



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

International Journal of Recent Scientific Research
Vol. 8, Issue, 2, pp. 15519-15523, February, 2017

**International Journal of
Recent Scientific
Research**

Review Article

PROTEOMICS – THE CHANGING EPOCH IN DENTISTRY

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ARTICLE INFO

Article History:

Received 06th November, 2015

Received in revised form 14th

December, 2016

Accepted 23rd January, 2017

Published online 28th February, 2017

Key Words:

Proteomics, Periodontal Diseases,
Molecular analysis, Protein Biomarkers

ABSTRACT

Proteins are polypeptides, which are the building blocks of all living beings, and are the main components of the physiological metabolic pathways of cells. All the structural and functional aspects of the body are carried out by protein molecules. Periodontal disease is an inflammatory disorder that damages tissues through complex interaction between bacteria and the host defence system. The onset, progression and severity of periodontal disease are mainly mediated by various protein molecules. The complex interactions of cells and matrix within compartmental groups, allow for a molecular understanding of the periodontium. The knowledge of various proteins involved in periodontal disease pathogenesis can be used in the diagnosis, prevention and treatment of periodontal diseases. Hence this article aims to review the potential proteomic approaches that may be applied to periodontal tissues and consider the opportunities that may arise in defining the expressed set of proteins in the periodontium.

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INTRODUCTION

Periodontal disease is considered as an inflammatory disorder that damages tissues through complex interaction between bacteria and the host defence system. The onset, progression and severity of periodontal disease are mediated by various protein molecules. Periodontal tissues constitutes multi-compartmental groups of interacting cells and matrices that render continuous support, attachment, proprioception and physical protection for teeth. (McCulloch CA, 2006) The knowledge of various proteins involved in periodontal disease pathogenesis helps in diagnosis, prevention and treatment of periodontal diseases. In periodontology, proteomes – the complete protein pool of an organism, are vital for understanding periodontal ligament physiology and regulation and to diagnose disease related protein markers.

Proteomics– the large scale analysis of proteins– has become one of the most important disciplines for gene function characterization, building functional linkages between protein molecules, and to comprehend the mechanisms of biological processes. “PROTEOMICS” is a relatively new field; and is defined as the total protein content of a cell or that of an organism. The term “proteome” a blend of “protein” and “genome” was coined by Marc Wilkins in 1996. (Marc R. Wilkins, 1996) Proteomic studies analyses the structure and function of various proteins and the protein-protein synergism of an organism. Any minor defects either in protein structure,

its function or alteration in expression pattern can be detected using proteomic techniques.

Recent progress in tissue isolation, protein separation, quantification, sequence analysis and structural interaction using proteomic techniques offers great promise for bringing about a change in periodontal physiology and pathology into the modern era. Hence this review targets at bringing into limelight, the potential proteomic avenues that may be applied to periodontal tissues and the opportunities that may arise in defining the expressed set of proteins in the periodontium.

Rationale of Proteomics

Apart from analysing the structure and function of biological systems, the objective of proteomics is to analyse the varying proteomes of an organism at different times, in order to highlight the differences between them. Among the many objectives of proteomics, the discovery of novel protein biomarkers has been a major driving force in the development of proteome characterization methodologies. (Lamba M, *et al* 2015)

Types of Proteomics

Proteomics in toto can be divided into three kinds as Structural Proteomics; Functional Proteomics; Expression Proteomics.

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Table 1 Types of Proteomics

Structural proteomics	Functional and interaction proteomics	Expression proteomics
The study of proteomics is based on structural information of total inventory for three dimensional images for all proteins in an organism.	The types of proteins that indicate the functions of proteins or how they are assembled into the molecular machines.	The quantitative study of protein expression between samples differing by some variable. Expression proteomics is quite useful in identifying disease-specific proteins

