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Emerging salivary biomarkers by mass spectrometry

Qihui Wang [a,b], Qiaoling Yu [a,c], Qingyu Lin [a], Yixiang Duan [a,*]

[a] Research Center of Analytical Instrumentation, College of Life Science, Sichuan University, Chengdu 610064, PR China
[b] College of Chemistry and Life Sciences, Chengdu Normal University, Chengdu 610041, PR China
[c] Department of Environmental and Food Engineering, Liuzhou Vocational and Technical College, Liuzhou 545006, PR China

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ABSTRACT

Human saliva, a multi-constituent oral fluid, has a high potential for early diagnosis of disease. Proteomic analysis of saliva holds promise as a non-invasive method that is advantageous over serum. This non-invasive diagnostic method represents developing trends in analytical and clinical chemistry. Significant technological advances in the field of proteomics during the last two decades have greatly facilitated the research toward this direction. However, these technologies still require integration and standardization of validation against accepted clinical and pathologic parameters. In this review, a summary of mass spectrometry-based technologies of saliva biomarker discovery, potential clinical applications, and challenges of saliva proteomics have been discussed, as well as latest technologies of validation and quantification of saliva biomarkers. It is likely that the use of saliva for early diagnostics of diseases will continue to expand thus providing a new approach of instrumental investigation for physiologic and physiological states. These novel biomarkers have obvious clinical utility that will help to diagnose many diseases at early stage.

Contents

Abbreviations: WS, whole saliva; SS, Sjögrens syndrome; 2-DE/MS, two-dimensional gel electrophoresis-mass spectrometry; LC–MS/MS, liquid chromatography tandem mass spectrometry; MALDI-ToF/MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SELDI-ToF/MS, surface-enhanced laser desorption/ionization time-of-flight mass spectrometry; LIT–Orbitrap, linear ion trap–Orbitrap; MudPIT, Multidimensional protein identification technology; SRM/MRM, Selected/multiple reaction monitoring assays; ELISA, Enzyme-linked immunosorbent assay; 2D-DIGE, 2D difference gel electrophoresis; ICAT, Isotope-coded affinity tagging; iTRAQ, Isobaric tags for relative and absolute quantification; OSCC, Oral squamous cell carcinoma.

* Corresponding author at: Research Center of Analytical Instrumentation, Sichuan University, 29 Wangjiang Road, Chengdu 610064, PR China. Tel./fax: +86 28 85418180.
E-mail address: yduan@scu.edu.cn (Y. Duan).

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1. Introduction

Saliva, non-invasive and stress-free alternative to blood, is widely accepted as a potential medium for clinical diagnostics. It is a readily accessible secretion that plays an important role in esophageal physiology, digestive process, gastric cell protection, and oral lubrication [1]. In addition, saliva also protects the oral cavity from foreign invaders, such as bacteria and viruses, by digestion and inhibition of their growth [2]. Therefore, saliva has attracted more and more attention.

Saliva is secreted primarily by the three major glands namely parotid gland, submandibular gland and sublingual gland [3]. Generally, salivary glands generate 1–1.5 L of saliva each day [4]. It contains approximately 99% water with minerals, nucleic acids, electrolytes, mucus and proteins such as amylase, cytokines, immunoglobulins, mucins and other glycoproteins [5]. It is one of the most complex, versatile, and important body fluids, supplying a wide range of physiological needs. Therefore saliva is also called the "mirror of the body" or "a window on health status".

The idea of using saliva in medical diagnosis was made in the second half of the 20th century [6]. At present, saliva represents an increasingly useful auxiliary means of diagnosis due to the use of novel approaches including proteomics, genomics, metabolomics and bioinformatics. Additionally, it has the advantages of being simple, non-invasive, easy to store, and inexpensive compared to blood.

Saliva may exchange with substances that compose blood. The mechanisms of transport of proteins and ions from blood into saliva were introduced: active transport, passive intracellular diffusion and extracellular ultrafiltration [7]. Some molecules as ligand receptor binding enter into saliva through active transport; Hydrophilic and small molecules enter saliva from blood capillaries through passive intracellular diffusion; Hydrophobic compounds enter into saliva through the gap junctions on the blood membrane (extracellular ultrafiltration). Therefore, saliva is functionally equivalent to blood in reflecting the physiological state of the body. Saliva consists of approximately 2000 proteins, and most importantly, about 597 of those proteins are also observed in the blood [8]. Therefore, salivary proteomics has demonstrated a great potential for clinical diagnosis.

In this review, we describe the mass spectrometry-based methods used for identification salivary biomarkers, saliva-test applications and their potential use in clinical diagnosis of various diseases.

