Salivary diagnostics powered by nanotechnologies, proteomics and genomics

David T. Wong, DMD, DMSc

ABSTRACT

Background. The ability to monitor health status, disease onset and progression, and treatment outcome through noninvasive means is a highly desirable goal in health care promotion and delivery. Oral fluid is a perfect medium to be explored for health and disease surveillance.

Methods. Two prerequisites exist before the goal of salivary diagnostics can be achieved: identification of specific biomarkers associated with a health or disease state and the development of technologies that can discriminate between the biomarkers. A recent initiative of the National Institute of Dental and Craniofacial Research has created a roadmap to achieve these goals through the use of oral fluids as the diagnostic medium to scrutinize the health and/or disease status of patients. This is an ideal opportunity to optimize state-of-the-art saliva-based biosensors for salivary biomarkers that discriminate between diseases.

Results. Seven technology groups are developing point-of-care salivary diagnostic technologies. Three groups are working together toward deciphering the salivary proteome.

Conclusion. These collective efforts and the convergence of salivary diagnostic technologies and the salivary proteome will present unparalleled opportunities to explore the diagnostic potential of saliva for oral and systemic diseases.

Key Words. Oral fluid; saliva; oral cancer; nanotechnology; proteomics; genomics.

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Systemic diseases, including cancer and cardiovascular, metabolic and neurological diseases, are challenging to diagnose without supplementing clinical evaluation with laboratory testing. Even with laboratory tools, definitive diagnosis often remains elusive.

Three roadblocks have prevented the realization of the potential of clinical diagnostics:

- lack of definitive disease-associated protein and genetic markers;
- absence of easy and inexpensive sampling methods that involve minimal discomfort;
- lack of an accurate, portable and easy-to-use diagnostic platform.

Saliva, a biofluid that is readily accessible via a totally noninvasive method, has long been recognized as addressing the second roadblock.[1] Because of the visionary investment by the National Institute of Dental and Craniofacial Research (NIDCR), the discovery of salivary biomarkers and the ongoing development of salivary diagnostic technologies will address the first and third roadblocks. It is safe to predict that the use of saliva for disease

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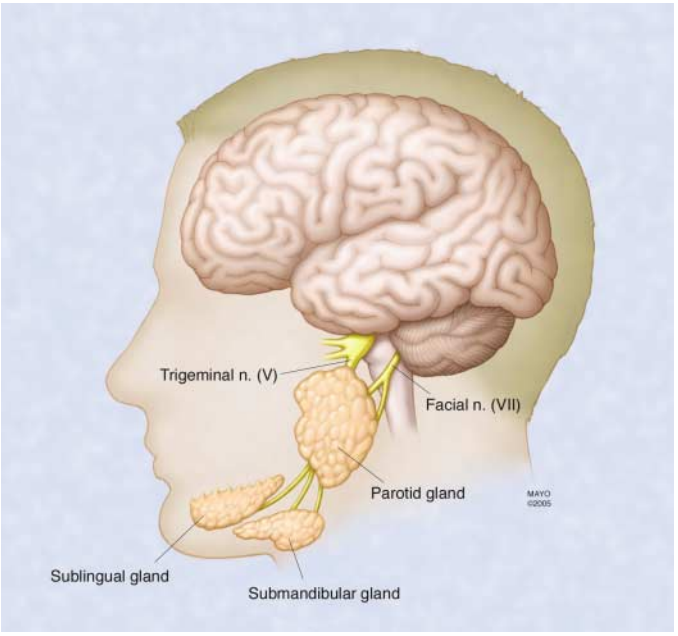

Figure 1. Anatomical locations of the three major salivary glands: parotid, submandibular and sublingual. n: Nerve. Reprinted from Forde and colleagues[2] with permission of Mayo Foundation for Medical Education and Research. All rights reserved.

diagnostics and health surveillance is about five years away.

This is an exciting time, as we are seeing the applications of salivary diagnostics for oral diseases, which will be followed soon by the application to high-impact systemic diseases, using highly informative panels of salivary proteomic and genomic biomarkers. This will enable researchers to bridge oral health research with systemic disease diagnostics via a biofluid that filters, processes and secretes itself from the vasculature that nourishes the salivary glands into the oral cavity (Figures 1[2] and 2[3]). Oral fluid is a perfect medium to be explored for health and disease surveillance. The translational applications and opportunities are enormous.

In this article, I explore current salivary diagnostic, research and developmental efforts for use in dentistry and medicine.

