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The role of natural salivary defences in maintaining a healthy oral microbiota

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ABSTRACT

Objectives: To provide an update on our current understanding of how saliva and its various constituents directly and indirectly affect oral bacteria and thereby play a role in the modulation and maintenance of a healthy oral microbiota and also the associations with symbiosis and dysbiosis.
Methods: The search for biomedical literature on saliva and its antimicrobial activities (years 1966 to 2017) was conducted in PubMed, Embase and Web of Science databases.
Results: This review underlines that saliva plays an essential role in shaping and maintaining the ecological equilibrium of the resident oral microbiota. Saliva contributes to the formation of the salivary pellicle, which covers the oral hard and soft tissues, and thereby determines the initial adhesion and colonisation of microorganisms. Saliva facilitates clearance of dietary carbohydrates and microorganisms from the oral cavity, but also supplies bacteria with nutrients through enzymatic breakdown of dietary starch and proteins and salivary glycoproteins. In addition, saliva comprises proteins such as mucins, which block the adherence of certain microorganisms to oral surfaces through binding and aggregating mechanisms. Saliva also provides antimicrobial activity through numerous proteins and peptides including mucins, lactoferrin, lysozyme, lactoperoxidase, statherin, histatins and secretory immunoglobulin A.
Conclusions: A balanced oral microbiome is important for the maintenance of oral health and symbiosis. Conditions associated with salivary gland hypofunction, impaired oral clearance, low salivary pH and altered salivary composition, often lead to perturbation of the function and composition of the oral microbiome causing dysbiosis, and an associated risk of oral disease.
Clinical significance: Saliva plays a significant role in keeping the relationship between the host and oral microbiota in a symbiotic state. In conditions with salivary gland dysfunction, the natural balance of the oral microbiome is often disturbed, leading to dysbiosis and associated risks of gingivitis, caries and fungal infection.

1. Introduction

The emergence of new genomic technologies, including next-generation sequencing and bioinformatics, has greatly increased our knowledge and understanding of the oral microbiome and its importance in health. The oral microbiota in health is very diverse and more than 700 different bacterial species have been identified in the oral cavity [1,2]. In each healthy individual, the number of resident species is estimated to be fewer, that is in the range of 250–300 different species, of which the genus *Streptococcus* being the most abundant [3–7]. The oral cavity offers a moist and warm environment with host-derived nutrients, such as dietary sugar, salivary proteins, glycoproteins and gingival crevicular fluid, which makes it a suitable residence for the growth of many different microorganisms, mostly bacteria but also fungi, viruses, archaea and protozoa [2,8,9]. The oral cavity offers several different niches for microbial colonisation such as teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, and hard and soft palate that display a site specific microbiota [3,10]. Moreover, the teeth are the only natural non-shedding surfaces in the human body, and thereby provide opportunities for extensive biofilm formation and microbial residence [2,11]. Similarly, dental restorations, fillings, fixed prosthodontics, dentures and implants display non-shedding surfaces that can influence biofilm formation and the composition of the resident oral microbiota [12–14].

The complex and diverse oral microbial ecosystem plays an important role in the promotion and maintenance of oral health. Several factors influence the formation and maintenance of the oral microbiota including the host (behaviour and defence mechanisms), the local

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environment and the microorganisms themselves, that is, their ability to adhere, co-aggregate, interact with other species as well as their virulence [2,15–17]. One of the factors that plays a very significant role in the oral homeostasis and symbiosis is saliva [16,18–20].

Saliva contributes to the formation of the acquired enamel pellicle and mucosal pellicle, which covers the oral hard and soft tissues, respectively, and thereby helps to modulate the initial adhesion and colonisation of microorganisms and shape the composition of the resident oral microbiota [4,21–25]. Furthermore, saliva facilitates clearance of microorganisms and dietary carbohydrates from the oral cavity, but it also supplies colonising bacteria with nutrients through breakdown of dietary starch, lipids and proteins and bacterial metabolism of salivary components, e.g. glycoproteins [20,25,27]. In addition, salivary mucins have the capacity to bind and aggregate microorganisms whereby their adherence and colonisation are inhibited. Saliva also provides antimicrobial activity through numerous proteins and peptides including mucins, lactoferrin, lysozyme, lactoperoxidase, statherin, histatins and antibodies (secretory immunoglobin A, sIgA) [2,17–20,25,26]. Moreover, saliva facilitates mastication, swallowing and speech, and takes part in initial digestion [26]. The objective of this review article is to give an update on our current understanding of how saliva and its various constituents directly and indirectly affect oral bacteria and thereby play a role in the modulation and maintenance of a healthy oral microbiota and also the associations with symbiosis and dysbiosis.

2. Saliva secretion and composition

Whole saliva is a complex mixture of fluids produced by three paired major salivary glands, i.e. the parotid, submandibular and sublingual glands, and numerous minor salivary glands located in the oral mucosa [28]. In addition, whole saliva contains components of non-glandular origin, such as desquamated oral epithelial cells, food debris, microorganisms, gingival crevicular fluid and blood-derived compounds (plasma proteins, erythrocytes and leucocytes). The levels in saliva of the two latter are dependent on the degree of periodontal and oral mucosal inflammation [27,29,30].

