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Review Article

Dentistry Section

Exploring the Scope of Salivary Proteomics in Periodontal Diagnosis: A Narrative Review

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BY-NC-ND

ABSTRACT

Periodontal disorders are among the most widespread chronic illnesses globally which impact the tooth-supporting tissues and have a multitude of ramifications for one's quality of life. The purpose of periodontal diagnostic methods is to offer meaningful information to clinicians about the type, location, and severity of periodontal disease so that disease monitoring and therapy may be established. Due to a lack of proper molecular diagnostic tools, their early detection is still difficult. Proteomics, the promising new "omics", has become an important complementary tool to genomics providing novel information and greater insight into biology. The value of multidimensional peptide resolving approaches in the characterisation of complicated proteomes cannot be overstated and thus can very much be used as diagnostic biomarkers in periodontal world. Since proteomics is considered more intricate and complicated than genomics, the sound and rich facts of proteins concerned in periodontitis can be applied in the analysis, avoidance and management of periodontal diseases. Despite several novel protein markers being recently enlisted by discovery proteomics through various methods such as Enzyme Linked Immunosorbent Assay (ELISA), immunoassays with Mass Spectroscopy (MS), Protein Topography and Migration Analysis platform (ProtoMap), Matrix Assisted Laser Desorption (MALDI) etc., their routine diagnostic application is hampered by the lack of validation platforms that can rapidly, accurately and simultaneously quantify multiple proteins in large populations. Furthermore, saliva contains a non invasively collected store of clinically relevant biomarkers, which, if properly employed, might aid in early illness detection and monitoring. Hence, in this context, this review aims to discuss the unravelling field of proteomics in brief and the scientific sphere of its development from laboratory to clinical practice and its implications in the field of periodontology.

Keywords: Biomarkers, Genes, Liquid chromatography, Mass spectrometry, Proteins, Proteolytic enzymes

INTRODUCTION

Proteomics is a relatively fresh 'postgenomic' discipline with enormous promise in the era of 'Molecular transition' in periodontal research [1,2]. The shift from non destructive established gingival inflammation to destructive periodontitis is characterised by alterations in molecular markers. This change is expected to be reflected not only in host reactions, but also in molecular alterations in the oral microbiota [3]. The molecular knowledge of periodontium is complicated by the intricate interactions of matrix and cells within compartmental groupings [4]. To accomplish so, it's critical to understand the science of heterogeneity of the periodontium, as well as the molecular underpinnings. Proteomics, genomics, metabolomics and biomarkers have all advanced in importance as tools for diagnosing periodontal disease and assessing treatment responses [5]. Periodontal proteomic markers range from a wide variety of potential biomarkers like Immunoglobulins (Ig) (IgA, IgG, IgM, sIgA), by-products of tissue breakdown (collagen telopeptides, proteoglycans, osteocalcin, fibronectin fragments and bone collagen fragments), Pyridinoline cross linked carboxyl terminal telopeptide of type I collagen (pyridinoline, deoxy-pyridinoline, N-telopeptides and C-telopeptide), osteopontin, host factors that include host response cells like monocytes, Polymorphonuclear leukocytes (PMNs), macrophages, IL-1B, TNF-α and PGE2, host cells like immune cells, and periodontal ligament fibroblasts along with host derived enzymes like aspartate aminotransferase, acid phosphatase, elastase, cathepsin B, Matrix Metalloproteinases (MMPs) and microbial factors from *Treponema denticola* (T.d), *Aggregatibacter actinomycetemcomitans* (A.a), *Porphyromonas gingivalis* (P.g), *Tanerella forsythia* (T.f), ions like calcium, lactoferrin, platelet activating factors and hormones like cortisol [6].

Wilkins MR et al., coined the term 'Proteomics' as a combination of the words "protein" and "genome" and proteomics is the study of the portion of the genome that is expressed [7]. This will fluctuate throughout time depending on the unique demands or stresses that a cell or organism must withstand. It is a science that seeks the systematic and large-scale examination of a cell', organism's complete protein composition in order to better comprehend the protein signature of physiological and pathological events [8]. Bone and tooth structural proteomics, as well as oral fluid diagnostics, are the two main areas where dental proteomics has shown promise [9].

