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A REVIEW OF SALIVA: SECRETION, COMPOSITION AND FUNCTION

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Abstract. *Saliva plays a central role in the complex physiological and biological processes that take place in the upper parts of the gastrointestinal tract in relation to ingestion and processing of the food. Human saliva has a number of physical, physicochemical and chemical agents that protect oral tissues against by various microorganisms and their metabolic products. Antimicrobial peptides with other innate defense molecules are fighting infection and control residents microbial populations throughout the oral cavity.*

1. INTRODUCTION

Saliva is a clear, slightly acidic mucoserous exocrine secretion. Whole saliva is a complex mix of fluids from major and minor salivary glands and from gingival crevicular fluid, which contains oral bacteria and food debris [1,2].

Mayor gland do produced more saliva than minor gland, but the quality of content endused the type of protection varies. the minor salivary gland are the most important because they have more protective components. The average daily flow of whole saliva varies in health between 1L and 1.5 L. Percentage contributions of the different salivary glands during unstimulated flow are as follows: 20% from parotid, 65% from submandibular, 7% to 8% from sublingual, and less than 10% from numerous minor glands. Stimulated high flow rates drastically change percentage contributions from each gland, with the parotid contributing more than 50% of total salivary secretions [3].

There is great variability in individual salivary flow rates. The accepted range of normal flow for unstimulated saliva is anything above 0.1 ml/min. For stimulated saliva, the minimum volume for the accepted norm increases to 0.2 ml/min. These numbers have been projected from research on general populations. Salivary flow is, a very individualized measurement and ideally should be recorded as a base reference after the age of 15 [3]. Any unstimulated flow rate below 0.1 ml/min is considered hypofunction [4]. In a 1992 study, the critical range separating persons with normal gland function from those with hypofunction was more precisely identified as unstimulated whole salivary flow rates between 0.12 and 0.16 ml/min [5]. If individualized base rates have been established, then a 50% reduction in flow should be considered hypofunction [6]. On average, unstimulated flow rate is 0.3 ml/min [3,5] with the average total for 16 hours of unstimulated flow (during waking hours) being 300 ml.

Salivary flow during sleep is nearly zero. Stimulated flow rate is, at maximum, 7 ml/min [3]. Stimulated saliva is reported to contribute as much as 80% to 90% of the average daily salivary production.

The secretion of saliva is controlled by a salivary center composed of nuclei in the medulla, [5] but there are specific triggers for this secretion. Three types of triggers, or stimuli, for this production are mechanical (the act of chewing), gustatory (with acid the most stimulating trigger and sweet the least stimulating), and olfactory (a surprisingly poor stimulus). Other factors affecting secretion include psychic factors such as pain, certain types of medication, and various local or systemic diseases affecting the glands themselves [2,5,7].

Salivary glands are innervated by both sympathetic and parasympathetic nerve fibers. Various neurotransmitters and hormones stimulate different receptors, different salivary glands, and different responses [8]. When sympathetic innervations dominate, the secretions contain more protein from acinar cells, whereas predominant parasympathetic innervations produce a more watery secretion [3]. Stimulation of 1 receptor often enhances and complements another receptor. Therefore, the separation of contributing stimuli and resulting secretory products is not absolute [8]. It must be emphasized that there is great individual variability in salivary stimulation and secretion from cell type to cell type, thereby affecting the content of saliva regionally and as a whole.

2. SALIVARY FUNCTION

Saliva serves multiple functions that are important for the maintenance of oral and systemic health. The fluid characteristics and viscoelastic properties of saliva are essential for the mechanical cleansing of the oral cavity, clearance of food debris and microorganisms, dissolution of tastants and dilution of hot, cold or spicy food, as well as for the lubrication and moistening of teeth and oropharyngeal mucosa, which facilitate the processes of chewing, bolus formation, swallowing and articulation of speech.

Saliva consist of more than 99% water and less than 1% solids such as proteins and electrolytes. [9,10,11]. Salivary constituent 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 depend of the flow rate [12]. This concentrations of sodium chloride bicarbonate and total calcium are higher and the concentrations of potassium and total phosphate are low in stimulated saliva compared to unstimulated saliva [9,10,11]. The physical chemical properties of the inorganic salivary components and hereby their role information of the oral surface biofilms and has on the resident oral microbiota.

