

## LECTURE-36

### Salivary Proteomics

#### Handout

#### PREAMBLE

Proteins are the effector molecules of cellular pathways and its over-expression or deficiency results in diseases. There are many diseases which are caused due to aberrant protein-protein interactions, which are indispensable for normal functioning of cells. Thus, proteomic profiling is very common approach to find out modulation of proteins due to any disease. For comparative proteomic analysis, samples are taken from the normal and diseased individuals. These samples typically include serum or plasma, tissue and cerebro-spinal fluid. Obtaining these samples need invasive procedures – like a lumbar puncture or sometimes surgery. Although these are useful sample but amount obtained is very less and involves invasive procedure. Therefore, newer samples need to be discovered for protein profiling, which can be obtained through non-invasive techniques. One such sample is saliva, which is abundant, non-invasive and very easily obtained without any harm to the patient. Thus, if a biomarker could be discovered in a bodily fluid like saliva or urine, it will be much easier to obtain samples to identify the occurrence and progression of disease in an individual.

## **OUTLINE**

I. Introduction

II. Collection and processing of saliva samples for diagnostic and research purposes

III. Methodology for protein extraction from saliva

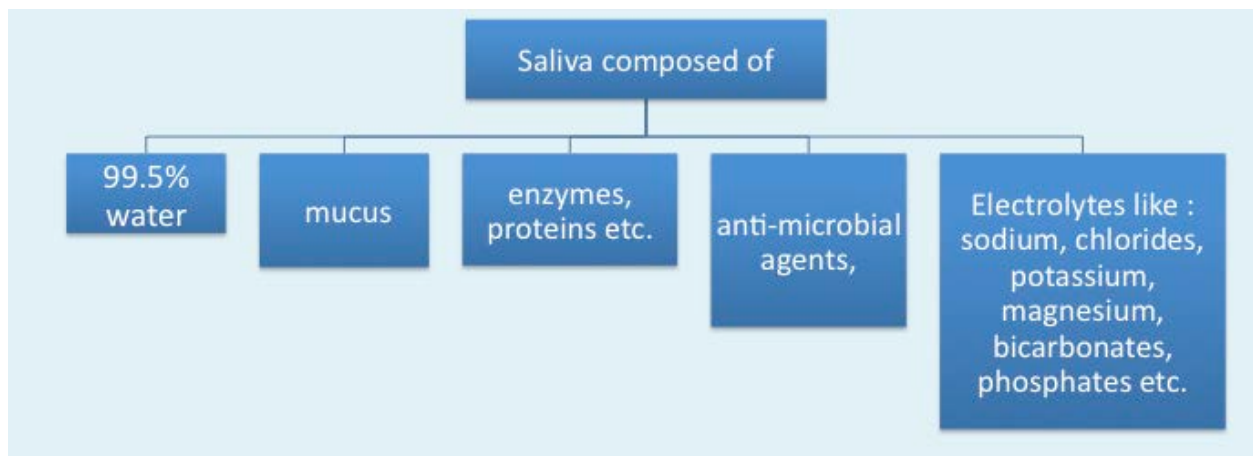
IV. Applications of salivary proteomics

V. Advantages and challenges

VI. Conclusions

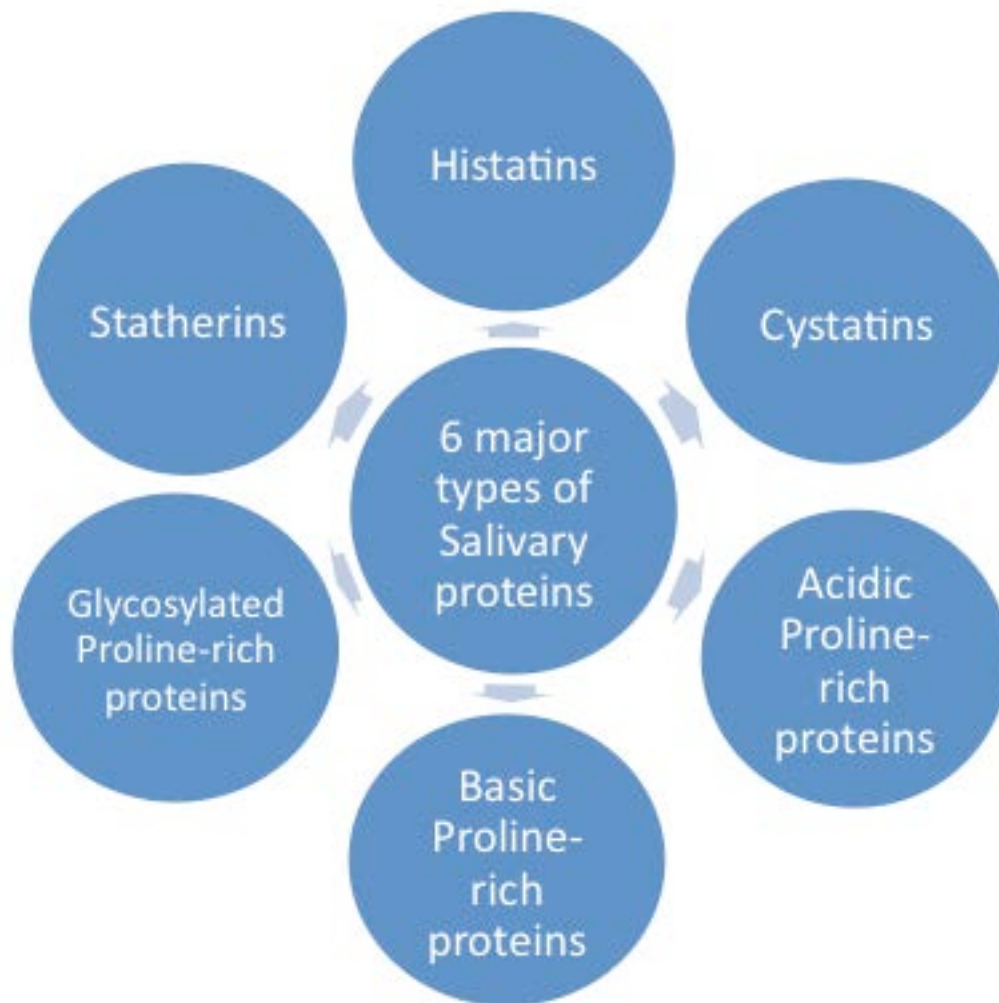
## I. INTRODUCTION

Saliva is a colorless fluid secreted by the salivary glands of the oral cavity. There are three main salivary glands in humans: the parotid glands, the sub-mandibular glands and sub-lingual glands, along with numerous small glands. Saliva is a fluid which helps to start the preliminary digestion of food as it has enzymes such as salivary amylase. The saliva composition is mainly as follows: 99.5% water, electrolytes, mucus, enzymes, anti-bacterial agents. The electrolytes include sodium, potassium, magnesium, bicarbonates, chlorides and phosphates etc. (Fig 1).



