Perio Tools: A Journey from Inaccuracy to Precision- A Mini Review
Ibrahim Fazal1*, Aysha Kaleem Pasha2, Khadijathul Irfana D1, Casius Cochikunnel1, Riya Ann Joseph3

1Postgraduates, Department of Periodontology, Ramaiah University of Applied Sciences, Bangalore 560054, Karnataka, India
2Postgraduate, Department of Oral and Maxillofacial Surgery, KVG Dental College and Hospital, Kurunjibhag, Sullia 574327, Karnataka, India
3Postgraduate, Department of Orthodontics and Dentofacial Orthopedics, Maharishi Markandeshwar College of Dental Sciences and Research, Haryana, India
4Intern, Ramaiah University of Applied Sciences, Bangalore 560054, Karnataka, India


Background: In the 21st century dentistry has not only reached an epitome of great inventions but also has established a great milestone in achieving efficiency, efficacy and technological advancement. Technology has not only helped dentistry to grow in its value but also has encouraged the dentists to introduce less traumatic procedures. The course of periodontal disease is obvious by the sporadic and intermittent pattern of disease activity and inactivity showing random or alternate exacerbation and remission. Although microbes were considered to be the primary etiology, they were insufficient to cause a disease all by themselves, environmental, systemic and host response were also a part of the etiotrophic factors. Hence, to understand these complexities various assessment tools and advanced diagnostic aids were developed and introduced in practice. The various chair side diagnostic aids include Advanced Periodontal probes, Advanced radiographic diagnosis, Advanced microbial analysis, advanced immunodiagnostic techniques and molecular biological assays, Advanced biomarker identification and genetic testing and Advanced Chair side diagnostic kits. This manuscript reviews the advancement in the recent chair side diagnostic kits which are frequently studied and relied upon.

Keywords: Periodontal instruments, periodontal probing, chairside instruments.

INTRODUCTION
In the 21st century dentistry has not only reached an epitome of great inventions but also has established a great milestone in achieving efficiency, efficacy and technological advancement. Technology has not only helped dentistry to grow in its value but also has encouraged the dentists to introduce less traumatic procedures and more accurate identification of disease.

Periodontology is a branch of dentistry which deals with the supporting investing tissues of the teeth. The course of periodontal disease is obvious by the sporadic and intermittent pattern of disease activity and inactivity showing random or alternate exacerbation and remission. A successful periodontal assessment is the key to a good supportive periodontal therapy. This success in the assessment is achieved by many innovative criteria and significant tools, which have been developed over a period of time for the sake of better and advance diagnosis and assessment. Earlier to 1970’s periodontitis was thought to be a slow progressive condition extending to a prolonged duration of time. Subsequently various models of disease progression came into existence and changed the concepts. Although microbes were considered to be the primary etiology, they were insufficient to cause a disease all by themselves, environmental, systemic and host response were also a part of the etiotrophic factors. Hence, to understand these complexities various assessment tools and advanced diagnostic aids were developed and introduced in practice. There is great obligation for novel researches in the arena of diagnosis to aid in the early recognition of the notorious underlying microbial flora and its role in the disease process along with the vulnerable sites for future breakdown. The advances in diagnosis are categorized as follows [1]:

Abstract

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*Corresponding author: Ibrahim Fazal
Postgraduates, Department of Periodontology, Ramaiah University of Applied Sciences, Bangalore 560054, Karnataka, India
1. Advances in assessment of gingival inflammation and loss of periodontal attachment.
2. Advances in radiographic diagnosis
3. Advances in microbial analysis
4. Advances in immunodiagnostic techniques and molecular biological assays
5. Advances in biomarker identification and genetic testing.
6. Advanced Chair side diagnostic kits.

For the purpose of easy analysis and understanding, each category of advances shall be discussed individually from the path of conventional to digital era.

