Saliva: An emerging biofluid for early detection of diseases

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ABSTRACT: The capability to assess physiological states, detect morbidity initiation and progression, and monitor post-treatment therapeutic outcomes through a noninvasive approach is one of the most desirable goals for healthcare research and delivery. Saliva, a multi-constituent oral fluid, has high potential for the surveillance of general health and disease. To reach the above goal through saliva-based diagnostics, two prerequisites must be fulfilled: (1) discovering biomarker(s) for different diseases among the complicated components of saliva, and (2) advancing sensitivity and specificity of biomarker(s) through persistent development of technologies. Under the support and research blueprint initiated by the National Institute of Dental and Craniofacial Research (NIDCR), salivary diagnostics has not only steadily progressed with respect to accuracy and availability, but has also bridged up-to-date nanotechnology to expand the areas of application. With collective efforts over several years, saliva has been demonstrated to be a promising bodily fluid for early detection of diseases, and salivary diagnostics has exhibited tremendous potential in clinical applications. This review presents an overview of the value of saliva as a credible diagnostic tool, the discovery of salivary biomarkers, and the development of salivary diagnostics now and in the future. (*Am J Dent* 2009;22:241-248).

CLINICAL SIGNIFICANCE: Saliva, like blood, contains an abundance of protein and nucleic acid molecules that reflects physiological status; however, unlike other bodily fluids, salivary diagnostics offer an easy, inexpensive, safe, and non-invasive approach for disease detection, and possess a high potential to revolutionize the next generation of diagnostics.

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Introduction

Early detection of disease plays a crucial role in successful therapy. In most cases, the earlier the disease is diagnosed, the more likely it is to be successfully cured or well controlled. Managing a disease, especially in the early stage, may dramatically reduce the severity of its impact on the patient's life, or prevent and/or delay subsequent complications. For example, in the case of ovarian cancer, which is the fifth most common malignancy and the fifth leading cause of cancer mortality in women in the US, the survival rate 5 years post diagnosis can reach 93% and 70% if tumors are detected in early stage I and II, respectively, but drops precipitously to 37% and 10% if the diagnosis is made in stages III and IV when the cancer is well established and spreading.[1] Type II diabetes, a serious metabolic disorder affecting more than 7% of American adults, could be well controlled solely by diet or changing lifestyle if symptoms were detected early enough.[2] People are aware of the importance of regular health check-ups; however, most systemic diseases are not diagnosed until morbid symptoms become apparent in the late phase. To overcome this challenge, medical researchers are devoted to finding molecular disease biomarkers that reveal a hidden lethal threat before the disease becomes complicated. These markers could be DNA, RNA, or protein molecules that act as indicators reflecting particular physiological states. In the past decade, scientists have demonstrated that human genetic alterations can be detected both intracellularly and extracellularly by molecular diagnostics.[3] In addition, abnormal nucleic acids and/or proteins have been identified in patients' bodily fluids such as blood, urine, and cerebrospinal fluid, and have been demonstrated to be effective biomarkers for diagnostic use.[4-6] Besides a significant impact on biological research, over the last decades molecular diagnostics have proved valuable in clinical applications.[7,8] Most systemic diseases such as cancer, cardiovascular, metabolic, and neurological diseases are very challenging to diagnose without supplementary clinical evaluation. Even with a complete work up, the diagnosis usually remains uncertain due to complications of the disease. Currently three major limitations have prevented people from recognizing the full potential of disease detection, and have seriously hampered the development of clinical diagnostics, namely:

1. Lack of definitive molecular biomarkers for specific diseases;
2. Lack of an easy and inexpensive sampling method with minimal discomfort; and
3. Lack of an accurate, easy-to-use, and portable platform to facilitate early disease detection.

Since 2002, the National Institute of Dental and Craniofacial Research (NIDCR) created opportunities to overcome these limitations by exploring oral fluids as a diagnostic tool for assessment of health and disease status. Saliva, an oral fluid that contains an abundance of proteins and genetic molecules and is readily accessible *via* a totally noninvasive approach, has long been recognized as the potential solution to limitation number 2.[9] Through the visionary investment by the NIDCR, the discovery of salivary biomarkers and ongoing development of salivary diagnostics technologies now provide promising solutions for limitations 1 and 3.

