Review Article

Current Update in Human Saliva and Its Role in Oral and Systemic Health and Diseases

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Abstract

Human saliva is a complex secretion containing abundance of biomolecules derived from salivary glands, mucosal and periodontal tissues, and oral microorganisms. Formation of saliva is involved with coupling of the nerve-mediated reflex to glandular secretion of salivary fluid and proteins. A variety of molecules including peptides, proteins, glycoprotein, lipids, metabolites, RNA, and genomic DNA can be found in saliva. These salivary molecules are derived from both local and systemic sources. Saliva has multifunctional roles in maintenance of oral health and supplies a variety of physiologically systemic needs including protection against tooth demineralization and microbial invasion, tissue lubrication, food perception, food digestion, and wound healing. Saliva can be an alternative source of other biofluids, because of the ease of obtainment, non-invasiveness and safety, and pleasantness of use. With an advanced-high throughput technology, a potential use of saliva as a diagnostic tool for oral and systemic diseases becomes essential for laboratory and clinical investigations with the aim of using saliva as a possible complementary examination with routinely diagnostic methods. The term “Salivaomics” was established recently to describe the information derived from studies in human saliva including: genomics and epigenomics, transcriptomics, proteomics, metabolomics and microbiomics. The aim of this review was to update the knowledge of human saliva regarding its role in oral and systemic health and diseases.

Key words: Diagnostics, Oral diseases, Saliva, Salivary gland, Systemic diseases

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Introduction

1. Physiology and Biochemistry of human saliva: Innervation of salivary glands

Human Saliva is primarily derived from 3 major salivary glands (parotid, submandibular, and sublingual glands) and minor salivary glands (Table 1). The acinar cells which produce saliva; and the ductal cells which modify and transfer saliva to the oral cavity are two major cell types of salivary glands.[1] Physiologically, production of saliva is controlled mainly by the autonomic nervous system (Fig. 1). In addition, the process of salivary secretion is supplied by arterioles surrounding salivary ducts and acinar cells. Taste buds and mechanoreceptors on the tongue can detect signals from food and tastants (salt, acid and etc.), whereas mechanoreceptors in oral mucosa and periodontal ligament receive the signals from chewing of food.[2] These taste and mechanical signals produce afferent signals in sensory nerves including the trigeminal (CNV), the facial (CNVII) and the glossopharyngeal (CNIX) nerves. The signals from CNVII and CNIX are transferred to the nucleus of solitary tract and relayed to the salivary centers including the superior and inferior salivary nuclei which are located in the medulla oblongata.[3] Efferent signals generated by the salivary nuclei are sent through the parasympathetic nerves to supply salivary glands. The submandibular and sublingual glands are supplied by efferent fibers from chorda lingual nerve to the submandibular ganglion. The parotid gland is supplied by efferent fibers from the tympanic branch of CNIX to the otic ganglion and postganglionic fibers in the auriculotemporal nerve. Minor salivary glands are supplied by parasympathetic nerve fibers in the buccal branch of the mandibular nerve, the lingual nerve and the palatine nerve.[2] The parotid and submandibular glands are also supplied by sympathetic efferent nerves arise from the thoracic spinal cord, whereas the sublingual and minor salivary glands obtain a sparse adrenergic innervation. It should be noted that nerves within the central nervous system innervated the salivary nuclei and this central neural activity contributed toward the resting rate of salivary secretion.[2,3]

Table 1 *Histologic and physical characteristics and main secretory product of human salivary glands*

Types of salivary glands	Histologic characteristics	Physical characteristics (main secretory product)
Major salivary glands		
Parotid glands	serous cells	Watery (amylase and PRRs)
Submandibular glands	mixed cells (mainly mucous cells)	Viscous (mucin)
Sublingual glands	mixed cells (mainly mucous cells)	Viscous (mucin)
Minor salivary glands		
Palatine glands	mucous cells	Viscous (mucin)
Buccal glands	mixed cells (mainly mucous cells)	Viscous (mucin)
Labial glands	mixed cells (mainly mucous cells)	Viscous (mucin)
Lingual glands	serous cells	Watery (lipase)

