ARCHIVES OF ORAL BIOLOGY 60 (2015) 863–874

Available online at www.sciencedirect.com

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Review

The functions of human saliva: A review sponsored by the World Workshop on Oral Medicine VI

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ARTICLE INFO

Article history:
Accepted 2 March 2015

Keywords:
Moistening and lubrication
Taste and smell
Tooth protection
Mucosal protection
Anti-microbial
Wound healing

ABSTRACT

This narrative review of the functions of saliva was conducted in the PubMed, Embase and Web of Science databases. Additional references relevant to the topic were used, as our key words did not generate references which covered all known functions of saliva. These functions include maintaining a moist oral mucosa which is less susceptible to abrasion, and removal of micro-organisms, desquamated epithelial cells, leucocytes and food debris by swallowing. The mucins form a slimy coating on all surfaces in the mouth and act as a lubricant during such processes as mastication, formation of a food bolus, swallowing and speaking. Saliva provides the fluid in which solid tastants may dissolve and distributes tastants around the mouth to the locations of the taste buds. The hypotonic unstimulated saliva facilitates taste recognition. Salivary amylase is involved in digestion of starches.

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http://dx.doi.org/10.1016/j.archoralbio.2015.03.004
0003–9969/

Saliva acts as a buffer to protect oral, pharyngeal and oesophageal mucosae from orally ingested acid or acid regurgitated from the stomach. Saliva protects the teeth against acid by contributing to the acquired enamel pellicle, which forms a renewable lubricant between opposing tooth surfaces, by being supersaturated with respect to tooth mineral, by containing bicarbonate as a buffer and urea and by facilitating clearance of acidic materials from the mouth. Saliva contains many antibacterial, antiviral and antifungal agents which modulate the oral microbial flora in different ways. Saliva also facilitates the healing of oral wounds. Clearly, saliva has many functions which are needed for proper protection and functioning of the human body.

Contents

1. Introduction

Saliva has a multitude of functions, many of which are described in two recent books.[1,2] In addition there have been several papers which have summarised a number of functions of saliva.[3–8] The World Workshop on Oral Medicine VI was held in Orlando, Florida, in April 2014, and one Group was asked to carry out a review of medication-induced salivary gland dysfunction. This was to include an updated and comprehensive assessment of the functions of saliva, since there have been several recent advances in our understanding of the role of saliva in oral health. This then was the objective of the present review.

2. Materials and methods

This paper was written by the Group on Medication-Induced Salivary Gland Dysfunction (MISGD) within the World Workshop on Oral Medicine VI (WWOM VI). The Group is comprised of five reviewers (AA, RJ, NN, YS and AlV), six consultants (senior experts in fields related to MISGD: DA, CD, JE, AMLP, GP and ArV), one research librarian (RM), a Group Head (AW), and two supervisors on behalf of the WWOM VI Steering Committee (SBJ and ARK). The mission of this Group was the preparation of systematic reviews of a variety of subjects related to MISGD. The research method was based on the policies and standards set forth by a Task Force for WWOM IV[9] and by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement,[10] adapted to the current review. Thus, the work was divided into 6 steps:

Step 1 – Scope definition: the current review covered the question: what are the functions of saliva?
Step 2 – Keyword selection: keywords were selected for the research question (see Table 1).
Step 3 – Literature search: the initial literature search was conducted, until June 2013, in the PubMed, Embase and Web of Science databases, based on our chosen keywords, was not limited in languages and allowed identifying potentially relevant records and developing a

Table 1 – Key words for 'Functions of Saliva'.

Keywords
Saliva AND Swallowing, Bolus formation, Lubrication, Buffering, Remineralisation, Tissue coating, Mucosal moistening, Food processing, Mastication, Chewing, Digestion, Starch metabolism, Fat metabolism, Enzymes, Taste, Antibacterial, Antimicrobial, Antifungal, Antiviral, Immunity, Clearance, Wound healing, Tissue repair, Dilution of strong tastants, Temperature modification of tastants, Speech, Denture wearing

comprehensive library. Group members were also encouraged to submit articles of interest located from referral or hand-searching. Information was gathered from reviews and books that have been published since 2008 and from original papers published in 2012–13. The search was completed by a hand search of the reference lists in the eligible papers, and after duplicates were removed, a total of 535 records were retained for Step 4.

Step 4 – Record screening for eligibility: supervised by the consultants, each record was screened by four reviewers (AA, NN, AlV and YS), and their eligibility determined, based on their title and abstract.

Step 5 – The reviewers analysed the titles, abstracts and the Materials and Methods sections of papers that were selected in Step 4 for: (1) type of study/publication (e.g. systematic review, etc.), (2) accurate and selective assessment of the relevance to the research question, and (3) level of evidence, i.e. the risk of bias in the paper. According to the degree of relevance and strength of methodology and evidence, papers were either excluded or retained for further in-depth analysis. This step was subdivided into two parts: (1) calibration among the reviewers as to the assessment requirements, and (2) actual review.

Step 6 – In-depth analysis: this was based on expert interpretation of the evidence. The consultants CD and AMLP and reviewer AV read and analysed the selected publications and drafted the current review. Additional key references relevant to the present review were also used because our key words and the restricted period of the literature search, although generating 535 references, did not identify ones which covered all known functions of saliva. Thus this is a narrative review, rather than a systematic review. The initial draft was reviewed, modified and subsequently approved by all coauthors. This review does not cover the role of saliva as a diagnostic fluid[11] since, strictly speaking, that is not a physiological salivary function!

