

## Proteomics or Genomics: A New Era in Periodontics

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### Abstract :

Proteins are the building blocks for both microorganisms and periodontium. Periodontitis is the result of complex interrelationship between infectious agents and host factors. The onset, progression and severity of periodontal disease are mainly mediated by various protein molecules. The study of proteins as biomarkers in periodontal diseases has increased attention during the last few years. The proteins involved in pathogenesis of periodontal disease can be used as biomarkers. The knowledge of various proteins involved in periodontal disease pathogenesis can be used in the diagnosis, prevention and treatment of periodontal diseases.

**Keywords:** Proteomics, Proteins, Periodontitis, Biomarkers.

### Introduction :

Proteins are the working parts of human cells. Almost every organic molecule in the body is either a protein or the result of a protein's activity. The word "proteome" is a blend of the words "protein" and "genome" created in the mid-1990s by Marc Wilkins, an Australian geneticist.<sup>1</sup>

The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system. This will vary with time and distinct requirements, or stresses, that a cell or organism undergoes.

The term "proteomics" was first coined in 1997 to make an analogy with genomics, the study of the genes. In simple terms, proteomics is defined as the study of all proteins including their relative abundance, distribution, posttranslational modifications, functions and interactions with other macromolecules, in a given cell or organism within a given environment and at a specific stage in the cell cycle.<sup>2</sup>

### Genomic or Proteomic :

Proteomics is a relatively new 'post-genomic' science with tremendous potential. After genomic and transcriptomics, proteomics is considered the next step in the study of biological systems. It is much more complicated than genomics mostly because while an organism's genome is more or less constant, the proteome differs from cell to cell and from time to time. This is because distinct genes are expressed in distinct cell types. This means that even the basic set of proteins which are produced in a cell needs to be determined.<sup>2</sup>

### Why Proteomics Better Than Genomics :

Proteomics gives a much better understanding of an organism than genomics. First, the level of transcription of a gene gives only a rough estimate of its level of expression into a protein. An mRNA produced in abundance may be degraded rapidly or translated inefficiently, resulting in a small amount of protein. Second, as mentioned above many proteins experience post-translational modifications that profoundly affect their activities; for example some proteins are not active until they become phosphorylated. Methods such as phosphoproteomics and glycoproteomics are used to study post-translational modifications. Third, many transcripts give rise to more than one protein, through alternative splicing or alternative post-translational modifications. Fourth, many proteins form complexes with other proteins or RNA molecules, and only function in the presence of these other molecules. Finally, protein degradation rate plays an important role in protein content.

Proteins are separated in two sequential steps according to isoelectric point and molecular weight. A singular advantage of this approach is that differentially posttranslationally modified forms of the same protein can be separated.

### Types of Proteomics :

Protein expression, or the quantitative measurement of the global levels of proteins, may still be done with two-dimensional gels. However, mass spectrometry has been incorporated to increase sensitivity, specificity and to provide results in a high-throughput format. A variety of platforms are available to conduct protein expression studies. The study of protein-protein interactions has been revolutionized by the development of protein microarrays.<sup>3</sup>

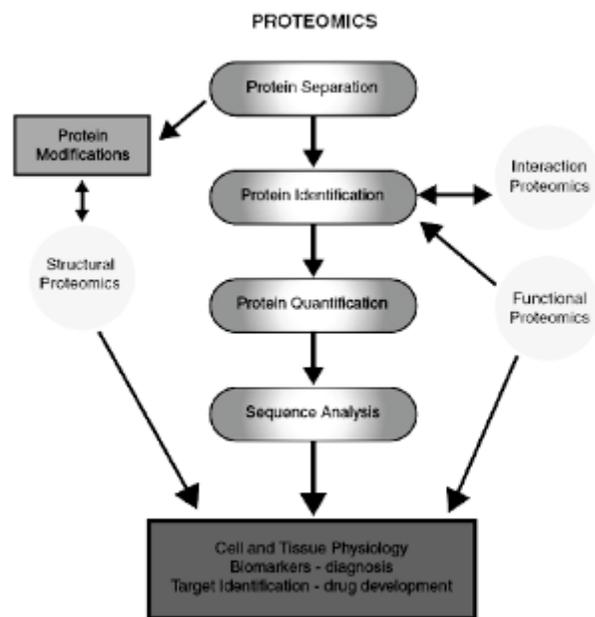
Fig 1. shows the sequence flow chart to illustrate the major steps from fractionated proteins, about to be separated and, ultimately, the determination of sequence analysis. Along