Analysis of the human salivary proteome has characterized about 3000 different proteins and peptides [13]. 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 compo- nents deriving from the minor glands (labial, palatine, buccal and lingual glands) [14], and from gingival crevicular fluid, e.g. α-defensins and products of mucosal exudates.

Salivary function can be organized into 5 categories that serve to maintain oral health and create an appropriate ecologic balance: (1) lubrication and protection, (2) buffering action and clearance, (3) maintenance of tooth and oral mucosa integrity, (4) antibacterial activity, and (5) taste and digestion.[15,16].

(1) THE ROLE OF LUBRICATION

The complex mix of salivary constituents provides an effective set of systems for lubricating and protecting the soft and hard tissues [17]. The best libricated components of saliva are muffins that are excreted from salivary glands. Mucins are complex protein molecules that are present predominantly in 2 molecular weight types [17,18] and formed by polypeptide chains that stick together. These mucins have the properties of low solubility, high viscosity, high elasticity, and strong adhesiveness. Any intraoral contact between soft tissues, between soft tissues and teeth, or between soft tissues and prostheses benefits from the lubricating capability of saliva supplied largely by these mucins [3]. Mastication, speech, and swallowing all are aided by the lubricating effects of mucins [18].

The lubricating and antimicrobial functions of saliva are maintained mainly by resting; saliva results in a flushing effect and the clearance of oral debris and noxious agents [19]. Saliva is a complex fluid, which influences oral health through specific and nonspecific physical and chemical properties [20]. Saliva contains numerous antimicrobial proteins that help protect the oral ecosystem from infectious agent [20]. Proteins can move from blood circulation into salivary glands through active transportation, passive diffusion, or ultrafiltration; some of which are then released into saliva and hence can potentially serve as biomarkers for diseases [21]. Saliva covers the oral hard and soft tissues with a conditioning film which governs the initial attachment of microorganisms, a crucial step in the setup of the oral microflora [22].

(2) BUFFERING ACTION AND CLEARANCE

Saliva buffers acids and its buffer capacity originates from the content of bicarbonate, phosphate and proteins [23, 24]. Salivary pH is maintained at a relatively constant physiological level, that is 6.5–7.4, by buffering dietary acids and acids derived from bacterial fermentation of carbohydrates and thereby diminishing the rate of tooth demineralization [23]. The concentration of bicarbonate in saliva, the salivary pH and the buffer capacity are highly dependent on the salivary flow rate, and they increase when the salivary flow rate increases and *vice versa* [23,25].

Salivary pH and the levels of calcium and phosphate are important factors for maintaining sa- liva supersaturated with respect to hydroxyapatite [26]. In the stimulated state, the bicarbonate buffer system is responsible for about 90% of the buffer capacity, whereas in the unstimu- lated condition, the phosphate concentration is nearly equal to the bicarbonate concentration and they contribute almost equally to the buffering capacity. At lower flow rates and salivary pH below 5, proteins constitute the major buffering capacity [23]. Saliva also contains certain proteins

including acidic proline-rich proteins, histatins, cystatins and statherins, which are among the first proteins that adhere to a clean enamel surface to initiate enamel pellicle formation. They display high affinity for hydroxyapatite as they bind calcium ions, and inhibit precipitation of calcium phosphate salts from saliva supersaturated with respect to hydroxyapatite, thus protecting the teeth from demineralization and calculus formation [27].

The ions in saliva, including calcium, are also important to the function of salivary α-amylase [28]. In addition, oral bacteria help to buffer saliva by breaking down urea to ammonia and carbon dioxide resulting in an increase in pH [28].