2. Specimen collection and process

Compared with blood, saliva collection is relatively easy and cost-effective [9]. It may not evoke an ethical issue in special populations. Q3 Non-invasiveness is one of the great advantages of saliva as a diagnostic medium, especially when repeated samples must be taken for particular examinations. It is convenient for the patient because samples can be collected at home. It is widely believed that different types of the collected saliva may give rise to different biomarkers.

2.1. Types of collected saliva

Saliva can be collected as whole saliva (WS) or the individual salivary gland saliva. Whole saliva includes secretions from both three major glands and minor glands as well as gingival crevicular fluid, cellular debris, bacteria, and many microbes [10]. It is most frequently Q4 studied for the evaluation of systemic diseases because of ease in collection, rapidness in obtaining and no need of specialized equipment. On the other hand, the collection of individual salivary gland saliva is much more difficult since a variety of sophisticated devices must be used. It provides controlled fluid and little influence from the other part of the oral cavity. Therefore, it primarily suits for the detection of gland-specific pathology.

Saliva can also be collected as unstimulated or stimulated saliva. On average, the flow rate of unstimulated saliva is 0.3 mL/min, stimulated flow rate is, at maximum, 7 mL/min [11]. Stimulated saliva is generally obtained by chewing on paraffin or gum, or by using citric acid or sour Q5 candy drops on the subject's tongue. In these stimulated method, citric acid elicits the largest volumes of saliva [12,13]. Unstimulated saliva represents an equilibrated condition, having less influence from salivary glands. Unstimulated saliva collection can be obtained by draining, spitting, and suctioning [14].

To date, a majority of diagnostic studies chose to use unstimulated WS [15–17]. However, stimulated WS is more suitable for diagnosis of some special diseases such as Sjögrens syndrome (SS). Because SS is a chronic disease affecting lacrimal, salivary and other exocrine glands, Q6 patients have difficulty producing enough saliva.

2.2. Saliva collection protocol

A common protocol used for collecting saliva sample is given below. In order to collect saliva, all the subjects are asked to abstain from eating, drinking, smoking, or from using oral hygiene products for at least 1 h prior to collection. Then their mouths were rinsed thoroughly with water. 1) For unstimulated whole saliva, it is dripped from the Q7 open mouth into a collecting cup. 2) For stimulated whole saliva, place a standard quantity of paraffin in the mouth and chew at a regular pace, then expectorate saliva periodically into a disposable plastic cup for a period of 5 min [18]. 3) For parotid saliva, it can be collected with a Lashley cup or a modified Carlson–Crittenden device. 4) For submandibular and sublingual glands, use a Block and Brotman collector for submandibular and sublingual gland secretions [19].

2.3. Specimen process

After collecting, the samples are centrifuged at 14 000 rpm for 20 min at 4 °C to remove insoluble materials, cell debris and food remnants. The supernatant is divided into 1 mL portions and frozen at −80 °C until laboratory analyses. Frozen saliva samples are then stored Q8 at −70 °C [15], or −20 °C [20]. Studies have indicated that storage at −80 °C are better than at −20 °C, especially for prolonged times [21], because saliva is one of the most complex body fluids, and storage at low temperature promotes peptide and protein stability. Another potential interference is high-abundance proteins, such as amylase, which may interfere in the detection of other low abundance proteins appearing in the disease state. Lower-abundance proteins such as cytokines, present at the pictograms level, may offer markers for clinical diagnostics. The immunoaffinity column can be used as a common method in preprocessing saliva in order to deplete high-abundance proteins [22]. Omer Deutsch et al. made an alpha amylase removing device Q9 through affinity adsorption to potato starch in a 1 mL plastic syringe Q10 with a 0.45 mm filter at the tip, and the removal efficiency is at least six-Q11 fold [23].

3. Mass spectrometry based proteomics techniques for identification biomarkers

Many diseases, especially for cancer, have caused huge amounts of deaths every year. Unfortunately, most of the cancers are hard to discover in the early stages [24]. Therefore, effective saliva biomarkers are urgently needed to be identified in the use for early diagnosis of diseases. With the advanced instruments and developed refined analytical techniques, proteomic technologies are widely used as useful and powerful approaches and provide tremendous opportunities for biomarker-related clinical applications. Due to its particular sensitivity and highly accurate mass measurement, mass spectrometry has become one of the core technologies for proteomics. A variety of MS techniques, such as two-dimensional gel electrophoresis–mass spectrometry (2-DE/MS), liquid chromatography tandem mass spectrometry (LC–MS/MS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), and surface-enhanced laser desorption/

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