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Review Article

Microchip Platforms in In Vitro Identification of Red Complex Periodontopathogens: A Systematic Review

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ABSTRACT

Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola—collectively known as the red complex periodontopathogens—have been widely analyzed in clinical samples from individuals with periodontitis. A lab-on-a-chip (LOC) is a compact device that consolidates multiple laboratory processes onto a single microchip or small platform. This systematic review investigates the utilization of LOC technology for detecting microorganisms within the red complex. The study followed PRISMA guidelines and involved searches across multiple databases. From 58 initially retrieved records, 10 met the inclusion criteria. All selected studies reported efficient, rapid, and accurate LOC systems capable of detecting and amplifying P. gingivalis, T. forsythia, and T. denticola. When compared with conventional methods, LOC-based detection required substantially fewer reagents. Moreover, amplification was achieved within approximately 2–8 minutes, and detection within about 2 minutes and 40 seconds, resulting in a total experimental time of roughly 11 minutes. The combination of miniaturization, precision, automation, and speed highlights LOC platforms as promising tools for the identification and amplification of red complex bacteria implicated in periodontal diseases.

Keywords: Lab-on-a-chip, Microchip, Microfluidics, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola

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Introduction

Periodontitis is a chronic inflammatory disease of the supporting tooth structures, leading to progressive tissue destruction. Its occurrence depends on multiple factors, including host susceptibility, the presence of pathogenic bacteria, and the reduction or absence of protective microbial species essential for periodontal balance. Although subgingival biofilm composition differs among individuals, disease progression is commonly linked to a microbial imbalance known as dysbiosis [1, 2]. Research has demonstrated a strong link between oral microbiome dysbiosis and the onset and advancement of periodontitis [3, 4].

Subgingival plaque obtained from periodontal pockets is frequently used to study microbial changes associated with the disease [2, 3]. Among these microbes, Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola have been the most extensively examined [5–7]. Owing to their frequent co-occurrence and strong connection with periodontitis, they are grouped as red complex bacteria, recognized for their virulence and capacity to induce chronic inflammation [8–10]. Consequently, managing these pathogens and restoring microbial balance are vital for controlling and preventing periodontal conditions [7, 10–12].

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Traditional bacteriological investigations include microscopy, infection assays, and various molecular and immunological techniques [13]. Although these approaches have enhanced understanding of host–microbe interactions, they present notable drawbacks [14]. Some species, such as T. denticola, remain challenging to culture. Ex vivo and in vitro models fail to fully mimic physiological environments [14, 15]. Moreover, molecular assays relying solely on DNA detection cannot differentiate viable from nonviable microorganisms, which can produce inaccurate outcomes where microbial viability is crucial [9–12]. Thus, laboratory and clinical results may not always align. Biofilm-related properties are also typically studied under oversimplified conditions that do not fully represent complex biological systems [14, 16].

Recent advances in lab-on-a-chip technology address many of these challenges and enhance fundamental bacteriological research, supporting future therapeutic innovations. The LOC platform is a miniaturized system that merges numerous laboratory operations—such as sample processing, separation, mixing, detection, and analysis—on a single chip. This integration offers multiple advantages, including lower reagent use, faster processing times, improved precision, and greater automation [14, 17, 18]. The adaptability of LOC systems has led to growing interest in their application across biomedical diagnostics, environmental monitoring, and biological research.

Microfluidic conduits, chambers, regulators, sensors, and detection systems are typically fabricated on a single compact chip through techniques such as lithography, etching, and bonding. These configurations enable highly precise control and handling of fluids, allowing the manipulation of minute quantities of samples and reagents [14, 17, 18]. The application of microfluidic technology for microbial detection has gained notable momentum in recent years. Devices with fluidic channels only a few micrometers wide provide rapid, high-throughput, and economical analytical possibilities [19].

Certain microfluidic devices—particularly droplet-based systems—have demonstrated reduced detection times by isolating individual bacteria and antimicrobial agents within small droplet volumes, notably in single-drug antimicrobial susceptibility evaluations [20]. Microchip platforms can produce results within minutes, facilitating the prompt identification of pathogens in clinical specimens. This speed is critical in emergency and intensive care environments where timely clinical decisions are essential. Furthermore, numerous microfluidic systems are capable of performing multiplex assays, enabling concurrent detection of multiple pathogens within a single test. Such functionality is advantageous for diagnosing co-infections or executing wide-spectrum microbial screening [17–20].

