
Polymerase chain reaction in exploring endodontic infections

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Abstract

Traditionally cultivation, microscopic and immunological based techniques were only the means by which endodontic microbiota were studied. Because of their several limitations in microbiological diagnosis, newer molecular genetic methods were introduced. Polymerase chain reaction -PCR is a widely spread advance in clinical diagnostic technology and known to be cornerstone in genome sequencing projects. Fortunately, PCR has been the focus of a great deal of research in recent years with the goal of exploring microbiota. Here we review the principles of PCR techniques, PCR derivatives and applications of Real-Time PCR in dental sciences.

Data sources: Studies published in the medical, dental and biological literature.

Conclusion: PCR has enabled the detection of bacterial strains which are phenotypically divergent or convergent in behaviour. PCR demonstrated a great range of diversity in endodontic microbiota.

Introduction

The Polymerase Chain Reaction (PCR) brought enormous benefits and scientific developments such as genome sequencing, molecular genetic analyses and the diagnosis of infectious disease¹. PCR enables the synthesis of nucleic acids through which a DNA segment can be specifically replicated in a semi-conservative way which generally exhibits excellent detection limits.²

In recent era, Real-Time PCR, has become increasingly important in clinical diagnostics and research laboratories because of its capability in generating quantitative results, whereas conventional PCR, displays only the qualitative results³.

The aim of the present paper is to outline the principles and applications of conventional PCR and Real-Time PCR techniques in endodontics.

Molecular Genetic Methods

Culture independent molecular genetic methods involve DNA amplification of 16s r-DNA followed by

cloning and sequencing. Unlike culture techniques, here both cultivable and uncultivable species are detected. Its higher sensitivity, specificity, accurate identification of microbes with ambiguous phenotypic behavior, including divergent and convergent strains and rapid diagnosis is advantageous in case of life threatening diseases. Large number of samples can be surveyed in epidemiological studies using molecular genetics.

The molecular methods are as follows:

- Broad range PCR(polymerase chain reaction) followed by sequencing DGGE (denaturing gradient gel electrophoresis) or Temperature gradient gel electrophoresis (TGGE)
- PCR followed by T-RFLP (Terminal restriction fragment length polymorphism) to analyze microbial community via finger printing technique.
- RT-PCR (Reverse transcriptase – PCR)
- Arbitrarily primed PCR (AP-PCR)
- Nested PCR
- Multiplex PCR
- Real time PCR.

Gene Targets for microbial identification

Microbial identification depends on the genes which are unique for each species. Ribosomes are intracellular particles composed of proteins and mRNA. Large subunit genes – 23s and 25s rDNA and small subunit genes – 16s and 18s r DNA are widely used for microbial identification, characterization and classification. 16s rDNA is the most useful target and 23S rDNA, a suitable alternative for bacterial identification.

PCR (POLYMERASE CHAIN REACTION):

PCR process was conceived by Karl Mullis in 1983.

The invitro replication of DNA is through:

- DENATURATION: Target DNA template is treated at 95°C to break the hydrogen bonds between the strands thus liberating single strands of DNA.
- PRIMER ANNEALING: Two short primers are annealed to complementary sequences on opposite strands of target DNA at 55-65°C.
- EXTENSION: Through the extension of each annealed primer a complementary new strand is

synthesized. Thermastable DNA polymerase in presence of excess deoxyribonucleoside triphosphate helps in this synthesis.

The exponential amplification of new DNA products confers 10-100 times extraordinary sensitivity in detecting target DNA.

PCR derived arrays:

- **NESTED PCR:** The Nested PCR uses the products of primary PCR amplification as a template in second PCR reaction with separate primer set. This result in increased sensitivity and specificity in detecting target DNA.

- **RT-PCR (Reverse transcriptase – PCR):** It is 2 step approach.

