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Polymerase Chain Reaction and Its Applications in Dentistry

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Abstract

A variety of molecular biology methods have become obtainable in the previous years and one of the most innovative of these techniques regarding nucleic acid analysis is the polymerase chain reaction (PCR), which was first described in 1985. PCR can be preceded by a reverse transcription (RT) reaction in order to produce cDNA from RNA (RT-PCR). RT-PCR provides the possibility to assess gene transcription in cells or tissues. The spotlight of this review is to describe the existing position of the DNA-based method PCR which has become a standard diagnostic and research tool in dentistry.

KEYWORDS: Polymerase chain reaction, Reverse transcriptase, DNA based techniques, Diagnostic tool

Introduction

The initiation of the polymerase chain reaction (PCR) drastically changed biological science from the time it was first discovered (Mullis, 1990). Initially it was allowed for specific recognition and fabrication of large amounts of DNA. Dr. Kary Mullis, who discovered the PCR assay, stated it “lets you pick the piece of DNA you’re interested in and have as much of it as you want” (Mullis, 1990).[1] PCR allows the amplification of specific regions of DNA more than a billion-fold and allows the manipulation of DNA for techniques such as cloning of genes.[2] The aim of this review is to illustrate the applications and recent status of the DNA-based method PCR which has become a standard investigative and study tool in dentistry.

Basic Concept of PCR

PCR is a simple, yet elegant, enzymatic assay that enables amplification of a specific DNA fragment from a complex pool of DNA.PCR requires 4 primary components: the thermostable DNA polymerase, nucleotide triphosphates (which serve as building blocks for the creation of DNA), sample DNA to be amplified, and gene-specific primers. The source of sample DNA can be either genomic DNA, isolated from cells or tissues, or DNA obtained from RNA samples through reverse transcription (RT).[3]

History of PCR

Polymerase Chain Reaction was developed in 1984 by the American biochemist, Kary Mullis. Mullis received the Nobel Prize and the Japan Prize for developing PCR in 1993. However the

basic principle of replicating a piece of DNA using two primers had already been described by Gobind Khorana in 1971. Progress was limited by primer synthesis and polymerase purification issues. PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications.[4]

PCR Cycle [1]

There are three major steps in a PCR which are repeated for 30 to 40 cycles. This is done on an automated Thermocycler, which can heat and cool the tubes containing the reaction mixture in a very short time.

1. Denaturation

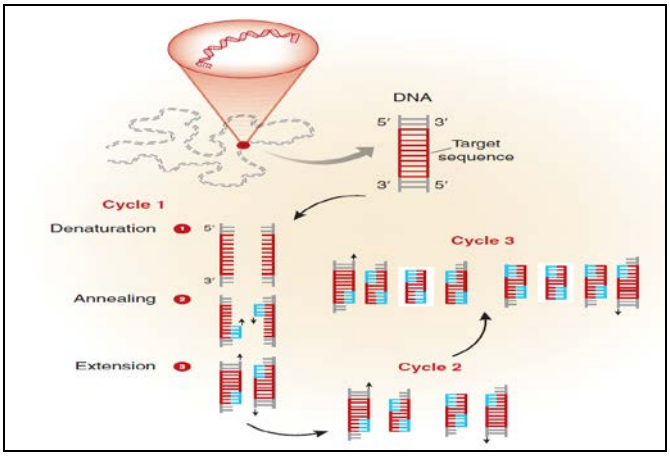
During denaturing, the 2 strands of the helix of the target genetic material are unwound and separated by heating at 90° to 95°C for 30-90 seconds.

