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Review Article

What do 'omic technologies have to offer periodontal clinical practice in the future?

M. M. Grant
Periodontal Research Group, School of Dentistry, University of Birmingham, St Chad's Queensway, Birmingham, UK

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Background and Objective: Periodontal diseases are the most common chronic inflammatory diseases of humans and a major cause of tooth loss. Inflammatory periodontitis is also a complex multifactorial disease involving many cell types, cell products and interactions. It is associated with a dysregulated inflammatory response, which fails to resolve, and which also fails to re-establish a beneficial periodontal microbiota. There is a rich history of biomarker research within the field of periodontology, but exemplary improvements in analytical platform technologies offer exciting opportunities for discovery. These include the 'omic technologies, such as genomics, transcriptomics, proteomics and metabolomics, which provide information on global scales that can match the complexity of the disease. This narrative review focuses on the recent advances made in *in vivo* human periodontal research by use of 'omic technologies.

Material and Methods: The Medline database was searched to identify articles currently available on 'omic technologies with regard to periodontal research.

Results: One hundred and sixty-one articles focusing on biomarkers of and 'omic advances in periodontal research were analysed for their contributions to the understanding of periodontal diseases.

Conclusion: The data generated by the use of 'omic technologies have huge potential to inform paradigm shifts in our understanding of periodontal diseases, but data management, analysis and interpretation require a thoughtful and systematic bioinformatics approach, to ensure meaningful conclusions can be made.

Dr Melissa M. Grant, Periodontal Research Group, School of Dentistry, University of Birmingham, St Chad's Queensway, Birmingham B4 6NN, UK
Tel: +44 121 237 2884
Fax: +44 121 237 2882
e-mail: m.m.grant@bham.ac.uk

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Periodontal diseases are the most common chronic inflammatory diseases of humans and a major cause of tooth loss (1). Diagnosis requires training, knowledge and dedicated clinical facilities, creating a need for those in nonspecialist and/or nondental environments (e.g. medical practice) for simple, objective diagnostic tools, to help identify patients with periodontitis. These would help in early diagnosis of disease onset, progression, or indeed resolution following treatment and may reduce both the healthcare and economic burdens arising from periodontitis, estimated as £2.78 billion in the UK in 2008 (2). Moreover, they may positively impact upon systemic inflammatory diseases, where periodontitis is recognized as a risk factor. The identification of biomarkers using 'omic technologies, such as genomics, transcriptomics, proteomics and metabolomics, could deliver such diagnostic tests.

The official National Institutes of Health (NIH, USA) definition of a

biomarker is 'a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'. Although this could be a physical trait, such as hair colour, for the purpose of this focused review of *in vivo* biomarkers of human periodontitis only molecular biomarkers and those determined in a genetic, proteomic or metabolomic profile will be discussed. As Bensalah *et al.* (3) have recently documented, six different types of biomarker can be differentiated, as follows:

- early detection of disease;
- diagnosis of presence or absence of disease;
- prognosis of disease outcome and possible patient stratification allowing for personalized medical interventions (particularly in periodontology for those at elevated risk of disease recurrence);
- prediction of treatment outcome;
- identification of patients who will respond well to a particular treatment; and
- surrogate end-points.

In addition, for a biomarker or a panel of biomarkers to be successfully employed within the clinical environment, they must also be objective, reproducible, easy to use, cheaper and with greater sensitivity, specificity and diagnostic accuracy than existing tests (3–5). These hurdles are made higher still by the need for potential biomarkers to achieve a status akin to the rigorous governance processes through which drugs must pass for licensing; there is, however, currently no such mechanism in place for such evaluations(3). Also in parallel to drug discovery is the process of validation through which biomarkers pass (Fig. 1).

In the past, the most useful biomarkers have either been found serendipitously or through careful evaluation of candidates generated through hypothesis-driven research (6). Many potential biomarkers are developed using preclinical *in vitro* models, and a few go onto the development of assays used in the evaluation of a small number of patients in the equivalent of phase 1 trials. Proof of biomarker efficacy cannot be established solely by statistics; there needs to be an evaluation akin to structured, phased trial testing (3,6). Such independent validation and efficacy determination in large community-dwelling populations, in the equivalent of phase 2 and 3 trials, is even scarcer than phase 1 studies. Thus, to mine the proverbial biomarker iceberg using these novel biomarker technologies, larger multicentre multi-'omic systems biology trials need to be performed.

