ejpmr, 2016,3(12), 185-189

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

POLYMERASE CHAIN REACTION & ITS APPLICATIONS IN DENTISTRY

Neha Vaid*[1], Priyanka Choudhary[2], Puja Bansal[3], Kalyani Bhargava[4] and Deepak Bhargava[5]

[1]Oral Pathologist, Greater Noida.
[2]Oral Pathologist, Greater Noida.
[3]Puja Bansal[3] (Reader), School of Dental Sciences, Sharda University, Greater Noida.
[4]Kalyani Bhargava[4] (Prof & Head), Inderprastha Dental College & Hospital, Sahibabad, Ghaziabad.
[5]Deepak Bhargava[5] (Prof & Head), School of Dental Sciences, Sharda University, Greater Noida.

Corresponding Author: Dr. Neha Vaid

(Oral Pathologist), H.No. 10/66, Old Campus, CCS-HAU Hisar.

Article Received on 24/09/2016 **Article Revised on 14/10/2016** **Article Accepted on 03/11/2016**

ABSTRACT

Various molecular biology techniques have become available in the last few years. One of the most revolutionary 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 focus of this review is to describe the current status 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 movie Jurassic Park and real life events like the J Simpson trial have captured public interest in molecular biology. The basis of molecular biology is the understanding of the structure and function of DNA, RNA, and proteins, as well as the techniques for manipulating these molecules.[1] Molecular diagnostics provides the necessary underpinnings for any successful application of gene therapy or biologic response modifiers. It is estimated that more than 5% of all laboratory testing is based on DNA or RNA analysis.[2] Currently, molecular diagnostics plays an important role in the clinical diseases, and inherited genetic disorders.[3] The molecular analysis of DNA, RNA, and protein derived from diagnostic tissue specimens has revolutionised pathology and led to the identification of a broad range of diagnostic and prognostic markers influencing our day to day decision making.[4] The success rate of many cancer treatments is largely dependent on the microscopically undetectable tumour cells (MRD) that remain in the body. Particularly in head-and-neck cancer, a more sensitive detection of MRD is of importance because residual tumour cells play a crucial role in the relatively high recurrence rate observed in these patients.[5] The application of immunologic research methods to histopathology has resulted in marked improvement in the microscopic diagnosis.[6] These genetic alterations have not only guided molecular studies in establishing the underlying genes involved, thereby yielding important pathogenetic information, but also have provided clinicians with a valuable tool to add to their diagnostic armamentarium.[7] The introduction of in vitro nucleic acid amplification methods, most notably, polymerase chain reaction(PCR) which amplifies minute amounts of DNA > 1 million-fold, triggered the development of novel sensitive technologies for the detection of tumour cells by molecular markers.[5] It is hard to exaggerate the impact of the polymerase chain reaction. From the daily practicalities of medical diagnosis to the theoretical framework of systematics, from courts of law to field studies of animal behaviour, PCR takes analysis of tiny amounts of genetic material-even damaged genetic material-to a new level of precision and reliability.[8] This review describes polymerase chain reaction and its application in dentistry.

HISTORICAL BACKGROUND

Polymerase chain reaction technique was first described by Khorana and colleagues in the early 1970, but brought to life and named as PCR in 1983 by Karry Mullis, who received the Nobel Prize for chemistry in 1994 for his work on PCR.[1] The DNA polymerase originally used for the PCR was extracted from the bacterium Escherichia coli. Although this enzyme had been a valuable tool for a wide range of applications and had allowed the explosion in DNA sequencing technologies in the preceding decade, it had distinct disadvantages [9] this enzyme is inactivated by the high temperatures and had to be replenished during every cycle after each denaturation step.[1]

Owing to this discovery, PCR was given the title of "Major scientific development of the year 1989"and Taq DNA polymerase as "Molecule of the year1989" by *Science*.[1]

WHAT IS PCR?

Complex organisms such as human beings possess DNA sequences that are uniquely and specifically present only in particular individuals. These unique variations make it possible to trace genetic material back to its origin, identifying with precision at least what species of organism it came from, and often which particular member of that species. Such an investigation requires, that enough of the DNA under study is available for analysis—where the role of PCR comes in.[8]

PCR is a simple, yet elegant, enzymatic assay that enables amplification of a specific DNA fragment from a complex pool of DNA. Only trace amounts of DNA are needed for PCR to generate enough copies to be analyzed using conventional laboratory methods. For this reason, PCR is a sensitive assay.[10]

PCR CYCLE[11]

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.

The process is repeated 30 to 40 times, each cycle doubling the amount of the targeted genetic material.