This review article was written to help the readers to grasp the principles of proteomics, a fascinating research horizon in periodontology that has the potential to be a vital tool for the early identification and prevention of oral pathologies that are genetically regulated directly or indirectly.

RATIONALE OF PROTEOMICS IN PERIODONTICS

Not only does proteomics shed new light on the complex interplay that periodontal pathogens on their hosts in both health and disease, it also brought prominence on the involvement of periodontal ligament fibroblasts and numerous disease-related protein markers, which provide insight into the periodontal ligament's physiology [10].

Is Proteomics Better than Genomics?

Proteomics is a significantly more sophisticated concept than genomics, owing to the fact that, while the genome remains essentially stable from cell to cell and throughout time, the proteome fluctuates. This is due to the fact that different cell types express different genes [4]. A major influence is the huge increase in potential complexity when switching from genome and transcriptome studies to proteome analysis. DNA and 4-nucleotide m-RNA's codes are translated into a significantly more sophisticated 20-amino-acid code, with primary sequence polypeptides of varying lengths folded into one of an incredibly large number of conformations and chemical modifications to produce a final functioning protein. Similarly, alternative splicing can result in several isoforms of the same protein

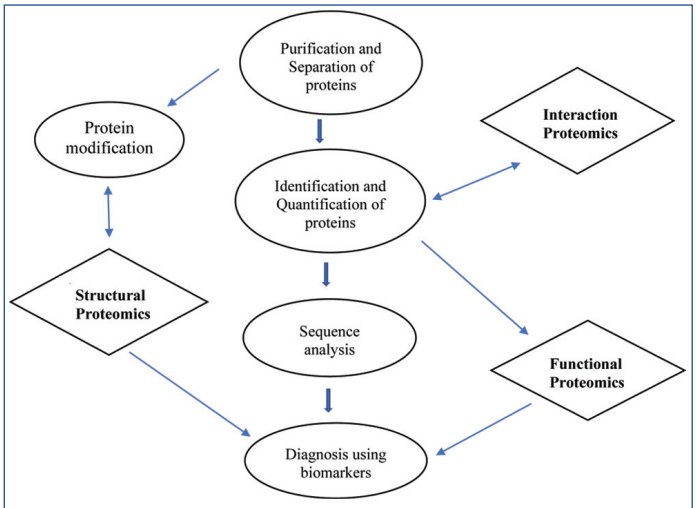
[11]. Previously, this was done by m-RNA analysis, however it was shown that this did not correspond with protein content [12]. It is now well understood that m-RNA does not necessarily result in protein [13]. The amount of protein output per unit of m-RNA is determined by the gene from which it is transcribed as well as the cell's existing physiological state [14].

Although variations are caused by m-RNA translation, many proteins are also subjected to a range of chemical changes following translation which would include methylation, nitrosylation, acetylation, phosphorylation, ubiquitisation, glycosylation and oxidation all of which are significant to the protein's functionality [15]. These post-translational alterations are studied using techniques like glycol-proteomics and phospho-proteomics. Some proteins go through all of these changes at the same time, indicating the level of complexity that can be encountered when investigating protein function and structure [16].

Proteomics is technology driven that involves the fractionation and separation of a substantial number of proteins derived from cells or tissues, that are identified and quantified using bio-informatics to better understand cell and tissue physiology, diagnose biomarkers, and develop drugs utilising target identification strategies [9].

TYPES OF PROTEOMICS

Different types of proteomics are depicted in [Table/Fig-1] [9].



[Table/Fig-1]: Types of Proteomics.

Structural Proteomics

Structural proteomics is a branch of biology that studies the association between protein sequence, structure, and function integrating structural data from a whole inventory of three-dimensional pictures for all proteins in an organism [17].

Functional Proteomics

Functional proteomics is a branch of biology that focuses on tracking and analysing the spatial and temporal features of molecular networks and flexus in viable cells [18].

