Proteomics for the periodontium: current strategies and future promise

Christopher A. McCulloch

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. At present there is no catalog of the total expression complement of matrix and cellular proteins in any of alveolar bone, cementum, gingiva and periodontal ligament. Further advances in our understanding of mechanisms of homeostasis and responses to trauma and infection of periodontal tissues will likely require a more complete catalog of the repertoire of expressed proteins. Currently used proteomics methods can provide global analyses of expressed proteins in specific mammalian cells and tissues. Recent progress in tissue isolation, protein separation, quantification, sequence analysis, and structural and interaction proteomics offers great promise for bringing periodontal physiology and pathology into the modern era. Yet remarkably few applications of proteomics to the analysis of periodontal tissues have been reported. This chapter reviews 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.

Challenges of the periodontium

Depending on one’s experimental viewpoint, the periodontium can be considered in many ways, 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. Detailed reviews of periodontal structure, cell and matrix structure and tissue dynamics have been provided earlier (13, 27, 34). Briefly, the complexities of periodontal tissue structure underlie the expression patterns of multiple cell types that are regulated by exquisitely well-integrated control systems (31). To maintain root attachment to alveolar bone during periods of remodeling and tooth movement, continuous adaptations of the periodontal ligament, cementum, bone and gingiva must be coordinated between all tissues. Currently, we have a limited understanding at the molecular level of how the remodeling of these different tissues is integrated, in part because the molecular basis of tissue heterogeneity is not well-defined. For example, Bosshardt, in a recent thoughtful review (4), has pointed out that cementum comprises multiple tissue types and that some of these subtypes have a structural similarity to alveolar bone. In the absence of a defined set of proteins that are actually present in these different
cementum types, and the interactions that these proteins exhibit in vivo, plus an understanding of their post-translational modifications, it will be difficult to understand what mechanisms determine cementum subtype development, formation and remodeling. The traditional approach to solving this problem would be to isolate single genes of interest and then determine the function of each gene in a serial fashion. This approach is effective but is not rapid. The availability of complete, well-described genome sequences for several eukaryotic organisms has provided an option for the ‘one gene–one protein’ hypothesis approach. An alternative approach which is embraced by modern proteomics analysis is to examine the whole set of expressed genes or proteins and assess their functional importance as a group (Fig. 1). In the context of tissue and cell complexity in the periodontium, the application of more global experimental approaches for determining expression profiles is timely. Proteomics methods may be able to substantially advance the field by, for example, facilitating the definition of cell and tissue subtypes on the basis of expressed proteins. A major challenge is to determine how the complement of expressed cellular proteins – the proteome – is organized into functional, higher-order networks and to develop global protein–protein interaction networks on a cellular or tissue level (10).

The proteomic armamentarium contains a wide range of technical approaches and no single technology is suitable for every single application. The different proteomic technologies exhibit various strengths and shortcomings. As the various steps of proteomic analyses require multiple technologies that are not intrinsically related, organization and integration of the analytical steps is a key point for future success. Further, unlike nucleic acids, which can be readily amplified (e.g. with polymerase chain reaction), no such method exists for proteins. Therefore the sensitivity of detection methods, particularly for low abundance proteins, is an important experimental consideration. Further, some of the most sensitive proteomic technologies such as the yeast two hybrid system (which detects binary protein interactions) exhibit reasonably high rates of false-negative and false-positive outcomes (38). Consequently, a good working knowledge of the strengths and pitfalls of proteomic component strategies is useful when considering specific experimental applications. Considered below are some of the critical steps and technologies currently in use in proteomics analysis.

