

# CLINICAL ASPECTS OF SALIVARY BIOLOGY FOR THE DENTAL CLINICIAN

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## Introduction

Saliva performs a multiplicity of roles within the oral cavity, and like many things in life, its importance is usually not appreciated until it is absent. Impairment of salivary parameters is commonly not recognized by clinicians (Walsh, 2000). Patients may present with a range of signs and symptoms which may be due to an underlying deficit in saliva production at rest. Reductions in saliva production during eating are much more apparent in terms of symptoms, and this generally brings it more to the patient's attention. In contrast, deficits in the production of saliva at rest can easily pass unnoticed. In elderly patients and medically compromised patients, where the use of multiple medications is common, salivary dysfunction is frequently seen.

The functions of saliva include:

- Lubricating the oral tissues (for swallowing and speech),
- Assisting the special sense of taste, by acting as a solvent for ions, and through proteins such as gustin,
- maintaining the health of the oral mucosa, through growth factors which promote wound healing, and cystatins, which inhibit destructive enzymes such as cysteine proteases,
- assisting in digestion, through amylase and lipase,
- dilution and clearing of material from the oral cavity,
- buffering acids from dental plaque and from consumed foods and drinks, and preventing erosion caused by episodes of prolonged exposure to weak acids (e.g. wines and black cola softdrinks) or short-term exposure to strong acids (e.g. reflux and vomiting).
- serving as a reservoir for ions (calcium, phosphorus, and fluoride) for remineralization
- controlling the oral microflora, through immunological (IgA), enzymatic, peptide and chemical mediators (Edgar et al. 1994; Walsh 2000, Brostek et al. 2006).

Salivary stimulation elicited reflexively by taste and mastication leads to an increase in the pH and buffering capacity (due primarily to elevated levels of bicarbonate), as well as supersaturation of saliva with calcium and phosphate. These factors influence the balance between enamel demineralization and remineralization. The importance of

stimulated salivary flow rate to the prevention of dental caries and dental erosion can be also explained by improved clearance of substrate due to more rapid movement of the salivary film, and, in the case of dental caries, greater activity of salivary antimicrobial mechanisms.

Reductions in the quantity of salivary secretions or changes in the properties of saliva are responsible for a host of related oral and dental problems which impact directly upon quality of life. These include:

- difficulties in eating and speaking
- alterations in taste (dysgeusia)
- increased plaque formation
- increased risk of dental caries, dental erosion, and periodontal diseases
- mucosal abrasions and mucosal irritation
- halitosis
- candidoses
- impaired retention of full dentures.

These oral problems may in turn influence medical status, in that patients lose interest in eating and may suffer from malnutrition as a consequence.

## Control of salivary secretion

Time of day has a substantial influence on the resting flow rate of saliva. The resting flow rate decreases during sleep and increases during the waking hours. The maximal resting salivary flow rate occurs during the mid-afternoon. Understanding this pattern is essential when assessing the resting flow rate in the clinical setting.

With a typical resting flow rate of 0.03 mL/minute, the total amount of saliva secreted during 8 hours of sleep will be only 15 mL, whilst during the two hours of stimulated flow during eating and 14 additional waking hours of resting flow contribute a further 700 to 1000 mL.

Autonomic parasympathetic and sympathetic nerves regulate salivary gland secretory activity. Taste, tactile stimuli from the tongue and oral mucosa, and proprioceptive stimuli from masticatory muscles and the periodontal ligament excite the inferior and superior salivary nuclei within the brain. These nuclei are also influenced by the cerebral cortex. These neurological influences underpin the effect of psychological status on resting salivary flow rates.

Stimulation of parasympathetic nerves causes the release of water and ions, but not proteins, whereas sympathetic stimulation causes the release of proteins packaged within acinar cells. These mechanisms work in partnership in

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controlling salivary flow.

Direct mechanical stimulation in and around the mouth is a powerful stimulus of salivation. For this reason, it is essential that the resting flow rate of saliva is assessed at the start of a consultation, before any dental procedures have been performed.

A range of hormones can influence salivary flow (and thereby its composition) by acting directly on acinar or ductal elements within the salivary glands. Antidiuretic hormone, which increases water reabsorption in the distal tubule in the kidney, has the same action on salivary gland ducts. These water-saving mechanisms underpin the dramatic effects of body hydration on resting salivary flow. Negative fluid balance and systemic dehydration decrease resting salivary flow. As a consequence, a reduced volume and increased viscosity of saliva in the anterior floor of mouth contribute to a sensation of thirst. However, thirst itself is an imperfect indicator of body fluid balance.

Female sex hormones can also increase resting salivary flow. This explains why resting salivary flow is often increased during pregnancy and decreased during menopause. Similarly, the male sex hormone testosterone is also known to increase resting salivary flow.

## Components of saliva

Saliva as a fluid is a composite of the secretions of major and minor salivary glands. Saliva also contains material derived from the gingival sulcus, a point which has diagnostic relevance in terms of salivary markers of periodontal destruction. The composition of saliva varies from site to site within the mouth of the one individual, and changes according to the time of day and proximity to meals. Its properties are affected by the level of hydration and general health of the individual.

Saliva can be considered a filtrate of the serum in as much as it is derived from the blood. It follows that the process of saliva production is linked to overall body fluid balance and that blood flow through salivary gland tissues (from branches of the maxillary and other arteries) has a major effect on the production of saliva. Water makes up approximately 99% of the volume of saliva, and serves as the solvent for the other components that make up saliva.

The total flow rate for saliva (both stimulated and unstimulated) ranges between 500 to 1500 mL per day in an adult, and the average volume of resting saliva present in the oral cavity is 1 mL.

The resting saliva is derived from the submandibular gland (60%), sublingual glands (5%), parotid glands (20%), and other minor glands (15%). Parotid saliva (also termed serous saliva) is high in bicarbonate ions and amylase, while submandibular gland secretion (mucinous saliva) is high in mucins and calcium. In fact, the concentration of calcium in submandibular saliva (3.7 mmol/L) is considerably higher than that in plasma (2.5 mmol/L) or in pooled whole saliva (1.35 mmol/L).

Because increased salivary flow causes the fluid environment of the oral cavity to become alkaline, there is a direct association between a more alkaline environment caused by increased salivary flow and mineralization of foci within supragingival plaque, leading to the formation of dental calculus. The increased calculus formation reflects not only the higher pH, but also the occurrence of highly ionised phosphate ions [ $\text{PO}_4^{3-}$ ] in the saliva and plaque resulting from breakdown of organic phosphates by salivary phosphatase enzymes. An example of this is seen in individuals with cystic fibrosis, who because of altered exocrine gland function have increased levels of  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  in their submandibular saliva. Such persons often show a marked tendency to form supragingival calculus.

