

## Proteomics- an emerging arena in periodontal diagnosis and treatment

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### Abstract

Proteins are the main components of the physiological metabolic pathways of cells. The onset, progression and severity of periodontal disease are mainly mediated by various protein molecules. The use of proteins as diagnostic biomarkers in periodontal diseases has increased over the last few years. Proteomics is the branch of molecular biology that involves study of protein properties on a large scale that helps in understanding the disease processes at protein level. Saliva and other oral fluids give clinically relevant information as they contain diagnostic protein biomarkers for periodontal diseases. These proteomic biomarkers incorporate various host and bacteria-derived enzymes, virulence factors and inflammatory mediators that are the main culprits for the key pathogenic processes in periodontal disease – inflammation, collagen degradation and bone resorption. In this review paper an attempt has been made to review the significance of proteomics in the diagnosis and assessment of progression of periodontal diseases.

**Keywords:** Proteins, Proteomics, Periodontitis, Biomarkers, Saliva, Gingival crevicular fluid.

### Introduction

The word "proteome" is a fusion of "protein" and "genome", and was termed by Marc Wilkins in 1996.<sup>(1)</sup> Proteins are the main components of the anabolic and catabolic pathways of cells. Proteome is the entire set of proteins, present in an organism and varies with time and distinct circumstances, that a cell or organism undergoes.<sup>(2)</sup>

The term "proteomics" emanated in 1997, analogous with genomics the study of the genes.<sup>(3)</sup> Proteomics is defined as the study of all proteins, in a given cell or an organism including their relative abundance, distribution, functions and interactions with other macromolecules.<sup>(2)</sup> Hence it is the study of protein properties at a large scale that helps in understanding disease processes, at the protein level.

### Historical background

Proteomics is one step ahead of genomics as it gives a precise estimate of abundance of proteins in an organism whereas genomics gives only a rough estimate of protein expression. Moreover, proteomics takes into account the post-translational modifications of proteins such as formation of phosphoproteins and glycoproteins. In 1975, O'Farrell demonstrated the separation of 1100 protein components from *Escherichia coli* by two-dimensional polyacrylamide gel electrophoresis.<sup>(4)</sup> In 1995, Fleischmann et al published the first complete genome sequence of *Haemophilus influenzae Rd*.<sup>(5)</sup> Also in 1995, Wasinger et al worked on the protein map of the smallest known self-replicating organism, *Mycoplasma genitalium*.<sup>(6)</sup> In 1997, Wilkins et al published the first book on proteome research.<sup>(7)</sup>

Proteomics can be divided into two different branches based on the application of the technology: 1)

Expression proteomics- It is the study of changes in protein expression in tissues, cells and body fluids and 2) Cell-map proteomics- It is the study of protein-protein interactions that lead to formation of protein complexes.

**Work flow:** It starts with a stepwise analysis of proteins in a particular sample that includes sample collection, protein extraction, and fractionation. Finally, there is mass spectrometry-based investigation of the clinical samples. The procedure follows a strict sample preparation and handling protocol. Sample collection consists of sample processing, collection and analytical strategies required to develop reproducible clinical proteomic assays. The proteins are then extracted from a mixture of lipids, metabolites and other non-proteinaceous compounds that are present in the sample.

Various approaches are used to disintegrate proteins before the mass spectrometry analysis. Methods used include one-dimensional SDS-PAGE, isoelectric focusing, OFF-GEL fractionation, capillary electrophoresis, two-dimensional gel electrophoresis, difference gel electrophoresis and liquid chromatography.<sup>(8-12)</sup> Protein mixtures can be converted into simplified peptide populations by the enzymatic digestion using trypsin or chymotrypsin.<sup>(13,14)</sup>

**Mass spectrometry:** The mass spectrometry analysis has become an imperative part of the study of proteomics. This technique helps in affirmation of known proteins and discovery of new proteins. Application of this technique to the study of proteomics involves two methods:

1. **Bottom-up/ shotgun proteomics:** It is the most common mass spectrometry -based method for studying proteins. It refers to the analysis of

proteins after their enzymatic digestion into peptides.