Cell and tissue fractionation

For analysis of dissected periodontal tissues, sections through periodontium or cultured periodontal cells, fractionation of cells and matrix followed by protein separation are the first steps required prior to protein analysis (Fig. 2). A major limitation for achieving successful fractionation is the production of an ideal homogenate in which organelles and other cellular constituents are released as a free suspension of intact, individual components. Compounding these difficulties is limited accessibility to mineralized tissues or the tissue in between mineralized tissues (e.g. periodontal ligament) without inclusion of adjacent ‘contaminating’ tissues. Further, a frequent problem is the formation of aggregates of cytoplasmic structures that may contain cytoskeletal polymers and various organelles. If organelles are trapped in cytoskeletal polymers, they may form large aggregates which tend to sediment. As aggregates frequently contain nuclei which release DNA during homogenization and cell lysis, significant loss of individual components of the homogenate may occur during the initial centrifugation step intended to remove nuclei. Moreover, as there are very large differences in the physical properties of extracellular matrices and cellular organization of the various types of periodontal tissues, homogenization conditions must be optimized for each tissue type or cell line.

For cultured cells, as an example, cells are cooled on ice, scraped into Ca²⁺/Mg²⁺-containing buffer to prevent cell breakage and the cells are collected by low-speed centrifugation prior to mechanical homogenization. The nuclei are removed by a second low-speed centrifugation step along with cell debris and some larger subcellular components. For more detailed analysis, nuclei can be separated from

---

**Fig. 1.** Simplified flow diagram showing the contrast between traditional, gene-to-protein analysis that has been used before global analysis of gene and protein expression became widespread. The global genomic and proteomic analysis approach considers that the phenotype is attributable to not just the expressed set of proteins but also their interactions, an important analytical strategy of proteomics.
pellets. The resulting postnuclear supernatant contains the cytosol and other organelles in suspension, which can be subsequently separated by gradient centrifugation. While several other techniques that rely on physical properties (e.g. electrical charge for free flow electrophoresis) or biological properties (e.g. ligand affinity for immunoabsorption) have been used for examining complex organelles, centrifugation is easily carried out in the lab and can be readily combined with analytical proteomics techniques. The postnuclear supernatant can be additionally fractionated by high-speed sedimentation / centrifugation (100,000 g), which separates the total membrane fraction from all soluble proteins using an ultracentrifuge or an airfuge. With this approach, cells can be fractionated into membranes, cytosol, and nuclei. The postnuclear supernatant can be further fractionated by density gradient centrifugation, an approach that separates membrane particles mainly by the ratio of their lipid to protein content.

**Protein separation**

Typically, proteomics involves separating the very large number of proteins in cells or tissue prior to analysis by mass spectrometry, followed by recognition and characterization with bioinformatics approaches (Fig. 2). Protein separations are performed at the protein or peptide level. Fractionation of samples can be performed according to a wide variety of parameters including post-translational modifications, or, for example, from their subcellular organelle localization. Pre-fractionation methods, including affinity, one- or two-dimensional gel electrophoresis, one- or two-dimensional chromatographic separation or ion-exchange, and reversed-phase resins, can demonstrate several hundreds of new species that are not detected in un-fractionated samples. Electrophoretic prefractionation protocols include electro-kinetic methods performed in free solution that utilize isoelectric focusing steps (33). Pre-fractionation of samples by removal of high abundance proteins can increase the dynamic range of detection and can facilitate detection of low abundance proteins. Subcellular fractionation (e.g. phagosomes; 11) can also be used to improve protein identification in organelles. It seems unlikely that a single chromatographic or electrophoretic procedure can resolve complex mixtures of peptides produced from a global proteolytic digest of a proteome. Consequently, combining two or more separation
procedures can improve the resolution of a larger number of peptides (16).

