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Review Article

Dentistry Section

Advanced Chairside Diagnostic Aids for Periodontal Diagnosis- A Review

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ABSTRACT

Periodontitis is an infection driven inflammatory disease caused by dental plaque accumulation that in turn causes microbial alterations which may lead to drastic consequences in the periodontium in susceptible individuals. Hence, the rationale of periodontal therapy is predominantly focused on the elimination or reduction of these periodontal pathogens. Despite following a wide range of preventive measures, controlling periodontal disease is challenging and treatment is usually initiated mostly after lesions become clinically detectable and tissues undergo irreversible damage. Microbiological diagnostic tests aid in the early detection of these lesions when they are still reversible giving an opportunity for non-invasive treatment. 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. Some of these diagnostic aids were solely for scientific purposes, while others were adapted and updated for therapeutic use. The current paper focuses on the practical utility of the rapidly expanding plethora of microbiological diagnostic aids highlighting the concerns surrounding their applications in periodontal diagnosis.

Keywords: Biolise, Biomarkers, Detection, Fluorescent in-situ hybridisation, Gingival crevicular fluid, Oraquick, Polymerase chain reactions, Saliva

INTRODUCTION

"Diagnosis is not the end, but the beginning of practice"-Martin H Fischer

In today's practice, a periodontal diagnosis is primarily based on information acquired from the patient's medical and dental records, radiographic, histological studies, and a thorough clinical examination [1]. Traditional clinical measures for periodontal diagnosis, including bleeding on probing, probing pocket depth, clinical attachment loss, and periodontal index, are sometimes of limited value since they are indications of previous rather than present disease activity [2]. The initially used anaerobic culture techniques that may be insufficient because some putative periodontal pathogens such as Spirochetes and *Tannerella forsythia* are either uncultivable or difficult to cultivate [3]. Consequently, since their quantification attempts were crude, the researchers concluded that the variations between health and illness "seemed too small to have diagnostic value" [4]. With the discovery of risk groups and uncertain pathology, the merits of objective testing for initial diagnosis for a periodontal patient have become evident [5]. But microbial aetiology and diagnostics are critical in establishing a definite diagnosis. So microbiological analysis, along with clinical monitoring enhances treatment quality and serves as a reference for further treatment strategies [1].

Many trials conducted have shown findings which served as a valuable model of microbiologic monitoring for diagnostic purposes and advancements in this area [6,7]. The review article encompasses the outline of the scope of periodontal diagnostic aids by briefing the current practices emphasising more on recent developments. [Table/Fig-1] shows the advances in microbiological techniques for early detection of periodontal pathogens [8,9].

Sl. No.	Type of test	Source	
		Saliva	**Gingival crevicular fluid**
1.	Microbiological tests	Culture methods	BANA (N-Benzoyl DL-Arginine 2- Napthylamide) test
		My Periopath	Perioscan
		Omnigene	Evalusite
		Chair Side Test (CST)	Diamond Probe/Perio 2000 system
			Institute for Applied Immunology (IAI) Pado test
			Toxicity Pre-screening Assay (TOPAS)
2.	Biochemical tests	Ofnaset	Periogard
		Electrotaxis-on-a-Chip (ETC)	Pocketwatch
		Oraquick	Periocheck
		Integrated Microfluidic Platform for Oral Diagnostics (IMPOD)	Prognostik
			MMP 8 Dipstick Test
			Biolise
3.	Molecular biology techniques	Polymerase Chain Reactions (PCR)	
		DNA Probes	
		Checkerboard DNA-DNA Hybridistion	
		Fluorescent In-Situ Hybridisation (FISH)	
		Terminal Restriction Fragment Length Polymorphism (T-RFLP)	
		454 Pyrosequencing	
		Supported Oligonucleode Ligation and Detection (SOLiD)	
4.	Genetic tests	My Perio id	
		Periodontal Susceptibility Test (PST)	

[Table/Fig-1]: Classification of microbiological diagnostic aids in periodontal clinical practice [8,9].
DNA: Deoxyribonucleic acid