About 90% of the total amount of saliva is produced by the major salivary glands and the remaining 10% by the minor salivary glands. However, the minor salivary glands play an important role in lubrication of the oral mucosa and also account for a large fraction of the secretion of salivary proteins [28]. The flow rate and the composition of saliva is dependent on various factors including the type and the size of gland which is activated [31], the time of day [32], state of hydration [33], nutritional state [34], the physical and chemical nature and duration of stimulus applied to activate the secretory reflexes [35], the emotional state of the individual [36] and gender [37]. Upon stimulation, the parotid gland (a purely serous gland) produces watery, amylase-rich saliva, whereas the submandibular (mainly serous), sublingual (mainly mucous) and the minor salivary glands (mainly mucous, except from the von Ebners' glands which are strictly serous) produce more viscous, slimy mucin-rich saliva [26–28].

3. Saliva flow and composition

Whole saliva, which constantly covers the hard and soft tissues of the oral cavity, is a complex, hypotonic and slightly acidic fluid [26–28,38]. In healthy persons, the total volume of saliva secreted per day is 0.5–1.0 litre, with an average of 0.6 L [39]. Saliva consists of more than 99% water and less than 1% solids such as proteins and electrolytes [26–28,38]. Under normal conditions, the mean unstimulated whole salivary flow rate is in the range of 0.3–0.4 ml/min. An unstimulated whole salivary flow rate of < 0.1 ml/min is considered pathologically low and designated hyposalivation. The mean chewing-stimulated whole salivary flow rates range from 1.5 to 2.0 ml/min, and flow rates below 0.5–0.7 ml/min are considered abnormal (hyposalivation) [37,40,41].

A normal flow of unstimulated and stimulated saliva is important to ensure sufficient and continuous lubrication of the teeth and oral mucous membranes, and also helps to prevent retrograde infection of the salivary glands with oral microorganisms via the salivary ducts [19,26,27,42]. Moreover, the fluid characteristics of saliva are essential for the mechanical rinsing of the oral cavity, for dissolving taste substances and transporting them to taste receptor sites, protection of the taste buds, food bolus formation, clearance of food debris and microorganisms, and facilitation of mastication and swallowing as well as speech. The moist environment is also important for the colonisation and growth of microorganisms on oral surfaces, which is addressed further below.

3.1. Salivary clearance

The process by which saliva dilutes and eliminates food substances such as sugars and acids from the oral cavity is referred to as salivary or oral clearance [43–46]. Before swallowing, the volume of saliva present in the oral cavity is on average 1.1 ml, and after a swallow, the residual volume of saliva is approximately 0.8 ml. This volume of saliva is spread out in a thin film, on average 0.1 mm thick. However, the thickness of the salivary film varies greatly between different parts of the oral cavity [44]. The clearance rate is determined by the salivary flow rate, the volumes of saliva in the mouth before and after swallowing and the swallowing frequency. It has been shown that low residual volume of saliva and high unstimulated and stimulated salivary flow rates facilitate the rate of clearance of fermentable carbohydrates and acids from food and drinks [43]. Saliva also plays an important role in clearance of desquamated epithelial cells from the oral cavity each of which carry about 100 microorganisms and for elimination of the microorganisms present in saliva [47]. One millilitre of human saliva from a healthy individual contains about 100 million bacterial cells. With a normal salivary secretion of 750 ml per day, about 8×10^{10} bacteria are shed from the oral mucosal surfaces per day, equivalent to 5–10 g of wet weight of bacterial cells [48]. Thus, saliva flow and subsequent swallowing promote removal of a significant number of bacteria and thereby play an important role in balancing the oral microbiome.

In patients with severe and persistent reduction in unstimulated and stimulated whole saliva flow rates due to e.g. medication-intake, radiation damage to the salivary glands or Sjögren's syndrome, the retention of food substances and microorganisms in the oral cavity is prolonged [49]. The consequence hereof is a shift in the oral microbiota favouring the growth of more aciduric and acid-tolerating bacteria, especially *Streptococcus mutans*, *Streptococcus sobrinus* and lactobacilli, which through fermentation of carbohydrates produce acids that increase the risk for dental caries [2,15,16,18,19,26,50]. It has been shown that the oral sugar clearance is markedly lower in patients with unstimulated whole saliva flow rates below 0.16 ml/min, which increases the risk of tooth demineralisation [51]. Moreover, during caries development the caries-associated species increase in numbers and the colonisation and growth of more beneficial species is inhibited [52]. A further consequence of reduced saliva flow is the increased number of *Candida albicans* which often leads to manifest oral candidiasis [50].

3.2. Inorganic salivary components

The inorganic salivary constituents include sodium, chloride, potassium, calcium, magnesium, phosphate and bicarbonate as well as trace elements. The composition of saliva, especially the concentration of various ions, is dependent on the flow rate [53]. Thus the concentrations of sodium, chloride, bicarbonate, total protein and total calcium are higher and the concentrations of potassium and total phosphate are lower in stimulated saliva compared to unstimulated saliva [26–28,38]. The physico-chemical properties of the inorganic salivary components and thereby their role in formation of oral surface biofilms and hence on the resident oral microbiota are addressed below.

