A View on Polymerase Chain Reaction as an Outstanding Molecular Diagnostic Technique in Periodontology

Adileh Shirmohammadi,1 Amirreza Babaloo,1 Solmaz Maleki Dizaj,2,3 Farzaneh Lotfipour,4,5 Simin Sharifi,2 Mohammad Ali Ghavimi,6 and Khadijeh Khezri7

1Department of Periodontics, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran
2Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3Department of Dental Biomaterials, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran
4Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
5Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
6Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran
7Deputy of Food and Drug Administration, Urmia University of Medical Sciences, Urmia, Iran

Correspondence should be addressed to Solmaz Maleki Dizaj; maleki.s.89@gmail.com and Farzaneh Lotfipour; farzaneh.lotfipour@gmail.com

Received 30 March 2021; Revised 11 June 2021; Accepted 13 July 2021; Published 20 July 2021

Academic Editor: Fernanda Faot

Copyright © 2021 Adileh Shirmohammadi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, and reproduction in any medium, provided the original work is properly cited.

Objectives. This study presents a discussion on the fundamentals of polymerase chain reaction (PCR) and its use as a diagnostic tool in periodontology. Materials and Methods. A computer-aided as well as hand-made search in PubMed and Scopus indexed journals (relevant to the topic) was done by keywords of molecular technique in periodontology, PCR, applications of PCR, and PCR in periodontics. Only the papers in the English language and outlining PCR and its association with periodontology were collected and utilized to provide a succinct review. There was no limitation for publication time. Results. The results of our search showed that PCR has turned into a standard in diagnosis in the field of periodontology. A variety of researches has demonstrated that its sensitive, and specific characteristics make it a quick and effective technique of recognition, identification, and quantification of microorganisms. Identification of various immunoinflammatory markers at the mRNA expression level as well as ascertaining gene-related polymorphisms can also be performed. Conclusions. The mechanisms of periodontal disease can further become clarified using PCR. Clinical Relevance. PCR as a diagnostic method can play a main part in the validation of the clinical diagnosis of periodontal disease indicating the reason, pathogenesis, clinical steps, progress, and prognosis of the disease.

1. Introduction

Periodontal disease includes a variety of diseases that are caused by inflammation. If this inflammation is not treated and eliminated, it can cause attachment loss, bone resorption, and eventually tooth extraction [1, 2]. Chronic periodontitis and aggressive periodontitis are two well-known types of soft and hard tissue disease around the teeth that cause the destruction of these tissues [3]. There are two types of classifications for this disease. The first is based on the amount of bone resorption supporting the tooth, divided into localized and generalized. The second is based on the severity of the disease, which in this regard is divided into three categories slight, moderate, and advanced [1, 3, 4]. In the new cataloguing (from 2018), clinical health is also considered, and periodontitis is categorized base on stages, ranging from stage 1 (least severe) to stage 4 (most severe). The risk and rate of disease progression have been categorized into three grades from the lowest risk of progression (grade A) to the highest (grade C). Then, the forms of the disease formerly
known as “chronic” or “aggressive” now grouped under a single category, “periodontitis”. The stage-based category is mainly reliant on the severity of disease at appearance as well as on the complexity of disease management. However, the grade-based category offers supplemental data about biological features of the disease [5].

Bacteria in dental plaque cause this chronic disease, and this disease can be seen from the slightest changes in the supporting tissues of the teeth to the severe destruction of the soft and hard tissues supporting the teeth. Bacterial plaques that cause the disease can include bacteria Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Tannerella forsythia. Still, these are not the only causes of the disease, but the environment and genetics can also predispose people to the disease [6]. As a result, periodontitis is a multifactorial disease, and the presence of pathogenic bacteria in dental plaque cannot cause disease alone. Other factors that help pathogens to cause and promote the disease include an increase in proinflammatory cytokines and a decrease in anti-inflammatory cytokines such as interleukin-10 and the presence of MMP, PGE2, TGF-β, and TIMPs [7, 8]. However, evidence suggests that the immune system’s response to periodontal disease is much broader.

Measurement of clinical attachment loss (connective tissue attachment loss on the root surface) and radiographic bone loss (alveolar bone loss) is performed for the identification of periodontal diseases in the clinical setting [4, 9]. However, clinical identification of the disease does not determine pathological mechanisms, the way the disease behaves, and advances, and nor does it dictate the prognosis of the disease.

