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Salivary Enzymatic Activity and Carious Experience in Children

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(بسم الله الرحمن الرحيم)

﴿فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَ لَا تَعْجل بِالْقُرْآنِ مِن قَبْل أَن يُقْضَىٰ إِلَيْكَ وَحْيُه وَ قُلْ رَبِّ زِدْنِي عِلْمًا﴾

(صدق الله العضيم)

طه: ١١٤

Dedication:

I dedicate this study :To all the amazing people I have met and those I have not who fight the dark days .To those who put me on the path of life ,made me calm and took care of me until I become old ;to my parents.

I dedicate this study: To my friends who support me throughout my study..

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Least but not least ,my lovely thank to many people who have cared about me and prayed for me through the five years of study.

LIST OF ABBREVIATIONS

(ABS)	α-Amylase-binding streptococcus
(IgA)	Immunoglobulin A
(IgG)	Immunoglobulin G
(LPO)	Lactoperoxidase
(SCN-)	oxidize thiocyanate ion
(CA VI)	Carbonic anhydrase VI
(GtfB)	Glucosyltransferase B
(sAA)	salivary alpha amylase
S. mutans	Streptococcus mutans
(HAP)	Hydroxyapatite
WSL	White Spot Lesion
(MMPs)	Matrix Metalloproteinase
APO A1	Apo lipoprotein A1
ECC	Early Childhood Caries
SECC	Sever Early Childhood Caries
(PRPs)	Proline-rich proteins
(aPRPs)	Acidic Proline-rich proteins

Abstract:

Saliva plays a significant role in oral health and tooth integrity. Salivary components reduce tooth surface exposure to demineralization, protect against teeth wear and aid in enamel remineraliza- tion. The properties and functions of saliva, as well as the role of saliva in oral health . The functions of saliva include lubricating the oral tissues protecting the oral soft tissues from abrasion during mastication, facili-tating the digestion of carbohydrates, antibacterial activity against foreign microorganisms, flushing the oral cavity to clear and remove food particles and debris from the tissues, and chemically maintaining an environment rich in calcium, phosphate and acid-buffering agents. The latter func-tion has been recognized as having the ability to reduce the incidence of dental caries..

1.Introduction:

Caries is a multifactorial infectious disease due to demineralization of tooth mineral caused by acid generated when cariogenic bacteria in the plaque on the teeth metabolize fermentable carbohydrates [1]. The prevalence of dental caries among Malaysian adults is 90% with more than ten teeth on average being affected which create social and economic burdens. The progression or reversal of dental caries was driven by imbalance of host resistant itself, microbial agents and environmental factors [2]. One of the main host factors affecting caries is saliva [3]. Saliva is composed of mainly H2O (99%), organic and inorganic ions (1%). Normal pH of saliva ranged from 6.4-7.4. The organic and inorganic ions are functional protein, digestive enzymes, ions, cells and antimicrobial substances. Saliva acts to protect teeth against caries by flushing away non-adherent bacteria and other debris which help in clearance of pathogens and to decrease the acid concentration on tooth surfaces. Given that saliva content can influence cariogenic process, measuring the levels of appropriate salivary component may provide information to predict caries risk. Therefore, any changes in composition of saliva can also provide potential tools to monitor caries. One of the salivary biomarkers is salivary alpha amylase. Salivary α-amylase has an important functions in the oral cavity affecting biofilm such as hydrolysis of dietary starch, binding to the tooth surface, and has high affinity binding to oral streptococci via specific surface- exposed α-amylase-binding proteins forming α-Amylase-binding streptococci (ABS) [4]. A study by Ahmadi et al, the present study found a positive correlation between alpha-amylase and caries prevalence [5]. This is also mentioned by a study by Youssef et al [6] who found that caries group exhibited a significant increase in amylase compared with caries-resistant group. The task is focused on caries host predictor detection to identify the patient caries risk group using saliva. Early detection of these biomarkers can help the clinicians to detect early risk of caries occurrence before irreversible damage is done. Thus, preventive measures by changing their oral habits may be reinforced effectively to the patients instead of the current approach of treatment using dental restorations. Current method available for caries risk assessment is via pH and bacterial Streptococcus mutans count in saliva. Another study had demonstrated the role of Streptococcus mutans in initiates dental caries and lactobacilli sp. in the progression of caries lesion [7]. However, the bacterial counts of these two bacteria, which have traditionally been considered the main etiological agents of dental caries, have proven to provide limited diagnostic value to predict the disease progression. This is because saliva is not a representative of the microbial community

at the disease site. Therefore, by measuring the salivary biomarkers may help to determine the caries risk group [8].

THE AIM OF THE STUDY:

The components of saliva, its functions in maintaining oral health and the main factors that cause alterations in salivary secretion will be reviewed, the importance of saliva in caries development and bacterial plaque formation will be discussed, and its role as an aid to diagnosing certain pathologies will also be discussed here.

2. Saliva & salivary gland:

2.1-Saliva:

Saliva is the mixed glandular secretion which constantly bathes the teeth and the oral mucosa. It is constituted by the secretions of the three paired major salivary glands; the parotid, submandibular and sublingual. It also contains the secretions of the minor salivary glands, of which there are hundreds contained within the submucosa of the oral mucosa and some gingival crevicular fluid.[9]

2.2-Salivary glands:

salivary glands are exocrine glands that produce saliva through a system of ducts. Humans have three paired major salivary glands (parotid, submandibular, and sublingual), as well as hundreds of minor salivary glands. Salivary glands can be classified as serous, mucous, or seromucous (mixed).[10]

2.2.1-The major salivary glands are:

1-Parotid gland: The two parotid glands are major salivary glands wrapped around the mandibular ramus in humans [11] .These are largest of the salivary glands, secreting saliva to facilitate mastication and swallowing, and amylase to begin the digestion of starches It is the serous type of gland which secretes alphaamylase[12] . The glands are located posterior to the mandibular ramus and anterior to the mastoid process of the temporal bone[13] .They produce 20% of the total salivary content in the oral cavity [14] .

2-Submandibular glands:

are a pair of major salivary glands located beneath the lower jaws, superior to the digastric muscles [15] .The secretion produced is a mixture of both serous fluid and mucus[16]. Around 70% of saliva in the oral cavity is produced by the submandibular glands, though they are much smaller than the parotid glands [17] .

3-Sublingual glands:

The sublingual glands are a pair of major salivary glands located inferior to the tongue, anterior to the submandibular glands [18] . The secretion produced is mainly mucous in nature, but it is categorized as a mixed gland [19] . About 5% of saliva entering the oral cavity comes from these glands [20]

FIGURE1: Major salivary gland.

2.2.2-Minor salivary glands:

Around 800 to 1,000 minor salivary glands are located throughout the oral cavity within the submucosa of the oral mucosa in the tissue of the buccal, labial, and lingual mucosa, the soft palate, the lateral parts of the hard palate, and the floor of the mouth or between muscle fibers of the tongue[21] . Their secretion is mainly mucous in nature and have many functions such as coating the oral cavity with saliva[22] .