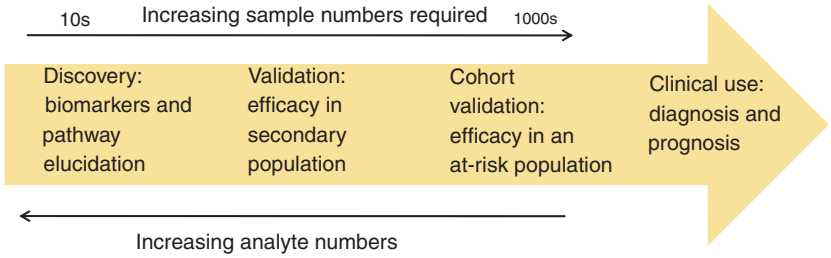

Fig. 1. The path through which biomarkers must travel to be useful for the clinician. 'Omic technologies can be used at all stages, but have most impact on the initial stages.

Samples available for *in vivo* studies of periodontal diseases include gingival crevicular fluid, plaque, saliva, biopsies, peripheral blood cells and plasma (Fig. 2). Several excellent reviews discuss these samples for targeted approaches to biomarker discovery (5,7–10). In particular, Loos & Tjoa (5) undertook a critical review of biomarkers in gingival crevicular fluid and found that only eight of 94 in the literature of the time fulfilled any of the criteria for biomarker status. These eight biomarkers were alkaline phosphatase (11–17), β-glucuronidase (15,18–26), cathepsin B (27–32), MMP-8 and MMP-9 (15,33–45), dipeptidyl peptidase II and IV (28,29, 31,46) and neutrophil elastase (15,24, 26–29,39,47–66). Potential novel biomarkers that have been described more recently using 'omics-driven discoveries are discussed below.

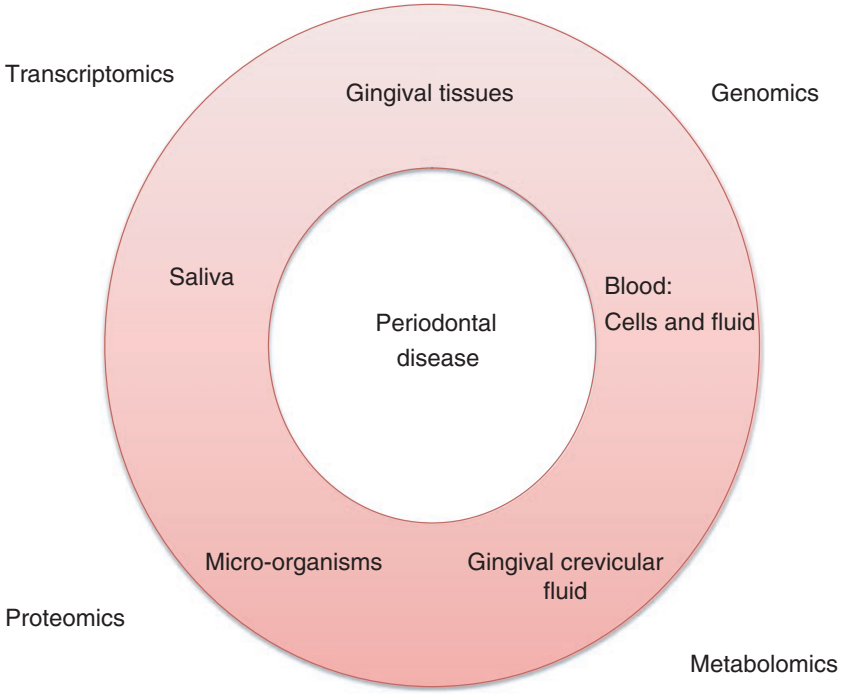

Fig. 2. The compartments available for studying periodontal disease using 'omic technologies.

