Review Article

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REAL - TIME PCR: AN IMPORTANT DIAGNOSTIC TOOL IN DENTISTRY

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ABSTRACT

Recent advancement in molecular methods has changed the detection and differentiation of microorganisms in a broad range of medical diagnostic fields, including virology, mycology, parasitology, microbiology and dentistry. Among these methods, Polymerase Chain Reaction (PCR) has evolved great benefits and allowed scientific advancements in the medical diagnostic fields. PCR is an excellent technique for the rapid detection of pathogens, including those difficult to culture. Along with conventional PCR techniques, Real Time PCR has emerged as a technological innovation and is playing an ever-increasing role in clinical diagnostics and research laboratories. Due to its capacity to generate both qualitative and quantitative results, Real- Time PCR is considered a fast and accurate platform.

KEYWORDS: Polymerase Chain reaction; real-time PCR; molecular methods

INTRODUCTION

POLYMERASE CHAIN REACTION (PCR)

It is an exponentially progressing synthesis of the defined target DNA sequences in vitro.It is technique that enables multiple copies of a DNA molecule to be generated by enzymatic amplification of a target DNA sequence. It was invented by Dr. Kary Mullis in 1983, received the Nobel Prize in Chemistry in 1993. It allows quick replication of DNA in vitro. Amplifies minute quantities of genetic material to millions of copies with in a few hours. Enables rapid and reliable identification of DNA fragments like-genetic markers, infectious agents, cancerous cells etc.[1]

Uses of Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a technique widely used in:

- ✓ Molecular biology
- ✓ Microbiology
- ✓ Genetics
- ✓ Diagnostics clinical laboratories
- ✓ Forensic science
- ✓ Environmental science
- ✓ Hereditary studies
- ✓ Paternity testing.
- ✓ Applications of PCR
- ✓ Cloning a Gene encoding a known protein
- ✓ Amplification of old DNA
- ✓ Amplifying cloned DNA from Vectors
- ✓ Rapid Amplification of cDNA ends
- ✓ Detecting Bacterial or Viral Infection
- ✓ Genetics Diagnosis
- ✓ Diagnosing inherited disorders
- ✓ Cystic fibrosis
- ✓ Muscular dystrophy
- ✓ Haemophilia A and B
- ✓ Sickle cell anaemia
- ✓ Diagnosing cancer
- ✓ Blood group typing.

Advantages of PCR

- ✓ Useful non- invasive procedure.
- ✓ Simplicity of the procedure.
- ✓ Sensitivity of the PCR.

Disadvantages of PCR

- ✓ False positive results (cross contamination).
- ✓ False negative results (e.g. rare of circulating fetal cells

Recent Advancement in PCR Method

Recently, a new method of PCR quantification

has been invented. This is called "Real-time PCR" because it allows the scientist to actually view the increase in the amount of DNA as it is amplified. The technological innovation of PCR, known as Real -Time PCR, has become increasingly important in clinical diagnostics and research laboratories due to its capacity for generating quantitative results. This technique allows accompanying the reaction and presentation of results in a faster and more accurate fashion than conventional PCR, which only displays the qualitative results.

Real -Time PCR

Real Time PCR is a technique in which fluoroprobes bind to specific target regions of amplicons to produce fluorescence during PCR. The fluorescence, measured in Real Time, is detected in a PCR cycler with an inbuilt filter flurometer.

Principles of Real -Time PCR

- Based on the detection and quantitation of a fluorescent reporter.
- The first significant increase in the amount of PCR product (C_T - threshold cycle) correlates to the initial amount of target template.

Applications of Real -Time PCR :

- Application in Molecular Diagnostics
- Clinical microbiology and Food microbiology
- Gene expression
- Viral quantitation
- Single Nucleotide Polymorphism (SNP) analysis
- Clinical oncology
- Cancer
- Analysis of cellular immune response in peripheral blood
- Chromosome aberrations.

Advantages of Real-time PCR

- Not influenced by non-specific amplification.
- Amplification and quantification can be monitored real-time .
- No post-PCR processing of products (high throughput, low contamination risk)
- Detection is capable down to a two-fold change .
- Confirmation of specific amplification by melting curve analysis.
- Most specific, sensitive and reproducible.
- Not much more expensive than conventional PCR (except equipment cost)

Disadvantages of Real-time PCR

- Not ideal for multiplexing.
- Setting up requires high technical skill and support .
- High equipment cost .
- Intra- and inter-assay variation.
- RNA lability.
- DNA contamination (in mRNA analysis).

Applications of Real -Time PCR in Dentistry

The literature reports the uses of Real-Time PCR for the study of factors involved in periodontal disease, dental caries, endodontic infections and oral cancer.

