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New Innovations in Periodontal Diagnosis: A Review

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ABSTRACT

Diagnosis is the identification of the nature of an illness or other problem by examination of the symptoms. The ability to correctly diagnose and assess periodontal disease has received considerable attention in the last decade. Traditional clinical diagnostic methods such as periodontal probing, probing attachment level and radiographs are suitable for most clinical situations but do suffer from a number of drawbacks such as clinical or radiological measurements of attachment loss are not precisely accurate and if not carried out very carefully can be misleading. Recent innovations in periodontal diagnostic techniques have overcome these limitations.

Introduction

Diagnosis is the identification of the nature of an illness or other problem by examination of the symptoms. The ability to correctly diagnose and assess periodontal disease has received considerable attention in the last decade.[1] Periodontal diagnostic procedures can potentially serve separate, but related functions such as screening, diagnosis of specific periodontal diseases, identification of sites or subjects at an increased risk of experiencing the progression of periodontal destruction, treatment planning and monitoring of therapy. To overcome the limitations of traditional clinical diagnostic methods such as periodontal probing and radiographs; innovations in periodontal diagnostic methods have been carried out. (Table 1)

The recent research in periodontal diagnosis is aimed at identification of disease process that if allowed to proceed unaltered, will result in future breakdown.

This review article is an attempt to provide a review on various recent diagnostic aids used in diagnosis of periodontal disease and their clinical applications in periodontal practice.

New Innovations in Clinical Diagnosis

Periodontal Probes

The word probe is derived from the Latin word Probo, which means "to test." For purpose of classification, currently available periodontal probes are divided into first, second and third generation probes (Philstrom 1992). [2] Fourth and fifth generation probes are yet in development stage. The First Generation Probes are manual probes like William's periodontal probe, UNC 15 probe, CPITN Probe, Michigan Probe Second generation probes are pressure sensitive probes. Gabuthuler and Hassell (1971) in a study designed to quantitate gentle probing, develop the first pressure sensitive probe. Van der Velden and De Vries (1978) developed a pressure sensitive etc periodontal probe. Vitek et al (1979) designed a leaf spring force controlled periodontal probe. This instrument delivers a force within 0.5 grams to a Michigan 'O' periodontal probe tip with terminal diameter of 0.35 ± 0.05 mm. Tromp et al (1979) designed periodontal probe to increase the reproducibility of pocket depth measurements. A constant torque spring was attached to a loose probe head which could rotate in a point bearing. [3] Vander Velden and De Vries (1980) modified the pressure sensitive periodontal probe in order to eliminate incorrect reading of the scale of probe. [4] Vine valley probe is an electronic pressure-sensitive probe introduced by Polson et. al. (1980) which was not sensitive to lateral forces not subject to error due to gravity. [5]

Hunter TPS Viva care periodontal probe [6] was explained by Hunter F. in the year 1994. The viva care probe is equipped with a 0.5mm ball tip with a tactile rim to minimize tissues trauma, and better detect irregularities on the root surface. The Yeaple probe [7] is a widely used tactile device and has been recommended as a reproducible quantification of a mechanical or tactile stimulus in the ADA Guidelines for the Acceptance of Products for the Treatment of Dentinal Hypersensitivity.

In spite of the advances in second-generation probes, other sources of errors, such as in reading the probe, recording data, and calculating attachment level, still needed to be addressed. Third generation probes were developed to help minimize these mistakes by using not only standardized pressure, but also digital read outs of the probes' readings and computer storage of data. These probes require computerization of the dental operatory and can be used by periodontists and academic institutions for research. These probes include Toronto Probe, Florida Probe, Foster- Miller Probe, Inter Probe and Peri Probe.

Fourth generation probes refers to three-dimensional (3D) probes. Currently under development, these probes are aimed at recording sequential probe positions along the gingival sulcus. [8] Fifth generation probes (research measuring device) being designed to be 3D and noninvasive: an ultrasound or other device is added to a fourth-generation probe.

Fifth-generation probes aim to identify the attachment level without penetrating it. The only fifth-generation probe available, the UltraSonographic(US) probe [9] devised by Hinders and Companion at the NASA Langley Research Center, uses ultrasound waves to detect, image, and map the upper boundary of the periodontal ligament and its variation over time as an indicator of the presence of periodontal disease.