3. Results and discussion

3.1. *Moistening and lubrication*

One of the main functions of the salivary glands is to provide a continuous flow of saliva into the oral cavity to maintain the oral mucosa in a moist condition so that it is less susceptible to abrasion and to remove micro-organisms, desquamated epithelial cells, leucocytes and food debris by the swallowing process.[12] A continuous flow of unstimulated saliva also helps to prevent retrograde infection of the salivary glands with oral microorganisms via the salivary ducts.[13] Several large studies have reported that the mean flow rate of unstimulated/resting whole saliva in healthy persons during the day is in the range of 0.3–0.4 mL/min but with a large standard deviation.[14–16] An unstimulated flow rate of <0.1 mL/min is considered evidence of salivary hypofunction.[17] However, it has been known for some time that the degree of xerostomia is not directly related to a decreased salivary flow rate as the subjective sensation of dryness has been claimed by persons with flow rates in the normal range.[18–20]

Salivary flow rate shows a circadian rhythm of high amplitude[21] with peak flow in the late afternoon, while the flow rate is extremely low during sleep,[22] which reduces the need to swallow during that time. Thus the time of day of saliva collection or flow rate measurement should be specified. The measured flow rate of whole saliva is actually the difference between the input of saliva from the salivary glands and possible loss of fluid, by absorption through the oral mucosa and by evaporation during mouth breathing,[23] if mouth breathing occurs during saliva collection. Absorption through the mucosa tends to occur because saliva is hypotonic at all flow rates and the oral mucosa is permeable to water. The total volume of saliva secreted per day has been estimated to be about 0.6 L.[24]

In some species without sweat glands, saliva functions as a substitute for sweat. For instance, when over-heated, a dog extends its tongue and loses heat by evaporation of saliva, whereas an over-heated rat spreads saliva over the ventral surface of its body.[25]

Although saliva is about 99% water, a very important difference between these two fluids is that saliva contains mucins which are synthesised by the submandibular and sublingual glands and by minor mucous glands present in the palatal, buccal and labial mucosa. Mucins, which are not present in parotid saliva or the secretions of von Ebner's glands, are heavily glycosylated glycoproteins and the two main ones in saliva are MUC5B and MUC7, the former being of high molecular weight and the latter of much lower molecular weight.[26] These mucins form a slimy, viscoelastic coating of all surfaces in the oral cavity and act as an important lubricant between opposing surfaces during such processes as mastication, swallowing and speaking. These three processes are very difficult for patients who have little or no salivary flow because of such conditions as Sjögren's syndrome, radiotherapeutic damage to the salivary glands or medication-induced salivary gland dysfunction.[17] MUC5B and MUC7 form part of the acquired enamel pellicle[27,28] and of the mucosal pellicle which forms a very thin layer attached to the surface of the oral mucosa. They are, of course, present in the salivary film (estimated mean thickness of 0.07–0.10 mm) which separates adjoining surfaces in the mouth.[29]

In vitro studies have shown that MUC5B is a more effective lubricant than MUC7.[30] In addition, a pilot study on 29 patients who had received radiotherapy for head-and-neck cancer showed that the 12 patients with mild or no xerostomia had higher levels of MUC5B in their submandibular saliva than the 17 patients with more severe xerostomia.[31] Thus MUC5B may help to reduce the subjective sensation of xerostomia (dry mouth). However, Yakubov[32] has pointed out that no single component is able to explain the lubricating ability of saliva and that it is likely to be due to a combination of mucins, glycosylated proline-rich proteins and some lower molecular weight proteins.

3.2. *Taste and smell*

The sensation of taste can only occur by the interaction of taste substances in solution with specific taste receptors present on taste buds. Thus saliva plays a critical role by providing the fluid in which solid tastants may dissolve and in

distributing the tastant around the oral cavity to the locations of the taste buds.[33] Once the tastant is closer to a taste bud it must diffuse through the salivary film which normally coats the oral mucosa. Taste buds are present around the mouth on the fungiform papillae and in the clefts of the circumvallate and foliate papillae on the tongue, on the soft palate, the epiglottis and the nasopharynx and oesophagus. However, in the past decade receptors which respond to various taste stimuli were discovered in many extraoral tissues, including the brain,[34] airway smooth muscle,[35] motile cilia of airway epithelial cells,[36] nasal epithelium,[37] digestive tract,[38,39] and testis.[40] Therefore these receptors would perhaps be more appropriately classified as chemosensory rather than taste receptors.