2. **Top-down proteomics:** It refers to the analysis of intact proteins, which are not enzymatically digested prior to mass spectrometry analysis. It requires less time for sample preparation but the analyses of intact proteins is tougher as compared to peptides analyses in bottom up proteomics.

The analysis is done with the help of a mass spectrometer. It comprises of an ion source, the mass analyzer, and the ion detector. The ionization of proteins and peptides is done by either electrospray ionization (ESI) or by matrix-assisted laser desorption/ionization (MALDI). The ions are then introduced into gas phase and their mass-to-charge (m/z) ratio is analysed by mass analyzer. Finally, the detector detects the population of ions at different mass-to-charge (m/z) ratios. Apart from the mass measurement, mass spectrometry also produces tandem MS (MS/MS) measurements that provide information regarding the amino acid sequence. The process requires peptide fragmentation from techniques such as collision-induced dissociation (CID), electron capture dissociation (ECD) or electron transfer dissociation (ETD). The interpretation of all spectra is done by several software packages and associated algorithms, such as SEQUEST, Mascot.<sup>(15)</sup>

**Application of proteomics in periodontics:** Proteomic technology has widespread applications in the field of periodontology, leading to disease diagnosis, prognosis and treatment planning.

The conventional methods employ subjective measures such as pocket probing depth, bleeding on probing etc. But most recently, the biomarker identification and quantification has provided more objective measures for periodontal disease diagnosis, risk determination, evaluating disease progression, monitoring of therapy outcomes, and drug discovery.

Proteins specific to periodontal diseases may be found in saliva, GCF, periodontal ligament fibroblasts and periodontal microbes.

**Salivary proteome:** Saliva is an easily available oral fluid that contains numerous proteins. Hu et al identified 309 distinct proteins in human whole saliva by using two-dimensional gel electrophoresis / mass spectrometry proteomic techniques.<sup>(16)</sup>

The salivary proteome can provide an excellent source for early detection of periodontal disease. The periodontopathic bacteria and their virulence factors produce an aggravated host response in periodontal tissues. There is a cascade of immune and inflammatory reactions resulting in release of mediators. Hence, these mediators as well as pathogenic virulence factors, most of which are proteins, hold importance as diagnostic biomarkers. Frodge et al demonstrated the elevated levels of salivary tumour necrosis factor (TNF $\alpha$ ) in

patients of periodontitis.<sup>(17)</sup> Ingman et al detected an elevated level of matrix metalloproteinase (MMP-8) in the saliva of subjects affected by periodontitis.<sup>(18)</sup> Higher levels of other MMPs, including MMP-2, MMP-3 and MMP-9, were also reported in the saliva of patients affected by periodontitis. Other biomarkers include interleukin (IL-1 $\beta$ ), IgA antibodies, cathepsin G, elastase and its inhibitors, C-reactive protein and enzymes such as aminotransferase, lactate dehydrogenase and alkaline phosphatase.<sup>(19-22)</sup>

**Gingival crevicular fluid (GCF):** GCF is an oral fluid which is procured from gingival crevice that contains components of serum as well as periodontal tissues. Various mass spectrometry techniques have been applied to target different proteins of GCF.

Baliban identified biomarkers in gingival crevicular fluid (GCF) samples from chronic periodontitis (CP) and periodontally healthy individuals by detailed proteomic analysis. 432 human (120 new) and 30 bacterial proteins were identified. The human proteins, angiotensinogen, clusterin and thymidine phosphorylase were identified as biomarkers only in periodontal health.<sup>(23)</sup>

Other biomarkers included neutrophil defensin-1, carbonic anhydrase-1 and elongation factor-1 gamma, associated with chronic periodontitis. Bacterial biomarkers comprised of 33 kDa chaperonin, iron uptake protein A2 and phosphoenolpyruvate carboxylase (health-associated) and ribulose biphosphate carboxylase, a probable succinyl-CoA:3-ketoacid-coenzyme A transferase, or DNA-directed RNA polymerase subunit beta (chronic periodontitis associated).