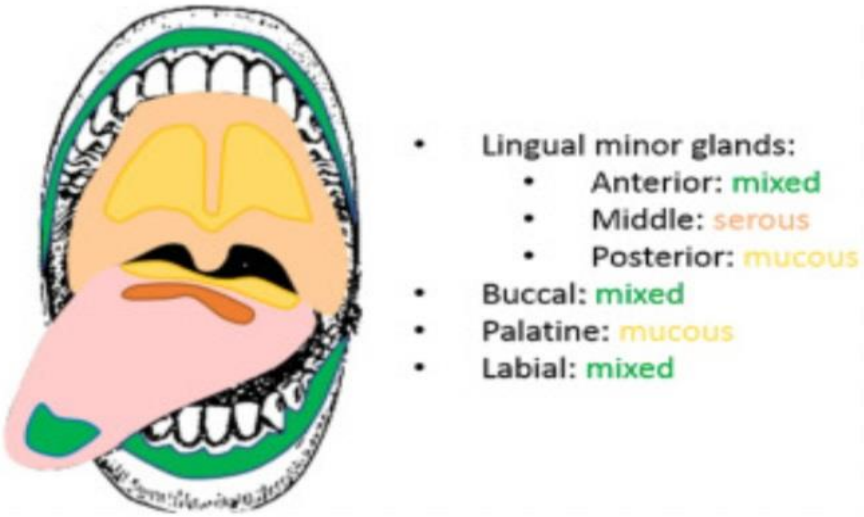

FIGURE 2:Minor salivary gland.

2.3-Composition and function of saliva :

Saliva is composed of a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia[23]. These components interact in related functions in the following general area:

(1) bicarbonates, phosphates, and urea act to modulate pH and the buffering capacity of saliva; (2) macromolecule proteins and mucins serve to cleanse, aggregate, and/or attach oral microorganisms and contribute to dental plaque metabolism; (3) calcium, phosphate, and proteins work together as an antisolubility factor and modulate demineralization and remineralization; and (4) immunoglobulins, proteins, and enzymes provide antibacterial action[24] .

Saliva is a very dilute fluid, composed of more than 99% water [25] . Saliva is not considered an ultrafiltrate of plasma[26]. initially, saliva is isotonic; it is formed in the acini, but it becomes hypotonic when it travels through the duct network [27] . The hypotonicity of unstimulated saliva allows the taste buds to perceive different

tastes without being masked by normal plasma sodium levels. Hypotonicity, eespcially during low-flow periods, also allows for expansion and hydration of mucin glycoproteins, which protectively blanket tissues of the mouth ; lower levels of glucose, bicarbonate, and urea in unstimulated saliva augment the hypotonic environment to enhance taste[28].

2.3.1-Flow rate:

Salivary flow rate exhibits circadian variation and peaks in the late afternoon; the acrophase[29] . Normal salivary flow rates are in the region of 0.3-0.4 ml/min when unstimulated and 1.5-2.0 ml/min when stimulated, although both rates have wide normal ranges[30]. Approximately 0.5 – 0.6 litres of saliva is secreted per day[31] . The contribution of the different glands to whole saliva varies according to the level of stimulation[32] . For unstimulated saliva, about 25% comes from the parotid glands, 60% from the submandibular glands, 7-8% from the sublingual gland and 7-8% from The minor mucous glands [33] . During sleep, flow rate is negligible[34] .For highly stimulated saliva the contribution from the parotids increases to an estimated 50%, the Submandibulars contribute 35%, the sublinguals 7-8% and 7-8% comes from the mmino Mucous glands [35] .

2.3.2-Oral fluid :

Which include all the fluid present in the mouth and it is made by Secretion of salivary glands, gingival crevicular fluid, food debris, microorganisms, Human cells, desquamated oral epithelia, transudate of the mucous membrane and Mucous from nasal cavity and pharynx, sometimes it may include acid from the Stomach in cases of gastric reflux[36].

2.3.3-Maintenance of pH:

Saliva is effective in helping to maintain a relatively neutral pH In the oral cavity, in the bacterial plaque, and on swallowing, in The esophagus as well[37] . In the oral cavity and the esophagus, the Major regulation of pH, especially during eating or drinking, is The salivary bicarbonate, the level of which varies directly with Flow rate [38] . In the bacterial plaque, where acid production is the natural Sequela to bacterial metabolism of carbohydrates, saliva helps Regulate pH in several ways[39] . Bicarbonate, phosphate, and Histidine-rich peptides act directly as buffers once they haveosmotic changes detected in hypothalamus or volemic changes Operating through the renin-angiotensin system of the kidney [40] . Thirst satiation and cessation of drinking are initiated by sensory Messages passing into the brain from taste receptors in the mouth[41] .

2.3.4-Buffer Capacity:

Saliva behaves as a buffer system to protect the mouth as follows:

1-It prevents colonization by potentially Pathogenic microorganisms by denying them Optimization of environmental conditions.

2-Saliva buffers (neutralizes) and cleans The acids produced by acidogenic Microorganisms, thus, preventing enamel Demineralization[42] .

Negatively loaded residues on the salivary Proteins work as buffers[43]. Sialin, a salivary Peptide, plays an important role in increasing The biofilm pH after exposure to fermentable Carbohydrates [44].Urea is another buffer present in total salivary Fluid which is a product of aminoacid and Protein catabolism that causes a rapid increase In biofilm pH by releasing ammonia and Carbon dioxide when hydrolyzed by bacterial Ureases[45]. Children with chronic renal Insufficiency present with less caries than Healthy children, due to the increased levels of Salivary urea[46] .Ammonia, a product of urea and aminoacid Metabolism, is potentially cytotoxic to gingival tissues. It is an important factor in the initiation Of gingivitis because it may increase the Permeability of the sulcular epithelium to other Toxic or antigenic substances in addition to the Formation of dental calculus [47] .The carbonic acid-bicarbonate system is th Most important buffer in stimulated saliva, while In unstimulated saliva it serves as the phosphate Buffer system [48] .

2.3.5-Salivary antibacterial systems:

Saliva contains a broad range of antibacterial agents[49].Immunoglobulin A (IgA) is a major component of saliva proteins, and is able to aggregate bacteria and prevent adhesion[50]. 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[51] . The contribution of gingival crevicular fluid to resting salivary flow is very small, in the order of 10-100 µL/hr[52] . The enzyme amylase can inhibit the growth of some species of bacteria[53] . Lysozyme breaks down the peptidoglycan in the cell wall of some Gram positive bacteria, including Streptococcus mutans[54]. Lactoperoxidase catalyzes the oxidation of salivary thiocyanate by hydrogen peroxide to the toxic molecule hypothiocyanite, which inactivates bacterial enzymes [55] . Histatins are histidine-rich proteins which inhibit the growth of Candida albicans and Streptococcus mutans[56].

3.Enzymes Definition & Salivary enzymes :

3.1-Enzymes Definition：

An enzyme is a biocatalyst, which enhances the rate of thermodynamically favourable biological reactions to several thousand to million folds[57] . Enzymes are highly specialized catalysts with extra ordinary catalytic power and also with remarkable specificity, catalysing almost all cellular reactions. Therefore they are known as the basis of life[58] .