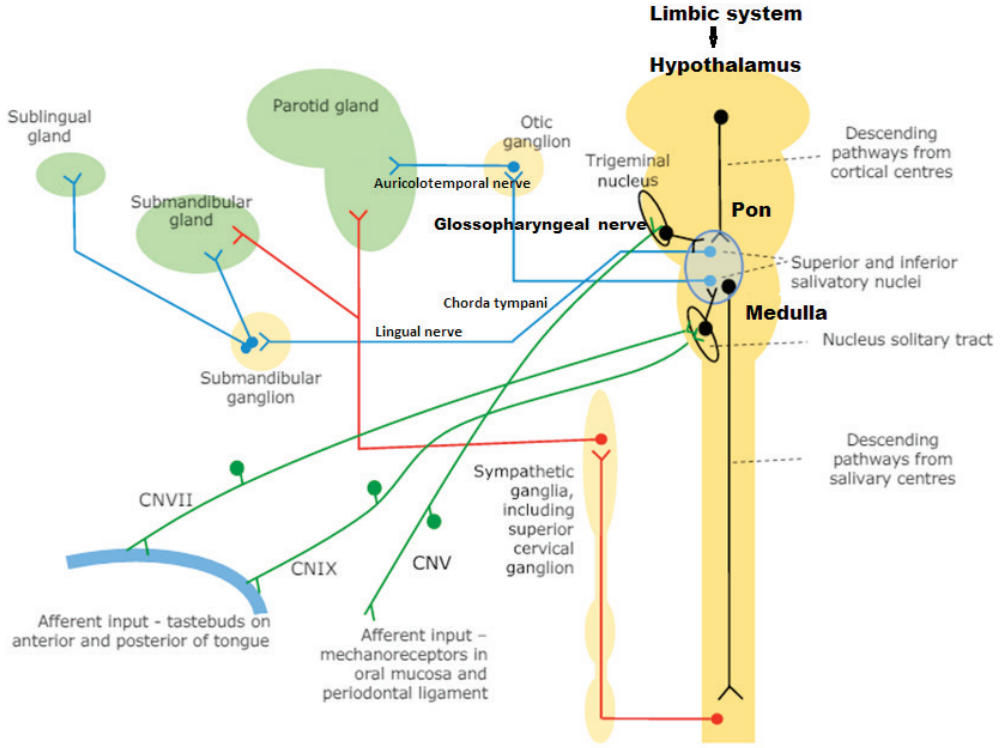

Figure 1 *Regulation of salivary secretion by the autonomic nervous system. (modified from: Proctor GB. The physiology of salivary secretion Periodontol 2000 2016;70:11-25. doi: 10.1111/ prd.12116)*

Production of saliva

Formation of saliva is involved with coupling of the nerve-mediated reflex to glandular secretion of salivary fluid (water and electrolytes) and salivary proteins.[4] Fluid secretion is regulated by the activation of muscarinic M3 receptors (with some contribution from M1 receptors) on acinar cells by acetylcholine released from parasympathetic nerves.[5,6] The intracellular mechanism is demonstrated by an elevation of cytoplasmic calcium concentrations, leading to the activation of chloride release.[7,8] Moreover, water molecules can diffuse through tight junctions and aquaporin channels on acinar cells, and some electrolytes such as salt (Na^+ and Cl^-) are actively transported by acinar cells into the acinar lumen.[9] Secretion of salivary proteins is regulated by the interaction between noradrenaline released from sympathetic nerves and beta 1 adrenoreceptors.[10] In addition, signaling from parasympathetic nerves can stimulate protein secretion by the release of vasointestinal peptides.[11] The intracellular mechanism is demonstrated by an increase in cyclic AMP which activates protein kinase A, resulting in exocytosis of protein storage granules and release of protein into the acinar lumen.[12] Saliva in the lumen is called primary saliva which is isotonic as compared with serum. Primary isotonic saliva becomes hypotonic saliva after passing through the striated ducts where the salt is reabsorbed[2] (Fig. 2).

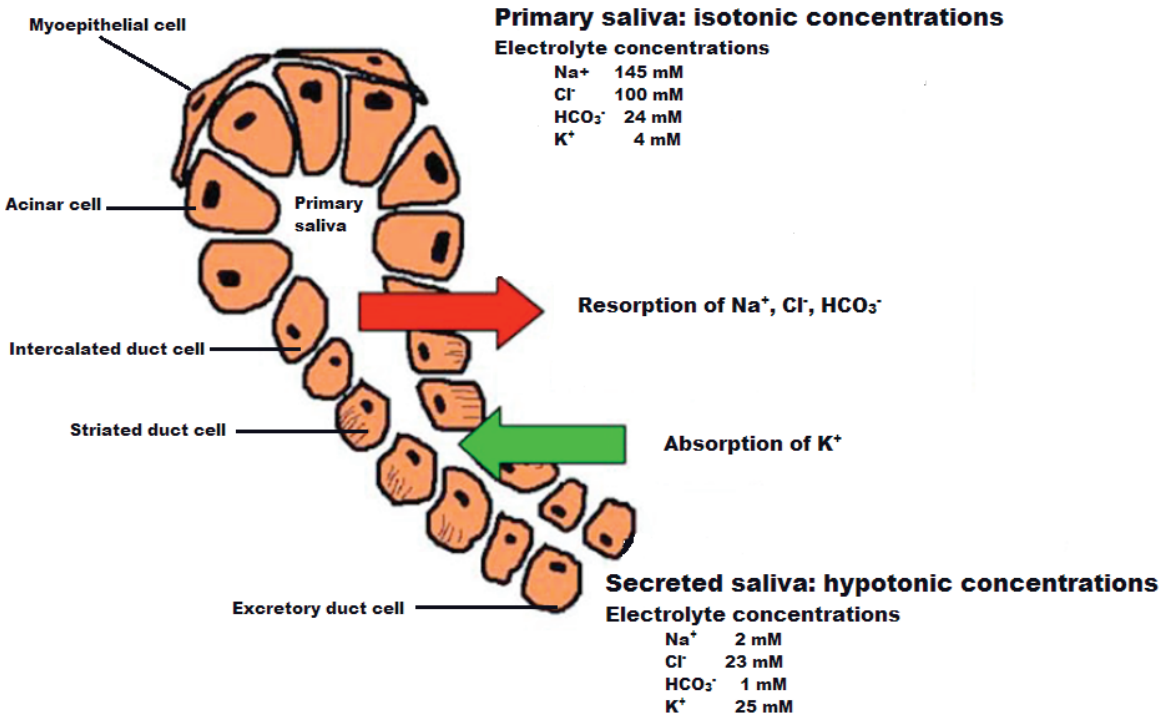

Figure 2 *Electrolyte concentrations of primary and secreted saliva. Primary saliva is isotonic when compared with plasma, whereas secreted saliva at the gland's orifice is hypotonic. (modified from: Aps JK, Martens LC. Review: The physiology of saliva and transfer of drugs into saliva. Forensic Sci Int 2005;150:119-31)*