Interactive Proteomics

Interactive proteomics focuses at physical interactions between proteins and generates disease and pathway-specific protein interaction networks. Proteins' intrinsic chemical and structural diversity, as well as their various expression levels and subcellular localisations, present unique hurdles for network investigation, necessitating the employment of a number of novel and inventive methodologies [19].

METHODS OF PROTEOMICS ANALYSIS

[Table/Fig-2] describes various methods of proteomic analyses [20-25].

S. No.	Type of proteomic analysis	Biomarkers detected
1.	Enzyme Linked Immunosorbent Assay (ELISA) [20]	Carbonic anhydrase 6, zinc-alpha-2-glycoprotein, Ig mu chain C region, prolactin-inducible protein, interferon-induced very large GTPase-1, S100A8, S100A9.
2	Immunoassays with Mass Spectroscopy (MS) [21]	Lactoperoxidase, leukotriene A4 hydrolase, adenylyl cyclase-associated protein-1, catalase, Rho GDP-dissociation inhibitor-2, S100P, MMP-9, neutrophil colleganase, neutrophil defensin, peptidoglycan recognition protein-1,gelsolin, profilin-1, lactotransferrin, calreticulin, plastin-2, fibrinogen alpha chain, α-2 macroglobulin, complement C3, α-2 HS-glycoprotein, ceruloplasmin
3	ProtoMap (Protein Topography and Migration Analysis Platform [22]	Aminoacyl-transfer ligase, GTP1/OBG family, MSS2, ERA/THDF family, RAN, RAS superfamily, GAG
4	MALDI (Matrix Assisted Laser Desorption/Ionization) [23]	Immunoglobulin (Ig) kappa variable 4-1, haptoglobin, MMP-8, 7 peptides.
5	Two-dimensional polyacrylamide gel electrophoresis [24]	Ig heavy chain V-III region, haemoglobin and albumin, cystatin, α-amylase
6.	Capillary Electrophoresis (CE) [25]	Alanine, taurine, pipecolic acid,leucine+Isoleucine, histidine, valine, tryptophan, glutamic acid, threonine, carnitine

[Table/Fig-2]: Various methods of proteomic analysis [20-25].

Enzyme Linked Immunosorbent Assay (ELISA)

The ELISA is a technique for identifying and quantifying proteins. The 'peptidomatrix' ELISA is a competitive assay that incorporates peptides that are either specific for a target protein or detected amongst protein's tryptic digestion byproducts [26]. Antibodies against small peptides that are expected to be released by tryptic digestion of cells are specific to the protein under investigation are used in this procedure [27]. The bound primary antibodies are detected using a tagged secondary antibody. To construct a calibration curve, antibodies are combined with soluble synthetic peptide in defined concentrations in the analysis itself. The signal is influenced by the peptide in solution interfering with the bound peptide. The signal will be maximum when no free peptide is present, and intermediate when a little level of available peptide is present and minimal when substantial amount of competitive peptide is present [28]. In a research conducted by Shin MS et al., saliva samples were collected from 207 individuals. Salivary S100A9 and S100A8 levels were significantly greater in those who did not have periodontitis, pertaining to shortgun proteomics, and salivary biomarkers S100A9 and S100A8 were validated by ELISA to be useful in evaluating for periodontitis, reducing periodontal morbidity and increasing periodontal health [20]. Another cross-sectional study conducted by Kim HD et al., recruiting 149 adults out of which 50 no or initial periodontitis (NIPERIO), 99 established periodontitis (PERIO) and compared S100A8 and S100A9 markers in saliva, blood and Gingival Crevicular Fluid (GCF). They concluded that even though S100A8 and S100A9 are found in body fluids like saliva, blood and GCF, it was considerably higher in PERIO group than in NIPERIO group. In addition to this, salivary S100A8 was positively correlated to blood S100A8 indicating that salivary S100A8 might be useful in screening periodontitis [29].