3.2-Salivary enzymes :

1-Amylase

2-Lysozyme

3- Lactoperoxidase

4- Alkaline phosphatase

5-Carbonic anhydrase VI (CA VI)

6-Lactate dehydrogenase

7-Glucosyltransferase B (GtfB)

Table 1: Oral enzymes and their function in saliva

Enzyme	function in saliva
Amylase (Alpha Amylase)	Helps digest starch, associated with bacterial plaque activity.
Lysozyme	Antimicrobial effect, destruction of bacterial cell wall.
Lactoperoxidase	Antimicrobial effect
Alkaline phosphatase	Effect on balance control of remineralization and demineralization
Carbonic anhydrase VI	Effect on neutralizing bacterial acids
Lactate dehydrogenase	Indicates tissue damage
Glucosyltransferase B	Basic role in pathogenesis and caries

3.2.1-Amylase:

Amylase in saliva helps digest starch[59] . Since the consumption of starch in the human Diet is so widespread, it is crucial to be aware of its association with salivary

amylase and its Pathogenicity following this interaction to understand the pathogenic properties of foods[60] . Alpha-amylase is the most abundant saliva enzyme and makes up 40-50% of the salivary glands Total protein [61] . This enzyme has several distinct biological functions that may allow or inhibit Tooth decay[62] . Although there is evidence of amylase-producing bacteria in dental plaque, Approximately 25% of plaque alpha-amylase activity is dependent on bacterial plaque activity[63] .

3.2.1.1-Alpha Amylase

salivary alpha amylase (sAA) is produced mostly in the parotid glands, some in the submandibular glands, and small portions also in the sublingual and small salivary glands[64] . The enzyme is exceptionally stable at high pH and temperature ranges[65] .The amylase activity in saliva has a diurnal profile pattern consisting of a distinct decrease within 60 minutes of awakening and then a steady increase of activity during the day[66] . The physiological function of salivary sAA is to initiate the breakdown of starch in the oral cavity, continuing in the acid-protected parts of the food bolus during the gastric passage. Pancreatic alpha amylase finishes the breakdown in the duodenum[67] . Here, the gut enzymes split the disaccharides to monosaccharides that may be absorbed by the intestine[68] . Salivary alpha amylase is present in the proteomics Of the acquired dental pellicle, forming a complex with mucins and retaining part of its enzymatic activity[69] . The amylase binds to several species of streptococci, facilitating their Adherence to dental surfaces [70] . It has been argued that sAA May provide breakdown products from starch that are metabolized by cariogenic bacteria and therefore is a factor in The caries etiology[71] .

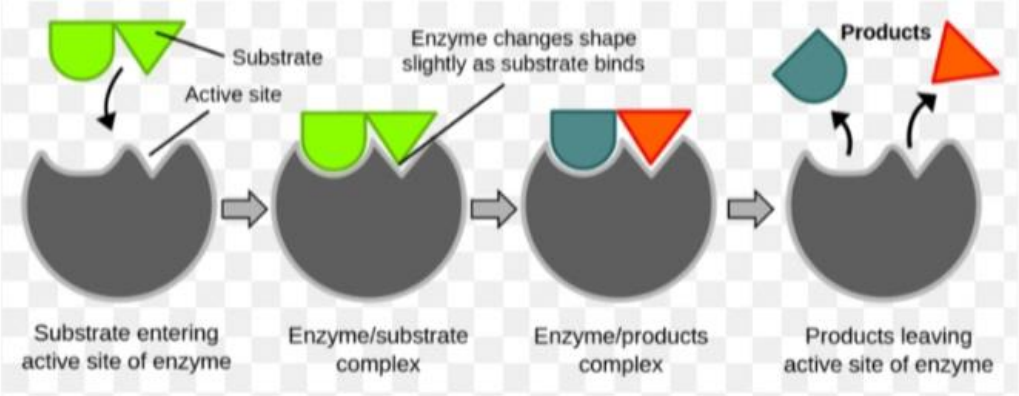

FIGURE 3: Amylase enzyme reaction.

3.2.2-Lysozyme:

Lysozyme is an enzyme with antimicrobial properties that can activate autolysis in Bacteria and destroy the bacterial cell wall [72] . Lysozyme use in toothpaste Has been shown to reduce Lactobacillus acidophilus and S. mutans colonies in children with ECC[73] . Studies have shown that lysozyme activity and levels increase in children with SECC; this increase is thought to be due to an increased immune response to caries[74] . One study studied children with ECC during treatment and found similar results, that lysozyme levels decrease as oral health improved [75] .

3.2.3- Lactoperoxidase (LPO):

This enzyme is one of the antimicrobial factors in saliva[76] . This enzyme is a significant factor in the nonspecific immune response in saliva[77] . Its primary function is to oxidize thiocyanate ion (SCN-) in the presence of hydrogen peroxide

(H2O2) to achieve its antimicrobial activity[78] . The presence of lactoperoxidase in toothpaste consumed by children with ECC has shown a significant reduction of Lactobacillus acidophilus and S. mutans colonies [79] . In proportion to the severity of caries, this enzyme's level in saliva increases to be useful in reducing the density of oral bacteria and clearing them from the oral cavity with bactericidal or bacteriostatic effects[80] .

3.2.4- Alkaline phosphatase :

The exact balance between remineralization and demineralization, which affects hard tooth tissue, depends on salivary calcium, phosphate, and alkaline phosphatase levels[81] . In one study, this enzyme's level in the CF group (caries-free) was significantly lower than in the ECC group[82].

3.2.5- Carbonic anhydrase VI (CA VI):

Studies have shown that CA VI penetrates saliva into the biofilm to facilitate the neutralization of bacterial acids[83] . A study on the relationship between this enzyme and ECC also found that this enzyme's level in children with ECC is significantly higher[84] .

3.2.6- Lactate dehydrogenase :

This enzyme is present in the cytoplasm of almost all tissues in The body [85] . Its main action is to catalyze the oxidation reaction of lactate to pyruvate[86] . This enzyme Is always inside the cell, and its extracellular presence can be a sign of necrosis or tissue damage[87] . This enzyme is also present in the mouth, and although the salivary glands produce it, its primary Source is the oral epithelium[88] .

3.2.7- Glucosyltransferase B (GtfB):

Glucosyltransferases are bacterial-derived enzymes that play a crucial role in the formation and pathogenesis of caries by synthesizing glucan polymers from sucrose and starch hydrolysis[89] .

