

Review Article

DNA Probes: Newer Diagnostic Aid in Periodontal Disease

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ABSTRACT:

Periodontal disease is a multi-factorial disease. Microorganisms and microbial products in oral cavity frame the main etiological factors through host and environmental factors also. They play a role in disease, microbial factors from the main etiopathological cause for the disease. DNA probe is the recent advancement in diagnosing periodontal disease. It is based on nucleic acid detection. It helps the simultaneous determination of the presence of a multitude of bacterial species in single or multiple clinical samples. In this method bacterial viability and epidemiologic research is not required. Various studies using this molecular method have considerably enhanced the understanding of the microbiology of periodontal disease.

Key words: DNA probe, Periodontal disease, Gingivitis, Nucleic acid, Diagnosis.

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INTRODUCTION:

Periodontal disease and dental caries are the two most common dental diseases prevalence in communities. In the recent times, etiology and pathogenesis of periodontal diseases are better understood. This has provided clinicians and researchers with newer diagnostic techniques and treatment strategies.¹

Periodontal disease is most widely prevalent in all populations across the globe. It is important to identify it and prevent it from recurrence. DNA probes seems to be the choice in identifying the disease at earlier stages.²

Limitations of Conventional Technique: Conventional technique depends on the presence or absence of clinical signs of inflammation, probing depth, pattern and extent of loss of clinical attachment and bone, presence or absence of miscellaneous signs and symptoms like pain, ulcers and presence of plaque and calculus. The traditional clinical assessments are clearly subjected to limitations. Traditional diagnostic tests are subjective, retrospective and they are not enough to detect small degrees of periodontal damage. They neither can identify susceptible individuals nor can differentiate between active and non active disease sites.

The new techniques for diagnosis of periodontal disease follows the below mentioned :

- a. Plaque bacteria
- b. Assessing metabolic changes associated with the identification of pathogenic bacteria.
- c. Assessment of host susceptibility
- d. Detection of tissue damage, necrosis or anatomical changes in the periodontium.³

DNA probes – These probes are single stranded pieces of nucleic acid, labelled with a specific tracer (isotope, enzyme or chromophore) that will hydrogen bond with complementary single stranded pieces of DNA or RNA under the appropriate conditions of pH, temperature and ionic strength. DNA probes work on the principle of the DNA base sequencing. The complementarity of DNA is based on the hydrogen bonding between the bases adenine and thymine, and guanine and cytosine, respectively. In ribonucleic acid (RNA), adenine hydrogen bonds to the base uracil. The temperature required to dissociate the double stranded DNA is dependent on the guanine to cytosine (G-C) content of DNA since the interaction between guanine and cytosine is stronger than between the bases adenine to thymine. This is due to the fact that guanine forms three hydrogen

bonds with cytosine as opposed to the two hydrogen bonds formed by adenine with thymine. The binding of target DNA to probe DNA generally occurs readily at a temperature which is 25°C lower than the temperature of the target DNA—probe hybrid.⁴

DNA Probe application:⁴

1. To study microbial distribution in plaque and on root surface
2. In identifying micro – organisms in infected root canals.
3. In forensic odontology having finger printing techniques.

Procedural application of DNA probes in periodontal prognosis:

1. **Procuring plaque samples:** Samples of plaque are taken with the help of sterile paper points and are put into Eppendorf tubes (plastic tube) for transportation. Multiple opinion are raised regarding the location and number of sites. According to Savitt et al, the sites which bleed on probing are the most likely to harbour periodontal pathogens like P.Gingivalis, P. Intermedius and Actinomycetemcomitans. The criteria for microbiological sampling are, the patient should not have previous periodontal therapy, with at least 10 teeth in one arch. A minimum of 2 sites with a pocket depth of 6 mm or more and who is not on antibiotics for 6 months.⁵
2. **Processing the sample in laboratory:** For the DNA probe analysis, the DNA molecules have to be denatured or split into 2 separate strands. This is done by treating the plaque specimen with a detergent to lyse the cells. This is then boiled in a high pH solution which renders the DNA in the denatured form. This denatured DNA is immobilized on nitrocellular filter. The filters are prehybridized with either EDTA or 0.5% Sodium dodecylsulfate for 1 hour at 65°C. This radiolabelled nucleic acid strands is then added to the immobilized specimen and hybridized in the same buffer by adding 10% dextran sulphate. This is then exposed to auto radiographic plates. Patient samples are then scored as percent of contrast.⁶
3. **Interpretation of data:** Results which are scored by comparing the signals of the test sample to the positive controls.

I.	A. Signals <10 ³	Non – detectable
	B. Signals > or equal to 10 ³ But < 10 ⁵	Positive
	C. Signals > or equal to 10 ⁵	High positive
II.	Negative < 6 X 10 ³ CFU	
	Low > or equal to 6 X 10 ³ but < 6 X 10 ⁵ CFU	
	Moderate > or equal to 6 X 10 ⁴ but < 6X 10 ⁵ CFU	
	High > or equal to 6 X 10 ⁵ CFU	

(CFU – Colony forming units)

Probe based Periodontal diagnostic kits:

Several supplemental diagnostic tests are now available to investigate progressive periodontal lesions. Some of them are:

1. **Omnigene:** They are used as DNA probe systems for a number of sub gingival bacteria. Probes are available for the detection of Actinomycetemcomitans, P.Gingivalis, P.Intermedia, Eikenella corrodens, Fusobacterium nucleatum, Campylobacter recturs, Treponema denticola and Treponema pectinovorum.⁷
2. **Microprobe corporation:** It is designed as an in-office nucleic acid probe assay for the semi quantitative detection of periodontal pathogens. The bacterial cells in patient plaque samples are lysed by heating in the presence of detergent. The extracted DNA is then placed in to the first well of multi-well cassette and then placed into a machine with a programmable robotic arm which shows digital display of current bacterial load.⁸
3. **Perio 2000:** Several pathogenic bacteria like T.denticola, P.gingivalis, P. intermedia and T.forsythia can produce sulphates, which produce significant levels of volatile sulphur compounds which can directly degrade periodontal structures aggravating periodontitis. The perio 2000 system is designed to display the sulphide level digitally at each site.⁹

CONCLUSION:

DNA probes in future promise to be the most specific reagents to identify periodontal diseases and should be considered the gold standard for periodontal pathogen identification. DNA probes have proved 100% effective in identifying A. Actinomycetem comitans and B. Intermedius at culture levels. Probe investigations identify these pathogens in samples which were cultured negative and showed better correlation between presence of a pathogen and clinical evidence of diseases.

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