BACKGROUND

A large number of diagnostic analytes have been shown to be present in saliva, including steroid hormones[2] and the HIV antibody.[4] For the past two decades, oral health researchers have been developing salivary diagnostic tools to monitor oral diseases (including periodontal diseases[5,6]), as well as for caries risk assessment.[7] These diagnostic advances range from genetic susceptibility analysis of interleukin-1 (IL-1) genetic alleles to the analysis of oral pathogens identified via lectin staining for caries risk assessment.[7] The current development of diagnostic biomarkers (via proteomic and genomic approaches) in conjunction with technological developments in salivary diagnostics will lead to the development of robust diagnostic tools for dentists to use in making clinical decisions and predicting treatment outcomes.

An increasing number of systemic diseases and conditions have been shown to be reflected diagnostically in saliva. Along with these developments are technology advancements that have overcome barriers to the widespread implementation of salivary diagnostics. These barriers include technological problems related to achieving high sensitivity, high specificity, miniaturization, high throughput (that is, assay a large number of samples concurrently), automation, portability, low cost, high functionality and speed; overcoming them has enabled researchers to detect and measure multiple disease markers.

Emerging technologies from a combination of miniaturization technologies (such as "lab on a chip") and discoveries from many fields are leading to saliva-based high-throughput, automated, portable, low-cost, more efficient and rapid biochemical analyses. Miniaturized saliva-based diagnostic technologies will enable the use of minute amounts of bodily fluids to yield critical patient information that reflects health and disease status. Such technologies will allow clinicians to achieve real-time and simultaneous assessment of multiple diseases.

VISION AND CHALLENGES

The postgenomic era provides opportunities for high-throughput approaches to genomics and proteomics. The novel technologies of miniaturization coupled with the highly parallel detection of disease create the possibility of developing radically new ways of detecting and diagnosing health and disease states in a person, even in remote or impoverished settings. These discoveries and technological advances in conjunction with the ability to diagnose disease through the use of a biofluid obtained noninvasively would offer a revolutionary change in medicine.

A great need exists for convenient and accurate point-of-care diagnostic tools that can be used in a noninvasive manner. This is of particular relevance in the developing world, where many health risks and illnesses remain poorly

defined and patients receive inappropriate treatment. In addition, little information is available about the burden of disease to guide population-wide health decisions.

The challenge of salivary diagnostics is to discover its potential and optimize engineering technologies for use with this biofluid. Figure 3 is a ven diagram illustrating that within the spectrum of human health and disease states, researchers envision that some of these states will be reflected diagnostically in saliva via proteomic or genomic information. However, which diseases will be reflected diagnostically in saliva remains to be determined. The lower right circle shows an example of the technology development platform needed to advance the point-of-care detection capability of saliva.

The challenge in making salivary diagnostics a clinical reality is establishing the scientific foundation and clinical validations needed to position it as a highly accurate and feasible technology that can achieve definitive point-of-care assessment of health and disease status. Inherent in this vision is the establishment of scientific and diagnostic biomarkers in saliva and the development of robust, simple-to-use biosensor technologies for reliable and valid clinical applications.

SALIVA AS A DIAGNOSTIC FLUID

The ability to use saliva to monitor a patient's health and disease states is a highly desirable goal for health promotion and health care research. However, only recently has there been an appreciation of how saliva can reflect virtually the entire spectrum of health and disease states.[1] These states include tissue levels of natural substances and a large variety of molecules