4.Dental Caries and the Carious Process:

Dental caries is essentially a dynamic process involving a microbial deposit, the dental biofilm on the tooth surface, which undergoes several metabolic reactions, resulting in chemical dissolution Of the tooth substance [90] . The dental biofilm is a community of metabolically active mi-Croorganisms that have adhered to the tooth surface[91] . Bacteria Within the biofilm metabolise fermentable carbohydrates from the Diet, and organic acids are produced, which dissolve the hydroxy-Apatite component of teeth[92] . The acidic environment selects for The microorganisms best able to withstand this low pH, therefore These organisms flourish and continue the carious process[93] .It is the frequency of carbohydrate consumption that plays such A pivotal role in the carious process[94] . Patients who repeatedly ingest High levels of carbohydrates have recurring drops in the salivary pH As the buffering capacity is overwhelmed[95] . The Critical pH is a key biochemical value in the carious process And is 5.5 for enamel, indicating the highest pH at which there is net Mineral loss of tissue from the tooth, i.e. the pH at which deminer-Alisation begins[96] . Dentine is composed of more organic

material and water, meaning it is more easily degraded in the carious process and so, the Critical pH is higher (more alkaline) at approximately 6.2[97] . S. Mutans is recognised as the principal species in caries due To its property of aciduricity, allowing it to tolerate acidic environments [98]. Thus, during the carious process, S. Mutans is able to thrive And multiply as a result of its high tolerance to the low pH environment[99]. As plaque matures and the microbiota adapts, there is a change From a Streptococcus-dominant to an Actinomyces-dominant bacterial community [100] .

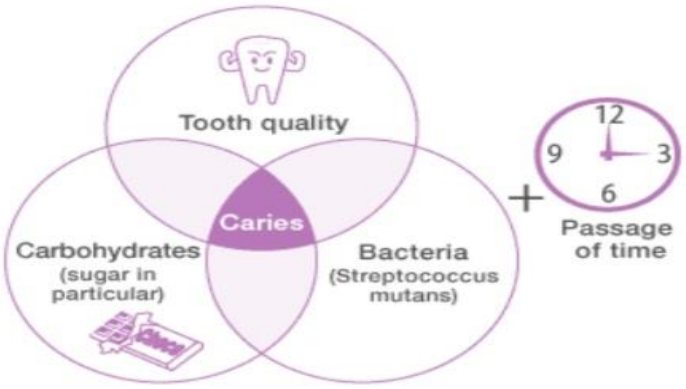

FIGURE 4: Mechanism of caries process.

4.1-Classification of dental caries :

1-Lesions may commonly be found in pits and fissures or on smooth surfaces. Smooth surface lesions may start on enamel (enamel caries) or on the exposed root cementum and dentin (root caries).

2-Primary caries is used to differentiate lesions on natural, intact tooth surfaces from those that develop adjacent to afilling, which are commonly referred to as recurrent or secondary caries. As such, the etiology of both is similar

3-Residual caries, as the term implies, is demineralized tissue that has Been left behind before a filling is placed.

4-An important classification is whether a lesion is cavitated or non-cavitated, As it impinges directly on the management of the lesion. A lesion considered to be progressing (the lesion would have developed Further at a subsequent examination if not interfered with) would be Described as an active carious lesion. In contrast to this is a lesion that may Have formed years previously and then stopped further progression. Such Lesions are referred to as arrested carious lesions or inactive carious lesions.

5-Rampant caries is the name given to multiple active carious lesions occurring in the same patient. This frequently involves surfaces of teeth that do not usually experience dental caries. These patients with rampant caries can be classified according to the assumed causality, e.g. bottle or nursing caries, early childhood caries, radiation caries or drug-induced caries.

6-Hidden caries is a term used to describe lesions in dentin that are missed on a visual examination but are large enough and demineralized enough to be detected radiographically.

4.2-Mechanism of caries process :

4.2.1-Remineralization:

Saliva controls the equilibrium of mineral gain and loss in an erosive or cariogenic oral environment[101] . 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 [10 2] . 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 [103] .

Saliva affords both static protective effects, which act continuously, and dynamic effects, which act during the Time-course of a challenge[104] . Salivary buffering and

sugar Clearance are important dynamic effects of saliva which Prevent demineralization [105] . Comparing these two effects, Buffering of acids is the most important as it is linked Directly with enhanced remineralization [106]. Fluoride in saliva (from dentifrices and dental materials, and derived from ingested foods and drinks) may promote remineralization and inhibit demineralization [107].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)[108]. A key point is that fluoride should be delivered to Enamel carious white spot lesions in moderate Concentrations to achieve the greatest remineralization[109]. 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[110].

4.2.2-Demineralisation:

Demineralisation begins with increased enamel porosity, which Can lead to cavitation and eventually tooth loss if untreated[111]. Organic acid production as a result of bacteria digesting sugars causes the pH of plaque to fall, resulting in Dissolution of HA into Calcium ions, Hydrogen Phosphate ions and Water, which begins the process of demineralisation within enamel[112]. Mineral loss from the enamel which leaches into the surrounding saliva and plaque is ultimately the cause of White Spot Lesion formation. The mineral is replaced by water, which Reduces the refractive index and so light cannot enter the enamel Rods as far before being scattered back towards the

surface . This reflection is responsible for the appearance of the WSL[113].Upon remineralisation, a dense layer of Calcium, Phosphate And Fluoride forms on the surface that has greater resistance to further demineralisation[114] . If demineralisation persists, a point Is reached where the enamel subsurface has been so reduced that The outer enamel surface cannot support or withstand any biting Forces, and so cavitation results[115] . Demineralisation of dentine results when the critical pH of 6.2 is reached, causing the dissolution of mineral[116] . Two dentine layers form described as an outer (infected) layer and inner (affected) layer[117] . Within the inner carious dentine, the collagen fibrils maintain their shape (and thus can be remineralised) but the collagen within the outer carious dentine (closest to the coronal tooth surface) changes shape due to its degradation [118] .