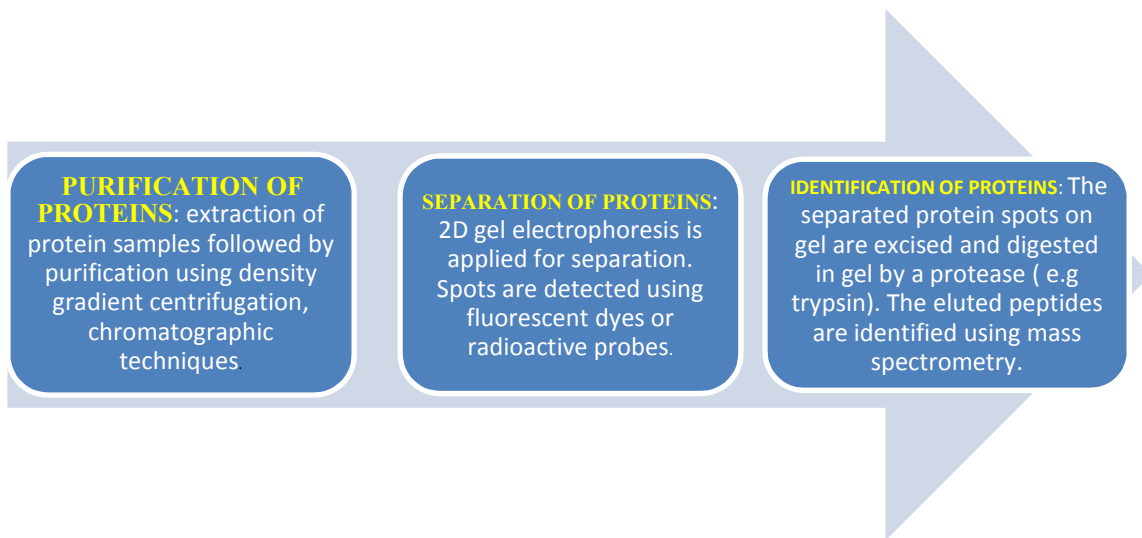
Techniques and Methods Involved In Proteomics Study

The primary step in all proteomic studies is the separation of a mixture of proteins. This is accomplished using Two Dimensional (2D) Gel Electrophoresis technique in which proteins are first separated based on their individual charges in 1D. The gel is then kept at 90 degrees from its initial position to separate proteins based on the difference in their size. This segregation occurs in second dimension hence the name 2D. The spots obtained in 2D electrophoresis are excised and further subjected to mass spectrometric analysis of each protein present in the mixture.

Proteomics in Dentistry

Proteomics is a relatively new postgenomic science with tremendous potential and has become one of the most important research frontiers in modern dentistry. The areas in which dental proteomics have been implemented are salivary diagnostics (i.e) oral fluid diagnostics or oral fluid biomarkers and proteomics of bone and enamel structures, especially dental enamel. (Shah N, 2013) With the help of human saliva, disease detection and surveillance of oral health can be done.

Table 2 Steps involved in the analysis of proteomes



Proteomic armamentarium comprises a broad array of technical approaches. The various methods of proteome analysis are tabulated

Table 3 The various methods of Proteome Analysis.

Methods of analysis	Uses
ELISA (Enzyme linked immunosorbent assay)	for isolation and quantification of proteins
IMMUNOASSAYS WITH MASS SPECTROMETRY (MSIA)	Determination of proteins that have undergone post translational modification.
PROTOMAP (Protein Topography and Migration Analysis Platform)	enables detection of changes in gel migration caused by proteolysis of posttranslational modification
MALDI (Matrix assisted Laser Desorption/ ionization)	rapid determination of proteins in particular mixtures
Two-dimensional poly acrylamide gel Electrophoresis	complex protein mixtures derived from biological samples
Non gel based proteome separation techniques	used to overcome the limitations of two dimensional electrophoresis
Capillary Electrophoresis	an alternative to both two dimensional electrophoresis for protein separation and to chromatography for peptide separation.

(Hu S, 2006) The comprehensive analysis and identification of the proteomic contents in the entire human system and ductal saliva is a fundamental step towards the discovery of saliva protein markers for human disease detection. (Sreedhar A, 2011) Various amounts of serum, serum products, gingival crevicular fluid, electrolytes, epithelial and immune cells, microorganisms, bacterial degradation products, lipopolysaccharides, bronchial products and other foreign substances are present in whole saliva. It comprises variety of periodontal proteomic markers from immunoglobulins to proteins effective for bone remodelling. It also has biomarkers specific for unique physiological aspects of periodontitis, in which the qualitative changes in the composition of these biomarkers could be diagnostic. Apart from saliva, gingival crevicular fluid, an inflammatory exudate from periodontal tissues contains many protein components and have been used as markers for periodontal diseases detection.