Besides the routine clinical test, diverse techniques of diagnosis are essential in confirmation of the clinical diagnosis. Despite having constitutive benefits, the conventional culture techniques have some disadvantages such as difficult sampling, more time needed for the results, and unable to recognize small quantities of microorganisms [10–13]. Dark field microscopy is incapable of identifying the immobile periodontal pathogens, and flowcytometry, immunofluorescence assay, etc. can yield false-positive results and cross-reactions [14]. Analysis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein is also performed by the methods in molecular biology. The DNA profiling genetic technique included restriction fragment length polymorphism (RFLP) that made use of diversities in homologous DNA sequences; it is arduous, long in duration, and costly, and a large sample is required. The nucleic acid probe is a nucleic acid molecule that has undergone unnatural synthesis and labeling for the identification of a particular organism accompanied by the restriction of cross-reactivity [14]. Hybridization is defined as the interconnection of complementary DNA strands to create double-stranded nucleic acids and the DNA-DNA hybridization technology with a chequered pattern utilized for epidemiological investigation, and ecologic researches require complicated laboratory equipment and high skills [14].

Polymerase chain reaction (PCR), as a vast diagnostic method, does not have the restrictions stated above and has the capability of recognizing even one copy of the explored DNA targets from clinical microbiologic samples [15–18].

There are complexities in regard to microbiota found in the subgingival area, and utilization of the microbiological methods for the identification and therapeutic management of progressive and resistant forms of periodontitis is dependent upon the diversity of plaque components [19].

A wide variety of advantages in the genetic investigation for research on gene expression has resulted from the evolution of PCR. Identification of the type and frequency of treatment is facilitated by genetic examination utilizing PCR to figure out whether an individual is vulnerable to periodontitis. Precise identification of bacterial strains with divergent phenotype, rapid analysis, ease of quantification, high precision, and least contamination are some main advantages of PCR-based methods. Researches focusing on PCR for assessing mRNA expression of different immune and inflammatory markers are beneficial in recognition of the pathological mechanism of periodontitis.

2. Search Methodology

The objective of this review includes a description of the fundamentals, benefits, uses, and restrictions of PCR in periodontology with their future outlooks. A hand-made and computer-aided search in PubMed and Scopus indexed journals pertinent to the topic was done by inputting molecular techniques in periodontology, PCR, applications of PCR, and PCR in periodontics. Sixty-nine articles on the specification of the PCR process along with its association with periodontology were selected to provide a succinct review among 260 articles explored.

3. PCR Application in Dentistry

Different types and applications of PCR are summarized in Table 1 and Table 2, respectively. PCR is also of paramount importance in different fields of dentistry. Scraping of the saliva, subgingival plaque, mouthwash, gingival tissue, blood, and buccal mucosa is utilized in PCR for the recognition of microorganisms, mRNA gene expression, and genetic polymorphisms of a diversity of inflammatory mediators in the dental practice [20, 21].

The rapid and sensitive detection of microorganisms in dental plaque samples by the nested PCR method [22], the study of gene expression for the oral anaerobe P. gingivalis in periodontal disease in vivo by real-time (RT) PCR [23], and examination the expression of the chemokines macrophage inflammatory protein-1 alpha, assay of subgingival overgrowth of pathogenic bacteria in periodontitis by hot-start PCR [24] and interferon-gamma inducible protein 10 and of their respective receptors, CCR5 and CXCR3, by means of reverse transcription-polymerase chain reaction (RT-PCR) techniques [25] have been used in dentistry.

Researches on PCR have well elucidated the knowledge of technology of the oral cavity [49]. Epidemiological researches on the ground of genetic polymorphisms, the microbiology of dental diseases, and their association with systemic diseases may be conducted. PCR is able to recognize pathogens involved in dental caries and describes the pathogenesis of dental caries [50]. Identification of microorganisms involved
Table 1: Different types of PCR techniques.

| Different types of PCR techniques | References |
|----------------------------------|------------|
| Nested PCR                       | [26]       |
| Multiplex PCR                    | [27]       |
| Real-time (RT) PCR               | [28]       |
| Genomic inverse PCR              | [29]       |
| Arbitrary PCR                    | [30]       |
| Assembly PCR                     | [31]       |
| Nanoparticle PCR                 | [32]       |
| Cold-PCR                         | [33]       |
| Photonic PCR                     | [34]       |
| Extreme PCR                      | [35]       |
| Ligation-mediated PCR            | [36]       |
| Methylation-specific PCR         | [37]       |
| Colony PCR                       | [38]       |
| Hot start/cold finish PCR        | [39]       |
| Core sample PCR                  | [40]       |
| Dial-out PCR                     | [39]       |
| Digital PCR                      | [41]       |
| In silico PCR                    | [42]       |
| Overlap-extension PCR            | [43]       |
| Quantitative PCR                 | [44]       |
| Solid phase PCR                  | [45]       |
| Suicide PCR                      | [46]       |

Table 2: Various applications of the PCR technique (including dentistry applications) [40, 47, 48].