There is considerable excitement surrounding the application of saliva-based diagnostics for oral diseases, and we believe that this will soon be followed by application of highly informative salivary biomarkers to other high-impact systemic disorders because saliva is composed of various molecules that are filtered, processed, and secreted from the vasculature that nourish the salivary glands (Fig. 1).[10,11] This realization will enable scientists to bridge oral health research with systemic

American Journal of Dentistry, Vol. 22, No. 4, August, 2009

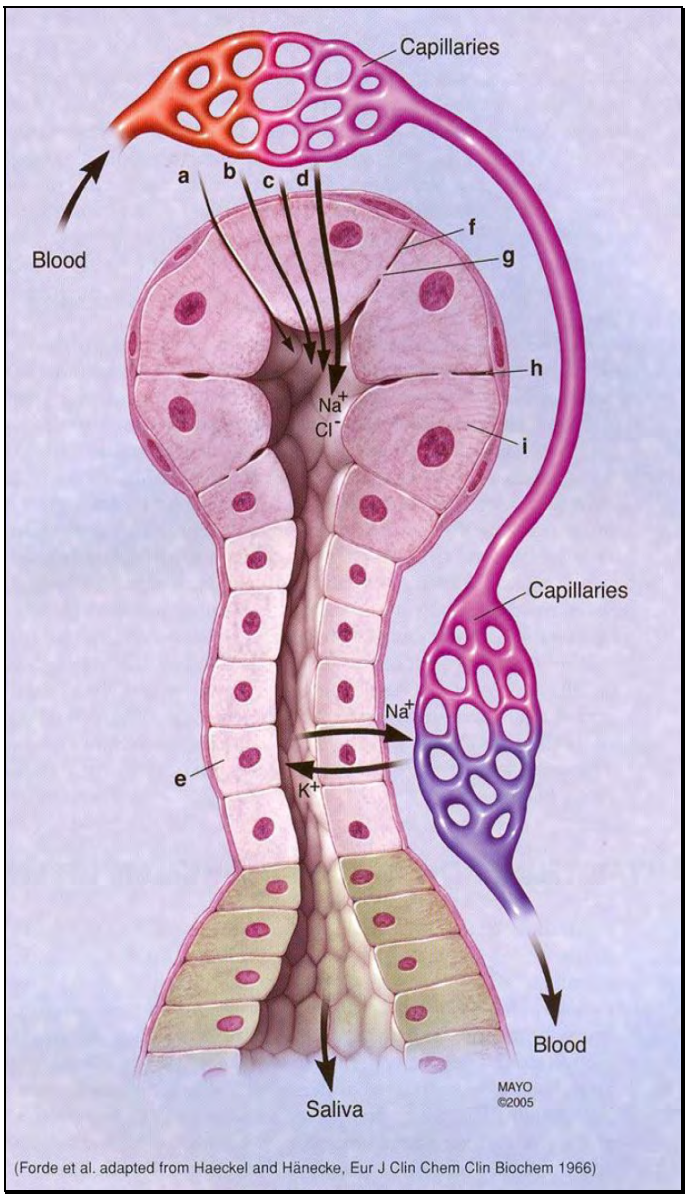

Fig. 1. Mechanism of molecular transport from serum into salivary gland ducts. A. Active transport.B. Passive diffusion. C. Simple filtration. D. Acinar cells actively pump sodium ions (Na+) into the duct. E. Duct cells pump Na+ ions back into blood. F. Cell membrane. G. Pore on the cell membrane. H. Intracellular space. I. Acinar cell. (This figure was adapted from Haeckel and Hanecke with permission of Walter de Gruyter GmbH & Co. Copyrighted and used with permission of Mayo Foundation for Medical Education and Research, all rights reserved).

disease diagnosis. With the additional advantages of an easy, safe, cost-effective, and non-invasive diagnostic approach, saliva shows high potential for monitoring general health and disease, with enormous translational values, and unparalleled opportunities for clinical applications.