## Salivary enzymes and mucins

Saliva is such a complex biological fluid that it is practically impossible to replicate it from individual components. Not surprisingly, the majority of its components are hydrophilic (water-loving), however some hydrophobic components are present. The most notable of these is the enzyme lipase, which is secreted the von Ebner's glands of the posterior tongue. Being hydrophobic, lipase can enter globules of fat, in which it breaks down fatty acids.

Saliva has a limited role to play in assisting digestion. The pre-digestive function of saliva is mediated by a number of enzymes, including amylase, lipase, and a range of proteases and nucleases. Amylase can break down starch and glycogen into smaller components such as limit dextrins and maltose. By breaking down complex carbohydrates which may adhere to the teeth, amylase may serve a limited protective role.

The mucins of saliva are glycoproteins with many short oligosaccharide residues on each molecule. They bind water by hydrophilic interactions, and this bound water is essential for maintaining hydration of the oral mucosa. Salivary mucins exist in both high and low molecular weight forms. Low molecular weight sulpho-mucins assist in clearing bacteria from the oral cavity by binding to and aggregating microorganisms. The levels of low molecular weight mucins (such as MG2) in resting saliva decrease with age.

The interplay between water and mucins has a dramatic effect on the viscosity of saliva, particularly for the secretions of the submandibular salivary gland. Reduced water results in a relative increase in the concentration of mucins, making the saliva more viscous in consistency and sticky in nature.

Mucins are essential for the lubricating functions of saliva. When salivary flow rates are low, wearing of mandibular dentures becomes a major problem because of trauma to the denture-bearing mucosa. With a full upper denture, commonly lack of retention (due to loss of cohesion) and chronic fungal infections are commonplace.

Salivary mucins serve other functions in addition to lubricating the oral cavity, and preventing dehydration of the

oral mucosa. Salivary mucins protect the mucosal surface and limit the extent of abrasion of the oral mucosal epithelial cells caused by normal masticatory function. An even coating of mucins also gives a smoother surface for the flow of air during speech.

### Salivary antibacterial systems

Saliva contains a broad range of antibacterial agents. Immunoglobulin A (IgA) is a major component of saliva proteins, and is able to aggregate bacteria and prevent adhesion. IgG and other immunoglobulins derived from the gingival crevice is also present in saliva, however little complement fixation is possible in saliva as levels of key complement components are too low. The contribution of gingival crevicular fluid to resting salivary flow is very small, in the order of 10-100  $\mu\text{L/hr}$ .

The enzyme amylase can inhibit the growth of some species of bacteria. Lysozyme breaks down the peptidoglycan in the cell wall of some Gram positive bacteria, including *Streptococcus mutans*. Lactoperoxidase catalyzes the oxidation of salivary thiocyanate by hydrogen peroxide to the toxic molecule hypothiocyanite, which inactivates bacterial enzymes.

Histatins are histidine-rich proteins which inhibit the growth of *Candida albicans* and *Streptococcus mutans*. Lactoferrin binds ferric ions and thereby inhibits bacteria from gaining the essential nutrient of iron. It can be degraded by some bacterial proteases. The related molecule apolactoferrin also exerts antimicrobial effects on a range of microorganisms, including *Streptococcus mutans*.

### Salivary buffer systems

In the healthy state, the pH of resting saliva is maintained in a narrow range between 6.7 and 7.4. The bicarbonate ( $\text{HCO}_3^-$ ) buffer system is the major buffer system present in saliva. As in the peripheral blood, the combination of sodium bicarbonate, carbonic acid, and gaseous carbon dioxide is an effective means of removing protons (hydrogen ions) from the system. When considering the dynamics of this buffer system, it should be remembered that saliva has a higher level of dissolved carbon dioxide than normal room air (5% vs. less than 1%), and is present in saliva both as bicarbonate ( $\text{H}_2\text{O} + \text{CO}_2 \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$ ) as well as dissolved  $\text{CO}_2$  gas.

The concentration of bicarbonate ion in resting saliva is approximately 1 mmol/L, and this increases to over 50 mmol/L on stimulation. As the concentration of bicarbonate ion increases, so does the pH and buffer capacity of the saliva. This is a key point in interpreting salivary diagnostic tests. Because of diurnal variations in resting flow rate, there are corresponding variations in levels of bicarbonate and thus in the pH and buffer capacity. The resting pH will be lowest during sleep and immediately upon waking, and then increases during the waking hours.

As bicarbonate levels in the saliva increase, this will not only

increase salivary pH and buffer capacity, and facilitate remineralization, but will also exert ecological effects on the oral flora. Specifically, higher salivary pH will suppress tendency for aciduric (acid tolerate) microorganisms to grow, particularly cariogenic mutans streptococci and *Candida albicans*.

Mastication of foods and chewing gum is a powerful stimulus of the secretion of sodium bicarbonate into the parotid saliva. Strong acids are powerful gustatory stimuli of salivary secretion, as can be seen clinically when residues of phosphoric acid or acidulated fluoride products contact the tongue. Salt, sweet and bitter are less effective stimulants of salivary secretion than acids. While a reflex of increased salivary flow in response to acids has an obvious protective function in normal individuals, the stimulatory effect of acids has little if any therapeutic value in patient care. In fact, frequent use of citric acid-based drops or sweets to stimulate salivary flow is contra-indicated in patients with salivary gland dysfunction because of the limited buffer capacity of their saliva.

Phosphate also contributes to the buffering capabilities of saliva, particularly in the resting saliva situation. A range of proteins in the saliva play a minor role in buffering. In addition to these proteins, peptides such as sialin assist by promoting the production of amines (which exert an alkalinizing effect) from enzymatic breakdown of salivary proteins and by oral bacteria. Similarly, urea in the saliva can be broken down to ammonia.

### Salivary components involved in the process of remineralization

Saliva contains a range of inorganic ions including calcium, phosphates, fluoride, magnesium, sodium, potassium, and chloride.

