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Saliva Functions, Collections and Manipulation for research purposes: A review article

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Abstract

This review paper elucidates the chemical composition of saliva and it roles in the oral cavity as lubrication and flushing action of the dentition to reduce the dental erosion. Update, Saliva also used as a diagnostic tools of different diseases. Additionally this review deals with the pH of the saliva and its function to control the pH of the mouth during the day through the buffering system. Also it highlight the main manipulation methods used for saliva collection during research purposes.

Introduction

Oral Cavity

According to the human anatomy, oral cavity defined as the orifice through which food and air pass in the body. The mouth releases to the out of doors on the lips and drains into the throat at the rear; its barriers are defined by using the lips, cheeks, palates, and glottis. It is distributed into divisions which include: vestibule, place between the cheeks and the teeth, and the oral cavity proper. The latter section is filled through the tongue; which is a massive muscle that is firmly attached to the floor of the mouth via the frenulum linguae. In addition to its chief role inside the consumption and primary digestion of food, the mouth and its structures are critical to the formation of speech (1).

The teeth are the mouth's main structures that tear and grind ingested food into small pieces suitable for digestion, and the tongue role is to that positions and mixes food and conveys sensory receptors for taste. Finally the palate which separates the mouth from the nasal cavity, and allowing separate paths for food and air (2).

The oral cavity and vestibule are totally lined by mucous membranes which containing several small glands that, together with the three pairs of major salivary glands, that bath the mouth with fluid, maintaining it moist and clean remnants of food and its debris. Specialized membranes shape each the gums (gingiva), which surround and help the teeth, and the surface of the tongue, on which the membrane is rougher in texture, containing many small papillae that keep the taste buds. The mouth's moist surroundings and the enzymes inside its secretions help to soften morsel, facilitating swallowing and starting the system of digestion (3)

The mouth of people and different vertebrates' also continent saliva. As saliva circulates inside the mouth cavity its gatherings up food debris, bacterial cells, and white blood cells. One to two liter of saliva are excreted every day into the human mouth (4).

Saliva

Saliva is the extreme abundant frame fluid, which produce by way of salivary gland and secreted in the oral hollow space for lubricate the oral mucosa, digestion of food, clean the mouth, help speaking, facilitated swelling, flushing dentitions and protect the them from acidity. It is a mixed frame fluid that consisted from 99% of water and minerals, mucin, enzyme (amylase enzyme which is accountable of first step of starch hydrolysis), protein, antibodies, blood, and inflammatory cells (5).

The most essential feature of saliva which live at mouth is maintained pH of oral hollow space with in rang of (6.8 - 7.4) for safe the teeth from acidity that result from activity of oral microorganism and food, which is the main cause of dental caries. pH of the dental plaque decreases when the host take a snack or meal that contains fermentable carbohydrates after that, the pH returns to the resting level by salivary secretion that act to buffer the acidity (6).

Saliva is a primary protection against dental caries, making mouth lubricant and healthy, causing bacteria to flush the mouth and fight acidity. It was recognized that a decrease in salivary flow rate as in Xerostomia (especially occurring in elderly people or after radiation therapy) was associated with an increased incidence of dental caries and periodontal diseases (7).

Source of Saliva and Secretion Mechanism

Saliva are excreted through salivary ducts from salivary glands which is controlled by sympathetic and parasympathetic division of autonomic nervous structure. The sympathetic innervation through advanced cervical ganglion which activated through the release of noradrenaline (norepinephrine), noradrenaline acts on alpha- and beta-adrenergic receptors at salivary gland. This outcomes in reduced secretion of saliva via acinar cells, increased protein secretion in saliva, and decreased blood draught to the glands. The sympathetic system innervation to salivary gland is far less essential than the parasympathetic innervation in terms of regulating and production of saliva (8).

The parasympathetic outflow is coordinated via centers within the medulla, and innervated through the facial and glossopharyngeal nerves. Sensory records from the nose, mouth, tongue, eyes and conditioned reflexes are integrated inside the brain that result in parasympathetic stimulation to occur, that results in the release of acetylcholine (ACh) from which act on M_3 muscarinic receptors on the salivary gland. This excite acinar cells which growth secretion of saliva in the mouth, excite duct cells that growth HCO^{-3} secretion, rise blood stream to the salivary glands, and contraction of myoepithelium that rise the rate of firing of saliva. Overall, accelerated parasympathetic stimulation results in an elevated go with the flow of watery saliva (7).

Saliva pH

pH a measure of hydrogen ion concentration, measure of the acidity or alkalinity of a solution. The pH scale, usually ranges from 0 to 14. Aqueous solutions at 25°C with a pH less than 7 are acidic, while those with a pH greater than 7 are basic or alkaline. A pH level of 7.0 at 25°C is defined as "neutral" because the concentration of H_3O^+ equals the concentration of OH^- in pure water. Very strong acids might have a negative pH, while very strong bases might have a pH greater than 14 (9).

