SALIVARY BIOMARKERS IN PERIODONTAL DIAGNOSIS: CURRENT CONCEPTS AND FUTURE TRENDS

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Abstract

Biomarkers are tell-tale molecules that could be used to monitor disease onset, health status, treatment response and outcome. Salivary biomarkers have been used in the past for diagnosis of various oral and systemic diseases. In recent times, there has been a great interest in using saliva as a diagnostic marker in periodontal field and many advances have been made in the use of salivary biomarkers; especially in the area of genomic and proteomic studies of saliva. This paper reviews the salivary biomarkers used in periodontics; and focuses on the newer trends in collection of saliva and the technological advances made in salivary biomarker diagnostics.

Key words: *Salivary biomarkers, Periodontal disease, Proteomic, Saliva, Tanscriptome*

A biomarker, or biological marker, is in general a substance used as an indicator of a biological state. In oral diagnostics, it has been a great challenge to determine biomarkers for screening, prognosis and evaluating the disease activity and the efficacy of treatment. An oral diagnostic tool, in general, should provide pertinent information for differential diagnosis, localization and severity of disease. Traditional diagnostic measures, such as visual examination, periodontal pocket depth, attachment level, and plaque index, bleeding on probing and radiographic assessment of alveolar bone loss are still used universally and still act as basis for oral diagnosis. For the past two decades, saliva has been increasingly evaluated as a diagnostic fluid for detecting breast cancer, oral cancer, caries risk, salivary gland diseases, periodontitis, and various systemic disorders.[1] It may reflect levels of therapeutic, hormonal, and immunologic molecules and can yield diagnostic markers for infectious and neoplastic diseases. Various mediators of chronic inflammation and tissue destruction have been detected in whole saliva of patient with oral diseases.[2]

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Newer advances in saliva as a biomarker

Collection of saliva:

Whole saliva is most frequently studied because its collection is easy, noninvasive and rapid to obtain without the need for specialized equipment. It can also be collected with or without stimulation. Un-stimulated whole saliva is commonly collected by the `draining' method where the subject's head is tilted forward so that saliva moves towards the anterior region of the mouth and the pooled saliva is drooled into a wide-bore sterile vessel. Stimulated whole saliva is generally obtained by masticatory action (i.e., from a subject chewing on paraffin) or by gustatory stimulation (i.e., use of citric acid or sour candy drops on the subjects tongue) and is expectorated into a tube. Unstimulated whole saliva is better for diagnostic applications because the foreign substances used to stimulate saliva tend to modulate the fluid pH and generally stimulate the water phase of saliva secretion, resulting in a dilution in the concentration of proteins of interest. To date, unstimulated whole saliva has been used in the majority of diagnostic studies.[3]

Over the last few years other promising devices have emerged that are based upon modifications to the traditional expectoration technique.

OraGene:
It is a more sophisticated way to collect saliva into a vessel to which is attached a screw-on cap containing a mixture of preservative buffers. Upon completion of the expectoration process, the cap is screwed onto the device releasing the preservative buffer, which drops into the saliva, is mixed by shaking and then acts to protect the integrity of the sample until processing and extraction can take place. It is the most widely used collection device.[3]

Saligene:
It is an alternative "spit-in-a-cup" technology, which has additional application as a collector for stool or swab specimens (when coupled with specific extraction kits for these alternate specimen types). In the Saligene device, subjects expectorate into a modified collection tube until a pre-determined volume has been reached. A screw-cap with attached plunger is screwed in place and the plunger when depressed causes a preservative/lysis buffer to flow into the collected saliva specimen. The sample of mixed preservatives and saliva is gently shaken then sent to a laboratory for further processing.[3]

Oracol:
This test kit consists of an absorbent foam swab (designed to collect up to 1 ml of saliva), centrifuge tube and cap. It is supplied sterile in batches of 500. This kit is universally used to collect data on measles, human immunodeficiency virus (HIV), hepatitis A and B, mumps and rubella.[3]

