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**REVIEW ARTICLE**

# ROLE OF SALIVARY PROTEOMICS IN ORAL DISEASES: A REVIEW

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**ABSTRACT**

Oral diseases are an imposing public health issue with a gradient trend of increase in incidence and morbidity rates worldwide. The diagnosis of disease state is challenging owing to the lack of sensitive and characteristic biomarkers in the serum and tissues. Salivary proteomic analysis represents a field for both diagnosis and treatment of various diseases and could be considered a new approach to the prevention of cancer, oral pathological conditions. The protein and nucleic acid molecules derived from oral tissues can be retrieved from the saliva. Thus salivary biomarker analysis provides a non-invasive methodology for early diagnosis, prognosis and monitoring treatment outcomes. This review aims to throw light on the characteristics of salivary proteins, methodology of detection and their role in oral diseases.



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## INTRODUCTION

Proteins are vital parts of living organisms, as they are the main components of the physiological metabolic pathways of cells. Proteomics is the large-scale study of proteins, particularly their structures and functions. The term "proteomics" was first coined in 1997 to make an analogy with genomics, the study of the genes. The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system (Larance and Lamond, 2015). The more extensive definition of proteomics encompasses protein studies with analyses such as mRNA analysis and genomics. The objective of a proteomic analysis is not just to identify proteins in a cell but also generates a wholesome three-dimensional (3-D) mapping of the constitutional cell and the proteins in their designated location (Graves and Haystead, 2002). In a typical proteomic approach, the segregation of individual proteins and their identification and visualization by employing a dedicated stain is a defining process.

### Salivary Proteomics

Salivary proteomic analysis represents an evolving field of biology, including both diagnosis and treatment of various diseases and could be developed as a new approach to address the prevention of cancer, oral pathological conditions (Scarano *et al.,* 2010). The most important goal is to distinguish between physiological and pathological conditions. Some salivary peptides/proteins are secreted by all the major glands, while secretion of others is gland specific (Tiwari, 2011) (Table1). The major families of salivary proteins are polymorphic and are complicated by individual insertions/deletions, tandem repeats and alternative splicing (Cabras *et al.,* 2006; De Jong *et al.,* 2010). Various post-translational modifications (PTMs), such as glycosylation, phosphorylation, and exo- and endo-proteolytic cleavages, occur before secretion. This dynamism is challenging for proteomic investigations of human saliva (Duan and Walther, 2015).

### Methodologies Employed in Proteomic Detection

Qualitative and quantitative differences of the proteins present in different samples may be assessed and disease biomarkers characterized by either top-down or bottom-up approach. Top-down approaches are based on the analysis of the naturally occurring intact proteome. Top-down 'solution'-based proteomic platforms are characterized by the separation of the naturally occurring proteome by chromatography coupled online to a high-resolution electrospray ionization mass spectroscopic (ESI-MS) detector. By means of high-resolution MS, the mass of the intact protein can be determined with high precision, as well as its fragmentation ions (Toby *et al.,* 2016; Boone *et al.,* 2016). While bottom-up platforms analyze the mixture of proteolytic fragments of a given proteome, connecting each detected fragment to the parent protein (Bruce

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*et al.,* 2010). On this basis, the main proteomics platforms comprise:

**Table 1. Origin of the proteins normally characterized in saliva**

| S. No. | Protein | Source |
|---|---|---|
| 1. | Proline-rich proteins (PRP)<br>acidic<br>basic<br>basic glycosylated protein | Major salivary gland |
| 2. | Salivary S type cystatin | Sub mandibular and sub lingual gland |
| 3. | α-amylases | Major salivary gland |
| 4. | Mucins | Major salivary gland |
| 5. | Histatins | Major salivary gland |
| 6. | Statherin and P-B peptide | Sub mandibular and sub lingual gland |
| 7 | α-defensins and β-thymosins | Gingival Crevicular fluid |
| 8. | Human serum albumin | Mucosal exudates<br>Oral microflora |