3.3. Salivary pH and salivary buffer capacity

The salivary buffer capacity includes the bicarbonate, phosphate and protein systems [54–57]. The role of salivary buffer systems is to maintain the salivary pH at a relatively constant level (i.e. 6.5–7) by buffering acids from dietary intake and acids produced by bacterial fermentation of carbohydrates, thereby decreasing the tooth demineralisation rate [26,27,56]. The concentration of bicarbonate in saliva and the salivary pH are highly dependent on the salivary flow rate, and the pH can, under normal physiological conditions, vary from 6.0 to 7.5. The bicarbonate concentration and thus the pH increase when the salivary flow rate increases and *vice versa*. The concentration of bicarbonate is highest in parotid saliva and lowest in the minor salivary glands [28,56]. There are even findings indicating that the labial salivary glands do not secrete bicarbonate upon stimulation [58].

The clearance rate of acids produced by oral bacteria varies between different oral sites, creating local micro-environmental conditions, i.e. niches with low pH. This can explain some of the site-specific differences in bacterial composition seen in the oral cavity [59–61].

Salivary pH and the levels of calcium and phosphate are important factors for the maintenance of saturation with regard to hydroxyapatite in the saliva. In addition, saliva contains proteins including acidic proline-rich proteins, histatins, cystatins and statherins, which display high affinity to hydroxyapatite as they bind calcium ions, and also inhibit precipitation of calcium phosphate salts from saliva supersaturated with respect to hydroxyapatite and thereby play a central role in the integrity of the teeth [62]. The ions in saliva, including calcium, are also important to the function of α-amylase in the oral cavity [63].

In addition to being part of the buffer capacity, contributing to keep the oral environment supersaturated with respect to calcium phosphates, phosphate is also a nutrient for the oral microbiota. Moreover, bacteria help to buffer saliva by breaking down urea to ammonia and carbon dioxide resulting in an increase in pH [64].

The salivary buffer capacity, and its ability to keep the pH within a neutral range, is important for the promotion and maintenance of the healthy microbial composition and consequently for symbiosis [16,65]. Consequently, salivary gland hypofunction is associated with changes in the composition of saliva, an impaired salivary buffer capacity and low pH conditions in the oral environment, resulting in a shift in the homeostasis of the healthy oral microbiota leading to dysbiosis and oral diseases [2,13,15,16]. Furthermore, before awareness of risk of oral diseases, patients with salivary gland hypofunction may have a frequent intake of fermentable carbohydrates like soft drinks and candy in order to alleviate the symptoms of oral dryness [56,66] which additional favour the growth of *S. mutans* and *Lactobacillus* and *Candida* species.

3.4. The salivary pellicle

The thin acellular film that forms on tooth surfaces upon exposure to the oral environment, is designated the acquired enamel pellicle. It mainly comprises adsorbed salivary proteins, but also non-salivary-derived carbohydrates, proteins and lipids [21,22]. The enamel pellicle is important for the maintenance of dental health due to its role in lubrication, mineral homeostasis of the tooth surfaces and in determining the composition of the initial colonising microorganisms which form the initial layer of dental plaque on the tooth surfaces [2,21,22,67]. Together with salivary ions, several salivary proteins such as proline-rich proteins, cystatins, mucins, lactoferrin, lysozyme and α-amylase and secretory IgA take part in the formation of the acquired enamel pellicle and interact with several oral bacteria on adsorption to hydroxyapatite [67–70].

The oral mucosa is also covered by a thin salivary pellicle comprising different salivary proteins, especially mucins [71–74]. The mucosal pellicle includes secreted soluble mucins (MUC5B and MUC7), membrane-associated epithelial mucins (MUC1), and to a lesser extent carbonic anhydrase IV, secretory IgA, cystatins and α-amylase [74]. Recent findings indicate that the fine structure of the mucosal pellicle differs from the enamel pellicle and due to its composition of larger glycoproteins retaining water, it can be considered as a hydrogel, with a lower tenacity than the enamel pellicle [74]. Maturation and turnover are influenced by the presence and variety of salivary proteins, by the salivary flow and the desquamating oral epithelium. Similar to the dental pellicle, the mucosal pellicle provides lubrication and a protective barrier against exogenous potential damaging substances and microorganisms [71,72]. About 80% of the oral cavity consists of oral mucosal surfaces, and thus exhibits an extensive area for bacterial attachment [75].

The microorganisms that colonise the oral cavity have developed various ways to bind to the salivary film on the oral mucosa and teeth [75]. Accordingly, several families of gram-positive bacteria produce surface proteins, including serine-rich repeat, antigen I/II, and pilus families that mediate adherence to a variety of salivary and oral bacterial receptors [75]. The serine-rich repeat bacterial surface adhesions, which are able to recognise carbohydrate (saccharide) moieties of glycosylated salivary components, are produced by streptococci, staphylococci, and lactobacilli [76].