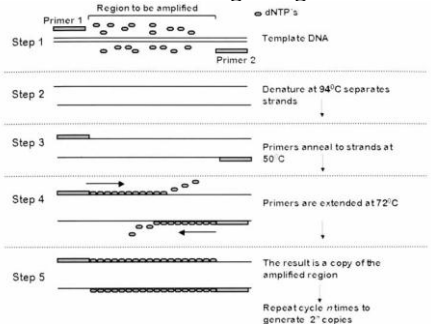

Fig 1. The basic steps for a single cycle of PCR. *Step 1:* The reaction mixture contains genomic (template) DNA with a target region to be studied, nucleotides (dNTPs), 2 primers, and buffer. *Step 2:* The mixture is heated to 94°C to separate the double strands. *Step 3:* When the temperature is then 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 2*n* copies of the DNA region of interest.[12]

VARIATIONS

A number of adaptations of the standard PCR have been developed in order to match the PCR to specific research needs.

1. **IN SITU PCR:** In the in situ adaptation, PCR is performed within intact cells, whether within tissue sections or on cytologic preparations. This technique has been used primarily for the detection of viral infections, such as HIV or HPV, in particular cell types.[13]
2. **ASYMMETRIC PCR:** It is a simple and effective method for the production of single stranded DNA suitable for direct sequencing. This can easily be sequenced directly, without the need for cloning or the establishment of DNA libraries.[1]
3. **NESTED PCR**: Nested PCR refers to the use of two sequential PCR reactions, with the primers used for the second PCR reaction being internal to those used for the initial PCR reaction.[13]
4. **AMPLIFICATION REFRACTORY MUTATION SYSTEM (ARMS)**: ARMS is a novel system using primers designed so that the 3' end coincides with a mutated nucleotide base, facilitating allelic discrimination.[14]
5. **REAL TIME PCR:** The recent development of "real-time" PCR (Q-PCR) added great advantages to traditional PCR. As the name indicates, this technique allows for the real-time quantitation of PCR product following each of the 40 amplification cycles.[11]

   Q-PCR is of great utility in the assessment of minimal residual disease following novel targeted therapy against specific molecular defects as well as bone marrow transplantation for myelogenous leukemia.[13] The method has proved valuable for the retrospective study of gene amplification in human tumors, including c-*erbB-2 (HER2/neu)* in salivary gland and breast tumors, *int-2* in thyroid cancers, and *CCND1* (cyclin D1) in breast carcinomas, oral cancers, and epithelial dysplasias.[15]
6. **TAQMAN PCR:** TaqMan PCR (5' nuclease assay) is a major advance in PCR. It was first described by Holland et al, who used the 5'-3' endonucleolytic activity of Taq DNA polymerase to detect target sequences during amplification by PCR. During

amplification Taq DNA polymerase, through its 5'-3' 2. endonucleolytic activity, cleaves the probe into fragments, separating the reporter molecule from the quencher, thus allowing its detection. The major advantage of this technique is its ability to detect specifically amplified DNA or RNA sequences at selected time points in the PCR, thereby allowing direct quantitative real time DNA and RNA detection.[1]

7. **REVERSE TRANSCRIPTASE PCR:** Another important application of PCR has been the detection and quantification of mRNA in cells. Because mRNA is short lived and unstable, the determination of its relative abundance is often difficult in tissue sections by using conventional methods of RNA analysis such as Northern blotting.[12]

RT-PCR is used to detect or quantify the expression of mRNA, often from a small concentration of target RNA. The template for RT-PCR can be total RNA or poly (A) selected RNA. RT reactions can be primed with random primers, oligo (dT), or a gene-specific primer (GSP) using a reverse transcriptase. RT-PCR can be carried out either in two-step or one-step formats. In two-step RT-PCR, each step is performed under optimal conditions. cDNA synthesis is performed first in RT buffer and one tenth of the reaction is removed for PCR. In one-step RT-PCR, reverse transcription and PCR take place sequentially in a single tube under conditions optimized for both RT and PCR.[16]

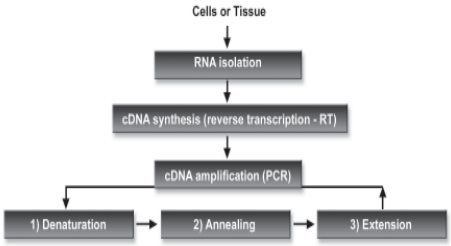

FIGURE 2- Schematic diagram of RT-PCR showing that RNA isolated from cells or tissue is used as substrate in reverse transcription for synthesis of cDNA that will serve as template for amplification by PCR[16]

ADVANTAGES of PCR

1. It is a simple technique to understand and use.[8]
2. It produces results rapidly.[8]
3. It is highly sensitive, with the potential to produce millions to billions of copies of a specific product for sequencing, cloning, and analysis.[10]
4. It can be used to analyze alterations of gene expression levels in tumors, microbes, or other disease states.[8]