THE ADVANCEMENT IN THE JOURNEY OF PERIODONTAL PROBES

First generation probes [2]

These are also known as conventional probes that do not control probing force or pressure and that are not suited for automatic data collection. The collection includes Williams periodontal probe, CPITN probe and UNC-15 probe.

The Williams periodontal probe was invented by periodontist Charles HM Williams in 1936 and is the prototype or benchmark for all first-generation probes. These probes have a 13mm thin stainless-steel tip and a 1mm diameter blunt tip end. The graduations on these probes are 1mm, 2mm, 3mm, 5mm, 7mm, 8mm, 9mm and 10mm. (The 4mm and 6mm markers are omitted to improve visibility and avoid confusion when reading the markers.) Probe tips and handles are closed at 130 degrees [3].

The Community Periodontal Index of Treatment Need (CPITN) was designed by Professors George S. Beagrie and Jukka Ainamo in 1978. CPITN probes are recommended for use when screening and monitoring patients with the CPITN index. The index and its probes were first described in World Health Organizations (WHO) Epidemiology, etiology, and prevention of periodontal diseases. Report of a WHO Scientific Group [4]. The FDI World Dental Federation/WHO Joint Working Group 1 has advised the manufacturers of CPITN probes to identify the instruments as CPITN-E (epidemiologic), which have 3.5-mm and 5.5-mm markings, and CPITN-C (clinical), which have 3.5-mm, 5.5-mm, 8.5-mm, and 11.5-mm markings. CPITN probes have thin handles and are lightweight (5 gm). The probes have a ball tip of 0.5 mm, with a black band between 3.5 mm and 5.5 mm, as well as black rings at 8.5 mm and 11.5 mm.

University of Michigan O probes have markings at 3 mm, 6 mm, and 8 mm. A modification of this probe with Williams markings also is available.

Second Generation Probes

Second generation instruments are pressure sensitive and allow for better standardization of probing pressure. Scientific literature showing that the probing pressure should be standardized and should not exceed 0.2 N/mm² led to the development of these probes. Second generation probes can be used in general dental offices as well as periodontal offices and do not require computerization in a dental setting.

These are also constant force probes which allow for improved standardization of probing. These include Pressure sensitive probe. In 1977, Armitage designed a pressure-sensitive probe holder to standardize insertion pressure and determine how precisely a 25-pound probe pressure affected insertion of connective tissue. In 1978, van der Velden developed a pressure-sensitive probe pressure with a connected cylinder and piston to an air pressure system, was modified with a pocket electronics depth reader with a single displacement transducer [3].

The True Pressure Sensitive (TPS) probe (Figure) is the prototype of the second-generation probes. Introduced by Hunter in 1994, these probes have a disposable probe head and a 0.5mm diameter hemispherical probe tip. A controlled probe pressure generally applies a pressure of 20 g. These probes have a visual guide and a sliding scale where two indicator lines meet at a given pressure [3].

The only shortcoming of constant pressure probes is inaccuracy in data readout and storage.

Third Generation Probes

Despite the advances in the second-generation probe, other sources of error, such as reading the probe, data recording and calculation of the level of attachments remained unaddressed. Third-generation probes were developed to help minimize these errors by using not only standardized pressure but also digital readouts of the probes’ readings and computerized data storage. This generation comprises computer-assisted direct data capture to decrease examiner bias and permits for greater probe accuracy. These probes necessitate computerization of the dental office and can be used by periodontists and academic institutions for research.

Third generation probe or Automated probes work on the principle of Computer assisted direct data capture which helps in reducing examiner bias and increasing probe precision. These include Toronto probe, Florida probe and Foster Miller probe.

Fourth Generation Probes

These are also known as Three dimensional probes which are currently still under development, these probes are aimed at recording sequential probe positions along a gingival sulcus. They are an attempt to extend linear probing in a serial manner to take into account the 3D pocket being examined.
Fifth generation probes

Despite all the advances in earlier generation probes, they continue to remain invasive and at times, can be painful to patients. Also, with earlier generation probes, the probe tip usually surpasses the junctional epithelium. Fifth-generation probes are being developed to eliminate these drawbacks. Probes are 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. Fifth generation probe are 3 Dimensional and Non-invasive working on the principle of ultrasound sensor detection along with the properties of a fourth-generation probes.

