reference
DSpSpEEKFLR (DSS/DR9)	Statherin	High affinity for HA, protection against enamel demineralization	Xiao et al. (2015)
DR9-DR9/DSS-DSS	Statherin	Amplified protection against enamel demineralization	Basiri et al. (2017), Grohe (2017), Valente et al. (2018)
DR9-RR14	Statherin, histatin 3	Protection against enamel demineralization and relatively strong antimicrobial property	Basiri et al. (2017), Valente et al. (2018)
SN15, SN_A15	Statherin	High affinity for calcium phosphate, high adsorption on HA	Raj et al. (1992)
DpSpSEEKC	Statherin	Remineralization of demineralized enamel	Yang et al. (2017)
DE-11	Statherin	Modulation of HA crystallization and remineralizaiton of artificial enamel caries	Wang, Wang, Li et al. (2018)
P-113	Histatin 5	Improved activity against *S. mutans*, and *S. sobrinus*	Sajjan et al. (2001), Cheng et al. (2018), Huo et al. (2011), Rothstein et al. (2001)
P-113D	Histatin 5	High resistance to proteolytic cleavage, improved antimicrobial activity	Sajjan et al. (2001)
Dhvar1-5	Histatin 5	Inhibition of *S. mutans* growth, resistance against degradation	Groenink et al. (2003), Helmerhorst, van't Hof et al. (1997)
hLF1-11	Lactoferrin	High antimicrobial activity against *S. mutans*	Sajjan et al. (2001), Seo et al. (2012)
HBD3-C15	HBD3	Antimicrobial activity against multispecies biofilm, inhibition of biofilm formation by *S. mutans*, longer half-life	Ahn et al. (2017) Gupta et al. (2015), Lee, Park et al. (2013), Lim et al. (2016)
C16LL-37	LL-37	Specificity for *S. mutans*, strong antibacterial activity, high stability	Chunxiao et al. (2016)
PGRPQ	PRP-1	Affect bacterial adhesion, proliferation and pH in biofilm formation	Drobni et al. (2006)
TRS23	Mucin 7	Potent bactericidal against *S. mutans*	Antonyraj et al. (1998)
MUC7 12-mer-L	Mucin 7	Antibacterial activity against *S. mutans*, inhibition of the formation of S. mutans biofilm	Wei et al. (2006)
MUC7 12-mer-D	Mucin 7	Antibacterial activity against *S. mutans*, inhibition of the formation of S. mutans biofilm	Wei et al. (2006)
MUC7 20-mer	Mucin 7	Antibacterial activity against *S. mutans*, inhibition of the formation of S. mutans biofilm	Wei et al. (2006)

research centers (Table 2). Insight into the mechanism of action, in addition to knowledge of the structure-function relationship of salivary proteins and peptides, makes it possible to design small, biologically active peptides that can be applied as natural biomaterials to fight against dental caries.

3.1. Salivary peptidomimetics with remineralization therapeutic potential against dental caries

Dental remineralization is the process of carrying minerals from the surrounding environment (saliva) into partially demineralized tooth structures. Since minerals from saliva or minerals supplied by other therapies need nucleation sites for precipitation and remineralization, a biomimetic technology was introduced to promote faster remineralization about a decade ago (Kirkham et al., 2007). As demonstrated in a variety of remineralization outcomes: visual inspection and scanning electron microscopy, QLF, photothermal radiometry and luminescence, microhardness, energy-dispersive x-ray spectroscopy analysis, and confocal laser scanning microscopy, laboratory studies demonstrated that bioactive peptide solutions could be introduced into enamel lesions to spontaneously create scaffolds capable of HA nucleation that enhances remineralization (Kirkham et al., 2007). Bioactive peptides may maintain or augment the functional properties of their native salivary proteins for the anti-caries application.