(3) MAINTENANCE OF TOOTH AND ORAL MUCOSA INTEGRITY

Maintaining tooth integrity is a third function of saliva, one that facilitates the demineralization and remineralization process. Demineralization occurs when acids diffuse through plaque and the pellicle into the liquid phase of enamel between enamel crystals. Resulting crystalline dissolution occurs at a pH of 5 to 5.5, which is the critical pH range for the development of caries [3]. Dissolved minerals subsequently diffuse out of the tooth structure and into the saliva surrounding the tooth. The buffering capacity of saliva greatly influences the pH of plaque surrounding the enamel, thereby inhibiting caries progression [30]. Plaque thickness and the number of bacteria present determine the effectiveness of salivary buffers. Remineralization is the process of replacing lost minerals through the organic matrix of the enamel to the crystals. Supersaturation of minerals in saliva is critical to this process. The high salivary concentrations of calcium and phosphate, which are maintained by salivary proteins, may account for the maturation and remineralization of enamel [2]. Statherin, a salivary peptide, contributes to the stabilization of calcium and phosphate salts solution, serves as a lubricant to protect the tooth from wear, and may initiate the formation of the protective pellicle by binding to hydroxyapatite.[3,6]. Proteins in the protective pellicle, such as statherin, histatins, cystatins, and proline-rich proteins, are too large to penetrate enamel pores. Therefore, they remain on the surface, bound to hydroxyapatite, to aid in controlling crystalline growth of the enamel by allowing the penetration of minerals into the enamel for remineralization and by limiting mineral egress [31,32]. The presence of fluoride in saliva speeds up crystal precipitation, forming a fluorapatite-like coating more resistant to caries than the original tooth structure. In that sense, small amounts of demineralization have been suggested as advantageous for the tooth because enamel components of magnesium and carbonate are replaced with the stronger, more caries-resistant fluorapatite crystals [3]. Fluoride in salivary solution works to inhibit dissolution of apatite crystals.

(4) ANTIBACTERIAL ACTIVITY

Immunologic and nonimmunologic antibacterial salivary content come from 2 different sources namely, plasma and ductal cells with different responses to stimulation and different content levels.

Salivary glands are exocrine glands, and, as such, secrete fluid containing immunologic and non-immunologic agents for the protection of teeth and mucosal surfaces. Immunologic contents of saliva include secretory IgA, IgG, and IgM. Non-immunologic salivary contents are selected proteins, mucins, peptides, and enzymes. Secretory IgA, the largest immunologic component of saliva, is an immunoglobulin produced by plasma cells in connective tissues and translocated through the duct cells of major and minor salivary glands. IgA, while active on mucosal surfaces, also acts to neutralize viruses, serves as an antibody to bacterial antigens, and works to aggregate or clump bacteria, thus inhibiting bacterial attachment to host tissues[33,34]. Other immunoglobulins present in saliva are in low quantities and probably come from gingival crevicular fluid [2]. It seems unlikely that host complement response could act generally in the oral fluid [3]. IgA itself does not activate complement[5], but oral fluids can be augmented by gingival crevicular fluid host complement components when gingivitis is present around existing teeth[1,15]. Non-immunologic antibacterial salivary contents such as proteins, mucins, peptides, and enzymes (lactoferrin, lysozyme, and peroxidase), all products of acinar gland cells, help protect teeth against physical, chemical, and microbial insults [35].

Antimicrobial peptides include: mucins, histatines, defensines, lactoferrin cathelicidins,, calprotrctin, lysozymes and oral peroxidase.

Mucins

The main role of mucins is in mechanical protection of mucosa, but the research showed their antimicrobial activity. Low molecular mucins of saliva in "*in vitro*" conditions show effect against different kinds of fungus (*Candida*

albicans, Cryptoccocus neformans), gram-positive (*Streptococcus mutans*) and gram-negative bacteria that cause periodontal deices (*Porfyromonas gingivalis*)[36, 37].

Because they are able to aggregate bacterial microflora, mucins represent the important factor of dental caries. Literature data show that low molecular mucins are more effective than high molecular ones. The indicator for this is that, high molecular mucins are predominant in saliva of caries sensitive persons, while in saliva of caries resistant persons higher concentration of low molecular mucins is established [38].

Histatines

The main source of histatines are salivary glands. The representatives of this family proteins are: histatine1, histatine 3, histatine 5. The specially articulated anti fungal effect in "*in vitro*" conditionals, has histatine 5 against different kinds of fungus (*Candida albicans,Candida krusei, Candida glabrata, Sacharmyces cervisiae, Cryptococcus neoformans*). These protein doesn't behave like classic antibiotic, by forming pores of ionic chanels in *c.albicans* membrane, but in its mechanisms of anti fungal effect there are a couple od phases: bonding for the specific receptors on membrane, transport trough membrane and entering the cell mobilization of ions K^+, Mg^{2+}, I ATP from the cell. The target for histatine to act inside *C.albicans* is mithochondria, where it inhibits respiratory chains [39].