2. Annealing

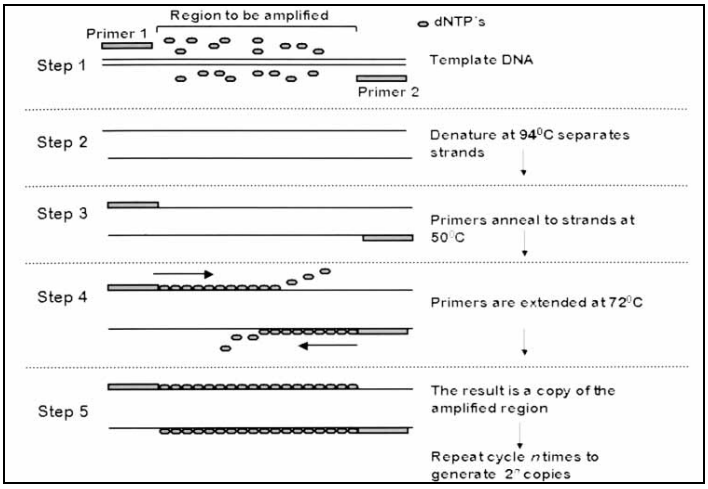
During annealing, or hybridization, oligonucleotide primers bind to their complementary bases on the single-stranded DNA. This step requires a much cooler temperature, 55°C for 30-120 seconds.

3. Extension

During polymerization (at 75°C for 60-180 seconds), the polymerase reads the template strand and quickly matches it with the appropriate nucleotides, resulting in 2 new helixes consisting of part of the original strand and the complementary strand that was just assembled.



Schematic presentation of the polymerase chain reaction principle



The basic steps for a single cycle of PCR. Step 1: The reaction mixture contains genomic (template) DNA with a target region to be studied, nucleotides (dNTPs), 2 primers, and buffer. Step 2: The mixture is heated to 94°C to separate the double strands. Step 3: When the temperature is then lowered to 50°C, the primers bind to the DNA template surrounding the region to be studied. Step 4: Raising the temperature to 72°C permits the nucleotides to be added along the template DNA. Step 5: The result of this one cycle is 2 copies of the region of DNA of interest. The steps are then repeated n times to generate 2n copies of the DNA region of interest.[5]

Advantages of PCR

It is a easy method to appreciate and use and produces results swiftly. It is extremely sensitive, and has the capacity to produce millions to billions of copies of a specific product for sequencing, cloning, and analysis. It can be used to scrutinize variation of gene expression levels in tumors, microbes, or other disease states.[6]

Disadvantages Of PCR

The ingredients necessary for PCR may be inadequate and depends on many complex, interrelated factors, like oligonucleotide primer size, annealing temperature and buffer salt concentration. Long DNA fragments (>300 base pairs) are difficult to amplify when the starting material is degraded (may be due to formalin fixation).[5]

Applications of PCR

Microbiology

The application of PCR has modernized the analysis and learning of infectious diseases and malignancies associated with microorganisms. Extensive number of infectious agents that can be detected by PCR, and has been used to detect organisms in blood, saliva, sputum, semen, and faeces, as well as in fixed tissues.[7]

Tumour Biology/Oncology

In oncopathology, PCR allows the analysis of mutations in oncogenes and tumour suppressor genes, the detection of minimal residual disease, clonality in identifying gene rearrangements, and in the assessment of loss of heterozygosity. An important application of RT-PCR has been in the detection and quantification of the transcripts of tumour-associated translocations.[8]

Other tumor defining tanslocations can be detected by RT-PCR including

1. t (15:17) in Acute promyelocytic leukemia
2. t (8:14) in Burkitt's lymphoma
3. t (2:5) in Anaplastic large cell lymphoma
4. t (11:12) in Ewing sarcoma and primitive Neuroectodermal Tumor.
5. t (2:13) in alveolar Rhabdomyosarcoma.

Forensic Pathology

PCR is used to identity mutilated corpses or decomposed human remains, in sex determination, in cases of disputed paternity, and in identifying perpetrators of crime.[9]

Human Genetics

PCR plays an important role in the identification of chromosomal disorders and hereditary diseases, including cystic fibrosis, Gaucher's disease, alpha-1 antitrypsin deficiency, haemophilia, and sickle cell anaemia. PCR can also be used to analyze fetal DNA for aneuploidy (the presence of extra chromosomes or the absence of chromosomes), trisomy 21, Turner's syndrome, Klinefelter's syndrome, and for sex determination.[9]