The salivary proteins and the formed AEP hinder the enamel from demineralization and also facilitate its remineralization process. The enamel-protective effects are based on key salivary phosphoproteins in the mineral regulation processes, such as statherin, histatin, as well as PRPs (Cochrane, Cai, Huq, Burrow, & Reynolds, 2010). As one of the principal salivary proteins, statherin has been previously demonstrated that its N-terminal part is responsible for the cited protein function (Raj, Johnsson, Levine, & Nancollas, 1992). The peptide consists of the 9 amino acids DSpSpEEKFLR termed DR9, has exhibited a higher affinity for HA and more efficient protection against enamel demineralization compared to other native statherin peptides (Xiao et al., 2015). In deed, the presence of a covalently linked phosphate group (at residues 2 and 3) in statherin peptides modulates the inhibitory effect on HA growth (Xiao et al., 2015). Basiri et al. further found that DR9-DR9 could amplify protection against enamel demineralization when compared to single DR9 or statherin, indicating that functional domain multiplication represented a strong protein evolution pathway (Basiri et al., 2017). Notably, after introduction of a specific amino acid sequence from histatin 3 (RR-14), the hybrid peptide DR9-RR14 demonstrated relatively strong protection when the antimicrobial property of these peptides (DR9, DR9-DR9, DR9-RR14, statherin, histatin 1) was tested against *C. albicans* and *S. mutans* (Basiri et al., 2017; Valente et al., 2018). Their findings provided a basis for the development of stable synthetic peptides for therapeutic use against dental caries. Recently, another study showed that synthetic peptides descended from statherin and histatin, DpSpSEEKFLR (DSS) and RKFHEKHHSHRGYR (RKF), exhibited inhibitory effect on crystal formation, with DSS-DSS showing the strongest effects while RKF showed no effect (Grohe, 2017). Additionally, the presence of the basic histatin sequence (RKF) showing antimicrobial effects could reduce the buildup of bacterial plaque (Grohe, 2017). Besides, the N-terminal 15-amino acid residue of statherin, known as SN15 (DpSpSEEKFLRRIGRFG) and its analog SN_A15 (DDDEEKFLRRIGRFG) exhibit a high affinity for calcium phosphate and therefore high adsorption on the surface of HA (Raj et al., 1992). Based on the initial six-peptide sequence of N-terminus of statherin, a cysteine-labelled peptide (DpSpSEEKC) and biomimetic peptide DE-11 extended by a mineralization hydrophilic tail composed of consecutive acidic amino acids capable of absorbing calcium and phosphate ions were synthesized and exhibited beneficial effects on the remineralization of demineralized tooth enamel in vitro (Wang, Wang, Li et al., 2018; Yang et al., 2017). However, the abovementioned biomimetic salivary peptides need to be further validated by the demonstration of enamel subsurface lesion remineralization in situ and then ultimately in randomized, controlled clinical trials. With modern peptide synthetic approaches, it is feasible to realize the incorporation of additional phosphoseryl residues or modification of peptides for better stabilization and delivery of bioavailable calcium and phosphate ions, and for control of enamel remineralization by forming scaffolds or templates to direct anisotropic crystal growth.

3.2. Salivary peptidomimetics with antimicrobial therapeutic potential against dental caries

S. mutans is considered the major etiologic agent involved in dental caries and forms biofilms on the tooth surface. In the past decades, antimicrobial agents targeting *S. mutans* have been developed as a means of caries prevention. A number of salivary proteins are involved in host defense and antimicrobial response in the oral cavity with a great deal of functional overlap. Histatins, defensins, cathelicidins, mucins, and PRPs have been recognized as the major oral antimicrobial agents in humans. As high manufacturing cost and low stability of full-length natural proteins with effective antimicrobial activity, there is increasing effort to design peptidomimetics using natural salivary proteins or peptides as templates for their applicability in the treatment of dental caries. In addition, being readily accessible for local application, the oral cavity might be particularly suitable for peptide therapy. Integration with bioinformatics tools, peptidomics techniques serve as a tool to disclose new potential antimicrobial peptides and peptidomimetics and further promote the development of novel peptide-based therapeutic approaches against dental caries. Based on the obtained information in the salivary peptidome, Trindade et al. performed bioinformatic analysis and predicted the antimicrobial activity of peptides using the AntiMicrobial Peptide Prediction tool available in Collection of AntiMicrobial Peptides website and found that five peptides resulting from human salivary P-B peptide exceeded the AMP probability of 0.8: (AMP probability 0.887, RIPPPPPAPYGPGIFPPPPPQP; 0.903, GRIPPPPPAPYGPGIFPPPPPQP; 0.991, FVPPPPPPPYGPGRIPPPPPAPY; 0.937, GPGRIPPPPPAPYGPGIFPPPPPQP; 0.987, GPYPPGPLAPPQPFGPGFVPPPPPPPY), highlighting the potential of human saliva to find new alternatives to antibiotics (Trindade & Amado, 2015).