Verofy:
Verofy is a unique platform technology that incorporates rapid and standardized saliva collection with high quality immune chromatographic test strips providing a system for delivery of immediate results in field or point-of-care locations. Verofy collects saliva from under the tongue by means of a proprietary absorbent material connected to either one or two immune chromatographic test strips located in the device housing and in fluid communication with the test strips. After approximately 1 to 2 minutes of saliva collection time, a sample volume adequacy indicator built into the device changes appearance, signifying that sufficient sample has been collected for testing. The device can then be removed from the mouth and allowed to run for an additional time (3-15 minutes depending upon the specific test). As for standard immune chromatographic tests, a line or series of test lines will appear on the test strips depending on the diseases or analytes being tested. In addition a control line will appear confirming the validity of the test and the appropriate function of reagents used in the tests. If a positive test result is obtained and a confirmation specimen is required, this can be collected by squeezing the absorbent collection pad through a plastic compression tube provided and into a standard 2 ml eppendorf centrifuge (or equivalent) collection tube. Once collected this sample is capped then archived or sent to a laboratory for suitable confirmation testing.[3]

Salivary Biomarkers in the Diagnosis of Periodontitis

Several biomarkers have been identified in saliva for diagnosis and disease prediction in periodontal field.[4,5,6] Kaufman et al categorized biomarkers into enzymes[7,8], immunoglobulins[9], other proteins like platelet activating factor[10], epidermal growth factor[11], vascular endothelial growth factor[12], etc., epithelial keratins[13], inflammatory cells, bacteria[14] and volatile sulfur compounds. Another classification of biomarkers includes dental biofilm derived, inflammatory, collagen breakdown and bone remodelling[3,15] and are summarized in Table 1. Many advances have been made with regard to salivary biomarkers in recent times.

DENTAL BIOFILM	INFLAMMATORY	COLLAGEN BREAKDOWN	BONE REMODELLING
Immunoglobulins (IgA, IgM, IgG)	β-glucuronidase	α2-macroglobulin	Alkaline phosphatase
Mucins	C- reactive protein	MMP-8	Osteoprotegerin
Lysozyme	Il-1β	MMP-9	Osteocalcin
Lactoferrin	Il-6	Aspartate aminotransferase	SPARC/osteonectin
Histatin	MIP 1α	Alanine aminotransferase	RANKL
Peroxidase	Tumor necrosis factor-α	TIMPs	β C-terminal type I collagen telopeptide
			C-telopeptide pyridinoline cross-links of type I collagen

Table 1: Demonstrates various salivary biomarkers of periodontal disease

IL: Interleukin; MIP: Macrophage inflammatory protein; MMP: Matrix metalloproteinase; RANKL: Receptor activator of NF-κB ligand; SPARC: Secreted protein, acidic, rich in cysteine; TIMP: Tissue inhibitors of metalloproteinase.

Recent Advances in salivary diagnostics:
Advancements in analytical techniques have enabled scientists to discover the specific biomarkers associated with human diseases. Salivary genome and epigenome, transcriptome, proteome and metablome projects and nanobiochip technology have given greater dimensions to saliva as a diagnostic tool. Currently, biologists, engineers, and dental practitioners are developing "lab-on-a-chip" platforms that use oral fluids in rapid tests to accelerate clinical decision making. Ultimately, these types of tests will lower the cost of health care because they eliminate the need for trained phlebotomists to draw blood. Lab-on-a-chip platforms will be able to perform multiple operations in non laboratory settings such as satellite clinics, field sites, or at home. Furthermore, these testing methods use smaller amounts of sample and reagents, further decreasing costs. Current diagnostic approaches that take days or weeks to obtain results are less effective than this new realm of point-of-care diagnostics that provide results within minutes or hours so the treatment plan can begin immediately.