Other non periodontal probes available are calculus detection probe [11], the diamond probe/Perio 2000 system [11] and the periotemp probe. [12] Periimplant probing [13] cannot be interpreted same as the natural teeth because of differences in

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the surrounding tissues that support implanted teeth. There is no equivalent fiber attachment around implants. Plastic or titanium probe tips should be used to avoid damage of the implant / tissue interface. If automatic probing is considered, the Florida probe is available with a titanium tip that will not hurt the implant, also, the inter probe system comes with disposable plastic tips.

Diagnosing tooth mobility

Mobility is determined clinically by luxating a tooth with a light force and observing its movement. The Miller index [14] is the most commonly used clinical method in which the tooth is held firmly between two instruments and moved back and forth. Mobility is scored from 0 to 3. Other indices for detecting tooth mobility are Glickman (1972) –for pathologic mobility, Wasserman et al. (1973) , Nyman et al (1975), Fleszar et al (1980) and Lindhe (1983) .There are various electronic devices used to measure tooth mobility such as Electronic transducer instrument, Microperiodontometer, Periodontometer, Custom-made acrylic clutches with the USAFSAM Periodontometer, Periotest. The Periotest [15] is a new instrument for the diagnosis of periodontal disease. The Periotest measure the reaction to a reproducible impact applied to the tooth ground. The Periotest value is a bio physical parameter. In the laser vibrometer method, the ratio between the maximum of the tooth displacement and the input force peak are considered as the mobility degree index. [16] Resonance frequency analysis is a new, noninvasive device based on the principles of resonance frequency analysis (RFA) has been developed to measure primary implant stability and to monitor implant stability over time. [17] Diagnosing oral malodor.

Halitosis is a general term denoting unpleasant breath arising from physiological and pathological causes from oral and systemic sources. Various volatile sulfur compounds (VSC) that can cause halitosis are hydrogen sulfide, methyl mercaptan and dimethyl sulphide. [18] The three primary methods of measuring genuine halitosis are organoleptic measurement, gas chromatography and sulphide monitoring. In Organoleptic rating, the expired air coming from the patient's mouth is sniffed and assessed whether or not this is unpleasant using an intensity rating, normally from 0 to 5 given by Rosenberg and Mcculloch. Sulphide monitoring can be done by using portable sulphide monitors such as Halimeter® (Rosenberg et al.), Oral Chroma™ (Miyazaki), Breathtron ® (Sopapornamorn).

Third method of measuring genuine halitosis is by the use of gas chromatography. Gas has provided a means by which a clinician could definitively quantify the precise levels of specific compounds present in someone's breath. Halicheck is a specialized Gas Chromatography test that measures the individual gases of bad breath. [19]

Additional or alternative methods of measuring genuine halitosis are BANA test, chemical sensors, quantifying β-galactosidase activity, salivary incubation test, ammonia monitoring, ninhydrin method, polymerase chain reaction, tongue sulphide probe, zinc oxide thin film conductor sensor, ora test and self assessment of oral malodour. [19]

Quantitative indicators of occlusal analysis

T scan occlusal analysis system and virtual dental patient are the two main indicators of occlusal analysis. The T-Scan occlusal analysis system (Tekscan) is a Microsoft compliant system that can record a given contact sequence in 0.01-s increments. This device is indicated in any situation where the bilateral simultaneous occlusal contact is necessary, for example, complete dentures, fixed or removable partial dentures, complete arch reconstruction solely using implants, complete arch reconstruction involving FPD, natural tooth occlusal equilibration, disclusion time reduction, occlusal splints and mandibular repositioning devices. Virtual dental patient is a recently introduced concept wherein the three-dimensional dental patient is assembled from the data scanned from the casts of a patient's dentition. [20]

New Innovations in Radiographic Assessment

Radiographs are a method of assessing the destruction of alveolar bone associated with periodontal disease, and they are widely available clinical tool that can be used for assessing the bone support. Conventional dental radiographs provide two dimensional images of three dimensional objects and reflect the anatomy of bone at that instant; they do not indicate whether bone loss is progressing view of the alveolar bone loss process, replicate measurements must be performed on standardized radiographs. Radiographic and nuclear medicine techniques have been developed to obtain a higher degree of sensitivity to minor bone changes. [21]

