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Salivary Diagnostics

In today's growing knowledge and use of genomics in medicine, a noninvasive method for disease diagnosis is a highly attractive goal. While blood diagnostic methods are common tests that evaluate a patient's health, tissue biopsies are generally performed to determine the presence and the extent of a disease. Biopsies are invasive because they require excision by a surgeon or surgical radiologist to remove some or all of the suspicious cells in question. Other biological fluids which are often used for the diagnosis of disease are urine and cerebrospinal fluid (Miller 2010). Unlike these methods which are invasive, saliva as a diagnostic medium seems promising. Saliva is a biofluid that is readily available and provides a completely noninvasive method to obtain a biological sample from a patient. Clearly, among the other diagnostic options, saliva is the least objectionable for patients. With the vast amount of research invested in the discovery of salivary biomarkers and the continuous development of salivary diagnostic technologies, the ability to easily monitor health status and disease onset through the use of an accurate and easy-to-use saliva platform is where this exciting field of research is heading.

One explanation for why research involving saliva as a diagnostic method did not get started until the early 1980s could be because saliva carries different meanings around the world (Strekfus 2002). The act of spitting in the United States, for example, is taken as an insult,

whereas in other cultures it is given as a blessing (Wong 2006). Regardless of the positive and negative social, psychological, and behavioral connotations that saliva possesses, saliva is like blood in that it contains many protein and RNA molecules. Both of these elements are encoded by genes. With the emergence of genomics, proteomics and nanotechnologies, salivary diagnostics has been transformed into a very dynamic field of research in the past twenty years. It has even evolved into such a sophisticated science that it is now considered a subset of the broad field of molecular diagnostics (Malamud 2011). Molecular diagnostics is a very important field of research because it plays major roles in drug development, personalized medicine (pharmacogenomics) and discovery of biomarkers.

The ability to monitor a patient's health status and a disease's onset, progression, and treatment outcome through noninvasive methods has been one overarching goal of molecular diagnostics. To make this goal a reality, there are three prerequisites that first need to be achieved (Lee 2009). Firstly, there needs to be specific biomarkers that are proven to be associated with health or certain disease states. Secondly, a viable noninvasive approach to detect and monitor these biomarkers needs to be implemented. Finally, advanced technologies need to be created that will accurately discriminate among the biomarkers. Because saliva has been recognized to be a noninvasive approach for sampling health and disease states through biomarkers, the National Institute of Dental and Craniofacial Research (NIDCR) has created a national initiative with a road-map to satisfy the remaining prerequisites and achieve the expansive goal which hopes to revolutionize modern diagnostic practices (Wong 2006).

Before a precise account of how salivary diagnostics is achieved, a brief review of the basic biology of saliva is needed. Saliva is the term generally designated for the fluid in the oral cavity. Yet, from the definition, saliva denotes only fluid originating from the salivary glands

(Amerongen 2007). The three main salivary glands that produce the fluid are the parotid, the submandibular and the sublingual (See Figure 1). Each type of gland secretes fluid with a defined composition and the amount in the whole sample varies due to whether the sympathetic or the parasympathetic linkages to the nervous system are activated. Also, it is important to keep in mind that small amounts of serum, blood plasma, from oral tissues is among the oral fluid when a person is healthy and it can increase substantially under some pathological conditions. The reason why saliva can be used as a medium for diagnosis is because it exchanges a wide variety of salivary proteins, inorganic and organic compounds from the serum (Liu 2012). Not only does the composition of the saliva change with respect to a single patient depending on nervous system activation, the array of salivary compounds have an equally wide range of concentrations. For example, α- amylase, which is involved in starch digestion, has concentration levels around the mg/ml level, whereas cytokines, a small cell-signaling protein molecule, is at the pg/ml range. With all this in mind, it is easy to see how saliva, although easy to obtain, poses a daunting task for researchers trying to develop standardized diagnostic techniques.

Another challenge for salivary diagnostics came when researchers tried looking for patterns among the variations seen among thousands of potential biomarkers. According to the National Institutes of Health, a biomarker is a "characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmaceutical responses to a therapeutic intervention" (Ilyin 2004). Ideally, biomarkers should be directly involved in the causal pathway of the disease because the closer the molecule is to the foundation of the disease, the more accurate predictions the biomarker will give (Bonassi 2001).