The 'omic technologies include genomics, transcriptomics, proteomics and metabolomics (Fig. 2), and each is discussed in a separate section below. Genes, transcripts, proteins and metabolites will all feed into the overall phenotype of periodontal disease (Fig. 3). It should be noted that, in contrast to genomics, transcriptomics, proteomics and metabolomics assess the temporal expression of genes rather than the static encoding of the genome. Thus, they take into account environmental influences, i.e. nurture as well as nature. As we progress from genomics to transcriptomics, proteomics and metabolomics, we also progress from what might happen to what really did happen, with transcriptomics being influenced by translation and activation, proteomics elucidating changes to global protein expression, splice variants of proteins and post-translational modifications, and metabolomics demonstrating end-products of reactions. There are further differences between these fields of study; the DNA, RNA and proteins studied in genomics, transcriptomics and proteomics, respectively, are very regular in structure, and this has greatly aided the discovery in these fields. In contrast, metabolomics encompasses many small chemicals with varying hydrophobicity–hydrophilicity, acidity–basicity and other physicochemical properties. This means that the level of complexity and analytical challenges are increased in metabolomics. However, taking into account the number of molecular species in each of the areas also challenges the investigator, because there may be over 30,000 genes and transcripts, over 100,000 proteins, including post-translational modifications, and over 6500 metabolites in the human (67); not one of these areas should be singled out as 'easier' than any other, and it should be noted that each level can be influenced by the others through feedback loops and regulatory mechanisms (Fig. 3). All these technologies and assessments can be applied to both the host and the microbiota in periodontitis. Here, only the host contributions are discussed; for excellent and recent reviews on microbiome advances please see work by Parahitiyawa *et al.*(68), Dewhirst *et al.*(69), Wade (70) and Pozhitkov *et al.*(71). So far, a lot of ground has been made on the microbiome associated with health, while advances comparing health and disease, such as those published in the field of gut microflora (72), are eagerly awaited.

Drawbacks of all the functional genomics technologies include confounding issues such as age, gender, diet, smoking and probably many more. Where dynamic range is a problem, the technology may be affected by the 'usual suspects' phenomenon (73), where similar chemical species are found in a variety of unrelated studies and reflect the fact that some situations/treatments affect central signalling or metabolic hubs within cells, for example affecting energy generation. This is a problem that can mask less obvious biological perturbations, but can be overcome with much larger study populations where general 'noise' can be removed and small changes can gain statistical significance due to increasing study power.

The following sections summarize the data already published using 'omics technologies. Table 1 summarizes the data-rich studies. Following the analysis by Loos & Tjoa (5) of the field of biomarkers, an attempt was made to stratify these studies according to their use in detection, classification, planning of treatment or monitoring of patients. However, though many show promise, none of the studies has yet matured to that level of usage, as most are still early though potentially valuable reports.

Genomics

Genomics is the study of whole genomes, i.e. all the DNA of a single organism. With improvements in sequencing, the dawn of genome-driven individualized medicine has arrived, where changes to multiple genes may be taken into account for diagnosis and treatment. With differences in more than 3 million nucleotides (0.1% of the whole genome) evident when comparing two individual genomes, it will, however, probably take many years before such differences can be mapped to disease correlations (74,75). However, for some time the changes in individual genes (gene polymorphisms) have been studied with reference to disease risk, severity and therapeutic outcome. These gene polymorphisms are highly prevalent in the population (76), and the most common type is the single nucleotide polymorphism (SNP), where an individual base pair is affected, by alteration within, insertion into or deletion from the DNA sequence. Where these changes fall in promoter regions, exons, introns or untranslated regions, will differentially affect gene products (75).

The influence of SNPs on periodontal disease was reviewed in 2006 by Takashiba & Naruishi (75). They highlighted that nearly half of the

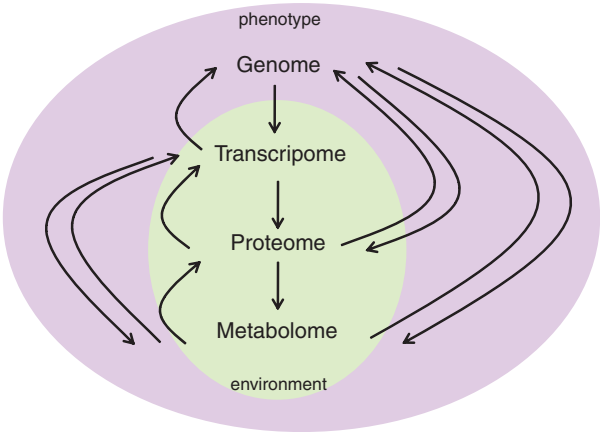

Fig. 3. The interplay of the different compartments studied by 'omic technologies.

