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Saliva: A Biosignature for Dental Caries

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Abstract

Dental caries curses major part of the world's population; rectified as a widespread multifactorial disease. As saliva is frequently flowing fluid along the teeth and other oral structures; which plays a major role in developing and progression of dental caries. Some organic and inorganic components may protect teeth from the occurrence of dental caries. This occurs via several functions, such as clearance of food debris and sugar, aggregation and elimination of microorganisms, buffering actions to neutralize acid, maintaining concentration with respect to tooth mineral, participation in formation of the acquired pellicle and antimicrobial defence. Unassuming attestation is available on the associations between dental caries and several salivary parameters, including flow rate, buffering capacity and abundance of mutans *streptococci*. Despite some controversial findings, the main body of the literature supports an elevated caries prevalence and/or incidence among people with a pathologically low saliva flow rate, compromised buffering capacity and early colonization or high titer of mutans *streptococci* in saliva. Saliva has the potential to be used in the early detection and diagnosis of caries. This is due to the abundant biomarkers present in saliva.

Keywords: Antimicrobial defence; *Streptococci*; Teeth

Introduction

Human saliva not only lubricates the oral tissues, making oral functions such as speaking, eating, and swallowing possible, but also protects teeth and oral mucosal surfaces in different ways. The lubricating and antimicrobial functions of saliva are maintained mainly by resting saliva [1]. Stimulation of saliva results in a flushing effect and the clearance of oral debris and noxious agents. However, the protective functions of saliva are not limited to the above-mentioned functions. Recent studies have revealed a large number of functions, mediated by both the inorganic and organic components of saliva that should be considered in assessments of the effects of human saliva on dental caries. Some of these studies have introduced a new approach to dental caries from being a bacterially induced multifactorial disease to a disease which may also be influenced by inherited salivary factors. Such genetically regulated salivary components may influence both the colonization and the clearance of micro-organisms from the oral cavity [2].

Host protective properties of saliva

It has been established that saliva plays a crucial role in reducing caries risk. This is due in large part to saliva's physical, chemical and antibacterial properties.

Physical protective qualities: Due to its water content and flow rate, saliva physically cleanses the oral cavity of food and debris. Unstimulated flow rates are approximately 0.3 to 0.4 ml/min, while stimulated flows are approximately 1.5 to 2 ml/min, although there are wide variations between individuals [3]. Most humans produce roughly 0.5 to 1 liter of saliva per day with 90% secreted from the major glands. Saliva also dilutes and removes organic acids from dental plaque (Figure 1).

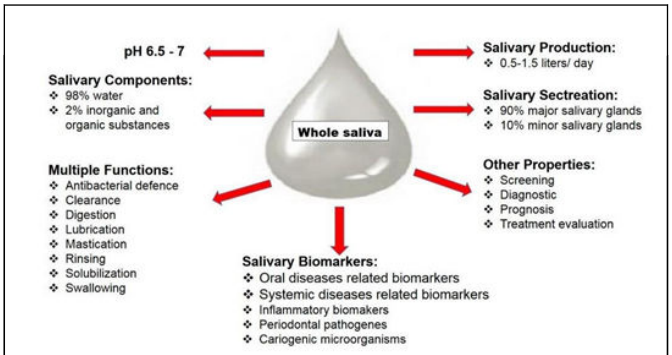

Figure 1: Different types of physical protective qualities.

Chemical protective qualities: Saliva contains a number of electrolytes and organic molecules that minimize decreases in local pH, creating an environment that favors remineralization. For example, sodium bicarbonate and phosphates, along with other salivary components, act as buffers or neutralizing agents in saliva. In addition, one salivary protein called sialin tends to raise salivary pH to neutral levels. Saliva is also supersaturated with hydroxyapatite, fluorapatite, and calcium and phosphate ions compared to the carbonated hydroxyapatite in enamel [4]. This supersaturation is maintained by the proline-rich proteins and statherins in saliva, and it increases the likelihood of remineralization via the incorporation of calcium and phosphate into enamel.

