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Proteomics-A New Dimension to Diagnosis

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Abstract: ***Proteins are vital parts of living organisms, as they are integral components of the physiological metabolic pathways of cells. 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. 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 proteome map, that is, complete catalogue of the matrix and cellular proteins expressed in alveolar bone, cementum, periodontal ligament, and gingiva, is to be explored for more in-depth understanding of periodontium. 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: Protein genomics, protein quantification, biomarkers

1. Introduction

Proteins are vital parts of living organisms, as they are the main components of the physiological metabolic pathways of cells. The word "proteome" is a blend of "protein" and "genome", and was coined by Marc Wilkins in 1996[1]

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[2] to make an analogy with genomics, the study of the genes.

In simple terms, proteomics is defined as the study of all proteins present in a particular cell or an organism in a given environment and at a specific stage in the cell cycle [3]Proteome analysis of bone and dental structure (enamel, periodontal ligament, and cementum) and oral fluid diagnostics (saliva and GCF) are the primary areas where dental proteomics has shown promising outcomes [3].

Periodontal tissues comprise multicompartmental groups of interacting cells and matrices that provide continuous support, attachment, proprioception and physical protection for the teeth. The periodontium is also specialized to minimize tissue damage arising from trauma and infection. The high level of tissue complexity generated by the multiple types of interacting cells and extracellular matrices, many of which are embedded in very small and difficult to-study compartments, has slowed research in periodontal physiology and pathology.[4] the complexities of periodontal tissue structure underlie the expression patterns of multiple cell types that are regulated by exquisitely well-integrated control systems [5].

The periodontium challenges including two extreme, hypothetical perspectives;

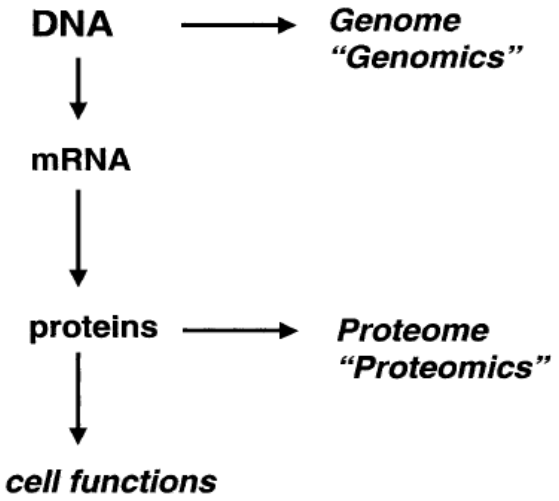
The periodontium is a powerful model system to examine homeostasis and integrative physiology in closely approximated soft and mineralized tissues, under the constant influences of microbial and physical challenges. The periodontium is a nightmarishly complex and almost impossible to study collection of poorly defined cell types and inadequately described matrix components; this mish-mash responds to ill-understood exogenous forces and infections that cause alterations to matrix proteins and epithelial barriers which are difficult to repair, let alone regenerate.

For both the optimist and the pessimist, a better understanding of what actually constitutes the total expressed set of cellular and matrix proteins would seem to provide a good beginning for future advances and for achieving a more in-depth understanding of the periodontium.

2. Genomics V/S Proteomics

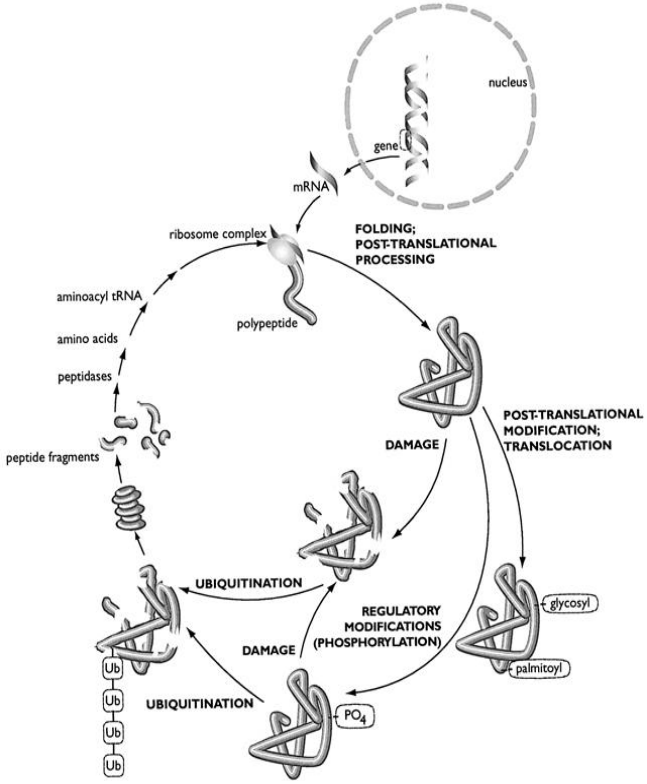
Proteomics is a relatively new 'post-genomic' science with tremendous potential. In contrast to gene expression studies employing oligonucleotide chips ('transcriptomics'), proteomics directly addresses the level of gene products present in a given cell state and can further characterize protein activities, interactions and subcellular distributions. Proteomics has beensuccessfully applied to areas as diverse as determining the protein composition of organelles, systematicelucidation of protein-protein interactions and the largescale mapping of protein phosphorylation in response to a stimulus. Scientists are very interested in proteomics because it 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.