As well as possible receptors for fatty acids, there are five recognised basic tastes and in order of their effectiveness as salivary stimulants they are sour, salt, bitter, sweet and umami (savoury taste), the last being exemplified by monosodium glutamate and 5′-ribonucleotides such as disodium 5′-inosinate.[40–42] Their effect in reflexly stimulating salivary flow is significant in light of the finding that bitter taste receptors expressed in the airways and digestive tract act as a second line of defence in expelling noxious substances from the body by way of initiating coughing or vomiting, respectively. Saliva, the first line of defence, dilutes any material taken into the mouth and materials with a noxious taste can be actively spat out before they reach the rest of the digestive tract. Certain polyphenols, such as tannins, found in red wines and several plant-based foods, have a bitter taste and an ability to bind to the oral mucosa and cause an astringent or puckering sensation. These effects are ameliorated by their binding to the basic proline-rich proteins and to the histatins in saliva to form insoluble compounds, which are less able to interact with taste receptors or with the oral mucosa.[43,44] More recently, it was shown that the degree of bitterness of 6-n-propylthiouracil (PROP) was associated with the salivary levels of two products of the PRB1 gene, namely II-2 peptide and Ps-1 protein.[45] The same group then showed that the salivary levels of II-2 peptide and Ps-1 protein were higher in the saliva of supertasters of PROP than in non-tasters and that addition of Ps-1 protein or arginine (which interact with PROP) to a test solution of PROP greatly increased the bitterness sensation in non-tasters but did not change it in supertasters.[46] These findings help to explain some of the marked genetically-controlled variability in taste sensitivity in different individuals.

The sour and salt receptors are ion channels for hydrogen and sodium ions, respectively, and whether there is more than one type of each receptor is currently not known. However, there are two types of receptors for sweet (coexpression of T1R2 and T1R3) and for umami (coexpression of T1R1 and T1R3) and 25 for bitter.[47] Many of these same receptors are also found in tissues around the body, where they may act as chemoreceptors.[48] The body may respond in various ways to ingestion of non-esterified fatty acids,[49] and oral taste receptors for these may now have been identified.[50] Non-esterified fatty acids may be present in foods or be formed by the action on triglycerides of lingual lipase, secreted by von Ebner's glands. In conditions such as Sjögren's syndrome the oral mucosa is very dry. This may damage the taste buds so that they exhibit elevated taste thresholds but they still maintain normal perception of supra-threshold intensities for sour, salt, bitter and sweet stimuli.[51]

The fluid secreted by the acinar cells of the salivary glands has an electrolyte composition very similar to that of an ultrafiltrate of plasma.[52] As that fluid passes through the various components of the salivary duct system, energy is expended to reabsorb virtually all the sodium, chloride, and bicarbonate, but not water, while secreting potassium.[53] Thus by the time the fluid reaches the opening of the main excretory duct, unstimulated saliva is hypotonic and its osmotic pressure is only about one sixth of that in plasma or that of saliva in the acinar regions. When flow rate is stimulated though, the main changes in electrolyte content are a marked increase in sodium and bicarbonate and a slight decrease in potassium,[53] which has a less salty taste than does sodium.

The hypotonicity of unstimulated saliva is very advantageous with respect to taste. Taste buds adapt quite rapidly to the taste of any solution in the mouth and if unstimulated saliva had the same concentrations of sodium and chloride as those in plasma (see Table 2), we would be unable to taste salty solutions more dilute than that of plasma. Since unstimulated saliva also contains glucose (sweet), bicarbonate (which would buffer acid solutions), and urea (the main bitter-tasting substance in saliva) but all at concentrations below their taste recognition thresholds,[54] as shown in Table 2, its composition facilitates the sensation of taste. Although saliva contains glutamate, which has an umami taste, its concentration is only about 18 μmol/L, whereas its taste detection threshold is 625 μmol/L.[42] The taste receptors appear to be continuously stimulated by the low concentrations of whatever tastants are present in unstimulated saliva.[33]

Table 2 – Relation of the concentrations of plasma and unstimulated whole saliva components to median taste recognition thresholds (all in mmol/L).

	Salt		Sour H^+	(HCO_3^-)	Sweet glucose	Bitter urea
	Na^+	Cl^-				
Plasma	145	101	4×10^{-5}	24	4.5	6
Saliva	6	16	1×10^{-4}	5	0.08	4
Test tastant	NaCl		HCl	$NaHCO_3$	Sucrose	Urea
Median taste recognition threshold	12		3	12	12	120

Data from Henkin et al.[54] and Dawes[12].

Aromas are detected by olfactory receptors in the nose and they may reach those receptors during the inhalation of air containing the aroma but also via the nasopharynx during the process of food consumption. For liquid products, Normand et al.,[55] developed a model with aroma release occurring by batch extraction for a few breaths after a swallow but then by continuous release for a short period from lubricated oral and pharyngeal mucosa.

More recently, Doyennette et al.,[56] have developed and tested (with four cheeses) a model of odour release from solid foods, which includes the masticatory process and its termination by swallowing. Saliva is very much involved in this process by its incorporation into the food bolus, by its melting action on certain foods, its warming or cooling action on some foods and its role in liquefying the food prior to swallowing. The longer the chewing period of each food bolus, the longer will be the time for odour release and the greater will be the incorporation of saliva, since the chewing process and gustatory stimulation both increase salivary flow in an additive manner, as seen by the higher flow rate while chewing flavoured gum, as opposed to chewing gum base.[57] A more liquefied bolus will also increase the contact area between the bolus and the oral and pharyngeal mucosa, thereby favouring flavour release.