Several components of saliva help maintain supersaturation of saliva with calcium and phosphate ions. Statherin inhibits precipitation and crystal growth of calcium phosphate. It is a phospho-protein with a strong affinity for calcium and for enamel and other apatite surfaces. Many of the key biochemical properties of human salivary statherin are possessed by phosphopeptides derived from casein (CPP). Statherin and CPP share partial sequence homology with phospho-proteins from mineralised tissues such as dentine and bone. Proline-rich proteins work in a similar fashion to statherin, and bind to the surface of calcium phosphate crystals to prevent their growth. Together with citrate, these proteins bind a considerable portion of the total calcium in the saliva, and help to maintain the correct calcium-phosphate ionic ratio. Citrate as an ingredient in many carbonated drinks and sports/energy drinks poses a major risk for dental erosion by binding calcium and depressing the concentration of free calcium ions in saliva.

Proline-rich proteins are a key component of pellicle, and bind strongly by their amino terminus to enamel. The trailing carboxyl terminus is the site of adhesion for some bacteria in

the early stages of plaque formation, and is also the binding site for tannins from the diet.

### Remineralization

Saliva controls the equilibrium of mineral gain and loss in an erosive or cariogenic oral environment. The importance of saliva in this role is demonstrated graphically in patients with salivary dysfunction, and in desalivated animals.

Protective properties of saliva that increase on stimulation on salivary flow include salivary clearance, buffering power, and degree of saturation with respect to tooth mineral. These benefits are maximized when saliva is stimulated after the consumption of fermentable carbohydrates, by reducing the fall in plaque pH leading to demineralization and by increasing the potential for remineralization. As shown by the studies of Edgar and colleagues at Liverpool, when gum is chewed to stimulate saliva after a carbohydrate intake, plaque acid production is neutralized, and incipient lesions in enamel are remineralized.

The site distribution of lesions of dental caries and dental erosion demonstrates the level of protection offered by the salivary film. Sites of predilection for dental caries and dental erosion are those where exposure to saliva is limited, such as fissures and proximal sites followed by cervical surfaces for dental caries.

Saliva affords both static protective effects, which act continuously, and dynamic effects, which act during the time-course of a challenge. Salivary buffering and sugar clearance are important dynamic effects of saliva which prevent demineralization. Comparing these two effects, buffering of acids is the most important as it is linked directly with enhanced remineralization.

Fluoride in saliva (from dentifrices and dental materials, and derived from ingested foods and drinks) may promote remineralization and inhibit demineralization (Edgar & Higham, 1995). Fluoride from the saliva accumulates in dental plaque. Low to moderate levels of fluoride (up to 40 ppm) inhibit glycolytic fermentation of sugars by dental plaque bacteria. This has been demonstrated by in vivo studies of enamel blocks when fluoride was added to sucrose at levels up to 5 ppm. Moreover, fluoride levels in resting saliva correlate well with the occurrence of caries arrest and reversal or regression (the conversion of disappearance of white spot lesions into sound enamel) (Woltgens et al. 1995).

Fluoride-releasing dental materials such as glass ionomer cements can contribute to fluoride levels in dental plaque and (potentially) in the saliva per se, however it appears that such materials reduce or prevent secondary caries primarily through the effects of fluoride on the surrounding tooth structure.

Remineralization can occur in the body of natural enamel caries lesions, especially when the surface layer is thin or lost. A key point is that fluoride should be delivered to enamel carious white spot lesions in moderate concentrations to

achieve the greatest remineralization. Topical applications of very high concentration fluoride products encourage the formation of an extremely dense surface layer on such lesions, effectively "locking in" the surface components. Once formed, the low permeability of this layer hinders further natural repair.

With the notable exception of phosphopeptides, all currently used topical fluoride agents deposit soluble fluoride as calcium fluoride (CaF<sub>2</sub>) on the surface of tooth structure or within lesions. This calcium fluoride serves as a source of fluoride for the subsequent formation of fluorapatite when the pH falls. Other fluoride sources include non-specifically-adsorbed fluoride, fluorapatite, and fluorohydroxyapatite. It is important to remember that fluorapatite is formed from CaF<sub>2</sub> when the pH drops, but is not formed during topical application. The reservoir effect gained from calcium fluoride formation is similar to that which occurs in phosphopeptide products, because their delivery system is also pH dependent. Using fluoride-containing phosphopeptides topically bolsters the capacity of the intra-oral fluoride reservoir. The capacity of this reservoir to supply ions for a prolonged period is crucial to the success of topical treatments in preventing and arresting dental caries and dental erosion.

### Clearance of substrates and acids

The term "Oral clearance" refers to the elapsed time between the introduction of a substance to the oral cavity and the moment when its presence there can no longer be detected. There are a number of ways to express oral clearance including half life (50% of the original concentration) and detection threshold (the time taken to reach a concentration at which the substance is no longer detected).

The oral clearance function of saliva can be demonstrated using clearance of a substrate such as sucrose or glucose. A simple and informative clinical procedure for assessing oral clearance by saliva is to assess glucose clearance using the detection threshold/minimal concentration method. The technique involves the use of an enzymatic reagent, impregnated in a paper strip (glucose urine analysis tape), which reacts with any glucose present. The glucose challenge can be provided by a glucose solution or a solid glucose sweet (such as a jelly bean). The test tape contains glucose oxidase, peroxidase and a chromogenic substance. In the presence of glucose, the tape changes colour as a result of oxidation of glucose and production of hydrogen peroxide.

Oral clearance of fermentable substrates and acids is affected greatly by the stimulated salivary flow rate. This influence is site-dependent, such that the most rapid clearance occurs in sites immediately adjacent to major salivary gland ducts. Assessing glucose clearance at several test sites such as the anterior floor of mouth (lingual to 31) and maxillary vestibule (labial of tooth 11) over a range of measurement times will reveal that clearance lingual to 31 occurs within 30 seconds in most individuals. In contrast, the labial maxillary site near the

midline typically requires 20 minutes to clear glucose completely.

Because the protective effects of saliva (including clearance) are increased greatly by stimulation, strategies for salivary stimulation should be considered as part of an overall preventive regimen for an at-risk patient. These may include eating patterns which lead to saliva stimulation, as well as the use of sugar-free chewing gum. In terms of eating patterns, the work of Geddes at Glasgow has shown that if a "meal" includes an item which contains carbohydrate such as sucrose, glucose, or fructose that can be fermented rapidly by the acidogenic microorganisms in dental plaque, there will be rapid acid production and the plaque pH will fall. Moreover, if one sugary item is followed by another, the demineralizing potential may be enhanced.