DISADVANTAGES of PCR

1. The ingredients necessary for PCR may be limited and depends on many complex, interrelated factors, like oligonucleotide primer size, annealing temperature and buffer salt concentration.[13]

2. Long DNA fragments (>300 base pairs) are difficult to amplify when the starting material is degraded (may be due to formalin fixation).[13]

APPLICATIONS OF PCR

MICROBIOLOGY

The use of PCR has revolutionized the diagnosis and study of infectious diseases and malignancies associated with microorganisms. The list of infectious agents that can be detected by PCR is extensive, and has been used to detect organisms in blood, saliva, sputum, semen, and faeces, as well as in fixed tissues.[12]

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.[12]

TUMOUR BIOLOGY/ONCOLOGY

In oncopathology, PCR has led to a better understanding of the pathobiology of malignancy, allowing the analysis of mutations in oncogenes and tumour suppressor genes (for example, c-myc, p53, ras), the detection of minimal residual disease (MRD), clonality (for example, B and T cell gene rearrangements in lymphomas), in identifying gene rearrangements (for example, t(14,18) in follicular lymphomas and the Philadelphia chromosome in CML), and in the assessment of loss of heterozygosity (allelic imbalance) particularly in colorectal and breast cancer.[1]

An important application of RT-PCR has been in the detection and quantification of the transcripts of tumour-associated translocations. Many neoplasms particularly hematopoitic malignancies contain specific chromosomal translocations. e.g. Philadelphia chromosome is a genetic alteration that is most commonly identified in chronic myeloid leukemia and a subset of acute lymphoblastic leukemia. RT-PCR is widely used in the diagnosis of genetic diseases and in the determination of the abundance of specific different RNA molecules within a cell or tissue as a measure of gene expression.[12]

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.

RT-PCR has also been used in dental research in order to assess the expression of mRNA for proteins involved in the development of periodontal disease.[16]

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.[1]

Table: 1 APPLICATIONS OF PCR IN DENTISTRY

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..[17]

PERIODONTAL DISEASE

PCR has revolutionized the understanding of periodontal pathogenesis. It is expected that PCR will be useful in the microbiological diagnosis of periodontal disease,[16] microorganisms like Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola & Aggregatibacter actinomycetemcomitans.[17] RT-PCR provides a new rapid diagnostic tool and opens the opportunity to detect small numbers of oral pathogens in clinical specimens, which are under the detection limit by culture technique.[16]

DENTAL CARIES

Among oral bacteria (Streptococcus cricettus, S. ratti, S. mutans, S. sobrinus, S. downei, S. ferus and S. macacae) of which S.mutans and S. sobrinus are more frequently isolated from the human oral cavity.[18] Although there have been diverse methods published to detect and identify cariogenic microorganisms, these are mostly time consuming and costly to carry out. Among these, there are direct microscopy, microbiological cultures, enzymatic tests, and DNA-based tests. Of these, tests based on Polymerase Chain Reaction (PCR) surge as an alternative to conduct epidemiological and clinical studies, due to its high sensitivity and specificity, besides its rapidity when obtaining results.[19] Igarashi T, Yamamoto AA, Goto N 1996 introduced Direct detection of *Streptococcus mutans* in human dental plaque by polymerase chain reaction.[20] Richard, et al 2002, accomplished a study with the aim of finding a reliable method for the identification of the species *Lactobacillus rhamnosus* among other oral lactobacilli. The authors described a DNA probe and a specific PCR primer able to discriminate *L. rhamnosus* from other *Lactobacillus* species.[16] 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.[18]

ENDODONTIC INFECTIONS

Porphyromonas gingivalis and Tannerella forsythia are anaerobic bacteria commonly involved in root canal infections. Saito et al (2009) concluded that the application of a real-time PCR methodology based on unique copy genes in primary endodontic infections has shown that T. forsythia can be highly prevalent and abundant in endodontic infections, whereas P. gingivalis is moderately frequent and less abundant, displaying 19-fold lower average levels than those observed for the former.[21] Molecular methods can be used to characterize the microflora associated with endodontic infections without the inherent biases of culture techniques.[16]

DETECTION OF VIRUSES

Parra and Slots (1996) determined the prevalence of human cytomegalovirus (HCMV), Epstein-Barr virus type 1 and 2 (EBV-1 and –2), herpes simplex virus, human papillomavirus and HIV in crevicular fluid from individuals with various forms of periodontal disease. Viral identification was performed using a PCR-based technique. This study provided evidence of human viruses in the crevicular fluid of many advanced adult periodontitis lesions.[17]

Future of PCR

PCR is the wave tool of the future in molecular biology. PCR technology not only overcomes the time-consuming

process using conventional culture & microscopic analysis, but also has increased sensitivity, specificity.[17] PCR is doing for genetic material what the invention of the printing press did for written material—making copying easy, inexpensive, and accessible. Thanks to PCR, we will be probing the genetic past, and peering into the genetic future, for many years to come.[8]

ACKNOWLEDGEMENT

We thank Dr. V.K. Hazarey, Dean, Govt. Dental College & Hospital, Nagpur and Dr. S.M. Gamvir, Professor & Head, Govt Dental College & Hospital, Nagpur for helping us in the review.