Immunoassays with Mass Spectroscopy (MS)

The MS is a powerful analytical tool for analysing the structure and chemical characteristics of individual molecules, as effectively as quantifying known materials and detecting novel chemicals within a sample [30]. It has been widely utilised as an analytical technique for the evaluation of proteins that have undergone post-translational alterations since its inception more than a century ago [31]. In MS, the ionisation source produces ions from the sample, which are then separated in a mass analyser so according to their m/z

ratio. Subsequently, the chosen ions are fragmented and analysed in a separate analyser. A detector that translates the ions into electrical signals is used to detect the ions issuing from the last analyser and determine their abundance and process the detector signals that would be sent to the computer while using feedback to control the device [32]. In 2013, Salazar MG and colleagues identified 344 human protein categories in whole saliva samples from 20 periodontally healthy and diseased subjects by adopting Liquid Chromatography (LC)-MS/MS spectra. There was a total 1.5-fold difference in abundance between controls and patients for 20 proteins indicating that the periodontally diseased condition was connected to the acute phase response and inflammatory process by functional annotation of proteins [32]. A study conducted by Guzeldemir-Akcakanat E et al., on molecular signatures of chronic periodontitis performed comparative proteomic analysis by LC-MS/MS and revealed that the most upregulated proteins in chronic periodontitis compared with controls were BP1, ITGAM, CAP37, PCM1, MMP-9, MZB1, UGTT1, PLG, RAB1B, HSP90B1. The integrative data from transcriptomes and proteomics revealed MZB1 as a potent candidate for chronic periodontitis [33].

ProtoMap (Protein Topography and Migration Analysis Platform)

The ProtoMap enables for the detection of post-translational modification proteolysis-induced changes in gel migration. The ProtoMap strategy is a degradomics method for screening protease substrates in complicated protein environments [34]. To isolate proteins, sodium dodecyl sulphate polyacrylamide gel electrophoresis is performed according to their relative mobility. The gel lanes would then be slided into horizontal sections, and trypsin will be used to digest individual gel slice. Using bioinformatics and LC -tandem MS, the extracted protein and peptides are sorted. The data is combined via software to make a peptograph, which is a visual depiction of sodium dodecyl sulphate polyacrylamide gel electrophoresis. [35]. Dix MM et al., found that the proteomic profiles comprised 91 known caspase substrates as well as 170 new proteins that were not previously documented to be disrupted during apoptosis. Remarkably, independent of the level of cleavage, the overwhelming bulk of proteolysed proteins yielded persistent fragments that correlate to distinct protein domains, implying that the synthesis of active effector proteins is a pivotal part of proteolytic apoptotic cascades [36]. In periodontology, it was recently confirmed that regulatory cross-talk occurs across proteases, either by direct cleavage or by targeting the appropriate inhibitors, regardless of protease type or traditional biochemical pathways [37]. The complicated activation of MMP-2 by membrane type-1 MMP (MT1-MMP, MMP14) at the cell surface, which needs adequate stoichiometric quantities of furin-activated MT1-MMP and TIMP2 to form a 1:1 complex, is detailed for the interplay between endogenous inhibitors and MMPs in the protease web [38]. Similarly, chemokine regulation has been recently observed by cysteine cathepsins, which activate non ELR chemokines such as SDF-1 (Stromal cell Derived Factor 1, CXCL12) [39]. As a result, knowing the N-terminus of a chemokine is critical when analysing a patient's health state.

MALDI (Matrix Assisted Laser Desorption/IONIZATION)

The MALDI aids in the fast identification of proteins in specific combinations [40]. An equal volume of CHCA matrix solution is combined with around 0.4 µ of the isolated peptide solution. (10 mg/mL-cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% trifluoroacetic acid) and allowed to dry. Protein identification data was collected via a 4700 proteomic analyser. A neodymium-doped yttrium aluminium garnet (Nd:YAG) laser with a 200 hz repetition rate is deployed to acquire both MS/MS spectra data. In MS, 1600 photons are often amassed for spectra. The ones with the strongest ion signals are chosen as MS/MS precursors and external calibration with trypsin autolysis peptides [41].