Table 1. Summary of data-rich 'omics studies

Approach	Study population(s)	Platform of evaluation	Number of chemical species investigated	Source of biological samples	Number of subjects in study	Conclusion	References
Genomics	Severe periodontitis, comparison to healthy subjects	TaqMan PCR	637 SNPs	Whole peripheral blood	$n = 22$ severe periodontitis patients, $n = 19$ control subjects	*GNRH1*, *PIK3R1*, *DPP4*, *FGL2*, and *CALCR* significantly associated with disease	114
	Aggressive periodontitis	Affymetrix Gene Chip Human Mapping 500K Array Set	Genome-wide association study of 500,000 SNPs	Whole peripheral blood and gingival tissue	$n = 141$, 142, 164 assessment in two cohorts by genome-wide association study and validated in a third cohort (affected teeth 2–6%)	*GLT6D1* was significantly associated with disease	116
Transcriptomics	Chronic and aggressive periodontitis	Affymetrix Human Genome U-133 A arrays	22,000 transcripts	Biopsies of gingival tissue	$n = 1$ localized chronic periodontitis, $n = 6$ generalized chronic periodontitis $n = 1$ localized aggressive periodontitis, $n = 6$ generalized aggressive periodontitis	Patients were clustered by pathogen presence rather than by disease type	117
	Chronic and aggressive periodontitis	Affymetrix Human Genome U-133 Plus 2.0 arrays	47,000 transcripts	Biopsies of gingival tissue	$n = 90$ (63 chronic and 27 aggressive) periodontitis patients	Gene ontology groups increased included apoptosis, antimicrobial humoral response, antigen presentation, regulation of metabolic processes, signal transduction and angiogenesis	118
	Chronic and aggressive periodontitis	Affymetrix Human Genome U-133 Plus 2.0 arrays	47,000 transcripts	Biopsies of gingival tissue	$n = 120$ (65 chronic and 55 aggressive) patients	Commonalities and differences were found between gene expression and bacterial species present	119
	Experimental gingivitis	Affymetrix Human Genome U-133 Plus 2.0 arrays	47,000 transcripts	Biopsies of gingival tissue	$n = 14$ healthy volunteers	Differences in gene ontology groups: neural processes, epithelial defences, angiogenesis and wound healing	121
	Periodontal disease compared with control	Agilent 2100 Bioanalyzer and a human inflammation microarray	160 genes	Biopsies of gingival tissue	$n = 12$ severe generalized chronic periodontitis, $n = 11$ control subjects	Activation of pathways regulating tissue damage and repair after treatment	122
	Refractory periodontitis	Affymetrix Human Genome U-133 A arrays	22,000 transcripts	Subepithelial connective tissue	$n = 7$ refractory periodontitis and $n = 7$ periodontally well-maintained patients	Increases in immune re sponse, tissue modelling and apoptosis in disease in refactory patients	123

Table 1. (Continued)

Approach	Study population(s)	Platform of evaluation	Number of chemical species investigated	Source of biological samples	Number of subjects in study	Conclusion	References
	Periodontitis patients undergoing treatment	Affymetrix Human Genome U-133 Plus 2.0 arrays	47,000 transcripts	Peripheral monocytes	$n = 15$ patients	Changes in innate immunity, apoptosis and cell signalling were seen	124
	Periodontitis	Affymetrix Human Genome U-133 A arrays	22,000 transcripts	Peripheral neutrophils	$n = 19$ patients	Type-1 interferon-stimulated genes were increased	127
Proteomics	Periodontitis in comparison to control	Electrophoresis and MS	Not given	Gingival crevicular fluid, serum and saliva	$n = 10$ periodontitis patients, $n = 4$ control subjects	S100A8 and S100A9 represented major differences between gingival crevicular fluid and saliva	134
	Periodontitis patients in maintenance phase	Electrophoresis and MS	66 proteins identified	Gingival crevicular fluid	$n = 12$ patients	Identification of serum and cell-derived proteins	135
	Aggressive periodontitis in comparison to control	Electrophoresis and MS	Not given	Saliva	$n = 5$ aggressive periodontitis patients, $n = 5$ control subjects	Six proteins were increased in saliva of periodontitis subjects, while five were decreased	136
	Generalized aggressive periodontitis	Quantitative MS	154 human, bacterial, fungal and viral proteins	Gingival crevicular fluid	$n = 5$ aggressive periodontitis patients, $n = 5$ control subjects	Human plastin-2 and microbial proteins increased in disease, annexin A1 increased in health	137
	Experimental gingivitis	Quantitative MS	202 human and bacterial proteins	Gingival crevicular fluid	$n = 10$ healthy volunteers	Identification of 186 proteins, including serum and cell-derived species, including plastin-2. Novel structural proteins for cilia and ribbon synapses found.	138
Metabolomics	Chronic periodontitis	MS	103 metabolites identified	Gingival crevicular fluid	$n = 22$ patients; samples collected included diseased and healthy sites	At diseased sites antioxidant, glutamine, di-and trisaccharide levels decreased; amino acids (except glutamine), choline, glucose, polyamines, purine degradation and urea cycle metabolites increased	147
	Localized aggressive periodontitis	MS	Seven diacylglycerol species	Neutrophils	$n = 11$ localized aggressive periodontitis, $n = 4$ asymptomatic family members	Increased diacylglycerol species in disease compared with control	148
Systems biology	Periodontitis	Data mining and cluster analysis	61 genes	*In silico*	Not relevant	Five leader genes (or hubs: *NFkB1*, *CBL*, *GRB2*, *PIK3R1* and *RELA*) identified	152