Other items eaten immediately before, during, or after the consumption of the sugary item can influence the plaque pH. If the non-sugary item stimulates salivary flow, it will have a pH-raising effect. The remineralizing potential may be enhanced if, for instance, calcium or fluoride is released from the food. This has relevance not only to foods but to food-derived dental products such as phosphopeptides. For example, when milk or cheese products are consumed at the end of a meal, the proteins they contain can buffer pH changes induced by acidic or acidogenic (fermentable) foodstuffs, and can also exert a topical effect through phosphopeptides. Ending an evening meal with a low fat cheese platter, or using low fat cheese sticks as a between meal snack, are examples of how a dietary pattern can be altered to increase the natural caries preventive actions of these foods.

### Gum chewing and salivary function

Chewing gum stimulates salivary flow, and thereby augments its protective properties (e.g. clearance, buffering, pH, and supersaturation with minerals). Chewing sugar-free gum elevates plaque pH and thus favours mineralisation. This natural repair can be enhanced by including phosphopeptides in the gum, as has been shown by Reynolds and colleagues who assessed demineralised slices of enamel mounted in intra-oral appliances (Reynolds 1987; Reynolds 1998).

During gum chewing, the flow rate peaks during the first minute. Beyond this point, a high flow rate can be maintained by continual chewing. Surprisingly, the flow rate is not increased dramatically by chewing faster, with a similar flow rate over a range of chewing frequencies between 35 and 130 chewing actions per minute, as shown by Dong and co-workers. Because a regular gum chewing habit causes a prolonged increase in the unstimulated salivary flow rate, chewing sugar-free gum is an important preventive oral health behaviour. It is important to stress that sugar (sucrose)-containing gums must be avoided as they are less stimulatory to salivary flow than sugar-free gums. Sugar-containing gums do not promote mineralisation but rather can be directly

cariogenic through sustained release of sucrose.

The inclusion of polyols such as xylitol into chewing gums improves the oral health benefits which can be gained by regular gum chewing. These polyols are unable to be fermented, and may directly inhibit plaque formation by biochemical effects on dental plaque microorganisms.

### Phosphopeptides and saliva

Human saliva contains low levels of phospho-proteins, and the major role of stabilising of ACP is performed by statherin. Bovine (cow) milk is the source material used for preparation of phosphopeptide products for dental use in humans, such as *Recaldent™* and *GC Tooth Mousse*.

Phosphopeptide products may be of three types:

- casein phosphopeptides alone (CPP),
- phosphopeptides with amorphous calcium phosphate (CPP-ACP), which contains 18% calcium ion and 30% phosphate ion on a weight basis,
- phosphopeptides with amorphous calcium fluoride phosphate (CPP-ACFP).

The latter is designed to provide all the essential building blocks of remineralization (calcium, phosphate, fluoride, water) localised at the tooth surface and within dental plaque. Importantly, as plaque enzymes such as phosphatases and peptidases slowly degrade CPP products, the net effect is a pH rise because of ammonia release. Use of CPPs with fluoride, or the inclusion of fluoride can impair phosphatase enzyme activity, maintaining the duration of action of the molecular complex.

CPP products (particularly CPP-ACFP) build upon the scientific foundation of components of milk in caries prevention. There is a considerable literature regarding fluoride compounds administered with calcium-rich food for dental caries prevention. It is, nonetheless, important to distinguish between fluoridated milk (where the bio-availability of the fluoride is low), and CPP-ACFP, where the bio-availability of the fluoride is high.

The anti-cariogenic actions of CPP products are mediated by topical effects, including:

- Modulation of levels of bio-available calcium and phosphate, by localising ACP in dental plaque to maintain supersaturation of free calcium and phosphate ion activities
- Buffering plaque pH changes
- enhanced remineralisation and reduced rate of dissolution of hydroxyapatite
- impairment of the adherence and growth of *Streptococcus mutans* and *Streptococcus sobrinus*.

CPP can bind up to 25 calcium ions, 15 phosphate ions and 5 fluoride ions per molecule, and can stabilise calcium phosphate in solution. Otherwise, in conditions of neutral or alkaline pH, clusters and nuclei of amorphous calcium phosphate (ACP) form which readily precipitate out of solution. Through their multiple phosphoserine residues, CPP can



sequester their own weight in calcium phosphate to form colloidal complexes. By binding to forming clusters of ACP via phosphoserine residues, CPP prevent these clusters growing to the critical size required for nucleation and precipitation (Reynolds, 1998). Complexes between casein phosphoproteins and amorphous calcium phosphate complexes (CPP-ACP) have been shown to exert anti-cariogenic effects in laboratory, animal, and human in situ caries models. These complexes localize ACP in dental plaque and substantially increase the level of calcium phosphate, which in turn serves as a reservoir for free calcium and phosphate ions. The net effect is that the plaque fluid (and saliva) is maintained in a state of supersaturation with respect to tooth enamel for both calcium and phosphate ions, which suppresses demineralization and enhances remineralization. This can be exploited clinically for prevention of dental caries and dental erosion.

### Saliva as a diagnostic fluid

Because it can be collected easily and non-invasively, saliva is an ideal fluid for the diagnosis of a range of conditions. The range of analytes includes:

- Microorganisms, such as *Streptococcus mutans*
- Markers of periodontal destruction
- Viruses, such as hepatitis C
- Antibodies to viruses, such as HIV
- Blood group substances
- Therapeutic drugs
- Alcohol and illicit drugs
- Steroid hormones, such as cortisol, oestrogen, progesterone, testosterone and aldosterone
- Heavy metals such as mercury, bismuth, and lead.

The relatively low protein concentration of saliva ensures that drugs and hormones which normally are bound to carrier proteins in the plasma are present in the unbound (free) form. Many lipid-soluble hormones can be found in saliva in amounts that are proportional to their concentrations in plasma. Saliva analysis allows for regular monitoring of the systemic levels of these hormones.

In patients with uncontrolled diabetes mellitus, the level of glucose (which is normally very low) is elevated substantially as spill-over of glucose from the plasma occurs. These patients tend to suffer from salivary dysfunction with a reduction in both resting and stimulated salivary flow rates, and in some patients bilateral parotid gland enlargement. The underlying mechanisms include negative fluid balance and auto-immune lymphocytic sialadenitis.

From the standpoint of forensic science, several components of the saliva are important analytes. The ABO blood group substances (which are carbohydrate-protein complexes) are found in the saliva of 80% of the population, who are known as "secretors". Levels of blood group substances in the saliva increase with the rate of secretion. The presence of blood group substances has allowed saliva residues to be used for

forensic identification of the blood type of their source. With the recent development of single cell DNA fingerprinting, desquamated mucosal epithelial cells, neutrophils and other leukocytes within the saliva provide the DNA which is an invaluable key to identification.

