TARGETED MOLECULAR DIAGNOSTICS IN ORAL LESIONS USING DNA HYBRIDIZATION PROBES

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Abstract
DNA probes are short, single-stranded and labeled complementary oligonucleotide sequences used to hybridize with the target gene or sequences. Knowledge about DNA probes, types, advantages, limitations, and also explore applications of DNA probes in oral pathology.

A DNA probe is a short, labeled DNA sequence that binds to a specific target DNA sequence, allowing researchers to detect, locate, and quantify specific genes or DNA sequences.[1]

STRUCTURE OF DNA PROBES:

A DNA probe typically consists of 3 main components:

1. **Target-specific sequence**: A short sequence (15-30 nucleotides) complementary to the target DNA.
2. **Label**: A detectable marker (e.g., radioactive isotope, fluorescent dye, or enzyme) attached to the probe.
3. **Linker or backbone**: A molecular backbone that holds the probe together.

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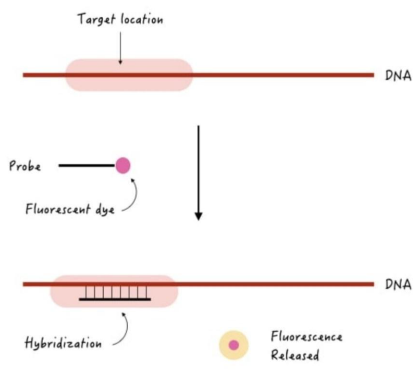
HOW DNA PROBES WORKS:

1. **Sample collection**: Oral tissue or cells are collected from patients.
2. **DNA extraction**: DNA is extracted from the sample.
3. **Denaturation**: Double-stranded DNA is denatured into single strands.
4. **Hybridization**: The DNA probe binds to specific oral disease-related genes or microorganisms.
5. **Detection**: The label attached to the probe is detected, indicating the presence of the target sequence.



A scheme of probe DNA hybridization.Cytogenetics, Genetic Education, Real-time PCR By Dr Tushar chauhan

TYPES OF DNA PROBES:

In oral pathology, DNA probes are used for the detection of various oral diseases, including infections (bacterial, viral, fungal), premalignant lesions, oral cancers, and other oral health conditions. Different types of DNA probes are employed based on their ability to detect specific genetic sequences of pathogens or abnormal cellular changes. Here are the main types of DNA probes used in oral pathology:

1. HYBRIDIZATION PROBES:

- **Definition:** These are DNA sequences designed to hybridize(bind) to complementary DNA sequences of pathogens or host cells. They are often labeled with radioactive or non-radioactive markers.
- Detection of specific bacteria in periodontal diseases (eg: Porphyromonas gingivalis, Tannerella forsythia).
- Detection of viral DNA (eg: HPV, HSV) in oral lesions or cancers.
- Identifying mutations in oral cancer cells(eg: mutations in tumor suppressor genes like p53).

2. FLUORESCENT PROBES:

- **Definition:** DNA probes labeled with fluorescent dyes that emit light when exposed to specific wavelengths. The fluorescence helps in the detection of specific sequences.
- Fluorescence in situ hybridization(FISH): Used for detecting genetic changes or chromosomal abnormalities in oral cancer or precancerous lesions.
- Detection of HPV types (especially high-risk strains like HPV-16 and HPV-18) associated with oral cancers.
- Identification of specific bacteria in periodontal tissues.

3. BIOTINYLATED PROBES:

- **Definition:** DNA probes are labeled with biotin, a small molecule that binds to avidin or streptavidin, which can then be linked to a detection system(eg: an enzyme or fluorescent molecule).
- Detection of pathogens like Candida species in oral infections.
- Used in conjunction with tissue sections or swabs from oral lesions for viral or bacterial detection.
- Used in southern blotting to identify specific bacterial or viral DNA in oral samples.

4. DIGOXIGENIN-LABELED PROBES:

- **Definition:** These probes are labeled with digoxigenin, a steroid molecule, which can be detected using specific antibodies. The use of digoxigenin avoids the use of radioactive isotopes.
- Used for detecting HPV DNA in oral lesions, particularly in cases where the

virus is implicated in the development of oral cancers.