Tang H et al., conducted a study in 2019 that acquired WUS, GCF and BS from 50 patients (17CP;17G;16PH) and subjected them to MALDI-TOF; MS. There were 91 peptides discovered, seven (1836.4, 1858.4, 1044.0, 1122.0, 1147.1, 1583.9, 3434.4 Daltons) of which showed statistically significant differences between the chronic periodontitis and periodontally healthy groups, and 10 of which showed significant upregulation in the gingivitis group. There were 48 peptide peaks discovered in the gingivitis group, three (1044.0,1122,4918.2 Daltons) of which were significantly distinct from the periodontally healthy group [42].

Two-Dimensional Polyacrylamide Gel Electrophoresis

It is used to separate complicated protein combinations from biological specimens [43]. For passive rehydration of 7 cm IPG strip 3-10 NL overnight at room temperature, an equal quantity of proteins soaked in rehydration buffer (8 M urea, 0.2M EDTA, 0.5 M dithiothreitol, NP-40, ampholyte, glycerol solution Ph 3-10 in 0.5 M Tris-Hcl) at 20°C, proteins are focused using equilibrium buffer I (6 M urea, 30% glycerol, 50 mM Tris-Hcl, 2% sodium dodecyl sulfate, Ph-8.8, 1% DTT) and equilibrium buffer II (6 M urea, 30% glycerol, 50 mM Tris-Hcl, 2% sodium dodecyl sulfate, 2% SDS Ph-8.8 and 2.5% iodoacetamide) The targeted proteins are separated on a 12.5% SDS-polyacrylamide gel electrophoresis after equilibrium upon electrophoretic separation onto 12.5% polyacrylamide gels. Silver staining is employed to monitor the gels and determine proteins [44]. Bergamini S et al., examined putative proteome changes generated by Chlorhexidine (CHX) and CHX-Anti-Discolouration System (ADS) using one- and two-dimensional gel electrophoresis in a study published in 2021. After resective bone surgery, curettage was conducted to acquire Tooth Surface Collected Material (TSCM) from three groups of patients that included placebo rinses, CHX, or CHX-ADS mouthwash. Before surgery at baseline circumstances, the proteome was analysed, and four weeks later, as CHX was administered (or not) as a chemical plaque control. Only after CHX treatment did changes in the TSCM proteome become apparent. After CHX-ADS treatment, no changes were seen. An ADS could prevent TSCM from developing and the antiseptic effect of CHX limits its potential to change bacterial cell permeability [45].

Non Gel Based Proteome Separation Techniques

Other approaches, notably as Multidimensional Liquid Chromatography (MUD-LC) in conjunction with Electrospray Ionisation MS (ESI-MS/MS) spectra, have acquired popularity as a result of the limitations of traditional proteomic approaches like not being accurate to identify minor spots and techniques like MALDI do not promote the detection of hydrophobic and basic peptides [46]. In these methods, complex protein combinations are processed in solution. One or more steps of capillary chromatography are used to fractionate the peptide mixtures which are then analysed by MS in a data-dependant manner. For differential protein expressed assessments, alternative or complementary MS-based techniques have been developed and are continually being refined [47]. They are predicted on stable isotopes (12C/13C/14N/15N/1H/2H) as well as differentially labelling perturbed and non perturbed protein isolates. Differential labelling will be workflow where peptide mixes are obtained by digesting a mixed protein sample and fractionation of peptide mixtures by chromatography MS/MS spectra analysis of the isolated peptides [48].

Classification of Techniques Based on Labelling Methods by Quantitative Proteomics [49]

A) Metabolic Isotopic Labelling- SILAC (Stable Isotope Labelling with Amino-acids)

Isotype-labelled proteins are produced by adding stable isotype labels to culture conditions during cell development. Mann and colleagues used a single isotopic amino-acid in cell culture. The mass disparity among both tagged and untagged proteins is

anticipated in this instance, making MS/MS spectra interpretation simpler [50] and it was considered as a straight forward, low-cost precise method [51].