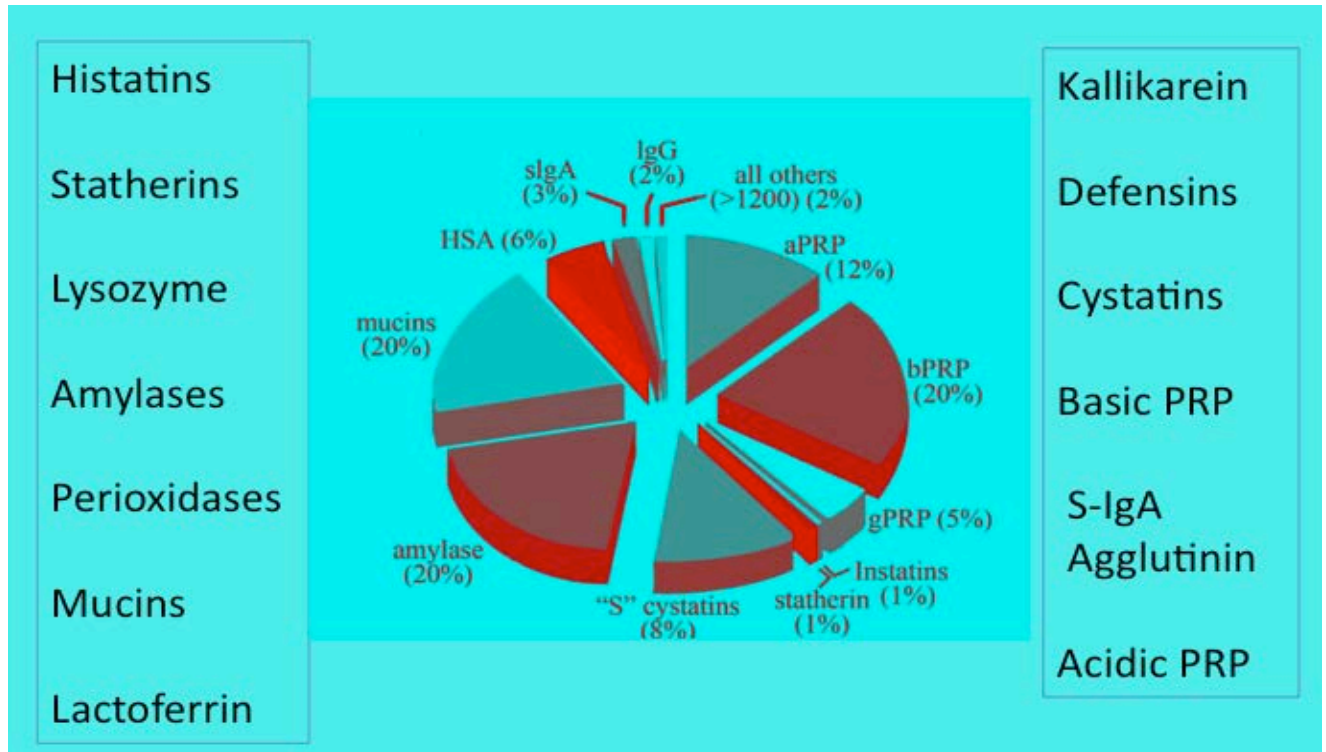
**Fig 1.** Composition of saliva

Many proteins have been detected in saliva and most abundant protein is salivary amylase. Other proteins which have been discovered in saliva include; Histatins, Statherins, Lysozyme, Amylases, Peroxidases, Mucins, Lactoferrin, Kallikarein, Defensins, Cystatins, Basic PRP, Peroxidase, S-IgA and Agglutinin etc. (Fig 2).



**Fig 2.** Six major types of salivary proteins: histatins, proline-rich proteins (acidic, basic and glycosylated), statherins and cystatins.

These salivary proteins have various functions such as digestion of food, antimicrobial activity, lubrication and protection of oral surfaces. Till date around 3000 proteins have been detected in saliva.



**Fig. 3.** Major proteins found in human saliva (E. Scarano et al. 2010)

### **II. COLLECTION OF SALIVA SAMPLES FOR DIAGNOSTIC AND RESEARCH PURPOSES**

Normally in adults, the amount of saliva produced is 1 – 1.5 L. Generally, this is produced at the rate of 1 – 3 mL per minute, when production of salivary fluid is stimulated. Saliva is mainly contributed by three salivary glands; the gingival fold, oral mucosa transudate, mucous layer of the nasal cavity and pharynx.

#### **Standard protocol for collection of saliva for diagnostic purposes**

1. Saliva collection is generally performed in the morning, optimally around 10-11 AM
2. The subject is not allowed to eat, drink or conduct oral hygiene procedures – such as brushing teeth at least 1 hour prior to the sample collection.
3. The subject is given drinking water for a mouth rinse.
4. After five minutes of the mouth rinse, the subject should be asked to spit whole saliva (WS) into a 50 ml sterile Falcon tube.
5. Talking should be ideally avoided before and during sample collection.
6. The subject should spit into the collection tube about once a minute for upto 10 minutes. At times, the subject is given citric acid solution rinse, and then the saliva is collected.
7. Ideally, around 5 ml sample should be collected at one time.

#### **Processing of samples after collection of saliva**

1. One important guideline to be strictly followed is that all collected samples must always be kept on ice prior to processing.

2. While collecting the sample, the tube should be placed on ice.
3. The first step in sample preparation is to add proteinase inhibitors. Thus, 0.2  $\mu$ L proteinase inhibitor cocktail is added per 100  $\mu$ l saliva.
4. Next,  $\text{Na}_3\text{VO}_4$  is added, and mixed gently.
5. Tubes are centrifuged at 2600xg for 15 minutes at 4°C.
6. After the spin, pellet and supernatant are stored at -80°C separately.