Families of gram-negative bacteria may exhibit pili, auto-transporters, and extracellular matrix-binding, which are important for host tissue recognition and formation of complex microbial communities. Fibrillar proteins are important mediators of the initial attachment of the periodontal pathogen *Porphyromonas gingivalis* to gingival epithelial cells through recognition of αvβ3 and α5β1 integrin receptors on the epithelial cell surface [77].

After initial attachment of microbial cells, an extracellular matrix is formed, supporting biofilm development, promoting microbial adhesion to surfaces and cohesion and influencing diffusion [78,79]. This matrix consists of a large number of hydrated extracellular polymeric substances including exopolysaccharides, proteins, lipids, nucleic acids, and lipo-oligosaccharides that are important for the growth and survival of microbial species [78]. Polysaccharides (glycans) produced by exoenzymes, glucosyltransferases, from *S. mutans* are the main components of the matrix of cariogenic plaque-biofilms and are also considered a virulence determinant of dental caries [79]. In cases where dietary sucrose is available in excess, glucosyltransferase activity is closely related to caries development. Some of the streptococcal exoenzymes have the ability to bind with the salivary pellicle, α-amylase and to the surfaces of streptococci and of other bacterial species [79].

The initial attachment of primary colonizers to oral surfaces presents new receptors for the subsequent adhesion of other bacteria. The binding of bacterial cells to pre-adherent cells on a surface is thought to be important for the recruitment of secondary colonizers to the oral biofilm.

Overall, the molecular composition and the physical and chemical properties of the salivary pellicle are important for the initial colonisation of microorganisms onto the oral surfaces. It is also important for the subsequent adhesion of other microorganisms, the interaction between species, including co-aggregation and co-adhesion that among others enables *C. albicans* to colonise surfaces already coated with streptococci [80].

3.5. Salivary organic components

Analysis of the human salivary proteome has characterised about 3000 different proteins and peptides [81]. More than 90% in weight of the about 3000 protein components are present in saliva from the parotid, submandibular and sublingual glands, belonging to the classes of acidic and basic proline-rich proteins, α-amylases, mucins, cystatins, histatins, statherin and host defence peptides and accounting for about 200 proteins and peptides. The remaining 10% in weight are components deriving from the minor glands (labial, palatine, buccal and lingual glands) [82], and from gingival crevicular fluid, e.g. α-defensins,

Table 1
Major salivary components with indirect and direct antimicrobial activities.

Component	Function	Target
Water	Oral clearance	Elimination of microorganisms, dietary sugars and acids by swallowing
Bicarbonate, phosphate and proteins	Buffering	Maintenance of a favourable pH in the oral cavity, i.e. in the neutral range
Salivary proteins	Formation of the acquired enamel and mucosal pellicle	Promoting adhesion of a number of microorganisms but also inhibiting adhesion and colonisation of others and potentially pathogenic microorganisms
Glycoproteins, lipids	Nutritional source	Providing nutrition for a number of microorganisms in order to keep the balance of the oral microbiome
Mucins, i.e. MUC5B, MUC7, MUC19, MUC1 and MUC4	Antibacterial Antifungal Antiviral Nutritional source	Lubrication and protection of oral surfaces against bacterial proteases Promoting agglutination/aggregation of a number of microorganisms Providing nutrition and promoting interactions between salivary proteins
Amylase	Antibacterial Hydrolysis of starch	Certain streptococci bind specially to amylase Providing nutrition for a number of bacteria
Lacto- and myeloperoxidases	Antibacterial Antifungal	Catalysation of the oxidation of thiocyanate to hypothiocyanite by hydrogen peroxide Gram-positive and -negative bacteria, Candida
Lysozyme	Antibacterial Antifungal Antiviral	Hydrolysis of the polysaccharide layer of the gram-positive bacterial cell wall Gram-positive bacteria, Candida, virus
Lactoferrin	Antibacterial Antifungal Antiviral	Binding and sequestering of iron, depriving microorganisms of iron Gram-positive and -negative bacteria, Candida, virus
Proline-rich proteins	Antibacterial Antiviral	Gram-negative bacteria, virus e.g. HIV
Statherin	Antibacterial Antifungal	Promoting aggregation of microorganisms Gram-negative bacteria, Candida
Cystatins	Antibacterial Antifungal Antiviral	Gram-positive and -negative bacteria, Candida, virus
Histatins	Antibacterial Antifungal Antiviral	Gram-positive and -negative bacteria, Candida, virus
Immunoglobulins, especially sIgA	Antimicrobial	Inhibition of microbial adhesion Enhancement of phagocytosis Aggregation of microorganisms in interactions with other salivary proteins

and products of mucosal exudates. Many of these proteins and peptides have antibacterial, antifungal and antiviral properties, others lubricate oral tissues, some help to maintain high salivary concentrations of calcium, some are digestive enzymes, and some catalyse the conversion of carbon dioxide to carbonic acids, and most of them act in concert and display multifunctionality [18–20,26,27]. The components of saliva with indirect and direct antimicrobial properties are listed in Table 1.