- ***Shotgun proteomics*:** This classical bottom-up strategy is typically based on the preliminary proteolytic digestion of the sample under analysis, followed by high-resolution mono- or multi-dimensional chromatography (or capillary electrophoresis) analysis coupled online with electrospray ionization (ESI) and huge number of fragmentation spectra can be analyzed automatically by devoted software such as Mascot and Sequest, which compare data with protein sequences available in databanks (Liu *et al.,* 2004; Cottrell *et al.,* 2011).
- ***2DE-based platforms*:** Protein profiling in these platforms is centered on a 2D electrophoretic separation. Typically, the identity of protein spots is obtained by individual in-gel proteolytic digestion and analysis of the digests using different mass spectroscopy. However, many small peptides or proteins with very acidic or basic can migrate outside the ranges of 2DE analysis. Moreover, the presence of highly abundant proteins can obscure the less abundant ones (Aebersold and Mann, 2003).
- ***MALDI-TOF (or TOF/TOF) mass spectrometry (MS) platforms*:** MALDI-TOF (or MALDI TOF/TOF) MS can be used offline after 2DE separation for the identification of the different spots evidenced as potential biomarkers in the 2D maps. In principle, the sensitivity of these platforms is very high, sample volume is reduced and detections at the low femtomole level can be achieved (Domon and Aebersold, 2006).

## Role of Proteomics In Oral Diseases

### *Salivary proteomics in Dental Caries*

Salivary defense systems including the salivary proteins play a significant role in maintaining the health of the oral cavity and preventing caries as stated by Mazengo *et al* and can be used to monitor the risk for caries (Jalil *et al.,* 1992; Jia-Yo *et al.,* 2010). Significant amount of salivary phosphopeptides (Proline rich proteins PRP1/3, histatin-1 & statherin) were associated with the absence of dental caries, emphasizing the importance of these peptides in the maintenance of tooth integrity. However, high numbers of peptide fragments were observed, suggesting a high proteolytic activity in caries susceptible individuals (Joseph *et al.,* 1985). In a recent study on early childhood caries, it was found that, a higher number of proline-rich protein bands significantly correlated among caries free subjects, substantiating the protective role of this protein, also a higher number of glycoprotein bands were observed in the whole saliva of subjects with early childhood caries (Kala Jessie *et al.,* 2010).

### *Salivary proteomics in Periodontal Diseases*

Variable amounts of serum products, GCF, electrolytes, epithelial and immune cells, microorganisms, bacterial degradation products, lipopolyscaaharides, and other foreign substances present in whole saliva makes it the best periodontal diagnostic tool (Imanguli *et al.,* 2008; Kaufman and Lamster, 2002). Saliva contains biomarkers specific for the unique physiological aspects of periodontitis, and qualitative changes in the composition of these biomarkers could be diagnostic. It contains a wide variety of periodontal proteomic markers from immunoglobulins to bone remodeling proteins. (Table 2)

**Table 2. Role of Proteins in Diagnosis and Treatment of Periodontitis**

| S.No | Protein | Role in Periodontitis |
|---|---|---|
| 1. | *Interleukin (IL) 1β* | ~pro-inflammatory cytokine<br>~induction of adhesion molecules<br>~correlated significantly with periodontal parameters<br>~combined levels of Il-1β and matrix metalloproteinase (MMP)-8 increased the risk of experiencing periodontal disease |
| 2. | Matrix metallo proteinases | ~ extracellular collagen matrix degradation<br>~ correlated significantly with periodontal activity<br>~ increased the risk of periodontal disease |
| 3. | *Immunoglobulin(IgG)* | ~ higher salivary concentrations of IgA, IgG and IgM specific to periodontal pathogens<br>~ levels are greatly reduced after periodontal treatment<br>~ noninvasive technique to identify individuals who have the potential to develop periodontal disease<br>~monitors treatment response |
| 4. | *Acid Phosphatase* | ~ positive correlation between salivary ACP and calculus formation. |
| 5. | *Alkaline phosphatase* | ~ adult periodontitis patients revealed the highest enzyme activities<br>~ increase associated with alveolar bone loss<br>~ useful marker for monitoring periodontal disease |
| 6. | *Esterase* | ~ significant, positive correlation between salivary esterase and calculus formation<br>~ higher in individuals with periodontal disease<br>~ periodontal treatment reduced its levels |
| 7. | *Lysozyme* | ~ low levels of lysozyme in saliva; more susceptibility to plaque accumulation |
| 8. | Lactoferrin | ~ up-regulated in mucosal secretions during gingival inflammation<br>~detected at a high concentration in saliva of patients with periodontal disease |