Generally, healthy individuals produce saliva 500-1500 ml per day, with approximate salivary flow rate of 0.3-0.5 ml/min.[13-15] (Table 2) During sleeping, the salivary flow rate is decreased due to reduction of autonomic stimulation from the higher centers[14] and this can make our teeth more susceptible to attack by oral microorganisms.[1] Several factors affect salivary flow such as chewing, taste, smell, psychological and hormonal status, age, medications and physical exercise.[16] The volume of saliva in the oral cavity before swallowing is different between individuals ranging 0.5-2.1 ml, whereas salivary volume after swallowing is ranging 0.4-1.4 ml.[17] The residual saliva is present as a film on mucosal surfaces and teeth with a variety of thickness depending on sites. The thickness of salivary film can be measured by the wetness of filter paper strips applied to various surfaces. It was demonstrated that the anterior tongue had the most thickness of salivary film (50-70 μm) followed by the buccal surface (40-50 μm) and the anterior hard palate (10 μm).[18] Salivary film of tooth surfaces is thinner than those on the oral mucosal surfaces. Clearance of substances in the mouth such as sucrose, acid, and bacteria is depended upon the rate of salivary secretion and the movement of salivary film over oral surfaces.[2] This clearance is important for prevention of tooth demineralization and maintenance of oral health.

Table 2 *Human salivary flow rate in the physiological condition*

Types of glandular saliva	Salivary flow rate	
	Resting stage (ml/min)	Stimulated stage (ml/min)
Whole mouth (mixed) saliva	0.35	2
Parotid saliva	0.1	1.05
Submandibular/subligual saliva	0.24	0.92
minor gland saliva	<0.05	<0.1

Compositions of saliva

A variety of molecules including electrolytes, peptides, proteins, glycoprotein, lipids, metabolites, RNA, micro RNA and genomic DNA can be found in saliva. These salivary molecules are derived from salivary glands, gingival crevicular fluid (GCF), serum transudate from the inflamed sites of oral mucosa, epithelial and immune cells and a large variety of microorganisms. Regarding salivary proteins, over 2,000 proteins in saliva were identified by proteomic approaches.[19] It is unclear why there are such differences in protein secretion among salivary glands. It was speculated that the location of each gland might be related to the secretory function of salivary glands. The orifice of parotid duct opposite to the upper molars might be essential to aid the chewing of food, whereas the orifice of submandibular/sublingual ducts located under the tongue is appropriate for distribution of saliva across the mouth by action of the tongue.[1] The concentrations of salivary proteins that are actively transported such as amylase, mucins, statherins and proline-rich proteins are not reduced in the stimulated stage.[20] However, the concentrations of salivary proteins that are not actively transported such as albumin are decreased in stimulated saliva.[21] A diversity of salivary proteins is not only from person to person but also within the same individual at different times of the day due to the contribution of different salivary glands.[2] Moreover, salivary compositions varies, depending on whether salivary secretion in the resting or stimulating stage. According to SDS-PAGE analyses, parotid saliva contains higher levels of alpha amylase and basic proline-rich proteins than those from submandibular and sublingual glands[1]. Saliva from submandibular and sublingual contains more mucins and cystatin. Minor glands also produce mucins and some proteins such as lipocalin and lingual lipase which are involved in food processing.[21] However, some salivary proteins are similar to all glands such as secretory IgA. In addition to salivary proteins, secreted vesicles such as exosomes are detected in saliva. Exosomes demonstrate cup-shaped appearance with 30-100 nm in diameter.[23] The surface of exosome consists of a lipid bilayer, and the

interior contains RNAs, proteins, carbohydrates and lipids. Exosomes can act as messengers intercellularly. These secreted vesicles are thought to be responsible for a variety of biological functions such as RNA processing and degradation, pathogen spread, tumor promotion and immune function.[24]

Properties and functions of saliva

Saliva is considered as a non-Newtonian fluid because the viscosity decreases with increasing shear and this allows saliva to be spread on the oral surfaces and to be retained and not easily washed off oral surfaces.[1] Saliva with hypotonicity has essential implications for the maintenance of taste buds and for their sensitivity to salt detection.[25] The saliva pH 6.2-7.6 is maintained by a bicarbonate (HCO_3^-) pH-buffering system. However, the higher concentrations of HCO_3^- in stimulated saliva are more effective in neutralization of dietary acids as compare with those in unstimulated saliva. The high concentrations of calcium and phosphate in saliva decrease demineralization of tooth enamel and promote reminalization of tooth surfaces.[26,27] Thiocyanate, iodide and nitrate in saliva demonstrate bacteriostatic activities which can inhibit bacterial growth.[28] Mucins are high molecular weight glycoproteins with an elongated structure that can form very large structures by self-aggregation, contributing significantly to viscous saliva.[29] The properties and effectiveness of saliva are mainly determined by secretions from major and minor salivary glands.[1] Saliva from each gland demonstrates differences in biochemical, physical and rheological properties. Parotid saliva contains no mucins (but has many glycoproteins) and has a viscosity closer to that of water with low concentrations of proteins, whereas submandibular and sublingual saliva demonstrate more viscoelasticity with higher concentrations of mucins.[30-33]