1. Periodontal Diseases

Real-Time PCR is an excellent tool for directly identifying periodontal pathogens in subgingival samples. Due to its sensitivity and specificity, it is also a rapid, efficient method for detecting, identifying and differentiating organisms, but appropriate standardization is necessary . Diverse molecular means are often used to identify periodontal pathogens, but PCR is considered the easiest and fastest method in clinical samples.PCR may soon become the ideal detection method for periodontal pathogens due to its greater ease of use in comparison to cultures associated with biochemical identification tests. It also demonstrates excellent detection limits with few cross-reactions under ideal conditions.[2]

2. Dental Caries

Real-Time PCR appears to be convenient for studying the epidemiology of disease in isolated individuals. It may prove useful in the identification of species associated with dental caries and their location in the ecological niches, thereby helping to clarify the progression of the carious process.PCR has the potential to replace conventional identification methods, such as biochemical and immunological tests.The discriminative power of PCR in the differentiation of *S. mutans* and *S. sobrinus* serotypes and lineages was investigated by Saarela *et al*. who found that PCR exhibited good results in differentiating *S. mutans* lineages and the technique is appropriate for epidemiological studies on this bacterium.[3]

3. Endodontic Infections

With the development of molecular methods based on the detection of specific genomic regions, it became possible to identify microbial

species in infected root canals that had never been seen by means of the conventional culture procedure. Using PCR, Bogen and Slots sought to determine the prevalence of *P. endodontalis*, *P. gingivalis*, *P. intermédia* and *P. nigrescens* in 20 with refractory periapical lesions. The authors concluded that bacteria that produce black pigment do not appear to constitute the majority of microorganisms in these cases. The relatively lower occurrence of these bacteria may help explain the relative stability and chronic nature of this condition. Studies have determined that bacteria are the greatest etiological agent in pulp and periradicular disease, fungi have also been associated to root canal infection.Baumgartner *et al.*, used PCR to assess the content of infected root canals as well as cellulite aspirations and abscesses of endodontic origin for the presence of *Candida albicans*.The results indicate that PCR is an extremely sensitive molecular method and can be used to identify *C. albicans* directly in samples of endodontic infection. PCR has been widely used to identify microbial species that are difficult or impossible to cultivate, as well as colonies within a species that exhibit a different phenotypic behavior and are therefore difficult to identify in culture procedures. PCR has a greater detection pattern than traditional microbiological identification methods and exhibits greater specificity under optimized conditions.Thus, the use of identification methods based on the knowledge of molecular biology has revolutionized medical microbiology and is broadening the horizons with regard to the actual profile of endodontic infection.[4]

4. Oral Cancer

One of the uses of PCR in dentistry is the detection of markers in the diagnosis and prognosis of some types of oral cancer.For this purpose, Real-Time PCR is a fast, easy method with a relatively low cost. Diagnoses, prognosis and treatment can be improved through the study and use of genetic markers identified by means of immunohistochemistry, PCR and other molecular biology procedures. Squamous cell carcinoma of the oral cavity is generally accompanied by other types of aerodigestive tract carcinomas, such as oropharyngeal and esophageal carcinoma. *Streptococcus anginosus* is a bacterium that may be isolated in different parts of the body and has been isolated in squamous cell carcinoma of the head and neck. Through real-time PCR, *S. anginosus* can be detected with greater sensitivity and specific approximation in squamous cell carcinomas of the oral cavity. Pre-malignant lesions of the head and neck have been studied extensively through genetic alterations. A genetic progression model has been established based on histological alterations that occur in the interior of the pre-malignant epithelium. HPV (Human Papillomavirus) has been employed in tumor progression in humans based on data from patients with cancer. Determining the moment of viral infections in these pre-malignant lesions could clarify the role of HPV in carcinogenesis and help guide future strategies for the prevention and early detection of squamous cell carcinoma of the head and neck. For such, a large number of studies have been conducted to detect the presence of HPV in head and neck epithelia through the use of a variety of laboratorial methods.Different techniques have been employed, including PCR, *in situ* hibridization, etc. Real-time PCR minimizes the risk of contamination, thereby becoming the ideal assay for HPV & DNA detection.[5]

CONCLUSION

In dentistry, molecular methods enhance knowledge regarding the diagnosis of infectious agents that lead to the maxillofacial infections, thereby favouring the assessment of patients at risk for conditions such as caries, periodontal disease, endodontic infections and oral cancer. The introduction of the Real-Time PCR technique was a revolutionary watershed for medicine and science. It has recently become a standard diagnostic and research tool in dentistry, permitting the early diagnosis of the oral diseases.

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