Abbreviations: MS, mass spectrometry; SNP, single nucleotide polymorphism; GLT6D1, glycosyltransferase 6 domain containin 1; NFkB1, nuclear factor of kappa light polypeptide gene enhanced in B cells 1; CBL, Cas-Br-M ectropic retroviral transforming sequence; GRB2, growth factor receptor-bound protein 2; RELA,v-rel reticuloendotheliosis viral oncogene homology A; PIK3R1, phosphoinistidite-3-kinase, regulatory subunit 1.

research in this area has focused upon cytokines, with the rest investigating human leukocyte antigens, immunoreceptors, proteases, structural molecules and other proteins. However, of the 140 papers they used for their review the majority focused on only six genes: interleukin 1 (*IL1*), tumour necrosis factor α (*TNF*α), Fcγ receptors, MMPs, cathepsin C and vitamin D receptor), indicating that this field is still in its infancy. Interleukin-1 SNPs were suggested to be more associated with environmental interactions, such as with smoking, than with susceptibility to periodontitis, whereas *TNF*α showed a lack of association with inflammatory periodontal disease. However, polymorphisms in Fcγ receptors tend to be associated with both aggressive and chronic forms of periodontitis. For the other genes mentioned above, limited evidence makes it difficult to relate SNPs to periodontitis. In the past 5 years since the review by Takashiba & Naruishi (75), there have been at least an additional 37 articles published concerning SNPs in cytokines (77–113). These small-scale studies of individual SNPs are no longer in a position to contribute anything new to the literature and are of limited value.

Moving into wider ranging analysis, Suzuki *et al.* (114) examined 637 SNPs in 19 healthy and 22 severe periodontitis cases, revealing five previously untargeted genes as potential markers for periodontitis.

Overall, the genetic basis of periodontitis accounts for approximately half the population variance in chronic periodontitis (115,116). There is a need to progress to large-scale genome-wide association studies, and the first of these has been published (116). Comparison of two cohorts of aggressive periodontitis patients independently identified 197 and 244 quality-controlled SNPs from 141 and 142 patients, respectively, examining 500,568 potential SNPs. However, when the results from both sets were compared, only one remained significant, which was subsequently validated in a third set of patients (n = 164). The gene identified was *GLT6D1*, which encodes for a glycosyltransferase 6 family protein. These enzymes are single-pass transmembrane proteins, which contribute to the synthesis of histo-blood-related antigens in the Golgi. *GLT6D1* was found to be highly expressed in the gingival connective tissues and may influence immune responses. Future studies using larger numbers of patients and control subjects may yield more associations; however, the acquisition of even one unknown gene that may predict periodontal disease is potentially of great value.