For analysis of complex protein mixtures derived from biological samples, two-dimensional polyacrylamide gel electrophoresis (28) remains an important technology. Proteins are separated in two sequential steps according to isoelectric point and molecular weight. The development of immobilized pH gradient strips for the first dimension of isoelectric focusing has improved the reproducibility and resolution of separations. A singular advantage of this approach is that differentially post-translationally modified forms of the same protein can be separated. Inter-gel comparisons following staining allow relative quantification of proteins between samples, a general method that has been facilitated by the use of fluorescence two-dimensional difference gel electrophoresis, which can improve reproducibility. In this approach, prior to two-dimensional SDS-PAGE separation, the samples are covalently labeled with different dyes (e.g. Cy3 and Cy5) and the samples are then mixed together and separated on the same two-dimensional SDS-PAGE gel. The gel can then be scanned at the specific excitation/emission wavelengths optimal for each dye, the images merged, and the differences of protein abundance computed. After separation, individual protein spots are excised, processed by in-gel proteolysis, and analyzed by mass spectrometry (e.g. matrix-assisted laser desorption ionization (MALDI)-mass spectrometry). The peptide fingerprint obtained from the MALDI-mass spectrometry can then be used for protein recognition.

In spite of the analytical power of two-dimensional electrophoresis, it is technically demanding, time-consuming, has a limited dynamic range and is not easily automated. Further, challenges of reproducibility, sensitivity, loss of protein during extraction, and failure of some hydrophobic, large or basic proteins to enter two-dimensional electrophoresis gels have limited the more general application of this technology. For proteins with extreme isoelectric points or molecular masses as well as for membrane proteins, two-dimensional electrophoresis gels have significant limitations (8). These factors prompted the development of nongel-based proteome separation techniques to overcome the limitations of two-dimensional electrophoresis while preserving the ability to resolve complex protein and peptide mixtures before mass spectrometry analysis. Capillary electrophoresis is an alternative to two-dimensional electrophoresis for protein separation and to chromatography for peptide separation. Two modes, capillary zone electrophoresis and capillary isoelectric focusing, can be applied to mass spectrometry-based proteomics. Capillary zone electrophoresis can be used as a rapid, final dimension before ionization for mass spectrometry. Capillary isoelectric focusing is better suited for first dimension separations in automated fractionation systems (35).

Mass spectrometry

Major advances in proteomics have relied in part on the improved ability of mass spectrometers to detect and characterize low amounts of proteins in biological samples. Mass spectrometer-based proteomic analysis is now being used more frequently in studies of interest to dental scientists including, for example, the analysis of Streptococcus mutans (20) and the analysis of osteoblastic differentiation (7). Mass spectrometry is particularly useful when limited sample material is available and when femtomole sensitivity is required. The general scheme for mass spectrometric analysis of an unknown mixture of proteins is to first separate the proteins from the biological sample (Fig. 3), digest the proteins (typically with trypsin), separate the peptides, and then analyze the proteins by mass spectrometry and sequence analysis.

Mass spectrometers comprise an ion source, one or several mass analyzers, and a detector. The principle of mass spectrometry is that gas-phase ions, produced in the ion source, are injected into the mass analyzer and discriminated based on their mass / charge \( m/z \) ratio. This ratio is determined on the basis of their movement in a vacuum while under the influence of electric or magnetic fields. Mass spectrometers continue to undergo rapid development, in part because of increased need for enhanced sensitivity, higher mass accuracy and resolving power, improved duty cycle and more efficient fragmentation of peptides (by tandem mass spectrometry). Electrospray and MALDI processes provide low-energy ionization methods for several different types of biological molecules such as peptides, proteins, oligonucleotides, and carbohydrates. MALDI instruments are robust and exhibit high sensitivity, and, when combined with tandem mass spectrometry methods and miniaturized peptide separation techniques, can produce significant improvements of overall sample resolution and enhanced sequencing of peptides. Electrospray ionization combined with tandem mass spectrometry is particularly useful for peptide sequencing. Several types of commercially
Available mass spectrometers combine electrospray or MALDI with a variety of mass analyzers. MALDI tandem mass spectrometry instruments are now increasingly used for sequencing of post-translationally modified peptides such as phosphopeptides.