When salivary flow is greatly reduced due to conditions such as Sjögren's syndrome and the oral mucosa is very dry, this may damage the taste buds so that they exhibit elevated taste thresholds.[33] In addition, patients who have received chemotherapy or head and neck radiation therapy usually experience alterations in taste and less frequently in smell which are difficult to treat, because of associated damage to the taste and smell receptors.[58,59] However, their taste thresholds usually return to normal within about one year and smell thresholds in a somewhat shorter time, although some patients may experience certain changes in taste quality.[59]

3.3. *Digestion*

The main protein and also digestive enzyme in saliva is α-amylase (1,4-glucan 4-glucanohydrolase), present as six isoenzymes which can split starch into maltose, maltotriose, maltotetrose, and some higher oligosaccharides[60] with an optimum pH about neutrality. It is primarily present in parotid saliva with concentrations in both submandibular and sublingual saliva being less than one quarter of those in parotid saliva[61] and with extremely low concentrations in minor salivary gland secretions.[62] Its physiological significance for starch digestion is uncertain, as once a swallowed food bolus is infiltrated with acidic gastric juice, the enzyme is inactivated. However, Woolnough et al.,[63] compared the reducing sugar concentration in various starch-containing foods which had either been chewed until ready for swallowing but then spat out and assayed immediately or chopped to a similar consistency without exposure to saliva. They found that with the former procedure up to 17% of the total starch was broken down whereas with the latter virtually none of the starch was broken down. However, when the expectorated food bolus was not assayed for up to 15 min, there was little further increase in reducing sugar release. Although amylase activity may supply a small source of sugars to the oral microorganisms when foods containing starch are being consumed, its main value may be to facilitate the dissolution of starch-containing food debris retained in the mouth after a meal or snack by forming more soluble products which can dissolve in the saliva. Since microorganisms in the mouth still survive quite well when there is no food taken by mouth, they certainly are not dependent on the products of food breakdown.

Lingual lipase was first identified in the serous glands of the rat tongue[64] and was shown to be present in rat saliva and act as a potent lipase in the rat stomach. The enzyme was found to be an important enzyme in the gastric contents of people with cystic fibrosis.[65] In 1988, however, Abrams et al.,[66] found that in the human stomach, a gastric lipase was considerably more active than lingual lipase. Indeed, in 1993 Spielman et al.,[67] described a method for collection of secretions of the glands of von Ebner in the human tongue and reported only very low levels of lingual lipase. Thus this enzyme, although apparently significant for lipid metabolism in the rat, does not appear to be of much importance in human fat digestion, even in infancy.[68]

As mentioned in the section on taste, saliva plays an important early role in digestion in that during the initial chewing of a portion of food, it contributes to the formation of a cohesive food bolus, covered by a mucin film, which facilitates the swallowing process. Patients suffering from salivary gland hypofunction usually have to keep drinking water as an inadequate substitute for saliva. In addition, saliva distributes tastants to the various taste receptors and pleasant tastes may contribute to the cephalic phase of gastric acid secretion.[69]

3.4. *Protection of the oral mucosa and oesophagus*

The volumes of saliva present in the mouth before and after a swallow average 1.1 and 0.8 mL, respectively.[70] Thus there is usually sufficient saliva present in the mouth to buffer the potentially damaging effects to the oral mucosa of a sip of very hot or very cold drinks. In addition, materials with a noxious taste can be spat out before they have potentially damaging effects on the teeth and oral mucosa. Because of its low bicarbonate concentration (about 5 mmol/L), unstimulated saliva does not have a strong buffering capacity against acid. However, the salivary bicarbonate concentration increases markedly with flow rate to values much greater than in plasma[53] and acid is in fact the most potent stimulus of salivary flow. Bicarbonate is an ideal buffer for saliva in that its reaction with hydrogen ions creates carbonic acid. Because saliva contains carbonic anhydrase VI,[71] this converts the carbonic acid to water and the volatile gas carbon dioxide and the latter is given off to the atmosphere. Thus, unlike other buffers, after reaction with acid there is no accumulation of the acidic form of the buffer. Vomiting is another possible source of acid in the mouth from gastric juice and this has a very low pH, in the region of 2. As part of the initial vomiting reflex, salivary flow is highly stimulated. In addition, as acid from gastric juice enters the mouth during the actual vomiting, it too causes a strong reflex stimulation of salivary flow but this increased saliva flow is only partially able to

buffer the hydrochloric acid entering the mouth. Thus individuals who vomit frequently because of bulimia are very likely to experience erosion of their teeth.

As mentioned previously, saliva helps to protect the oral mucosa and the oesophagus by softening and lubricating the surface of hard foods which would otherwise tend to scratch the surface of these tissues as they are chewed and swallowed. The oesophagus contains 600–700 mucous glands that secrete bicarbonate and mucous to form a protective surface layer 95 ± 12 μm thick.[72] It is of interest that this thickness is similar to that of the mean thickness of the salivary film in the mouth.[29] Salivary mucin from swallowed saliva may also contribute to the surface mucous layer of the oesophagus.