Defensives

Defensives shows antimicrobial activity because they are able "to kill" all kind of gram-positive and gram-negative bacteria, the fungus (C*andida albicans)* as well some viruses (*Herpes simplex*) [40,41].

The mechanism od antibacterial defensives can be divided into couple of phases:

1. Electrostatic connection between defensives as a cations at the surface of bacteria cell membrane, which has anon characteristics.

2. The increasing of permeability of bacteria membrane is achieved in two ways: the first one is to form ionic channels with dimension depend of the type of the cell; the second is called "*carpet model*" which means aggregation of those peptides with positive electrified parts of membrane and in this way formation of a transit path for their pass.

3. Disturbance in a protein synthesis in bacteria cell [41].

Because of the great potential in "killing" bacteria, defensives are popularly called "*natural antibiotics*". In some studies is suggested the possibility of their use in oral disease therapy [41].

Lactoferrin

Lactoferrin is an important component for unspecific antimicrobial mucosa protection, because it demonstrates bacteriostatic and bactericidal effect towards gram-positive and gram-negative bacteria. It has an outstanding affinity in bond with ferritin, so it made it inaccessible for bacteria and so they are deprived of these bio element necessary for them. The phenomena is cold "*nutritive immunity*"and these is a way that lactoferrin prevents the growth and reproduction of bacteria [42].

Lactoferrin demonstrated antiviral activity, because in (*in vitro*) conditions it could inhibit replication of viruses. However, research show that lactoferrin primary stops virus infection of host cells and to the smaller extend, it inhibits the replication of viruses. Lactoferrin achieves the prevention of the infection of the host cells in two ways:

1. By direct bonding of lactoferrin to the virus (hepatitis C virus, polyvirus, rotavirus, herpes simplex virus and human immunodeficiency virus (HIV)).
2. By bonding of lactoferrin to the host cells, especially for those biomolecules in the structures of plasma membranes witch serve for viruses as a receptors or coreceptors (HSPGs) [43].

Lactoferrin has the same effect like *Candida albicans* bacteria. It refers to ferrous bonding as a direct interactions of lactoferrin and it peptides with this fungus, which provoke disturbance in porousness of it membranes [44].

The patients with progressive periodontal decease where one of the causes is *Actinobacillus actinomycetemcomitans*, negative correlation is found between the number of those pathogens and concentration of lactoferrin in saliva. After the appropriate periodontal disease therapy the level of lactoferrin in saliva and gingival fluid is significantly decreased. This shows that lactoferrin can be sensitive biomarker for the parodontopathy level and the efficiency of therapy applied [45].

Calprotectin

Calprotectin has antimicrobial effect which is achieved by zinc bonding, and that is why, microorganisms are the deprived of this essential element and their survival can be prevented [46]. The presence of calprotectin is proved in saliva. The main source of these saliva proteins are the gum fluid mucosa transudate and gum keratinocytes. These protein is included in unspecific antimicrobial protection of oral environment because of its antibacterial and anti fungal effects. The increased concentration of calprotectin in saliva is proved in some of oral diseases, so it could be considered as a valid marker for those diseases [47]. In addition of direct antibacterial effect of calprotectin the data shows that this defensive protein has a role in protection of oral mucosa from bacterial colonization. This multifunctional protein lessens the possibility of bacterial bonding for the epithelia cells on the oral mucosa [48].

Lysozime

Lysozyme is part of the innate salivary defense 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 [49]. 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 [50]. Lysozyme of saliva presence are important factor of unspecified environment. A couple of studies show that concentration of saliva with accumulation of dental plaque and appearance of gingivitis in children and young. The other studies pointed out an increase of lysozyme in saliva from the population with oral candida, in addition to its importance in defense of oral environment against bacteria and fungi, it also inhibited the adherence of bacteria streptococcus mutant and anguish for the acquired dental periclle, with lessons accumulation of dental plaque.