Various applications of the PCR technique

- Diagnosis therapy (cancer, diabetes, metabolic, obesity, congenital diseases, neurological disorders, cardiac, etc.)
- The ability to amplify RNA
- Nucleic acid detection assays
- Mycology-parasitology
- Clinical bacteriology and virology
- Organ transplantation
- Agricultural sciences
- Studying and understanding the disease state
- Dentistry (periodontal diseases, dental caries, oral cancer, and endodontic infections)
- Genetic and genomic studies
- Insert analysis
- Molecular systematic evolution
- Tissue typing
- Biomarker
- Phytopathology
- PCR fingerprinting

4. PCR Technique in Periodontology

4.1. Detection of Microbial Pathogens. The PCR method having more precision, sensitivity, and rapidity is made use of for the recognition and measurement of periodontal bacterial count [19, 53]. Q-PCR or real-time PCR containing primers with specificity in regard to species is able to quantify individual microbial species and total bacterial count in dental plaques precisely [54]. This technique with its precision and sensitivity is a beneficial test for researches on the etiology of periodontal diseases.

Zhou et al. examined the sensitivity and specificity of real-time PCR for periodontal pathogens detection. They used eight periodontal bacteria including *A. actinomycetemcomitans*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *P. ginvialis*, *Prevotella intermedia*, *Streptococcus mutans*, and *T. forsythia* microorganisms. For sensitivity assay, tenfold serial dilution of purified bacterial-specific DNAs was used while specificity assay (specificity of the primer/probe set) was tested by running real-time PCR in parallel against other bacterial-specific DNA. The detection limits of examined bacteria were *S. mutans* 14.31 fg, *P. intermedia* 3.33 fg, *T. forsythia* 0.15 fg, *E. corrodens* 0.40 fg, *F. nucleatum* 0.09 fg, *C. rectus* 1.04 fg, *P. ginvialis* 180 fg, and *A. actinomycetemcomitans* 4.18 fg presenting high sensitivity. No cross-reaction was detected with any of the bacteria, representing high specificity of the method. They concluded that the established test for the quantification of periodontal pathogens using real-time PCR method may be applied in epidemiological assays and as an assistant process for clinical diagnosis of periodontal disease [55].

Numerous periodontal pathogens such as *P. ginvialis*, *A. actinomycetemcomitans*, *T. forsythia*, *P. intermedia*, *Prevotella nigrescens*, *Parvimonas micra*, *Capnocytophaga ochracea*, *Capnocytophaga sp*., *Eubacterium*, *C. rectus*, and *Capnoctytophaga* have been recognized in plaque samples found in subgingival areas [56]. The quantity of *P. ginvialis* and *A. actinomycetemcomitans* resembled one another in patients with periodontitis as well as control groups, but it was only *A. actinomycetemcomitans*, which was associated with the disease [57]. Identification of *Mycobacterium tuberculosis* in cases where gingiva has increased in size as well cases of osteomyelitis has also been performed by PCR technique [58].

Gram-positive organisms such as *Peptostreptococcus* and *Filifactor, Desulfobulbus* and *genera Megasphaera*, classes or phylotypes of *Atopobium*, *Campylobacter*, *Catonella*, *Deferribacteres*, *Diafiltraster*, *Eubacterium*, *Selenomonas*, *Tannerella*, *Streptococcus*, and *Treponema*, the level of which increases in periodontal diseases, have lately been detected by opened PCR/sequencing methods [57].

PCR is intended to conduct researches to measure the frequency of herpes simplex virus, human papillomavirus, HIV, human cytomegalovirus, and Epstein-Barr virus type I and II (1 and 2) in the gingival crevicular fluid of patients with different forms of periodontal disease [59, 60]. The use of hot-start PCR was concluded to reveal that the herpes virus might show direct hurt or compromise the resistance in endodontic infections is possible [51]. PCR techniques are capable of recognizing genetic markers for oral malignancies and serve as a diagnostic tool as well as aid in treatment prognosis [52].
of the periodontium to allow subgingival overgrowth of pathogenic bacteria in periodontitis [24].