Most salivary functions are specific for the surface (teeth or mucosa). For example, mineralization, buffering and clearance of remaining food debris and microorganisms are important for teeth, whereas the functions of saliva such as solubilization of tastants, maintenance of taste buds, wetting food and repair of tissues are essential for oral mucosa.[34] However, some salivary functions such as lubrication and removal of microorganisms are important for both teeth and oral mucosa. Regarding food digestion, when food is broken down into smaller particles during chewing, saliva incorporates into food particles to make them stick together, resulting in bolus formation before passing through the throat.[35] In addition, salivary amylase is most active in digesting maltose and helpful at converting nonsoluble complex polysaccharides into smaller soluble molecules. These help to dissolute food particles stuck on tooth surface and to reduce the availability of substrates for oral microbial growth.[1]

According to other proteins found in human saliva, PRRs are phosphorserine-containing proteins that bind hydroxyapatite on tooth surface and bind calcium in saliva to prevent the

calculus formation.[36] Statherin is considered to be the most surface active protein in saliva and functions as a lubricant of tooth surfaces, during chewing, to protect the teeth from wearing.[37] Histatin is found in enamel pellicle and inhibit crystal growth of calcium phosphate salts.[38] Regarding salivary defense proteins, they can be classified as: salivary antibodies, salivary antimicrobial peptides and salivary innate defense proteins.[39] Considering salivary function in taste recognition, several proteins should be addressed such as leptin, ghrelin, insulin, neuropeptide Y, peptide YY and carbonic anhydrase (gustin).[40] Several salivary proteins promote the healing of oral wounds such as epidermal growth factors, secretory leucocyte protease inhibitor, histatin and trefoil factors.[41]

2. Roles of human saliva in health and diseases

Saliva is one of body fluids with biological functions for the maintenance of oral health and supply a variety of physiologically systemic needs. In the mouth, saliva plays important roles in tissue lubrication and moistening, protection of teeth and oral mucosa, immune defense against pathogens, taste and smell, mastication, swallowing and wound healing. Most of these functions are dependent upon the interaction between salivary fluid and oral surfaces with different texture such as soft mucosal epithelial surfaces with various keratinization, along with the hard surfaces of teeth.[1,2] Saliva from minor salivary glands is important for maintaining a mucin-rich layer adjacent to oral mucosa. Regarding systemic needs, saliva plays an essential role in digestion by lubrication and protection of esophageal mucosa. Additionally, salivary analysis is beneficial for evaluating physiological and pathological conditions in humans. The value of saliva as a diagnostic tool for oral and systemic diseases is particularly due to its origin, composition, functions and interactions with other organ systems.[42] This section is a review of the published articles on the roles of saliva in the context of physiological and pathological processes of the human body.

2.1 Role of saliva in maintenance of oral and systemic health

Moistening and lubrication

A continuous flow of saliva into the mouth maintains the moist condition of oral mucosa that protects them from abrasion and facilitates the removal of microorganisms, desquamated epithelial cells, leucocytes and food debris from oral mucosa by swallowing.[34] Healthy individuals have approximate salivary flow rate of 0.3-0.5 ml/min.[13-15] Salivary flow rate is associated with a circadian rhythm. The highest flow rate is observed in the afternoon, whereas the lowest rate is detected during sleeping.[14] When the salivary flow rate of unstimulated saliva is less than 0.1 ml/minute, salivary hypofunction should be taken into account. Besides moistening the oral mucosa, saliva plays an important role as a lubricant, present in the salivary film, coating all surfaces in the mouth. The estimated thickness of this film is about 0.07-0.10 mm.[43] Salivary

mucins (MUC5B and MUC7) combined with glycosylated PRP and other glycoproteins and proteins are important for lubrication by forming a slimy viscoelastic coating of all surfaces in the mouth to facilitate mastication, swallowing and speaking.[34] In patients with Sjögren's syndrome or the patients who receive radiotherapy for head and neck cancer, salivary hypofunction is usually detected with the low salivary flow rate that causes the dryness of oral mucosa. These patients usually have difficulties in mastication, swallowing and speaking due to the lack of saliva. Previous study demonstrated that the patients who received radiotherapy with more severe xerostomia had lower levels salivary MUC5B as compared with those who had less xerostomia.[44]

Mucosal tissue protection and wound healing

Oral and oesophageal mucosa are exposed to a variety of food and substances during eating and drinking. Some substances or acid may have damaging effects on oral and oesophageal mucosa. Saliva is capable of buffering against these noxious substances. Bicarbonate plays a major role in buffering by reacting with hydrogen ions, resulting in formation of carbonic acid. Salivary carbonic anhydrase VI transforms carbonic acid into water and the volatile carbon dioxide and the latter is evaporated into the oral environment without accumulation of acid forms.[45] Another source of acid in the oral cavity is derived from vomiting. Vomiting reflex brings hydrochloric acid from gastric juice into the mouth. As vomiting starts, salivary flow is increased. Thus, bicarbonate concentration increases with salivary flow rate can be able to buffer this strong acid. However, the buffering capacity of saliva against hydrochloric acid is limited in bulimic individuals due to frequent vomiting. Saliva also protect the mucosal tissues of oral cavity and oesophagus by softening and lubricating hard food which may irritate the mucosal surfaces during the processes of chewing and swallowing. In addition, when swallowing, salivary mucins are transferred to oesophageal area and may help to form mucous layers on oesophageal surfaces.[34]