Beginning in 2003, with funding from the NIDCR, vast amounts of research into the

identification of the proteins in human saliva has been accumulated through different proteomic approaches (Wong 2008). This analysis has helped the search for salivary biomarkers because creating a comprehensive catalog of saliva proteins using these proteomic approaches is the first step to identifying potential protein biomarkers for a wide range of diseases. One proteomic technique is high-resolution liquid separation which can be accomplished through liquid chromatography or mass spectrometry (Liu 2012). Other proteome research has been achieved through two-dimensional gel electrophoresis which separates the protein components first, then analysis by mass spectrometry follows. Using two-dimensional gel electrophoresis, as opposed to liquid chromatography, identified 1050 more proteins in saliva. Additional new methodologies have also proved useful in the identification of small salivary proteins. Among the new techniques to be used are surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) and high performance liquid chromatography (HPLC) (Hardt 2005). Today, the Online Salivary Proteome Knowledge Base totals over 1,100 catalog entries (http://hspp.dent.ucla.edu/cgi-bin/spkbcgi-bin/main.cgi). From this ongoing collection of information, it has proved that the molecular components of the saliva are not the same as the proteins found in human plasma. Dr. David Wong, head of the Salivary Proteome Project, hopes that the comprehensive list of all salivary components will create a sort of "periodic table" of the parotid, submadibular and subligual secretory components, which will then aid in the acquisition of disease pathogenesis and evaluation through the use of saliva as a diagnostic medium (Wong 2006).

Even though researchers believe there is much more to be learned from the salivary proteome, many research groups are already using the published salivary proteome data and applying it to diagnostic research. First, salivary diagnostics has been shown to detect antibodies that are produced due to an immunological response to infectious diseases. The detection of

dental caries and periodontal diseases have been accomplished by detecting either the bacteria that play significant roles in the disease or monitoring the increase in immunoglobulin levels which heightens as pathogens are present in the oral cavity. Studies have also demonstrated successful and reproducible diagnosis of HIV using specific antibodies as biomarkers.Saliva based tests can determine the presence of HIV types 1 and 2 with 99% accuracy in a matter of twenty minutes (Lima 2010). This is an amazing accomplishment, especially in areas where the traditional test would take two weeks to get results and one third of the patients would never return to receive the information. Other infectious diseases that have been identified by salivary diagnostics are *Helicobacter pylori*, associated with infection of the mucosal surfaces causing peptic ulcers and cancrum, Hepatitis and Dengue, a viral disease transmitted by a type of mosquito that can lead to hemorrhagic fever or dengue shock syndrome (Kaufman 2002).

Through the analysis of human DNA with biomarkers, salivary diagnostics has also been able to detect some types of neoplasias (Lima 2010). Neoplasia is the abnormal proliferation of cells. A previously reported study evaluated that the use of the CD44 protein found in saliva is a potential biomarker for head and neck cancer and can be effective for the detection of this particular type of cancer at any stage of its progression. Recent studies have also detected a number of different biomarkers that are able to detect pancreatic, breast and ovarian cancer with high specificity and sensitivity (Lima 2010). Additional studies are required to determine their diagnostic value in comparison with other, more established, diagnostic tests, but this has proved to be a promising outlook for the future of salivary diagnostics.

Biomarkers for autoimmune and cardiovascular diseases are also being sought after. One autoimmune disease that has been widely studied for salivary biomarkers is Sjögren's Syndrome. Sjögren's Syndrome is a chronic autoimmune disease that is characterized by

dysfunction of salivary glands, keratoconjunctivitis and other serological abnormalities (Lima 2010). Researchers have found specific concentrations of cytokines associated with patients with Sjögren's Syndrome. In the future, these may prove to be useful in the identification and progression of the disease. Lastly, work has been done that concerns cardiovascular diseases, which are a leading cause of death worldwide (Adam 1999). Biomarkers found in saliva, for example amylase, have been used for the monitoring of post-operative patients who had cardiovascular surgery (Lima 2010). Also, Adam showed that low levels of salivary amylase before the patient has cardiovascular surgery is associated with an increase in mortality. These studies show that salivary diagnostics have to ability to evaluate a patient's general health and monitor the progress of a disease, but many of these studies are still at the initial stages.