<table>
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<tr>
<th>Generation</th>
<th>Advantage</th>
<th>Disadvantage</th>
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<tr>
<td>First Generation</td>
<td>• Tactile sensitivity&lt;br&gt;• Rounded tip: minimal tissue trauma&lt;br&gt;• Probe can be inserted even if presence of sub gingival calculus is detected.&lt;br&gt;• Color coding for easier and quicker identification of readings&lt;br&gt;• Inexpensive and readily available.</td>
<td>• The probe force is not controlled so the tip of the probe may extend beyond the base of the pocket.&lt;br&gt;• May encounter errors while visualizing the readings.&lt;br&gt;• Chairside assistant is required to note the readings to the chart.</td>
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<tr>
<td>Second Generation: Pressure Sensitive</td>
<td>• Probing forces are standardized.&lt;br&gt;• Pressure remains constant.&lt;br&gt;• Patient comfort and compliance.</td>
<td>• Reading performed manually, and an assistant is required to document the readings.&lt;br&gt;• Probe tip may pass beyond the junctional epithelium in inflamed sites.</td>
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<tr>
<td>Third Generation: Florida Foster-Miller</td>
<td>• Probing forces are standardized.&lt;br&gt;• Reading errors and documentation errors are eliminated.&lt;br&gt;• Printout of the data from the computer can be used for patient education.</td>
<td>• Decreased tactile sensitiveness.&lt;br&gt;• The probe force is not controlled so the tip of the probe may extend beyond the base of the pocket.&lt;br&gt;• After inflammation has resolved, probe may not penetrate beyond the long junctional epithelium, leading to underestimation of the pocket depth.</td>
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<tr>
<td>Fourth Generation:</td>
<td>• 3d Probe&lt;br&gt;• Sequential positions for probing are measured.</td>
<td>• Under development.</td>
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<td>Fifth Generation:</td>
<td>• A noninvasive probe that offers painless probing no question of probe passing beyond the junctional epithelium, as ultrasound waves detect image and map the upper boundary of periodontal ligament.&lt;br&gt;• Pre-determined guidance path.&lt;br&gt;• Provides the condition of the gingival tissue.</td>
<td>• Expensive&lt;br&gt;• Operator needs to understand the images provided by the computer&lt;br&gt;• Requires a learning curve</td>
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Advances in radiographic diagnosis

Digital subtraction radiography

In 1935 Zeidse des Plantes demonstrated a technique which he attributed as a photographic technique. According to this technique, the images which are not required in a radiograph are reduced so that the changes in the radiograph can be precisely detected. Digitalization was achieved by taking a picture of radiograph using video camera. This technique facilitated both qualitative & quantitative visualization of even minor density changes in bone by removing the unchanged anatomic structures from image.

CCD- Direct Digital Image, Acquired Using a Charge Coupled Device, Complementary Metal- Oxide Semiconductor, or Other Electronic Device [5]

In this method, the intensity of the radiation in the x-ray beam is measured directly by an electronic device consisting of a large number of light-sensitive elements. The output of these elements is transferred to the computer as an electric signal and digitized in the frame grabber board. A scintillation layer is put on top of the sensor array. X-ray photons are converted into light photons, increasing the efficiency of the detector. The size of the electronic sensors has been considerably smaller than a No. 2 dental film, but currently sensors

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with an active area that approaches the dimensions of standard intraoral film are available.

CMOS: Complementary Metal Oxide Semiconductor [6]

Complementary Metal oxide Semiconductor based sensors are now finding their way into intraoral sensory stems. Their advantage is design integration, low power, manufacturability, low cost and ability to benefit from the high-volume manufacturing capacity already in place to support the CMOS semiconductor industry. CMOS sensors also permit the integration of control circuit, including ADC, directly into the sensor but although they perform well in bright light conditions their performance in low light conditions or with the rigorous demands of medical imaging systems. They have more fixed pattern noise and use some of the chip.