PROPERTIES OF SALIVA AS A DIAGNOSTIC FLUID

Saliva is a clear, slightly acidic (pH = 6.0-7.0) and complex biological fluid composed of secretions from major salivary glands: the parotid, submandibular, and sublingual glands, as well as multitudes of minor glands including labial, buccal, lingual, and palatal tissues. In general, human salivary glands produce about 1-1.5 L of serous and mucinous saliva daily by combining water, salts, and an abundance of molecules from the blood with a cocktail of salivary proteins in the oral cavity to give rise to the multi-constituent whole saliva.[12]

Since the advantages of saliva as a diagnostic tool were revealed, the use of saliva for surveillance of disease and general health has become a highly desirable goal in healthcare research and promotion. However, the full power and potential of saliva in medical applications was only recently recognized when saliva was shown to reflect the spectrum of health and disease states and to offer distinctive advantages over serum.[13,14] Like blood, saliva is a complex fluid containing a variety of enzymes, hormones, antibodies, antimicrobial constituents, and growth factors.[15,16] Many of these enter saliva from the blood by passing through the spaces between cells by transcellular (passive intracellular diffusion and active transport) or paracellular routes (extracellular ultrafiltration).[17-19] Therefore, most compounds found in blood are also present in saliva, thus saliva is functionally equivalent to serum in reflecting the physiological state of the body, including emotional, hormonal, nutritional, and metabolic variations. There has been concern that although saliva contains diverse components with diagnostic properties, their low concentration compared with levels in the blood[20] may prevent salivary diagnostics from being clinically practical; however with the development of new and highly sensitive techniques (*e.g.*, molecular diagnostics, nanotechnology), the low concentration of analytes in saliva is no longer a limitation. Today, a growing number of proof-of-principle assays have been established using saliva to monitor diseases or bodily conditions such as HIV infection,[21,22] immune responses to viral infections (*e.g.*, hepatitis A, B, and C),[23-25] systemic levels of drugs,[17] and the detection of illicit drug use.[26,27]

One of the main advantages of saliva as a diagnostic tool is that sample collection is easy and noninvasive, thus dramatically diminishing discomfort associated with blood collection and privacy issues associated with urine collection. Salivary constituents vary depending on the harvesting method and the degree of salivary flow. The different methods for collecting saliva can be classified according to whether they use stimuli. Stimulated saliva is commonly collected by inducing masticatory action on paraffin wax or chewing gum (*i.e.*, absorbent method; Fig. 2) to increase the salivary flow rate. This method obviously affects the quantity and pH of the saliva, and is generally only used in patients who have difficulty producing enough saliva. Unstimulated saliva is collected without exogenous facilitation, and its flow rate is mostly affected by the degree of hydration. The three most common approaches for collection of unstimulated saliva are draining, spitting, and suction methods.[28] Irrespective of the method used, subjects should be instructed to clean the oral cavity before collection by rinsing the mouth thoroughly with water to avoid contaminants.

There are compelling reasons for exploring saliva as a diagnostic tool. It clearly meets the demands for an inexpensive, noninvasive, and easy-to-use screening method. As a diagnostic specimen in the clinic, saliva has many advantages in terms of collection, storage, shipping, and voluminous sampling; all of these processes can be carried out very economically compared with serum or urine. Saliva is also easier to handle during diagnostic procedures than blood because it does not clot, thus re-