MICROBIOLOGICAL TESTS

Culture Methods

Historically, culture techniques were widely employed in research aiming at defining the composition of the subgingival microflora

and are still regarded as the gold standard in periodontics for deciding the microbial diagnosis [10]. Colonies of *Aggregatibacter actinomycetemcomitans* ((*A.actinomycetemcomitans*) grow on Dentaid-1 medium [11], *Porphyromonas gingivalis* (*P.gingivalis*) and *Prevotella intermedia* (*P.intermedia*), *Prevotella nigrescens* (*P.nigrescens*), grow on blood agar medium supplemented with haemin and menadion [12] and *Tanerella forsythia* (*T.forsythia*) grow on blood agar medium [13]. The ability to get relative and absolute counts of cultivated species is the major advantage of this approach. The disadvantages include the capacity to only grow live bacteria, the difficulty of cultivating treponema species, lower sensitivity, and detection limits averaging only 10^3-10^4 cells [10]. The most significant disadvantage is that they need specialised laboratory equipment and skilled staff, in addition to being time consuming and costly. In a study conducted by Bernardi S et al., where culture and culture independent methods were combined to identify the new microbial composition of halitosis, culture- independent methods revealed 50 species including *Streptococci* and *Prevotella* while the culture method identified 47 species that include *Vilonella rogosae* that was never isolated from the tongue biofilm of halitosis so far [14].

My Periopath

My Periopath analyses saliva samples to determine the type and concentration of relevant bacteria causing periodontal infections. It detects oral infections early, allowing periodontal therapy to be personalised to the individual [15]. It detects active periodontal disease by detecting the bacterial load by estimating high risk pathogens like *A.actinomycetemcomitans*, *P.gingivalis*, *T.forsythia*, *T.denticola*, moderate risk pathogens like *Eubacterium nodatum* (*E.nodatum*), *Fusobacterium*.nucleatum (*F.nucleatum*), *P.intermedia*, *Campylobacter rectus* (*C.rectus*), *Parvimonas micros* (*P.micros*) and low risk pathogens like *Eikenella corrodens* (*E.corrodens*), *Capnocytophaga sputigena* (*C.sputigena*) [16].

Omnigene

It is a genetic nucleic acid probe. Purified Deoxyribonucleic Acid (DNA) fragments can detect *P.intermedia* and *P.gingivalis*, but not *A.actinomycetemcomitans*. The DNA probe test for *P.gingivalis* may provide false negative findings. Omnigene has applied the principles of genetic engineering to develop species specific DNA probe tests for 8 periodontal pathogens (*P.gingivalis*, *P.intermedia*, *A.actinomycetemcomitans*, *F.nucleatum*, *E.corrodens*, *C.rectus*, *T.forsythia*, *T.denticola*). Subgingival plaque samples are collected from the patients and sent through the mail for analysis. Results are transmitted to the practioner by phone, fax or mail [17]. A study done by Lim Y et al., using falcon tubes and Omnigene tubes revealed that the saliva microbiome profiles are minimally affected by collection method [18]. Omnigene is used for COVID-19 saliva sample collection granted by Food And Drug Administration's Emergency Authorisation Use [19].

Chair-Side Test (CST)

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 CST and results (positive signals for every pathogen/control) are visually detected by eye. It has lower sensitivity than Quantitative-Polymerase Chain Reaction (Q-PCR) but the sensitivity and specificities of the test are not yet clearly defined [9].

N-benzoyl-DL-arginine-2-napthylamide (BANA) Analysis

The BANA helps in the detection of *P.gingivalis*, *T.denticola* and *T.forsythia* enzymatic action [3]. It is a quick and relatively accurate test performed chairside in 15 minutes. A stock solution of BANA is diluted in Sorensen buffer 1:100 to yield a working solution of 0.67mM BANA in the Standard BANA method. This is added to 100 µL of various bacterial or plaque suspensions, incubated overnight and colour was developed by the addition of 50 µl of fast garnet solution. Results are read as negative (yellow), weakly positive (yellow-orange) or positive (orange-red) [20]. It can be positive in clinically stable areas also because it identifies only a small number of species, and negative results do not rule out the presence of many other periodontal microbes. According to Morita and Wang, the BANA Test had a significant link with the sulphur levels of hydrogen sulphide and methanethiol present in the gingival sulcus, demonstrating that the BANA Test can be employed for assessing the plaque's sulphur levels *in-vivo* [21]. It had a clear association with organoleptic scores collected from the saliva, tongue and the entire mouth, not so much with Volatile Sulphur Compounds (VSCs) according to Kozlovsky A et al., [22].