During daily activity such as eating, drinking and speaking, oral mucosa is susceptible to a variety of chemical and mechanical injuries, leading to development of oral wounds. Saliva has beneficial effects on promoting wound healing. First, saliva provides a suitable humid condition that facilitates the survival and functions of immune cells during healing processes.[41] Second, saliva consists of several components that are essential for wound healing. For examples, saliva contains tissue factor which is attached to the membrane of exosomes derived from epithelial cells. This tissue factor accelerates the coagulating process during haemostasis.[46] Third, saliva has various antimicrobial peptides and molecules that prevent oral wounds from microbial infections. Fourth, saliva contains several growth factors such as epidermal growth factor (EGF), transforming growth factor (TGF) and vascular endothelial growth factor (VEGF).[47-49] These molecules are

important for proliferation of oral keratinocytes. Finally, other salivary molecules have a variety of wound healing effects: trefoil factor 3 (TFF3) and histatin promote would closure[50,51]; SLPI inhibits microbial protease activity[52] and leptin (anti-obesity hormone) promotes angiogenesis.[53] The role of salivary proteins in wound healing encourages upcoming research for development of new medications for tissue regeneration.

Food perception and food digestion

In the oral cavity, food perception is derived from its texture, taste and aroma. Saliva is the first fluid that mixed with food. It is worth noting that the complexity of saliva components interacting with food may play a role in food perception.[54] Taste sensation is produced by the interaction between tastants which are non-volatile molecules and taste bud receptors localized in the tongue papillae. It was suggested that secretory fluid from von Ebner glands (minor salivary glands) that bathed taste bud receptors might involve in taste perception.[54] In addition, salivary enzymes and hormones are suggestive for the protection or modulation of taste receptor cells. Gustin (also known as carbonic anhydrase 6) which is a Zinc-binding protein is reported as a trophic factor affecting the taste buds.[55] It was reported that salivary cAMP and cGMP was decreased in individuals with hypogeusia and these molecules might play a role as a growth factor in taste buds.[56,57] Hormones detected in saliva such as leptin and ghrelin were demonstrated and implied for modulation of taste perception.[58] The sour receptors are hydrogen ion channels and the salt receptors are sodium ion channels. In the mouse model, it was demonstrated that the receptors for bitter, sweet and umami were G-protein-coupled receptors.[59]

Smell or aromatic sensation is produced by the interactions between volatile molecules (odorants) and olfactory receptors in the nasal cavity. The odorants are migrated from the mouth to the nasal cavity via the nasopharynx. Previous results were demonstrated the role of saliva on the retronasal aroma perception of wine.[60] Recent evidence suggested that saliva from obese individuals suppressed the release of aroma compounds from wine.[61] Thus, interactions between saliva and food or beverage have a significant role in food preference, food perception and therefore personal diet selection. In patients with Sjögren's syndrome, the dryness of oral mucosa may pathologically affect on taste buds, leading to decreased taste threshold sensitivity. However, the taste performance of these patients with stronger taste stimuli was not impaired.[62] In patients with head and neck cancer who receive chemotherapy or radiotherapy, they usually have altered food perception, possibly due to damage of taste and smell receptors.[63]

Saliva participates in the initial digestion of a variety of food components. Salivary α-amylase is thought to be the main digestive enzyme in the mouth. This enzyme is largely detected in parotid saliva, whereas less than one quarter of those in parotid saliva is found in

submandibular and sublingual glands. Salivary amylase concentration is very low in minor salivary glands.[64,65] The enzymatic activity of salivary α-amylase is specific for the cleavage of the α-(1,4)-glycosidic bonds of polysaccharides.[66] The role of α-amylase in the oral cavity for starch digestion remains uncertain.[67] However, it plays a major role in the initiation of bioadhesive process, facilitation of carbohydrate metabolism, and bacterial adherence at the tooth surface.[68] It remains a matter of interest why humans have such high amounts of salivary amylase. It is thought that the main value of salivary α-amylase is to facilitate the dissolution of starch containing debris retained in the oral cavity after meal, leading to formation of more soluble products which are dissolved in saliva.[34]