### Clinical testing of saliva parameters

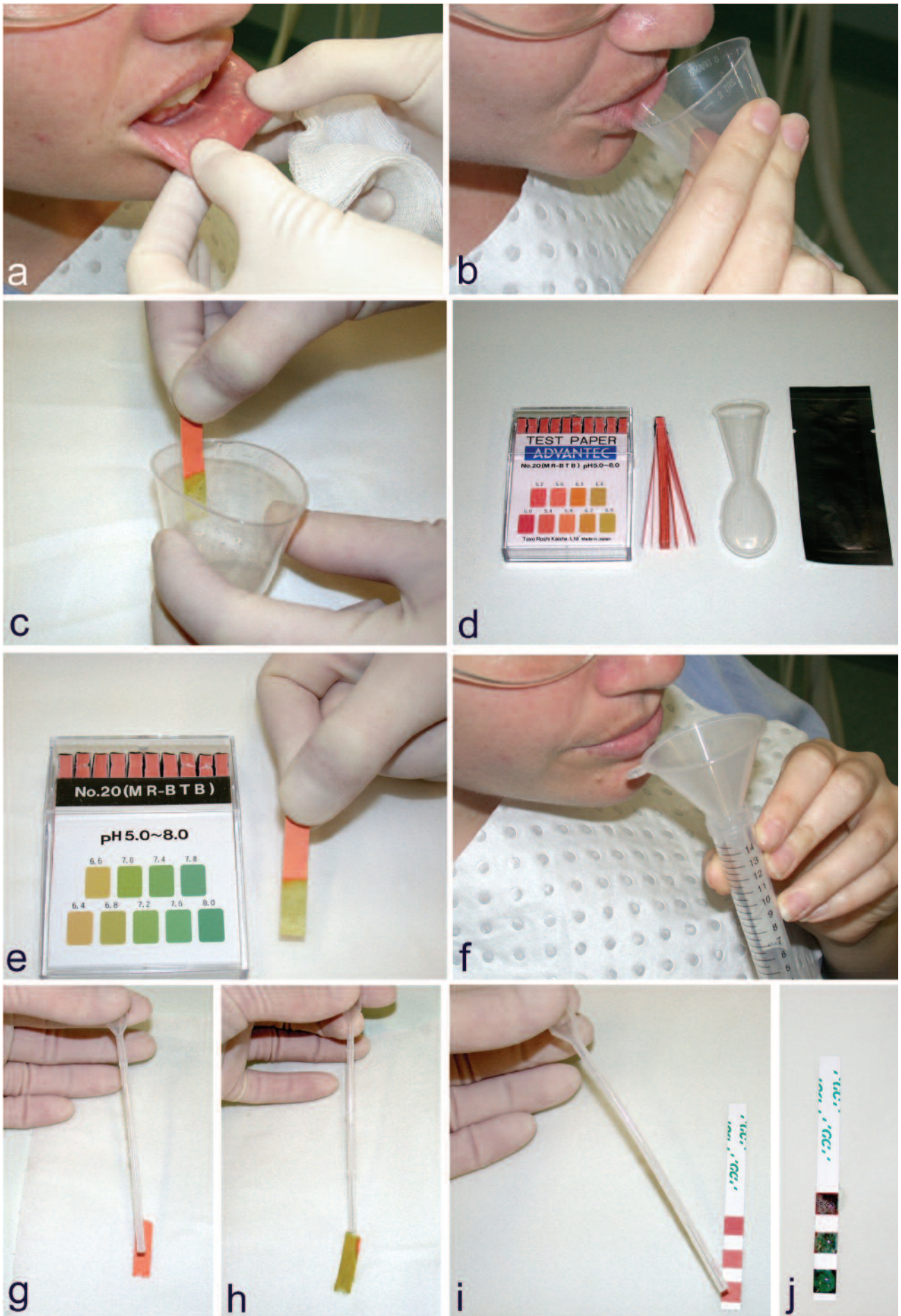
While the composition of saliva is complex, its properties in relation to defending the oral hard tissues can be assessed at the chairside in a relatively simple manner using the *GC Saliva Check Buffer kit* (Walsh, 2001). At the same appointment, an assessment of dental plaque maturity and plaque fermentation can be made using the *GC Saliva Check + pH test kit*, providing a comprehensive approach to patient assessment (Walsh, 2006).

A widely used systematic method for assessing salivary parameters follows a sequence to check the physical and chemical properties of saliva which relate strongly to the risk of dental erosion and dental caries. The test sequence is divided into two parts, the first of which assesses the resting parameters, and the second which assesses the stimulated parameters.

#### Resting saliva

The first phase of the test is to assess the production of saliva at rest. While a number of sensitive technologies have been developed which can measure the output of individual minor salivary glands, a visual screening method is rapid and effective, and produces a result which is of immediate value to the clinician. After gently blotting the inside surface of the lower lip, the surface of the everted lip can be examined for the presence of droplets of saliva. Approximately, each square centimeter of the lower lip will contain the duct of one minor salivary gland, and each of these produces one visible droplet every minute when functioning at the normal resting salivary flow rate. Patients can be screened for the presence or absence of these droplets. Younger patients may have droplets evident on the lip after as little as twenty or thirty seconds. Regardless of the patient's age, if droplets cannot be seen after sixty seconds, the resting salivary flow is below the normal range, and this should be investigated, for example is the patient's fluid balance negative because of low water intake, or the excessive intake of agents which cause fluid loss, for example caffeine from tea and coffee or alcohol.

The second phase of the test is to examine the viscosity of the saliva at rest. This also uses a simple visual method rather than complex testing apparatus. Where the resting saliva is dysfunctional, its appearance changes dramatically, and it begins to appear bubbly, frothy and sticky. Normal resting saliva has a clear watery appearance. These changes reflect organizations of the mucins and glycoproteins within the saliva as the amount of water varies. By examining resting saliva in the floor of the mouth, and in the vestibule, a simple subjective



**Fig.1.** An overview of the saliva testing process. **A**, Assessing the resting flow rate using the droplet time. **B**, Collecting resting saliva. **C**, Testing the pH of resting saliva. **D**, Components used for testing pH and buffer capacity, comprising pH test paper, a collection vessel for saliva, and buffer test strips (packed in foil). **E**, Measuring the pH of the resting saliva. **F**, Collecting stimulated saliva into a graduated container. **G** and **H**, Applying stimulated saliva to pH test paper using a pipette. **I** and **J**, Testing buffer capacity using test pads impregnated with acid.

assessment can be made as to whether this saliva is (a) clear and watery, (b) bubbly or (c) white and frothy. It will be noticed that in the latter case, the saliva will also have a glutinous sticky character.