Transcriptomics

The field of transcriptomics involves the study of mRNA production by cells in particular conditions. Unlike proteomics and metabolomics (see next section), this is typically studied in cell populations and thus in periodontal investigations either uses biopsies of relevant oral tissues or peripheral blood leukocytes rather than oral fluids such as gingival crevicular fluid and saliva, which can be studied using proteomic and metabolomic platforms. There are two major advantages that this technique provides: (i) the ability to amplify the expressed gene products; and (ii) the stability and uniformity of the platforms employed in identification of interesting and/or novel chemical species. This is reflected in the far greater number of articles reporting transcriptomic studies than proteomic and metabolomic studies. Over the last 5 years, Papapanou *et al.* have analysed whole-tissue transcriptomes from the excised papillae of healthy and diseased patients in an attempt to reclassify periodontal disease biologically rather than clinically (117–119). A pilot study, however, could not differentiate between chronic and aggressive forms of periodontitis (117), whereas comparison of diseased and healthy papillae from patients with advanced periodontitis did detect differences in gene ontology groups for apoptosis, antimicrobial humoral responses, antigen presentation, regulation of metabolic groups, signal transduction and angiogenesis. The authors commented that the papillae are composed of a variety of cell types, These differences in composition may give rise to different transcriptome profiles and contribute to the heterogeneity of results. However, it was possible to identify genes that have not previously been linked with periodontal diseases, such as *CXCL6* (granulocyte chemoattractant protein 6; 117,120). In their latest paper, Papapanou *et al.* (119) correlated the transcriptomes of chronic periodontitis patients with the subgingival microflora in those patients/sites. This interesting study coupled the two key drivers of periodontal disease expression, the host and microbial factors, to determine whether species of bacteria can cluster the large number of genes differentially expressed in periodontal disease, thus yielding information on how bacterial species might influence host gene expression. Gingival biopsies were also taken by Offenbacher *et al.* (121) to investigate the temporal changes in gene expression during experimental gingivitis. Again, large numbers of genes were differentially expressed and novel gene ontology groups were reported, including those of neural processes, epithelial defences, angiogenesis and wound healing.

Beikler *et al.* (122) investigated gene expression changes in periodontal tissues before and after treatment using a semi-targeted human inflammation microarray. They concluded that those gene profiles that were altered the most indicated an activation of pathways that regulate tissue damage and repair. Kim *et al.* (123) examined subepithelial connective tissues from healthy control subjects and periodontal patients. They found that these tissues also demonstrated transcriptomic increases in the immune response, tissue remodelling and apoptosis genes.

Looking at how periodontitis affects the peripheral blood system, Papapanou *et al.* (124) took monocytes from periodontal patients undergoing treatment and examined mRNA expression using Affymetrix arrays. They found that a third of patients had substantial changes in genes relevant to innate immunity, apoptosis and cell signalling, and concluded that periodontal therapy had a systemic anti-inflammatory effect. Matthews *et al.* (125,126) have previously reported that neutrophils

from periodontitis patients are both hyper-reactive to stimulation by *Fusobacterium nucleatum* or Fcγ receptors and also show baseline hyperactivity with respect to production of reactive oxygen species. Following these discoveries, the same group (127) used neutrophils from periodontitis patients to determine what genes were affected. They found significant increases in type-1 interferon-stimulated genes, and this led to the discovery that patients had significantly greater concentrations of circulating interferon-α, which, upon successful periodontal treatment, decreased to the same levels as in nondiseased control subjects. They concluded that periodontitis is a complex disease, where increases in interferon-α may be one component of a distinct molecular phenotype in neutrophils, triggered potentially by viral priming or autoimmune responses. This latter concept is new to periodontology and may help explain the association between periodontitis and rheumatoid arthritis (128–130).

Advances have been made using transcriptomic approaches, but there is a need to bring together the established data sets and also to conduct much larger, wide-ranging studies that can take into account possible changes in cell type within periodontal tissues, to pinpoint genes that may be useful in differentiating between disease types and address the criteria for biomarker research previously stated.

Proteomics

Proteomics, the study of all the proteins in a given sample, was revolutionized by advances in mass spectrometry in the 1990s. It became possible to identify the constituent protein species within biological samples, and now many studies have used an ever-expanding and complex array of techniques that are both qualitative and quantitative in their outputs. A feature of many biological/clinical samples is that they exhibit a very wide dynamic range of constituent protein species, for instance in plasma that range is six orders of magnitude. Without the advantages that DNA and RNA amplification strategies offer, it is often not possible to examine the entire proteome, and it is frequently necessary to try and remove or separate the most abundant proteins from a sample (e.g. albumin) prior to analysis. However, proteomics does address changes to proteins, such as splice variants and post-translational modifications. Targeted approaches to look at panels of cytokines, such as using the bead-based Luminex platform, allow examination of proteins of low concentration, but such presumptive approaches are not discussed here.