Perioscan

Perioscan employs the BANA principle, which involves the presence of enzymes capable of degrading N-benzoyl DL-arginine 2- napthylamide to identify *T.denticola*, *T.forsythia* and some Capnocytophaga species that generate trypsin-like proteases in dental plaque. A sterile curette is used to obtain subgingival plaque samples, which are then placed on a BANA-containing plastic strip. A dampened parallel strip containing Evans black dye is folded and positioned in contact with the other strip for 15 minutes at 55°C. After incubation, the card is unfurled and examined for the presence of blue colour on the dye containing strip [20]. It is simple to read, easy to understand and has high sensitivity. However, it has lower examiner reproducibility as the outcomes are quantifiable and based on the operator's evaluations of the calorimetric end point [23].

Diamond Probe/Perio 2000 System

The PERIO 2000 device incorporates periodontal probe capacity and ability of VSCs identification 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 VSCs may actively deteriorate periodontal surfaces, their evaluation can reveal information regarding the microbial burden sub-gingivally. 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 [24].

Toxicity Prescreening Assay (TOPAS)

With the aid of bacterial toxins and proteins that signal the presence of pathogens, a novel TOPAS test kit identifies levels of bacterial toxins and elevated levels of human and bacterial inflammatory proteins. It can differentiate active and inactive periodontal destruction [25] by assessing the severity of inflammation based on the intensity of blue colour produced which is proportional to the amount of proteins present in Gingival Crevicular Fluid (GCF). It is a basic, painless technique that may be completed in seven minutes by any medical expert.

Evalusite

The formation of antigen-antibody complexes by the incorporation of reporter enzymes unique to bacteria aids in the identification of periodontal destruction activity. The technique aids 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 [26]. It produces greater findings as samples are taken from deeper pockets rather than shallow pockets [27]. It is a multistage evaluation with an ill-defined calorimetric end point. The studies conducted by Boyer BP et al., 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 $>10^4$ cultivable counts [28].

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 [29]. This assay encrypts the rRNA using oligonucleotide probes that are complementary to conserved 16s RNA segments and operates as a subunit of the bacterial ribosome [30]. A Study conducted by Leonhardt A et al., in chronic periodontitis patients, the detection frequencies were evaluated using the Pado test 4.5 and the Checkerboard DNA-DNA hybridisation 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 [31].

BIOCHEMICAL TESTS

Oral Fluid Nanosensor Test (OFNASET)

It is a swift, automated point-of-care device that measures DNA gene transcripts, electrolytes, proteins, enzymes such as Horse Radish Peroxide (HRP), and cancer biomarkers in saliva utilising microfluidic and Micro Electro Mechanical Systems (MEMS). Detects Thioredoxin, Interleukin (IL)-8 like salivary proteomics and salivary biomarkers like ODZ, IL-8, IL-1β, SAT [32]. This detection system makes use of oligonucleotide capture as well as detector probes or antibodies that adhere to RNA or antigens present in saliva [33]. In the initial study done by Gau V et al., IL-8 was detected [34].

ElectroTaxis-On-A-Chip (ETC a Lab-On-Chip System)

Microfluidic lab-on-chip helps to demonstrate clinical biomarkers in physiological settings with a relatively small sample size, which is particularly beneficial to the patient or point of care. Complex immunological assays can be performed with minimal reagent costs, a small sample size and reduced analysis periods using ETC technique [35]. It aids in the detection of several periodontal biomarkers including IL-1, MMP-8, CRP as well as biomarker-based identification of cancer from whole saliva [36]. Furthermore, because the accuracy of the ETC assay is heavily reliant on the size and homogeneity of its component sensor beads, the beads are sieved to create a consistent collection of microspheres 280+-10μm [37]. A micro-Total Analysis System (μTAS) developed by Sandia National Lab (USA) based on microelectromechanical technology, a recent version of lab-on-chip technology, is used to detect MMP-8 in saliva. An optical system with a fluorescence-labeled anti-MMP-8 antibody is being used in test, which is then followed by electrophoresis. The introduction of reagent integrators, soft lithography, injection moulding, photolithography, hot embossing, laser micromachining to fabrication process, surface modification using polydimethylsiloxane (PDMS), PDMS- Polytetrafluoroethylene (PTFE) and integration with other technologies like optics by using gallium-nitride-blue-light emitting diode, acoustic phenomenon to control fluidic operations, electricity and magnetism. 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 [38].