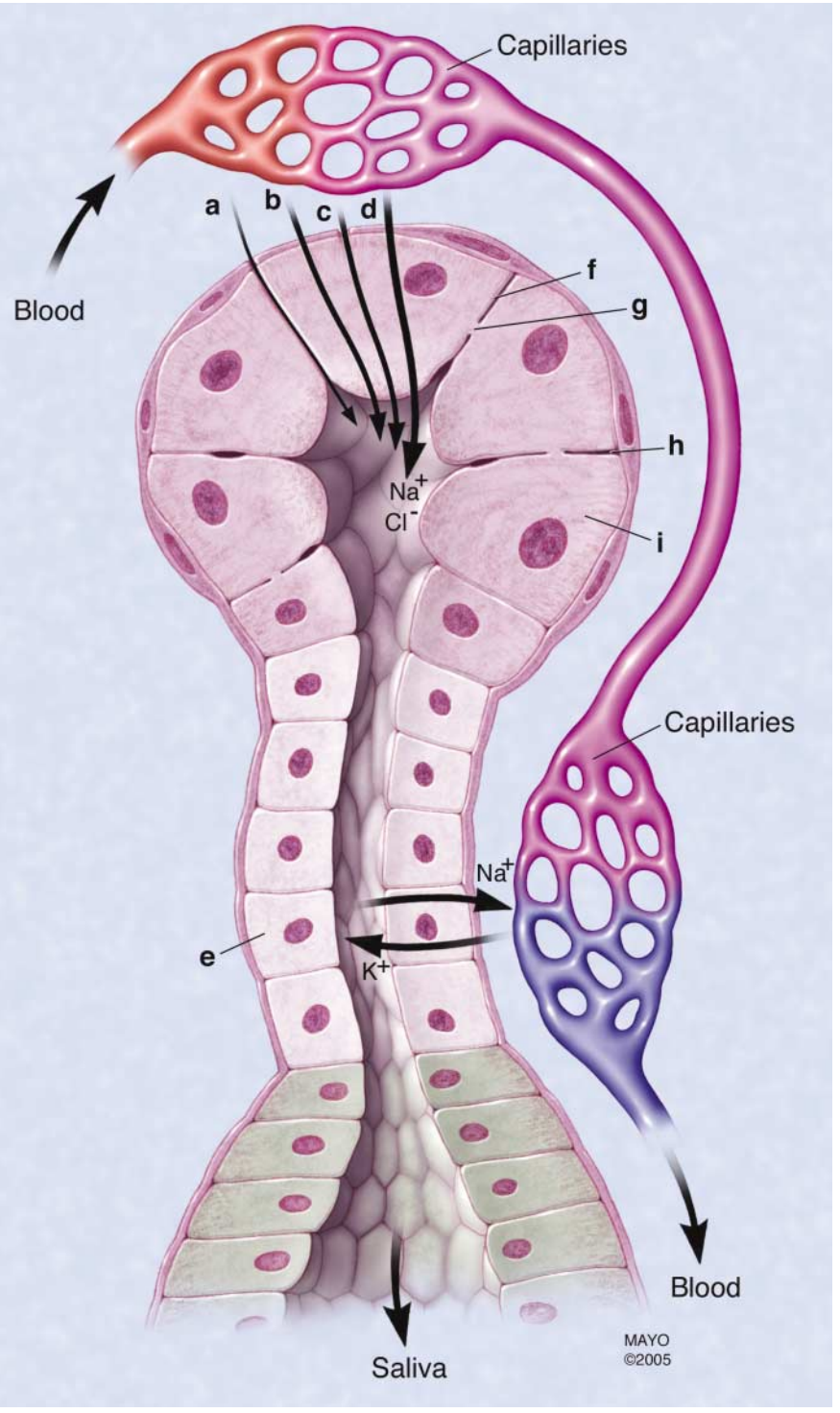

Figure 2. Mechanisms of transport of proteins and ions from serum into salivary gland ducts. a: active transport of selected compounds. b: passive diffusion of lipid soluble compounds. c: simple filtration of selected compounds. d: acinar cells actively pump sodium ions (Na+) into the duct, followed by water. e: duct cells pump Na+ back into blood, producing hypotonic saliva. f: cell membrane. g: pore of cell membrane. h: intracellular space. i: acinar cell. Cl–: chlorine ion. K+: potassium ion. Reprinted with permission of Mayo Foundation for Medical Education and Research. All rights reserved. (This figure was adapted from Haeckel and Hanecke[3] with permission of Walter de Gruyter GmbH & Co.)

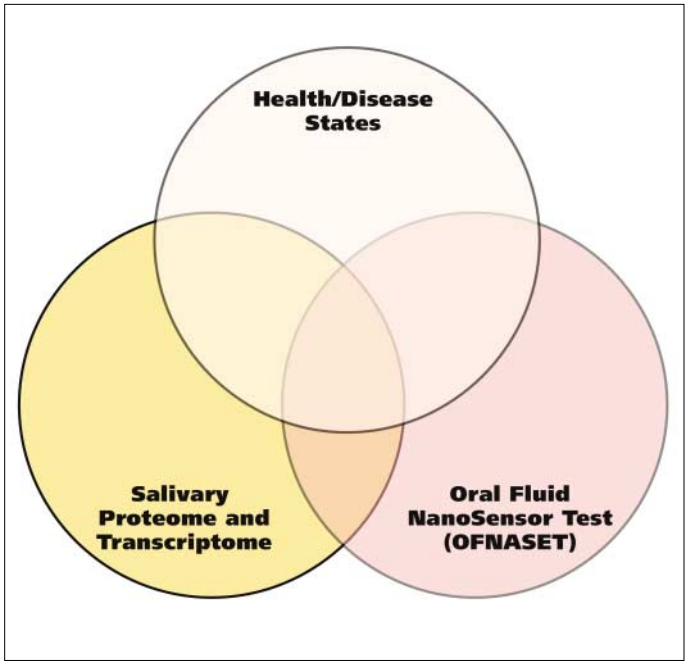

Figure 3. Manifestation of disease markers in saliva and their detection by salivary diagnostic biosensors.