In the study of periodontal diseases, many proteomic approaches have been used. Top-down whole-protein approaches to identify low molecular weight proteins have investigated the presence of human neutrophil peptides (131–133) in gingival crevicular fluid. However, the use of bottom-up approaches, where proteins are digested to individual peptides prior to identification by tandem mass spectrometry techniques, has yielded many more novel insights into the periodontitis proteome. Kojima *et al.* (134) separated gingival crevicular fluid proteins by two-dimensional electrophoresis and then identified proteins of interest by mass spectrometry. The addition of two-dimensional electrophoresis introduced a method to assess protein levels quantitatively between diseased and healthy subjects, although intra-individual variation swamped the slight trend for more calprotectin subunits in periodontitis patients. Use of liquid chromatography–mass spectrometry techniques to study periodontitis has recently been reported. Ngo *et al.* (135) examined gingival crevicular fluid samples by electrophoresis and liquid chromatography tandem mass spectrometry (LC-MS/MS) to identify 66 proteins, which included a large number of serum and cell-derived proteins, reflecting the dual origin of the fluid. Wu *et al.* (136) compared saliva proteomes from generalized aggressive periodontitis patients and control subjects using a similar technique. Whole saliva yielded differences in highly abundant proteins, such as albumin and amylase, which were increased in the diseased samples, illustrating perhaps the need for prefractionation to dissect deeper down into the proteome. Quantitative LC-MS/MS has been used by Bostanci *et al.* (137) and by Grant *et al.* (138) to investigate gingival crevicular fluid profiles from patients with generalized aggressive periodontitis and volunteers undergoing experimental gingivitis, respectively. Both studies, as with Ngo *et al.* (135), found proteins of both serum and tissue origins and, more specifically, found changes in common, previously uninvestigated proteins, such as neutrophil plastin-2, an actin-bundling protein involved in Fcγ receptor stimulation. With the inclusion of a quantitative aspect, these studies allow for a more detailed investigation, where bioinformatic tools may be able to find composites of proteins that could be used as biomarkers. To date, however, these biomarkers have not been validated.

Degradomics is a specialized field of proteomics employing all of the techniques previously described. It assesses the proteases at work within the tissues which may be involved in substrate processing, yielding activated or inactivated substrates, altering functionalities or localizations, by looking at protease activity and cleaved proteins. This is of particular interest to periodontology, where there is a large amount of tissue destruction by either the host or pathogen efforts. Protease activities have been found in periodontitis, for example the cleavage of interleukin-8 by *Porphyromonas gingivalis* protease gingipains (139,140). The truncated interleukin-8 increases in chemotactic activity and the ability to stimulate the oxidative burst in neutrophils (139). Another protease that has been studied is cathepsin K (141), demonstrating a correlation with the severity of periodontal disease by defining the targets of this protease. Large-scale unbiased approaches have yet to be employed for the evaluation of protease activities or substrates in periodontitis, and they will be of great value when completed. For information of the state of the field, please see recent reviews by Impens *et al.* (142) and Morrison *et al.* (143).

Metabolomics

Metabolomics is a discipline that studies the quantities of all chemicals except DNA, RNA and proteins within a sample. No one experimental technique can analyse all chemical structures. Thus, samples need to be analysed by a battery of techniques and separated by their chemical and physical properties and identified, principally, by nuclear magnetic resonance and mass spectrometry. There is a vast number of potential metabolites, and targeted approaches have elucidated some changes (22,144–146), but there are very few articles that report on tackling the global metabolome in periodontal disease. Barnes *et al.* (147) used gas and liquid chromatographic separations coupled to mass spectrometry to investigate gingival crevicular fluid samples from 22 chronic periodontitis patients, stratified for healthy, gingivitis and periodontitis sites. They identified 103 metabolites in comparison to a chemical reference library, finding that levels of metabolites from gingivitis sites fell between healthy and periodontitis sites. At disease sites, in comparison to healthy sites, antioxidant, glutamine and di- and trisacchride levels were decreased, whereas amino acids (except glutamine), choline, glucose, polyamines and purine degradation and urea cycle metabolites were increased. This study has expanded our knowledge of the sources of oxidative stress, which is already acknowledged as being of particular importance in periodontal disease by the potential increase in activity of the xanthine oxidase–reactive oxygen species axis (147). Nuclear magnetic resonance-based approaches have not, as yet, been described for human gingival crevicular fluid. This may be due to the larger concentrations of samples required.

Lipidomics is a particular subspeciality of metabolomics that investigates the role of lipids in cellular function, because they integrate signalling and metabolic processes. The most common technique employs mass spectrometry, particularly using MS^n where $n > 1$ and multiple sequential mass spectrometry events continue analysis of one chemical species of interest. Recently, Gronert *et al.* (148) used a lipidomics approach to identify and quantify diacylglycerol species in neutrophils from localized aggressive periodontitis patients, following a transcriptomics analysis that had identified diacylglycerol kinase from neutrophils as not being expressed, in comparison to disease-free control subjects. Metabolomics is an area that could and should see intensive research to provide a clearer understanding of periodontitis. It will be able to reveal information about the host and host–microflora interactions which may yield specific small molecule targets that have been overlooked by other techniques.