**Periodontal pathogens:** Xia et al found that a total of 385 proteins were over expressed in intracellular *Porphyromonas gingivalis* as compared to the extracellular controls.<sup>(24)</sup> Zilm et al drew the proteomic portrait of *Fusobacterium nucleatum*.<sup>(25)</sup>

**Periodontal ligament fibroblasts:** Characterization of periodontal ligament fibroblast proteome has been done for understanding periodontal ligament physiology and regulation. A total of 117 proteins corresponding to 74 different gene products have been identified by total protein analysis of PDL cells.<sup>(26)</sup>

**Proteomics of dental enamel:** Recently isolation and characterization of enamel matrix and dentin proteins has been accomplished. The matrix proteins associated with enamel formation are dentin sialophosphoprotein (*DSPP*, a gene normally linked with dentin formation), the structural enamel proteins– amelogenin (*AMELX*), enamelin (*ENAM*), and ameloblastin (*AMBN*) – and a matrix metalloproteinase, enamelysin (*MMP20*). Zilm et al did the proteomic identification of proteinase

inhibitors, *fetuin A* and *alpha1-antichymotrypsin*, in the porcine enamel matrix derivative (EMD).<sup>(27)</sup>

**Mesenchymal stem cells:** The science of tissue engineering has finally emerged and has opened up a new vista for treating various pathological conditions. This technology mainly includes stem cell procurement, storage, differentiation and transplantation, requiring different proteins.

Park presented a list of mesenchymal stem cell (MSC) proteins for producing a reference map of proteome.<sup>[28]</sup> However future possibilities lie in expanding the list of (MSC) proteins further with known as well as unknown gene products.

**Proteomic technology for drug discovery:** One of the most sought after application of proteomics is the identification and development of potential new drugs for the treatment of diseases. This is done by using the genome and proteome information for identifying proteins associated with a disease, which are then analysed by computer software to be used as targets for new drugs. For example, if a certain protein is associated with a disease, its three dimensional structure guides the designing of drug to inhibit the action of that protein. This is done by a computer technique called the "virtual ligand screening".

Hence, in future, custom made drugs could be prepared for an individual. Attempts have been made to target and inactivate the HIV-1 protease by identification of new drugs. The HIV-1 protease is a necessary enzyme for HIV and therefore, it could be one of the most effective routes of killing HIV.<sup>(2)</sup>

In spite of limited practical usefulness as of now, we hope that there is a promising future for some gingival crevice fluid components to be of use primarily at the patient level, should be available in the form of chair-side or home use dip stick tests. This enables the patients to self-perform the test and indicates the risk for periodontal tissue loss.

## Conclusion

Proteomics is a relatively new post-genomic science with tremendous potential and consists of mass screening of proteins and the analysis of various genomes via their protein complements. The diagnosis of dynamic phase of disease, identifying patient at risk for periodontal disease, and focusing on early identification of microbial confront to host are tranquil for clinical investigations been increasing interest in exploring protein biomarkers to get optimal, best possible, novel, and non-invasive approaches.

This has definitely aided in developing sophisticated molecular assays for the detection of biomarkers leading to early diagnosis, determining risk in healthy, monitoring progression, response to treatment and outcome of the periodontal disease. Proteome analysis of microbes, tissues (enamel,

periodontal ligament) and oral fluid diagnostics (saliva and GCF) are the primary areas of interest to periodontists, proteomic analysis of gingival crevicular fluid may lead to the discovery of novel biomarkers for periodontal disease which, after proper validation, could reach clinical applications.

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