### **III. Methodology for protein extraction from saliva**

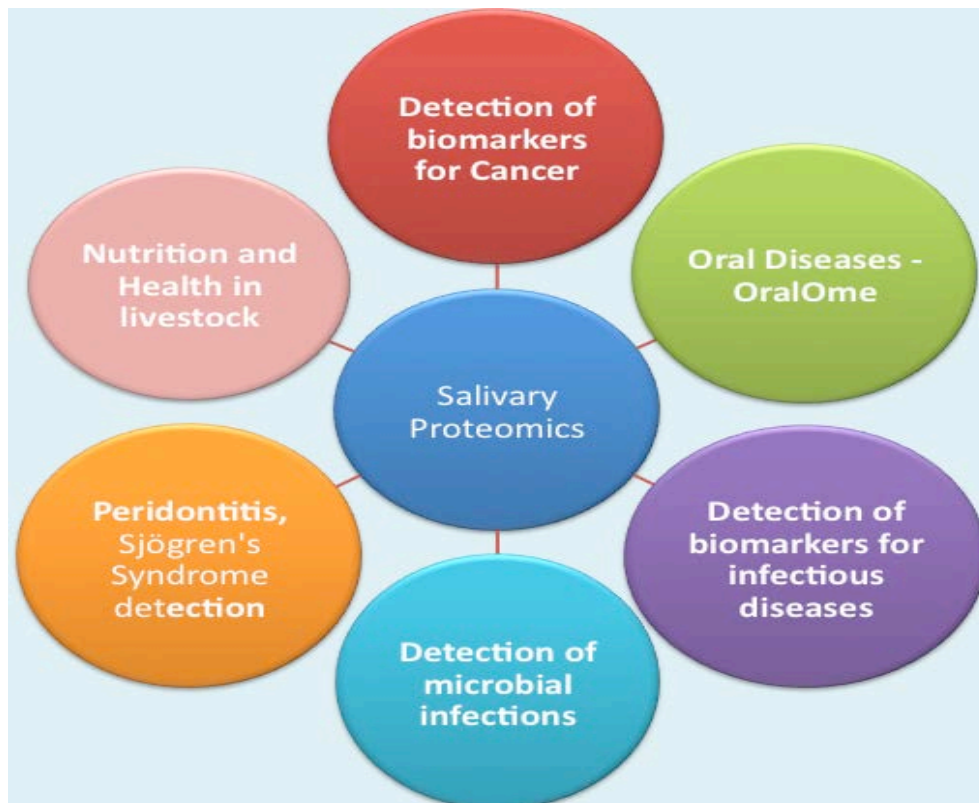
The methodology for extraction of proteins from saliva is similar to any other protein extraction protocol. The samples are centrifuged to remove the presence of any impurity. The remaining sample is then subjected to TCA-Acetone precipitation.

In saliva samples, amylase is the most abundant protein. Thus, many researchers first use the depletion protocol for depletion of amylase from the saliva sample.



### **IV. Applications - current progress in diagnostics using saliva proteomics**

The main reason for studying the proteomic profile of saliva is for the discovery of biomarkers. The protein signatures that help in distinction between healthy and diseased conditions is investigated. The proteins in saliva are precipitated and proteomic analysis using gel-based (2DE, DIGE) or gel-free mass spectrometry techniques is performed. Various examples demonstrate how salivary proteome has been investigated for different applications (Fig 4).



**Fig 4.** Various fields in which salivary proteomics has been utilized.

### **(a) Diagnosis of oral diseases**

The proteins in saliva can be monitored for identifying the oral diseases. In this respect, much progress has been made for identification and prognosis of the oral disease-periodontitis. Periodontitis affects the bones and ligaments supporting the teeth. This has led to the formation of the OralOme, a complete collection of proteome of the saliva. The OralOme helps detect the source of the proteins, and also find out which proteins are secreted only under diseased conditions. It is a comprehensive database to facilitate discovery of biomarkers in saliva for various diseases. Also, much progress is being made to identify dental diseases, like dental carries using proteomic markers in saliva.