Oraquick

The test is a simple, qualitative, FDA approved, single step ELISA for the detection of HIV-1/2 antibodies that utilises a test device and a vial that contains premeasured amount of PBS solution. The assay contains two zones viewed through a result window. The test (T) zone contains a synthetic gp41 peptide and the control (C) zone containing a anti human IgG [39]. One of the major limitations is that it detects more serious infection during the acute phase of HIV and they require a confirmation via second ELISA or ideally western blot [40].

Integrated Microfluidic Platform for Oral Diagnostics (IMPOD)

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. To evaluate analyte concentrations in pre-treated saliva samples, it incorporates sample pre-treatment (filtering, enrichment, and mixing) alongside electrophoretic immunoassays. Photoinitiated polymerisation is employed to coat the channel surfaces with the help of linear polyacrylamide that undergoes cross-linking in-situ [41]. It rapidly measures MMP-8, IL-6, TNF-α in saliva from healthy and periodontally diseased subjects [42]. In IMPOD, off-chip sample intubation and reporter binding steps can be discarded since the analyte trapping happens in the volume near the membrane [41]. More research into the recognition and confirmation of these biomarkers is currently underway.

Periogard

Periogard Periodontal Tissue Monitor was designed on the findings of Aspartate Aminotransferase (AST) levels in GCF. It includes a tray with two test wells for each tooth and the required reagent. A filter paper strip is used to collect GCF, which is subsequently placed in a tromethamine hydrochloride buffer. The sample is treated with a substrate reaction mixture including 1-aspartate and α-ketoglutaric acid for 10 minutes. α-ketoglutaric acid and aspartate are catalysed to oxaloacetate and glutamate in the presence of AST. The intensity of the colour of fast red dye is proportional to the AST activity of the sample [43]. It can indicate 2-3 mm attachment loss or significant alveolar bone loss over a particular time period. Using increased AST levels to detect cell death and tissue failure is a scientifically solid rationale. This method usually identifies a small proportion of sites, generally 5-10% as deteriorating. There is no doubt that these sites are degrading, but the approach misses many more sites [44].

PocketWatch™

AST levels differ in periodontally diseased and healthy sites. PocketWatch™ is a reliable technique that can measure AST levels in order to validate the clinical outcomes in chronic periodontitis patients preoperatively. The enzyme AST catalyse leads to the formation of sulfinyl pyruvate which decomposes and releases inorganic sulfite naturally and rapidly. This inorganic sulfite ion shines in pink colour 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 dependant [45]. A study done by Sánchez-Pérez A et al., to detect the presence of AST in peri-implant crevicular fluid with and without mucositis using PocketWatch and compared inflamed tissues with healthy tissues in-situ, concluded that the implant position could be responsible for this difference [46].

Periocheck

Facilitates in the identification of GCF derived enzyme behaviour (matrix metalloproteinases and neutral protease enzymes). It is the quickest chairside test for detecting neutral proteinases, with high sensitivity and specificity. However, due to saliva contamination, the interproximal sites cannot be sampled, the assay is not unique for

polymorphonuclear leukocyte (PML) collagenase and can require enzymes of bacterial origin. A study conducted by Hemmings KW et al., concluded that Periocheck had 88% sensitivity and 61% specificity, Periocheck had a 50.4% clinical impact after therapy, whereas Perioscan had a 52% clinical effect. As a result, diagnostic kits didn't properly represent clinical assessments of periodontal disease [47].

Prognos-Stik

Prognos-stik was launched in 1993, it detects serine proteinase and elastase in GCF samples. Elastase is a protein that is secreted from the lysosomes of PMN leukocytes and accumulates in areas of gingival inflammation. Elevated elastase levels in GCF may be symptomatic of active disease states. The GCF is collected on filter paper strips impregnated with buffered elastase substrate which is labelled with a fluorescent indicator. During the reaction period of 4-6 minutes, the substrate on the test strip is cleaved by elastase and releases a fluorescent visible indicator [48].

Biolise

Hermann JM et al., designed a test for detecting elastase activity in GCF. GCF and sample buffer or elastase standards are centrifuged at 5000 rpm for five minutes in a test tube. Then, 10 µL of this volume is pipetted into a microtiter plate containing 90 µL (PH -8.1) assay buffer. The test plate is then pipetted with 50l of a 10^{-3} M solution solution of the fluorogenic substrate MeO-Succ-ala-ala-pro-val-7-amino-4-methylcoumarin. The plates are then covered with a removable film and incubated at 25°C for six hours. Human Leukocyte Elastase (HLE) standards are included on each microtiter plate. Biolise software is used to directly calculate the Elastase activity of the samples [49].