Thus the application of proteomics in dentistry is vast and many novel proteomic technologies are yet to be explored in dental sciences. The study of proteins at the molecular level will help in applying these new methods thereby promoting the

evolution of treatment in all dental specialities and act as an eye opener to what is still unknown in oral health and disease.

Proteins of the Periodontium

Periodontal tissues comprise multi-compartmental groups of interacting cells and matrices that provide continuous support, attachment, proprioception and physical protection for the teeth. Complete understanding of the mechanisms of homeostasis and response to infection of periodontal tissues will require a complete repertoire of expressed proteins. Proteins present in periodontium are involved in various functions such as cell matrix adhesion and signalling as well as regulating the diffusion of nutrients, waste products, and soluble signalling molecules. (Thesleff I *et al*, 1995) The proteins present in the gingiva and the periodontal ligament form the intermediate filament network which is responsible for maintaining the structural integrity of the periodontium. Reinchenberg *et al* analysed the protein components in periodontal ligament fibroblasts and found 117 proteins originating in 74 different genes. (Reichenberg E *et al*, 2005) Later, Yang H Y and his co-workers, applied quantitative proteomic approach to analyse and compare the proteins whose expression was altered in gingival soft tissue and alveolar bone following tooth extraction. (Hee-Young Yang *et al*, 2013) Suchetha A *et al* also analysed the proteins present in gingiva and periodontal ligament of periodontium, and concluded that there would be morphological and functional changes in the molecular components of the periodontally affected patients and a thorough knowledge of different proteins was essential to understand the physiology and pathology of the periodontal tissues. (Suchetha A *et al*, 2014)

Apart from gingiva and periodontal ligament proteins, Azarte H *et al* in his study revealed the presence of 56-kDa and 43-kDa as major protein bands in the cementum. The lack of cementum markers was a hurdle to understand the molecular mechanisms that regulate periodontal regeneration. (Arzate *et al*, 2015) To overcome this Alvarez-Perez *et al* identified one such differently human expressed gene, termed cementum protein-23. (Alvarez-Pérez *et al*, 2006) Later, Arzate H *et al* again did a study in which they had highlighted the recent advances of cementum and cementum attachment proteins and their role in formation, repair and regeneration. Their studies concluded that a detailed knowledge of the biology of cementum is essential for understanding how the periodontium functions, for identifying pathological issues and for developing therapies for periodontal repair and regeneration. (Arzate H *et al*, 1996)

Practical Applications of Proteomics

Proteomics has extensive applications in all aspects of life sciences including several practical applications. One of the most promising advances from the study of human genes and proteins is identification of plausible novel drugs for disease management. This relies on the information of genome and proteome to identify proteins associated with a disease. The basis of new drug discovery is to inactivate proteins involved in disease, which is performed with the help of 3D structure of the protein associated with the 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. (Sreedhar A *et al*, 2011)

Proteomic Biomarkers

Periodontal disease is a bacteria-induced chronic inflammatory disease affecting the soft and hard supporting structures encompassing the teeth. (Kathariya R *et al*, 2010) Assessment of periodontal disease is based on clinical parameters and radiographs. Though efficient, these traditional techniques are limited, as the current status of the disease alone can be determined. They do not have the capacity to diagnose susceptible patients who are at risk for disease progression. Development in the use of oral fluids as biological samples for measuring the present disease state, has made saliva and other oral-based fluids the vanguard of technology. Oral fluids comprises of local and systemic mediators of periodontal disease, (Mandel ID, 1990) which comprises of microbial, host response, and markers specific for bone resorption.