- Detection of EBV and HSV DNA in oral lesions such as hairy leukoplakia or cold sores.
- Used in Northern blotting or in situ hybridization to study gene expression in oral cancer tissue.

5. <u>TAQMAN PROBES:</u>

- **Definition:** These probes are used in quantitative PCR (qPCR). They consist of a fluorescent dye and a quencher, which are separated when the probe binds to its target and is cleaved during PCR amplification, resulting in fluorescence.
- Quantitative detection of viral loads in oral lesions, such as HPV or HSV, in oral cancers or recurrent infections.
- Quantification of bacterial pathogens in periodontal diseases, enabling the detection of pathogen load in patients with periodontitis.
- Monitoring antimicrobial resistance in oral pathogens(eg: detection of resistance genes in Streptococcus mutans).

6. <u>MOLECULAR BEACON PROBES:</u>

- **Definition:** These are hairpin-shaped DNA probes that fluoresce when bound to their target DNA. The stem structure brings the fluorophore and quencher close together, preventing fluorescence until the probe binds to its target.
- Detection of specific mutations or bacterial DNA in oral cancer samples.
- Real-time PCR assays for detecting periodontal pathogens such as Aggregatibacter actinomycetemcomitans and Treponema denticola.
- Used for quantifying viral load in oral HPV infections associated with oral cancers.

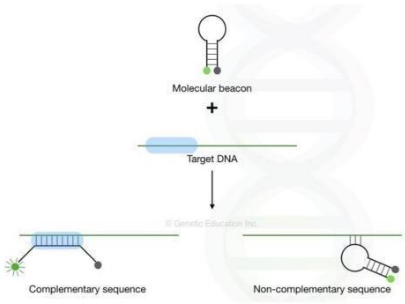


Illustration of molecular beacon probe chemistry.Cytogenetics, Genetic Education, Real-time PCR, By Dr Tushar Chauhan

7. <u>LOCKED NUCLEIC ACID(LNA) PROBES:</u>

- **Definition:** LNA probes are modified oligonucleotides with enhanced binding affinity due to their unique sugar modification. This modification makes them more stable and allows for higher specificity in binding.
- Used for high-sensitivity detection of HPV in oral cancers.
- Detection of bacterial species implicated in periodontitis, such as Porphyromonas gingivalis or Fusobacterium nucleatum.
- Detection of genetic mutations related to oral cancer(eg: mutations in the p53 gene).

8. <u>ARRAY-BASED PROBES (DNA MICROARRAYS):</u>

- **Definition:** DNA probes are immobilized on a solid surface in a grid format to detect multiple targets simultaneously in a sample.
- Used to analyze the oral microbiome and detect a variety of bacterial, viral, or fungal pathogens in a single test.
- Gene expression profiling of oral cancer tissues to identify biomarkers associated with malignant transformation or therapeutic targets.
- Analysis of genetic mutations in oral cancer samples to identify potential targets for personalized treatments.

9. PCR PRIMER-PROBE SYSTEMS: (eg: Scorpion and Primer-Probe Assays)

- **Definition:** These systems combine primers and probes into a single molecule, which enhances the efficiency of PCR amplification and detection.
- Detection of specific genetic sequences in oral cancer cells, such as mutations in tumor suppressor genes or oncogenes.
- Detection of bacterial DNA related to dental caries or periodontal diseases.
- Identifying viral infections such as HPV or HSV in oral lesions using real-time PCR.

TECHNIQUES UTILIZING DNA PROBES:[5]

1. **Southern Blotting**: Detects specific DNA sequences.
2. **Dot Blotting**: Detects specific DNA sequences in oral tissues.
3. **In Situ Hybridization (ISH)**: Localizes specific DNA sequences in oral tissues.
4. **Fluorescence In Situ Hybridization (FISH)**: Detects genetic mutations in oral cancer.
5. **Polymerase Chain Reaction (PCR)**: Amplifies specific DNA sequences for detection.
6. **DNA Microarray Analysis**: Analyzes multiple genes simultaneously.