The equation for calculating pH was proposed in 1909 by Danish biochemist Søren Peter Lauritz Sørensen:
As pH = -log[H^+]

Where log is the base-10 logarithm and [H^+] stands for the hydrogen ion concentration in units of moles per (L) solution. The term "pH" comes from the German word "Potenz," which means "power," combined with H, the element symbol for hydrogen, so pH is an abbreviation for "power of hydrogen (10).

Saliva has a pH normal variety of 6.2-7.6 with 6.7 being the common pH. Resting pH of healthy mouth shouldn't fall beneath 6.3. In the oral cavity, the pH is maintained near neutrality (6.7-7.3) via saliva.(10)

Saliva buffering system

The saliva contributes to maintenance the pH by mechanisms. First one, is the draught of saliva removes carbohydrates that would be metabolized by way of bacteria and eliminates acids produced by microorganism. While the second one, acidity from beverages and foods, as well as from bacterial waste products, is neutralized through the buffering action of saliva (11).

A critical salivary pH equal to 5.5 was reported in dental studies, a further lower in pH of the saliva reason dental decay by change association of calcium and phosphate, this pH considers dangerous on causation and progression dental enamel (12).

The drop in salivary pH after consumption of food gets neutralized over a period of 15–60 min, by salivary fluid typical function. The pH can be brought down by flushing the mouth with antibacterial wash or plain water as its most commonly and effortlessly accessible everywhere (13).

Saliva as buffer solution

Buffering solution is an aqueous solution consisting of a mixture of weak acid and conjugated base, and its pH change very little when a small amount of strong acid or base is adding to it. Saliva is the buffering solution inside the mouth. This buffering capacity is built on several mechanisms such as the phosphate system and the carbonic acid / bicarbonate system. In unstimulated saliva (resting salivary flow), the concentration of inorganic phosphate is high while the concentration of carbonic acid / bicarbonate system is low, whereas the higher concentration of carbonic acid / bicarbonate system is the most effective buffer in stimulated saliva (14).

Saliva buffering mechanism

The phosphate buffer system is due to the ability of the secondary phosphate ion, $(HPO_4)^{-2}$, to bind a hydrogen ion and form a primary phosphate ion $(H_2PO)^{-4}$. Hence the phosphate buffer has the potential to be an effective buffer in the mouth (15).

Bicarbonate content in the saliva range from five mmole in resting (unstimulated) saliva to about 24 mmole in stimulated (salivary discharge is helped by gustatory stimuli or mechanical or pharmacological agents) saliva.

Bicarbonate acting largely to neutralize acids produced of bacteria by carbohydrate digestion within the mouth or acids from the stomach. In activated saliva, such as during eating greater bicarbonate $(HCO)^{-3}$ is produced as a derivative of cellular metabolism and which diffuses into dental plaque and facilitates neutralize the increased quantity of acid (H^{+}) produced through oral microbes (16).

Saliva as a diagnostic tool

Most clinical research are based totally on determination of blood biomarker; however, this approach are invasive, painful and consider aggressive to certain patient. While saliva is simplest to be accumulated and retain many disorder-signaling biomarkers that can be used for detect normal and disease in human. Additionally, as an alternative to urine due to suspected metabolic adulterations of the main analyte (17).

Usage of saliva as sample is recall as easy and noninvasive manner that may be easily storage and transport. Saliva sampling is painless method that is particularly useful for patients at when accumulating blood samples are hard along with hemophiliac sufferers, neonates, elderly humans, and disabled human beings and others. Patients who require frequent clinical monitoring with a couple of sampling over the day or numerous days may be blessings from saliva sampling. Dissimilar to blood, saliva collection does not require specialized equipment or skilled personnel, it continue little or no danger in any respect of cross infection amongst patients and offer minimal exposure of healthcare body of workers to blood-borne diseases such as HIV and hepatitis (16, 17).

Sampling of saliva has become a critical source of disease detection, by the usage of modern strategies and chemical equipment, recently there may be an observable growth within the use saliva for laboratory investigations. A big range of compounds were found in saliva which do not forget as relatively informative and discriminatory. These compounds may be use in early detection and analysis of diseases; for conform analysis; and in monitoring illness progression and/or treatment outcomes. (18)

Handling of saliva sample

There are many techniques for saliva gathering. Passive drool technique, maximum researchers pick to use unstimulated, complete saliva accumulated by the passive drool technique in order to hold consistency in the sort of sample gathered. Absorbent tool approach, swabs can be positioned inside the mouth to accumulate saliva for expanded ease of use. Studies with kids or other individuals that have issue with the passive drool approach also may additionally require participant-specific swab-primarily based series methods. However, swabs are limited to certain analytes. All SalivaBio swabs are crafted from the same non-toxic, inert polymer which is assured for consistency throughout all lots, making it perfect for longitudinal and multi-participant institution studies (19).