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[6].



Biochemical context of genomics and proteomics.

The life cycle of a protein



The life cycle of a protein

3. Differences between Protein Chemistry and Proteomics

3.1 Protein Chemistry and Proteomics

Protein Chemistry	Proteomics
Individual Proteins	Complex Mixtures
Complete sequence analysis	Partial sequence analysis
Emphasis on structure and function	Emphasis on identification by database matching
Structural biology	Systems biology

3.2 Types of Proteomics

Structural Proteomics

Study of proteomics is based on structural information of total repertoire for three dimensional images for all proteins in an organism. This arises from analysis of unknown proteins such as protein bound ligand or cofactor and is useful for functional description [4]. The identification of all proteins on a genome wide scale, determining their structural-functional relationships, and describing three-dimensional structures are the important hurdles in structural proteomics [31].Functional and evolutionary protein relation which were not visible at sequence level are now possible with the advent of structural proteomics[32].

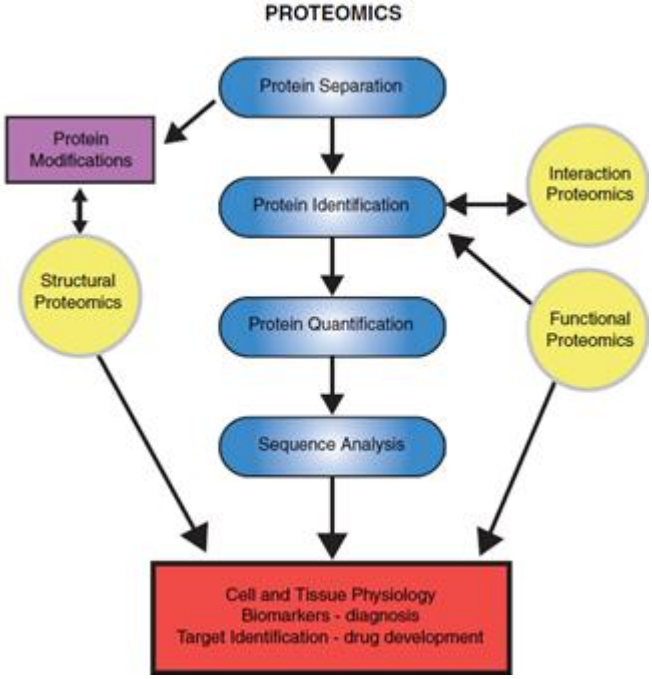
Interaction Proteomics

The functions of biological systems are dependent on interactions between their components. These interactions are ultimately determined by genetic elements and selection processes [33].Thesequencing of complete genomes provides information on the proteins responsible for cellular regulation. The different technique used for this includes yeast two-hybrid system, microassays, and affinity purification. This technology has been used for many different biological systems including, for example, identification of novel matrix metalloproteinase substrates that act to regulate inflammation[34].

Functional Proteomics

Types of proteins that indicate the function of proteins or how they are assembled into the molecular machines and functional networks that regulate cell behaviour[35] determine the functional proteomics. It is "focused to monitor and analyse the spatial and temporal properties of the molecular networks and fluxes involved inthe living cells" [36]. It concentrates on the following two issues [37]:
(i) elucidation of biological functions of unknown proteins,
(ii) cellular activity at molecular level

3.3 Types of Proteomics and Steps of Sequence Analysis



4. 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[7,8] and proteomics of bone and enamel structures, especially dental enamel [9]. Human saliva contains proteins that can be informative for disease detection. 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 [10,11].