MMP-8 Dipstick Test

The immunochromatography concept is used in this test. It contributes to the development of two monoclonal antibodies against distinct MMP-8 isotopes. It helps in the detection of *C.rectus*, *P.gingivalis* and *F.nucleatum*. A frequent problem in microbial testing is the potential for differences in results obtained by different laboratories and techniques. Dipsticks are rough and unsuitable for sensitive small volume samples such as GCF obtained with paper strips. The concentration of the tested biomarker can vary greatly in a small sample, this change is represented by a line on the test stick which is difficult to see with the naked eye [50]. According to the study conducted by Sorsa T et al., MMP-8 analysis has proven to be a sensitive and an objective biomarker as an indicator of health, pathologic (MMP-8 helps in patient-specific diagnostic analysis in periodontitis, peri-implantitis and helps in monitoring cardiovascular diseases), pharmacological (therapeutic intervention including doxycycline medication as an MMP-inhibitor) responses [51].

MOLECULAR BIOLOGY TECHNIQUES

Polymerase Chain Reaction (PCR)

In 1993, PCR was used for the first time to identify periodontal pathogen P.gingivalis in oral plaque samples [52]. PCR is an invitro technique that allows for amplification, examination of genes and their RNA transcripts derived from GCF, peripheral blood, skin, hair and semen. The inclusion of DNA polymerase, DNA primers and nucleotides is determined for most assays. Derivative products of classical PCR such as Reverse Transcriptase-PCR (RT-PCR), Quantitative-PCR (Q-PCR), Multiplex-PCR, Nested-PCR, Realtime-PCR, Allele-specific PCR were developed and eventually played a crucial role in periodontology [53]. In the plaque analysis in 2005, open ended PCRs were used to map the whole bacterial spectrum [54]. In 2016 Coffey J et al., detected *A.actinomycetemcomitans*, *F.nucleatum*, *P.gingivalis*, *T.denticola* and *T.forsythia* using multiplex-PCR with the help of TaqMan oligos and TaqMan probes [55]. In 2018, qRT-PCR done by Assem M et al., detected proliferation in periodontal ligament Stem cells and Gingival Stem cells is higher in non-diabetic patients that helps to understand autologous regenerative therapy [56]. In a study done by Al-Ahmed A et al., a higher abundance of Bacteroides, F.*nucleatum*, putative red complex like *P.gingivalis* and *T.forsythia* in peri-implantitis group and yellow complex like *Vielonella parvula* (*V.parvula*) in healthy implant group using 16s rRNA gene cloning [57]. Due to its extremely high detection level it can detect and amplify even 5-10 cells. In ideal conditions, there is less cross reactivity and more species can be witnessed simultaneously. However, high cost, high degree of experience requirement, non specific binding of primers to identical sequences of template DNA and error proneness are its fundamental drawbacks [58].

DNA Probes

Modern DNA technology allows for the detection of complex nucleic acid sequences that can be used to classify bacterial organisms. In a nutshell, the strategy makes use of the fact that DNA is enzyme digested, yielding specific fragments of single strands that are representative of individual organisms. These fragments are radiolabelled to compose a "DNA library" for potential experiments. It is a more sensitive and precise test. Numerous species can be identified with a single examination and each species can be detected with as little as 10^4 cells [59]. They help in the detection of *A.actinomycetemcomitans*, *T.forsythia*, *P.intermedia* and *T.denticola* in less than 40 minutes. It is utilised to monitor the location prior to and after therapy to evaluate the desired effect of reducing subgingival infections to undetectable levels, as well as to analyse microbial profiles during the treatment [60]. A novel attempt by Silva NLC et al., understanding periodontal status, vascular permeability and platelet aggregation changes in rat models using DNA probes submitted to hypercholesterolemic diet, demonstrated a higher number of species with a more diverse biofilm in periodontitis group. However, this method is restricted to the periodontal infections for which the particular DNA probes are developed [61].