B) Chemical Labelling- ICAT (Isotope Coded Affinity Tags)

The Aebersold group created Isotope Coded Affinity Tags (ICAT) reagents which are the most widely used approach in quantitative proteomics [52]. The 'heavy' as well as 'light' of the ICAT reagent were used to chemically mark protein sulph-hydryl groups. An endo-protease digests both materials after they've been mixed together. The peptide is isolated and fractioned using MUD-LC reversed-phase microcapillary chromatography employing strong cation exchange where LC is widely utilised. The MS/MS spectra analysed using an ESI tandem mass spectrometer and peptide sequences are found using an automated database search which allows MS data to calculate relative protein abundance [53].

C) iTRAQTM (isobaric Tagging for Relative and Absolute Quantification)

This is a collection of amine-specific stable-isotope reagents that can concurrently label peptides in up to four separate biological samples, allowing for relative and absolute quantification from MS/MS spectra [54]. This approach makes use of isobaric tags, which may be cleaved during collision-induced dissociation to produce an isotope series (reporter ions) that represents the quantity of a single peptide of known mass from up to four separate samples. The benefit of iTRAQTM over other isotope tagging technologies is that the label is cleaved in MS/MS before quantification, removing competing untagged isobaric species from the equation [55].

D) SELDI-TOF (Surface Enhanced Laser Desorption/Ionisation-Time Of Flight)

Protein profiling: SELDI-TOF-MS can be employed to analyse complex protein mixtures segregated by on-chip retentate chromatography [56]. Prefractionated biological fluids are deposited onto numerous chemically modified supports-protein chip arrays with specified chromatographic characteristics (anionic, cationic, hydrophilic, hydrophobic, ion metal chelating). The second phase involves co-crystallising immobilised proteins with just a matrix on a target surface and obtaining MS spectra utilising a customised mass analyser, which is MS spectra acquisition, a SELDI-TOF mass spectrometer [57]. The SELDI-TOF MS integrates retention and MS, contrasting LC-MS which is focused on elution. It does not, in most cases allow for the direct identification of proteins that could be used as disease biomarkers. Eventually, peak correlations are conducted utilising multifactorial bioinformatic software tools during data analysis [58]. This approach works best with protein and protein fragments that are fewer than 20 kilodaltons in size. Despite the fact that it is limited by the same dynamic range as other MS technologies, the approach provides a quick and repeatable analytical tool for comparing protein expression profiles [59].

E) Capillary Electrophoresis (CE)

Capillary electrophoresis is a peptide separation strategy that offers an alternative to both 2D-electrophoresis and chromatography [4]. Due to the inherent fast analysis durations, minimal sample and reagent utilisation, high performances and a variety of flexible separation modes, CE has become extremely promising and advantageous in many proteomic applications, despite being dominated by LC methods [60]. In a review by Dolník V various advancements in capillary coatings, detection methods, separation modes, integration to microfluidic chips and bio-analysis application since the introduction of CE have been described [61]. In the field of proteomics, the development of CE using principally two detection technologies, MS and LIF has resulted in biologically relevant data [62]. Sugimoto M et al., used capillary electrophoresis time of flight MS to undertake a comprehensive metabolite of saliva samples derived from pancreatic, breast cancers, periodontitis and healthy controls, the profiles revealed that majority of the metabolites observed in malignancies had higher quantities than those detected in persons with periodontal disease and control subjects. Thus, we can predict susceptibility to disease using quantitative data for the metabolites like alanine, taurine, pipecolic acid, leucine + Isoleucin, histidine, valine, tryptophan, glutamic acid, threonine, carnitine and their combinations [25].