Digital radiography is a method in which application of computer technology has allowed for image acquisition, manipulation, storage, retrieval and transmission (teleradiography) and remote sites in digital format. [22] Digital subtraction radiography was introduced to dentistry by Ruttimann et al 1981, Webber et al 1982 and Grondahl et al 1983. Serially obtained digital images can be super imposed and the resultant composite viewed on a video screen. Changes in the density and / or volume of bone can be detected as lighter areas (bone gain) or dark areas (bone loss). [23]

To overcome the drawbacks of conventional radiography various three dimensional imaging techniques have been developed. Computed tomography [22] was first invented in 1972 by Godfrey Horeusefield. This technique is able to produce an axial cross sectional image of the head by narrowly collimating a moving beam of x rays. Tuned aperture computed tomography (TACT) is built on the basic principles of tomo synthesis: by shifting and combining a set of basis projections, arbitrary slices through the object can be brought into focus. [22] Cone beam computed tomography [23] is a radiographic technique in which patient volume can be scanned in a single rotation. The simplified design of CBCT units also allows for a considerable cost saving relative to medical CT units. Local Computed Tomography is a form of Cone Beam Computed Tomography and uses a small field resolution detector to generate a limited high resolution three dimensional volume. Optical Coherence Tomography generates cross-sectional images of biological tissues using a near infrared light sources. [22]

Other recent techniques include computer assisted densitometric image analysis (CADIA), dentascan, xeroradiography, photodensitometric analysis technique, magnetic resonance imaging (MRI), ultrasound and nuclear medicine (bone scanning). CADIA is a system in which a video camera measures the light transmitted through radiograph and the signals from the camera are converted into gray scale images.[24] DentaScan (General Electric) is a CT software program developed to automatically reformat the oblique cross-sectional images. [25] Xeroradiography, which requires only about one-third of the dose required for conventional radiographs, is a valuable alternative to conventional radiography for detecting carious lesions, calculus deposits and periodontal disease. [26] Photodensitometric analysis technique is based on absorption of a beam of light by the radiographic film, which also shows the image of an aluminium scale and transformation of the density readings into millimeters of aluminium equivalents. [27]

Table 1 . New Innovations in Periodontal Diagnosis

New innovations in clinical diagnosis	Periodontal probes Diagnosing tooth mobility Diagnosis of oral malodour Indicators of occlusal analysis
New innovations in radiographic diagnosis	Digital radiography Digital subtraction radiography Three dimensional imaging methods Other recent techniques Implant site imaging
New innovations in microbiologic diagnosis	Microscopic identification Bacterial culture Enzymatic assays Immunoassays Diagnostic assays based on molecular biology techniques
New innovations in characterizing host response	Host response in periodontal disease Diagnostic biomarkers in saliva Diagnostic biomarkers in GCF

Table 2. GCF Components in Periodontal Disease Diagnosis

GCF Molecules	Commercial test kit
Collegenase and related molecules	Prognos-Stick
Cathepsin-Like Activities and Neutral Proteases	Perio-check
Alkaline Phosphatase	
ß-Glucuronidase and Arylsulfatase	
Aspartate aminotransferase	PerioGard Pocket watch
Cytokines	
Metabolites of arachidonic acid	
Antibodies	

MRI uses nonionizing radiation and to acquire an MRI image, the patient is placed in strong magnetic field. [28] Ultrasound is one of the advanced imaging techniques which use sound waves for viewing the normal and pathological conditions involving bone and soft tissue of the oral and maxillofacial region. [29] Nuclear medicine technique detects alterations in bone metabolism secondary to bone resorptive as well as formative diseases (Williams 1992), by using a radio labelled bone seeking radiopharmaceutical such as technetium-99m. [30]

Implant site imaging [31] involves use of radiographic imaging to assist in the therapy, preoperative treatment planning, surgical procedure, and in the postoperative assessment and ongoing implant function. New generation radiographic techniques have been developed for implant site imaging. Helical CT is a new generation radiographic technique in which radiation is detected by a highly sensitive crystal or gas detector, which is then converted to digital data. This data is stored and manipulated by computer software to produce a grey-scale image and then multiplane sections are reconstituted. Simplant is a computer based image software programme where it is possible to produce images of implants and their restorative components which can then be placed within the 'CT scan'. Scan ora is a new generation sophisticated tomographic device similar to conventional dental panoramic tomograph (DPT) machines, but with facilities to generate high quality sectional images. Computerized digital radiovisiography (CDR) provides an alternative medium to produce an image. Here, the detectors are solid state; hence the doses can be greatly reduced.