APPLICATION OF DNA PROBES IN THE FIELD OF ORAL PATHOLOGY:

I. Oral cyst diagnosis:[6]

1. Detection of Microbial agents in cysts:

- Certain oral cysts, such as radicular cysts, may be associated with bacterial infections. DNA probes can detect bacterial DNA, such as Actinomyces or Treponema species, which are implicated in the pathogenesis of odontogenic cysts.

2. Molecular diagnosis of Developmental cysts:

- DNA probes can identify genetic markers involved in developmental cysts, such as PTCH1 mutations in Gorlin-Goltz syndrome, which is associated with Keratocystic odontogenic tumors(KCOT).

3. Distinguishing between Cysts and Tumors:

- DNA probes can identify molecular differences between cystic lesions and cystic neoplasms (eg: odontogenic keratocysts vs. Ameloblastomas), assisting in differential diagnosis.

4. Detection of HPV in cysts:

- DNA probes targeting Human Papilloma Virus(HPV) can be used to detect viral DNA in cystic lesions with suspected viral etiology.

5. Study of Genetic Mutations:

- DNA probes help identify mutations or chromosomal alterations in cystic lesions. For example:
  - PTCH1 mutations in keratocystic odontogenic tumors(KCOT).
  - Genetic analysis of dentigerous cysts to study oncogenic potential.

6. Early detection of Malignant Transformation:

- Some cysts, such as odontogenic keratocysts, have a higher recurrence rate and a potential for malignant transformation. DNA probes can identify molecular markers indicative of such risks, aiding in early intervention.

II. Oral tumor detection:[7]

1. Detection of Genetic Mutations in Oral cancer:

- DNA probes can identify mutations in tumor suppressor genes or oncogenes commonly associated with oral cancers, such as:
  - p53: Frequently mutated in oral squamous cell carcinoma(OSCC).
  - EGFR: Over-expressed in many oral cancers.
  - RAS mutations: Associated with tumor progression.

2. Detection of Viral Oncogenes:

- Human Papilloma Virus(HPV): DNA probes can detect high-risk HPV types (eg: HPV-16, HPV-18) implicated in a subset of OSCCs.
- Epstein-Barr Virus(EBV) :Associated with lymphoepithelial tumors and nasopharyngeal carcinoma.

3. Differentiating benign from malignant tumors:

- DNA probes can identify specific genetic profiles or molecular markers that distinguish benign tumors like fibromas from malignant lesions like OSCC.

4. Monitoring Tumor Progression and Recurrence:

- DNA probes can detect residual tumor DNA or circulating tumor DNA(ctDNA) in saliva or blood, helping monitor disease progression and recurrence after treatment.

5. Detection of Chromosomal Abnormalities:

- DNA probes used in fluorescence in situ hybridization(FISH) can detect chromosomal aberrations, such as:
  - Loss of heterozygosity(LOH) at specific loci.
  - Amplification of oncogenes.

6. Epigenetic Analysis:

- DNA probes can identify methylation changes in tumor suppressor genes (eg: p16, RASSF1), which are common in oral cancers.

7. Identification of Tumor Margins:

- DNA probes can help define molecular margins during surgical resection by detecting tumor-specific DNA in surrounding tissues.

III. Premalignant lesion detection:[8]

1. Detection of Genetic Mutations:

- DNA probes can identify mutations in genes commonly altered in premalignant lesions, such as:
  - p53: A tumor suppressor gene frequently mutated in oral epithelial dysplasia.
  - Cyclin D1 Amplification: Associated with progression to malignancy.

2. Identification of Epigenetic Changes:

- DNA probes can detect hypermethylation of tumor suppressor genes, such as:
  - p16INK4A
  - MGMT
  - RASSF1A
- These changes are early markers of malignant transformation.

3. Viral Oncogene Detection:

- DNA probes can detect high-risk Human Papilloma Virus (HPV) types(eg: HPV-16, HPV-18) that may contribute to the development of premalignant lesions.

4. Monitoring Molecular Alterations:

- DNA probes can identify loss of heterozygosity (LOH) at chromosomal loci (eg: 3p, 9p, 17p), which are associated with progression from dysplasia to carcinoma.