Histatins exhibit both bacteriostatic and bactericidal effects on *S. mutans* in vitro at physiological concentrations in both healthy and carious physiological environments (Mackay, Denepitiya, Iacono, Krost, & Pollock, 1984). A variety of derivatives of histatins have been evaluated for their therapeutic potential against dental caries. P-113 is a C-terminus amidated histatin 5 derivative (histatin 5 residues 4–15, AKRHHGYKRKFH) that appears to possess improved activity against *S. mutans* and *S. sobrinus* (Rothstein et al., 2001; Sajjan et al., 2001). While the D-enantiomer, P-113D, retains antimicrobial activity with high resistance to proteolytic cleavage due to the stereospecificity of proteinases (Sajjan et al., 2001). Huo et al. reported that P-113 displayed antibacterial activity against dental cavity-inducing *S. mutans* through an intracellular mechanism involving DNA binding (Huo et al., 2011). Strikingly, a recent study established an effective protocol to generate large amounts of P-113 for future clinical investigation using an analogue and antagonist of human CXCL8, hG31 P, as a novel fusion partner in *Escherichia coli* for expressing and purifying P-113 (Cheng et al., 2018). The reduced cost of synthesis and its reported activity make P-113 an ideal candidate for further clinical studies for caries indications. Additionally, the C-terminal fungicidal domain of histatin 5 called Dh5 (residues 11–24) has also been used as a scaffold to design histatin analogues (Helmerhorst, van't Hof, Veerman, Simoons-Smit, & Nieuw Amerongen, 1997). As native histatin 5 is weakly amphipathic, analogues Dhvar 1 and 2 were designed to improve amphipathicity in α-helical conformation by substitution of the histidine and lysine residues on the hydrophobic face (Helmerhorst, van't Hof et al., 1997). While the net cationic charge of Dhvar 3 and 4 was further increased by substitution of glutamic acid with lysine. Dhvar 1 and 2 exhibited a 6-fold increased antimicrobial activity over Dh5 and inhibited the growth of three caries-associated strains of *S. mutans* on agar, whereas Dhvar 3 and 4 were more resistant to degradation after incubation with different *S. mutans* (Groenink et al., 2003).

The synthetic hLF1-11 peptide (GRRRRSVQWCA) is a derivative corresponding to the N-terminal eleven residues of salivary lactoferrin. The hLF-11 peptide shows antimicrobial activity against both gram-positive and gram-negative bacteria and various fungi. Moreover, cationic residues (-RRRR-) of the hLF-11 were rather flexible to be suitable for the interaction with the anionic bacterial membrane. The hydrophobic region positioned approximately perpendicular to the cationic residues, enabled the peptide to bind to the membrane interior (Seo, Won, Kim, Mishig-Ochir, & Lee, 2012). In particular, hLF1-11 could penetrate the bacterial cell membranes and accumulate in the cytoplasm in *S. mutans* through DNA binding (Huo et al., 2011). Thus, peptide hLF1-11 might be attractive and valuable candidates for effective antimicrobial therapies to combat dental caries.

As a 45 amino acid cationic peptide with three anti-parallel β-sheets and three disulfide bonds, HBD3 possesses broad-spectrum bactericidal activity against gram-positive, gram-negative bacteria, and fungi through pore formation and membrane disruption (Lee, Chang et al., 2013). Numerous studies have suggested that a 15 amino acids peptide consisting of the C-terminal cationic amino acid region of HBD3 (HBD3-C15, GKCSTRGRKCCRRKK) exhibits the antimicrobial activity of native HBD3, yet has a lower manufacturing cost and a longer half-life (Gupta, Singh, & van Hoek, 2015; Lim et al., 2016). Also, the synthetic peptide was found to be sufficient for its antimicrobial activity against *E. faecalis*, *C. albicans*, and multispecies biofilm (Lee, Park et al., 2013; Lim et al., 2016). More recently, Ahn et al. found that HBD3-C15 could potentiate the bactericidal and anti-biofilm activity of calcium hydroxide and chlorhexidine digluconate (Ahn, Kim, Kum, Yun, & Han, 2017). Besides, HBD3-C15 alone or in combination with calcium hydroxide or chlorhexidine digluconate showed antimicrobial activity by inhibiting the biofilm formed by *S. mutans* and other caries-associated bacteria such as *E. faecalis* and *S. gordonii* (Ahn et al., 2017). Based on the function of regulating host immunity of HBD, HBD3-C15 is also expected to improve human immune functions that are essential for antimicrobial humoral immune responses.