Evaluation of the microbial extent can be performed following a variety of treatment approaches, thereby making it an index for effective treatment in chronic and aggressive periodontitis [61].

It is also used to examine the relationship of systemic diseases including coronary heart disease, pregnancy complications, diabetes, chronic renal disease, osteoporosis, and respiratory disease with periodontitis by recognizing the quantity of disease-causing agents of the periodontium in different tissue samples such as subgingival plaque, thrombi, carotid endarterectomy, coronary atherosclerotic plaque, aortic valves, placenta, maxillary sinus tissue/wash samples [62, 63].

Diagnostic examinations including the MicroDent® Test, ParoCheck® kits, MyPerioPath® Test, and oral DNA® utilizing multiplex PCR scheme are available on the market for the assessment of microbiota in subgingival plaque samples, and they offer essential details with respect to prevention strategies for healthy individuals and treatment plans for “vulnerable” patients [64].

4.2. Detection of Peri-implantitis. PCR has been able to recognize periodontal pathogens such as A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythensis, and Treponema denticola at peri-implantitis sites [65]. Fungi like Candida species were recognized at peri-implantitis and disease-free implant sites and did cocolonization with P. micra and T. forsythia [66]. The uncultured phyla Chloroflexi, Synergistetes, and Tenericutes and the organisms P. micra, Pseudoramibacter alactolyticus, Peptostreptococcus stomatis, and Solobacterium moorei which are linked to peri-implantitis were also recognized [67].

This method is also influential in the recognition of bacteria involved in peri-implantitis prior to loading of implant for the prevention of peri-implantitis [68]. Opportunistic pathogens like E. faecalis have been recognized by Q-PCR in the circumstance of failed implants recommending removal of the prosthetic appliance and regular disinfection of the implant surface and implant/abutment interface [69].

The positive outcome of dental implant procedures was highly related to a negative TGP (Genetic Test for Periodontitis) specifying polymorphisms of −889 IL1A gene and +3953 IL1B gene utilizing PCR, whereas there existed no relationship between success and a positive result [69].

4.3. Detection of Immuno-inflammatory Markers. During an imbalance in the oral cavity, the subgingival biofilm stimulates the liberation of proinflammatory cytokines and enzymes originating from the host leading to tissue disintegration. PCR has turned into the standard in protein recognition in periodontic point of view, with microbial antigens, extracellular matrix proteins, and cytokine recognition being of paramount value. The examination of genetic expression of P. gingivalis virulence factors was done with the use of Q-PCR [70]. mRNA expression of adhesion molecule (ICAM-1) was determined in E. corrodens as a periodontal pathogen that infected epithelial cells using real-time PCR, and it was found to grow in number after being in contact with N-acetyl-D-galactosamine adherence lectin of E. corrodens [71].

The ratio was observed to grow using semiquantitative PCR gene expression of receptor activator of NF-KB ligand (RANKL) to osteoprotegerin (OPG) in periodontitis [71].

When Q-PCR was used, there was a relationship between the expression of matrix metalloproteinases and RANKL and the expression of interleukin-1β, TNF-α, IF-gamma, severe inflammatory reaction, and alveolar bone loss. However, IL-4, IL-10, TIMPs, and OPG expression led a reduction of cellular infiltration and alveolar bone loss [72].

There was a relationship between smoking and mRNA expression of IL-1 β with the use of PCR in patients with periodontitis [73]. The expression of specific microRNA species in inflammation of the periodontium targeted and modulated cytokine mRNA using quantitative microRNA PCR assay offers insights to alter periodontal inflammation [74]. Quantitative mRNA expression of different growth factors; toll-like receptors (TLRs), NOD2, and NALP3; and signaling mediators CD14, MYD88, and TIR-domain-containing

| Advantages of PCR                                                                 | Limitations of PCR                                                                 |
|----------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Precise identification of bacterial strains with divergent phenotype             | The enormous cost (high test and equipment cost)                                  |
| The ease of quantification                                                       | Need to achieve high technical skills                                            |
| The ability to quantify multiple targets in the clinical specimen                | Altering the specificity of amplified PCR product                                |
| Quality control                                                                   | False-positive/False-negative results                                           |
| Precision                                                                        | There are limitations to creating a high sterile environment                     |
| One-minute method for rapid analysis with larger sample size                      | DNA contamination                                                                |
| Rapid analysis                                                                    | Low ability to detect between closely related and also highly recombinant species |
| The ability to search for various organisms or genes in one reaction             | There are limitations in multiplex PCR mixing different primers                 |
| Least contamination                                                               | Capable of contaminating other reaction vials                                    |

Table 3: Some main advantages and limitations of the PCR technique [48, 83, 84].
adapter-producing interferon-beta was also specified by RT-PCR [73].