The third phase of the test is to assess the pH of the saliva at rest. This is done by asking the patient to expectorate the remaining saliva in their mouth into the cup provided in the test kit. A single piece of pH test paper is dipped into this, and once wet, is removed immediately and the colour compared to the reference chart. This assessment must be done while the paper is still moist so that the most accurate result can be achieved. Saliva which has a neutral pH at rest will give a green reading on the test. Values below neutral pH will show orange and yellow, and the values which are near the critical pH of dental hard tissues will be orange to red.

#### Stimulated saliva

Having now assessed the patient's saliva at rest, the clinician can move on to examine the properties of the stimulated saliva. It is necessary to obtain a sufficiently large sample of stimulated saliva by having the patient chew a small piece of paraffin wax. The saliva which is collected in the cup is then used for the assessments.

The first of these is the quantity of saliva produced in a time period which allows calculation of the stimulated salivary flow rate. Patients with normal flow rates will produce a sufficiently large sample in two minutes to allow measurement. Where the patient's stimulated flow rate is suppressed, a five minute collection period should be used. It is important to leave the patient in privacy when collecting this sample. The volume can then be assessed and the flow rate per minute determined. Flow rates less than 0.7mL/minute indicate dysfunction in stimulated saliva production. This can be due to severe dehydration, or more frequently to damage to the glands, e.g. by infiltration of cells from the immune system. This can occur in a number of conditions, including chronic hepatitis C infection, HIV infection and a range of autoimmune conditions such as Sjogren's syndrome.

The fifth test is to measure the pH of the stimulated saliva. This is done by dipping a small piece of pH test paper into the sample, and scoring the colour against the reference chart while the paper is still wet. In general, the pH of the stimulated saliva should be at least one full pH unit above that of the saliva at rest. This is so because of the increased concentration of bicarbonate ion present in parotid saliva which is the dominant component of the stimulated pools of saliva which has been collected.

The sixth and final component of the testing program is to assess the buffering capacity of the stimulated saliva. This is done using a series of buffer test pads. The buffer test strip is removed from its foil pack and a small disposable pipette used to draw up saliva from the collection cup. One drop of saliva is then dispensed on to each of the three test pads, and any

excess saliva is drained away by placing the strip on its side onto some absorbent paper or disposable tissue. The colour of the test pad after five minutes can then be compared with the reference chart, and scored as either green, blue, red or partial results between these colours. A score is then calculated with green providing the highest score, and red the lowest. Patients whose buffering abilities are normal show a very high score. Where patients show depressed buffering capacity, a careful review of the other results is necessary, since patients with inflamed or damaged salivary glands tend to show depressed flow rate when stimulated, depressed pH when stimulated, and also depressed buffering capacity when stimulated. One situation where buffering capacity can be reduced, but other parameters are normal, is in the early stages of pregnancy.

#### Clinical applications of saliva testing

One can use the Saliva Check Buffer test kit in a range of clinical situations. In patients who present with cervical dental hypersensitivity, one may see several areas where there is cervical tooth loss from dental erosion. Such a patient may have a resting salivary pH of 5.6 which is near the critical threshold for demineralization, while the stimulated pH at 7.6 is normal, as is their buffer capacity. One would be prompted to look very carefully at the hydration levels of such a patient.

In a patient who has accelerated tooth wear, the resting pH may be rather low, for instance near 5.4. If the stimulated pH was also low, for example 5.8, and the buffer capacity below normal, this indicates a more complex problem which affects the gland tissues, such as organic salivary gland disease.

Where there is dramatic loss of tooth structure, this could occur by wear of softened tooth structure. The resting salivary pH may be low, and near the critical threshold. If the stimulated pH was normal and the buffer capacity also normal, one would concentrate on occupation, recreation and medication in the first instance. Closer examination of the maxillary incisors would provide a clue as to the presence of reflux disease.

In a patient with incipient root surface caries, the resting flow rate may be low, and the resting pH may also be low (e.g. at 5.6), while the stimulated pH could be more near the normal range (at 6.8). In such a patient, low buffer capacity would suggest major salivary gland disease, such as Sjogren's syndrome, which is not uncommon in elderly female patients.

Similarly patients with cervical lesions which encircle the teeth, a low flow rate, high viscosity, and low resting pH (such as 5.8) would not be unexpected. If there was also a low buffer test result, this would imply damage to the functional capacity of the salivary glands. A cause of this is lymphocytic sialadenitis associated with diabetes mellitus.

Even in younger patients, saliva testing can give important information to assist their clinical management. In patients in their 20's who show an increase in their caries rate, such as this 25 year old woman, encircling lesions on the canines and premolar teeth signify an acidic salivary environment at rest.



Salivary profiling may show a low resting salivary pH just above the critical threshold, and lower than normal buffer capacity. These problems may reflect the use of legal or illicit medicines, including anti-depressants or methadone.

Saliva testing helps to point the clinician in particular directions and away from others, and it provides a means to assess changes over time as various factors in the patient's lifestyle are altered.

### Recognition of salivary dysfunction

Salivary dysfunction is a common problem, and is common undiagnosed orodental disorder. It is important to note that symptoms of the patient are not a completely reliable indicator of salivary gland function. In other words, the absence of symptoms does not necessarily indicate normal salivary gland function, as some patients may not be aware of diminished output until the flow rate is less than half the normal rate.

Many major medical disorders are associated with xerostomia (dry mouth), and these are usually recognised by health professionals. However, other more common factors in the aetiology of xerostomia are often overlooked, particularly medication usage. While several hundred medications are recognised to induce xerostomia, this is not always listed as an adverse effect in prescribing guides. Self-administered and over-the-counter medications such as expectorants and decongestants) are particularly important in this context.

Since multiple medication usage (poly-pharmacy) is common amongst medically compromised and elderly patients, it is prudent to examine routinely all such patients for salivary dysfunction. Moreover, in the elderly, salivary dysfunction as a side effect of medications is more common because of delayed metabolism and clearance of drugs by the liver and kidney respectively.

The ability to identify a particular aetiological factor (e.g. a medication, or inadequate fluid intake) may in turn suggest an approach for treating the problem at its source.