Antibacterial properties: Saliva contains several proteins with different types of antibacterial properties. The mucins are sulfated glycoproteins that trap, aggregate, and clear bacteria. The enzymes

called amylases break down food particles that stick to teeth, reducing the bacterial build- up that can lead to decay.

Antimicrobial proteins in saliva

Innate defence factors: The innate defence factors identified in saliva have been extensively studied in vitro, and they express different antimicrobial properties. The modes of action of these molecules differ vastly, suggesting a long evolution during which the oral cavity has been exposed to a large variety of bacteria, fungi, viruses, and other noxious substances, e.g., mutagenic and carcinogenic substances, as well as H_2O_2. The data obtained so far are mainly from in vitro studies, and there is only limited information on how these molecules act in vivo. It is well-known that many antimicrobial proteins in saliva interact in vitro with each other [5]. The interactions result in additive, synergistic, or inhibitory effects on mutans *streptococci*, lactobacilli, or fungi. The main oral innate defence factors are the peroxidase systems, lysozyme, lactoferrin, and histamins. In vitro, these proteins are known to limit bacterial or fungal growth, interfere with bacterial glucose uptake or glucose metabolism, and promote aggregation and, thus, the elimination of bacteria. It should be emphasized that, in addition to the antimicrobial action of both salivary peroxidase and myeloperoxidase systems, one of the main purposes of these systems is to eliminate H_2O_2, which is highly toxic for mammalian cells.

Specific defence factors and dental caries: The immunoglobulins, IgG, IgM, IgA, and secretory IgA (sIgA), form the basis of the specific salivary defence against oral microbial flora, including mutans *streptococci.* The most abundant Ig in saliva, as in all other human secretions, is dimeric sIgA, which is produced by plasma cells located in the salivary glands. Two IgA subclasses are present in saliva; IgAl forms the major component of Igs, although the relative amount of IgA2 is higher in saliva than in other secretions [6]. In human beings, IgG, mainly of maternal origin, is the only detectable Ig in the saliva of neonates. Salivary IgA is absent at birth but is readily detectable in infants at the age of only one week. The IgG concentration decreases to nondetectable levels after some months but appears again after tooth eruption. Low concentrations of IgG can be detected in stimulated parotid saliva, but most of the IgG detected in whole saliva enters the mouth from the gingival crevicular fluid, thus originating from sera. The formation of specific IgAs in saliva correlates with the colonization of bacteria in the oral cavity. In most children over three years of age, salivary IgAs against mutans *streptococci* can be detected, and their amount increases with the length of exposure. Salivary Igs can bind to the salivary pellicle, and they are found also in dental plaque [7].

In the oral cavity, Igs act by neutralizing various microbial virulence factors, limiting microbial adherence, and agglutinating the bacteria, as well as by preventing the penetration of foreign antigens into the mucosa. IgGs are also capable of opsonizing bacteria for phagocytes, which are reported to remain active in dental plaque and saliva. Phagocytosis may be especially important in modifying microbial flora during tooth eruption when high amounts of IgGs and neutrophils exist in close contact with the teeth. The role of salivary Igs in dental caries formation is still a matter of debate. There are some experimental data suggesting a protective role of the anti-streptococcal IgGs, mainly measured from serum, against caries and colonization of S. mutans in early childhood and in adults, but also contradictory results exist.

Saliva and blood

Like saliva, blood is a complex bodily fluid known to contain a wide range of molecular components, including enzymes, hormones, antibodies, and growth factors. While cells, tissues, stool, and other alternatives are routinely pursued, blood serum or plasma is traditionally and most frequently the source of measurable biomarkers. Although life-saving in many instances, the procedures required to collect and eventually analyze blood samples can often be expensive, problematic, and physically intrusive. Employing salivary fluids as a medium for biomarker development and evaluation alleviates subject/patient discomfort through the provision of a noninvasive method of disease detection. Comparatively, saliva carries many advantages over blood, including the following:

- Collection is undemanding. While blood sampling requires highly trained personnel, saliva procurement can be done by anyone, including self-collection.
- The procedure is noninvasive. Sample procurement is painless, reducing the discomfort most individuals endure from biopsies and repeated blood draws, while encouraging others to participate in timely medical evaluations and screenings [8].
- Samples are safer to handle. Salivary secretions contain factors that inhibit the infectivity of HIV, resulting in extremely low or negligible rates of oral transmission.
- Samples are easier to ship and store. Saliva does not clot and requires less manipulation than blood.
- The procedure is economical. Saliva is easily collected, shipped, and stored, resulting in decreased overall costs for patients and health care providers.