5. Differentiation Between Low- and High-Risk Lesions:

- DNA probes help classify premalignant lesions based on molecular markers, distinguishing lesions with a higher likelihood of malignant transformation.

6. Assessing Field Cancerization:

- DNA probes can detect molecular alterations in clinically normal tissue adjacent to premalignant lesions, helping identify areas of “field cancerization”.

7. Salivary Diagnostics:

- DNA probes can detect circulating tumor DNA (ctDNA) or epigenetic changes in saliva, offering a non-invasive method for monitoring premalignant lesions.

IV. Oral Cancer Detection:[9]

1. Detection of Genetic Mutations:

- DNA probes are designed to identify key mutations in genes associated with oral cancer, such as:
  - p53: Frequently mutated in oral squamous cell carcinoma(OSCC).
  - Cyclin D1(CCND1): Over-expressed in oral cancers.
  - EGFR: Amplified in many cases of OSCC, Associated with aggressive behavior.

2. Epigenetic Alteration Detection:

- DNA probes can identify hypermethylation of tumor suppressor genes like p16, RASSF1, and DAPK. These changes are early indicators of malignant transformation.

3. Identification of Viral Oncogenes:

- High-risk human papillomavirus (HPV) types, particularly HPV-16 and HPV-18, are detected using DNA probes, as they play a significant role in HPV-positive oral cancers.

- Epstein-Barr virus(EBV) can also be detected in certain subtypes of oral cancers.

4. Loss of Heterozygosity (LOH) Analysis:

- DNA probes can detect LOH at chromosomal regions such as 3p, 9p, and 17p, which are early molecular events in oral cancer progression.

5. Early Detection of Field Cancerization:

- DNA probes can identify molecular alterations in the surrounding clinically normal tissue, indicating areas prone to developing malignancies.

6. Microbial Detection:

- DNA probes can detect microbial pathogens, such as Porphyromonas gingivalis, associated with oral cancer progression.

7. Monitoring Tumor Recurrence:

- Circulating tumor DNA (ctDNA) in blood or saliva can be detected using DNA probes to monitor disease recurrence post-treatment.

V. Detection of Fungal Infections:[10]

1. Identification of fungal pathogens:

- DNA probes can specifically detect fungal species commonly associated with oral infections, including:

➢ Candida albicans: The most prevalent species causing oral candidiasis.

➢ Candida glabrata, Candida tropicalis, and Candida krusei: Increasingly observed in resistant infections.

➢ Aspergillus spp. or Histoplasma capsulatum: Rare but significant in immunosuppressed patients.

2. Rapid Diagnosis of Candidiasis:

- DNA probes allow for early and precise identification of Candida species, even in cases with low fungal loads or atypical presentations.

3. Detection of Anti-fungal Resistance Genes:

- DNA probes can identify genetic mutations responsible for resistance to anti-fungal agents, such as azoles or echinocandins, guiding appropriate therapy.

4. Differentiation Between Species:

- Traditional method like culture may not differentiate between closely related fungal species. DNA probes can distinguish between C.albicans and non-albicans candida species, which have different treatment protocols.

5. Monitoring of Recurrence:

- Post-treatment, DNA probes can detect residual fungal DNA, helping to monitor for recurrence or incomplete eradication.

6. Non-Invasive Salivary Diagnostics:

- DNA probes can analyze saliva samples to detect fungal DNA, providing a non-invasive diagnostic option.

VI. Detection of Viral Infections:[11]

1. Detection of HPV in oral lesions:

- HPV, particularly high-risk types(eg: HPV-16, HPV-18), is linked to the development of oral squamous cell carcinoma (OSCC).
- DNA probes can detect the presence of HPV DNA in oral mucosal lesions, including leukoplakia, oropharyngeal cancers, and other premalignant conditions.

2. Identification of Herpes Simplex Virus (HSV):

- HSV (type 1 and 2) is responsible for oral lesions like cold sores and can be detected using DNA probes, especially in immunocompromised patients where HSV infections may be recurrent or severe.
- DNA probes identify HSV DNA in oral swabs or biopsy specimens, aiding in precise diagnosis.