REFERENCES

1. O'Leary JJ, Engels K, Dada MA. Origins of. .The polymerase chain reaction in pathology. J Clin Pathol 1997; 50: 805-10
2. Netto GJ, Saad RD Diagnostic molecular pathology. An increasingly indispensable tool for the practicing pathologist. Arch Pathol Lab Med (2006); 130: 1139-1348.
3. Netto GJ, Saad R. Diagnostic molecular pathology, part 2: Proteomics and clinical applications of molecular diagnostics in hematopathology. BUMC PROCEEDINGS 2005; 18: 7–12.
4. Fend F, Raffeld M Laser capture microdissection in pathology. J Clin Pathol 2000; 53: 666–72.
5. Vida M. M. van Houten, Maarten P. Tabor, Michiel W. M. van den Brekel, et al. Molecular Assays for the Diagnosis of Minimal Residual Head-and Neck Cancer: Methods, Reliability, Pitfalls, and Solutions. Clin Cancer Res 2000; 6: 3803-16.
6. Jordan RCK, Daniels TE, Greenspan JS, Regezi JA. Advanced diagnostic methods in oral and maxillofacial pathology. Part II: Immunohistochemical and immunofluorescent methods. OOO (2002); 93(I): 56-74.
7. Bridge JA, Cushman-Vokoun AM. Molecular Diagnostics of Soft Tissue Tumors. Arch Pathol Lab Med (2011); 135: 588-601
8. Vida M. M. van Houten, Maarten P. Tabor, Michiel W. M. van den Brekel, et al. Molecular Assays for the Diagnosis of Minimal Residual Head-and Neck Cancer: Methods, Reliability, Pitfalls, and Solutions. Clin Cancer Res 2000; 6: 3803-16.
9. Powledge TM. The polymerase chain reaction. Adv Physiol Educ (2004); 28: 44–50.
10. Methods in Molecular Biology, Vol. 226: PCR Protocols, Second Edition. Edited by: J. M. S. Bartlett and D. Stirling © Humana Press Inc., Totowa, NJ:1-6
11. Garibyan L, Avashia N. Polymerase Chain Reaction. Journal of Investigative Dermatology (2013); 133: 1-4.
12. Netto GJ, Saad RD, Dysert PA. Diagnostic molecular pathology: current techniques and clinical applications, part I. BUMC Proceedings (2003); 16: 379–83
13. Jordan RCK, Daniels TE, Greenspan JS, Regezi JA. Advanced diagnostic methods in oral and Maxillofacial Pathology. Part I: Molecular methods. OOO 2001; 92(6): 650-69.
14. Anderson's pathology. Ivan Damjanov, James Linder; 1: 206-209.
15. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, Liotta LA. Laser Capture Microdissection. Science (1996); 274: 998-1001.
16. Santos CF, Sakai VT, Machado MA, Schippers DN, Greene AS. Reverse transcription and polymerase chain reaction: principles and applications in dentistry. J Appl Oral Sci.(2004); 12(1): 1-11
17. Patil SH, Bhat KG, Lotlekar PS, Hombal LV. Polymerase Chain Reaction. An innovative Tool in periodontal Diagnosis. Jaypee Journals: 60- 66
18. Marcela Agne Alves Valones1; Rafael Lima Guimarães1; Lucas André Cavalcanti Brandão1;Paulo Roberto Eleutério de Souza2, Alessandra de Albuquerque Tavares Carvalho1; Sergio Crovela. principles and applications of polymerase chain reaction in medical diagnostic fields: a review. Brazilian Journal of Microbiol (2009): 40: 1-11
19. Luis Eduardo Carmona, OD1, Niradiz Reyes, PhD2, Farith González, OD. Polymerase Chain Reaction for detection of *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque of children from Cartagena, Colombia. Colomb Med. 2011; *42:* 430-7
20. Igarashi T, Yamamoto A, Goto N. Direct detection of *Streptococcus mutans* in human dental plaque by polymerase chain reaction. Oral Microbiol and Immunol 1996; 11(5): 294-98.
21. Cheon K, Moser SA, Wiener HW, Whiddon J, Momeni SS, Ruby JD, Cutter GR, Childers NK. Characteristics of Streptococcus mutans genotypes and dental caries in children. Eur J Oral Sci 2013; 121: 148–155.