**Quantification and sequence analysis**

The comparison of different proteomes using two-dimensional electrophoresis is technically demanding and can be invalidated by intergel variability. One approach to reduce this variation is to separate two or more protein samples labeled with different fluorescent dyes in one single gel (two-dimensional difference gel electrophoresis [39]). Relative quantification methods also employ the differential labeling of each set of proteins or peptides obtained from two different groups of cells with light and heavy isotopes of the same chemical reagent, followed by mass spectrometry analysis (Fig. 4). This technique permits the relative quantification of basic, hydrophobic or large proteins that are normally excluded from two-dimensional electrophoresis techniques. Two labeled samples (e.g. $^{15}$N or $^{14}$N) are combined, separated by liquid chromatography and analyzed by mass spectrometry, which permits estimation of the relative abundance levels of the proteins (18). Isotopic labels can be added during cell culture or at the protein or peptide level. An important *a priori* assumption for this approach is that at the outset of the experiment, the structurally similar, isotopically labeled peptides will have identical ionization efficiencies during mass spectrometric analysis. In the mass spectrum, the light- and heavy-labeled peptides appear as doublets, and the peak heights of the doublet peaks are proportional to the relative abundance of the protein derived from each condition or treatment. In mass spectrometry analyses, the signal intensity arising from a specific peptide varies as a function of the chemical and physical properties of the analyte and the solvent. Consequently signals from two peptides, even from the same protein, will exhibit different intensities. For relative quantification, only direct comparisons of chemically similar structure are meaningful. The stable isotope-labeling approach can also be used for detecting the numbers of labeled amino acid residues and can facilitate protein recognition. The isotope-coded affinity tag approach (e.g. 36) uses a label that contains a thiol group that reacts with cysteine residues, hydrogens (light) or deuteriums (heavy) for relative quantification, and a biotin group that is detected during the affinity extraction step by an avidin group attached to a column. Proteins from two different conditions are labeled with either the heavy or the light isotope. The mixture is digested and separated by affinity extraction prior to tandem mass spectrometry analysis. Although stable isotopes offer considerable promise for relative quantification, it is possible that the isotope ratio analysis of protein concentration between samples does not necessarily relate directly to protein expression (18).

Tandem mass spectrometry facilitates the sequencing of proteins and peptides and has become an

---

**Fig. 3.** Prior to analysis by mass spectrometry, biological samples must be properly prepared and undergo protein separation, protease digestion, peptide separation and analysis. This flow diagram emphasizes the preliminary analytical steps prior to mass spectrometry (adapted from [12]).
important method for protein recognition, structure elucidation, and the characterization of post-translational modifications. In contrast to the Edman degradation technique, which is useful for N-terminal sequencing of large amounts of relatively pure peptides, tandem mass spectrometry can be used for sequencing peptides in mixtures or for sequencing of proteins blocked at the amino terminus. Peptide fragmentation by dissociation induced through collision during tandem mass spectrometry allows the discrimination of complete or partial peptide sequences. Following collision-induced activation, peptides are fragmented along the backbone or in their side chains, thereby producing ions that are attributed to the amino acid sequence. However, it can be challenging to interpret an incomplete tandem mass spectrometry spectrum from fragmentation of a weak peptide ion signal for sequencing.

MALDI-Time of Flight-based methods for identifying membrane and other low-abundance proteins have been described in which several stable isotope-labeled amino acid precursors are selected to mass-tag, in parallel, cultured cells. The labeled residues are recognized by characteristic isotope patterns in MALDI-TOF mass spectrometry spectra, which in turn provides information about amino acid compositions. Proteins can be accurately identified through a single peptide with its $m/z$-value and partial amino acid composition (29).