4.4. PCR in Genetic Polymorphism. There is an association between one’s vulnerability to periodontitis and genetic factors [75]. The relationship of recognized genetic polymorphisms with phenotypes for particular patient groups currently seems to offer the most inspiring use of genetic factors in the treatment of periodontitis.

Periodontal diseases are influenced by genetic polymorphisms with a number of single-nucleotide polymorphisms (SNPs) taking place in the gene coding for cytokines, receptors, and immune cells linked to the intensity and vulnerability of periodontitis. PCR was utilized for the identification of an altered gene on chromosome 11 which reduced cathepsin C activity leading to Papillon-Lefèvre syndrome [76].

PCR has been applied in linkage and segregation analysis of genetic tests in periodontal disease. Various researches have been conducted with the use of PCR investigating the effect of IL-1 gene polymorphism as an intensity determinant on periodontal diseases in a variety of populations and ethnic groups [77].

It was concluded that there was a relationship between TLR-4 gene polymorphism and chronic periodontitis, whereas such a relationship was not found in regard to TLR-9 [78, 79]. Polymorphisms in the Fc gamma receptor gene and MPO-463G/A gene were investigated making use of allele-specific and RT-PCR, respectively, which were concluded to be associated with periodontitis [80]. Other different polymorphisms such as the IL-10 gene, chemokine ligand (CCL5 and CCR5) gene, and OPG gene have been linked to inflammation of the periodontium applying PCR amplification [81, 82]. Table 3 shows the advantages and disadvantages of the PCR method.

5. Conclusion and Future Outlooks

The primary step to develop efficacious treatment modalities is the recognition of microbial pathogens correlated to periodontitis. PCR may turn into the optimal recognition tool for disease-causing agents of the periodontium in the near future outstanding to its intrinsic capability of specificity and sensitivity. Examinations of oral diseases can integrate inbred polymorphisms with microbial outlines inside the mouth and might also consist of analyses of gene expression and proteomic data calculated in saliva or other oral tissues. PCR as a vast analytical technique can show a chief share for the validation of the clinical diagnosis of periodontal disease representing the cause, pathogenesis, clinical stages, progress, and prognosis of the disease. As a main example, PCR methods based on amplification of the bacterial gene suggest great applications in clinical samples.

The PCR is an innovator turning point in the arenas of science and medicine, and today, it has turned into a standard analytic and investigation instrument in periodontology. Delving into the pathological mechanisms responsible for the onset, progression, and treatment of the periodontal diseases could remarkably aid in providing active approaches for prevention and treatment in addition to declining the risk issue for pertinent systemic situations. The upcoming of PCR is encouraging in creating more significant understanding beginning from recognition of disease-causing agents of the periodontium to effective treatment approaches. Standardization of the PCR-based methods and their protocols through research laboratories and clinical trials are desired to advance the quality of tested data in the arena of periodontology. Identification of the type and frequency of treatment is facilitated by genetic examination utilizing PCR to figure out whether an individual is vulnerable to periodontitis. The real-time PCR technique is appropriate for microbial examination in individual diagnosis, treatment programs, and control of patients with periodontitis. In-house real-time PCR as an inexpensive technique should be restricted for large studies.

Data Availability

The raw/processed data required to reproduce these findings can be shared at this time.

Conflicts of Interest

The authors state that they have no conflict of interest.

Authors’ Contributions

All authors contributed to the drafting and scientific revision of the manuscript.

Acknowledgments

The present paper was derived from a thesis provided by the Vice-Chancellor for Research at Tabriz University of Medical Sciences (number 65367), which the authors would like to appreciate the financial supports of them. The Vice-Chancellor for Research at Tabriz University of Medical Sciences provided financial support.

References

[1] S. Offenbacher, “Periodontal diseases: pathogenesis,” Annals of Periodontology, vol. 1, no. 1, pp. 821–878, 1996.