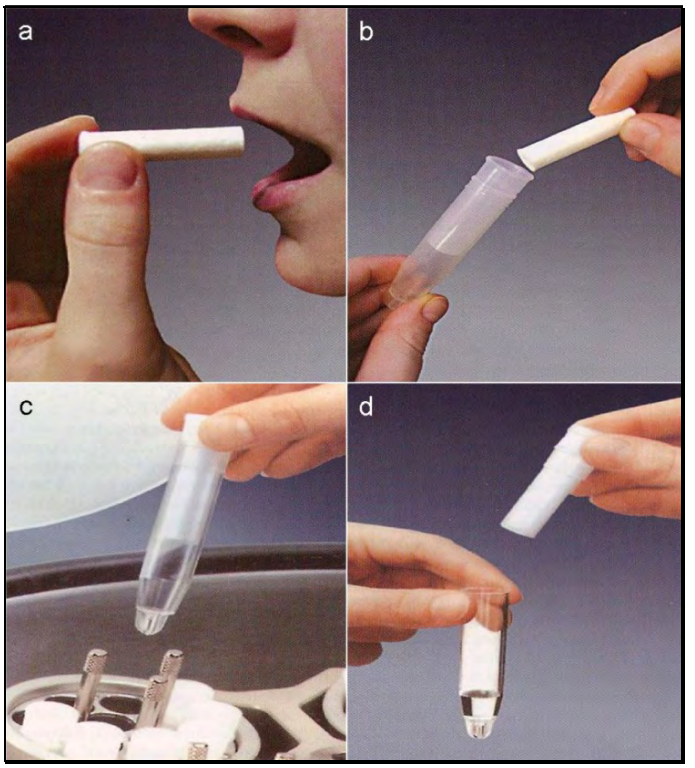

Fig. 2. Collection of whole saliva by the Salivette (absorbent) method. (a) Saliva is collected by chewing a cotton wool swab. (b) The swab containing saliva is placed in the tube of Salivette. (c) Centrifugation of the assemblage. (d) Saliva is separated from the swab and is ready for analysis.

ducing the number of manipulations required. Moreover, for healthcare professionals, a salivary test is safer than using serum, which is more likely to expose operators to blood-borne diseases. For the patients or examinees, the noninvasive collection approach could dramatically reduce anxiety and discomfort, and increase their willingness to undergo health inspections that will greatly increase the opportunity to monitor their general health over time and to diagnose morbidities in the early stage.

SALIVARY DIAGNOSTICS

Background

For the past two decades, salivary diagnostic approaches have been developed to monitor oral diseases such as periodontal diseases[29,30] and to assess caries risk.[31] Recently, due to the combination of emerging biotechnologies and salivary diagnostics, a large number of medically valuable analytes in saliva are gradually unveiled and some of them represent biomarkers for different diseases including cancer,[32-34] autoimmune diseases,[35,36] viral diseases,[23-25,37] bacterial diseases,[38,39] cardiovascular diseases,[40] and HIV.[21,22] These developments have extended the range of saliva-based diagnostics from the simple oral cavity to the whole physiological system. Thus, saliva-based diagnostics is on the cutting edge of diagnostic technology, and may offer a robust alternative for clinicians to use in the near future to make clinical decisions and predict post-treatment outcomes.

Vision and challenges

Over the past decade, salivary diagnostics have received increasing attention as a growing number of high-impact systemic diseases (*e.g.*, cancer, cardiovascular, and metabolic diseases) and physiological conditions were shown to be accurately reflected by the composition of saliva, motivating scientists from academia, government, and industry to invest resources into saliva-based diagnostics. Most of these efforts have concentrated on technological advancement and assay development in order to identify disease-signaling biomarkers and meet the criteria of salivary diagnostics. A good diagnostic method should have the characteristics of high sensitivity, specificity, and functionality, and meet the requirements of high throughput, portability, and low cost for subsequent clinical application. For salivary diagnostics, many of these goals have been met through engaging diverse technology fields including biology, chemistry, physics, and engineering. Today, the improved efficiency and accuracy of genomic and proteomic biomarker discovery technologies are turning salivary diagnostics into a clinical and commercial reality. Among the newly developed technologies, the miniaturization technology known as "lab-on-a-chip" provides a new avenue for point-of-care diagnostics since it is able to detect multiple biomarkers in parallel and allows simultaneous assessment of multiple disease conditions. Furthermore, because miniaturization allows diagnosis to be performed outside of the laboratory, such as in the home, this new technology may further enhance the healthcare delivery, reduce health disparities, and improve access to care. Such new technologies in association with the saliva-based approach, which is painless, inexpensive, easier, and safer than approaches based on serum or urine, can significantly impact molecular diagnostics.

There is a soaring need for convenient and simple point-of-care diagnostic tools in developing countries, where many health risks and illnesses remain poorly defined and medical resources including well-trained professionals are sadly lacking. In addition, information concerning the burden of disease, guidelines for health maintenance, and disease management are seriously inadequate, resulting in severe spread of disease and high death rates. Under these circumstances, salivary diagnostic technologies that offer easy, cost-effective, noninvasive ways to prevent disease dissemination will have enormous impact.

The greatest challenge of salivary diagnostics is to identify disease diagnostic markers and successfully translate these research efforts from the laboratory into the clinic. To empower salivary diagnostics to become an approach for health surveillance, we have established robust scientific platforms for saliva biomarker discovery, validated potential candidates, and developed point-of-care technologies for high throughput, efficiency, and accurate clinical applications.