Though most biomarkers in oral fluids exhibit inflammatory mediators, specific collagen downgrading and bone turnover-related molecules have become apparent measures of periodontal disease. In oral diagnostics, it has been a great challenge to determine biomarkers for screening and predicting the early onset of disease or evaluating the disease activity and the efficacy of therapy. An oral diagnostic tool should provide relevant information for differential diagnosis, localization of disease and severity of infection. It should serve as a basis for treatment planning and act as a means of assessing the effectiveness of therapy. Thus keeping these criterias in mind researchers have made an effort to understand the underlying science of the disease, with an aim to find improved ways to diagnose and treat the disease before any serious outcome. Human saliva in oral diagnostics is of great importance and researchers are concentrating on it. Saliva is considered as an important Periodontal diagnostic tool since variable amounts of blood, serum and its products, GCF, epithelial cells as well as immune cells, microorganisms, products obtained from bacterial degradation, lipopolysaccharides, bronchial products and other foreign substances are present in whole saliva. (Schenkels LC *et al*, 1995) Saliva, has high potential for the surveillance of general health and disease. It represents a promising diagnostic fluid for the screening of various oral diseases. It is a fluid that comprises of constituents of exocrine glands and gingival crevicular fluid (GCF). (Kathariya R *et al*, 2010) Moreover, saliva is effortlessly available and easily collected without any cumbersome procedure. Matrix Metalloproteinases (MMP 2 and 9), Immunoglobulin (Ig), Esterases, Lysozyme, Lactoferrin levels in saliva are valuable for predicting the progression of periodontitis. Cytokines like C- reactive protein, pentraxin-3, TNF, and other interleukins which are involved in disease pathogenesis are essential for diagnosing periodontal diseases. (Lamba M *et al*, 2015) Apart from this, numerous proteomic markers, like acid phosphatase, alkaline phosphatase, (Masakazu N, Slots J, 1983) histatins, kallikreins, cystatins, kininogens, aminopeptidases, glucosidase, galactosidase and glucuronidase, and various bone remodelling proteins (Osteopontin, Osteonectin, Osteocalcin) are noted in the diagnosis of periodontal disease. (Kathariya R *et al*, 2010) Apart from periodontal diseases, salivary defence systems which comprises of salivary proteins play a significant and vital role in maintaining the health of the oral cavity and preventing caries. (Mazengo MC *et al*, 1996) Significant amount of salivary phosphopeptides (PRP1/3, histatin-1 and

statherin) were associated with the absence of dental caries, affirming the value of these peptides in nurturing tooth integrity. (Vitorino R *et al*, 2005)

Proteomics in gingival crevicular fluid (GCF)

Apart from saliva, studies were done in the GCF of healthy individuals and periodontitis patients to study biomarkers. (Young-Jin Choi *et al*, 2009) GCF is an inflammatory exudate collected at the gingival margin. Around 90 various components in GCF have been evaluated during periodontal pathogenesis. (Loos BG *et al*, 2005) Of the numerous constituents in GCF, the major portion comprise soft tissue inflammatory events, of which only a few are regarded as biomarkers specific for alveolar bone destruction. (Kinney J S *et al*, 2007) Grant *et al* identified 186 human proteins in gingival crevicular fluid samples from periodontally healthy and experimental gingivitis sites using LC-MS/MS. (Grant MM *et al*, 2010) These proteins included circulating blood proteins, enzymes, cytoskeleton-related proteins, immunity related proteins, AMPs, inflammation related proteins, lipid-related proteins. The components in gingival crevicular fluid are similar to serum components. More proteins were discovered when a urea buffer was used to extract gingival crevicular fluid proteins as opposed to phosphate buffer saline. Thus multiple components in gingival crevicular fluid can be analysed using mass spectrometry and this approach may be useful for the diagnosis of periodontal diseases.