Biomarkers: Biomarkers exist in a variety of different forms, including antibodies, microbes, DNA, RNA, lipids, metabolites, and proteins. Alterations in their concentration, structure, function, or action can be associated with the onset, progression, or even regression of a particular disorder or result from how the body responds to it. A collection of reliable and reproducible biomarkers unique to certain maladies is often referred to as a biomarker or molecular signature. Understanding and evaluating the significance of an individual's biomarker signature can be useful in determining the presence, location, and even likelihood of disease. Thus, biomarkers serve as a valuable and attractive tool in the detection, risk assessment, diagnosis, prognosis, and monitoring of disease [9].

Microbial biomarkers

As proposed by Haffajee and Socransky, there are 3 major points to take into account when determining the efficacy of microbial salivary diagnostics. First, in order for microbes to be considered disease-specific biomarkers, they must be associated directly with, but not necessarily the cause of, the condition in question. Next, if microbial biomarkers truly reflect health status, their regression or eradication should coincide with a positive therapeutic outcome. In other words, as a patient's condition improves, the concentration or detectability of corresponding biomarkers should diminish. The last consideration, and perhaps most meaningful, is whether microbial markers can be used to assess the risk of disease. If so, could a saliva-based microbial profile serve as a predictive indicator of disease, and is there a healthy profile to strive for? With regard to these issues, what is most exciting about oral microbial diagnostics is its potential utility beyond evaluating pathologies of the oral cavity. As discussed below, microbial and immunologic salivary profiles may be indicative not

only of local disease but also of systemic maladies and infectious disorders [10].

The oral microbiome

The human oral cavity is a diverse habitat composed of teeth, gingival sulci, the tongue, hard and soft palates, the buccal mucosa, and tonsils. Each structure is colonized by bacteria and continuously bathed in saliva. Interestingly, studies have shown that salivary bacteria, including those shed from dental caries, may be surrogate indicators of disease useful in patient diagnosis, monitoring, and overall health evaluation. With this in mind, a great deal of work has been done to define the human oral microbiome. Established by the NIH, the human microbiome Project aimed to characterize the microbiological flora of several anatomical regions in healthy adult subjects, including the oral cavity. Even though certain studies report that 700 to 1,200 bacterial species reside in the mouth, investigators using Next-Generation Sequencing (NGS) suggest that this number could be as high as 10,000. While this is intriguing, further studies are required to clarify these numbers, as it is not clear whether such a large range of species truly colonize the oral cavity or are simply environmental transients [11].

Although most individuals harbor only 75 to 100 of the predominant bacterial species known to inhabit the oral cavity, 35% to 50% of those have yet to be cultivated. Ironically, recent analyses of sublingual plaque deposits indicate that many “uncultivable” specimens may actually be associated with oral health or disease. Fortunately, there are ancillary means by which to detect and monitor these and other species, using genomic analysis. Currently, most laboratory techniques, including NGS, bacterial microarrays, DNA hybridization, PCR, and quantitative PCR (qPCR), are employed in pursuit of specific questions as opposed to elucidating diagnostic values. Typically, the development of reliable disease markers follows the establishment of an association between specific bacterial species and specific diseases. Thus far, most studies utilizing the aforementioned methods have focused on certain oral sites, including subgingival plaque, tongue epithelial scrapings, and buccal mucosa, to determine the role of bacteria in oral health and disease. The following sections discuss early culture-based methods as well as contemporary molecular methods as they apply to salivary diagnostics and microbial biomarker development.