TECHNOLOGIES FOR DISCOVERY OF SALIVARY BIOMARKERS

Since saliva contains many components also found in serum, and has several advantages over serum as described above, saliva is therefore a unique bodily fluid for the development of molecular diagnostics. However, its potential was frequently underestimated due to technological barriers that did not meet the requirements necessary to screen saliva containing complex constituents with low abundance.[41] Emerging technologies developed in the last decade, especially for the analysis of proteins and nucleic acids, have given a new horizon to overcome these technological challenges. Recent studies in salivary protein research have shown that, in addition

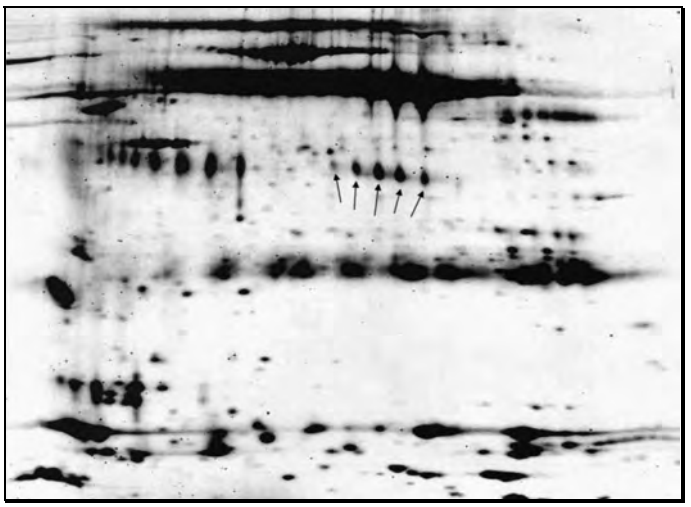

Fig. 3. 2D gel electrophoretograms of human whole saliva proteins. Proteins were separated in the 1st dimension immobilized pH gradients (IPG) followed by SDS-PAGE. Speckles were visualized by SYPRO Ruby stain. The arrows indicate five isoforms of carbonate dehydratase VI precursor. Targets of interest will be identified by subsequent MS analysis.

to the major salivary protein families, saliva contains hundreds of minor proteins or peptides that are present in low concentrations but may play an important role on the discrimination of diseases. Besides proteomes, the salivary transcriptomic technology, the second salivary diagnostic alphabet, further advanced the diagnostic potential of saliva for medical applications.

Discovery of salivary biomarkers by proteomic technology

The proteome is the protein complement of the genome, and proteomics is analysis of the portion of the genome that is expressed. The proteomes in bodily fluids are valuable due to their high clinical potential as sources of disease markers. In principle, a global analysis of the human salivary proteomes can provide a comprehensive spectrum of oral and general health. Furthermore, analysis of salivary proteomes over the course of complications may unveil morbidity signatures in the early stage and monitor disease progression.

Proteome-based approaches have been applied over the last three decades to monitor changes in protein expression. Generally, protein expression is primarily analyzed by one- or two-dimensional polyacrylamide gel electrophoresis (PAGE). To resolve the complex composition of saliva, 2-D PAGE allows separation not only of different molecules with similar molecular weights, but also of different modification patterns or isoforms of the same protein (Fig. 3). Along with the development and introduction of mass spectrometry (MS), the PAGE-separated proteins can be more accurately characterized and identified, leading to a wider range of applications for proteomic assays. Proteins that are primarily identified by MS can be further characterized by ionization methods such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). Moreover, coupling ESI and MALDI with mass analyzers, such as quadrupole/linear ion trap, time-of-flight (TOF), quadrupole TOF (QTOF), fourier transform ion cyclotron resonance (FT-ICR), and the OrbiTrap, may improve the sensitivity, resolution, accuracy, and efficiency of protein sequence determination. To date, MS technology has yielded advanced insight into the characteristics of salivary proteomes, and provided strong evidence supporting the use of saliva as a diagnostic tool.[42]