Dentists use the panoramic mode to image the entire set of teeth and parts of the jaw in a single view for placement of dental implants and assessment of TMJ disorders, sinus infections and other problems.

CADIA- Computer Assisted Densitrometric Image Analysis System [7]

Computer Assisted Densitrometric Image Analysis System was introduced by Urs Brägger et al., 1988. It comprises of a video camera which measures the light transmission through a radiograph converting the signals into grey scale images. The Camera is interfaced with computer and image processor for storage and mathematic manipulation of image offering an objective method for studying alveolar bone changes quantitatively with high degrees of sensitivity, accuracy and reproducibility.

Computed tomography [8]

Godfrey Hounsfield and Allan MacLeod Cormack in the year 1979 made a device which consisted of a x ray tube emitting finely collimated x-ray beam directed through the patient to a series of scintillating detectors or ionizing chambers. Detectors form a continuous ring and x-ray tube moves in a circle with in the ring. CT machines use a rotating fan beam to image one thin slice.

ADVANCES IN MICROBIAL ANALYSIS

Historically, culture techniques were the widely accepted method in research aiming at defining the composition of the subgingival microflora. Microbiological diagnostic tests help in the detecting these lesions early and providing an opportunity for non-invasive treatment protocols.

Microscopy, bacterial culture, immunological assays like Evalusite, Fluorescent In-Situ Hybridisation (FISH), Oraquick, enzymatic assays like Perioscan, Periogard, Pocketwatch, Periocheck, Matrix Metalloproteinase (MMP) dipstick test, Biolise, and molecular biology techniques like Polymerase Chain Reactions (PCR), Terminal Restriction Fragment Length Polymorphism (T-RFLP), 454 Pyrosequencing, Supported Oligonucleode Ligation and Detection (SOLiD) have been among the techniques employed.

According to Bernardi S et al., [10] a combination of culture and culture independent methods help in the rapid identification of new microbial composition of halitosis. In the study, culture-independent methods revealed 50 species including Streptococi and Prevotella while the culture method identified 47 species that include Vilonella rogosa that was never isolated from the tongue biofilm of halitosis so far.

ADVANCES IN BIOMARKER IDENTIFICATION

The term "markers of disease" basically consists of three categories;

1. Indicators of current disease activity;
2. Predictors of future disease progression;
3. Predictors of initiation of future disease at a currently healthy site.

Biomarkers are more appropriately defined as quantifiable and measurable biologic parameters that serve as indicator for health and physiology-related assessments.

Integrated Microfluidic Platform for Oral Diagnostics (IMPOD) is a clinical point-of-care diagnostic test that involves a monolithic disposable cartridge designed to perform in a compact analytical equipment to identify an oral disease biomarker in human saliva which helps in evaluating the analytic concentrations in pre-treated saliva samples alongside electrophoretic immunoassays.

In this technique, Photoinitiated polymerisation is coated to the channel surfaces with the help of linear polyacrylamide via cross-linking. It spontaneously measures MMP-8, IL-6, TNF-α in saliva from healthy and periodontally diseased subjects.


Microfluidic lab-on-chip is used to demonstrate clinical biomarkers in physiological settings with a relatively small sample size particularly beneficial to the patient. It gives a benefit of economical angle where the assays can be performed with minimal reagent costs with a small sample size and reduced analysis periods using ETC technique aiding in the detection of several periodontal biomarkers including IL-1, MMP-8, CRP and biomarker-based identification of cancer from whole saliva.

This can be used in drug screening, high throughput screening, immunosensing and binding assays, microfluidic cell culture, cellular environmental...
control, single cell analysis, manipulating and sorting individual cells.