The main cause of damage to the oesophageal mucosa is from gastro-oesophageal reflux disease (GORD). This tends to occur when there is an increased frequency of lower oesophageal sphincter (LOS) relaxation, a decrease in the tone of LOS, delayed gastric emptying, impairment within the crural diaphragm component of the LOS, or compromised oesophageal motility.[72] During GORD, hydrochloric acid and the proteolytic enzyme pepsin, which has an optimum pH of 1.8–2.3, diffuse through the mucin layer and damage the cells lining the oesophagus. In certain conditions, bile may also be present as well as acid and pepsin and its detergent effect increases damage to the oesophageal mucosa. The greatest susceptibility to GORD is immediately after meals, because the peristalsis of the oesophagus and the passage of each food bolus tend to remove part of the mucous layer. After a meal, saliva continues to be secreted as ''unstimulated saliva'' and with each swallow acts to buffer any acid transported into the oesophagus by GORD and promotes its return to the stomach. The mucin in saliva may also help to replenish the lining mucous layer. In addition, acid in the oesophagus elicits an oesophago-salivary reflex secretion of more saliva[72] which will have a higher buffering capacity than that of unstimulated saliva because of its higher flow rate.

Another protective factor is the polypeptide epidermal growth factor (EGF) which is secreted in saliva.[73–75] Although in rodents it is in highest concentration in submandibular saliva, in humans it is in highest concentration in parotid saliva.[76] The oesophageal mucosal cells contain luminal receptors for EGF, which can diffuse from the saliva, through the mucous coat of the oesophagus, and promote the proliferative activity of the mucosal cells.[72,75]

More recently, it has been recognised that the refluxed fluids of GORD may extend beyond the oesophagus up into the larynx and pharynx.[77] They may be detected by miniature pH probes, and by the identification of pepsin in saliva and exhaled breath condensate by use of specific immunoassays. The higher the unstimulated salivary flow, the less likely are gastric fluids to reach into the upper oesophagus. However, during sleep, when salivary flow is extremely low and gravitational effects on fluid in the oesophagus are reduced, there will be less protection against the damaging effects of GORD.

The oral mucosal pellicle was first described by Bradway et al.,[78] as being due to adsorption of salivary proteins and their attachment to mucosal cells by transglutamination. More recently several salivary proteins, including the mucins MUC5B and MUC7, have been identified in mucosal pellicle[28,79] but its thickness has been estimated to be only between 0 and 0.1 μm.[79] Nevertheless, this thin mucinous layer can presumably act as a lubricant during contacts between the teeth and oral mucosa or between different types of oral mucosa such as those of the tongue and cheeks. Because the entire surface layer of cells of the oral mucosa has been estimated to be replaced every 3 h,[80] this may explain why some cells that had just been exposed to the oral fluids may not show any mucosal pellicle.[79] The composition of the oral mucosal pellicle will presumably determine the types of microorganisms which can attach to the oral mucosa. Because of the fairly rapid turnover of the cells in the oral mucosa, it is not possible for thick layers of biofilm to accumulate on them.

3.5. *Tooth protection*

Saliva plays many roles in protection of the teeth against abrasion, attrition, erosion, and dental caries.

3.5.1. *The acquired enamel pellicle*

One major role of saliva is its participation in the formation of the acquired enamel pellicle (AEP), the properties of which have been extensively discussed by Hannig and Joiner.[81] The AEP is primarily a protein layer which covers all surfaces of the enamel and the underlying dentine or cementum when these have become exposed by loss of enamel, although the presence of certain lipids has also been reported.[82] When first named,[83] the AEP was assumed to be derived only from salivary proteins but, with the aid of the technique of proteomics, pellicle formed in vivo over a 2-h period on meticulously polished enamel has now been shown to contain up to 130 different proteins, with 89 of these being present in three or more studies.[27] Surprisingly, only 14.4% are of salivary gland origin, the others being from cells (67.8%) and plasma (17.8%). However, the bulk of the protein in the AEP may be derived from salivary proteins. The cellular proteins appear to be derived from oral epithelial cells desquamated into saliva and because of the relatively rapid turnover of the entire surface layer of cells, these are readily available. The plasma proteins will mainly be derived from gingival crevicular fluid. Lee et al.,[84] reported that calcium- or phosphate-binding proteins such as histatin 3 and statherin tended to form a higher proportion of the proteins which initially attach to the enamel whereas proteins with recognised protein–protein interactions formed a higher proportion of the total protein of the pellicle at later stages. The thickness of the pellicle has been estimated to vary from about 0.3–1.1 μm at different locations in the mouth,[85] depending on their susceptibility to abrasive forces.

The pellicle begins to reform within seconds of a clean enamel surface being exposed to saliva,[81] so that after enamel has been etched with acid (which removes the pellicle and a few μm of enamel) prior to placement of a composite resin, it is important that the enamel not come in contact again with saliva before application of the resin, since the resin will not bond well to pellicle-covered enamel. However, this remarkable ability of saliva to reform a new layer of proteins almost instantaneously on an exposed enamel surface makes the pellicle an excellent renewable lubricant. In fact the pellicle has been shown to reduce the frictional coefficient between

opposing teeth by 20-fold.[86] The pellicle thus provides important protection against loss of enamel by both attrition (caused by tooth to tooth contact) and abrasion (caused by contact of foreign objects such as food or a toothbrush with enamel).[87]

The possible roles of enamel pellicle in protection of the teeth against acid erosion, such as by acting as a diffusion barrier both to inward movement of hydrogen ions and outward movement of calcium and phosphate ions, have recently been discussed.[88,89] While it may have a minor role in this process, because it is so thin (mostly <1 μm) it can provide little protection by its buffering capacity against strong acids such as the hydrochloric acid in gastric juice.