Many of these systemic diseases described above, including cancers and cardiovascular, metabolic and neurological diseases are very challenging to diagnose without laboratory tests (Wong 2006). But, the future hopes of salivary diagnostic research is to be able to test these diseases and obtain results with the same, or better, sensitivity and specificity. Although the proteomic components of saliva have been studied first, genomic targets have recently been shown to be highly informative and discriminatory biomarkers (Pfaffe 2011). Finding genomic targets will be another future aim for analyzing salivary biomarkers. Yet, the future of salivary diagnostics hinges on the ability of screening technologies to be able to analyze a wide spectrum of salivary biomarkers with very high accuracy.

One often cited criticism of using saliva as a diagnostic medium is that biomarkers in saliva are available in too low of amounts to be easily detectable. However, this is "no longer a limitation," according to Wong, who cites that the technology development for salivary diagnostics has increasingly sensitive detection techniques (Wong 2006). Engineers at the UCLA

School of Engineering are pioneering the development of nanoelectromechanical systems (NEMS) biosensors that are able to produce high levels of sensitivity and specificity for biomarkers. They have been able to differentiate among an analyte sample down to the single molecule level. The group is working on the completion in upcoming years of a "lab-on-a-chip" prototype that will be able to quickly analyze saliva samples for research and for clinical applications. The envisioned product, called the Oral Fluid NanoSensor Test (OFNASET), seen in Figure 2, is a handheld, automated, and easy-to-use system that will be able to quickly and simultaneously detect multiple salivary protein and nucleic acid targets (Wong 2006). This detector system is the idealized goal because it can easily be used in the office of a dentist or another health care provider and deliver quick disease screening results.

In the past, the functional value of saliva testing was thought to outweigh the actual diagnostic possibilities. But, recent evidence regarding saliva as a diagnostic medium for diseases such as HIV, various forms of cancer, and other autoimmune and cardiovascular disease has shown that salivary diagnostics is a viable research endeavor (Wong 2006). However, some major obstacles must not be ignored. Many of the biomarkers for these diseases were individually able to detect diseases, but for saliva to live up to it's diagnostic expectations, upon one saliva sample the technology should be able to detect multiple biomarkers for multiple diseases (Liu 2012). Although proteomic and nanoelectromechanical system technologies are in their early stages, it is very likely that new breakthroughs will be made in the future that will increase the sensitivity and selectivity of salivary diagnostics among multiple disease biochemical markers. While there are skeptics who believe that saliva is an inferior diagnostic medium compared to blood or urine, many researchers are very optimistic of the future of salivary diagnostics and expect that one day saliva will be a complementary tool in health

monitoring and early detection of disease.

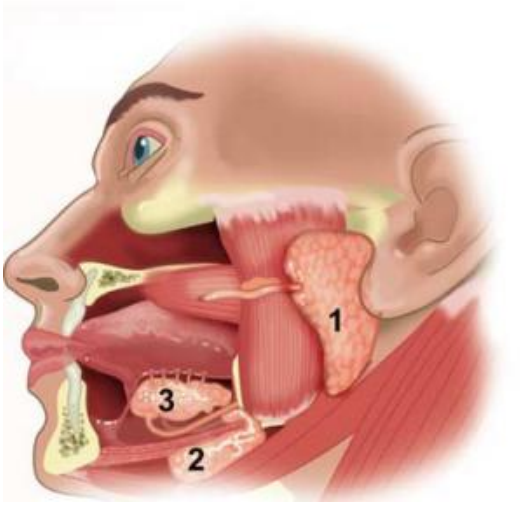

Figure 1: The anatomical location of the three large salivary glands: parotid glands (1), submandibular glands (2), and sublingual glands (3) (Amerongen 2007).

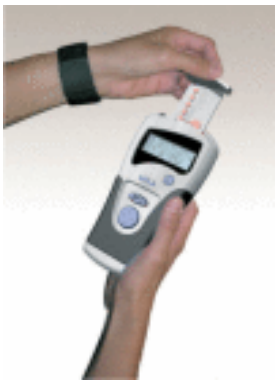

Figure 2: Oral Fluid NanoSensor Test (OFNASET) is a handheld, automated, and easy-to-use system that will be able to quickly and simultaneously detect multiple salivary protein and nucleic acid targets (Wong 2006).

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