Tooth protection

To protect the teeth from abrasion, attrition, erosion and dental caries, saliva form a thin acellular organic film on tooth surface, called salivary pellicle[69] (also called acquired enamel pellicle: AEP).[70] According to proteomic studies, it was reported that enamel pellicle contained approximately 130 proteins, 67.8 % derived from desquamated oral epithelial cells, 17.8 % from plasma proteins and 14.4 % from salivary glands.[71] Although the major components of salivary pellicle are proteins, carbohydrates and lipid are also detected in the pellicle.[70] Formation of enamel pellicle requires a highly selective adsorption process where macromolecules from saliva adsorb onto enamel. It was reported that PRPs, statherin and histatins were proteins which initially attached to the enamel, followed by protein aggregation through protein-protein interactions.[72] The thickness of salivary pellicle is varied ranging from 0.3-1.1 μm depending on locations in the oral cavity and on their susceptibility to abrasive forces.[73,74] Formation of salivary pellicle can be affected by several factors such as salivary flow and clearance, oral bacteria and biologic reactions such as enzymatic cross-linking and protein degradation.[75] Salivary pellicle can protect teeth against attrition and abrasion by acting as a lubricant which reduces frictional forces between opposing teeth.[76,77] For protection of teeth against acid erosion and dental caries, salivary pellicle functions as a diffusion barrier between the inward movement of hydrogen ions and the outward movement of calcium and phosphate ions.[78] It was suggested that components in salivary pellicle determined the types of microorganisms that formed the initial layer of biofilm (also known as dental plaque) on the tooth surfaces.[34] In addition, it was demonstrated that the rate of acid clearance from biofilm was opposite in relation to the salivary film velocity. Thus, the regions of teeth in the mouth where the salivary film velocity is low are more vulnerable to caries progression.[79] Besides protective effects of salivary pellicle against dental caries, saliva contains urea which is considered to be an anticariogenic component.[80] Therefore, persons with hyposalivation are more susceptible for development of dental caries due to the loss of salivary protective components.

Saliva and its role in oral immunity

The mouth is one of the most heavily colonized compartments of our body because of its moist, warmth and rich nutrient. Saliva is considered to be a major determinant of the oral environment which affects the colonization and growth of microorganisms.[81] There are plenty of salivary defense proteins in saliva. Although the concentrations of these molecules are low in whole saliva, it was suggested that the effects of salivary defense proteins were synergistic and produced an efficient defense complex system which participated in innate and acquired immune response in the oral cavity.[39] There are several defense properties of salivary proteins such as: microbial agglutination/surface exclusion[82]; lysis of microbial membranes[83]; anti-fungal property[84]; anti-viral property[85] and immune regulation[86]. It is suggested that salivary defense proteins contain multi-functions and their biologic effects may be overlapped[81]. According to their functional characteristics, salivary defense proteins can be classified as: salivary antibodies; antimicrobial peptides; salivary innate defense proteins.

Salivary antibodies

There are two major classes of antibodies found in saliva: IgA (90-98 %) and IgG (1-10 %). Salivary IgA is mainly dimeric and complexed with secretory component (also called secretory IgA: sIgA), whereas IgG is monomeric. Other antibodies such as IgM, IgD, and IgE can be detected in saliva with very low concentrations.[87] sIgA are mainly produced by specific plasma cells located in the salivary glandular stroma and some plasma cells found in oral mucosa. Salivary IgG is primarily from serum IgG entering the oral cavity via the gingival crevicular fluid (GCF). In addition, monomeric IgA and IgG found in saliva may derived from mucosal transudate or acinar ultrafiltration.[88] Salivary antibodies are the first line of defense in the mouth. There are several functions of salivary antibodies such as: agglutination and surface exclusion of antigens in saliva, mucus layer of oral epithelial cell and the acquired enamel pellicle on the tooth surfaces[39]; phagocytosis and antigen presentation[87]; cytokine production and activation of degranulation[89,90] and antibody catalyzed ozone formation.[91]

Antimicrobial peptides

Antimicrobial peptides are defined as any protein which its size is smaller than 100 amino acids with molecular mass values ranging between 3.5 and 6.5 kDa.[92] Antimicrobial peptides are mostly cationic peptides and their mechanism of action is that they bind the negatively charged surface of microbial membranes, resulting in pore formation and disruption of membrane integrity.[93] The most abundant antimicrobial peptides found in saliva are histatins, defensins and cathelicidin LL-37.[81] Histatins belong to a family of metal-binding peptides enriched in positively charged amino acids including histidine, arginine and lysine. At least 12 histatins were detected in human saliva. Among salivary histatins, histatin-1,

histatin-2, histatin-3 and histain-5 are most important. Histatins demonstrate potent antibacterial, antifungal and wound-healing activities. These properties, combined with the ability to inhibit collagenases and cysteine proteases, indicate their potential use in the treatment of several oral diseases.[94] Defensins in humans are classified into two subtypes: alpha-defensins and beta-defensins.[92] Salivary alpha-defensins are produced by neutrophils, whereas beta-defensins are produced by mucosal cells. Defensins exhibit multi-microbicidal activities, including antibacterial, antifungal and antiviral activities. Moreover, defensins exert a variety of immune activation and immune modulation.[81] Accumulating data in the past decade demonstrate that defensins have extended functions such as modulators of the innate and adaptive immune system, angiogenesis and wound healing.[95,96] Human cathelicidin, namely human cationic antimicrobial peptide 18 kDa: hCAP18, belongs to a member of proteins with a highly conserved N-terminal domain and an antimicrobial C-terminal domain. LL-37 is a cleaved part of hCAP18 after proteolysis by proteinase 3 from neutrophils.[81] LL-37 has a wide functional repertoire that includes direct antimicrobial activities against various types of microorganisms. LL-37 also plays a role in angiogenesis, wound healing and the regulation of apoptosis.[97,98]