3.5.1. Salivary mucins

Salivary mucins are large, highly glycosylated proteins mainly produced by the mucous acinar cells of the submandibular, sublingual and the minor salivary glands [19,83–85]. The specific types of mucins that have been characterised in the oral cavity include MUC5B, MUC7, MUC19, MUC1, and MUC4 [85]. As mucins are hydrophilic and contain much water, they are effective in lubricating and maintaining a moist surface on the teeth and the oral mucosa. Thus they provide an effective protective barrier against desiccation and against penetration of potential damaging substances to epithelial cells, and against proteases produced by bacteria in the dental plaque [83–85]. The variability of the complex oligosaccharide side chains in the mucins provides wide possibilities for interactions with oral surfaces, oral microorganisms and other salivary proteins such as IgA, lactoferrin, and lysozyme, thereby altering their properties and ability to modulate the microbial colonisation in the oral cavity [83,86–90]. Salivary mucins may serve as carriers for salivary proteins and transport them throughout the oral cavity, in order to enhance their retention in the salivary pellicle, and/or protect proteins from proteolytic degradation through the formation of complexes. However, mucins are also the predominant and most accessible source of sugar for bacterial growth.

MUC5B, the primary gel-forming mucin in the oral cavity, is secreted by mucous cells in the submandibular, sublingual, palatal and labial salivary glands [91,92]. It has been demonstrated that MUC5B affects the interactions between microbial species by promoting coexistence of *S. mutans* and *Streptococcus sanguinis*, reducing the attachment and biofilm formation of *S. mutans*, and by shifting cells from the biofilm into the single cell (planktonic) state, and thereby influencing the composition of the oral microbiota [89,93]. Moreover, MUC5B limits the virulence of *C. albicans* by reducing the formation of hyphae, which are associated with the host cell invasion [94]. This can contribute to explain why opportunistic pathogens like *C. albicans*, can exist in the oral microbiota as non-pathogenic residents without causing manifest oral candidiasis. Salivary mucins also reduce HIV-1 infection of T cells [95]. The levels of MUC5B as well as amylase have also been shown to be reduced in patients with hyposalivation of different aetiologies, but were not associated with changes in the levels of various bacteria and *C. albicans* in saliva cultures [96].

MUC7 is smaller than MUC5B, and it is a non-gel-forming mucin produced by both mucous and serous salivary acinar cells, except by parotid and lingual serous cells [97,98]. Although MUC7 is less efficient as lubricant, it is notably more efficient in bacterial agglutination and clearance than MUC5B and therefore an important part of the salivary non-immune defence system [99,100]. Thus, MUC7 directly binds to microorganisms to facilitate their removal by swallowing. MUC7 also binds to acidic and basic proline-rich proteins, statherins and histatin 1 and through the formation of these protein complexes, it protects them from proteolysis, modulates their function and activity and also serving as a delivery system for distribution of secretory salivary proteins throughout the oral cavity [99].

MUC1 mucins are membrane-associated glycoproteins, which are present in the superficial part of the oral epithelium, particularly on the buccal and labial epithelial surfaces, but also line the ducts of the parotid, submandibular, and minor salivary glands [73,101–106]. MUC1 is assumed to play a role in cell signal transduction and in binding secreted salivary proteins, and thereby contributes to the formation of the mucosal pellicle [102,105–107]. A recent study has found higher levels of MUC5B and MUC1 in saliva of adolescents with high

number of carious lesions compared with those having fewer lesions. The authors suggested that MUC1 acts as a scaffold for MUC5B, and when MUC1 is shed into saliva then MUC5B will follow, leaving the enamel pellicle with less MUC5B and thereby more susceptible to adherence of *S. mutans*, which may cause demineralisation [108].

MUC4 is a transmembrane mucin secreted by the human submandibular gland [105,109]. It is assumed to play a role in cell-cell and cell-extracellular matrix interactions and in cell signalling [85]. It has recently been shown that low levels of MUC4 in saliva and in gingival crevicular fluid and high levels of metalloproteases7 are associated with periodontitis, which indicate that MUC4 is degraded by bacterial proteolysis. Moreover, it indicates that reduced salivary levels of MUC4 may result in impaired ability to agglutinate and cleanse oral pathogens, leading to formation of a biofilm of virulent bacteria and continuous inflammatory response in patients with periodontitis [110].

3.5.2. Salivary amylase

Alpha-amylase is one of the most abundant enzymes of human saliva, and it is also present in the salivary pellicle and dental plaque [18,19,21,24,67,111–114]. It is mainly secreted from the serous acinar cells in the parotid glands and to a lesser extent from the serous cells in the submandibular glands [21,27,38,112,113]. Salivary α-amylase breaks down ingested starch by cleavage of the α-1,4-glycosidic linkages of starch molecules into maltose, maltotriose and dextrins. Salivary α-amylase is active at a pH above 6, and it is inactivated in the acidic environment in the stomach [18–20,26,27,38]. Maltose can be fermented by oral bacteria, and hydrolysis of maltotriose leads to additional glucose for metabolism by bacteria in dental plaque. The resulting lactic acid production lowers the pH within the biofilm, which contributes to tooth demineralisation and development of carious lesions [113]. Amylase also facilitates the dissolution of starch-containing food debris retained in the oral cavity after a snack or meal by forming more soluble compounds which can dissolve in the saliva.