New Innovations in Microbiological Diagnosis

In recent years, increasing evidence has appeared indicating that different periodontal conditions are associated with different microbial colonization patterns. The identification of certain bacterial species associated with active periodontal disease has led to the development of several types of diagnostic tests to identify the presence of these microorganisms such as microscopy, bacterial culture, enzymatic essays, immunoassays, nucleic acid probes and polymerase chain reaction assays.

Microscopy, particularly dark field and phase contrast microscopy has been used to demonstrate differences in the distribution of bacterial morphotypes that occur in sub gingival plaque in a coronal to apical direction but the major shortcoming is that the method cannot discriminate between individual bacterial species. [32] Bacterial culture technique is the only current method capable of identifying new species, determining the in vitro antimicrobial susceptibility of periodontal pathogens and able to identify and provide a quantitative measurement of viable microorganisms. However, this technique has serious limitations, mainly; the difficulty in recovering even cultivable species when they are found in low numbers. [32]

Proteolytic enzymes found in the putative periodontopathogens could serve as a marker molecule for these organisms leading to the development of enzymatic essays. BANA test (Perioscan) is based on the principal that several putative periodontopathic bacteria possess a trypsin-like enzyme(s) that can cleave a variety of synthetic substrates that have arginine attached to a chromophore. [32]

Immunologic assays employ antibodies that recognize specific bacterial antigens to detect target microorganisms. This reaction can be revealed using a variety of procedures, including Direct and indirect immunofluorescent microscopy assays (IFA), Flow cytometry, Enzyme-linked immunoabsorbent assay (ELISA), Membrane assay, and Latex agglutination. Direct IFA employs both monoclonal and polyclonal antibodies conjugated to a fluorescein marker that binds with the bacterial

antigen to form a fluorescent immune complex detectable under a microscope. Indirect IFA employs a secondary fluorescein-conjugated antibody that reacts with the primary antigen-antibody complex. [32]

Cytofluorography or flow cytometry for the rapid identification of oral bacteria involves labelling bacterial cells from a patient plaque sample with both species-specific antibody and a second fluorescein-conjugated antibody. ELISA is similar in principle to other radioimmunoassays, but an enzymatically derived color reaction is substituted as the label in place of the radioisotope. The intensity of the color depends on the concentration of the antigen and is usually read photometrically for optimal quantitation. Latex agglutination is a very simple immunological assay based on the binding of protein to latex. A membrane immunoassay has been recently marketed (Evalusite).
Molecular biology techniques for diagnosing the periodontal disease are divided into three main cateogaries namely polymerase chain reaction (PCR) based methods, DNA DNA hybridization methods and sequencing methods. PCR based methods [33]include single target PCR applications, multiplex PCR and Real time PCR. Single target PCR applications include the use of species-specific or phylotype-specific PCR primers in highly stringent, individual PCR reactions to establish the prevalence of target species in plaque samples of healthy subjects and of those with periodontal disease. Multiplex PCR is an expansion of single target PCR methodology in which more than one pair of species- specific primers is used in a single PCR assay and that permits multiple species to be detected simultaneously. Such assays have been used to detect A. actinomycetemcomitans, T. forsythia and P. gingivalis at the same time. The MicroDent Test is a commercially available method using multiplex PCR that tests for five oral species and has been used to compare the microbial profiles of subgingival plaque samples in oral health and periodontitis. Real time PCR , also referred to as quantitative PCR, quantitative reverse transcription PCR, reverse transcription quantitative PCR and kinetic PCR, is a method used to quantify the copy numbers of DNA in clinical samples. There are two types of real-time PCR, namely an intercalator-based method and a probe-based method. MyPerioPath from OralDNAlabs is a commercially available service that utilizes TaqMan PCR to determine the presence and the microbial profile of 13 putative periodontal pathogens from oral specimens provided by clinicians. [33]