Salivary innate defense proteins

A variety of saliva proteins demonstrates an inhibitory effect on microbial growth such as lysozyme, lactoperoxidase, lactoferrin and statherin.[81] Salivary MUC5B plays an important role in modulating the bacterial attachment to the dental pellicle, whereas salivary MUC7 entraps and agglutinates bacteria, fungi and viruses.[99] Cystatins are cysteine protease inhibitors that demonstrate the anti-bacterial and anti-viral properties and also have an immunomodulatory function.[100] Salivary agglutinin (SAG), lung glycoprotein-340 (gp-340) and deleted in malignant brain tumors-1 (DMBT-1) are identical proteins which belong to the scavenger receptor cysteine-rich (SRCR) superfamily of proteins.[101] SAG demonstrates antibacterial and antiviral properties, and also exert certain immune activation and immunomodulation.[102,103] Proline-rich proteins (PRPs) are able to bind a variety of microorganisms such as bacteria, fungi, viruses and participate in the microbial clearance.[99] In addition, PRPs act as a first line of defense against tannins.[104] Secretory leukocyte proteinase inhibitor (SLPI), an approximate 12 kDa nonglycosylated cationic protein, is considered as an important regulator of innate and adaptive immunity and as a component of tissue regeneration. In addition, SLPI has been recognized as a molecule that benefits the host because of its anti-proteolytic, anti-microbial and immunomodulatory activities.[105,106]

Other salivary defense proteins

Extracellular heat shock protein 70 (HSP70) was demonstrated in human saliva[107]

and it was suggested that this protein had the dual role of extracellular heat shock protein as both chaperone and cytokine, also called chaperokine.[108] Extracellular HSP70 has been demonstrated to play a cytostimulatory role by enhancing proinflammatory cytokine and chemokine synthesis; up-regulating co-stimulatory molecule expression; enhancing the maturation of dendritic cells; and promoting antitumour surveillance.[109] Adrenomedullin (AM) is a pluripotent hormone-like cationic peptide which can be found in whole saliva and GCF.[110] AM demonstrates antimicrobial activity.[111] Recently it was reported that salivary AM was significantly increased in aggressive periodontitis patients.[112] Adiponectin (APN) is an adipocyte-specific secretory protein and APN is considered as an important immunomodulatory cytokine.[113] Evidence indicated that APN could be detected in human saliva and these findings might be implicated in the regulation of local immune responses.[114] However, the nature and the biologic functions of APN in human saliva are not well understood. Parotid secretory protein (PSP) belongs to the palate, lung and nasal epithelium clone (PLUNC) family of mucosal secretory proteins. Regarding the structural prediction, it was proposed that PSP played a role in host-defense, including the recognition of LPS.[115] Recent study identified an anti-inflammatory peptide domain of PSP and indicated that PSP was a lipopolysaccharide-binding protein.[116]

2.2 Role of saliva research in an OMIC era

OMIC is defined (https://en.wikipedia.org/wiki/Omics) as the study aiming at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms. As previously described, saliva contains a variety of biomolecules such as DNA, mRNA, microRNA, peptides, proteins, glycoproteins and metabolites. In recent years, saliva has been considered as a mirror that reflects our body in term of physiologic and pathologic conditions due to its composition and functions.[117] Several physiologic and pathologic states influence on alterations of salivary components and functions.[118] The collection of saliva is simple, non-invasive and low cost. These reasons make saliva a highly desirable biofluid for development of biomarkers to detect oral and systemic diseases, to assess disease prognosis and to monitor the appropriate treatment.[119,120] The term "Salivaomics" was established recently to describe the information and knowledge derived from OMIC studies in human saliva including: genomics and epigenomics; transcriptomics; proteomics; metabolomics and microbiomics.[121]

Salivary genomics and epigenomics

DNA found in saliva is derived from human DNA (70 %) and oral microbial DNA (30 %).[122] It was reported that the quantity and quality of salivary DNA was comparable to blood-derived DNA and salivary DNA could be genotyped, amplified and sequenced.[123] Saliva-based DNA analysis was performed in several oral diseases

such as dental caries[124]; periodontal diseases[125]; oral cancer[126]; oral lichen planus.[127] Saliva-based DNA assays were used for the detection of systemic diseases such as cancers,[128] infectious diseases[129] and autoimmune diseases such as Sjögren's syndrome.[130] In addition, another application of salivary genomics can be beneficial for personal identification in forensic investigations.[131] Epigenomics is the study of the complete set of epigenetic modifications (such as DNA methylation, histone and non-histone chromosomal protein methylation, acetylation, sulfation and phosphorylation) on the genome which is environmentally regulated and capable of adaptation via chemical and structural modification.[132,133] Although human epigenome has been actively investigated in the fields of biology and medical sciences, epigenetics in dental and oral sciences is at the early stages. Regarding epigenetic investigations in oral diseases, one previous study demonstrated significant differences in salivary DNA methylation profiling between patients with oral cancer and control subjects.[134] In addition, it was suggested that the epigenetic mechanisms were involved in chronic inflammation of periodontal and dental pulp tissues.[135,136] Besides oral diseases, it was reported that the DNA methylation profile of saliva reflected facioscapulohumeral muscular dystrophy (FSHD) status.[137]