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**Fig. 1 Types of Proteomics and steps of sequence analysis**

with the major analytical components shown along the mid-line, analysis of posttranslational modifications, as well as functional and interactive proteomics, provides a rich treasure trove of future possibilities for biologists and, in particular, scientists focusing on the periodontium.<sup>3</sup>

#### **Structural Proteomics :**

Structural proteomics includes the identification of all the proteins on a genome-wide scale, determining their structure-function relationships, and describing three-dimensional structures of the proteins. Structural genomics attempts to map the total repertoire of protein folds in the hope of providing three-dimensional images for all proteins in an organism and to infer protein functions.

#### **Interaction and Functional Proteomics<sup>3</sup> :**

The functions of biological systems are dependent on interactions between their components. These interactions are ultimately determined by genetic elements and selection processes. The sequencing of complete genomes provides information on the proteins responsible for cellular regulation, but it does not indicate the function of proteins or how they are assembled into the molecular machines and functional networks that regulate cell behavior.

The regulation of cell metabolism involves protein interaction domains which regulate the association of polypeptides with each other and with phospholipids, small molecules, or nucleic acids. Several large-scale proteomics technologies have been developed to generate comprehensive, cellular protein-protein interaction maps.

#### **Proteomics and Dentistry :**

The two primary areas which dental proteomics have really shown are salivary diagnostics i.e. oral fluid diagnostics or oral fluid biomarkers and proteomics of bone and enamel structures, especially dental enamel.<sup>4</sup>

Human saliva contains proteins that can be informative for disease detection and surveillance of oral health. Comprehensive analysis and identification of the proteomic contents in human whole and ductal saliva is a necessary first step toward the discovery of saliva protein markers for human disease detection in particular for oral cancer and Sjogren's syndrome.<sup>5,6</sup>

Specific salivary proteomic biomarkers have been identified for three key features, namely the pathogenic process-inflammation, collagen degradation and bone turnover. Recently, by using proteomic approach, a reference proteome map of human whole saliva allowing for the resolution of greater than 200 protein spots in a single two-dimensional polyacrylamide gel was deduced. Fifty-four protein spots, comprised of 26 different proteins, were identified using N-terminal sequencing, mass spectrometry, and/or computer matching with protein database. Ten proteins, whose levels were significantly different when bleeding had occurred in the oral cavity, were isolated. These 10 proteins include -1-antitrypsin, apolipoprotein A-I, cystatin A, SA, SA-III, and SN, enolase I, hemoglobin chain, thioredoxinperoxiredoxin B, as well as a prolactin-inducible protein. The proteomic approach identifies candidates from human whole saliva that may prove to be of diagnostic and therapeutic significance.<sup>7</sup>

#### **Proteomics and Periodontics :**

Periodontal ligament fibroblast protein expression has been studied using immunological methods, although this technique is limited to previously identified proteins for which specific antibodies are available. A total of 117 proteins have been identified from PDL fibroblasts which can serve as a reference map for future clinical studies as well as basic research.<sup>8</sup>

#### **Periodontal Pathogens :**

Periodontal diseases are still worldwide human ailments, resulting in a high level of morbidity and an economic burden to the society. Proteomics offers a new approach to the understanding of holistic changes occurring as oral microorganisms adapt to environmental change within their habitats in the mouth. *Porphyromonas gingivalis* is a periodontal pathogen, is known to undergo a transition from its commensal status in healthy individuals to a highly invasive intracellular pathogen in human patients suffering from periodontal disease. Extensive proteomic research is

done on *P. gingivalis*.

- Whole cell quantitative proteomics, along with mutant construction and analysis were conducted to investigate how *P. gingivalis* adapts to species community. The results have confirmed that some 403 proteins were down regulated and 89 proteins were upregulated. The proteins such as HmuR which is up-regulated can be necessary for community structure.<sup>9</sup>
- Whole-cell proteomic analyses were conducted to investigate the changes from an extracellular to intracellular lifestyle for *Porphyromonas gingivalis* and found that a total of 385 proteins were over expressed in internalised *P. gingivalis* relative to controls.<sup>10</sup>
- Hendrickson EL et al found that there is shift in the production of cytotoxic fatty acids by intracellular *P. gingivalis*, which suggests that the interior of host cells provides a more energy rich environment compared to the extracellular milieu.<sup>11</sup>
- Yoshimura M et al carried out a similar study on proteome analysis of *P. gingivalis* which was placed in subcutaneous chamber of mice showed that PG1385 protein is involved in the virulence of these bacteria.<sup>12</sup>

The results of these studies suggest that adaptation to an epithelial cell environment induces a major shift in the expressed proteome of the organism.