Biological Relevance of Proteomics in Periodontology

1. Periodontal ligament fibroblasts

The cytoskeleton proteins vimentin, tubulin, actin as well as stress folding enzymes, metabolic enzymes, membrane trafficking proteins, cellular mobility proteins and chaperones are found in fibroblasts. Their biological significance is to maintain the functionality and homeostasis of periodontal ligament fibroblasts [63]. Xiong J et al., compared the cell surface proteome of human Periodontal Ligament Stem Cells (hPDLSC) to that of human fibroblasts in a 2016 study. The MS was used to identify protein 'spots'. Four proteins previously linked to mesenchymal stem cells were tested for validity: Sphingosine kinase-1, Annexin A2, CD73 and CD90. CD73 and CD90 were found to be strongly expressed by human PDLSC and gingival fibroblasts, but not by keratinocytes, in a flow cytometry study, suggesting that these antigens may be involved in wound healing and could be leveraged to distinguish between epithelial and mesenchymal cell lineages. Gingival fibroblasts and human PDLSC had annexin A2 on their cell surfaces, while human keratinocytes did not exhibit the protein. Sphingosine kinase-1 expression on the other hand was detected by immunocytochemical examination in all of the cell types studied. These proteomic-analysis lay the groundwork for further defining the PDLSC surface protein expression profile to better understand the cell population and develop new purification processes for these stem cell variants [16].

2. Periodontal ligament cells undergoing mineralisation

Nuclear proteins, cell membrane attached proteins, cytoskeleton proteins and cytoskeleton linked proteins have all been identified. The biological significance of these proteins is that they help to maintain periodontal tissue homeostasis [64]. Tandem mass tags quantitative proteomics was used in work by Li J et al., to identify the temporal protein expression pattern during osteogenic differentiation of hPDLSC on day 0, 3 and 14 of testing the extracted samples with total RNA and proteins of osteogenic hPDLSC and Defence Proteins (DFPs) [65]. Integrated Pathway Analysis Software was used to undertake pathway enrichment analysis employing the Kyoto Encyclopedia of Genes and Genome database, accompanied by predicted activation. The hub protein SOD2 was evaluated by Western blotting in interaction networks of Oxidative Phosphorylation and Redox-Sensitive (OXPHOS) signalling pathways. A total of 1024 DEPs were identified out of which SOD2, a key component of the sitrulin pathway shows a steady rise during osteogenesis. This research revealed a dynamic regulating mechanism of hPDLSC osteogenesis, which could open up new avenues for periodontal regeneration research [65].

3. Periodontal pathogens

The PG1089, PG1385 and PG2102 are the proteins responsible for *Porphyromonas gingivalis* (*P. gingivalis*) pathogenicity [66]. Cytoplasmic proteins regulated by acidic pH such as anthranilate synthase, NADP- specific glutamate dehydrogenase and alkaline pH regulated proteins such as fructose bisphosphonate aldolase, pyruvate kinase and enolase has been discovered in *Fuscobacterium nucleatum* (*F.nucleatum*) [67]. Ali Mohammed MM et al., in 2021 conducted research into the interactions between the salivary

component of the proteome and the task is to uncover proteins involved in biofilm formation in P.gingivalis and F.nucleatum. The synthesised proteomes of P.gingivalis and F.nucleatum, cultured in biofilm or planktonic culture, as much as mono and dual species models, were investigated using LC tandem mass spectrometry. They discovered substantial variations that include 593 *P.gingivalis*, 1069 *F.nucleatum* and 797 of all proteins being quantified under both biofilm and planktonic conditions with Label-Free Quantification (LFQ) with intensities of 7%,0.4% and 14%, respectively. Also, LFQ intensities over 90% of the significantly modified P.gingivalis proteins had their levels drastically dropped [68].

4. Relevance of salivary proteomics in periodontitis subjects

Haptoglobin, prolactin inducible protein, parotid secretory protein and S100 proteins were found in saliva [69]. They are linked to host defense and being exposed as novel possible biomarkers for monitoring periodontitis disease activity. Salivary indicators were explored to predict and prevent periodontitis in a study undertaken by FARR H et al., [10]. The salivary proteome of participants with chronic periodontitis and periodontal health was studied using mass spectrometry. Ten periodontally healthy and 30 chronic periodontitis patients had their whole saliva stimulated and combined into five healthy control samples and 15 chronic periodontitis samples. Thioredoxin, androgen regulated protein, cystatin- SA, histatin-1 and fatty acid binding protein were discovered to correlate substantially with signs of periodontal attachment apparatus loss and inflammatory process in diseased patients, contributing to the identification of disease predictors or biomarkers for chronic periodontitis [10]. Periodontal biomarkers discovered using proteomic analysis are mentioned in [Table/Fig-3] [20,42,70-73].