introduced into the body for therapeutic, dependency or recreational purposes; emotional status; hormonal status; immunological status; neurological effects; and nutritional and metabolic influences.

A major drawback to using saliva as a diagnostic fluid has been the notion that informative analytes generally are present in lower amounts in saliva than in serum.[8] With new and highly sensitive techniques, however, the lower level of analytes in saliva is no longer a limitation. Almost anything one can measure in blood, one can measure in saliva. Saliva has been used reliably to detect HIV 1 and 2, as well as viral hepatitis A, B and C.[9,10] It also can be used to monitor a variety of drug levels, including those of marijuana, cocaine and alcohol.[1]

Compelling reasons exist to use saliva as a diagnostic fluid. It meets the demands for inexpensive, noninvasive and easy-to-use diagnostic methods. As a clinical tool, saliva has many advantages over serum, including ease of collection, storing and shipping, and it can be obtained at low cost in sufficient quantities for analysis. For patients, the noninvasive collection techniques dramatically reduce anxiety and discomfort and simplify procurement of repeated samples for monitoring over time. Saliva also is easier to handle for diagnostic procedures because it does not clot, thus lessening the manipulations required.

DEVELOPMENT OF TECHNOLOGIES FOR SALIVA-BASED DIAGNOSTICS

In 2002, NIDCR initiated a research effort in the area of salivary diagnostics, and progress is being made toward developing technologically viable systems that are suitable for commercialization. NIDCR funded seven awards for the development of microfluidics and microelectromechanical systems (MEMS) for salivary diagnostics (Box). MEMS are integrated systems composed of mechanical elements, sensors, actuators and electronics on a common silicon substrate developed through microfabrication technology. These systems use small sample and reagent volumes coupled with integrated detection methods to perform an analysis. The seven NIDCR-supported awards focused on the development of microfluidic and MEMS technologies for measuring proteins, DNA, gene transcripts (mRNA), electrolytes and small molecules in saliva.

Eric Anslyn, PhD, at the University of Texas (UT) at Austin has developed a sensor array platform suited for point-of-care diagnostics; it is based on a micromachined bead-based platform that is amenable to multianalyte detection and quantitation. The UT research group teamed with experts in salivary diagnostics at the University of Kentucky, Lexington, for the collection and analysis of clinical samples.

Daniel Malamud, PhD, MA, and his research team at New York University, New York City, are involved in the identification and characterization of antibacterial and antiviral molecules, as well as assay development, using oral fluid as a noninvasive diagnostic method. This research team is involved in designing a point-of-care detection system for the identification of bacterial and viral pathogens.

Anup Singh, PhD, Sandia National Laboratories, Livermore, Calif., is developing microfluidic systems for the analysis of biological molecules. His research group is developing an integrated microfluidic system for simultaneous detection of multiple analytes in saliva and in other oral fluids.

David Stahl, PhD, University of Washington, Seattle, is involved in studies of microbial community structure and function in many habitats, including the human mouth. Dr. Stahl's group is developing DNA microarray-based technology for the rapid and unambiguous detection of microbial biomarkers in oral fluids.

David Walt, PhD, Tufts University, Medford,

BOX

NIDCR*-funded salivary diagnostic technology development and salivary proteome research groups.