Another strategy to accelerate bacterial isolation involves implementing antibiotic susceptibility testing on an inertial microfluidic chip, followed by RNA-based hybridization detection [21]. Additionally, after a 3-hour incubation, molecular diffusion was employed to detect bacterial growth on hydrogel matrices with gradient drug concentrations, allowing for the estimation of minimal inhibitory concentration values [22].

It is crucial to underscore the importance of antimicrobial susceptibility testing prior to prescribing antibiotics, even though the process is often viewed as intricate and time-consuming. Consequently, achieving efficient point-of-care diagnostics remains challenging. In this regard, lab-on-a-chip (LOC) technology offers promising potential for enhancing the detection of red complex microorganisms linked to periodontal disease, enabling faster and more individualized diagnostic and therapeutic approaches. However, this area has not yet been thoroughly examined through a systematic review.

Based on these considerations, the objective of this systematic review is to evaluate the application of microchip-based platforms in the identification of red complex microorganisms.

Materials and Methods

Search strategy

This analysis adhered to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [23]. The literature search covered multiple databases—PubMed/MEDLINE, SCOPUS, and SCIELO—as well as gray literature sources. Up to June 2023, searches included MeSH terms and keywords such as lab-on-a-chip, microfluidics, organ-on-a-chip, microchip platforms, microphysiological systems, bioassays, antimicrobial susceptibility test, biofilms, P. gingivalis, T. forsythia, and T. denticola, without language restrictions. Boolean operators (OR, AND) were used to combine the following terms: “lab-on-a-chip” OR “organ-on-a-chip” OR “microfluidics” OR “microphysiological systems” OR “bioassays” OR “microchip platforms” OR “sensors” OR “biofilms” AND “P. gingivalis” AND “T. forsythia” AND “T. denticola.”

The review protocol was registered in the Open Science Framework database under the identifier: osf.io/y52fn.

Inclusion and exclusion criteria

Eligible studies included in vitro research utilizing microfluidic or lab-on-a-chip technologies, combined where applicable with 3D modeling and/or bioprinting for organ-on-a-chip applications.

Excluded materials encompassed other systematic reviews, meta-analyses, general reviews, conference abstracts, brief reports, case studies, patents, and publications lacking essential details about device fabrication or experimental setup.

Research question

This systematic review aims to answer the following question: Can microchip platforms support the detection of microorganisms within the red complex?

P: Experiments involving P. gingivalis, T. forsythia, and T. denticola

I: Microchip or lab-on-a-chip platforms

C: Control-based comparative experiments

O: Identification of red complex microorganisms

Review process

Two independent reviewers screened article titles and abstracts for eligibility. In cases of disagreement, a third reviewer (GJ) served as the adjudicator. The inter-rater reliability was quantified using Cohen's Kappa statistic, yielding a significant agreement level exceeding 93%.

Data extraction

Data from selected studies were organized into a comparative table. Each reviewer independently extracted and then cross-verified information. The compiled table summarized details such as microfluidic methodology, materials used for fabrication, bacterial strains and culture conditions, and key findings.

Risk of bias assessment

The risk of bias was assessed using a standardized quality appraisal tool for in vitro research [24]. Each study was evaluated for factors including research objectives, sampling strategy, control group design, methodological clarity, operator details, randomization, measurement reliability, data analysis, and blinding procedures. Studies were then rated for overall quality as low (9–12), moderate (5–8), or high (1–4) risk of bias.

Results and Discussion

From the 58 studies initially identified through electronic databases, 37 were excluded for not meeting inclusion requirements—mainly for not examining red complex microorganisms, focusing exclusively on genes or proteins, or lacking integration of microfluidic systems. Additionally, six duplicate entries were eliminated. Following a full-text review, five more papers did not satisfy the eligibility criteria. Consequently, 10 studies were ultimately included in this systematic review [25–34] **(Figure 1)**.