**Structural proteomics**

There are important hurdles in structural proteomics that include the identification of all the proteins on a genome-wide scale, determining their
structure-function relationships, and describing three-dimensional structures of the proteins (22). In any newly sequenced genome, 30–50% of genes encode proteins with unknown molecular or cellular function (41). Some structural information arises from analysis of unknown proteins in which a protein-bound ligand or cofactor is discovered. These data are useful for functional description since the nature of the ligand, the ligand-binding site, and the disposition of catalytic residues can be inferred. Another approach involves comparing newly determined structure to the structural databases, an analysis that can show a structural similarity which is not obvious from sequence analysis. Further, structures can be inferred by identifying local structural motifs (e.g. helix-turn-helix motif), or distinct regions with conserved residues on the surface of an unknown protein that can function as enzyme catalytic sites, or possible protein–protein interaction sites. Technologies are under development to characterize unknown proteins in parallel rather than individually. Many new structures suggest biochemical functions that can be determined experimentally. Protein structures are usually solved experimentally by X-ray crystallography or nuclear magnetic resonance spectroscopy. However, a detailed understanding of three-dimensional space obtained from these techniques is limited. Computational methods such as comparative approaches and molecular dynamic simulations are now used as alternatives to predict three-dimensional structures and the dynamic behavior of proteins (22). New structural motifs have been discovered that are involved in enzymatic catalysis or in binding ligands or other macromolecules such as DNA and RNA. The efficiency with which function is deduced from structure can be further improved by the integration of structure with bioinformatics and other experimental approaches, such as screening for enzymatic activity or ligand binding (41). In addition to sequence-based methods, other approaches can be used to predict gene function, including obtaining information about the temporal, spatial, and physiological regulation of proteins; the proteins with which they interact; the phenotype of the gene knockout; biochemical activities; post-translational modifications; and protein structural analysis. 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. These new structures have revealed many unexpected functional and evolutionary relationships that were not visible at the sequence level (42).

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 (5). 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 (30).

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 (10). One of the most commonly used technologies is the yeast two-hybrid system, an ex vivo assay that detects binary physical interactions. This technology has been exploited for many different biological systems including, for example, identification of novel matrix metalloproteinase substrates that act to regulate inflammation (25).

For the yeast two-hybrid analysis, the interaction between a ‘bait’ fusion and a ‘prey’ fusion reconstitutes a functional transcription factor, which in turn activates reporter genes or selectable markers. Homodimeric and heterodimeric interactions can be detected, but because of the bimolecular complex that is formed, it is not possible to assess cooperative interactions between more than two proteins (9). The yeast two-hybrid system is economical and can be scaled up to improve throughput (e.g. screening a predefined library of full-length proteins against itself either in the form of clone arrays or pools) but is not without its limitations. Because the interaction occurs in the yeast nucleus, some types of proteins may be excluded, such as integral membrane proteins, and because ectopically expressed proteins do not consistently exhibit post-translational modifications appropriately, binary interactions between post-translationally modified proteins may be missed. To overcome some of these drawbacks, modifications of the yeast two-hybrid system have been developed to detect interactions between membrane proteins (e.g. the Ras recruitment system [2] and the split-ubiquitin system [37]).
Protein microarrays (or chips) allow the in vitro detection of binary interactions of varying types including protein–protein, protein–lipid and antibody–antigen interactions. Typically, proteins are covalently attached to a solid support (e.g. a microscope slide) and screened with fluorescently labeled probes (e.g. proteins). Protein chips provide a novel and versatile method to examine protein function in a highly efficient manner. In view of the chemical and structural complexity of the proteome, it is perhaps not surprising that the development of protein chips has been technically daunting. In spite of these challenges, protein chips have been used to map interactions of proteins with various other molecules, and to identify potential disease biomarkers, especially in the area of cancer biology (19). Snyder and colleagues have assembled a high-density yeast proteome microarray which comprises 5800 GST–HisX6 fusion proteins spotted on microscope slides at high density (43). This microarray was used for identifying new calmodulin- and phospholipid-binding proteins. Such protein arrays can also be used for examining inter-domain interactions. Protein array technologies also employ antibodies for analysis of cluster of differentiation antigens of discrete cell types. The creation of high-density antibody microarrays using phage display (1) could conceivably be used for characterization of the proteome. Until recently, the yeast two-hybrid system was the major global approach for examining protein–protein interactions. However, the development of powerful and sensitive, high-throughput mass spectrometry methods have enabled the detection of peptides in the femtomolar range; this methodology in turn allows calculation of the molecular masses of proteins in proteomics (17). But the detection of post-translationally modified proteins in proteomics (17). But the detection of post-translational modifications using mass spectrometry-based methods is technically difficult as, unlike the conventional recognition of proteins that requires the recognition of only a few peptides, the identification of a post-translational modification of a protein requires the specific recognition of the particular peptide or proteins that are modified. Post-translational modification and processing of a protein causes changes to molecular mass relative to the molecular weight calculated from the unmodified amino acid sequence of the protein. For example, phosphorylation of a serine residue increases the molecular weight of a protein by 80 Da. This increase of mass could in theory be measured by mass spectrometry of the intact protein (9).