Periodontal biomarkers In saliva	Proteomic analysis performed	Observations
S100A8, S100A9 [20]	Shortgun proteomics	Increase in the biomarkers
Haptoglobin [42]	MALDI-TOF-MS	Biomarkers increase with peptide sequences of HYEGSTVPEK and AVGDKLPECEADDGCPKPPELAHGYVEHSVR
α-antitrypsin Immunoglobulin κvariables 4-1 Immunoglobulin heavy constant γ 2 [70]	MALDI-TOF-MS followed by nano LC/ESI-MS/MS	Increase in the biomarkers
Haemopexin β 2 Glycoproteins Plasminogen α & β Fibrinogen [71]	30 minutes Multiplexed LC-MRM method	Increase in the biomarkers
Apolipoprotein H [71]	30 minutes Multiplexed LC-MRM method	Increased biomarker. Truly novel and non invasive approach to distinguish between chronic periodontitis and aggressive periodontitis at early stages.
Resistin MMP-8 Galactin-3 [72]	LC-MS	Increase in the biomarkers Decreases but its specific role in chronic periodontitis is yet to be determined
Periodontal pathogens [73]	Non targeted short gun proteomics combined with label-free quantitative proteomics	Detection of *Aggregatibacter actinomycetemcomitans, Campylobacter rectus, F.nucleatum, P.gingivalis, Prevotella oralis, Streptococcus anginosus, Treponema denticola, Veilonella dispar, Streptococcus oralis*

[Table/Fig-3]: Periodontal biomarkers discovered using proteomic analysis [20,42,70-73].
MALDI-TOF-MS- Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectroscopy
LC/ESI-MS/MS- Liquid Chromatography- Electrospray Ionization- Mass Spectroscopy (MS)
LC-MRM- Liquid Chromatography- Multiple Reaction Monitoring
LC-MS Liquid Chromatography- Mass Spectroscopy (MS)
MMP- Matrix Metalloproteinases

Periodontal Challenges

The periodontium is a complex tissue structure that necessitates a better understanding of the whole variety of cellular along with matrix proteins for future advancements. Bosshardt DD undertook an in-depth investigation into cemental subtypes concluding that some of them are structurally similar to alveolar bone [74]. It's exceptionally difficult to understand the unique mechanism that drives cemental subtype growth, synthesis, and remodelling since this precise collection of proteins contained in any of these complexes as well as their post-translational adaptations, is unknown. To solve this difficulty, researchers used a standard gene to protein technique which involves isolating important genes first, then discovering their functions. Another proteomic method examines the full set of expressed gene products, including their function [8]. As a result, it is evident that proteomics is becoming a more complex field, and it has only been possible to analyse the proteome since the development of new MS techniques in the mid-1990s. Because proteins are abundant in a cell and their function is dependent on not only their abundance and post-translational changes, their cell location and association with other proteins, all of which can change in a fraction of a second, more specific tests are required to eliminate errors in proteomic analysis for a prompt diagnosis [75].

Future Perspective

The discovery of prospective novel medications for chemotherapeutic purposes is one of the most exciting implications of studying human genes and proteins. When a protein is associated in a disorder, its three-dimensional structure seems to provide relevant data that can be used to develop drugs that interfere with the protein's action. Furthermore, 'Virtual Ligand Screening,' a computer technique for fitting thousands of tiny molecules within the protein's three-dimensional structure, scores the standard of match up to numerous locations in the protein having the purpose of whether bolstering or deactivating the protein's function [76].

CONCLUSION(S)

Proteomics and gene expression knowledge will help to improve periodontal disease diagnosis and therapy. Its utility in dentistry, however, will hinge on how skillfully oral healthcare providers assimilate it in and out of their practices, considering it necessitates a solid grasp of human genetics as well as the adoption of novel diagnostic and therapeutic technology. Given the complex nature of periodontal disorders, the development of effective diagnostic methods for periodontitis prevention and treatment will require a mix of salivary proteomic profiles, microbial and immune response biomarkers, as well as genetic and epigenetic variables in larger populations.

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