### *Salivary proteases as biomarkers for Premalignant and Malignant oral lesions*

Clinicians lack tests which easily and reliably distinguish pre-malignant oral lesions from those already transitioned to malignancy. Numerous candidate proteins were discovered in saliva of patients with oral lesions, however, not all proteins have biomarker ability in diagnosing oral cancers. Bioinformatic analysis of exfoliated epithelial cells from subjects' saliva revealed increased myosin and actin abundance in those with malignant lesions with sensitivity and specificity rivaling other noninvasive oral cancer tests as confirmed by western blotting (Liu *et al.,* 2011). Five candidate biomarkers-M2BP, MRP14, CD59, profilin and catalase were successfully validated using immunoassays on an independent set of OSCC patients and matched healthy subjects. They concluded that patient-based saliva proteomics is a promising approach to searching for OSCC biomarkers. The discovery of these new targets may lead to a simple clinical tool for the noninvasive diagnosis of oral cancer (Yang *et al.,* 2006). IL- 6 and IL-8 are involved in the pathogenesis of OSCC, and have been linked with increased tumor growth and metastasis, hence its levels could serve as informative biomarkers for OSCC in saliva (Loo *et al.,* 2010). A new study published by researchers at the UCLA School of Dentistry substantiates the effectiveness of measuring the microRNAs present in saliva to detect OSCCs. MicroRNAs are the molecules produced by cells that simultaneously assess the behavior of multiple genes and control their activity.

Oral lichen planus (OLP) is a chronic inflammatory mucosal disease with a cell-mediated immunological pathogenesis. Saliva from patients with OLP comprised various proteins. A total of 31 protein spots representing 14 proteins with at least two-fold difference in abundance between OLP and controls were identified. Among these, the expression of urinary prokallikrein was increased while soft palate, lung and nasal epithelium carcinoma associated protein (PLUNC) was decreased in all samples of OLP (Rahul Kathariya, 2010). In an another study it was found that the levels of salivary CD44s and CD44v5 (Isoforms of CD44) from OLP patients were significantly higher than those from controls (Maie *et al.,* 2004). Thus these proteomics may serve as new biomarkers which play a role in inflammation and immune response of OLP. IFN-γ and IL-4 levels in whole unstimulated saliva screened by ELISA in OLP patient showed a low-level IFN-γ but high-level IL- 4 expression profile in saliva, with a lower ratio of salivary IFN-γ/IL-4 compared to healthy controls. Imbalance of Th1/Th2 cytokines with Th2- profile in saliva may be involved in OLP. Thus salivary IL-4 level may be a fine biomarker reflecting the severity of OLP (Rahul Kathariya, 2010).

### *Salivary proteomics for Sjögren's syndrome (SS)*

SS is a systemic autoimmune disease in which immune cells attack and destroy the salivary and lachrymal glands. Mass spectrometry analysis showed 16 down-regulated and 25 up-regulated proteins in primary SS patients compared with matched healthy controls (Rigante *et al.,* 2008). These proteins reflected the damage of glandular cells and inflammation of the oral cavity system in patients with primary SS. It was also found that whole saliva contains more informative proteins, peptides, and mRNA, as compared with gland specific saliva that can be used in generating candidate biomarkers for the detection of primary SS (Shen *et al.,* 2007).

### Conclusion

The increasing number of studies devoted to the analysis of human saliva under physiological and pathological conditions will allow specific salivary biomarkers of systemic and local pathologies to be disclosed in the near future. Moreover, information obtained by proteomics studies will provide clues and stimuli for the comprehension of the roles of the different families of salivary proteins in the oral cavity. The future of proteomics of bodily fluids, and hence of saliva, is strongly linked to the improvement of the instrumental performance, and in the next few years the number of components detected in saliva is likely to increase.

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