Salivary amylase not only facilitates bacterial fermentation of carbohydrates and adherence of bacteria to oral surfaces, it also binds specifically to certain oral bacterial species. Thus, amylase can complex with sIgA in the salivary pellicle to form a binding receptor for *S. sanguinis* [115]. In addition, *Streptococcus gordonii* and *Streptococcus mitis* encode specific amylase binding proteins (adhesins) [116,117]. Through these various mechanisms, salivary amylase plays an important role in modulating the adhesion, co-adhesion and colonisation of microorganisms, and in supporting the host-microbiome symbiosis. The function of salivary amylase may be compromised in conditions associated with salivary gland hypofunction, impaired oral clearance and saliva buffering, where low pH in the biofilm lead to a shift in the balance of the microbiota towards a more acid-tolerating and acid-producing and thus potentially cariogenic microbiota and dysbiosis [16].

3.5.3. Salivary peroxidase systems

The salivary peroxidase systems comprise lactoperoxidase, myeloperoxidase, thiocyanate (SCN^-) ions and hydrogen peroxide [118]. Salivary peroxidases are produced in the parotid and submandibular glands [119]. Peroxidases catalyse the oxidation of thiocyanate (SCN^-) to hypothiocyanite ($OSCN^-$) by hydrogen peroxide [118,120,121]. Lactoperoxidase and thiocyanate are natural constituents of saliva, whereas hydrogen peroxide originates from bacterial metabolism in the oral cavity [120,121]. Myeloperoxidase is a leukocyte-derived protein, and the concentration of this enzyme in saliva reflects gingival and mucosal inflammation [122]. The salivary peroxidase systems exert antimicrobial activity and protect host cells and proteins from the potentially damaging effects of hydrogen peroxide. The hypothiocyanite inhibits important bacterial metabolic processes and exerts antimicrobial effects on *S. mutans*, lactobacilli, yeasts, several gram-negative species including periodontal pathogens, e.g. *P. gingivalis* and *Prevotella intermedia*, and certain viruses [123–126]. Hypothiocyanite also inhibits bacterial lyases related to the production of oral malodour [127].

In order to enhance the antimicrobial effects of saliva, the lactoperoxidase system and other proteins have been added to oral health products [128–135]. Studies have shown that regular use of lactoferrin and lactoperoxidase-containing tablets, or toothpaste, mouth rinse or gel containing peroxidase system as well as colostrum results in a shift in the microbial ecology that may contribute to improvements in oral health, including oral malodour and gingival conditions [130–134]. On the other hand, a study by Kirstilä et al. [135] on the effects of a lactoperoxidase-system-containing toothpaste (Biotene™), found no effect on salivary flow rate, peroxidase activity, thiocyanate/hypothiocyanite, bacterial counts or on the dental plaque levels compared with the placebo toothpaste. However, the toothpaste was only used for a very limited period of two weeks. In addition, with new technologies including 16S rRNA gene high-throughput sequencing, proteomics, transcriptomics and metabolomics, allowing in depth analysis, it is more likely to identify differences. Thus, a recent randomised clinical study, comparing the use of fluoride toothpaste containing enzymes and proteins (Zendium™) and a fluoride toothpaste without these ingredients for a 14-weeks period, showed a shift in the ecology of the oral microbiome at species level after the use of the toothpaste with natural enzymes and proteins. Accordingly, 12 taxa associated with gum health including *Neisseria* species had increased, whereas 10 taxa including *Treponema* species associated with periodontal disease had decreased [133]. These results have recently been supported by larger clinical studies, demonstrating that persons having used a fluoride toothpaste with enzymes and proteins for 3 months and at least one year, respectively, had better gingival state than persons having used a fluoride toothpaste without these enzymes and proteins [134,136].

3.5.4. Salivary lysozyme

Lysozyme is part of the innate salivary defence mechanisms. The lysozyme present in whole saliva originates from the major and minor salivary glands, and to a minor extent from gingival crevicular fluid, and salivary leukocytes. Lysozyme is present in the salivary pellicle as well as in the dental plaque [21,137–141]. Lysozyme exerts enzymatic activity via hydrolysis of the β-1,4-glycosidic bonds between N-acetylmuramic acid and N-acetyl-d-glucosamine in the polysaccharide layer of the gram-positive bacterial cell wall. Apart from this well-known bacteriolytic activity, and a highly cationic protein, lysozyme also has the ability to aggregate oral bacteria, e.g. streptococci, thereby affecting their adherence to the oral surfaces and promoting clearance of microorganisms from the oral cavity. In addition, lysozyme can activate bacterial autolysins which destroy the bacterial cell walls [65,141–143]. A recent study on the relation between the salivary microbiome, salivary metabolome and host-related biochemical salivary parameters in healthy young adults showed that low salivary pH and high lysozyme activity are associated with high amounts of streptococcal phylotypes and membrane-lipid degradation products which indicate an ecological shift towards a dysbiotic state [144]. It is most likely that the lysozyme activity has increased in response to dysbiosis. Furthermore, it has been demonstrated that lysozyme exerts antifungal as well as antiviral activities [145–148].