Nucleic acid based assays include nucleic acid hybridization performed on colony lifts or in dot or slot-blot assays. DNA Probe [34] involves the technique in which DNA obtained from pure cultures is enzyme-digested, resulting in specific fragments of single strands that are representative of individual species. These fragments are then radiolabeled and serve as a "DNA library" for future tests. DNA DNA hybridization methods [35] include two techniques i.e. Fluorescence in situ hybridization and checkerboard hybridization. Fluorescence in situ hybridization (FISH), or more specifically whole-cell hybridization, can be used to quantify, determine the spatial configuration and demonstrate the morphology of individual bacterial cells in complex natural communities, such as dental plaque. Checkerboard hybridization involves two methods; one utilizing whole genomic DNA probes that are hybridized to sample DNA on the membrane; and the other utilizing labeled 16S ribosomal RNA amplicons that are hybridized to 16S ribosomal RNA-based probes that are on the membrane. This latter method has been referred to as reverse-capture, 16S ribosomal RNA- based oligonucleotide checkerboard hybridization.

Oligonucleotide microarray technology [35] is developed as an extension of the 16S ribosomal RNA-based, reverse-capture DNA–DNA checkerboard hybridization, in order to examine the complex oral microbial diversity in a single hybridization reaction on glass slides. The ParoCheck DNA chip targets 20 oral bacterial species and has been used to determine the microbial profiles of clinical samples. The Phylochip can detect up to 32,000 16S ribosomal RNA phylotypes. Next generation sequencing[35] is the newest technology for high-throughput genomic analysis using a pyrosequencing platform. The three main technologies for next-generation sequencing are 454 pyrosequencing, SoLiD and Illumina/Solexa methodology.

New Innovations in characterizing Host Response

Host responses, which are primarily directed to defending the host against fulminating infections, are also likely result in some of the local tissue destruction that we know as periodontal disease.

Saliva as diagnostic fluid in Periodontal Diseases

Recently, the combination of emerging biotechnologies and salivary diagnostics has extended the range of saliva-based diagnostics from the oral cavity to the whole physiologic system. Salivary constituents that have been studied as potential diagnostic biomarkers for periodontal disease mainly include locally produced proteins of host and bacterial origin (enzymes, immunoglobulins and cytokines), genetic / genomic biomarkers such as DNA and mRNA of host origin, bacteria and bacterial products, ions, steroid hormones and volatile compounds.

Specific salivary proteomic biomarkers have been identified for three key features of the pathogenic processes in periodontal disease inflammation, collagen degradation and bone turnover. Host-derived MMPs are considered to be key initiators of the extracellular matrix degradation associated with periodontal diseases. MMP-8, which is primarily derived from polymorphonuclear leukocytes during active stages of periodontitis, has been identified as a major tissue destructive enzyme in periodontal disease. Similar diagnostic power has also been shown for IL- 1b and TNFa. Other enzymes and growth factors in gingival crevicular fluid and saliva have been examined for their utility in periodontal disease diagnosis, and include lactate dehydrogenase, aspartate amino transferase and alkaline phosphatase, TNFα, epidermal growth factor, transforming growth factor alpha and transforming growth factor beta, platelet-derived growth factor, platelet-activating factor, vascular endothelial growth factor and hepatocyte growth factor.
Development of point-of-care technologies for saliva-based diagnostics has great potential in the use of oral fluid for point-of-care testing. Researchers are designing lab-on-a-chip prototypes. These handheld, automated, easy-to-use and integrated systems will enable simultaneous and rapid detection of multiple salivary protein and nucleic acid targets. [36]

Analysis of GCF and Risk of Progression of Periodontitis

Gingival crevicular fluid (GCF) is an exúdate that can be harvested from the gingival sulcus or periodontal pocket using filter paper strips or capillary tubes.Various components of GCF play a role in diagnosis of periodontal disease.(Table2) [37]

Summary and Conclusion

Throughout history, scientists with an interest in periodontal diseases have applied new findings in the basic biological sciences in an attempt to understand the nature of this complex group of diseases in more detail. Concepts in periodontal diagnostics have evolved in order to keep pace with advances in microbiology, biochemistry, immunology, molecular biology, genetics and connective tissue biology.

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