Checkerboard DNA-DNA Hybridisation

Socransky SS et al., introduced the checkerboard DNA-DNA hybridisation technology for studying the oral microbial community. This approach leads to quicker processing of a substantial proportion of plaque samples. It doesn't need bacterial viability and is extremely useful in epidemiological studies [62]. As a result, checkerboard hybridisations are expected to gradually replace cumbersome culture approaches. Naqvi AZ et al., estimated the impact of Decosahexaenoic Acid (DHA) therapy on subgingival plaque microbiota using this technique and detected 40 periodontal bacterial species at baseline out of which *P.gingivalis* was isolated from DHA + aspirin group that may lead to biofilm alteration with reversal of dysbiosis [63].

Fluorescence In-Situ Hybridisation (FISH)

The FISH developed from non fluorescent in-situ Hybridisation, which Gall and Pardue reported in 1969 based on the complementary binding of a nucleotide probe labelled with a reporter molecule to a particular target nucleic acid sequence within cellular compartments. It is simple, rapid, easily adaptable and has high sensitivity, with the ability to detect a single bacterial cell. FISH has been used to detect and identify species in periodontitis and caries [64]. The abundance and distribution of *P.gingivalis*, *P.intermedia*, *T.forsythia* or *Fusobacterium* etc., were analysed. The relevance of questionable oral species such as Selenomonas or Filifactor alocis for periodontitis was investigated using FISH as well [65]. A study done by Bhat KG et al., on the application of FISH-stained *A.actinomycetemcomitans* were identified, counted from the smear and quantified, 98.7% chronic periodontitis showed the presence of *A.actinomycetemcomitans* [66].

Terminal Restriction Fragment Length Polymorphism (T-RFLP)

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. DNA extracted is fragmented using Restriction Endonucleases (RE), subjected to polyacrylamide or agarose gel electrophoresis → denatured using NaOH, blotted and the labelled probe is attached to a charged membrane →hybridised and visualised using genomic analysis and genetic disease analysis [67]. It is relatively simple but is a lengthy technique with high labour requirements, high quality and wide quantity of DNA requirements, working with radioisotopes is needed on a regular basis, certain probes are not possible based on organisms, so many polymorphisms may be present for a short probe and the cost of production is very high. It aids in evaluation of multiple bacterial communities, fast comparison of their structure and diversity of ecosystems [68]. This property aids in rapid diagnosis of periodontal diseases. In a study conducted by Ding YJ et al., T-RFLP had more detail and higher sensitivity as compared to traditional culture techniques [69].

454 Pyrosequencing

Several metagenomic research employing 16s rRNA gene cloning techniques offered information on bacterial communities, although the findings were limited due to a lack of clone numbers [70]. Hence, next generation sequencing, such as 454 pyrosequencing, has transformed bacterial diversity research on the oral microbiota [71]. DNA is fragmented and PCR creates millions of identical copies of each fragment, split across thousands of wells, incubated with DNA polymerase. ATP is generated by the action of ATP sulfurylase on adenosine 5' phosphosulfate. This ATP takes part in luciferase mediated conversion of luciferin to oxyluciferin that emits light which is picked by the detector and then used to infer the number and type of nucleotides added [72]. A study conducted by Park OJ using 454 FLX Titanium Pyrosequencing helped to detect *P.gingivalis, F.nucleatum, T.denticola* species in periodontitis and *Streptococcus, Capnocytophaga, Leptotrichia* and *Haemophilus* in healthy and gingivitis subjects [73].

Supported Oligonucleotide Ligation and Detection (SOLiD)

SOLiD instrumentation was released by Applied Biosystems in 2007. The sample preparation is similar to 454 pyrosequencing technology [74]. The applications of SOLiD include whole genome resequencing, targeted resequencing, transcriptome research (including gene expression profiling, small RNA analysis and whole transcriptome analysis) and epigenome (like chromatin immunoprecipitation- CHiP following by high throughput DNA sequencing and methylation) [75]. The drawbacks of SOLiD include biased sequence coverage in AT-rich repetitive sequences and it requires a long run times i.e., six days [76].

Future Directions

Centralisation of laboratory procedures for sampling patients and processing samples includings methods for identifying essential pathogens, determining antibiotic susceptibility, conducting well controlled clinical trials can benefitperiodontal care and motivate clinicians to provide effective antimicrobial therapy.

CONCLUSION(S)

Chairside point-of-care diagnostics can be an effective method in diagnosis of periodontal infections. The nature of subgingival microflora and the degree of pathogenic microorganisms can vary between subjects and also between sites. Hence, it can be established that no one treatment may be suitable for every individual. Further research is required regarding these diagnostic tools before their introduction into clinical practice.

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