Enamel is composed of crystals of an impure hydroxyapatite of approximate composition $(Ca)_{10}(PO_4)_6(OH)_2$. Because saliva contains both calcium and phosphate ions and most of the time is supersaturated with respect to hydroxyapatite,[90] without the presence of the pellicle there would be a tendency for crystals of hydroxyapatite to grow on the tooth surface, causing the teeth to become larger with time. Although the pellicle inhibits the enlargement of the enamel surfaces by crystal deposition, it is sufficiently permeable that it allows calcium and phosphate ions to diffuse through it and help to remineralise early subsurface caries lesions when the conditions that promoted the lesion development (such as an acidic plaque from frequent sugar consumption) have been reduced.[90] However, when an enamel surface has been eroded by acidic fluids, as soon as the acid is removed, a new acquired pellicle reforms on exposure to saliva. The presence of this pellicle is why the lost enamel can never be reformed (for the same reason that healthy teeth do not enlarge continuously in the mouth).

Since the pellicle covers the entire tooth surface, its composition will play a role in determining the types of microorganisms which form the initial layer of dental plaque (oral biofilm) on the teeth. Conversely, microorganisms which cannot attach to the AEP, to the mucosal pellicle, or to other microorganisms already attached to these structures, will not be able to colonise the mouth.

3.5.2. *Salivary clearance*

One of the most important functions of saliva is to facilitate the removal of food, drinks and food debris from the mouth. The process of swallowing can be represented as the operation of an incomplete syphon,[12,91] as following a swallow, a residual volume of saliva is left in the mouth. The volumes of saliva in the mouth before and after a swallow average are 1.1 and 0.8 mL, respectively,[70] so between meals only a relatively small fraction of the saliva in the mouth is swallowed with each swallow. In terms of oral health, the most important materials to remove from the mouth as soon as possible after intake are fermentable carbohydrates such as sucrose and glucose and acidic foods or drinks. Computer modelling and experiment have shown that clearance of these materials is facilitated by a low residual volume and high unstimulated and stimulated salivary flow rates.[12] Thus people with very low unstimulated salivary flow rates, due to radiation damage to the salivary glands for treatment of head and neck cancer, Sjögren's syndrome, or medication-induced salivary gland dysfunction, have very low salivary clearance rates. They are particularly susceptible to dental caries because of prolonged retention of sugar in their mouths and sugar is a relatively weak stimulus of salivary flow. In contrast, acid is the strongest stimulus of salivary flow and a good response to acid is often retained in patients with hyposalivation.[92]

For anticariogenic substances such as the fluoride in dentifrices, a more prolonged retention in the mouth is desirable for them to have maximal effect and thus the mouth should not be rinsed thoroughly with water after tooth brushing.[93]

Saliva is also important for clearance of desquamated epithelial cells from the mouth which each carry about 100 microorganisms[80] and for removal of the microorganisms which are free in saliva.

3.5.3. *Salivary inorganic components, erosion and dental caries*

The main inorganic components of saliva involved in tooth protection are calcium, phosphate and bicarbonate. The calcium and phosphate concentrations are such that over a wide range of flow rates, whole saliva is supersaturated with respect to hydroxyapatite, the main mineral in enamel and dentine.[90] Thus in the absence of food or drink in the mouth, there is no tendency for the teeth to dissolve in saliva. In fact the reverse is true but growth of hydroxyapatite crystals on the tooth surface is inhibited by the acquired enamel pellicle. This tendency to precipitate hydroxyapatite may be represented by Eq. (1).

$$\underset{\text{Solid}}{Ca_{10}(PO_4)_6(OH)_2} \leftarrow 10\underset{\text{Solution}}{Ca^{2+}} + 6PO_4^{3-} + 2OH^- \quad (1)$$

However, if the pH of saliva is decreased in a certain location by the presence of acid, hydrogen ions remove PO_4^{3-} and OH^- ions as shown in Eqs. (2) and (3).

$$PO_4^{3-} + H^+ \underset{pK_3=12.3}{\rightarrow} HPO_4^{2-} + H^+ \underset{pK_2=7.2}{\rightarrow} H_2PO_4^- + H^+ \underset{pK_1=2.1}{\rightarrow} H_3PO_4 \quad (2)$$

$$OH^- + H^+ \rightarrow H_2O \quad (3)$$

Then, if the PO_4^{3-} and OH^- concentrations decrease such that the ionic product falls below the solubility product for tooth mineral, the direction of Eq. (1) is reversed so that the tooth tends to dissolve in the acidified saliva, which shows as erosion of the tooth.