Cells Studied By Proteomic Analysis	*Proteins Identified*	*Relevance/Significance*
PDL fibroblast[12]	Cytoskeleton proteins-actin,tubulin, vimentin; cellular motility protein; membrane trafficking protein; chaparonone; stress and folding protein; metabolic enzymes.	Related to PDL fibroblast function and homeostasis
PDL cell undergoing mineralization13	Cytoskeleton proteins; cytoskeleton associated proteins; nuclear protein; cell membrane bound protein.	Maintain Periodontal tissue homeostasis
Oral squamous cell carcinoma (OSCC) specimen14	Ubiquitin-cross reactive protein (UCRP) of IFN stimulated gene (ISG) family.	May help in the development of novel biomarkers for OSCC pathogenesis
Streptococcus mutans15	Surface proteins; Intrinsic membrane proteins	They are similar to proteins present in other gram positive bacteria.
P. gingivalis[16]	PG1089; PG1385; PG2102	PG1385 is involved in Pg Virulence
F. nucleatum[17]	Various cytoplasmic proteins eg. Pyruvate kinase; enolase; flavodoxin; adenylate kinase etc.	Cellular biosynthesis; maintenance of homeostasis; may be important in the organism persistence during the transition from health to disease.
Saliva in periodontitis subjects[18]	S100 proteins; haptoglobulins; prolactin induced protein; parotid secretary proteins.	Associated with host defence; New potential biomarker for monitoring disease activity in periodontitis.
Minor labial salivary gland from primary sjogren's syndrome (pSS) patients and non SS patients[11]	Heat shock proteins; carbonic anhydrase; enolase; vimentin; alpha defensin and carmodulin	Alpha defensin and carmodulin are exclusively found in pSS patients; may be used as a biomarker of prognostic and diagnostic value

4.1 Proteins of Periodontium

Periodontal proteomic markers range from salivary protein markers like Immunoglobulin G to bone remodeling protein markers [25]. These can be specific/nonspecific. Specific markers are immunoglobulinswhich characterize the presence of chronic or aggressive periodontitis. Among nonspecific markers are enzymes, proteins,mucins, histatin, lactoferrin, lysosomal peroxidase, and so forth. In addition, blood, GCF, serum, serum products, electrolytes, microorganisms, epithelial and immune cells, bacterial degradation products, lipopolysaccharides, and periodontal fibroblasts can be used for proteome analysis [38]. Biomarkers specific for periodontitis and any change in their composition could be diagnostic [25]. Comprehensive analysis and identification of proteomic contents in saliva, GCF, periodontal fibroblasts, and periodontal microbes are a necessary first step towards the discovery of periodontal protein markers for periodontal disease.

4.2 Proteomics and Periodontics:

Periodontal ligament fibroblast protein expression has beenstudied using immunological methods, although this technique is limited to previously identified proteins for which specific antibodies are available. Atotal 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[12]. A recent study by Holly McKnight et al (2014) made four matched pairs of human gingival fibroblasts (hGFs) and human periodontal ligament fibroblasts (hPDLFs)were cultured. Before confluence, membrane-bound and –associated proteins from cells of the fourth passage were extracted. The processed protein samples were evaluated using capillary-liquid chromatography-nanospray tandem mass spectrometry. Global protein identification was performed on an orbitrap mass spectrometer equipped with a microspray source operated in positive ion mode. Proteome software was used to validate protein identifications derived from tandem mass spectrometry sequencing resultsand concluded that distinct differences in the cellular protein catalog may reflect the dynamic role and high energy requirements of hGFs in extracellular matrix remodeling and response to inflammatory challenge as well as the role of hPDLFs in monitoring mechanical stress and maintaining tissue homeostasis during regeneration and remineralization[29].

4.3 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. Porphyromonasgingivalis 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 isdone on Pgingivalis.

- Whole cell quantitative proteomics, along with mutantconstruction 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[22].

- Whole-cell proteomic analyses were conducted to investigate the changes from an extracellular to intracellular lifestyle for Porphyromonasgingivalis and found that a total of 385 proteins were over expressed in internalised P. gingivalis relative to controls[24].
- Hendrickson EL at 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[24].
- 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[16]
- The results of these studies suggest that adaptation to an epithelial cell environment induces a major shift in the expressed proteome of the organism.

The major challenge for research workers in periodontology is to embrace proteomics approaches when appropriate and start to apply them to critical, unresolved questions such as molecular and biologic understanding for the various cell populations of periodontium. Thus a more in-depth knowledge of cellular and matrix protein component of periodontium provides an excellent commencement for future advances [19].