Nano-Biochip technology:
The tools of nano materials and microelectronics for the practical implementation of miniaturized sensors are suitable for a variety of important applications. There are two types of systems been created, the first is based on a micro bead array, wherein micro-pits within a silicon wafer are populated with a variety of chemically sensitized bead `microreactors'. This sensor system is based on a bio-micro-electromechanical systems

Table 2: DEMONSTRATES VARIOUS SALIVARY BIOMARKERS CLASSIFIED INTO PROTEOMIC, GENOMIC AND MICROBIOLOGICAL BIOMARKERS

Proteomic Biomarkers			Genomic Biomarkers	Microbial biomarkers	Other markers
Immunoglubilins	Ca protectin	Kininase	Cathepsin C gene Mutation	*Aggregatibacter Actinomycetemcomitan-s*	Calcium
Acid phosphatase	Caprylate esteraselipase	Caprylate esteraselipase	Collagen gene Mutation	*Campylobacter rectus*	Cortisol
Alkaline phosphatase	Cathepsin B	Lactotransferr-in	IL-1 polymorphisms	*Mycoplasmas*	Hydrogen sulfide
Aspartate Aminotransferase	Cystatins	Lysozyme	TNF Polymorphisms	*Prevotella intermedia*	Picolines
Aminopeptidases	Elastase	MMP 1, MMP 2, MMP 3	IL-10 polymorphisms	*Pepiostreptococcus Micros*	PMNs
Beta-galactosidase	Epidermal growth Factor	MMP-8, MMP-9 MMP-13		*Prevotella nigrescens*	Pyridine
Beta-glucosidase	Esterase	ICTP		*Treponema denticola*	
Beta-glucuronidase	Fibronectin	Myelcperoxd-ase		*Tannerella forsythia*	
CRP	CD14	Lactate dehydrogenase		*Porphyromonas Gingivalis*	
Alpha-glucosidase	Gelatinase	Osteocalcin		*Treponema socranskii*	
Histatin	Kallkrein	Osteonectin			
Mucins	Peroxidase	Osteopontin			

platform, and may be described as a `chemical processing unit' in analogy to the central processing unit that serves as the brains for computer chip. Instead of handling electrical signals passing through conductors, as in the case of traditional circuits, the Nano- Biochip technology processes fluids so as to provide a digital fingerprint that can be correlated with the local chemical environment, detecting pH, electrolytes, metal cations, sugars, toxins, proteins and antibodies. Building on this technology, a second class of miniaturized sensor system has been pioneered that contains beads within etchings of stainless steel plates and utilizes a membrane capture element integrated into a fluidics structure. These membrane microchip ensembles have been adapted to service cell, spore, and bacteria separation and biomarker identification applications. Importantly, the performance metrics of these miniaturized sensor systems have been shown to closely correlate with established macroscopic gold-standard methods, making them suitable for use as subcomponents of highly functional detection systems for the analysis of complex fluid samples, such as saliva, for a variety of analyte systems. The development of a point of care (POC) device that contains a modular and miniaturized sensor system, universal analyzer with functional integrated mechanical/optical interfaces, and flexible microchip architecture can service the future needs of clinicians and the research communities.[16]

In this POC device, saliva (100–300 μl) is placed into the salivary collection/delivery module, and then delivered into the Nano-Biochip. The injection-molded cartridge is `creditcard' size and encloses the array Nano-Biochip where complex fluorescent immunoassays are performed. Here, a network of fluidic components ensures the complete transfer and process of saliva samples to the multiplex bead array to provide quantitative information of target biomarkers of disease. The sample introduction requirements are consistent with the use of saliva or finger-prick quantities of blood that can be directly introduced into the sample introduction port. Detection reagents are stored dry on a conjugate pad embedded within the biochip, and are reconstituted as needed, through the release of a pre-packed buffer contained in biochip-integrated pouches.