1st step: Reverse transcriptase converts RNA into S-S complementary DNA

2nd step: PCR primer, DNA polymerase and nucleotides are added to create 2nd strand of C-DNA. Newly formed double stranded C-DNA is subjected to conventional PCR reaction

- **MULTIPLEX-PCR:** PCR arrays are concentrated on detection of single species but in multiplex PCR two or more sets of primer specific for different targets are introduced into same reaction tube. Multiplex PCR minimizes time and expenditure.

- **PCR BASED MICROBIAL TYPING:** This method is used for clonal analysis of microorganisms e.g. Arbitrarily primed PCR (AP-PCR) Random amplified polymorphic DNA (RAPD) determine whether two isolate of the same species are related. It tracks the origin of microorganisms infecting the given site and furnishes highly specific DNA profiles with no known prerequisite DNA sequence.

- **REAL TIME PCR:** Conventional PCR assays are mostly qualitative but real time PCR is quantitative as it continuously measures the amplification products throughout the reaction. This is a sensitive method for the accurate quantification of individual species, which could be very relevant for the diagnosis of pathogens and genetic diseases. There is greater sensitivity, reproducibility, precision, rapid analysis and ease of quantification with real time PCR. SYBR-GREEN probes, TaqMan probes, Molecular Beacon are different probes in real time PCR.

- **BROAD RANGE PCR:** In broad range PCR primers are complimentary to conserved regions of particular gene which is shared by group of microorganisms. Any kind of bacteria sample can be detected and identified. Initially bulk nucleic acids are extracted from a sample, then 16S-rDNA is isolated via with oligonucleotide primer for conserved regions of the gene. PCR products are cloned into plasmid vector. Cloned genes are sequenced individually and

submitted to database for identification.

- **DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE):** It's a method to determine the diversity of different microorganisms living in diverse ecosystems. The procedures include extraction of DNA of same length but with different base pair sequence, amplification of 16S- rDNA using broad range primers is done and then DGGE will analyse the PCR products in which electrophoresis of PCR amplified 16S rDNA fragment in polyacrylamide gels containing DNA denaturants (urea + formamide). As PCR products migrate in this gel and get partially or fully denature. Partially denaturation cause decreased electrophoretic mobility of DNA molecule. DNA bands in DGGE are visualized by using ethidium bromide, SYBR – GREEN or silver staining. Temperature gradient gel electrophoresis (TGGE) uses same principle as DGGE except gradient is temperature not chemical denaturants.

- **TERMINAL RFLP:** This measures the size polymorphism of terminal restriction fragments from a PCR amplified marker. The rDNA from different species in a community is PCR amplified using PCR primer labeled with fluorescent dye and obtained PCR products digested with restriction enzyme endonucleases resulting in different fragment length appropriate for sizing on high resolution sequencing gels (ABI) gel or the use of fluorescent labeled primer limits the analysis to terminal fragments thus simplifies banding pattern and analysis of complex communities as each band represent each species. By the application of automated DNA sequence technology, T-RFLP has greater resolution than gel based profiling technologies DGGE/TGGE.

PRINCIPLES AND APPLICATIONS IN DENTISTRY

The recognition of the universality of the genetic code in living organisms has been essential to the development and application of genetic technologies. Different clinical samples include blood, sweat, semen, strands of hair and saliva. Saliva is a potential source of genetic material for diagnostic tests in oral and systemic diseases. saliva sampling mandatory in routine dental exam. Furthermore, as a medium where biologically active proteins and exogenous substances are found, saliva is also a source of patient DNA as well as cariogenic and periodontopathogenic microorganisms. The literature reports the uses of PCR for the study of factors involved in periodontal disease, dental caries, endodontic infections and oral cancer.

Dental Caries

PCR method gives specific quantitative determination of group mutans, which encompasses cariogenic 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 cavity⁸. Species characterization based on DNA testing is currently widely accepted, as phenotyping is a reflection of gene expression⁹. This method allows a quick, exact determination of unknown quantities of this bacterium and provides an efficient means for evaluating the risk of caries in patients as well as monitoring the efficiency of preventative and therapeutic measures¹⁰.