Systems biology

Systems biology is the integration of multiple 'omics platforms and data through the reconstruction of the complex networks involved (149). These complex networks characterize particular systems, often cells, but in periodontitis it would need to address the whole disease, i.e. interactions not of one cell type but of many and also with the micro-organisms present in the disease state. Advances in network inference and analysis in other diseases, such as obesity, diabetes and atherosclerosis, are already highlighting that it may be necessary to target multiple (10–50) genes, in different tissues, simultaneously to treat a disease effectively (150). Such an approach would yield a holistic overview of the disease milieu. The complementary information from the different 'omic technologies needs to be co-ordinated and integrated, and several strategies are being progressed in other research areas (151). This still remains a major challenge to the periodontal field, and there is still the requirement for fundamental understanding of the mechanisms taking place so that the data can be appropriately modelled. The first report has been published in the field. Using an *ab initio* bioinformatic approach, Covani *et al.* (152) predicted five leader genes from an investigation of 61 genes potentially involved in periodontitis, using published articles as the source of data. These genes were *NFkB1*, *CBL*, *GRB2*, *PIK3R1* and *RELA*, and are predominantly involved receptor-mediated signalling and may reflect the stimulation of the host inflammatory–immune system by bacteria in periodontitis.

Use of holistic approaches will have the advantage that they will address the synergistic qualities of multiple bacterial challenges and multiple cell types present at the diseased lesion. The bacterial challenge, in particular, should not be overlooked, with so many so-called unculturable bacteria being present (153). Microbiome strategies to study the thousands of bacteria present will unite with the 'omic technologies (154). Nibali *et al.* (155) have already termed the interaction between host genetic factors, such as SNPs, and the oral microbiome as 'infectogenomics'.

Even before it was termed systems biology, scientists were creating networks, graphs or maps of the interaction of genes through regulation, proteins through protein–protein interactions and signalling cascades and metabolites through metabolic maps. Systems biology now needs to rely on the computational modelling of quantitative large-scale data sets that can be produced by 'omic technologies. Biological and artificial networks can be inferred and used to understand differences between states of systems, for instance, health and disease. Although many approaches can be used, there is now a drive towards making these rigorous with challenges such as those from DREAM (Dialogue on Reverse Engineering Assessment and Methods; 156), and problems of integration of different data sets have been overcome by advances in the field (157,158). At the heart of systems biology is a cycle of data and knowledge–*in silico* modelling–hypothesis generation–experimental validation that leads back to more data and knowledge (159). In the periodontal field we have lots of knowledge, such as the investigations into transcriptomics data sets that have thrown up some initial gene ontology information (117,121,124) that could demonstrate where dislocations in networks might occur. Integration and combination of overlapping data sets and creation of networks might yield interesting

results, such as those found in other fields. For example, network inference and module analysis of yeast exposed to the DNA-damaging agent methyl methane-sulfonate demonstrate how pathways and complexes are reconfigured to cope with this stress (160); and in analysis of hereditary ataxias, proteins affected by the gene mutations clustered to functionally related pathways (161). In the future, systems approaches should be built into the design of studies, and a concerted effort to join together the information already gained will greatly enhance the understanding of the multiple contributions of cell types and underlying biology to periodontitis. This will require a large effort from the research community and the inclusion of experts in systems biology, but it will open many new avenues of research and knowledge.

Conclusion

To conclude, as yet the 'omic technologies have not yielded validated biomarkers for periodontal disease, but they are identifying new routes for research to follow in relation to disease pathogenesis. It is unrealistic to think that one biomarker will be found; there is no more 'low hanging fruit' (5). Periodontitis is acknowledged as a complex inflammatory disease, initiated by a plaque biofilm and with multiple component causes, and it is therefore much more likely that there is a multiplicity of biomarkers which together can differentiate between health and disease or between disease onset and progression, improve the prognosis of disease outcomes and possible patient stratification allowing for personalized medical interventions, identify disease resolution/healing, predict treatment outcomes, identify patients who will respond well to a particular treatment or provide surrogate end-points. The use of use 'omic techniques will play an important role in their discovery.

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