SALIVARY DIAGNOSTIC TECHNOLOGY DEVELOPMENT

University of Texas at Austin
Eric Anslyn, PhD
"www.cm.utexas.edu/directory/eric_anslyn/"

New York University, New York City
Daniel Malamud, PhD, MA
"www.nyu.edu/dental/research/faculty/malamud.html"

Sandia National Laboratories, Livermore, Calif.
Anup Singh, PhD
"www.ca.sandia.gov/chembio/microfluidics/staff/singh.html"

University of Washington, Seattle
David Stahl, PhD
"www.stahl.ce.washington.edu/index.html"
Paul Yager, PhD
"http://faculty.washington.edu/yagerp/"

Tufts University, Medford, Mass.
David Walt, PhD
"chem.tufts.edu/faculty/walt/"

University of California, Los Angeles
David Wong, DMD, DMSc
"www.saliva.bme.ucla.edu/"

SALIVARY PROTEOME

University of California, San Francisco
Susan Fisher, PhD
"www.salivarium.ucsf.edu/"

The Scripps Research Institute, La Jolla, Calif.
John Yates, PhD
"fields.scripps.edu/public/project/saliva/"

University of California, Los Angeles
David Wong, DMD, DMSc
"www.hspp.ucla.edu"

* NIDCR: National Institute of Dental and Craniofacial Research.

Mass., is using a bead-based fiber-optic–based detection platform to investigate salivary diagnostics in the context of end-stage renal disease, asthma and opportunistic infections.

My research group at the University of California, Los Angeles (UCLA) is developing point-of-care microfluidics systems that can permit concurrent detection of multiple salivary analytes, including proteins and nucleic acids. The UCLA technology permits salivary analyte detection at high levels of sensitivity and specificity, without the need for a polymerase chain reaction for nucleic acid detection or an enzyme-linked immunosorbent assay for protein detection. Clinical applications include oral and breast cancer and metabolic diseases (for example, type 2 diabetes).

Paul Yager, PhD, University of Washington, is developing an integrated microfluidic system for rapid, inexpensive and simultaneous measurement of multiple analytes in saliva that uses a simple disposable polymeric laminate format. The goal of this program is to detect low levels of hormones, drugs, metabolites, specific pathogens and markers of oral cancer, as well as protein markers of systemic disease.

Although these academic developments are in progress, none of these salivary diagnostic technologies will become a practical and clinical reality without strong partnerships with industry early in the developmental stage. Challenges include integration of individual components, validation, regulatory approval and, finally, bringing technologies to the marketplace. This challenge has sparked a new NIDCR initiative for the development and validation of technologies for saliva-based diagnostics whereby researchers will team with industrial partners to further develop functional prototypes and test their robustness for clinical applications. The anticipated outcome of this initiative is the commercialization of saliva-based diagnostic technologies optimized for the detection of highly sensitive and specific salivary biomarkers for human diseases.

DIAGNOSTIC MOLECULAR TARGETS IN SALIVA

In 2003, NIDCR funded three awards for studies aiming to comprehensively identify and catalog human salivary proteins from the three major salivary glands (Box). The research community envisions that the human salivary proteome will be a resource to help elucidate disease pathogenesis and evaluate the influence of medications on

the structure, composition and secretion of all salivary constituents.

The three NIDCR-supported programs use an array of technologies, not only to identify all of the peptides and proteins in salivary fluid, but also to gain knowledge about their function. Each of the programs uses modern mass spectrometry, protein chemistry and protein separation tools to uncover the identity of the high- and low-abundance proteins. However, each of the three groups contributes something unique—and ultimately complementary—to the collective efforts.

Susan Fisher, PhD, and her group at the University of California, San Francisco, perform detailed analyses of the combined submandibular/sublingual secretions and parotid salivary samples. The group will use methods to elucidate glycoproteins and phosphoproteins, which will add to the functional aspects of the catalog.

The group led by John Yates, PhD, The Scripps Research Institute, La Jolla, Calif., will compile a salivary protein catalog by implementing novel protein fractionation methods along with state-of-the-art mass spectrometry technologies. The group is teamed with Jim Melvin, DDS, PhD, and his group at the University of Rochester, N.Y., and they will create a comprehensive library of antisalivary antibodies, using bacteriophage display, to build multiple probes for each salivary component. These antibody or immune affinity reagents will permit detection and purification of specific salivary proteins, which will, in turn, permit a high-throughput approach to the characterization of each protein's posttranslational modification state, degree of interaction with other salivary components and affinity for an in vivo tooth model (hydroxyapatite).

The UCLA group, which I lead, is a collaboration between the dental schools at UCLA and the University of Southern California (USC). Clinical cores (facilities) at the two schools will identify and develop resources for the clinical acquisition of saliva from the separate parotid, submandibular and sublingual glands. The proteomics core at UCLA, led by co-director Joseph Loo, PhD, uses bottom-up and top-down sequencing methods to compose a catalog for each of the segregated glands. Paul Denny, PhD, and his group at USC lead the efforts to understand the function of the proteins by characterizing the glycoproteome of salivary proteins.