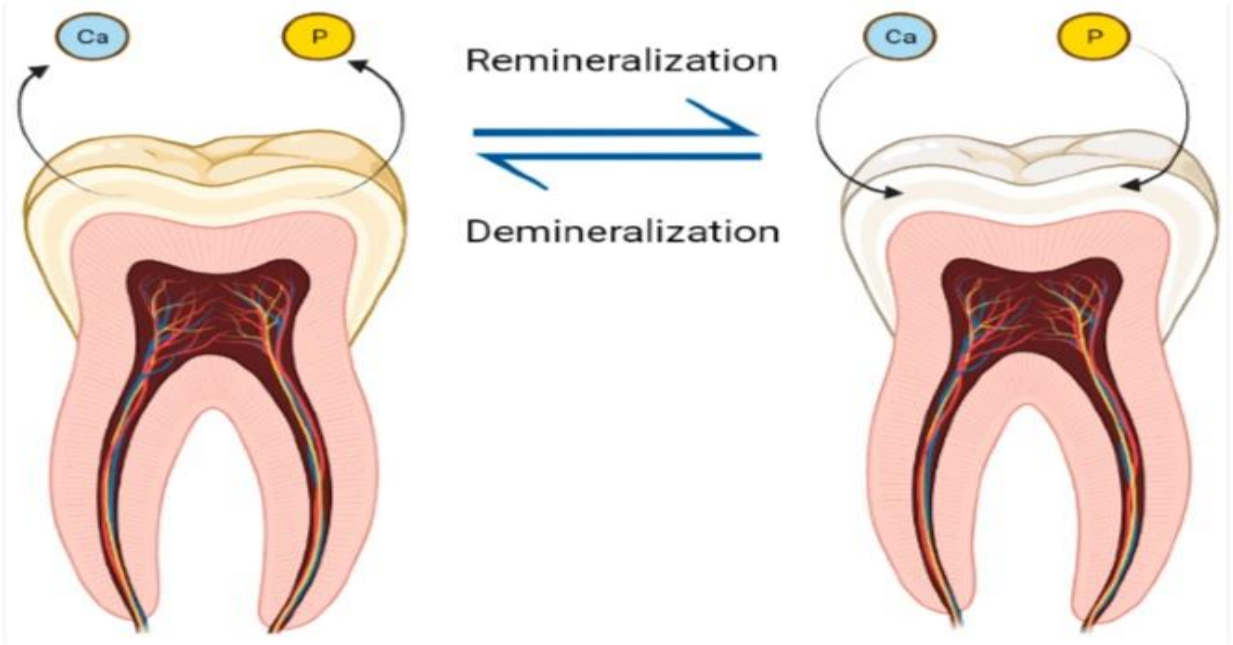

FIGURE 5:Demineralization and Remineralization of teeth.

4.3-Saliva and dental caries:

Evidence shows that saliva from caries-free individuals has higher values of the following: (Compared to caries prone).

1. The increased rate of flow increased pH and increased buffering.

2. Higher calcium and phosphorous concentrations.

3. Higher ammonia concentration.

4. High concentrations of ATP and fructose diphosphate.

5. Increased aldolase activity and O2 uptake of bacteria.

6. Increased opsonin activity.

7. Increased general antibacterial activity.

8. Increased antibacterial activity specific to lactobacilli and streptococci.

9. Higher number of intact leukocytes.

10.Difference in proportion of epithelial cells to leukocytes.

Table 2:Salivary variables measured for caries risk assessment

Variable	Caries Risk Assessment
Flow rate	**Low flow rate is associated with increased caries and high flow rate is related to reduced caries risk.**
Buffering capacity	**Higher buffering capacity indicates better ability to neutralise acid and therefore more resistance to demineralisation.**
Salivary mutans streptococci	**>105 CFU/ml saliva indicates increased risk.**
Salivary Lactobacilli	**>105 CFU/ml saliva indicates frequent carbohydrate consumption and therefore increased risk.**
Fluoride ions	**Higher ambient levels of fluoride ions in saliva are associated with use of fluoride products or with water fluoridation.**
Ca and P ions	**Higher levels associated with less caries.**

4.4-The enzymes involved in the carious process:

Matrix Metalloproteinases (MMPs) are categorised as Zinc-dependent, host-derived proteolytic enzymes, which are important In the degradation of the organic matrix of dentine in the carious Process[119]. Research has found that pH changes in the carious lesion activate MMPs[120] . MMPs only function at neutral pH and so the salivary buffer systems neutralise the acidic pH, allowing the MMPs to become activated to

degrade the organic matrix of dentine .The activation of MMPs is also dependent on the presence of Zinc[121]. However, interestingly, it has been reported that as well as this, Zinc can influence the signalling pathway of MMPs and result in dentine remineralisation [122]. This makes Zinc an attractive element to exploit clinically as a therapeutic agent for the remineralisation of tooth tissue[123] .Zinc-leaching dental materials such as amalgams, Zinc Phosphate cement, Calcium Hydroxide and Zinc-Oxide Eugenol cements are thought to inhibit the demineralisation of dentine and promote remineralisation[124].

4.5-Role of a-amylase in dental Caries :

Because starch is wide Spread in the human diet, a knowledge of its Relationship with salivary a-amylase and the subsequent Cariogenic Potential of this interaction is important to Understanding the Cariogenicity of foods[125] . Numerous Studies of the cariogenicity of Starch-containing foods Have been performed using a variety of in vitro And in Vivo measurements of pH or tooth demineralization, Animals Model systems, as well as cross-sectional and Longitudinal clinical Studies[126] . Most of the in vitro and Animal model systems, however, Involved the use of Mutans streptococci as the cariogenic organism[127] . It is Known that mutans streptococci do not produce endogenous a - Amylase nor do they bind Salivary a-amylase [128]. Thus, the evaluation Of the cariogenic potential of starch-containing foods in these Model Systems may not accurately reflect in vivo conditions.Although there is Evidence for the presence of a-Amylase-producing bacteria in dental plaque , it is generally accepted That Most plaque a-amylase activity is of salivary origin [129] . In An Earlier study, approximately 25% of the total plaque a-amylase activity Was found to be bound to plaque bacteria[130] . More recent studies have Shown that only about 20 to 60% of a-amylase Bound to S. gordonii Remain in active form[131] . Starch metabolism by Oral bacteria appears To require salivary ca-amylase[132] .Stephan and Hemmens

(1947) found That starch, in Comparison with mono- and disaccharides, was the Poorest Substrate for acid formation by pure cultures of Plaque bacteria[133] . When the same bacteria were mixed With saliva and then exposed to Starch, however, acid was produced at a rate comparable with glucose, Presumably due to processing of the starch by salivary a-amylase [134]. Interestingly, in vitro studies have demonstrated That bacteria coated with a-amylase produce acid following starch exposure[135] . Thus, it is likely that a-amylase specifically bound to plaque bacteria may foster dietary starch Hydrolysis with subsequent acid formation in close Proximity to the tooth[136] .

5.Salivary proteins and peptides in children :

The substances in saliva can contain a lot of health Information. Therefore, the use of saliva to diagnose Various types of oral diseases has been in the spotlight since it is non-invasive, easy to work with, and Inexpensive[137] . Salivary proteomics can also lead to the Discovery of diagnostic biomarkers[138] . Numerous Proteomics studies have been performed on the saliva of children with ECC and control groups without The disease. In 2014 observed that the protein content of saliva in The group of patients with S-ECC has increased com- Pared to the control group[139] . In 2017 found a marked difference in salivary peptides Between children with and without ECC relapse after Treatment, and salivary proteins in children without ECC relapse appear to be more sensitive to treatment [140] .Comparing the protein peaks in The saliva of children with ECC and the control group, Studies have shown higher levels of APO A1, SPTAN1 , histatin-rich and PITSLRE protein kinase beta SV1 isoform in the former group[141] . Therefore, it Is suggested that these proteins be used as diagnostic biomarkers[142] .three peptide peaks were Also found that are lower in people with ECC than in Healthy people[143] . These three peptides can also be used As diagnostic markers of ECC:

5.1-Proline-rich proteins (PRPs):

PRPs are important oral Glycoproteins involved in remineralizing tooth enamel And regulating the oral cavity's microbial flora[144] . Acidic PRPs (aPRPs) are involved in the formation of dental Pellicles and enhance the adhesion of S. mutans to

Hydroxyapatite (HAP) surfaces[145] . They also maintain calcium homeostasis in saliva[146]. In 2010 using electrophoresis analysis showed that the Number of PRP bands in non-caries groups was significantly higher than in ECC groups[147] .