3.5.5. Salivary lactoferrin

Lactoferrin is an iron-binding glycoprotein secreted by the serous acinar cells of major and minor salivary glands. The lactoferrin in whole saliva also originates from neutrophil granulocytes and from the gingival crevicular fluid and levels are high in infection and inflammatory conditions [149–151]. Lactoferrin binds and sequesters iron and consequently deprives the microorganisms such as bacteria, yeasts and parasites of iron, which is essential for their growth. As a cationic protein, lactoferrin can also promote adherence and aggregation of certain microorganisms as well as contribute to breakdown of microbial cell membranes. It has been shown the iron-free form of lactoferrin

(apo-lactoferrin) mediates agglutination of oral *S. mutans, S. sobrinus, Streptococcus rattus, S. sanguinis, P. gingivalis* and *Aggregatibacter actinomycetemcomitans*, whereas the iron saturated form only agglutinates *S. mutans* indicating that lactoferrin possess strong iron-binding independent bacteriolytic properties [152,153]. Apart from the antibacterial effects on cariogenic bacteria and periodontal pathogens [128,154], lactoferrin also exerts antifungal activity e.g. against *C. albicans* and *Candida krusei* [155,156] and antiviral activity, e.g. against herpes simplex virus, most likely via neutralisation of the virus by direct binding and/or by blocking specific host cell glycosaminoglycans used by viruses for adhesion [157]. Human lactoferrin is multifunctional displaying extensive antimicrobial, anti-inflammatory and immunomodulatory activities. It plays a central role in regulating the oral microbiota and the inflammatory state of the oral mucosa, keeping the host-microbiome in symbiosis [155,158]. In dysbiosis, where the biofilm is allowed to accumulate and not regularly disturbed by for example by tooth brushing, certain pathogenic bacteria are able to flourish. The associated local inflammation (gingivitis) provides iron from gingival bleeding and thereby supports the growth of *P. gingivalis*, which requires iron from haem. If the dysbiotic state continues and resolution of the chronic resolution fails, the condition may progress to periodontitis in susceptible persons, depending on various genetic, environmental and lifestyle factors. In dysbiosis, levels of salivary lactoferrin are increased in an attempt to resolve the inflammation and restore symbiosis [159].

3.5.6. Salivary proline-rich proteins

Proline-rich proteins (PRPs) are produced by the parotid and submandibular glands, and constitute 25–30% of all proteins in saliva [160–162]. Salivary PRPs are divided into acidic, basic and glycosylated PRPs. PRPs bind to the surface of teeth and oral mucosa and are involved in the formation of the initial salivary pellicle. Especially acidic PRPs have the ability to inhibit spontaneous precipitation of calcium phosphate salts and thereby contribute to protection of the teeth. PRPs are also able to precipitate tannins and thus contribute to the sensation of astringency [163–165]. Especially glycosylated PRPs bind and agglutinate oral bacteria, such as *Fusobacterium nucleatum,* allowing them to be removed from the oral cavity through swallowing [166]. Acidic PRPs have been shown to promote adhesion of specific bacteria to dental surfaces, e.g. *Actinomyces viscosus* and *S. gordonii* [167–169]. Moreover, human parotid basic PRPs exhibit anti-HIV-I activity [170]. Thus, salivary PRPs have an impact on biofilm colonisation and the interaction between bacterial species, whereby they help to maintain a balanced microbiota. Basic and glycosylated PRPs also promote adhesion of *C. albicans* thus play a role in their colonisation onto oral surfaces [171]. However, it is unclear whether this mechanism actually is important in balancing the whole oral microbiome or it increases the risk of developing oral candidiasis.

3.5.7. Statherin

Statherin is present in saliva of the parotid and submandibular glands. Statherin is a phosphoprotein that binds hydroxyapatite and contributes to the formation of the enamel pellicle. Statherin inhibits primary or spontaneous precipitation of calcium phosphate from the dental surfaces and thus being important for the integrity of the teeth [172]. However, statherin is also known to promote the adhesion of *A. viscosus* to tooth surfaces and to possess specific binding sites for *P. gingivalis* fimbriae [173,174]. In addition, statherin induces transition of *C. albicans* hyphae to yeast and may thus contribute to the oral defence against oral candidiasis [175]. The binding of statherin to mucins forms protein complexes that can protect the proteins against microbial proteolytic activity and also promote aggregation of microorganisms that can be eliminated by swallowing [176].