3. Detection of Epstein-Barr Virus (EBV):

- EBV is associated with oral hairy leukoplakia and certain forms of oral cancers. DNA probes can detect EBV DNA in saliva, oral lesions, or biopsy samples.
- It is especially useful in immunocompromised patients, where EBV infections can be more persistent and severe.

4. Cytomegalovirus (CMV) Detection:

- CMV, a member of the herpes virus family, is another viral agent that can cause oral lesions, especially in immunocompromised individuals. DNA probes can identify CMV DNA in oral fluids or biopsy specimens.

5. Monitoring HIV in the Oral Cavity:

- HIV can lead to various oral manifestations, including oral candidiasis, periodontitis, and Kaposi's sarcoma. DNA probes can detect HIV DNA in oral tissues, assisting in monitoring viral load and diagnosing associated oral conditions.

6. Non-Invasive Detection through Saliva:

- DNA probes offer the advantage of detecting viral DNA in saliva, providing a non-invasive alternative to tissue biopsy for viral infection diagnosis.

7. Quantification of Viral Load:

- DNA probes can quantify viral DNA, which helps assess viral replication and inform treatment decisions, especially in cases of persistent or recurrent infections.

VII. Detection of Bacterial Infections:[12]

1. Identification of Periodontal Pathogens:

- DNA probes can detect specific periodontal pathogens that are commonly associated with periodontal diseases, including:
  - Porphyromonas gingivalis
  - Treponema denticola
  - Tannerella forsythia
- These pathogens are part of "red complex", which is strongly linked to periodontitis.

2. Detection of Bacteria in Dental Caries:

- DNA probes can identify Streptococcus mutans, Streptococcus sobrinus, and Lactobacillus spp., which are key contributors to dental caries(tooth decay).
- DNA probes enable detection of these bacteria even in early stages of infection, allowing for prompt intervention.

3. Detection of Mixed Bacterial Infections:

- The oral cavity harbors a diverse microbiome, and DNA probes can identify a wide array of bacteria simultaneously in mixed infections, such as in abscesses or chronic infections, where multiple bacterial species are present.

4. Identification of Antibiotic Resistance Genes:

- DNA probes can detect specific antibiotic resistance genes in oral bacteria, providing information on the potential resistance to commonly used antibiotics. This is crucial for guiding effective antimicrobial therapy, especially in periodontal infections that are difficult to treat.

5. Molecular Profiling of Oral Microbiome:

- DNA probes can analyze the oral microbiome by detecting bacterial DNA in saliva or plaque samples. This is particularly useful in understanding the microbial composition in individuals with dysbiosis (an imbalance of oral bacteria) that can contribute to disease development.

6. Non-Invasive Diagnostics:

- Saliva, gingival crevicular fluid, or oral swabs can be used to collect samples for DNA probe analysis, making it a non-invasive diagnostic method.

VIII. Periodontal Disease Detection:[3]

1. Identification of Periodontal Pathogens:

- DNA probes can detect specific bacterial species implicated in periodontal diseases, such as:
  - ➢ Porphyromonas gingivalis
  - ➢ Aggregatibacter actinomycetemcomitans
  - ➢ Treponema denticola
  - ➢ Tannerella forsythia
  - ➢ Fusobacterium nucleatum
- These bacteria are part of the “red complex” and “orange complex” associated with periodontal disease progression.

2. Quantification of Pathogenic Load:

- DNA probes not only identify pathogens but also quantify their abundance, aiding in assessing disease severity and risk.

3. Early Detection of Periodontal Infections:

- DNA probes can detect subclinical levels of pathogens before significant tissue damage occurs, enabling early intervention.

4. Evaluation of Treatment Outcomes:

- Post-treatment use of DNA probes can monitor reductions in bacterial load, helping assess the effectiveness of scaling, root planing, or antimicrobial therapies.

5. Risk Assessment:

- DNA probes help identify individuals at higher risk for periodontal diseases based on their microbial profile.

6. Detection of Antibiotic Resistance Genes:

- DNA probes can identify genetic markers for antibiotic resistance, guiding personalized antimicrobial therapy.