5.2-Salivary mucins:

Salivary mucins are large, highly Glycosylated proteins secreted by submandibular acinar cells[148] . The mucin family in human saliva is divided Into two types: High molecular weight weighing about 1000 kDa and low molecular weight weighing about 150-200 kDa[149] . Mucins found in the oral cavity include MUC5B, MUC7, MUC19, and MUC1. These glycopro-Teins prevent the demineralization of teeth by acid-Producing microbes[150] . Mucins exert their protective Role by binding to microorganisms, preventing their Accumulation, attachment, and influencing tooth Enamel.[151]

5.3-Lactoferrin:

Lactoferrin is an iron-binding cationic Glycoprotein with a molecular weight of about 80 kDa[152] . It is secreted by serous acini in the major and minor Salivary glands and acts effectively against S. mutans, Fungi, parasites, and viruses[153] . Salivary lactoferrin can Also originate from neutrophil granulocytes and gingival crevicular fluid[154] . With its N-terminal's help, this Glycoprotein can bind to the bacteria and kill them Directly by destroying the bacterial wall[155] . Also, as a Cationic glycoprotein, lactoferrin can prevent the accumulation of microbes[156] . Previous studies have shown That salivary lactoferrin levels are associated with ECC, With salivary lactoferrin levels decreasing as the patient recovers from ECC[157].

Conclusion :

ECC-related salivary proteins and peptides are PRPs, salivary mucins, Lactoferrin, immunoglobulins, TLRs, Lysozyme, Histatins, Statherin, Defensins, Calprotec-tin, and Cytokines. ECC-related enzymes are Amylase, Lysozyme, Lactoperoxidase, Alkaline phosphatase, Car-bonic anhydrase VI, Lactate dehydrogenase, and Gluco-syltransferase B. Immunity factors affecting ECC include IgA(sIgA), IgG, IgM, Salivary mucins, Lactoferrin, TLRs, Histatins, Statins, Defensins, Calprotectin, Lysozyme, Lactoperoxidase, Cytokines and interleukins, Cathelici-din (LL-37), Agglutinin, Cysteine,andNeutrophils.

References :

1.Z Strużycka. The oral microbiome in dental caries. Pol J Microbiol. (2014);63(2):127-35.[1]

2.R. Esa, A. L. Ong, G. Humphris, and R. Freeman. The relationship of dental caries and dental fear in Malaysian adolescents: a latent variable approach. BMC Oral Health. (Dec. 2014);14(1):19.[2]

3. J. D. Featherstone and B. W. Chaffee. The evidence for caries management by risk assessment (CAMBRA®). Advances in dental research. (Feb. 2018);29(1):9-14.[3]

4.A. E. Nikitkova, E. M Haase, and F. A. Scannapieco. Taking the starch out of oral biofilm formation: molecular basis and functional significance of salivary α-amylase binding to oral streptococci. Appl. Environ. Microbiol. (15 Jan. 2013);79(2):416-23.[4]

5.F. Ahmadi-Motamayel, M. T. Goodarzi, Z. Jamshidi, A. Mahdavinezhad, and N. Rafieian. Evaluation of salivary and serum alpha amylase level in dental caries of adolescence.Brazilian Dental Science. (7 Jul. 2016);19(2):40-6.[5]

6.E. K. WM and A. R. Youssef. Salivary Biomarkers in Caries Affected and Caries Free Children. International Journal of Dentistry Oral Science. (2016):3(10), 348-352. [6]

7.A. Chokshi, P. Mahesh, P. Sharada, K. Chokshi, S. Anupriya, and B. K. Ashwini. A correlative study of the levels of salivary Streptococcus mutans, lactobacilli and Actinomyces with dental caries experience in subjects with mixed and permanent dentition. Journal of oral and maxillofacial pathology: JOMFP. (Jan. 2016);20(1):25.[7]

8.A. Mira, A. Artacho, A. Camelo-Castillo, S. Garcia-Esteban, and A. Simon-Soro. Salivary immune and metabolic marker analysis (SIMMA): a diagnostic test to predict caries risk. Diagnostics. (Sep. 2017);7(3):38.[8]

9.Rajesh, E., & Masthan, K. M. K. (2020). Embryology and development of salivary gland. *European Journal of Molecular & Clinical Medicine*, *7*(10), 764-770.[9]

10.Ghannam, M. G., & Singh, P. (2019). Anatomy, head and neck, salivary glands. [10]

11.Aimbetov, T. D., & Aliyeva, M. Z. (2017). The reaction of mast cell in the parotid gland on chain alcohol intoxication. European Journal of Natural History, (2), 3-6.[11] [12] [13][14]

12.De Paula, F., Teshima, T. H. N., Hsieh, R., Souza, M. M., Nico, M. M. S., & Lourenco, S. V. (2017). Overview of human salivary glands: highlights of morphology and developing processes. The Anatomical Record, 300(7), 1180-1188.[15][16][17]

13.Treuting, P. M., & Dintzis, S. M. (2012). Salivary glands. In Comparative Anatomy and Histology (pp. 111-120). Academic Press.[18] [19] [20]

14.Khandekar, S., Dive, A., Munde, P., & Wankhede, N. D. (2015). Pleomorphic adenoma of the buccal salivary gland. Journal of Oral and Maxillofacial Pathology: JOMFP, 19(1), 111. [21] [22]

15.Hemashree, J., Gayathri, R., & VishnuPriya, V. (2018). Variations in buffering capacity and total protein concentration of saliva in patients with and without periodontitis. Drug Invention Today, 10(8).[23] [24]

16.Emsies, J. G. (2019). Saliva as a Diagnostic Biological Fluid and the Human Salivary Proteome. State University of New York at Albany .[25][26][27][28]

17.Lee, Y. H., Auh, Q. S., & Park, H. K. (2023). Determination of Xerostomia with Cutoff Value for Salivary Flow Rate using Machine Learning Algorithm.[29][30][31]

18.Strojan, P., Hutcheson, K. A., Eisbruch, A., Beitler, J. J., Langendijk, J. A., Lee, A. W., … & Ferlito, A. (2017). Treatment of late sequelae after radiotherapy for head and neck cancer. Cancer treatment reviews, 59, 79-92.[32][33] [34] [35]

19.Monedeiro-Milanowski, M., Monedeiro, F., Ligor, T., & Buszewski, B. (2022). Saliva and Related Specimens as a Source of Volatile Biomarkers. Volatile Biomarkers for Human Health: From Nature to Artificial Senses, 100.[36]

20.Kumar, B., Kashyap, N., Avinash, A., Chevvuri, R., Sagar, M. K., & Shrikant, K. (2017). The composition, function and role of saliva in maintaining oral health: A review. Proteins, 220, 140-640.[37] [38] [39][40][41]

21.Abruzzo, A., Vitali, B., Lombardi, F., Guerrini, L., Cinque, B., Parolin, C., … & Luppi, B. (2020). Mucoadhesive buccal films for local delivery of lactobacillus brevis. Pharmaceutics, 12(3), 241.[42] [43] [44]