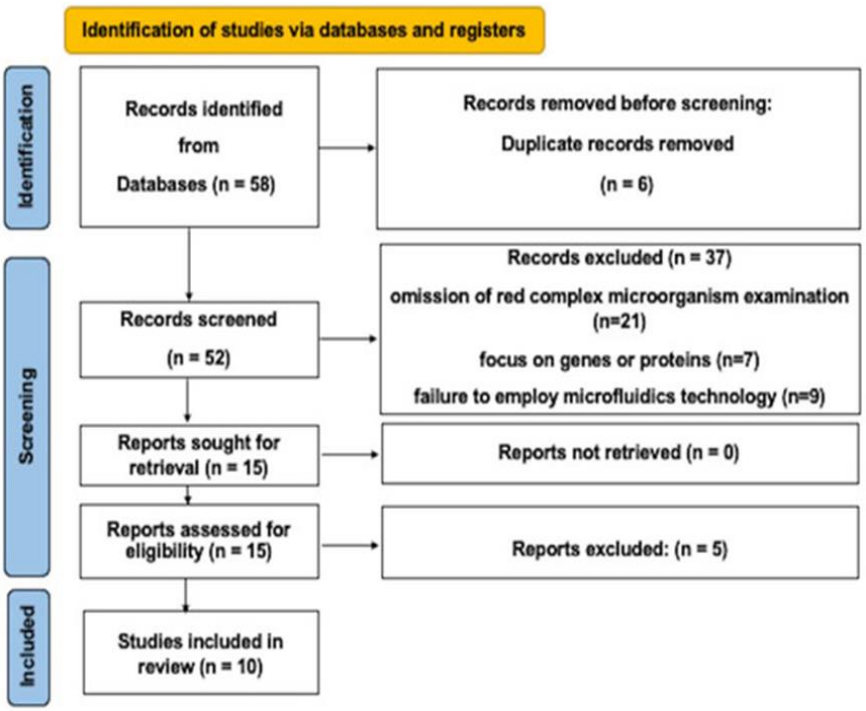

Figure 1. Diagram illustrating the selection process.

Table 1 outlines the characteristics of the included studies, which spanned from 2010 [25] to 2023 [30, 32].

Table 1. Key attributes of the included studies.

Objective of Microchip Application	Fabrication Materials	Bacterial Cultivation, Strains, and Incubation Parameters	Key Findings	Citation
Technique employing fluidic electrodes and hydrodynamic focusing for bacterial detection in purified water.	Features 3D electrodes with variable spacing; syringe pump ensures precise flow regulation; automated computer-controlled pump delivers bacterial sample and KCl electrolyte.	Bacterial sample compressed into three conductivity-differentiated layers via KCl sheath and electrode fluid on microfluidic platform; impedance profiles assessed for concentration sensitivity.	Microfluidic design achieves lowest detection limit of 10^3 cells mL^{-1} in deionized water; P. gingivalis quantifiable from 10^3 to 10^9 cells mL^{-1}; sensitivity adjustable via sample-to-KCl velocity proportion.	[25]
Portable integrated biochip combining continuous-flow PCR and electrophoresis for accelerated pathogen identification.	Comprises novel pumping mechanism, dual aluminum heaters, polycarbonate continuous-flow PCR-electrophoresis chip produced via injection molding.	P. gingivalis (ATCC 33277), T. denticola (ATCC 35405), T. forsythia (ATCC 43037) sourced from Microbiologics Inc.; PCR mix circulated at controlled rates; benchmarked against T100 thermal cycler conventional PCR.	DNA amplification completed in 2′31″; PCR product detection in 3′43″; minimal bacterial load amplified: 125 cfu/μL.	[26]
Finite element modeling of PCR fluid dynamics using water substitute to optimize channel geometry ratios.	Polycarbonate chip with straight/curved microchannels across dual heating zones.	T. denticola (ATCC 35405) from Microbiologics Inc.; utilized 10× TBE buffer, SpeedSTAR HS DNA Polymerase, hydroxyethyl cellulose, 100 bp ladders.	16S rDNA conserved regions of T. denticola amplified in 8 min via portable continuous-flow PCR chip.	[27]
Continuous-flow PCR microfluidic platform for rapid target gene amplification.	SU-8 photoresist spin-coated silicon wafer; prebaked 65°C/5 min, soft-baked 95°C/15 min; photolithography patterned via mask.	P. gingivalis (ATCC 33277), T. denticola (ATCC 35405), T. forsythia (ATCC 43037) from Microbiologics Inc.; sterile tip centrifuged in 100 μL PBS at 10,000 rpm/10 min; 2 μL supernatant as PCR template.	P. gingivalis/T. denticola genes amplified in 3′48″; T. forsythia (641 bp) requires ≥8′25″; multiplex PCR identifies only P. gingivalis after 11′20″; demonstrates substantial continuous-flow PCR speed superiority.	[28]
Array-based continuous-flow PCR microfluidic system for multiplex amplification.	Syringe pump-driven PCR solution through array chip with dual aluminum heaters.	Primer specificity validated via conventional thermal cycler/electrophoresis for P. gingivalis (F: GCGCTCAACGTTCAGCC; R: CACGAATTCCGCCTGC), T. denticola (F: TAATACCGAATGTGCTCATTTACAT; R: TCAAAGAAGCATTCCCTCT TCTTCTTA), T. forsythia (F: GCGTATGTAACCTGCCCGCA; R: TGCTTCAGTGTCAGTTATACCT).	Shortest times: P. gingivalis 2′07″, T. denticola 2′51″, T. forsythia 5′32″; simultaneous triple amplification in 8′05″; enables high-throughput DNA processing for field applications.	[29]