Early culture-based microbial diagnostics

Microbial salivary diagnostics is not a novel concept. Over 20 years ago, saliva-based tests were developed for *Streptococcus mutans* and *Lactobacillus* spp., two known etiological agents of dental caries. Dip slide tests for lactobacilli debuted in 1975, followed by cariescreen SM, an analysis that used agar-coated slides to detect and quantify salivary *S. mutans*. A similar test, called dentocult SM strip mutans, by orion diagnostica, quantifies *S. mutans* by incubating saliva-dipped test strips in selective broth media for 48 h. A software program called cariogram evaluates the results, along with host dietary habits, plaque amount, and fluoride use, to calculate the relative risk of developing dental caries. Likewise, the caries risk test, a currently available diagnostic tool, simultaneously detects *S. mutans* and lactobacilli in saliva. This test, which has also been used to evaluate the relative risk of caries, utilizes blue mitis salivarius agar selective medium with bacitracin and Rogosa agar to detect *S. mutans* and lactobacilli, respectively. Although some studies have questioned their validity, these tests provide objective data used in clinical practice and research to detect bacteria and monitor health or disease status.

Molecular microbial diagnostics

As previously discussed, there is a clear rationale for using culture-based methods for risk assessment for dental caries. However, investigations drawing on culture-independent techniques are now producing evidence indicating the significance of molecular microbial analysis in identifying oral pathologies. Recent studies employing quantitative 16S rRNA gene sequencing found several putative pathogens in the saliva of periodontitis patients in comparison to healthy controls. Another investigation evaluating the synergy of microbial and molecular analyses found that biomarkers alone were insufficient discriminatory analytes, and only a combination of the microbial and molecular values could reasonably discern healthy from diseased subjects. Further studies have identified malodorous and caries-active subjects by using Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis, deep sequencing, or human microbe identification microarrays (HOMIM), which are 16S rRNA-based microarrays capable of detecting 300 oral bacterial species, including those not yet cultivated.

Transcriptomics

As stated above, studies have shown that salivary secretions not only harbor RNA molecules but also may be a highly promising source of discriminatory biomarkers. To that end, recent investigations have identified more than 3, 000 species of mRNA and over 300 miRNAs in the salivary fluids of healthy and diseased subjects, suggesting the possibility that transcriptomic analysis may yield valuable information regarding the condition of the body. With this in mind, a number of investigations have reported the identification of salivary biomarkers for Sjogren's syndrome and a number of cancers. While further analyses need to be performed, these outcomes suggest a substantial role for the salivary transcriptome as a viable and noninvasive source of disease-specific biomarkers.

Proteomics

Human saliva contains a large collection of diverse proteins, each with distinct biological functions. While some aid in digestion and lubricating oral cavity, others help to maintain homeostasis and oppose pathogenic bacteria. Although its proteomic content is estimated to be only 30% that of blood, saliva is actively being investigated as a rich source of protein biomarkers capable of discerning healthy from diseased subjects. To that end, numerous studies have revealed discriminatory protein profiles for oral cancer, diabetes, periodontal disease, AIDS, and mammary gland carcinoma.

Conclusion

Known to affect mammalian development, cellular differentiation, and carcinogenesis, DNA methylation induces cells to maintain or alter unique characteristics by controlling and modulating gene expression. Curiously, several investigations are now reporting saliva-based genomic methylation analyses discerning Oral Squamous Cell Carcinoma (OSCC) and Head and Neck Squamous Cell Carcinoma (HNSCC) patients from their respective controls. Additionally, a number of studies have explored local and global epigenetic alterations with regard to age, suggesting the possibility of saliva-based predictive screenings for age-related diseases. Another

interesting aspect of salivary methylomics is its potential role in forensic science and body fluid identification. As evidenced in a recent study, 5 tissue-specific differentially methylated regions (tDMRs) were distinguished via bisulfite sequencing using pooled DNA from blood, saliva, semen, menstrual blood, and vaginal fluid. Though preliminary, these results are promising and lay the groundwork for future genomewide DNA methylation analyses. Future applications may include the use of this technology as a standard forensic technique in the determination of unknown host bodily fluids.

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