Protein modifications

Post-translational modifications of proteins have the capability of generating considerable complexity and heterogeneity of gene products (17). Following synthesis, many cellular proteins are reversibly or permanently modified to control protein activity and protein–protein interactions. These modifications include deamidation, acylation, phosphorylation, glycosylation, ubiquitination, proteolytic processing, fatty and addition of glycosylphosphatidylinositol lipid anchors. Combinations of affinity-based enrichment and extraction methods, multidimensional separation technologies and mass spectrometry are particularly attractive for systematic investigation of post-translationally modified proteins in proteomics (17).
individual peptides and comparison to the corresponding expected molecular masses. In the final step, the modified amino acid residues are determined by sequencing using tandem mass spectrometry. This methodology is complicated by the intrinsic complexity of the post-translationally modified protein, its physicochemical characteristics, and the size of modified peptides, which in turn lowers their detection efficiency by mass spectrometry (9). Notably, more complete analysis of an amino acid protein sequence can be obtained with individual or sequential proteolytic digestions using different enzymes. Further, two-dimensional electrophoresis can often resolve similar but differentially modified forms of a specific protein.

Differential incorporation of stable isotopes with the use of metabolic or chemical labeling allows relative quantification of post-translational modifications between two or more different treatments or conditions by mass spectrometry (17). A novel proteomic approach has been developed for identifying substrates of tyrosine kinases in signaling pathways. This method is based on in vivo labeling of proteins with $^{12}$C-labeled or $^{13}$C-labeled tyrosine. Stable isotopic labeling in cultured cells provides definitive identification of tyrosine kinase substrates, since peptides derived from true substrates produce a unique signature in mass spectrometry analyses, an approach that simplifies high throughput studies (15).

**Current applications of proteomics to oral tissues**

As described above, proteomics can provide comprehensive and systematic information about proteins in a wide array of tissues and organs. As proteins are functional molecules, methods to determine their expression, by both quantitative and qualitative means, are thought to be essential for an in-depth understanding of tissue function. Despite the considerable analytical power offered by proteomics methods, only a relatively small set of papers has been published on periodontal cell and matrix proteins. Indeed there has been more published work using proteomics methods on the periodontal microbiota. As far as oral microorganisms are concerned, only one method of proteome analysis, two-dimensional electrophoresis, has been used to any extent (23), specifically for production of reference maps of the functional proteome of *S. mutans* (20) and of the outer membrane subproteome of *Porphyromonas gingivalis* (40). In studies of chick facial development at the first branchial arch between 3 and 5 days, ~8% of the total proteins identified by two-dimensional electrophoresis underwent detectable change (24), indicating that this technology could be used to identify potentially important proteins involved in critical stages of facial development. Proteomic studies have also been conducted on human saliva using a combination of liquid chromatography, electrospray tandem mass spectrometry and two-dimensional electrophoresis, experimental approaches that led to the identification of 309 separate proteins (14).

For cells that are important in the function of periodontal tissues, proteomic analysis has been applied to fibroblasts (3, 21), osteoblasts (7) and for rare cells like osteoclasts (6). 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. Current proteomics analyses have the capacity to provide new insights into the repertoire of expressed proteins and some inkling of their interactions, at a more global level than previously considered. 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. However, 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 (26).

**References**