7. Salivary Diagnostics:

- DNA probes can detect periodontal pathogens in saliva, offering a non-invasive approach for screening and monitoring.

IX. Dental Caries Detection:[13]

1. Detection of Cariogenic Bacteria:

- DNA probes are used to identify and quantify bacteria implicated in dental caries, such as:
  - Streptococcus mutans: A primary pathogen associated with caries.
  - Lactobacillus spp. : Contribute to lesion progression due to acid production.
  - Actinomyces spp. : Plays a role in root caries.

2. Understanding Oral Microbial Ecology:

- DNA probes can detect the entire microbial community in the oral cavity, providing insights into the complex biofilm associated with caries development.

3. Early Diagnosis of Caries:

- DNA probes enable the detection of cariogenic bacteria before visible clinical or radiographic evidence of caries, facilitating early intervention.

4. Monitoring Caries Risk:

- DNA probes can assess the levels of cariogenic bacteria in saliva or plaque, helping identify individuals at higher risk of developing caries.

5. Evaluating Effectiveness of preventing Measures:

- Post-treatment monitoring of bacterial load using DNA probes can determine the effectiveness of interventions like fluoride therapy or antimicrobial agents.

ADVANTAGES OF DNA PROBE:

1. High specificity and sensitivity.
2. Rapid detection of oral pathogens.
3. Ability to detect multiple pathogens simultaneously.
4. Non-invasive sampling methods (e.g., saliva, oral rinse).
5. Early detection of oral diseases (e.g., cancer, periodontitis).
6. Monitoring of treatment efficacy.
7. Research applications (e.g., epidemiology, pathogenesis).
8. Improved diagnostic accuracy.
9. Reduced risk of false negatives or false positives.
10. Potential for automated analysis.

LIMITATIONS OF DNA PROBE:

1. **Specificity**: May bind to non-target sequences.

2. **Sensitivity**: May not detect low-copy-number targets.
3. **Probe design**: Requires knowledge of target sequence.
4. **Hybridization conditions**: Temperature, pH, and salt concentration can affect binding.
5. **Cost**: Can be expensive, especially for specialized probes.
6. **Interpretation**: Requires expertise in molecular biology and oral pathology.
7. Limited availability of probes for certain oral pathogens.
8. Potential for false positives or false negatives.
9. Requires specialized equipment and training.

CLINICAL IMPLICATIONS:[15]

1. Early detection and diagnosis of oral cancer.
2. Identification of high-risk patients for oral cancer.
3. Monitoring of oral cancer treatment efficacy.
4. Detection of periodontal pathogens and tailored treatment.
5. Diagnosis of fungal and bacterial infections.
6. Personalized medicine for oral diseases.
7. Improved patient outcomes and prognosis.

Clinical applications include:

- Oral cancer screening.
- Periodontal disease diagnosis and treatment.
- Oral infection diagnosis and treatment.
- Saliva-based diagnostics.

FUTURE DIRECTIONS:[16]

1. **Point-of-care diagnostics:** Development of portable, user-friendly devices for rapid DNA probe-based testing.
2. **Multiplexing:** Simultaneous detection of multiple pathogens or genetic markers.
3. **Nanotechnology:** Integration of DNA probes with nanomaterials for enhanced sensitivity and specificity.
4. **Artificial intelligence:** Application of AI algorithms to analyze DNA probe data and improve diagnostic accuracy.
5. **Personalized medicine:** Use of DNA probes to tailor treatment plans to individual patients' genetic profiles.
6. **Epigenetic analysis:** Investigation of epigenetic changes associated with oral diseases using DNA probes.
7. **Saliva-based diagnostics:** Development of DNA probe-based tests for oral diseases using saliva samples.

CONCLUSION:

DNA probes are revolutionizing the diagnostic capabilities in oral pathology,enabling early detection, accurate identification of pathogens, and personalized approaches to treatment. They are essential tools in oral pathology for detecting bacterial, viral, and fungal infections, as well as genetic mutations in oral cancers and premalignant lesions. The choice of probe type depends on the specific infection or genetic alteration being investigated, with options ranging from traditional hybridization probes to advanced real-time PCR systems.

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