Care should be taken not to exclude a diagnosis of xerostomia simply because the flow rate was within the normal range at one time-point. Measurement of salivary resting flow rate may be performed simply visual parameters, as outline below. Alternatively, the flow may be estimated by timed collection of unstimulated saliva into a calibrated receptacle. A resting flow rate below 0.3 mL/min may be regarded as indicative of xerostomia. Care should be taken to identify factors which may influence a particular recording (e.g. smoking, exercise, a recent meal or other oral activity). This is of prime importance with medications, since there will be periodic fluctuations in salivary resting flow in accordance with the pharmacokinetics of the medication. Thus, xerostomia may be most marked in the period following absorption and distribution of the drug, and resting flow rates may return to normal levels prior to the next dose being taken.

Visual assessment of resting saliva is a simple and time-

efficient means for screening patients for salivary dysfunction. Subjective visual evidence of xerostomia, such as pooling and frothing of saliva in the sublingual regions ("beer froth" appearance), and the appearance of white mucinous strands of saliva residues on the oral mucosa is informative in making the diagnosis. Because saliva becomes more viscous and mucinous in nature as the resting flow reduces, a simple test of saliva viscosity is useful. Viscosity can be graded using the so-called "web" test. In this procedure, a tongue blade or dental mirror is used to lift pooled saliva from the floor of the mouth or buccal mucosa. As the instrument is withdrawn a web is formed, which when stretched will eventually break. Normal saliva can maintain a vertical salivary web for a small distance (2-5 cm), whereas the web distance for the viscous saliva of the xerostomic patient can be as much as 15 cm.

### A clinical protocol for systematic patient assessment

#### 1. Listen to symptoms

- Oral dryness during the waking hours
- Oral dryness on waking
- Lack of lubrication during eating, talking or swallowing
- Salivary web formation during swallowing
- Altered taste perception
- Impaired retention of full upper dentures
- Impaired lubrication of lower dentures
- Mucosal irritation from foods and dental home care products
- Other potentially related complaints such as halitosis

#### 2. Listen to the history

- Duration and severity of symptoms
- Known exacerbating and relieving factors
- Medical conditions associated with salivary dysfunction
- Other medical conditions
- Prescribed medications
- "Over the counter" medications
- Past medical treatments
- Past dental treatment
- Use of home care products

#### 3. Listen to the patient's lifestyle

- Patterns of fluid intake
- Dietary patterns for fermentable carbohydrates
- Preferred snacking patterns
- Intake of caffeine
- Intake of alcohol
- Intake of acidic foods and drinks
- Intake of nicotine
- Intake of illicit substances
- Patient's occupation
- Patient's recreational habits
- Major stressful events in the patient's life

#### 4. Look for Signs

##### *Soft tissue changes*

- Dryness of the vermilion border of the lip
- Dryness of the oral mucosa
- Loss of filiform papillae of the tongue
- Cratering and fissuring of the tongue
- Increased plaque formation on the tongue
- Related mucosal pathology such as oral candidal infections
- Absence of saliva in response to gland palpation

##### *Hard tissue changes*

- Increased caries rate (particularly cervical caries)
- Increased rate of non-carious loss of tooth structure by dental erosion
- Multiple teeth with cervical dentinal hypersensitivity from dental erosion
- Failure to form supragingival calculus from plaque in the lower incisor region
- Increased plaque accumulation on the teeth and appliances

#### 5. Identify Causal factors

##### *Dehydration*

- Inadequate fluid intake
- Strenuous physical activity
- Swimming
- Outdoors occupation
- Dehydrating work environment
- Driving/travelling long distances
- Caffeine (black cola softdrinks, energy drinks, coffee, tea, etc.)
- Alcohol
- Polyuria in uncontrolled diabetes mellitus

##### *Salivary gland pathology*

- Head and neck or total body irradiation
- Lymphocytic sialadenitis in HIV, hepatitis C, and diabetes mellitus
- Primary Sjogren's syndrome
- Secondary Sjogren's syndrome associated with connective tissue diseases including
  - rheumatoid arthritis,
  - sarcoidosis,
  - systemic lupus erythematosus,
  - scleroderma,
  - dermatomyositis, and
  - polymyositis.
- Graft-vs-host disease in bone marrow transplant recipients

##### *Medical conditions*

- Psychological stress
- Depressive illnesses
- Chronic renal failure

- Menopausal hormone imbalance
- Thalassemia major
- Chronic protein-energy malnutrition

##### *Side effect of recreational drugs*

- Nicotine
- Alcohol (dehydration, liver cirrhosis)
- Cannabis
- Opiates (heroin, methadone, narcotics, etc)
- Amphetamines

##### *Medications*

- Anti-convulsants
- Anti-emetics
- Anti-nauseants
- Anti-Parkinsonian agents
- Anti-psychotics
- Anti-depressants (TCA, SSRI)
- Anti-pruritics
- Anti-histamines
- Anti-hypertensives
- Anti-spasmodics
- Anti-neoplastic agents
- Anxiolytics
- Cardiac antiarrhythmics
- Expectorants
- Decongestants
- Diuretics
- Narcotic analgesics
- Monoamine oxidase inhibitors
- Sedatives
- Systemic bronchodilators
- Skeletal muscle relaxants
- Tranquillisers

With regard to medications, several hundred medications are recognised to induce salivary dysfunction, and this important side effect is not always listed as an adverse effect in prescribing guides. Over-the-counter medications (e.g. expectorants and decongestants) are particularly important.

Since multiple medication usage (polypharmacy) is common amongst elderly and medically compromised patients, it is prudent to examine routinely all such patients for salivary dysfunction, even in the absence of overt caries or other pathology. It is prudent to note the time when medications suspected of contributing to xerostomia are taken, as salivary effects will typically be most marked in the period following absorption and distribution of the drug, while resting flow rates may return to near normal levels prior to the next dose being taken. Care should be taken not to exclude a diagnosis of xerostomia simply because the flow rate was within the normal range at one time point. This false negative diagnosis is most likely to occur when patients are seen at times of the day

when the flow is highest (mid-afternoon) because of the circadian rhythm.