In some cases, however, simply discriminating up and/or down regulation of the expression of specific proteins may not directly reflect the circumstances of physiological states or disease progression. This is because biological functions of proteins may change due to posttranslational modifications that occur without alteration of protein level.[43,44] It has been demonstrated that many functional alterations of proteins result from posttranslational modifications such as phosphorylation, glycosylation, acetylation, and methylation.[45,46] These post-translationally modified proteins may represent signatures in some diseases such as autism spectrum disorder[47] and cervical cancer.[48] To evaluate the potential of posttranslationally modified proteins as diagnostic biomarkers, dendrimer-associated MS/MS, MALDI-MS, and targeted HPLC-ESI-MS/MS provide comprehensively analytical methodologies for proteins with different types of posttranslational modifications.[49,50]

As of January 2009, over a thousand salivary proteins have been identified from major salivary glands.[42] For most of these proteins, their expression in saliva is quite distinct from that in serum or tear, and have already demonstrated clinical diagnostic values for diseases manifested in the oral cavity. For example, Sjögren's syndrome (SS), a chronic autoimmune disorder that is clinically recognized by dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca), is associated with changes in specific salivary constituents, such as an increase in inflammatory proteins (*e.g.* α-enolase, carbonic anhydrase I and II, salivary α-amylase fragments) and decrease in acinar proteins (*e.g.*, lysozyme C, polymeric immunoglobulin receptor (pIgR), calgranulin A) compared with the profile in non-SS individuals.[35] Other research efforts showed that saliva is an important tool for the detection of oral squamous cell carcinoma (OSCC). Three tumor markers (Cyfra 21-1, tissue polypeptide antigen (TPA), and cancer antigen CA125) are significantly elevated in saliva when compared to the patients' sera.[51] Recently our laboratory has discovered and validated a highly discriminatory panel of salivary biomarkers for oral cancer detection. Five salivary proteins (M2BP, MRP14, profilin, CD59, and catalase) were shown to be able to discriminate oral cancer with greater than 90% clinical accuracy.[34] Besides SS and OSCC, salivary proteomic constituents are also capable of detecting high-impact systemic disorders. For example, measurement of antibodies to HIV in saliva has been shown to be as accurate as measurement in serum, and the salivary assay has been commercialized as a product called OraQuick. Moreover, early studies suggest that measurement of salivary CA125 and epidermal growth factor may have diagnostic potential for ovarian cancer[52] and breast cancer,[53] respectively.

Current efforts to elucidate the proteomes from whole saliva or individual glandular (*e.g.*, parotid, submandibular and sublingual) saliva have progressed rapidly along with development of MS and protein separation techniques. A central salivary protein database has been established by the UCLA research team (www.hspp.ucla.edu) in which we have assembled acquired proteomic data and exchanged research

results with groups worldwide. The integration of up-to-date information is ongoing, and extensive comparisons between proteins in saliva and other bodily fluids are under construction. This comprehensive categorization of salivary proteomes will be an important resource for researchers who are studying protein chemistry, especially in the fields of oral biology and salivary diagnostics, and will be helpful for analyzing how the expression of salivary proteomes changes with different diseases and hence identifying corresponding disease-related salivary biomarkers.

Discovery of salivary biomarkers by transcriptomic technology

In addition to salivary proteome, in 2004 we discovered the salivary transcriptomes (RNA molecules) that are unusually stable in saliva.[54] They included mRNA molecules that cells use to convey the instructions carried by DNA for subsequent protein production. This discovery presented a second diagnostic alphabet in saliva and opened a door to another avenue of salivary transcriptomic diagnostics. Although the salivary transcriptome is an emerging concept, we have established a robust platform at UCLA for salivary RNA studies including automated extraction, purification, amplification, and high-throughput microarray screening. Importantly, we have also developed statistical and informatics tools that are tailored for salivary biomarker discovery and validation. Also, Early Disease Research Network (EDRN), an entity within the National Cancer Institute (NCI), has just completed an independent validation study of salivary RNA biomarkers for oral cancer detection. This investigation confirmed a clinical translational value of salivary RNA for oral cancer detection. In the past 5 years, research into the nature, origin and characterization of salivary mRNA has been actively pursued.[33,35,55]