ADVANCES IN GENETIC TESTING

MyperioID [12]

MyperioID identifies the genetic susceptibility of the patient to periodontal diseases through salivary samples. These tests significantly help in diagnosing patients with high risk of periodontal destruction.

Omnigene [13]

It is a genetic nucleic acid probe with purified DNA fragments having an ability to detect *P.intermedia* and *P.gingivalis*, but not *A.actinomycetemcomitans*. Omnigene works on the principles of genetic engineering to develop species specific DNA probe tests for 8 periodontal pathogens which are *P.gingivalis*, *P.intermedia*, *A.actinomycetemcomitans*, *F.nucleatum*, *E.corrodens*, *C rectus*, *T.forsythia*, *T.denticola*.

In this technique subgingival plaque samples are collected from the patients and sent for analysis. The results are transmitted to the practitioner by phone, fax or mail.

According to Lim Y et al., [14] falcon tubes and Omnigene tubes reveal about the saliva microbiome profiles which are minimally affected by collection method. In recent days, Omnigene has also been used for COVID-19 saliva sample collection.

Terminal Restriction Fragment Length Polymorphism (T-RFLP) [15]

T-RFLP is a molecular biology technique that uses the location of a restriction site nearest to a labelled end of an amplified gene to profile microbial populations. The extracted DNA is fragmented using Restriction Endonucleases (RE), subjected to polyacrylamide or agarose gel electrophoresis, further denatured using NaOH where blotted and the labelled probe is attached to a charged membrane which further hybridises and visualises using genomic analysis and genetic disease analysis. It is relatively simple but is a long-lasting technique with high labour requirements, high quality and wide quantity of DNA requirements, working with radioisotopes on a consistent time frame, there are a lot of polymorphisms present for a short probe and the cost of production is very high. It also aids in evaluation of multiple bacterial communities, fast comparison of their structure and diversity of ecosystems leading to a rapid diagnosis of periodontal disease. In a study conducted by Ding YJ et al., T-RFLP had more detail and higher sensitivity as compared to traditional culture techniques.

Supported Oligonucleotide Ligation and Detection (SOLiD) [16]

SOLiD instrumentation was released by Applied Biosystems in 2007. The sample preparation is similar to 454 pyrosequencing technology. SOLiD comprises of whole genome resequencing, targeted resequencing, transcriptome research and epigenome. The drawbacks of SOLiD include biased sequence coverage in AT-rich repetitive sequences and it requires a long run times, six days.

ADVANCED CHAIR SIDE DIAGNOSTIC KITS

Chair-Side Test (CST) [17]

Research conducted in 2019 introduced a new chairside test can detect five periodontal pathogens, *A.actinomycetemcomitans*, *P.gingivalis*, *T.forsythia*, *T.denticola*, *P.intermedia*. Samples are analysed with Chair Side Test and results are detected by visual method. It has lower sensitivity than Quantitative-Polymerase Chain Reaction (Q-PCR) but the sensitivity and specificities of the test undefined.

Diamond Probe/Perio 2000 System [18]

The PERIO 2000 device incorporates periodontal probe capacity with an ability of identification of Volatile Sulphur Compounds in periodontal pockets. Various pathogenic microorganisms such as *T.forsythia*, *P.gingivalis* and *P.intermedia* produce large amounts of VSCs as a result of serum protein degradation i.e., Methionine and Cysteine. While Volatile Sulphur Compounds actively deteriorate periodontal surfaces, their evaluation can reveal information regarding the microbial burden subgingivally. It is similar to a standard periodontal probe; however, it has a special microsensor in its tip that detects the amount of bacterial development at individual tooth tips right before gum bleeding occurs.

EvaluSite [19]

The mechanism involves formation of antigen-antibody complexes by the incorporation of enzymes unique to bacteria. These enzymes help in the identification of periodontal destruction activity. This technique is mainly used in the diagnosis of *A.actinomycetemcomitans*, *P.intermedia* and *P.gingivalis* bacterial antigens. The sample is placed in the kit, especially adopts a sandwich type ELISA, and if the test organism is identified, a pink spot emerges. Because such sample dilution is insignificant, multiple paper points can be compiled in a single sample tube. It produces greater findings as samples are taken from deeper pockets rather than shallow pockets. It is a multistage evaluation with an ill-defined calorimetric end point.