4.4 Need for a Periodontal Disease Indicator

Thediagnosis of dynamic phase of disease, identifying patientat risk for periodontal disease, and focusing on early identificationof microbial con front to host are tranquil for clinical investigations [3, 19, 20]. So there has been increasing interestin exploring protein biomarkers to get optimal, best possible,novel, and noninvasive approaches for the above stated causes [3, 19]. Thus the knowledge of periodontal disease indicator is a must to ultimately improve the clinical management of periodontal patients [3]. The roadblocks that have prevented the realization of periodontal diagnostics [21] are
(i) lack of definitive disease-associated protein and genetic biomarkers,
(ii) expensive sampling method,
(iii) lack of an accurate, easy-to-use diagnostic platform.

The novel expertise of miniaturization coupled with corresponding/ analogous disease detection creates fundamental ways of detecting and diagnosing disease state by studies employing transcriptomics (oligonucleotide chips) known as the field of genomics. Genomics only can directly address the level of gene products present in cell state and has limited applications. During the last few years, protein as a biomarker in periodontal disease has gained confirmation. The study of proteome, that is, composition, protein-protein interaction, systemic elucidation of protein, extracellular matrix interaction, and posttranslational modification, is in forefront of oral diagnosis. Proteomics thus provides systematic/ comprehensive information about proteins in various tissues and organs [4] to have an excellent beginning for future advancements in the field of diagnostics.

4.5 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, lipopolyscaaharides, bronchial products and other foreign substances are present in whole saliva. Matrix Metalloproteinases (MMP 2, 39), 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 [25].

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 [26]. Numerous proteomic markers, like acid phosphatse, alkaline phosphatase, histatins, cystatins, kallikreins&kininogens, aminopeptidases ,aspartate transaminase, glucosidase, galactosidase and glucuronidase and various bone remodeling proteins (Osteopontin, Osteonectin, Osteocalcin) are well known in periodontal diagnosis[25].

5. Proteomics and Tissue Engineering

Tissue engineering has evolved in recent years, into effective tool for treating various pathological conditions. This technology mainly includes stem cell procurement ,storage , differentiation and transplantation which is done by using specific bio markers i.e. proteins [6].
A review by Hye Won parkpresents an expandable list of MSC proteins which will function as a starting point for the generation of a comprehensive reference map of their proteome. The acquired protein list of MSC and the effective mass spectrometric tools used in this research constitutes a useful inventory, which facilitates the identification of the normal proteomic pattern as well as changes in activated or suppressed pathways occurring during proliferation, differentiation, or other experimental conditions[27].

5.1 The Future of Proteomics

Several possibilities for further application of proteome mapin biotechnology and health care applications, especially in the field of diagnostics, exist. Huge amount of research activity has already been done to expose the role of oral and salivary fluids in oral diagnostics. Recent advances in HIV diagnosis, for example, OraSure, OraSure Technologies, Bethlehem, Pennsylvania, which collects HIV-1 antibodies

from gingival tissues using oral mucosal transudate, are entirely based on proteome analysis.

5.2 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 handheldand 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 [28].

5.3 Genetic single nucleotide polymorphisms

Several researchers have focused on genetic single nucleotide polymorphisms in the study of periodontal disease. A genetic susceptibility test is available for severe chronic periodontitis (Interleukin Genetics, Waltham, Massachusetts). It works by detection of two types of IL-1 genetic alleles, IL-1α + 4845 and IL-1β+ 3954 [30]. Individuals identified as "genotype positive," or are found to have both of these alleles, are more likely to have the phenotype of overexpression of this gene.

5.4 Computer Technique

Acomputer 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 [6].

5.5 Development of Newer 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. Amolecule 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 [6].

6. Conclusion

The use of proteomics and gene expression will advance the diagnosis and treatment of various oral pathological conditions. In periodontium, many but not all expressed proteins are tissue-specific and the function of various proteins is modulated by multiple factors, including interactions with other proteins and modifications arising from attached phosphates, sulfates, carbohydrates, and lipids.Proteomics canprovide comprehensive and systematic information about proteins in a wide array of tissues and organs. An important challenge that needs to be met by research workers in periodontology is to embrace proteomics approaches when appropriate, and start to apply them to critical, unresolved questions such as the biological basis for the heterogeneity in gingival, bone, and cementum cell populations. 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.