At present, the main strategy for identification of salivary transcriptomic biomarkers is through microarray technology. Although it has been demonstrated that the 3'-based array employing poly-dT priming and two rounds of *in vitro* transcription (IVT) amplification works well for profiling salivary transcripts, some pitfalls still need to be overcome. For instance, much information is lost because approximately 50% of salivary RNA molecules are fragmented,[55] therefore they do not carry the poly-A tail and are not protected against degradation. Furthermore, the random priming approach in the RNA amplification may cause an additional shortening of the fragments resulting in further loss of RNA molecules during the procedure. To address these issues, we have recently made a significant improvement to saliva transcriptomic screening using an emerging 3'-poly(A)-independent amplification technology to recover all salivary RNA fragments (ExpressArt TRinucleotide mRNA Amplification Kit[a]), followed by profiling all fragments on the Affymetrix All Exon Array (AEA) platform. This novel approach allows investigation of the salivary transcriptome at a higher resolution level *via* detection of individual exons. Theoretically, the increased resolution could detect more genes and hence increase the opportunities to discriminate disease markers. So far we have defined the salivary exon core transcriptome (SECT), which contains 1,370 probe sets representing 851 unique genes that are present in more than 85% of the tested saliva samples.[56] Pilot studies of oral and pancreatic cancers using AEA are consistent with previous results using conventional assays (*i.e.*, Affymetrix's Human Genome U133A assay), and show that the AEA does indeed expand the numbers of sequences and genes that can be detected. Quantitative real-time PCR (qPCR) is currently the gold standard for quantification of nucleic acids. It is perfectly appropriate for validation of transcriptomic biomarkers after profiling by microarray, and it is not restricted by the length of the RNA, even for fragmented RNA. However, low amounts of RNA in saliva tremendously hinder their performance in qPCR. To overcome this problem, a new multiplex reverse transcriptase-PCR-based pre-amplification approach was developed that allows accurate quantification of over 50 targets from one reaction. This method dramatically increases the capacity of quantitative analysis that it extends approximately six-fold for the magnitude of target input,[56] and is tailored to the short nature of salivary RNA. It also offers good time- and cost-effectiveness by performing simultaneous reverse transcriptase reactions for different targets, allowing a small volume of pre-amplification product to be used for subsequent qPCR measurement.

Our studies of salivary mRNA biomarkers from patients with primary T1/T2 OSCC showed promising results and demonstrated the diagnostic and translational potential of the salivary transcriptome.[33] Data combining microarray profiling and qPCR validation showed seven mRNA whose expression levels in patients were elevated at least 3.5-fold compared with matched healthy counterparts. These mRNAs are transcripts of DUSP1, H3F3A, OAZ1, S100P, SAT, IL-8, and IL-1β. In the initial study, the combination of these biomarkers presented 91% sensitivity and specificity (ROC = 0.95), displaying a high credibility for discrimination of OSCC. To further validate the salivary transcriptomic biomarkers for oral cancer detection, we compared saliva and blood transcriptomes from the same patients with respect to their capability for disease discrimination. The study showed that a group of five transcriptomic biomarkers in serum can be consistently validated and distinguished OSCC with 91% sensitivity and 71% specificity (ROC = 0.88).[57] The salivary transcriptome is a more discriminatory tool for oral cancer detection than the serum transcriptome. So far, over 220 additional oral cancer patients have been tested and the clinical accuracy of the salivary mRNA biomarkers holds up at > 82% (Wang *et al.*, unpublished data), indicating they are among the most discriminatory panels for OSCC screening to date.

DEVELOPMENT OF POINT-OF-CARE TECHNOLOGIES FOR SALIVARY DIAGNOSTICS

In September 1999, the NIDCR initiated a research workshop aimed at applications of microfluidics and micro/nanoelectromechanical system (MEMS/NEMS) to saliva-based diagnostics. MEMS/NEMS is an integrated system that consists of a central unit for processing data (*i.e.*, microprocessor) and several other components that connect with the outside interface, such as microsensors. In principle, multiple analytes in a drop of saliva could be simultaneously measured and ana-

American Journal of Dentistry, Vol. 22, No. 4, August, 2009

Table. Commercial products for saliva-based diagnosis in the USA.