[2] N. Abolfazli, S. Jabali, F. S. Saber, Z. Babaloo, and A. Shirmohammadi, “Effect of non-surgical periodontal therapy on serum and salivary concentrations of visfatin in patients with chronic periodontitis,” Journal of Dental Research, Dental Clinics, Dental Prospects, vol. 9, no. 1, pp. 11–17, 2015.

[3] G. C. Armitage, “Development of a classification system for periodontal diseases and conditions,” Annals of Periodontology, vol. 4, no. 1, pp. 1–6, 1999.

[4] A. Shirmohammadi, M. T. Chitsazi, and A. Lafti, “A clinical comparison of autogenous bone graft with and without autogenous periodontal ligament graft in the treatment of periodontal intrabony defects,” Clinical Oral Investigations, vol. 13, no. 3, pp. 279–286, 2009.

[5] H. Meng, “2018 world new classification of periodontal and peri-implant diseases and conditions,” Zhonghua Kou Qiang yi Xue za zhi= Zhonghua Kouqiang Yixue Zazhi= Chinese Journal of Stomatology, vol. 54, no. 2, pp. 73–78, 2019.
-deoxyguanosine and 5-hydroxycytosine at the codon 176 of the p53 gene of hepatitis C-associated hepatocellular carcinoma patients,” International Journal of Molecular Sciences, vol. 21, no. 18, pp. 6753, 2020.

[37] S. H. Sabi, O. F. Khabour, K. H. Alzoubi, C. O. Cobb, and T. Eissenberg, “Changes at global and site-specific DNA methylation of MLH1 gene promoter induced by waterpipe smoking in blood lymphocytes and oral epithelial cells,” Inhalation Toxicology, vol. 32, no. 3, pp. 124–130, 2020.

[38] Y. Wang, D. Xu, D. Liu et al., “A rapid and effective colony PCR procedure for screening transformants in several mammal mushrooms,” Mycobiology, vol. 47, no. 3, pp. 350–354, 2019.

[39] M. A. Shaheen Shahzad, S. Sikandar, and I. Afzal, “Polymerase chain reaction,” Genetic Engineering: A Glimpse of Techniques and Applications, vol. 13, 2020.

[40] S. Rajalakshmi, “Different types of PCR techniques and its applications,” International Journal of Pharmaceutical, Chemical & Biological Sciences, vol. 7, no. 3, 2017.

[41] L. Dong, J. Zhou, C. Niu et al., “Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR,” Talanta, vol. 224, p. 121726, 2021.

[42] B. Banaganapalli, N. A. Shaik, O. M. Rashidi et al., In Silico PCR, Essentials of Bioinformatics, Volume I, Springer, 2019.

[43] R. S. Hilgarth and T. M. Lanigan, “Optimization of overlap extension PCR for efficient transgene construction,” MethodsX, vol. 7, p. 100759, 2020.

[44] C. Jian, P. Luukkonen, H. Yki-Järvinen, A. Salonen, and K. Korpela, “Quantitative PCR provides a simple and accessible method for quantitative microbiota profiling,” PLoS One, vol. 15, no. 1, article e0227285, 2020.

[45] W. H. Chin, Y. Sun, J. Høgberg, T. Q. Hung, A. Wolff, and D. D. Bang, “Solid-phase PCR for rapid multiplex detection of Salmonella spp. at the subspecies level, with amplification efficiency comparable to conventional PCR,” Analytical and Bioanalytical Chemistry, vol. 409, no. 10, pp. 2715–2726, 2017.

[46] P.-E. Fournier and D. Raoul, “Suicide PCR on skin biopsy specimens for diagnosis of rickettsioses,” Journal of Clinical Microbiology, vol. 42, no. 8, pp. 3428–3434, 2004.

[47] J. M. Bartlett and D. Stirling, PCR Protocols, Springer, 2003.

[48] R. Maheshwari, J. T. Kshirsagar, and N. Lavanya, “Polymerase chain reaction: a molecular diagnostic tool in periodontology,” Clinical, Cosmetic and Investigational Dermatology, vol. 18, no. 5, pp. 632–636, 2014.

[49] S. Bizzarro, B. G. Loos, M. L. Laine, W. Crielaard, and E. Zaura, “Subgingival microbiome in smokers and non-smokers in periodontitis: an exploratory study using traditional targeted techniques and a next-generation sequencing,” Journal of Clinical Periodontology, vol. 40, no. 5, pp. 483–492, 2013.