Although the phosphate in saliva acts as a buffer against acid, the phosphate concentration falls as flow rate is stimulated by acid and becomes too low to have much buffering effect. However, the bicarbonate in saliva acts as an excellent buffer, as discussed previously, since its concentration increases markedly with flow rate in the secretions of the major salivary glands[53] but not in those of the minor glands.[62]

The concentration of fluoride in saliva is only just over 1 μmol/L[94] but is sufficient to keep the saliva supersaturated with respect to any fluorapatite formed in the tooth as a result of intake of food, drink or oral health products containing fluoride. Although saliva is normally supersaturated with respect to hydroxyapatite and fluorapatite, these minerals do not precipitate out in the saliva, as this contains certain proteins, such as statherin, having the ability to bind to the surfaces of very small hydroxyapatite and fluorapatite crystals

and greatly retard their growth.[95] In addition, the average half-time of saliva in the mouth when a person is awake is only 2.2 min,[12] so this gives little time for mineral to precipitate out before the saliva is swallowed. During sleep the salivary flow rate is extremely low[22] but as the bicarbonate concentration and pH also decrease as flow rate decreases, the saliva would be expected to be much less supersaturated with respect to tooth minerals at that time.

Dental caries begins by acid dissolution of tooth mineral, initiated by acidogenic microorganisms in dental plaque which has been exposed to fermentable carbohydrate.[96] Individuals with hyposalivation are more susceptible to dental caries because of the loss of the many protective factors in saliva.[7,97] The reduced flow rate retards oral sugar clearance and also adversely affects salivary buffering of plaque acid. At a low flow rate the salivary bicarbonate concentration is reduced, causing a fall in salivary pH, and although the phosphate concentration is increased at a low flow rate, its effectiveness as a buffer is reduced since the salivary pH will be less than the pK_2 value for phosphate (see Eq. (2)), so that most phosphate is already in the $H_2PO_4^-$ form and cannot take up an extra hydrogen ion until the pH is close to the pK_1 value.

When fermentable carbohydrates such as sucrose are consumed, they diffuse into dental plaque where certain microorganisms can convert them into lactic and other organic acids. This causes a temporary reduction in the pH of the plaque with a subsequent rise as the acids diffuse out into the overlying salivary film and some base is produced.[98] This fall and rise in pH is termed the Stephan curve, after the person who first described it. If the pH of the fluid phase of dental plaque falls below the critical pH (the pH at which the fluid is just saturated with respect to tooth mineral) the tooth will tend to dissolve and a caries lesion will be initiated or progress. The critical pH depends on the composition of the fluid phase of plaque but it is considered to be in the range of 5.2–5.6.[90,99] The velocity with which the salivary film moves over dental plaque is a major factor influencing the rate at which acid formed in dental plaque can diffuse out into the overlying salivary film. Computer modelling and use of a physical model have shown that the rate of acid clearance from plaque is inversely related to the salivary film velocity.[100] Since the velocity of the salivary film varies markedly in different regions of the mouth,[101] sites with a low film velocity will be more susceptible to caries progression, which explains the increased caries in individuals with a very dry mouth.

Another important anticariogenic component of saliva is urea, the concentration of which is slightly less than that in blood. Certain microorganisms in dental plaque secrete urease which converts urea into two molecules of ammonia and one of carbon dioxide. As ammonia is a stronger base than carbon dioxide is an acid, this tends to raise the plaque pH.[98] In fact it has been calculated by computer modelling that if saliva did not contain urea, the minimum pH in the Stephan curve would be deeper by an additional 0.5 units.[102]

3.5.4. *Anti-bacterial, anti-viral, anti-fungal and some other effects of salivary proteins*

Saliva contains a large number of proteins and peptides which have been shown to have anti-bacterial, anti-viral and anti-fungal effects.[103–107] However, since the mouth is known to contain about 700 different species of microorganisms, it is clear that these antimicrobial factors are not present in sufficient concentration to eliminate the members of the normal flora. For instance, α-amylase is a growth inhibitor of *Porphyromonas gingivalis*, a periodontal pathogenic bacterium,[108] but this organism still survives in the mouth. It is possible though that antibacterial factors, in conjunction with good oral hygiene, function to maintain the proportion of the harmful oral flora at levels sufficiently low for oral health to be maintained. In addition, they may maintain the oral flora at sufficiently low levels that systemic infection with oral microorganisms rarely occurs. They may, of course, prevent some pathogenic microorganisms from ever colonising the mouth at all.

There are several cationic proteins in saliva including histatin, statherin and alpha and beta defensins. The most abundant histatins are histatin 1, 3 and 5, the last being a derivative of histatin 3, and they inhibit the growth of *Candida albicans*, an opportunistic oral fungus.[109] Statherin's primary function may be to inhibit the crystallisation of calcium phosphate from saliva but it also inhibits the growth of anaerobic bacteria.[103] Alpha defensins are secreted into saliva by neutrophils which enter the mouth via the gingival crevice, while beta defensins are secreted by epithelial cells. Beta defensins subtypes 1 and 3, but not 2, have been found to be increased in rat parotid glands (but not the submandibular or sublingual glands) in which lipopolysaccharide has been injected into the duct system to elicit an inflammatory action.[110] The defensins may have antibacterial effects and may also have antiviral effects.[110–112]

There are a number of bacterial agglutinins in saliva which agglutinate microorganisms, thereby facilitating their removal by swallowing and possibly inhibiting their attachment to oral surfaces. These include the mucin MUC7, proline-rich proteins, and salivary agglutinin, the last of which is identical with GP340[104] and DMBT1.[113]

Lactoferrin is a chelator which has high affinity for iron (Fe^{3+}) and the removal of this essential metal inhibits the metabolic activity of several pathogenic microorganisms.