### **(b) Saliva for diagnosing cancers**

Saliva has been used to diagnose cancers such as oral cancers and head and neck cancer. For breast cancer, the biomarkers screened from saliva are Vasclular Endothelial Growth Factor (VEGF), human epidermal growth factor receptor 2, epidermal growth factor (EGF) and carcinoembryogenic antigen (CEA). The salivary c-erbB-2 levels correlated strongly with breast malignancy in women (Streckfus et al. 2005). For oral squamous cell carcinoma, the salivary proteases have been used for detection of the disease. Tumor-derived exosomes and microvesicles in head and neck cancer have been suggested as useful source for biomarker discovery (Principe S, 2013). Also, the efforts are being taken to use proteomic profiling to distinguish between the malignant and non-malignant oral lesions, which develop on OSCC. The proteomic profile of patients with oral squamous cell carcinoma (OSCC) revealed higher levels of

salivary transferrin levels that correlated with size and stage of tumor (Jou et al. 2010). The salivary transcriptome has also been analyzed for diagnosing pancreatic cancer. These studies have provided potential targets, which could be potentially screened for diagnosis of cancer.

### **(c) For identifying microbial infections**

Certain types of microbes leave certain trails, which can be identified and infection can be tracked. Some viral or bacterial infections lead to release of specific factors from the host cells. The bacteria-derived enzymes; collagen-degrading enzymes, proteases, aminopeptidases and peptidases have been detected in proteomic profiling. Thus, either the host-derived factors in response to inflammation or the bacteria-derived enzymes can serve as biomarkers in saliva for infections. This also helps in identifying the stage of infection and thus, helps to decide the therapeutics to be administered depending on stage of infection.

### **(d) Salivary proteomics for identification of Sjögren's syndrome**

Sjögren's syndrome (SS) is an auto-immune disease in which salivary glands and lachrymal glands are destroyed by the host's immune system. Mass spectrometric analysis has been performed on salivary samples, and definite up-regulation and down-regulation of certain proteins has been reported. These proteins are potential targets for early disease detection and disease progression.

### **(e) Animal health and nutrition in cattle and livestock animals**

Saliva biomarkers can be used to monitor the disease status of animals. The proteomic analysis can also be used to make necessary diet changes or changes in environmental conditions to maintain optimum health of the animals. This will help in keeping track of animal physiology.

### **(f) Salivary proteomic analysis for infectious diseases**

Salivary proteomics can be used to identify even infectious diseases caused by pathogens. One area in which much progress has been made is Malaria. One of the studies showed that the inflammatory and erythrocyte-derived proteins were highly up-regulated in malaria-positive patients. A proteomic profiling of depleted saliva samples from uncomplicated *Plasmodium falciparum* malaria children was performed and three proteins, PFL0480w, PF08\_0054 and PFI0875w, were identified in malaria patients (Huang et al. 2012). Thus, saliva can be clinically used for malaria biomarker detection.

**V. ADVANTAGES AND CHALLENGES**

<b>ADVANTAGES</b>	<b>CHALLENGES</b>
Completely non-invasive, painless procedure, no injections or surgery is required.	Very low amount of protein is present in saliva.
Extremely cost-effective as sample collection requires no complex procedure or treatment on patient.	Composition of saliva varies with day and night time.
Abundant sample and there is no restriction on sample amount, which aids in protein profiling.	Composition of saliva varies with physiological conditions and biochemical changes.
Samples can be obtained on demand at any time without any delay.	The composition of saliva has been observed to be quite different in children and adults.
Patient health is not compromised in any way.	The content of saliva changes with incoming oral stimuli – like eating, drinking and even brushing teeth or flossing.

<p>Fresh sample can be obtained whenever required, without the help of a skilled technician.</p>	<p>Saliva has many other components other than proteins like food, microorganisms, dead cells, which may require extra washing steps to remove from required sample.</p>
<p>Easy handling of sample.</p>	<p>Quantity of saliva obtained during one sample collection is low.</p>

### **VI. CONCLUSIONS**

- Saliva has great potential to be used for diagnostics. It can be obtained easily, non-invasively and whenever required.
- It has been used accurately to diagnose many diseases such as dental problems, oral cancers and other infectious diseases like malaria.
- Even though saliva proteomics is a field with vast possibilities; however, there are some challenges need to overcome like low amount of proteins in saliva, presence of high-abundant proteins and variations in the composition of saliva.
- With advancement in proteomic technologies such as mass spectrometry, now comprehensive profiling of salivary proteome in various diseases will be possible. Targets identified from salivary proteomics will play a central role for early diagnostics.

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