[50] S. M. Jan, F. Y. Khan, M. A. Bhat, and R. Behal, “Primary tuberculous gingival enlargement-a rare clinical entity: case report and brief review of the literature,” Journal of Indian Society of Periodontology, vol. 18, no. 5, pp. 632–636, 2014.

[51] G. Bogen and J. Slots, “Black-pigmented anaerobic rods in closed periapical lesions,” International Endodontic Journal, vol. 32, no. 3, pp. 204–210, 1999.

[52] C. Murdoch-Kinch, “Oral medicine: advances in diagnostic procedures,” Journal of the California Dental Association, vol. 27, no. 10, pp. 773–780, 1999.

[53] M. Riggo, T. Macfarlane, D. Mackenzie, A. Lennon, A. Smith, and D. Kinane, “Comparison of polymerase chain reaction and culture methods for detection of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in subgingival plaque samples,” Journal of Periodontal Research, vol. 31, no. 7, pp. 496–501, 1996.

[54] S. R. Lyons, A. L. Griffen, and E. J. Leys, “Quantitative real-time PCR for Porphyromonas gingivalis and total bacteria,” Journal of Clinical Microbiology, vol. 38, no. 6, pp. 2362–2365, 2000.

[55] X. Zhou and J. Li, “Sensitivity and specificity of real-time PCR for periodontal pathogens detection,” in Conference: IADR/AADR/CADR General Session and Exhibition 2013, Seattle, Wash., USA, 2013.

[56] A. Bringuier, S. Khelaifa, H. Richet, G. Aboudharam, and M. Drancourt, “Real-time PCR quantification of Methanobrevibacter oralis in periodontitis,” Journal of Clinical Microbiology, vol. 51, no. 3, pp. 993–994, 2013.

[57] G. A. Sánchez, A. B. Acquier, A. De Couto, L. Busch, and C. F. Mendez, “Association between Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis in subgingival plaque and clinical parameters, in Argentine patients with aggressive periodontitis,” Microbial Pathogenesis, vol. 82, pp. 31–36, 2015.

[58] G. Bakutra, B. Manohar, and L. Mathur, “Tuberculous osteomyelitis affecting periodontium: a rare case report,” Journal of Indian Society of Periodontology, vol. 19, no. 5, pp. 578, 2015.

[59] A. Pucar, J. Milasin, V. Lekovic et al., “Correlation between atherosclerosis and periodontal putative pathogenic bacterial infections in coronary and internal mammary arteries,” Journal of Periodontology, vol. 78, no. 4, pp. 677–682, 2007.

[60] I. Saygun, S. Şahin, A. Özdemir et al., “Detection of human viruses in patients with chronic periodontitis and the relationship between viruses and clinical parameters,” Journal of Periodontology, vol. 73, no. 12, pp. 1437–1443, 2002.

[61] A. S. Rodrigues, D. S. Lourenço, L. G. Lima Neto et al., “Clinical and microbiologic evaluation, by real-time polymerase chain reaction, of non-surgical treatment of aggressive periodontitis associated with amoxicillin and metronidazole,” Journal of Periodontology, vol. 83, no. 6, pp. 744–752, 2012.

[62] S. Paju, J. M. Bernstein, E. M. Haase, and F. A. Scannapieco, “Molecular analysis of bacterial flora associated with chronically inflamed maxillary sinuses,” Journal of Medical Microbiology, vol. 52, no. 7, pp. 591–597, 2003.

[63] K. Nakano, H. Nemoto, R. Nomura et al., “Detection of oral bacteria in cardiovascular specimens,” Oral Microbiology and Immunology, vol. 24, no. 1, pp. 64–68, 2009.

[64] T. Do, D. Devine, and P. D. Marsh, “Oral biofilms: molecular analysis, challenges, and future prospects in dental diagnostics,” Clinical, Cosmetic and Investigational Dentistry, vol. 5, pp. 11, 2013.

[65] P. L. Casado, I. B. Otazu, A. Balduino, W. de Mello, E. P. Barboza, and M. E. L. Duarte, “Identification of periodontal pathogens in healthy periimplant sites,” Oral Microbiology and Immunology, vol. 51, no. 3, pp. 993–994, 2013.

[66] F. Schwarz, K. Becker, S. Rahn, A. Hegewald, K. Pfeffer, and B. Henrich, “Real-time PCR analysis of fungal organisms and bacterial species at peri-implantitis sites,” International Journal of Implant Dentistry, vol. 1, no. 1, pp. 1–7, 2015.