Salivary transcriptomics

Salivary transcriptomics is the study of mRNA and microRNA in saliva. MicroRNAs are defined as a group of small and noncoding RNAs that are transcribed but not translated into proteins.[138] Accumulated data demonstrated that more than 3,000 mRNAs and 300 microRNAs were detected in human saliva of healthy and diseased individuals.[139,140] These findings imply that analyses of salivary transcripts may be beneficial for diagnosis, prognosis and treatments. Regarding oral diseases, salivary transcripts have been intensively investigated in patients with oral squamous cell carcinoma.[141,142] Moreover, analyses of salivary transcriptome can be applied for other malignancies in lung and pancreas.[143,144] However data of salivary microRNAs on other oral diseases are limited due to the lack of suitable endogenous controls for normalisation of salivary microRNAs.[145]

Salivary proteomics

Salivary proteomics is the study of entire protein content in saliva. More than 2,000 peptides were detected in human saliva.[146] Although the majority of proteins found in saliva are derived from salivary glands, numbers of proteins from blood plasma can be detected in saliva.[147] These findings imply the potential use of saliva for determination of health status.[148] It should be noted that salivary proteins were more susceptible to degradation as compared with serum proteins.[149] Thus, during the process of saliva collection, stabilization of salivary proteome with protease inhibitors should be performed.[150] The mass spectrometric technology has been intensively used for protein identification and

quantification of post-translational modifications on salivary proteins.[120] However, this technology requires integration and standardization of validation against accepted clinical and pathologic parameters[151]. Regarding oral diseases, our previous study using MALDI-TOF/TOF mass spectrometry demonstrated the unique patterns of salivary proteomic mass signals in patients with oral cancer, periodontitis and oral lichen planus.[152] In addition, salivary proteomic biomarkers have been investigated in oral squamous cell carcinoma,[153] chronic periodontitis,[154] dental caries,[155] oral lichen planus[156] and other systemic diseases such as Sjögren's syndrome,[157] diabetes mellitus,[158] systemic sclerosis,[159] breast cancer,[160] gastric cancer.[161] Among these identified molecules, some biomarkers were increased, whereas others were decreased or varied, depending on diseases.[162] Thus, defining potential salivary biomarkers among differently expressed proteins in a variety of human diseases is challenging and it requires appropriate strategies that remain to be validated.

Salivary metabolomics

Salivary metabolomics is the study of entire metabolites in saliva including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols and carbohydrates.[120]. Although salivary metabolomics is still in the beginning stages, accumulated studies reveal that metabolites in saliva can identify health status and differentiate diseased patients from healthy individuals.[163,164] According to metabolomic approaches such as a capillary electrophoresis time-of-flight mass spectrometer, salivary metabolites were identified in both oral and systemic diseases such as oral cancer, periodontal disease, breast cancer and pancreatic cancer.[165] Moreover, an integrated separation approach of reversed phase liquid chromatography and hydrophilic interaction chromatography combining with time of flight mass spectrometer identified 14 potential salivary metabolites in patients with oral squamous cell carcinoma.[166] Another study using an ultraperformance liquid chromatography combined with quadrupole/time-of-flight mass spectrometric technique demonstrated that combination of three salivary metabolites (including phenylalanine, valine and lactic acid) differentiated patients with oral squamous cell carcinoma from healthy individuals.[167] It is thought that salivary metabolomics may help to identify salivary biomarkers which are beneficial for medical screening, disease diagnosis and monitoring.[148]

Salivary microbiomics

Salivary microbiomics is the study of entire microorganisms in saliva. Using the next generation sequencing technique, it was reported that there were more than 10,000 microbial species in oral microbiome.[168] Microorganisms in saliva are derived from biofilms on oral tissues and the composition of the oral microbiome differs from one intraoral site to another, reflecting in part the host response and immune

capacity at each site.[169] Accumulated data demonstrated that changes in oral microbiome had been correlated with several oral and systemic diseases such as dental caries,[170,171] periodontal disease,[172] oral cancer,[173,174] oral lichen planus,[175] Crohn's disease,[176]pancreatic cancer[177] and HIV infection.[178] Although explanation for the association between alterations in oral microbiome and human diseases remains unclear, continued work in this area could provide additional insight into the complexity of human health and disease. Moreover, utilization of emerging microbiomic techniques to characterize the patterns of healthy and diseased microbiome across different time points would create large-scale data sets for studying the role of oral microorganisms in oral and systemic diseases.

Conclusion

This review article provides a comprehensive knowledge of human saliva and its role in health and diseases. It is essential to understand how saliva is formed so that we can make appropriate interpretations of how alterations in the composition of saliva are related with physiological or pathological conditions. Saliva is an accessible biofluid that contains components derived from the mucosal surfaces, gingival crevices, tooth surfaces and microorganisms of the oral cavity. We are now at the beginning of a new era using human and microbial whole-genome sequencing as a platform for personalized medicine.[179,180] Abundance of potential biomarker molecules in saliva make them more applicable for detection of oral diseases and systemic disorders. The development of advanced OMIC technology has shown insights toward an understanding of human saliva as a mirror reflecting our health status. In the near future, salivary biomarkers will be applied to the early detection and make significant health care decisions as to risk assessment, diagnosis, prognosis and monitoring of treatments with specific outcomes.[120,133] For future perspective of saliva research, the challenge is to translate large-scale information of salivary OMICs to predict an individual's outcomes in relation to health and diseases.

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