#### Salivary proteomics for Periodontitis:

Saliva is considered as an important Periodontal diagnostic tool since variable amounts of blood, serum, serum products, GCF, electrolytes, epithelial and immune cells, microorganisms, bacterial degradation products, lipopolysaccharides, bronchial products and other foreign substances are present in whole saliva. Matrix Metalloproteinases (MMP 2, 3, 9), Immunoglobulin (Ig), Esterases, Lysozyme, Lactoferrin levels in saliva are valuable for predicting the progression of periodontitis. Numerous other salivary proteases have also been used as diagnostics biomarkers. Various cytokines like C- reactive protein, pentraxin-3, TNF, various other interleukins which are involved in its pathogenesis have come handy in diagnosing periodontal diseases.<sup>7</sup>

Young-Jin Choi et al studied the GCF in healthy individuals and Periodontitis patients to study biomarkers. He identified azurocidin in the GCF, but not in the saliva, as an upregulated protein in the periodontitis patients. He concluded that azurocidin could be a potential biomarker candidate for the early detection of inflammatory periodontal destruction by gingivitis and some chronic periodontitis. Azurocidin may have an inhibitory role in osteoclast differentiation and, thus, a protective role in alveolar bone loss during the early stages

of periodontitis.<sup>13</sup>

Melissa M. Grant et al studied the 21-day experimental gingivitis model. The model was designed to enable the study of both the induction and resolution of inflammation. Across the course of experimentally induced gingivitis, He identified 16 bacterial and 186 human proteins. Although abundances of the bacterial proteins identified did not vary temporally, *Fusobacterium* outer membrane proteins were detected.<sup>14</sup>

#### Proteomics And Stem Cell Research:

Large scale mesenchymal stem (MSC) cell proteome analyses have been emphasized in recent MSC research. A review by Hye Won park presents an expandable list of MSC proteins which will function as a starting point for the generation of a comprehensive reference map of their proteome. This proteomic and transcriptomic analyses may allow us to obtain new and hopefully fundamental insights into the protein expression, regulation, and cellular biology of MSC.<sup>9</sup>

#### The Future Of Proteomics :

##### Customized Drugs :

One of the most promising developments to come from the study of human genes and proteins has been the identification of potential new drugs for the treatment of disease. This relies on genome and proteome information to identify proteins associated with a disease, which computer software can then use as targets for new drugs. For example, if a certain protein is implicated in a disease, its 3D structure provides the information to design drugs to interfere with the action of the protein. A molecule that fits the active site of an enzyme, but cannot be released by the enzyme, will inactivate the enzyme. This is the basis of new drug-discovery tools, which aim to find new drugs to inactivate proteins involved in disease. As genetic differences among individuals are found, researchers expect to use these techniques to develop personalized drugs that are more effective for the individual.

##### Development of Biomarkers :

The two main research frontiers for application of proteomics in dentistry are salivary diagnostics, or oral fluid biomarkers, and proteomics of bone and enamel. While saliva is accessible and its collection is totally noninvasive, its use in clinical diagnostics has only recently been demonstrated. One team of researchers at UCLA, and others, has shown that oral fluid harbors the same composition of disease biomarkers as blood, but in smaller quantities. These scientists have developed, with support of the National Institute of Dental and Craniofacial Research, a molecular sensor that provides the basis for future development of the "Oral Fluid NanoSensor Test (OFNASET)." OFNASET is predicted to be a handheld

and easy-to-use instrument that clinicians can use to rapidly detect complex salivary protein and nucleic acid targets. The result will be the ability to clinically detect oral cancer before oral signs and symptoms.<sup>15</sup>

### Computational Method :

A computer technique which attempts to fit millions of small molecules to the three-dimensional structure of a protein is called "virtual ligand screening". The computer rates the quality of the fit to various sites in the protein, with the goal of either enhancing or disabling the function of the protein, depending on its function in the cell. A good example of this is the identification of new drugs to target and inactivate the HIV-1 protease. The HIV-1 protease is an enzyme that cleaves a very large HIV protein into smaller, functional proteins. The virus cannot survive without this enzyme; therefore, it could be one of the most effective protein targets for killing HIV.<sup>2</sup>

### Limitation:

As protein expression and post-translational modifications are dynamic processes, particularly in the periodontium, identification and quantification of proteins alone are not sufficient to understand functional changes. New technologies will be needed to enable combinations of metabolic labeling and identification as well as quantification and measurement of synthesis rates. Also Proteomics experiments conducted in one laboratory are not easily reproduced in another.