Other salivary proteins, such as the cystatins (cysteine-containing phosphoproteins) and secretory leucocyte protease inhibitor (SLPI), act as protease inhibitors and their actions inhibit the ability of microorganisms to metabolise salivary proteins to amino acids. Lysozyme is derived from the salivary glands, crevicular fluid and salivary leukocytes. It is strongly cationic and damages microbial cell walls by hydrolysing the β(1–4) bond between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of the bacterial cell wall. Unstimulated submandibular saliva has been shown to inhibit the HIV-1 virus, even when diluted several-fold,[114,115] and this may explain why HIV infection is not considered to be transmitted by the oral route. Saliva contains two peroxidases, namely salivary peroxidase and myeloperoxidase.[117] Using hydrogen peroxide, they are able to convert salivary thiocyanate into hypothiocyanite ($OSCN^-$), a more potent oxidising and antibacterial agent than hydrogen peroxide. In inflamed gingival crevices, myeloperoxidase is able to produce hypochlorite (OCl^-), a chemically more powerful oxidant and antibacterial agent which may cause tissue damage.[116]

The main antibody to oral microorganisms in saliva is secretory IgA (sIgA) which is induced in IgA B lymphocytes in the gut-associated lymphoid tissue. These cells locate in the salivary glands where they form an IgA dimer and also produce a joining chain. This dimer plus joining chain is taken up by the poly-IgA receptor (also termed secretory component) on the surface of acinar cells prior to its release into saliva. Secretory component is a small, heavily glycosylated molecule which makes the IgA dimer less susceptible to bacterial proteolytic enzymes than is the single molecule IgA. However, the functional significance of sIgA is uncertain, as persons with a hereditary lack of IgA do not appear to be more susceptible to oral disease, although they may show an increase in IgM in their saliva.[106] In addition, small amounts of IgG and IgM may enter whole saliva via the gingival crevices.

Certain of the miRNAs in saliva have antiviral activity and saliva has been used as a treatment of ophthalmic herpes zoster.[117] Saliva also contains three transcobalamins[118] which bind vitamin B12 and may facilitate its uptake across the wall of the GI tract. Castagnola and his colleagues have shown that the protein composition of saliva does not remain constant with age but changes markedly, particularly in infants and children[119] and much work remains to be done in this area to understand the significance of the changes with age.[7]

3.5.5. *Wound-healing factors*

Like other parts of the body, the mouth is susceptible to wounds of various types, ranging from cheek biting to tooth extraction, and saliva plays important roles in wound healing, many of which have been well described by Brand et al.[120] Studies on pigs have shown that the mouth heals much more quickly from wounds than does the skin[121] and a major factor is that the salivary mucous layer keeps the oral mucosa from becoming desiccated. Tissue factor from salivary exosomes accelerates haemostasis and it is presumed that the many antibacterial factors in saliva[103] are usually sufficient to prevent infection of an oral wound. Saliva also contains various growth factors, including EGF, as mentioned previously, transforming growth factor alpha, trefoil factor 3[122] and vascular endothelial growth factor (VEGF). The latter is one of the main angiogenic growth factors and is also involved in reepithelialisation and regulation of extracellular matrix.[123] Other factors, including SLPI, inhibit protease activity and have anti-inflammatory and antimicrobial activity. The histatin 1 in saliva also stimulates the migration of epithelial cells and fibroblasts,[124] which facilitates the closure of wounds. More recently, Umeki et al.,[125] showed that leptin, an anti-obesity hormone present in saliva,[126] promotes wound healing by stimulating angiogenesis.

Exposure to stress is known to reduce the rate of wound healing[127] which is dependent on activation of oral keratinocytes. Saliva is known to contain catecholamines[128] and Steenhuis et al.,[128] showed that keratinocytes contain α_{2B}- and β_2-adrenergic receptors which, when activated, impair oral keratinocyte migration. Thus the higher levels of catecholamines in stress may cause delayed healing by their inhibitory action on oral keratinocytes. Melatonin is synthesised in the pineal gland and in the gastrointestinal tract and passes into the circulatory system. It is present in saliva at levels 15–33% of those in plasma[129] and may have many functions in the mouth, such as being a scavenger of free radicles, acting as an indirect antioxidant and being beneficial against periodontal disease.[129] In contrast, leptin, a hormone secreted by fat cells, particularly in persons with obesity, has been shown to interfere negatively with the regenerative capacity of isolated human periodontal ligament cells.[130]

4. Conclusion

In conclusion, saliva is a remarkably complex fluid with a large number of properties and functions which are indispensable for both oral and general health.

Funding

No financial support by non-university sources.

Competing interests

None declared.

Ethical approval

Not required.

Authors' contributions

All authors were involved in selection of the key words for the review. The reviewers, supervised by the consultants, assessed the quality of the identified articles. The first three authors wrote the first draft of the review and the others subsequently provided constructive input.

Acknowledgements

Our sponsors: American Academy of Oral Medicine, Biocosmetics, patients of Dr. David Sirois, European Association of Oral Medicine, Johnson and Johnson, The Oral Cancer Foundation, Unilever, and Elsevier are gratefully acknowledged for their unrestricted educational grants to help us hold the 2014 WWOM VI meeting in Orlando. The Wrigley Company for support of the attendance of CD at the Orlando meeting.

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