All processing steps are conducted within the micro fluidic network of the biochip via actuation inside the analyzer without human intervention. These features eliminate the need for external fluidics, such as pumps, tubing and connectors. Therefore, the integrated system has the potential to reduce cost and reduce the risk for leaks and contamination. The assay is processed entirely through a 5–15 min sequence that is programmed in the main controller board. The flexibility of the control software allows for modifications to be made through an assay builder interface. Control over the flow rate, incubation time and reagent wash, is achieved by the actuation of stepping motors that direct the fluid flow through the depression of the fluid pouches. The sample is directed to an on-chip waste reservoir, which provides a safe containment of bio-hazardous fluids. The entire biochip can be discarded as solid waste after the assay, facilitating biohazard waste management. Together, these essential features serve to facilitate the transition from chips-in-a-laboratory to a lab-on-a-chip, and offer significant opportunities for POC technology needs.[16]

The salivary genome and epigenome

Salivary genetic and epigenetic analysis provides insight into the presence of invading pathogens, and aberrant gene transcription profiles that directly reflect pathological genetic processes pertaining to, for example, cancers. The salivary genome consists of DNAs representing the genome of the individual, oral microbiota and infecting DNA viruses[15]. The quality and yield of DNA that can be obtained from saliva is relatively good compared with blood and urine, can be used for genotyping, amplification or sequencing[17], and can be stored long-term without significant degradation[18]. As such, salivary DNA is a robust

analyte for diagnostics, but is limited to reflecting presence or absence of specific genes, alteration to sequences (mutation) and methylation status, and cannot provide information on upregulation and down regulation of gene expression[19].

The Human Oral Microbe Identification Microarray (HOMIM) has recently been developed comprising an oligonucleotide microarray based on 16S rRNA, and has allowed profiling and monitoring of changes in the oral microbiota[20]. Alterations in the oral bacterial profile have been found to correlate with several diseases, including pancreatic, oral and lung cancer, colonic neoplasia and extra-colonic malignancy, cardiovascular and cerebrovascular disease, and preterm birth, and these findings are being explored as potential salivary diagnostic approaches.[19]

The salivary transcriptome

mRNAs and miRNAs are secreted from cells into the extracellular milieu and can be found in bio-fluids that are distant to the cellular sources[21]. In a diseased state, transcription of specific mRNAs and miRNAs is altered. Although the validity of salivary RNAs for the development of biomarkers with diagnostic potential initially received some criticism[22], this approach is now widely accepted. However, the precise sources of salivary RNAs and other molecules remain unclear.

Standard procedures for salivary mRNA analysis previously required low temperatures and time-consuming and costly mRNA isolation, which impeded clinical application. Now, simple methods of stabilizing mRNA in saliva samples have been developed, allowing room temperature storage without stabilizing agents, and sample analysis without further processing: so-called 'direct-saliva-transcriptomic-analysis'[23].

Microarray technology has allowed high-throughput saliva analysis and is the current gold standard for identifying saliva transcripts. The salivary transcriptome is profiled using microarrays and validated with qPCR. However, due to the low concentration of some biomarkers and occasional small sample volume, further innovations in technology have been required. Hu *et al.*[24] has overcome these limitations by two developments: a universal mRNA-amplification method for microarray discovery, and a multiplex pre-amplification method for qPCR validation. Moreover, the multiplex pre-amplification method allows the simultaneous detection of many transcripts, providing cost-effective screening and quantitative measurement using a relatively small amount of pre-amplified product. In the following sections, we discuss advances in transcriptomic analysis of mRNAs and miRNAs using saliva samples.

mRNA- Characterization of mRNA profiles in body fluids provides insights into gene transcription in normal and disease states. Salivary mRNA biomarkers have been pursued for a number of diseases, including Oral Squamous Cell Carcinoma (OSCC), primary Sjogren's syndrome, pancreatic cancer and ovarian cancer, and they have proved to be sensitive, specific and accurate.