### Conclusion :

The use of proteomics and gene expression will advance the diagnosis and treatment of various oral pathological conditions. Advances in tissue engineering, drug delivery, gene therapy and biopharmaceuticals will present new therapeutic opportunities. However, its application into the field of dentistry depends on how best oral health care practitioners will incorporate this into their practice as it requires a thorough knowledge of human genetics and application of new diagnostic and therapeutic technologies.

### References :

1. Wilkins HR, Pasquali C, Appel RD, Ouk, Golaz O, Sanchez JC, Van JX, Golley AA, Hughes G, Smith IH, Williams, Hochstrasser DF. From Proteins to Proteomes: Large Scale Protein Identification by Two-Dimensional Electrophoresis and Amino Acid Analysis. *Nature Biotechnology* 1996; 14 (1): 61-65.
2. Sreedhar A, Shobha P, Sapna N, Santhosh K. Proteomics - The New Periodontics. *Journal of Dental Sciences and Research* 2011; 2(2):1-5.
3. Breault MR, Collins PH. Regenerative therapy. The future of Periodontics. *NY State Dental Journal* 1995; 61(10): 34-37.
4. Van Dyke TE, Serhan CN. Resolution of inflammation: a new paradigm for the pathogenesis of periodontal diseases. *J Dent Res* 2003; 82: 82-90.
5. Hasturk H, Kantarci A, Ohira T. RvE1 protects from local inflammation and osteoclast mediated bone destruction in periodontitis. *Faseb J* 2006; 20:401-3.
6. Serhan CN, Brain SD, Buckley CD. Resolution of inflammation: state of the art, definitions and terms. *Faseb J*. 2007; 21: 325-32.
7. Kathariya R, Pradeep A. Salivary proteomic biomarkers for oral diseases: a review of literature. *Archives of Oral Sciences & Research*. 2010;1(1):43-49.
8. Reichenberg E, Redlich M, Cancemi P, Zaks B, Pitaru S, Fontana S, Pucci-minafra, Palmon A. Proteomic analysis of protein components in periodontal ligament fibroblasts. *J Periodontol* 2005; 76, 1645-53.
9. Hye Won Park, Jun-Seop Shin, Chan-Wha Kim .Proteome of mesenchymal stem cells. *Proteomics*.2007; 7: 2881-94.
10. Xia Q, Wang T, Taub F. Quantitative proteomics of intracellular Porphyromonas gingivalis. *Proteomics*2007; 7(23): 4323-37.
11. Hendrickson EL, Xia Q, Wang T, Lamont RJ, Hackett M. Pathway analysis for intracellular Porphyromonas gingivalis using a strain ATCC 33277 specific database. *BMC Microbiol*2009; 1(9):185.
12. Yoshimura M, Ohara N, Kondo Y, Shoji M, Okano S, Nakano Y, Abiko Y, Nakayama K. Proteome analysis of Porphyromonas gingivalis cells placed in a subcutaneous chamber of mice (2008). *Oral Microbiol Immunol*. 23(5): 413-8.
13. Young-Jin Choi, Sun-Hee Heo, Jae-Mok Lee, Je-Yoel Cho. Identification of azurocidin as a potential periodontitis biomarker by a proteomic analysis of gingival crevicular fluid. *Proteome Science* 2011; 9(42):2-11.
14. Grant MM, Creese AJ, Barr G, Ling MR, Scott AE, Matthews JB . Proteomic Analysis of a Noninvasive Human Model of Acute Inflammation and Its Resolution: The Twenty-one Day Gingivitis Model. *Journal of Proteome Research* 2011; 9:4732-4744.
15. UCLA.Human Salivary Proteome Project Website. Available at: [hssp.dent.ucla.edu/OFNASET.htm](http://hssp.dent.ucla.edu/OFNASET.htm). Accessed Dec. 22, 2011.

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