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. Examples of bacterial species that were only detected in canals through molecular methods and that are currently considered important endodontic pathogens include *Treponema denticola*, *Dialister pneumosintes*, *Tannerella forsythia*, *Treponema socranskii* and *Prevotella tanneriae*¹¹. Based on current studies, molecular methods can be used to characterize the micro-flora associated to endodontic infections. Using real time PCR the prevalence of *P. endodontalis*, *P. gingivalis*, *P. intermedia* and *P. nigrescens* were seen¹¹. The relatively lower occurrence of these bacteria may explain the relative stability and chronic nature of the root condition.

Although studies have determined that bacteria are the greatest etiological agent in pulp and periradicular disease, fungi (*Candida albicans*) have also been associated to root canal infection.

Therefore, the use of highly sensitive techniques is welcome in the study of endodontic infections, decreasing the risks for potentially important species to pass unnoticed during sample analysis. Although qualitative results do not lack significance, the use of quantitative molecular assays, like the real-time PCR, can allow inference of the role of a given species in the infectious process while maintain high sensitivity and the ability to detect fastidious or uncultivable microbial species.

Conclusion

PCR technology has enabled the detection of bacterial species which are phenotypically divergent in behavior. Both PCR and real-time PCR have

dramatically changed the field of basic science and diagnostic medicine. These technologies are now a well-established, indispensable part of research and clinical laboratories. Its simplicity, sensitivity and specificity together with its potential for high output and the ongoing introduction of new chemistries, more reliable instrumentation and improved protocols, has made real-time PCR the benchmark technology for the detection and comparison of RNA levels.

References

- Speers D.J., Ryan S., Harnett G., Chidlow G. Diagnosis of malaria aided by polymerase chain reaction in two cases with low-level parasitaemia. *Inter. Med. J.* 2003;33(12):613–615
- Spolidorio D.M.P., Spolidorio L.C. Técnicas básicas de biologia molecular. In: ESTRELA C., editor. *Metodologia científica-Ciência-Ensino-Pesquisa*. São Paulo: Artes Médicas; 2005.
- Morillo J.M., Lau L., Sanz M., Herrera D., Silva A. Quantitative real time PCR based on single copy gene sequence for detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *J. Periodont. Res.* 2003;38(5):518–524.
- Silva L.A.F., Passos N.S. Coleta de amostras biológicas em locais de crimes para estudo de DNA. Maceió: UFAL; 2002. DNA Forense.
- Morillo J.M., Lau L., Sanz M., Herrera D., Silva A. Quantitative real time PCR based on single copy gene sequence for detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *J. Periodont. Res.* 2003;38(5):518–524.
- Richard B., Groisillier A., Badet C., Dorignac G., Lonvaud-Funel A. Identification of salivary *Lactobacillus rhamnosus* species by DNA profiling and a specific probe. *Res. Microbiol.* 2001;152(2):157–165.
- Santos C.F., Sakai V.T., Machado M.A.A.M., Schippers D.N., Greene A.S. Reverse transcription and polymerase chain reaction: principles and applications in dentistry. *J. Appl. Oral Sci.* 2004;12(1):1–11.
- Yoo S.Y., Kim P.S., Hwang H.K., Lim S.H., Kim K.W., Choe S.J., Min B.M., Kook J.K. Identification of non-mutans *Streptococci* organisms in dental plaques recovering on mitisalivarius bacitracin agar medium. *J. Microbiol.* 2005;43(2):204–208.
- Truong T.L., Menard C., Mouton C., Trahan L. Identification of mutans and other oral *Streptococci* by random amplified polymorphic DNA analysis. *J. Med.*

Microbiol. 2000;49(1):63–71.

10. Rupf S., Kneist S., Merte K., Eschrich K. Quantitative determination of *Streptococcus mutans* by using competitive polymerase chain reaction. *Eur. J. Oral Sci.* 1999;107(2):75–81.

11. Kim Y., Flynn T.R., Donoff R.B., Wong D.T.W., Tood R. The gene: the polymerase chain reaction and its clinical application. *J. Oral Maxillofac. Surg.* 2002;60(7):808–815.