3.5.8. Salivary cystatins

Cystatins are cysteine-containing phosphoproteins, which have proteinase inhibiting properties, thereby controlling proteolytic activity from the host e.g. leucocytes during inflammatory processes or from microorganisms [177,178]. A number of different proteoforms of cystatin A, cystatin B, cystatin S, cystatin SN, cystatin SA and cystatin D have been detected in human saliva [177–180]. They take part in the formation of the salivary pellicle and can also affect calcium phosphate precipitation. It has been shown that cystatins have a variety of antimicrobial properties. Thus, cystatin SA exerts antibacterial activity against the periodontal pathogen *A. actinomycetemcomitans* and cystatin S can partially inhibit proteolytic enzymes released from *P. gingivalis* but this activity appears not to be linked to their enzyme-inhibiting properties [178,179,181]. Moreover, cystatins also appears to have strong antifungal properties as patients with endocrine autoimmune disease with chronic mucocutaneous candidiasis also suffer from cystatin SA1 deficiency [182].

3.5.9. Salivary histatins

Histatins are a family of cationic peptides produced by ductal cells of the parotid, sublingual and submandibular salivary glands [183,184]. Histatins readily absorb to hydroxyapatite and contribute to formation of the acquired enamel pellicle, thereby playing a role in bacterial colonisation on tooth surfaces. The presence of intact histatins in the pellicle indicates that once absorbed they are capable of avoiding proteolytic degradation by microorganisms [188]. All three major human histatins, i.e. histatin-1, -3, and -5, exhibit antifungal activity towards *C. albicans*, but histatin-5 appears to be the most efficient [183,185–188]. Histatins also exert antibacterial and antiviral properties [189–191].

As cationic peptides, histatins can bind to negatively charged microbial cell membranes, promote aggregation and integrate into the lipid bilayer of the cell membrane. The latter is assumed to result in formation of ion channels, transmembrane pores, membrane leakages and membrane breakage, eventually leading to microbial cell death [187,192,193]. Histatin-5 also exhibits specific binding sites for metal ions, zinc and copper. Consequently the microorganisms are deprived of these ions, which are important for the function of their enzymes, and thereby for their growth [194,195]. Histatin-5 has also been shown to inhibit enzymatic activity of bacteria involved in periodontal disease, e.g. *P. gingivalis* and *Bacteroides gingivalis* [191,196]. Together with statherins, histatin-1 modulates the adhesion of *S. mutans* to dental surfaces presumably through competitive inhibition of the adsorption of salivary high molecular weight glycoproteins [197].

3.5.10. Salivary immunoglobulins

The two major antibody classes present in human saliva are secretory IgA (sIgA) and IgG [140,198,199]. The dimeric IgA is produced by plasma cells in the stroma of the salivary glands, and then transported through the glandular epithelial cells by the polymeric Ig receptor, i.e. the membrane secretory component. At the apical surface of the epithelial cell, sIgA is exocytosed after cleavage of the Ig receptor [199,200]. The major part of salivary IgG derives from blood through passive leakage via the gingival crevicular fluid, and only a minor part originates from the salivary glands. The monomeric (non-secretory) fraction of IgA in whole saliva is small (15% of total salivary IgA) and mainly enters the oral cavity via the gingival crevicular fluid or mucosal transudate [199,201]. The fractions of salivary IgM, IgD and IgE are small, and they predominantly derive from gingival crevicular leakage and the levels of IgM in saliva are correlated both to the serum IgM concentration and periodontal inflammation [199].

In the oral cavity, the most important defence mechanism of sIgA seems to be binding to antigens in the saliva, in the oral mucosa and in the acquired enamel pellicle; an activity designated immune exclusion [199]. Although the secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes, there are a number of bacteria which enzymatically breakdown parts of the sIgA isoform, sIgA, e.g. *S. sanguinis* and *S. mitis* and periodontal pathogens

such as *P. gingivalis*, *Prevotella* and *Capnocytophaga* species [202,203]. The antimicrobial properties of sIgA also include inhibition of the microbial adhesion to mucosal and dental surfaces and enhanced elimination of microorganisms, e.g. *S. mutans*, from the oral cavity by agglutination. Thus the salivary immunoglobulins act in concert with innate defence mechanisms, but the formation of salivary antibodies response to e.g. streptococci is also important for the early colonisation of oral surfaces [202–204].

4. Conclusion

Saliva is a key component in maintaining a balanced oral microbiome and it exerts a large variety of functions that help to promote oral health. The importance of the complex interaction between host, saliva and the oral microbiota becomes evident when the salivary flow is reduced, and the composition is altered, leading to dysbiosis and the risk of associated oral diseases such as dental caries, gingivitis and oral fungal infections. Salivary constituents provide an important nutritional source for several microorganisms, and the complex interaction of many salivary inorganic and organic constituents is essential for the maintenance of a balanced and beneficial microbiota and for symbiosis. A large number of proteins and peptides have been detected in saliva and some of their functions and complex interactions with the oral microbiome have yet to be clarified. The availability of advanced technologies provides promising possibilities for studying the interplay of the host and microbiome, and consequently identifying risk predictors in the salivary proteome for common oral diseases such as dental caries or periodontal disease, in addition to identifying measures to help prevent dysbiosis. This paper has reviewed some of the most prominent constituents of saliva, and how they directly and indirectly contribute to modulate and balance the oral microbiome.

Conflict of interest

The authors declare that they do not have any conflict of interest.

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Informed consent

This manuscript is a review of literature and does not include any study participants, and consequently no informed consent was required.

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