Oral Peroxidase

Oral peroxidase is saliva enzyme with consist of two peroxidase enzymes: saliva peroxidase 80% and mieloperoxidase 20%. Saliva peroxidase is secreted from the main saliva glands mostly parotid gland. The role of peroxidase is to catalyze reaction between H_2O_2 (product of oral bacterial metabolism) and tiociyant ions. The product of these reaction is hipotiociyant acid and hipotiocianates, witch blocade sulfurhydric groups of bacterial enzymes, glycolyse, hexokinase, aldolases and pyruvate kinase. The enzymes show antibacterial effect against of number of gram-positive (Streptococcus mutant) and gram-negative bacteria (P.Nucleatum, P. gingivalis, Prevoteles, Actinobacillus, actinomycetem comitans). Besides the role of unspecific antimicrobial protections of oral environment, these enzyme also contributes effective elimination of H_2O_2 from oral environment.

Mielooperoxidase is HEM-dependent enzyme witch is part of leukocytes (neutrofiles and monocytes). In the presence of H_2O_2 and mieloperoxidase a compex enzyme-substrate is formed and it has the ability to oxidase iodides and chlorides, making toxic products. Because of the great diffusion of chorions in biological systems, its oxidation gives the hypochloric acid (HOCL). This acid has expressive oxidative capacity and during the reaction it makes products witch have, not only bacterial capacity but they also participate in demolishment of uninfective substance toxin and inflammatory mediators. [51]

In order to enhance the antimicrobial effects of saliva, the lactoperoxidase system and other proteins have been added to oral health products [52,53]. 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 [54,55,56]. On the other hand, a study by Kirstilä et al. [57] on the effects of a lactoperoxidase-system-containing toothpaste (BioteneTM), 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, proteins and 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 [57]. 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 [57].

Amilase

Alpha-amylase is one of the most abundant enzymes of human saliva, and it is also present in the salivary pellicle and dental plaque [58,59]. 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 [60,61]. Salivary α-amylase breaks down ingested starch by cleavage of the α-1,4-glycosidic lin- kages 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 [58,59]. Maltose can be fermented by oral bacteria, and hydrolysis of maltotriose leads to ad- ditional 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 le- sions [61]. 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* [62]. In addition, *Streptococcus gordonii* and *Streptococcus mitis* encode specific amylase binding proteins (adhesins) [63]. 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 [64].

(5) TASTE AND DIGESTION.

Saliva plays an important role in the digestive processes of taste, initial breakdown of foods, chewing, bolus formation and swallowing [65].

During mastication, or the act of chewing, food is broken down into smaller fragments and mixed with saliva. The food particles are thereby lubricated and softened and exposed to digestive enzymes, processes which are essential for the formation of a food bolus suitable for swallowing [66]. Mastication, which requires involvement of the teeth, the masticatory muscles, the temporomandibular joint and the tongue, facilitates the subsequent gastrointestinal absorption of food particles. Mastication is under the control of the central pattern generator located in the brain stem, which is regulated by the extensive sensory inputs evolving from the oral cavity during ingestion and chewing of food, in order to constantly adjust the act of chewing to the food properties (texture) and facilitate formation of a bolus ready for swallowing [68].

The optimal moment for swallowing appears to occur when the cohesive forces between the food particles in the bolus are strongest. The cohesiveness and adhesiveness are determined by the food particle size, the liquid in the food and the salivary secretion [68]. The most prominent salivary enzyme is α-amylase, which breaks down starches to soluble maltoses and dextrins by cleaving the α-(1- 4) glycosidic bonds [69]. This breakdown to simple hexoses occurs in two phases. The luminal phase starts in the oral cavity with the initial digestion of starch by salivary α-amylase, and the second phase occurs in the upper small intestine as pancreatic α-amylase reaches the chyme. Salivary α-amylase is considered to be of minor significance in polysaccharide digestion due to its rapid inactivation in gastric acid and its pH optimum at 6.8, but short-chain glucose polymers in the diet may stabilize the enzyme and allow maintenance of activity at acid pH during the first period in the stomach [70]. Furthermore, that salivary amylase plays a significant role in gastric digestion.

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