Proteomics and Periodontal Pathogens

The oral environment contains various colonies of micro-organisms comprising of bacteria, fungi, protozoa, and viruses. Oral ecology studies have reviewed the complexity of the interactions that these micro-organisms have with their host in both health and disease. In spite of this, dental caries and periodontal diseases are still worldwide oral diseases, resulting in a high level of morbidity among humans. (Sreedhar A *et al*, 2011) Proteomics offers a new approach to comprehending these holistic changes, as oral micro-organisms adapt to environmental change within their habitats in the mouth. Various microorganisms are present in subgingival plaque, of which only few play a etiological role in the pathogenesis of periodontal diseases in the susceptible host. Specific microorganisms involved in the periodontal pathogenesis are *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Aggregatibacter actinomycetemcomitans*. (Socransky S S, 1970) BANA activity (benzoyl-DLarginine-naphthylamide) was exhibited by the members of the “red complex” of periodontal pathogens (*T. forsythia*, *P. gingivalis*, and *T. denticola*) are strongly correlated with periodontal activity. (Loesche W L, 1992)

Oral Antimicrobial Peptides

Antimicrobial peptides (AMPs) are a wide-ranging class of host-defence molecules that act early to contest against microbial invasion and challenge. In the oral cavity, the salivary glands produce the AMP's and the microorganisms and pathogens have easy access to the oral cavity and the rest of the body via epithelium and the gastrointestinal tract. (Khurshid Zohaib, 2015) The oral epithelial cells, salivary glands and neutrophils secrete at least forty-five identifiable antimicrobial gene products that are found in saliva. A wide

range of AMPs with heterogenous functions have been identified in the oral tissues and secretions. The defensive role played by these peptides against microbes entering the oral cavity results in competent fight against infections. AMPs have favourable potential to be used against oral microbes to control their growth and biofilm formation. AMPs are expected in the future to be used as models for designing effective oral microbial antibiotics. (KhurshidZohaib, 2015)

The Role of Biomarkers in the Clinical Management of Periodontitis

Currently, there are no dependable tests to diagnose and predict progression of periodontitis. The traditional diagnostic procedures give an expression of severity and, reflect previous disease activity but not current disease activity and they do not diagnose susceptible individuals who may be at risk of future periodontitis. (Pihlstrom B L, 2005) The requirement for reliable biomarkers to differentiate progressive periodontitis from normal biological processes is considered ideal to diagnose periodontitis at an earlier or even preclinical stage, to initiate preventative pre-treatment, and also to conduct epidemiological studies. Thus the concept of a biomarker arose from the ability to monitor health status, disease susceptibility, advancement, and treatment outcome with respect to a number of common medical conditions. (Wong D T, 2006)

Proteomics and Tissue Engineering

Tissue engineering has evolved in recent years, as efficient means for treating various pathological conditions. This scientific knowledge mainly constitutes stem cell procurement, storage, differentiation as well as transplantation which is performed by using specific biomarkers such as proteins. However, the absence of knowledge regarding these prospective markers for stem cells and their specific differentiation remains a considerable limitation for these applications. (Sreedhar A *et al*, 2011) In future, the proteomic and transcriptomic analyses may pave way to obtain new and hopefully fundamental insights into the protein expression, and cellular biology of mesenchymal stem cell. (Xia Q *et al*, 2007)

Future Trends in Proteomics

Several prospects exist for future application of proteome map in biotechnology and health care applications, especially in the field of diagnostics. Humongous amount of research activity has been performed to expose the role of oral and salivary fluids in oral diagnostics. Genetic single nucleotide polymorphisms are reviewed by researchers in the study of periodontal disease. As protein expression and post-translational modifications are dynamic processes, pertaining to the periodontium, identification and quantifying of proteins alone are not sufficient to comprehend functional changes. Proteomics experiments conducted in one laboratory are not easily reproduced in another. New technologies will be needed to enable combinations of metabolic labeling and identification as well as quantification and measurement of synthesis rates.

CONCLUSION

In conclusion, proteomics is a remarkable tool that can revolutionise the treatment of oral disease in the context of identifying the risk factors, prompt diagnosis, prevention and control, lead to changes in treatment philosophy in all aspects

of dental sciences. Understanding the interaction networks requires systematization and analysis of a huge amount of information emerging from experimental studies. In this context, proteomics is a promising tool to change the practice of dentistry. In this integration there is still much to be discovered to fill the gaps of our knowledge in oral science. With the future of proteomics still remaining in the womb of time, further research is required to investigate deeper regarding the application and integration of this new field. Further, proteins being an universal component of all biological functions of the body, the scope of proteomics in health and especially the field of periodontology is expected to scale greater heights.

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