miRNA- miRNAs are short (19 to 25 nucleotides) RNA transcripts associated with post-transcriptional regulation by the RNA-induced silencing complex. These RNAs have been well characterized and found to play roles in cell growth, differentiation, apoptosis, pathogen-host interactions and stress responses and immune function, and are found in saliva. miRNAs are differentially expressed in several cancer cell types compared with normal cells, with observed differences that range from ten to over a hundredfold. Changes in mRNA levels in cancer cells are comparatively small, and miRNA has been found to be more useful for characterizing solid tumor types than mRNA. Therefore, miRNA cancer biomarkers are potentially very powerful, and if miRNA-based approaches also reveal similarly differential profiles using saliva samples they may become very useful in salivary diagnostics.

The salivary proteome

The use of surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry (MS), which allows rapid, high-

throughput detection of proteins in minute sample volumes without pre-processing, has been reported for several diseases[25]. Recently, analysis of saliva for protein biomarker discovery has mainly been performed using two-dimensional difference gel electrophoresis (2D-DIGE) coupled with MS[26], and liquid chromatograpy- MS (LC-MS) based techniques[27]. Despite these advances, the discovery and validation of salivary protein biomarkers provide challenges. Proteins generally have short half-lives, although there can be large differences in observed stability. Both the nature of peptides and the milieu of the oral cavity make salivary proteins vulnerable to degradation. Thus, protein-based salivary diagnostics require immediate processing and/or analysis of saliva samples, or the use of freezers and costly protease inhibitors. In a research setting, these requirements can be easily met. However, in clinical settings protein stabilization without freezers and specialized machinery may be required.

Several different classes of salivary protein biomarkers have been reported. Endothelin-1, a vasoconstrictor, was reported as a potential biomarker for OSCC development in patients with oral lichen planus. Interleukins IL-8 and IL-1β, glycoprotein M2BP (thought to be involved in natural killer cell activity enhancement, tumor suppression, interleukin production and cell aggregation), profilin (actin-binding protein, important for temporal and spatial control of actin microfilament growth), CD59 (complement regulatory protein), MRP14 (a protein involved in regulating myeloid cell function) and catalase (catalyses the conversion of hydrogen peroxide to water and oxygen) have been reported as salivary biomarkers for oral cancer[28], and immunoglobulins have long been described as salivary biomarkers for HIV infection.[19]

The salivary metabolome

The metabolome is the complement of small-molecule metabolites. Similar to the transcriptome and proteome, the metabolome changes continually and any single profile is a snapshot reflecting gene and protein expression. Metabolomic investigations can generate quantitative data for metabolites in order to elucidate metabolic dynamics related to disease state and drug exposure.[29]

Using capillary electrophoresis time-of-flight MS, Sugimoto *et al*. identified metabolites for detecting oral, breast and pancreatic cancer, and periodontal disease. Multiple logistic regression models yielded area under curve (AUC) of 0.865 for oral cancer, using a profile consisting of three oral-cancer-specific metabolites: taurine, piperidine and a peak with a mass-to-charge ratio (*m*/*z*) of 120.0801. A breast cancer profile consisting of taurine and lysine achieved an AUC of 0.973. Periodontal diseases achieved an AUC of 0.969 using a panel of only two markers, but the specific markers were not reported. However, a limitation of metabolomics compared with genomics, transcriptomics and proteomics is the inability at times to specifically identify the differentially expressed metabolites.[30]

Emerging salivary diagnostic tools for periodontal disease detection and therapeutic efficacy monitoring