22.Ahmed, R. F. (2016). The study of salivary biomarkers levels in uncontrolled type 1 diabetic patients (Doctoral dissertation, Baghdad University).[45] [46]

23.Nayyar, A. S., Khan, M., Deosarkar, B., Deosarkar, S. B., Chalapathi, K. V., Kartheek, G., & Kartheeki, B. (2018). Saliva: newer avenues in the era of molecular biology, diagnostic and prognostic application. Journal of Medical Sciences, 38(1), 7.[47]

24.Dos Santos, D. R., Fiais, G. A., de Oliveira Passos, A., Dos Santos, L. F. G., Kayahara, G. M., Crivelini, M. M., … & Chaves-Neto, A. H. (2022). Effects of orchiectomy and testosterone replacement therapy on redox balance and salivary gland function in Wistar rats. The Journal of Steroid Biochemistry and Molecular Biology, 218, 106048.[48]

25.Van‘t Hof, W., Veerman, E. C., Amerongen, A. V. N., & Ligtenberg, A. J. (2014). Antimicrobial defense systems in saliva. Saliva: Secretion and functions. [49][50][51]

26.Glovsky, T. E., & Iwasaki, L. R. (2021). Markers of Paradental Tissue Remodeling in the Gingival Crevicular Fluid and Saliva of Orthodontic Patients. Biological Mechanisms of Tooth Movement.[52]

27.D Syafriza, H Sutadi, A Primasari… - … Science Development …, 2021 - atlantis-press.com.[53] [54] [55] [56]

28. Bornscheuer, U. T., Huisman, G. W., Kazlauskas, R. J., Lutz, S., Moore, J. C., & Robins, K. (2012). Engineering the third wave of biocatalysis. Nature, 485(7397), 185-194. [57]

29.Deponte, M. (2013). Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Biochimica et Biophysica Acta (BBA)-General Subjects, 1830(5), 3217-3266.[58]

30.Parada, J., & Santos, J. L. (2016). Interactions between starch, lipids, and proteins in foods: Microstructure control for glycemic response modulation. Critical reviews in food science and nutrition, 56(14), 2362-2369.[59] [60]

31.Gao, X., Jiang, S., Koh, D., & Hsu, C. Y. S. (2016). Salivary biomarkers for dental caries. Periodontology 2000, 70(1), 128-141.[61]

32.Salehabadi, N., Moallem Savasari, A., & Nahvi, A. (2022). Salivary Proteins, Enzymes and Immune Factors Associated With Early Childhood Caries: A Narrative Review. Journal of Pediatrics Review, 10(4), 305-314.[62] [63]

33.Hensten, A., & Jacobsen, N. (2019). Salivary alpha amylase as a stress biomarker. OSP J Dent Sci, 1(1), 1-6.[64] [65]

34.Ghiciuc, C. M., Cozma-Dima, C. L., Pasquali, V., Renzi, P., Simeoni, S., Lupusoru, C. E., & Patacchioli, F. R. (2011). Awakening responses and diurnal fluctuations of salivary cortisol, DHEA-S and alpha-amylase in healthy male subjects. Neuroendocrinol Lett, 32(4), 475-480 .[66]

35.Ahsan, H. (2019). Biomolecules and biomarkers in oral cavity: Bioassays and immunopathology. Journal of Immunoassay and Immunochemistry, 40(1), 52-69.[67]

36.Machado Jr, D. Z. (2019). Mass Spectrometry-Based Proteomics Analysis Of Bioactive Proteins In EMD That Modulate Adhesion Of Gingival Fibroblast To Improve Bio-Integration Of Dental Implants (Doctoral dissertation, The University of Western Ontario (Canada)).[68] [69]

37.Hensten, A., & Jacobsen, N. (2019). Salivary alpha amylase as a stress biomarker. OSP J Dent Sci, 1(1), 1-6 .[70] [71]

38.Octiara, E., Sutadi, H., Siregar, Y., & Primasari, A. (2022). The use of Lysozyme Toothpaste to Prevent Early Childhood Caries (Ecc) in 2 Years Old Children. Journal of International Dental and Medical Research, 15(2), 623-629.[72] [73]

39.Salehabadi, N., Moallem Savasari, A., & Nahvi, A. (2022). Salivary Proteins, Enzymes and Immune Factors Associated With Early Childhood Caries: A Narrative Review. Journal of Pediatrics Review, 10(4), 305-314 .[74][75]

40.Maddu, N. (2019). Functions of saliva. In Saliva and Salivary Diagnostics. Inchcape.[76][77]

41.Magacz, M., Kędziora, K., Sapa, J., & Krzyściak, W. (2019). The significance of lactoperoxidase system in oral health: Application and efficacy in oral hygiene products. International journal of molecular sciences, 20(6), 1443.[78][79][80]

42.Salehabadi, N., Moallem Savasari, A., & Nahvi, A. (2023). Cytokines, Minerals, Total Antioxidant Capacity, Nitric Oxide and Salivary Characteristics as Biomarkers Associated with Early Childhood Caries; A Narrative Review. Journal of Pediatrics Review, 0-0.[81] [82]

43.Borghi, G. N., Rodrigues, L. P., Lopes, L. M., Parisotto, T. M., Steiner-Oliveira, C., & Nobre-dos-Santos, M. (2017). Relationship among α amylase and carbonic anhydrase VI in saliva, visible biofilm, and early childhood caries: a longitudinal study. International journal of paediatric dentistry, 27(3), 174-182.[83] [84]

44.Granchi, C., Bertini, S., Macchia, M., & Minutolo, F. (2010). Inhibitors of lactate dehydrogenase isoforms and their therapeutic potentials. Current medicinal chemistry, 17(7), 672-697.[85] [86]

45.De La Peña, V. A., Dios, P. D., & Sierra, R. T. (2007). Relationship between lactate dehydrogenase activity in saliva and oral health status. Archives of oral biology, 52(10), 911-915.[87] [88]

46.Salehabadi, N., Moallem Savasari, A., & Nahvi, A. (2022). Salivary Proteins, Enzymes and Immune Factors Associated With Early Childhood Caries: A Narrative Review. Journal of Pediatrics Review, 10(4), 305-314.[89]

47.Bouzidi, F. (2022). Mecanismos bioquímicos da formação da cárie: revisão narrativa (Doctoral dissertation).[90][91]

48.Lei, C., Jiyao, L., Hockin HK, X., & Xuedong, Z. (2016). Demineralization and remineralization. Dental Caries: Principles and Management, 71-83.[92]

49.Marsh, P. D. (2012). Contemporary perspective on plaque control. British dental journal, 212(12), 601-606.[93]

50.Vallabh, M. (2016). Evaluation of Salivary pH, Flow Rate and Buffer Capacity in Children with Protein Energy Malnutrition-A Comparative Study (Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India)).[94] [95][96]

51.Damé-Teixeira, N., Parolo, C. C. F., & Maltz, M. (2017). Specificities of caries on root surface. In Root caries: From prevalence to therapy (Vol. 26, pp. 15-25). Karger Publishers.[97]