The envisioned outcome of the NIDCR program is a single human salivary proteome compiled with input from all three groups. Each group will contribute bioinformatics specialists, who will develop computational methods for the maintenance of a stable, comprehensive, fully classified and accurately annotated protein sequence knowledge base. This database, with extensive cross-references and querying interfaces of structural and functional proteomics projects, will be important for understanding the genetic and biological mechanisms causing human disease. This first comprehensive list of all salivary secretory components will help create the so-called "periodic table" of the parotid, submandibular and sublingual secretory components, which will help in the elucidation of disease pathogenesis and evaluation of the influence of medications on the structure, composition and secretion of all salivary constituents.

UCLA COLLABORATIVE ORAL FLUID DIAGNOSTIC RESEARCH CENTER

The UCLA School of Dentistry is engaged in both technology development and salivary proteome initiatives for salivary diagnostics. During the past three years, we have established the UCLA Collaborative Oral Fluid Diagnostic Research Center to develop the platform for using nanotechnology/microtechnology to detect salivary protein and genomic biomarkers for point-of-care applications for high-impact human diseases.

Technology development. For salivary diagnostic technology development, the group partnered with engineers at the UCLA School of Engineering, who are pioneers in the development of MEMS and nanoelectromechanical systems (NEMS) biosensors that exhibit high levels of sensitivity and specificity for analyte detection, down to the single molecule level.[11,12] The research consortium established a firm commitment toward the development of MEMS/NEMS biosensors for the real-time, ultrasensitive and ultraspecific detection of salivary diagnostic analytes. The group envisions that in about two years, "lab-on-a-chip" prototypes will be available for research, as well as for patient applications.[13]

Oral Fluid NanoSensor Test. The envisioned product is called the Oral Fluid NanoSensor Test (OFNASET). The OFNASET is a handheld, automated, easy-to-use integrated system that will enable simultaneous and rapid detection of multiple salivary protein and nucleic

acid targets (Figure 4).[14] This salivary biomarker detector can be used in the office of a dentist or another health care provider for point-of-care disease screening and detection.

To fully utilize the diagnostic potential of saliva, one needs to comprehensively decipher and catalog its diagnostic components. Comparison of such a catalog in healthy patients with that in a diseased population will reveal diagnostic signatures that can discriminate between healthy people and people with disease. The salivary proteome presents one such resource. The UCLA group has to date identified 309 proteins in human saliva.[15,16] In addition, the group has begun to make translational discoveries into the salivary proteome for patients with oral cancer (S. Hu, T. Yu, Y. Xie, et al., unpublished data, 2006) and for those with Sjögren's syndrome (S. Hu, S. Leong, Y. Xie, et al. unpublished data, 2006).

The UCLA laboratory recently discovered that discriminatory and diagnostic human mRNAs are present in the saliva of healthy people and people with disease. The salivary transcriptome offers an additional valuable resource for disease diagnostics. The first report of the salivary transcriptome demonstrated that the normal salivary transcriptome consists of about 3,000 mRNAs.[17] Of particular importance is that of the 3,000 mRNAs, 180 are common between healthy subjects, constituting the normal salivary transcriptome core (NSTC).

To demonstrate the diagnostic and translational potential of the salivary transcriptome, the UCLA group profiled and analyzed saliva from patients with oral cancer. Four genes from the NSTC (IL-8, ornithine decarboxylase, spermidine acetyltransferase and IL-1β) were able to discriminate and predict whether a saliva sample was from a patient with cancer or from a healthy subject, with a sensitivity and specificity of 91 percent each (receiver operator characteristic [ROC] = 0.95) (Figure 5). The group has validated these salivary transcriptome biomarkers for oral cancer detection in approximately 300 subjects. The behavior of these salivary transcriptome biomarkers is consistent—that is, their levels are significantly higher in the saliva of patients with oral cancer than in the saliva of matched control subjects.

Although the UCLA group used oral cancer as the first proof-of-principle disease for salivary transcriptome diagnostics, data soon will be available for systemic diseases. These data, although

Figure 4. Oral Fluid NanoSensor Test, a handheld, automated, easy-to-use integrated system that will enable simultaneous and rapid detection of multiple salivary protein and nucleic acid targets.

early and exploratory, provide sufficient rationale for pursuing the research and demonstrate the urgent need to explore fully salivary transcriptome diagnostics for major human disease translational applications.