The study conducted by Boyer BP et al., [20] concluded that the results of the analysis of the sample site with the EvaluSite test in all cases demonstrated the percentage of deep pockets with positive results was higher than in shallow sites for all three microorganisms tested. In addition, the sensitivity and specificity of the immunoassay test results corresponded most closely to culture results observed at a threshold of >104 cultivable counts.
Institute for Applied Immunology (IAI) PADO Test

4.5

Four periodontal pathogens A. actinomycetemcomitans, T. forsythia, T. denticola and P. gingivalis can be detected using Pado RNA probe test. This assay encrypts the RNA using oligonucleotide probes that are complementary to conserved 16s RNA segments and operates as a subunit of the bacterial ribosome. A Study by Leonhardt A et al., [21] in chronic periodontitis patients, the detection frequencies were evaluated using the Pado test 4.5 and the Checkerboard DNA-DNA hybridization approach. While the Pado test detected four periodontal bacteria in 36.6% of cases, the Checkerboard test detected them in all patients. The detection frequencies observed for this test show a poor sensitivity and the test appears to undercount the number of positive sites/individuals exposed as a result of a large number of false negatives.

Pocket Watch [22]

AST levels fluctuate in healthy sites and periodontally involved sites. Pocket Watch is a technique that can measure AST levels in order to validate the clinical outcomes in chronic periodontitis patients preoperatively. The enzyme AST catalyze leads to the formation of sulfanyl pyruvate which decomposes and releases inorganic sulfite naturally and rapidly. This inorganic sulfite ion shines in pink color due to Rhodamine B dye after reacting with Malachite Green (MG) that changes it from green to a leuco state. The amount of MG conversion is proportional to the concentration of AST. It counts the number of cells that already have died and the extent of the disrupting pockets. However, the evaluation is subjective and technique dependent.

A study done by Sánchez-Pérez A et al., [23] to detect the presence of AST in peri-implant crevicular fluid with and without mucositis using Pocket Watch and compared inflamed tissues with healthy tissues in-situ, concluded that the implant position could be responsible for this difference.

Toxicity Prescreening Assay (TOPAS) [24]

TOPAS is a chairside test kit for indirectly detecting bacterial toxins and bacterial proteins in a gingival infection. The principle behind this test is based on the detection of actively dividing and growing pathogens assessed through the metabolic activity of these organisms in the crevicular fluid. This test can be used to know difference between an active and an inactive periodontal disease as indicated by the change in the color intensity scale of the test based on the fact that metabolic activity increases as the concentrations of these toxins increases.

CONCLUSION

The pool of periodontal diseases consists of a wide range of microbial infections which are frequently observed in the patient flow. With most common being generalized gingivitis. Apart from regular commonly seen gingivitis and periodontitis there are certain other diseases which occur quiet frequently but are also marked as one of the rarest of rare and severe periodontal diseases which not only effect the patient at a very young age but also have a drastic effect of depriving them from the remaining teeth in a very short period of time. An accurate diagnosis of such diseases is a foundation for the success of further treatment. The existing diagnostic methods suffices the purpose but with the arrival of new and advanced chairside test kits of periodontal disease monitoring of specific sites is now possible. Great amount of research activity is being undertaken to investigate the role of oral fluids as a medium for diagnostic purposes in various fields. Although, challenges remain ahead, the use of saliva and GCF based oral fluid diagnostics are promising in the diagnosis of periodontal diseases and predicting periodontal treatment outcomes. Although, challenges remain ahead, the use of saliva and GCF based oral fluid diagnostics are promising in the diagnosis of periodontal diseases and predicting periodontal treatment outcomes.

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