## 6. Measure Parameters

### *Salivary resting flow*

- Visually assess lower lip labial gland secretion
- Assess resting salivary volume in the oral cavity (pooling)
- Inspect salivary viscosity (frothy, bubbly, sticky)
- Measure resting salivary pH using pH paper or pH meter  
(N.B. It is important to bear in mind the influence of factors which can affect the resting flow rate, such as position (sitting or supine), proximity to meals/eating, time of day (diurnal variation, medication intake), anxiety level, smoking, and recent physical activity)

### *Stimulated salivary flow*

- Estimate flow rate by volume collected over a defined period
- Measure stimulated salivary pH using pH paper or pH meter
- Determine buffer capacity using challenge strips with weak acids
- Assess levels of pathogens by solid phase immuno-assay

## 7. Manage Problems

### *Ensure adequate hydration*

- Limit intake of caffeine, alcohol, and other diuretics
- Ensure adequate water intake
- Use an oral hydrating gel
- Apply lip balm regularly
- Sleep on the side to avoid mouth breathing at night
- Construct a denture with an internal reservoir

### *Increase the pH and buffer capacity of saliva*

- Gain extrinsic bicarbonate via mouthrinse or toothpaste
- Increase intrinsic bicarbonate via chewing a sugar-free gum
- Rinse thoroughly after ingesting acidic foods and drinks

### *Promote remineralization*

- Use a phosphopeptide chewing gum (Recaldent) or topical gel (Tooth Mousse Plus or Tooth Mousse) at home
- Use a fluoride dentifrice (1000 or 5000 ppm) for toothbrushing
- Tooth surface protection (exposed root surfaces) with Fuji VII
- Use a dentifrice as a self-applied topical agent for early cervical and proximal lesions
- Use home fluoride gel (1.23% neutral NaF) in high risk patients
- Apply fluoride varnish at recall appointments

- Use minimal intervention approaches for caries removal (e.g. ART)
- Use GIC (Fuji IX, Fuji VII) to restore lesions as part of the overall caries control strategy

### *Suppress cariogenic microorganisms*

- Dietary modification
- Reduce the frequency of cariogenic between-meal snacks
- Reduce levels of acidic foods and drinks between meals
- Use a xylitol-containing chewing gum
- Apply chlorhexidine gel intermittently (e.g. weekly)

### *Improve oral hygiene*

- Regular toothbrushing (twice daily)
- Regular interdental cleaning (at least daily)
- Use a detergent (sodium lauryl sulphate)-free dentifrice if mucosal burning occurs

### *Assess Outcomes*

- Review salivary parameters at recall appointments
- Monitor salivary levels of mutans streptococci
- Monitor caries increment
- Look for caries arrest and reversal
- Look for changes in the mineralizing potential of saliva

## Preventive home care programs for patients with salivary dysfunction

Frequent use of bland rinses (saline or sodium bicarbonate) and rehydrating or mucosal protective products (such as Laclede Oral Balance gel) can provide relief from symptoms of oral dryness. Many patients with profound xerostomia suffer from mucosal irritation, and are unable to tolerate the use of some commercial rinses, which may contain alcohol or flavouring agents. *GC Dry Mouth gel* stabilizes oral pH in the neutral range and relieves symptoms of oral dryness. Many other products used for oral dryness have an acidic pH, which presents an erosion risk in xerostomic patients.

Salivary stimulants, such as pilocarpine, may also be employed, but these can exert problematic gastro-intestinal side effects (Edgar & Higham, 1995).

It is essential that sugar-containing or acidic (citrus) sweets as salivary stimulants be discouraged as these will accelerate dental caries in the hyposalivated state. Patients should be encouraged to apply a lip lubricant at regular intervals.

Use of sugarless chewing gums (e.g. Recaldent) is of major importance for promoting salivary function. The antibacterial, buffering, and lubricant qualities of saliva improve with increasing flow rates, such that stimulating the flow with gums improves the protection afforded to the oral cavity by the salivary secretions (Imfeld, 1999).

Other ingredients in chewing gums, such as xylitol and phosphopeptides, provide additional preventive benefits and assist in maintaining oral health.

Home use of a neutral sodium fluoride gel or high fluoride dentifrice on a daily basis will maintain salivary fluoride concentrations and inhibit mineral loss from dental hard tissues. Neutral fluoride gel may be applied at home on a toothbrush. In general, acidulated fluoride products are contraindicated for patients with salivary dysfunction, as they may cause dental erosion, dentine hypersensitivity, or mucosal irritation.

Xylitol and casein phosphopeptides exert modest suppressive effects on mutans streptococci, however additional antimicrobial targeting is worthwhile in patients with very high caries activity.

Chlorhexidine gluconate gel (0.2 %) is the agent of choice for chemical plaque control in patients with salivary dysfunction, since this agent possesses proven anti-plaque, anti-gingivitis, and anti-caries activity. It causes profound inhibition of cariogenic mutans streptococci. The gel formulation is preferred as this is alcohol-free. The alcohol content of some chlorhexidine rinses is problematic because mucosal burning. Chlorhexidine gel (0.2 %) does not cause mucosal burning, and can be applied easily with a toothbrush.

Topical CPP-ACP preparations (*GC Tooth Mousse and Tooth Mousse Plus*) are available as topical thick pastes for clinical use in tray carriers or brushed directly onto the teeth. In addition to their use in preventing dental caries or dental erosion, these preparations are well suited to the management of cervical dentinal hypersensitivity, a condition which is often associated with reduced salivary flow or pH.

The thick creamy consistency of CPP-ACP products is well suited for self-application by patients. Importantly, CPP-ACP is completely safe if ingested, which is an important consideration for products which will be used by patients in their home.

Antifungal therapy should be considered in xerostomic patients who have a history of recurring oral candidosis.

Chlorhexidine gel exerts mild anti-fungal effects and can be used together with alkalinizing mouthrinses (such as sodium bicarbonate) to suppress levels of candida species in the oral cavity.

If dedicated topical antifungal agents are used, care must be taken that the preparation is free of sucrose or other fermentable substrates. Suspensions or lozenges of nystatin or amphotericin are used commonly for treatment of candidoses.

Tissue surfaces beneath dentures and the denture surface itself typically are heavily contaminated with fungal organisms.

The denture surfaces can be cleaned and then the denture immersed for a short period in dilute sodium hypochlorite to reduce the fungal load. In addition, antifungal creams (such as miconazole) or suspensions can be directly applied to the fitting surface of a denture, and the denture then put in the mouth to give a sustained effect. Severe denture-related fungal infections require expert attention.

Regular dental review (at least three monthly) is important for ensuring that oral hygiene is maintained at an adequate standard, and dental caries and other conditions are controlled. Maintenance dental sessions should include oral hygiene reinforcement, removal of plaque, and surface treatments with fluoride varnish or phosphopeptides. Adequate monitoring will ensure that the oral health status is maintained, and the need for extensive restorative work is minimised.

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## Disclaimer

*Prof LJ Walsh played a major role in the development of the GC Saliva-Check Buffer test kit. However he has no commercial interest in this product.*