52.Krzyściak, W., Jurczak, A., Kościelniak, D., Bystrowska, B., & Skalniak, A. (2014). The virulence of Streptococcus mutans and the ability to form biofilms. European Journal of Clinical Microbiology & Infectious Diseases, 33, 499-515.[98] [99]

53.Takahashi, N., & Nyvad, B. (2011). The role of bacteria in the caries process: ecological perspectives. Journal of dental research, 90(3), 294-303.[100]

54.Walsh, L. J. (2007). Clinical aspects of salivary biology for the dental clinician. International Dentistry South Africa (Australasian Edition), 2(3), 16-20.[101] [102][103]

55.Kelly, R. S., Kelly, M. P., & Kelly, P. (2020). Metabolomics, physical activity, exercise and health: A review of the current evidence. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1866(12), 165936.[104][105][106]

56.Walsh, L. J. (2007). Clinical aspects of salivary biology for the dental clinician. International Dentistry South Africa (Australasian Edition), 2(3), 16-20.[107] [108][109]

57.Ten Cate, J. M., & Buzalaf, M. A. R. (2019). Fluoride mode of action: Once there was an observant dentist… Journal of dental research, 98(7), 725-730.[110]

58.Sundararaj, D., Venkatachalapathy, S., Tandon, A., & Pereira, A. (2015). Critical evaluation of incidence and prevalence of white spot lesions during fixed orthodontic appliance treatment: A meta-analysis. Journal of International Society of Preventive & Community Dentistry, 5(6), 433 .[111][112]

59.Shaw, K. (2015). The Effect of Post Brushing Mouthrinses on Salivary Fluoride Retention, And, The Effect of Varying Fluoride Concentration on Remineralisation of Bovine Enamel in Vitro (Doctoral dissertation, University of Liverpool).[113]

60. Elkassas, D., & Arafa, A. (2014). Remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. Journal of dentistry, 42(4), 466-474[114]

61..Banerjee, A., & Watson, T. F. (2015). Pickard's guide to minimally invasive operative dentistry. OUP Oxford.[115]

62.Caries, W. I. (2017). Dental caries: etiology, clinical characteristics, risk assessment, and management. Sturdevant's Art & Science of Operative Dentistry-E-Book, 40.[116] [117]

63.Toledano, M., Osorio, E., Aguilera, F. S., Osorio, M. T., Toledano, R., López-López, M. T., … & Osorio, R. (2023). Dexamethasone and zinc loaded polymeric nanoparticles reinforce and remineralize coronal dentin. A morpho-histological and dynamic-biomechanical study. Dental Materials, 39(1), 41-56.[118]

64.Bedran-Russo, A. K., Pauli, G. F., Chen, S. N., McAlpine, J., Castellan, C. S., Phansalkar, R. S., … & Leme, A. A. (2014). Dentin biomodification: strategies, renewable resources and clinical applications. Dental materials, 30(1), 62-76.[119] [120]

65.Chaussain, C., Boukpessi, T., Khaddam, M., Tjaderhane, L., George, A., & Menashi, S. (2013). Dentin matrix degradation by host matrix metalloproteinases: inhibition and clinical perspectives toward regeneration. Frontiers in physiology, 4, 308.[121][122]

66.Osorio, R., Osorio, E., Cabello, I., & Toledano, M. (2014). Zinc induces apatite and scholzite formation during dentin remineralization. Caries research, 48(4), 276-290.[123] [124]

67.Seow, W. K. (1998). Biological mechanisms of early childhood caries. Community dentistry and oral epidemiology, 26(S1), 8-27.[125] [126]

68.Lemos, J. A., Quivey Jr, R. G., Koo, H., & Abranches, J. (2013). Streptococcus mutans: a new Gram-positive paradigm?. Microbiology, 159(Pt 3), 436.[127]

69.Scannapicco, F. A., Bhandary, K., Ramasubbu, N., & Levine, M. J. (1990). Structural relationship between the enzymatic and streptococcal binding sites of human salivary α-amylase. Biochemical and biophysical research communications, 173(3), 1109-1115.[128][129]

70.Fiehn, N. E., & Moe, D. (1983). A-amylase activity in supragingival dental plaque in humans. European Journal of Oral Sciences, 91(5), 365-370.[130]

71.Holt, S. C., Kesavalu, L., Walker, S., & Genco, C. A. (1999). Virulence factors of Porphyromonas gingivalis. Periodontology 2000, 20(1), 168-238.[131]

72.Manjushree, R., Anandakrishna, L., Ks, K. P., & Shetty, A. K. (2022). Evaluation of Salivary Components and Dental Plaque in Relation to Dental Caries Status in Type 1 Diabetes Mellitus. International Journal of Clinical Pediatric Dentistry, 15(Suppl 2), S121.[132] [133][134]

73.Scannapieco, F. A., Torres, G. I., & Levine, M. J. (1995). Salivary amylase promotes adhesion of oral streptococci to hydroxyapatite. Journal of dental research, 74(7), 1360-1366.[135][136]

74.Liu, J., Tang, Y., Cheng, Y., Huang, W., & Xiang, L. (2023). Electrochemical biosensors based on saliva electrolytes for rapid detection and diagnosis. Journal of Materials Chemistry B, 11(1), 33-54. [137] [138].

75.Laputková, G., Schwartzová, V., Bánovčin, J., Alexovič, M., & Sabo, J. (2018). Salivary protein roles in oral health and as predictors of caries risk. Open life sciences, 13(1), 174-200.[139]

76.Zhou, X., Li, H., Zhu, C., Yuan, C., Meng, C., Feng, S., … & Zheng, S. (2021). Analysis of salivary proteomic biomarkers for the surveillance of changes in high-risk status of early childhood caries. BMC Oral Health, 21, 1-10.[140]

77.Salehabadi, N., Moallem Savasari, A., & Nahvi, A. (2022). Salivary Proteins, Enzymes and Immune Factors Associated With Early Childhood Caries: A Narrative Review. Journal of Pediatrics Review, 10(4), 305-314.[141][142] [143][144][145] [146] [147][148] [149]

78.Frenkel, E. S., & Ribbeck, K. (2015). Salivary mucins in host defense and disease prevention. Journal of oral microbiology, 7(1), 29759.[150][151]

.Bloat, E., Eker, F., Kaplan, M., Duman, H., Arslan, A., Saritaş, S., … & Karav, S. (2022). Lactoferrin for COVID-19 prevention, treatment, and recovery. Frontiers in Nutrition, 9.[152]

79.Olsen, I., & Singhrao, S. K. (2021). Low levels of salivary lactoferrin may affect oral dysbiosis and contribute to Alzheimer's disease: A hypothesis. Medical hypotheses, 146, 110393.[153] [154]

80.Marsh, P. D., Do, T., Beighton, D., & Devine, D. A. (2016). Influence of saliva on the oral microbiota. Periodontology 2000, 70(1), 80-92.[155][156]

81.Salehabadi, N., Moallem Savasari, A., & Nahvi, A. (2022). Salivary Proteins, Enzymes and Immune Factors Associated With Early Childhood Caries: A Narrative Review. Journal of Pediatrics Review, 10(4), 305-314.[157]