Serum versus salivary transcriptome. Because saliva is not a mainstream diagnostic fluid, researchers at UCLA compared the clinical accuracy of saliva with that of blood RNA biomarkers for oral cancer detection. They found four informative RNA biomarkers that have a sensitivity and specificity of 91 and 71 percent, respectively (ROC = 0.88). As explained above, the four salivary oral cancer biomarkers had a collective ROC value of 0.95[18]; this demonstrates clearly that for oral cancer detection, salivary transcriptome diagnostics have a slight edge over serum.[19] This example also points out an important fact with regard to the discovery and validation of biomarkers for disease diagnostics: the power of salivary biomarkers to discriminate and detect disease likely will be based on a panel rather than on a single biomarker.

Advantages of transcriptome markers. There are advantages to using transcriptome markers to detect disease. The marker discovery

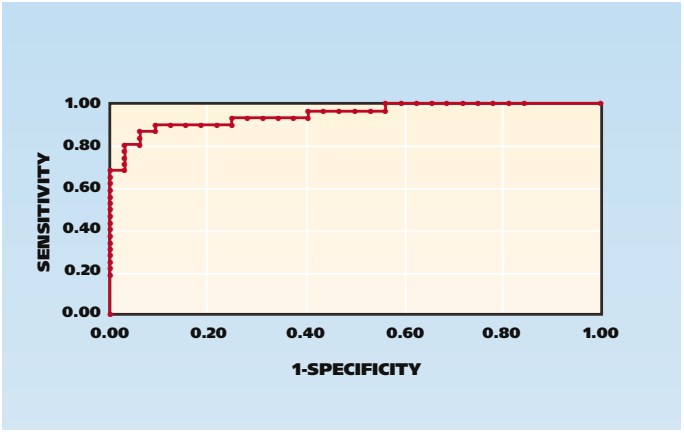

Figure 5. Receiver operator characteristic (ROC) curve analysis for the predictive power of combined salivary mRNA biomarkers. The final logistic regression model included four salivary mRNA biomarkers: interleukin-8, ornithine decarboxylase, spermidine acetyltransferase and IL-1β. Using a cutoff probability of 50 percent, the author achieved a sensitivity of 91 percent and a specificity of 91 percent by ROC. The calculated area under the ROC curve was 0.95.

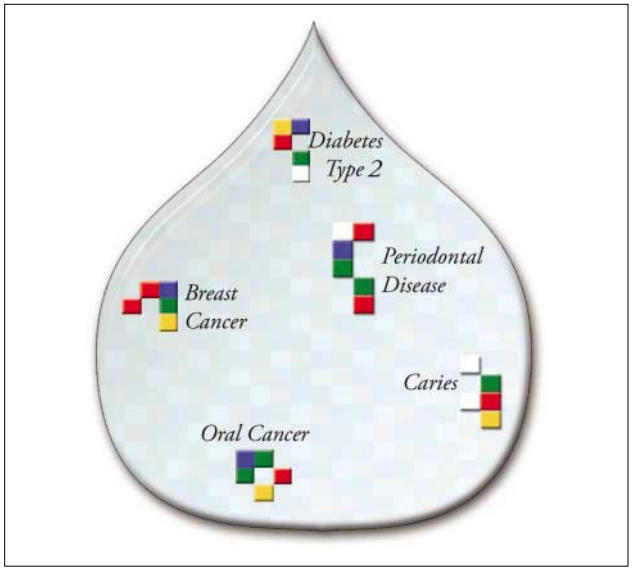

Figure 6. Envisioned signatures for oral cancer, breast cancer, type 2 diabetes, periodontal disease and caries. Adapted with permission of Wong from the UCLA Human Salivary Proteome Project.[15]

process is high-throughput involving the use of genomewide microarray platforms. While the human salivary proteome is still about two years away from being compiled, the salivary transcriptome of healthy subjects has been completed.[17] As a biomarker, RNA is as robust and as informative as any other analyte. Thus, salivary transcriptome offers the combined advantages of high-throughput marker discovery via a noninvasive biofluidic method and high patient compliance. Highly diagnostic salivary RNA signatures have been identified for oral cancer and for two other major human systemic diseases.

SCIENCE AND THE FUTURE

The functional value of saliva has long been thought to outweigh its diagnostic possibilities. Recent evidence regarding saliva as a diagnostic tool for diseases such as HIV, various forms of cancer, diabetes, arthritis and heart disease has shown that much more information is contained in saliva than was previously thought. With the abundance of information that may be contained within, saliva might play an even greater role in people's daily lives than it does today. Scientists are transitioning from viewing saliva as a diagnostic outcast in comparison with blood or urine to viewing it as a valuable biofluid.