Consider the following criteria before selecting the appropriate saliva collection method: participant age & species validated for analytes of hobby number of samples required sample quantity required single vs. multi-analyte and/or DNA evaluation self vs. assisted collection biobanking vs. discarding samples (20).

After collection sample should be freeze at or below -20°C (temperature of a household freezer) immediately after collection. If freezing is not possible, refrigerate immediately at 4°C and maintain at this temperature for no longer than necessary (ideally less than 2 hours) before freezing at or below -20°C to minimize degradation and to prevent bacterial growth. While in SalivaBio Swab Collection, samples can be frozen in the swab for up to 6 months with no decline in levels. However, if it is known ahead of time that samples may need to be stored for longer than 6 months, we recommend expressing the saliva out of the swab either by centrifugation or squeezing through a syringe immediately after collection, and storing the expressed saliva in cryovials, ideally at -80 °C (21).

Samples may be saved at -80 °C for numerous years; the precise time has now not yet been decided and can vary with the aid of analyte. However, many samples that have been stored properly for over four years have shown very little degradation (22).

Assays must be achieved using best clean saliva, avoiding the pellet formed at the lowest of the tube, best to centrifuge sample before freezing. When pipetting viscous solutions inclusive of saliva, greater accuracy is acquired via aspirating slowly to avoid the formation of bubbles. Vortex and re-centrifuge tubes following each freeze-thaw cycle in view that additional precipitates may additionally expand upon refreezing. If samples will be used for genetic analysis, it is crucial to preserve the cell sediment at the bottom of your whole saliva pattern or contained in the swab device (23).

Saliva and oral health

The residences and functions of saliva have been studied significantly for extra than sixty years. This complicated bio-fluid plays an essential role within the maintenance of

oral health. Saliva is constituted by way of water, organic and inorganic components that have biological features crucial for homeostasis of the oral cavity. Saliva secretion is controlled via the self-sufficient nervous device with the extent produced varying in accordance to the type and depth of stimulation (24).

Saliva is an essential part of body health. Saliva is crucial because it, keeps your mouth moist and comfortable, helps you chew, taste, and swallow, Fights germs on your mouth and stops terrible breath, has proteins and minerals that shield teeth and prevent enamel decay and gum disease, helps maintain dentures securely in place (25).

An extra saliva quantity is produced before, in the course of and after food, where a decrease salivary production is occurred through sleep. Adequate salivary go with the flow and composition are identified as critical for lubrication and protection of soft and hard oral tissues. Soft tissues protection is supplied towards desiccation, penetration, ulceration, and potential cancer-causing agents by using mucin and anti-proteases. A principal protective feature results from the salivary position in stabilizing the ecological balance within the oral cavity thru clearance, aggregation and decreased adherence by way of both immunological and non-immunological means in addition to direct antimicrobial activity (26).

With its buffer ability, saliva is efficient in preserving pH in the oral cavity and contributes to controlling the pH of the dental biofilm and the integrity of the enamel surface. Salivary protection of enamel integrity relies upon on mechanical cleaning and enamel re-mineralization, for those reasons' individuals with impaired salivary synthesis and secretion may have problems in eating, swallowing and emerge as vulnerable to oral diseases including mucosal infections and dental caries (27).

Saliva and Dental Caries

Tooth decay is result from the action of mouth microorganism, meals, substrate and time. mouth microorganism especially Streptococcus mutant which is a normal flora of the mouth, it had the ability to stick to the tooth surface because it structure contain a capsule that facilitate it adhesion to tooth enamel lead to form biofilm at which other bacteria are trapped lead to dental plaque which is the building block of dental caries. Bacteria fermented meals substrate (particularly sucrose rich food) that shape product which rise acidity of the mouth. Dietary habits especially intake of carbohydrates wealthy meals or terrible oral hygiene are associated with high danger of dental carries (28).

Dental caries is a process of demineralization of tooth enamel and dentine (which means damage to the tooth substance) as a result of chemical processes through interactions between bacteria and food that cause the pH of the saliva to decrease (29).

The etiology of dental caries is associated with four main factors. These factors are bacteria, time, sensitive tooth surface, and carbohydrates that can be fermented. There are certain behavioral and social factors along with these factors that are likely to increase the risk of caries. These include deprived oral hygiene, age, unsuitable tooth brushing habits, plaque and sugar-containing beverages (30).

It is very challenging to Prevent dental caries, as the prevalence of the ailment is very high in general population and it takes place in economically deprived humans who cannot afford the commercially to be had oral hygiene products. Even although caries is thought to be an infectious disorder for decades, little or no effort has been done to apply this record clinically (31).

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