Test purpose	Marker(s)	Product Name
Substance abuse	NIDA 5 Blood panel + barbiturates, methamphetamines, benzodiazepines, methadone	Intercept[b]
Substance abuse	NIDA 5 Blood panel	SALIVASCREEN[c] 5 Professional
Blood alcohol	Ethanol	Q.E.D. Saliva Alcohol Test[b]
HIV	HIV antibody	OraQuick ADVANCE[b] Rapid HIV-1/2 Antibody Test[b]
HIV	Anti-HIV immunoglobulin (IgG antibody -For confirmatory test)	Orasure HIV1 Western Blot[b]
Insurance & toxicology assays	Cocaine metabolite, cotinine, cannabinoids, opiates, phencyclidine	MICRO-PLATE EIA[b]
Hormones	Estradiol, progesterone, testosterone, DHEA, cortisol	ZRT Saliva Test[d]

Fig. 4. Oral Fluid NanoSensor Test (OFNASET), a point-of-care, automated, easy-to-use integrated system that will enable simultaneous, accurate, and rapid detection of multiple salivary protein and nucleic acid biomarkers.

lyzed through highly sensitive biosensors. This workshop comprised scientists from academia, government, and industry, and covered multidisciplinary fields including oral biology, chemistry, instrumentation, engineering, and clinical sciences. To proceed with the development of saliva diagnostic technologies, in 2002 the NIDCR funded seven projects that explored different point-of-care systems to detect salivary analytes and provided an overall profile that correlated with a particular disease state: electrochemical sensing,[59,60] on-chip PCR/RT-PCR,[61] microsphere-based nano-bio chip,[62,63] microsphere-based optical fiber array,[64,65] high-throughput DNA microarray (*i.e.*, validation of the first-generation DNA chip), surface plasmon resonance optical system,[66] and microchip electrophoretic immunoassay.[67] In 2006, four groups were awarded another 5 years of support to further develop the respective point-of-care technology for prototype production, analysis, and clinical validation. UCLA is one of the four funded groups. The UCLA Collaborative Oral Fluid Diagnostic Research Center, partnered with engineers at the UCLA School of Engineering, developed a MEMS-based electrochemical detection platform that is capable of real-time, ultrasensitive, ultraspecific multiplex detection of salivary protein and RNA biomarkers.[68] This envisioned product has been labeled the Oral Fluid NanoSensor Test (OFNASET) (Fig. 4). The OFNASET 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 doctor's office, but also in any other healthcare station to perform an instant point-of-care diagnosis.

SALIVARY DIAGNOSTICS – A NEW INDUSTRY IN A PROSPECTIVE FUTURE

The value of saliva as a diagnostic tool has long been disregarded until the advantages of saliva-based approaches was recognized in the past decade, and led to an evolution from treating saliva as a diagnostic worthlessness to promoting salivary diagnostics. Regarding diagnostic capability, the gap between saliva and other bodily fluids, such as blood, urine, and cerebral spinal fluid, is closing, primarily due to rapid technology development, scientific validation of diagnostic analytes, and advocacy by the NIDCR.

Salivary diagnostics would enable clinicians to monitor diseases frequently and easily and would have impact on the future medical research and therapy. In addition to previously mentioned oral cancer and Sjögren's syndrome, systemic disorders may be reflected diagnostically in saliva as well. At present, we have promising preliminary results showing that saliva can be used to detect lung cancer, pancreatic cancer, breast cancer, and type II diabetes; however, for each disease, we need further scientific validation, as well as to benchmark the diagnostic capacity of saliva against other bodily fluids. These studies are ongoing and will undoubtedly remain a major focus of investigation in the future.

Based on the abundance of promising research efforts and the fact that research into salivary diagnostics is currently a priority at NIDCR, saliva-based diagnostics present unparalleled opportunities for research and commercialization opportunities. We foresee that more products to be commercialized; either as new inventions or based on products shown in the Table. With the current rate of progression, salivary diagnostics can become a key player in routine health monitoring in the near future and enable the early detection of disease using a simple and effective assay. Thus, salivary diagnostics will not only save lives, but also preserve the quality of lives that have been saved.

a. AmpTec GmbH, Hamburg, Germany.
b. Orasure Technologies, Inc., Bethlehem, PA, USA.
c. Craig Medical Distribution, Inc., Vista, CA, USA.
d. ZRT Laboratory, Beaverton, OR, USA.

Acknowledgements: This study was supported by the Felix & Mildred Yip Endowment Professorship Fund (D.T.W.) and PHS grant T32 DE 007296-12 (Y.-H.L.).

Disclosure statement: Dr. David Wong is co-founder of RNAmeTRIX Inc., a molecular diagnostics company. Dr. Lee has no conflict of interest.

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