There are compelling reasons to use saliva as diagnostic fluid to monitor the onset and progression of periodontal diseases. In the past 5 years, through a series of initiatives by the National Institute of Dental and Craniofacial Research, the use of saliva for translational and clinical application has emerged at the forefront. Most relevant to periodontal diseases are the emerging toolboxes of the salivary proteome and the salivary transcriptome for early detection, disease progression and therapeutic monitoring. Using these emerging technologies, we have shown that salivary proteins and RNAs can be used to detect oral cancer and Sjogren's syndrome. The stage is now poised to use these technologies for translational and clinical applications in periodontal diseases. Table 3 summarizes various salivary biomarkers classified into proteomic, genomic and microbiological biomarkers that can be utilized for periodontal diagnostic purpose.[16, 31]

Newer aids for measuring salivary biomarkers:

Significant advances are in development for the screening of periodontal diseases. The current method of diagnosing periodontitis is through assessment of clinical parameters and radiographs; however, this is not necessarily the most efficient method for early diagnosis. If periodontal diseases are detected early, treatment can be easier and less painful for the patient. Left untreated, periodontal diseases may lead to systemic problems such as cardiovascular disease and diabetes. Therefore, early screening for periodontal diseases is essential during dental examinations.

MyPerioID and My PerioPath:
Two DNA-based saliva tests are available- MyPerioPath and MyPerioID. My PerioPath uses a saliva sample to identify the type and concentration of the specific bacteria that cause periodontal diseases. MyPerioID test also uses saliva to determine a patient's genetic susceptibility to periodontal diseases and which patients are at higher risk of more serious periodontal infections. Both tests require the shipping of saliva samples to a laboratory for results.[3]

Oral Fluid Nano Sensor Test:
The University of California, Los Angeles (UCLA) Collaborative Oral Fluid Diagnostic Research Center, partnered with engineers at the UCLA School of Engineering, developed a micro electromechanical system based electrochemical detection platform that is capable of real-time, ultrasensitive, ultraspecific multiplex detection of salivary protein and RNA biomarkers. This envisioned product has been labeled the Oral Fluid Nano Sensor Test (OFNASET). It is a point-of-care, automated, and easy-to use integrated system that will enable simultaneous and precise detection of multiple salivary proteins and nucleic acids. In addition, this system is portable and could be used not only in the doctors' office, but also in any other healthcare station to perform an instant point-of-care diagnosis. The OFNASET technology platform combines cutting edge technologies, such as self-assembled monolayers bionanotechnology, cyclic enzymatic amplification, and microfluidics, with several well-established techniques including microinjection molding, hybridization-based detection, and molecular purification. The intended use of the OFNASET is for the point of care multiplex detection of salivary biomarkers for oral cancer. It analyzes saliva for the presence of four salivary mRNA biomarkers (SAT, ODZ, IL-8, and IL-1b) and two salivary proteomic biomarkers (thioredoxin and IL-8).[3]

Electronic Taste Chips:
Several study groups have reported elevated serum CRP levels in periodontitis patients. The higher the levels of CRP in periodontitis patients, the more severe the disease, even with adjustments for external factors. The biomarker CRP is an acute phase reactant and a well-accepted indicator of inflammation. Numerous clinical studies have established elevated serum CRP as a strong, independent risk factor for the development of cardiovascular disease (CVD). CVD has also been associated with oral infections (i.e. periodontal diseases) and there is evidence that systemic CRP may be a link between the two. Clinical measurements of CRP in serum are currently performed with "high sensitivity" CRP (hsCRP) enzyme-linked immunosorbent assay (ELISA) tests that lack the sensitivity for the detection of this important biomarker in saliva. Salivary CRP may represent a novel approach for diagnosing and monitoring chronic inflammatory disease, including CVD and periodontal diseases.
Chemically sensitized bead microreactors within the lab-on-a-chip system were recently applied for measurement of CRP and other biomarkers of inflammation in saliva, demonstrating significantly lower detection level (by > 3 decade orders of magnitude) for CRP than high-sensitivity CRP ELISA methods, allowing for measurement of inflammatory biomarkers related to select disease states.7 Currently, researchers at Rice University in Houston, Tex, are developing a lab-on-a-chip

system using a new detection system for measuring analytes in saliva based on an electronic taste chip (ETC). The ETC methodology was compared with the standard laboratory technology (ELISA) for measuring CRP in saliva, and displayed a 20-fold lower limit of detection than the ELISA. With this technique it was possible to quantitate the difference in CRP levels between healthy individuals and patients with periodontal diseases. Additional studies confirmed the ability of the ETC platform to simultaneously monitor several additional biomarkers. The prospect of a commercially available ETC lab-on-a-chip platform that can detect multiple biomarkers for early diagnosis of periodontal disease is promising.