A recombinant peptide C16LL-37 based on salivary peptide LL-37 and a peptide derived from *S. mutans* competence stimulating peptide (C16) exhibited obvious specificity for *S. mutans* with strong antibacterial activity and high stability (Chunxiao et al., 2016). This targeted antimicrobial peptide shows good potential in anti-caries application. Bacterial proteolysis of salivary PRPs may release a network of peptides, which could support oral commensal bacteria and inhibit pathogens. Drobni et al. found the release of a pentapeptide, RGRPQ, from PRP-1 (a prevalent allelic variants of aPRPs) upon proteolysis by *S. gordonii*, may constitute a caries resistance factor and affect bacterial adhesion, proliferation and local pH in the biofilm formation (Drobni et al., 2006). Moreover, after incubation with *S. gordonii*, the RGRPQ peptide could cause an increment in the pH that was dependent on the N-terminal Arg residue. Consequently, future studies on the host-bacterium commensalism related to salivary PRPs may shed light on new strategies to interfere with the biofilm formation and protect against dental caries. Interestingly, a tandem repeat 23-residue sequence, (TRS23 (145–167) TTAAPPTPSATTPAPPSSSAPPE) of mucin 7 has been shown to be a potent bactericidal agent for *S. mutans*. The bacterial colonization, facilitated by the adsorbed salivary mucins on tooth surface, could be partly controlled and cleared by proteolytically degraded proline-rich peptides of salivary mucin 7 before the colonized organisms turn into pathogens (Antonyraj, Karunakaran, & Raj, 1998). In addition, several cationic peptides derived from the N-terminal region of mucin 7, including mucin 7 12-mer-L (RKSYKCLHKRCR), 12-mer-D (D amino acid isomer), and 20-mer (LAHQKPFIRKSYKCLHKRCR), exhibited antibacterial activity against a number of oral bacteria especially *S. mutans* in biofilms (Wei, Campagna, & Bobek, 2006). Remarkably, these peptides also displayed inhibitory effects on the formation of *S. mutans* biofilm on the vertical surfaces, which are closer to natural oral conditions.

4. Conclusions and future perspectives

Dental caries is an unevenly distributed, preventable disease, posing considerable burdens on economy and quality-of-life. It is of vital

importance to identify those who are at highest risk for disease and to enable limited resources to be targeted toward individualized, aggressive and pro-active interventions to prevent dental caries. As presented in this paper, salivary proteins and their derivatives play a concerted role in protecting the teeth integrity and counteracting the caries process. Based on current literature, we cannot yet determine if certain salivary proteins are predictive of future caries, but we have shown biologically active proteins with differential levels in caries-susceptible and caries-free individuals, which are worth studying as biomarkers of caries risk assessment for further validation in different populations and by independent groups. Meanwhile, rather than focusing on single salivary protein as caries-risk indicator, more efforts in salivary research should be made to elucidate the complex interplay among different salivary proteins, which may be a more promising way in identifying caries-susceptible individuals. In addition, synthetic peptides and peptide analogues derived from salivary proteins have surged to the forefront of research efforts since the last decade, many of which show promise for antimicrobial and/or remineralization therapeutic potentials against dental caries. On one hand, for the application of biomimetic management of early caries, the bioactive peptides derived from salivary proteins, alone or in combinations, can be further developed as a potential alternative or incorporated into oral health care products. On the other hand, for the extensive lesions or secondary caries, it is expected to allow dentists to adopt a more tooth-preserving approach through utilizing these peptides as additive of filling materials. Furthermore, the successful management also depends on the conduction of clinical trials to evaluate their efficacy and effectiveness that should be the research priority in the near future.

Author contributions

K.W. drafted the original paper, and X.D.Z., W.L., and L.L.Z. conceived of the overall study and reviewed the manuscript. All authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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