References

[1] Marc R. Wilkins, et al. "From Proteins to Proteomes: Large Scale Protein Identification by Two-Dimensional Electrophoresis and Arnino Acid Analysis". Nature Biotechnology (1996); 14 (1): 61–65.
[2] P. James (1997). "Protein identification in the post-genome era: the rapid rise of proteomics". Quarterly reviews of biophysics 30 (4): 279–331.
[3] H. Khashu, C. S. Baiju, S. R. Bansal, and A. Chhillar, "Salivary biomarkers: a periodontal overview," *Journal of Oral Health & Community Dentistry*, vol. 6, pp. 28–33, 2012.
[4] C. A. McCulloch, "Proteomics for the periodontium: current strategies and future promise," *Periodontology 2000*, vol. 40, no.1, pp. 173–183, 2006.
[5] Pitaru S, McCulloch CA, Narayanan SA. Cellular origins and differentiation control mechanisms during periodontal development and wound healing. J Periodontal Res 1994: 29: 81–94.
[6] SreedharA ,ShobhaPrakash , Sapna N. , Santhosh Kumar: **Proteomics - The New Era of Periodontics.** Journal of Dental Sciences and Research Vol. 2, Issue 2, Pages 1-5.
[7] Vitorino, R., Lobo, M. J., Ferrer-Correira, A. J., Dubin, J. R., Tomer, K. B., Domingues, P. M. & Amado, F. M. (2004) Identification of human whole saliva protein components using proteomics. Proteomics 4, 1109–1115.
[8] Walz, A., Stuhler, K., Wattenberg, A., Hawranke, E., Meyer, H. E., Schmalz, G., Bluggel, M. &Ruhl, S. (2006) Proteome analysis of glandular parotid and submandibular–sublingual saliva in comparison to whole human saliva by twodimensional gel electrophoresis. Proteomics 6, 1631–1639.