Oraquick:
It is an antibody test that provides results in 20 minutes. The blood, plasma or oral fluid is mixed in a vial with developing solution, and the results are read from a sticklike testing device. Usually detects HIV 1 and HIV 2. The Oraquick rapid saliva HIV test is definitely the way to go. It is a toothbrush-like device with a fabric swab on the head and a small plastic stem. The stem naps off to avoid contamination, while the swab is inserted into a tube of testing fluid. The testing tube is easily sealed and ready for transport to a testing laboratory. By using a toothbrush-like device that is inserted into the mouth instead of drawing blood, this testing method makes an already high-anxiety screening a little more comfortable.

Integrated Microfluidic Platform for Oral Diagnostics (IMPOD):
A clinical point-of care diagnostic test that enables rapid quantification of an oral disease biomarker in human saliva by using a monolithic disposable cartridge designed to operate in a compact analytical instrument was reported. This microfluidic method facilitates hands-free saliva analysis by integrating sample pretreatment (filtering, enrichment, mixing) with electrophoretic immunoassays to quickly measure analyte concentrations in minimally pretreated saliva samples. Rapid (< 10 min) measurement of levels of the collagen cleaving enzyme MMP-8 in saliva from healthy and periodontally diseased subjects can be achieved using 20 µl of saliva. Based on this, a portable diagnostic device called the IMPOD was developed. An early clinical study in which the hand-held IMPOD was used to rapidly (3–10 min) measure the concentrations of MMP-8 and other biomarkers in small amounts (10 ml) of saliva has been reported.
Table 3 summarizes the various products and their uses

PRODUCT NAME	PURPOSE
MyPerioID	determine a patient's genetic susceptibility to periodontal diseases and which patients are at higher risk of more serious periodontal infections
My PerioPath	identify the type and concentration of the specific bacteria that cause periodontal diseases
Oral Fluid NanoSensor Test	simultaneous and precise detection of multiple salivary proteins and nucleic acids.
Electronic Taste Chips	detects multiple biomarkers for early diagnosis of periodontal disease
OraQuick	an antibody test that provides results in 20 minutes, usually detects HIV 1 and HIV 2
Integrated Microfluidic Platform for Oral Diagnostics	rapidly (3–10 min) measures the concentrations of MMP-8 and other biomarkers in small amounts (10 ml) of saliva

Table 3: Demonstrates various products and their uses for measuring salivary biomarkers

Conclusion

While the future of periodontal disease diagnosis using salivary diagnostics looks promising, obstacles to these approaches may appear in the clinical setting. Validation of novel periodontal diagnostics will need to be benchmarked with existing gold standards of disease, such as alveolar bone levels and clinical attachment levels, in large patient populations. Acceptance by dentists and treatment clinicians is also necessary and may prove difficult. The dental community is not familiar with mass screening of populations for oral and systemic diseases. If more efficient periodontal therapy can be delivered, clinicians will be more likely to utilize new diagnostic approaches. Procedural reimbursement is also another issue that needs to be addressed. A greater emphasis must be placed on clinician education in diagnostics, disease-risk and disease prevention through the public health sector before diagnostics will be integrated into routine clinical periodontal practice. Although challenges remain ahead, the use of saliva based oral fluid diagnostics appear promising for future application to diagnose periodontal diseases and to prognosticate periodontal treatment outcomes

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