[67] T. Koyanagi, M. Sakamoto, Y. Takeuchi, M. Ohkuma, and Y. Izumi, “Analysis of microbiota associated with periimplantitis using 16S rRNA gene clone library,” Journal of Oral Microbiology, vol. 2, no. 1, p. 5104, 2010.
T. Ito, M. Yasuda, H. Kaneko, H. Sasaki, T. Kato, and Y. Yajima, “Clinical evaluation of salivary periodontal pathogen levels by real-time polymerase chain reaction in patients before dental implant treatment,” *Clinical Oral Implants Research*, vol. 25, no. 8, pp. 977–982, 2014.

P. Vaz, M. Gallas, A. Braga, J. Sampaio-Fernandes, A. Felino, and P. Tavares, “IL1 gene polymorphisms and unsuccessful dental implants,” *Clinical Oral Implants Research*, vol. 23, no. 12, pp. 1404–1413, 2012.

C. E. Shelburne, R. M. Gleason, G. R. Germaine et al., “Quantitative reverse transcription polymerase chain reaction analysis of _Porphyromonas gingivalis_ gene expression in vivo,” *Journal of Microbiological Methods*, vol. 49, no. 2, pp. 147–156, 2002.

D. Liu, J. Xu, L. Figliomeni et al., “Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction,” *International Journal of Molecular Medicine*, vol. 11, no. 1, pp. 17–21, 2003.

G. Garlet, C. Cardoso, T. Silva et al., “Cytokine pattern determines the progression of experimental periodontal disease induced by Actinobacillus actinomycetemcomitans through the modulation of MMPs, RANKL, and their physiological inhibitors,” *Oral Microbiology and Immunology*, vol. 21, no. 1, pp. 12–20, 2006.

A. Meenawat, V. Govila, S. Goel et al., “Evaluation of the effect of nicotine and metabolites on the periodontal status and the mRNA expression of interleukin-1β in smokers with chronic periodontitis,” *Journal of Indian Society of Periodontology*, vol. 19, no. 4, pp. 381–387, 2015.

R. Perri, S. Nares, S. Zhang, S. Barros, and S. Offenbacher, “MicroRNA modulation in obesity and periodontitis,” *Journal of Dental Research*, vol. 91, no. 1, pp. 33–38, 2012.

B. S. Michalowicz, D. Aeppi, J. G. Virag et al., “Periodontal findings in adult twins,” *Journal of Periodontology*, vol. 62, no. 5, pp. 293–299, 1991.

T. C. Hart, P. S. Hart, D. W. Bowden et al., “Mutations of the cathepsin C gene are responsible for Papillon-Lefèvre syndrome,” *Journal of Medical Genetics*, vol. 36, no. 12, pp. 881–887, 1999.

L. Quappe, L. Jara, and N. J. López, “Association of interleukin-1 polymorphisms with aggressive periodontitis,” *Journal of Periodontology*, vol. 75, no. 11, pp. 1509–1515, 2004.

N. Schröder, D. Meister, V. Wolff et al., “Chronic periodontal disease is associated with single-nucleotide polymorphisms of the human TLR-4 gene,” *Genes & Immunity*, vol. 6, no. 5, pp. 448–451, 2005.

N. Ashok, S. Warad, N. B. Kalburgi, S. Bilichodmath, P. S. V. Prabhakaran, and B. Tarakji, “Toll-like receptor 9 gene polymorphism in chronic and aggressive periodontitis patients,” *Journal of Indian Society of Periodontology*, vol. 18, no. 6, pp. 723–727, 2014.

K. Debabrata, B. Prasanta, N. Vineet, G. Anshul, S. Arindam, and D. Satadal, “Aggressive periodontitis: an appraisal of systemic effects on its etiology-genetic aspect,” *Journal of Indian Society of Periodontology*, vol. 19, no. 2, pp. 169–173, 2015.

Y. S. Shih, E. Fu, M. M. Fu et al., “Association of CCL5 and CCR5 gene polymorphisms with periodontitis in Taiwanese,” *Journal of Periodontology*, vol. 85, no. 11, pp. 1596–1602, 2014.

L. Chai, Y. Q. Song, and W. Leung, “Genetic polymorphism studies in periodontitis and Fcγ receptors,” *Journal of Periodontal Research*, vol. 47, no. 3, pp. 273–285, 2012.