[9] Hu JC, Yamakoshi Y, Yamakoshi F, Krebsbach PH, Simmer JP. (2005) proteomics and genetics of dental enamel. Cell Tissues Organs, 181(3-4): 219-31.
[10] Hu S, Loo JA, Wong DT. (2007) Human saliva proteome analysis. Ann N Y Acad sci. 1098(mar); 323-9.
[11] Y Fleissig, O Deutsch, E Reichenberg, M Redlich, B Zaks, APalmon, DJ Aframian (2008). Different proteomic protein patterns in saliva of Sjögren's syndrome patients. Oral Diseases 15(1), 61-8.
[12] Reichenberg E, Redlich M, Cancemi P, Zaks B, Pitaru S, Fontana S, Pucciminafra I, Palmon A (2005). Proteomic analysis of protein components in periodontal ligament fibroblasts. Journal of periodontology 76, 1645-53.
[13] L. Wu, X. Wei, J. Ling, L. Liu, S. Liu, M. Li, Y. Xiao (2009). Early osteogenic differential protein profile detected by proteomic analysis in human periodontal ligament cells. J Periodont Res; 44: 645–56.
[14] Lang-Ming Chi, Chien-Wei Lee, Kai-Ping Chang, Sheng-Po Hao, Hang-Mao Lee, Ying Liang, ChuenHsueh, Chia-Jung Yu, I-Neng Lee, Yin-Ju Chang, Shih-Ying Lee, Yuan-Ming Yeh, Yu-Sun Chang, Kun-Yi Chien and Jau-Song Yu (2009). Enhanced Interferon Signaling Pathway in Oral Cancer Revealed by Quantitative Proteome Analysis of Microdissected Specimens Using 16O/18O Labeling and Integrated Two-dimensional LC-ESI-MALDI Tandem MS. Molecular & Cellular Proteomics 8, 1453-74.
[15] Len AC, Cordwell SJ, Harty DW, Jacques NA (2003). Cellular and extracellular proteome analysis of Streptococcus mutans grown in a chemostat. May; 3(5):627-46.
[16] Yoshimura M, Ohara N, Kondo Y, Shoji M, Okano S, Nakano Y, Abiko Y, Nakayama K. Proteome analysis of Porphyromonasgingivalis cells placed in a subcutaneous chamber of mice (2008). Oral MicrobiolImmunol 23(5), 413-8.
[17] Peter S. Zilm, Christopher J. Bagley, Anthony H. Rogers, Ian R. Miln and Neville J. Gully (2007). The proteomic profile of Fusobacteriumnucleatum is regulated by growth pH. Microbiology, 153, 148–59.
[18] Haigh BJ, Stewart KW, Whelan JRK, Barnett MPG, Smolenski GA, Wheeler TT (2010). Alterations in the salivary proteome associated with periodontitis. J Clin Periodontol;37: 241–7.
[19] R. KathariyaandA. R. Pradeep, "Salivaryproteomicbiomarkers for oral diseases: a review of literature," *American Overseas School of Rome*, vol. 1, pp. 43–49, 2010.
[20] P. B. Patil and P. B. R. Saliva, "A diagnostic biomarker of periodontal diseases," *Journal of Indian Society of Periodontology*, vol. 15, pp. 310–317, 2011.
[21] D. T. Wong, "Salivary diagnostics powered by nanotechnologies, proteomics and genomics," *Journal of the American Dental Association*, vol. 137, no. 3, pp. 313–321, 2006.
[22] Hye Won Park, Jun-Seop Shin, Chan-Wha Kim .Proteome of mesenchymal stem cells. Proteomics.2007; 7: 2881-94.
[23] Xia Q, Wang T, Taub F. Quantitative proteomics of intracellularPorphyromonasgingivalis. Proteomics2007; 7(23): 4323-37.
[24] Hendrickson EL, Xia Q, Wang T, Lamont RJ, Hackett M. Pathwayanalysis for intracellular Porphyromonasgingivalis using a strain ATCC33277 specific database. BMC Microbiol2009; 1(9):185.
[25] Kathariya R, Pradeep A. Salivary proteomic biomarkers for oraldiseases: a review of literature. Archives of Oral Sciences & Research. 2010;1(1):43-49.
[26] 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.
[27] Hye Won Park, Jun-Seop Shin and Chan-Wha Kim (2007). Proteome of mesenchymal stem cells. Proteomics; 7, 2881- 94.
[28] UCLA.Human Salivary Proteome Project Website. Available at: hspp.dent. ucla.edu/OFNASET.htm. Accessed Dec. 22, 2011.
[29] Holly McKnight,* W. Patrick Kelsey,* Deborah A. Hooper,* Thomas C. Hart,†and Angelo Mariotti.Proteomic Analyses of Human Gingival and Periodontal Ligament Fibroblasts. J Periodontol 2014;85:810-818.
[30] G. Greenstein and T. C. Hart, "Clinical utility of a geneticsusceptibility test for severe chronic periodontitis: a critical evaluation," *Journal of the American Dental Association*, vol. 133, no. 4, pp. 452–459, 2002.
[31] A. F. Yakunin, A. A. Yee, A. Savchenko, A. M. Edwards, andC. H. Arrowsmith, "Structural proteomics: a tool for genomeannotation," *Current Opinion in Chemical Biology*, vol. 8, no. 1,pp. 42–48, 2004.
[32] H.-L. Liu and J.-P. Hsu, "Recent developments in structuralproteomics for protein structure determination," *Proteomics*,vol. 5, no. 8, pp. 2056–2068, 2005.
[33] G. Cesareni, A. Ceol, C. Gavrila, L. M. Palazzi, M. Persico, andM. V. Schneider, "Comparative interactomics," *FEBS Letters*,vol. 579, no. 8, pp. 1828–1833, 2005.
[34] G. A.McQuibban, J.-H. Gong, E. M. Tam, C. A. G.McCulloch,I. Clark-Lewis, and C.M. Overall, "Inflammation dampened bygelatinase a cleavage of monocyte chemoattractant protein-3,"*Science*, vol. 289, no. 5482, pp. 1202–1206, 2000.
[35] T. Pawson and P. Nash, "Assembly of cell regulatory systemsthrough protein interaction domains," *Science*, vol. 300, no.5618, pp. 445–452, 2003.
[36] J. Godovac-Zimmermann and L. R. Brown, "Perspectives formass spectrumetry and functional proteomics," *Mass SpectrometryReviews*, vol. 20, no. 1, pp. 1–57, 2001.
[37] M. Monti, S. Orr`u, D. Pagnozzi, and P. Pucci, "Functionalproteomics," *ClinicaChimicaActa*, vol. 357, no. 2, pp. 140–150, 2005.
[38] L. C. P. M. Schenkels, E. C. I.Veerman, andA.V.N.Amerongen,"Biochemical composition of human saliva in relation to othermucosal fluids," *Critical Reviews in Oral Biology and Medicine*, vol. 6, no. 2, pp. 161–175, 1995.