The advantages of using salivary testing for diagnostic purposes are its containment of highly diagnostic disease biomarkers, its noninvasive nature and the ability to obtain quick and reliable results.[20] The research community increasingly is viewing saliva as an emergent diagnostic fluid, the result of which is the potential to extract more data than is possible currently with other diagnostic methods. However, there may be cultural and behavioral perceptions against using saliva; these barriers will need to be overcome with time.

CONCLUSION

The NIDCR initiatives and current research efforts are closing the gap rapidly between the use of saliva and other biofluids (blood, urine, cerebrospinal fluid, tears, nipple aspirate) for disease diagnostics. Scientific data to establish a benchmark for the diagnostic value of saliva in comparison with that of other biomedia will be necessary to assess the disease discriminatory value of saliva. It may well turn out that, similar to the UCLA finding that saliva is more accurate than blood in detecting oral cancer,[18,19] saliva will outperform other biomedia in the diagnosis of other diseases as well (Figure 6). ■

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1. Mandel ID. Salivary diagnosis: more than a lick and a promise. JADA 1993;124(1):85-7.
2. Forde M, Koka S, Eckert S, Carr A, Wong DT. Systemic assessments utilizing saliva, part I: general considerations and current assessments. J Prosthodontics 2006;19(1):43-52.
3. Haeckel R, Hanecke P. Application of saliva for drug monitoring: an in vivo model for transmembrane transport. Eur J Clin Chem Clin Biochem 1996;34:171-91.

4. Reynolds SJ, Muwonga J. OraQuick ADVANCE Rapid HIV-1/2 antibody test. Expert Rev Mol Diagn 2004;4:587-91.
5. Kornman KS, Crane A, Wang HY, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997;24(1):72-7.
6. Socransky SS, Haffajee AD, Smith C, Duff GW. Microbiological parameters associated with IL-1 gene polymorphisms in periodontitis patients. J Clin Periodontol 2000;27:810-8.
7. Baughan LW, Robertello FJ, Sarrett DC, Denny PA, Denny PC. Salivary mucin as related to oral *Streptococcus mutans* in elderly people. Oral Microbiol Immunol 2000;15(1):10-4.
8. Miller SM. Saliva testing: a nontraditional diagnostic tool. Clin Lab Sci 1994;7(1):39-44.
9. Hodinka R, Nagashunmugam T, Malamud D. Detection of human immunodeficiency virus antibodies in oral fluids. Clin Diagn Lab Immunol 1998;5:419-26.
10. Mortimer PP, Parry JV. Detection of antibody to HIV in saliva: a brief review. Clin Diagn Virol 1994;2:231-43.
11. Huang TJ, Shalci A, Ho D, Zhang YH, McCabe ER, Ho CM. Rapid bacterial diagnosis: MEMS-based DNA detection. Paper presented at: Pediatric Academic Societies' Annual Meeting; May 4-7, 2002; Baltimore.
12. Soong RK, Bachand GD, Neves HP, Olkhovets AG, Craighead HG, Montemagno CD. Powering an inorganic nanodevice with a biomolecular motor. Science 2000;290:1555-8.
13. St. John MA, Li Y, Zhou X, et al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2004;130:929-35.
14. UCLA Collaborative Oral Fluids Diagnostic Research Center. Available at: "www.saliva.bme.ucla.edu/". Accessed Jan. 25, 2006.
15. UCLA Human Salivary Proteome Project. Available at: "www.hspp.ucla.edu". Accessed Jan. 25, 2006.
16. Hu S, Xie Y, Ramachandran P, et al. Large-scale identification of proteins in human salivary proteome by liquid chromatography/mass spectrometry and two-dimensional gel electrophoresis-mass spectrometry. Proteomics 2005;5:1714-28.
17. Li Y, Zhou X, St. John MA, Wong DT. RNA profiling of cell-free saliva using microarray technology. J Dent Res 2004;83(3):199-203.
18. Li Y, St. John MA, Zhou X, et al. Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res 2004;10:8442-50.
19. Li Y, Elashoff D, Oh M, et al. Serum circulating human mRNA profiling and its utility for oral cancer detection. J Clin Oncol (in press).
20. Dreifus C. A bloodless revolution: Spit will tell what ails you. New York Times April 19, 2005:F2.