Applications of PCR in Dentistry [9]

- **Detection of periodontal pathogens***: Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola & Aggregatibacter actinomycetemcomitans*
- **Detection of cariogenic pathogens***: Streptococcus mutans and Streptococcus sobrinus*
- **Detection of microorganisms involved with endodontic infections**
- **Detecting viruses present in host cells***:* To detect *human papillomavirus and hepatitis C virus, and also in other studies that suggest virus involvement in the etiology of periodontal disease*
- **Detection of useful markers** in diagnostic and prognostic of some types of oral cancer
- Quantitative estimation of different microorganisms

Recent advances in PCR

Recently, a newer method for PCR quantification has been invented. This is called "REAL TIME PCR"- this allows the scientists to view the increase in the amount of DNA as it is amplified. In this technique, fluoroprobes bind to specific target region of amplicons to produce fluorescence during PCR.

Conclusion

In dentistry molecular methods enhance knowledge regarding the diagnosis of infectious agents that lead to oral and maxillofacial infections, thereby favoring the assessment patients at the risk for conditions such as caries, periodontal diseases, endodontic infections and oral cancer. PCR has become a standard and research tool in dentistry permitting early diagnosis of oral diseases.

References

1. Garibyan, L., Avashia, N. Polymerase Chain Reaction. J. Invest. Dermatol. 2013, 133 (3), e6
2. Mullis, K.B. and Faloona, F.A.: Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Erraymol. 155 (1987) 335-350.
3. Ishmael FT, Stellato C. Principles and applications of polymerase chain reaction: basic science for the practicing physician, *Ann Allergy Asthma Immunol*, 2008, vol.101(pg.437-43).

4. Joshi M, J.D. Deshpande. Polymerase chain reaction: methods, principles an application. IJBR 1 [5] [2010]81-97
5. Jordan RCK, Daniels TE, Greenspan JS, Regezi JA. Advanced diagnostic methods in oral and maxillofacial pathology. Part I: molecular methods. Oral Maxillofac Pathol 2001 Dec; 92(6): 650- 69
6. van Houten VMTabor MPvan den Brekel MW et al Molecular assays for the diagnosis of minimal residual head-and-neck cancer: methods, reliability, pitfalls, and solutions. *Clin Cancer Res*.2000;6:3803-3816.
7. G. J. Netto, R. D. Saad and P. A. Dysert, 2nd, Diagnostic molecular pathology: current techniques and clinical applications, part I, Proc. (Bayl. Univ. Med. Cent.), 2003, 16, 379–383.
8. O'Leary JJ, Engels K, Dada MA. Origins of. .The polymerase chain reaction in pathology. J Clin Pathol 1997; 50: 805-10 .
9. Vaid N et al. Polymerase chain reaction & its applications in dentistry. *Ejpmr, 2016,3(12), 185-189*
10. Sargaiyan V et al. Real time PCR: An important diagnostic tool in dentistry. IJOCR Apr-Jun2015;volume3issue2.

Table 1 Inherited disease that can be screened for using PCR[9]

- Thalassemia
- Antitrypsin deficiency
- Cystic fibrosis
- Gaucher's disease
- Haemophilia
- Huntingdon's disease
- Lesch-Nyhan syndrome
- Muscular dystrophy
- Osteogenesis imperfecta
- Porphyria
- Phenylketonuria
- Sickle cell anaemia
- Tay Sachs disease

Table2: Microorganisms that can be detected by PCR[9]

Viruses
Adenovirus
Cytomegalovirus
Epstein-Barr virus
Hepatitis A, B, C
Herpes simplex virus
HIV I and II
Human herpesvirus 7, 8
Human papillomavirus
HTLV-1
Lassa virus
Measles virus
Rotavirus
Bacteria
Mycobacterium tuberculosis
Mycobacterium paratuberculosis
Mycobacterium leprae
Borrelia burgdoferi
Legionella pneumophilia
Listeria monocytogenes
Chlamydia trachomatis
Helicobacter pylori
Protozoa
Toxoplasma gondii
Plasmodium fakiparum