Dual-layer droplet continuous-flow PCR chip minimizing hydrodynamic resistance.	T-junction droplet generator, serpentine PCR channel, outlet reservoir; PDMS prepolymer:curing agent 10:1 poured into duplicate mold.	P. gingivalis (ATCC 33277) from Microbiologics Inc.; SYBR Green PCR Mastermix; primers synthesized by Sangon Biotech.	P. gingivalis amplification in 11′16″ with positive droplet fluorescence; reduces long-channel resistance, accelerating droplet PCR timelines.	[30]
Integrated continuous-flow PCR with on-chip electrophoresis for comprehensive analysis.	Dual PDMS layers (10:1 prepolymer:curing agent) bonded via plasma oxidation.	Detected P. gingivalis (TGTAGATGACTGATGGTGAAAACC), T. denticola (AAGGCGGTAGAGCCGCTCA), T. forsythia (GCGTATGTAACCTGCCCGCA); PCR solution injected at chip inlet.	Target genes amplified in 11 min; accurate PCR products confirmed via integrated electrophoresis.	[31]
Multiplex circular array continuous-flow PCR chip for parallel processing.	Soft lithography/plasma bonding; mask-printed microchannels; 10.1 cm silicon wafer with thick negative photoresist baked 95°C/25 min.	P. gingivalis (ATCC 33277), T. denticola (ATCC 35405), T. forsythia (ATCC 43037) from Microbiologics; primers from Sangon Biotech.	12-sample parallel amplification; on-site fluorescence detection; 5 µL PCR volume; serpentine channel transit: 5.38 min.	[32]
Chair-side disk microfluidic platform for point-of-care testing.	Polycarbonate foils microthermoformed via hot embossing at Hahn-Schickard; PDMS mold and cover foil heated above glass transition with forced air shaping.	Contained P. gingivalis, T. forsythia, T. denticola; 920 µL saliva enzymatically lysed via GenElute™ Kit, silica-extracted; 16S rRNA multiplex qPCR quantified.	Triple pathogen detection <3 h; ~90% concordance with lab reference for positive/negative calls.	[33]
In vitro anti-P. gingivalis antibodies for biosensor-based real-time clinical detection.	Amine-coupled antibodies immobilized on Biacore® 1000 CM-5 sensor chip.	P. gingivalis ATCC 33277 anaerobically grown 37°C in fastidious broth; lymphocytes stimulated with 10^9 cfu/mL heat-killed cells at 37°C in L-15/10% FBS.	Surface plasmon resonance shows antibody incubation attenuates bacterial signal; validates real-time periodontitis diagnostics and passive immunization potential.	[34]

The analyzed reports employed various microfluidic techniques for the detection and amplification of P. gingivalis, T. denticola, and T. forsythia, as summarized in **Table 1**. Each study demonstrated that these microchip-based systems provided efficient, fast, and dependable detection of periodontal pathogens. Compared to conventional diagnostic approaches, lab-on-a-chip (LOC) technologies required significantly smaller reagent volumes. The amplification of P. gingivalis, T. denticola, and T. forsythia was typically achieved within 2–8 minutes, with detection completed in about 2 minutes and 40 seconds, for a total runtime of roughly 11 minutes [26–30]. In addition, multi-PCR methods shortened amplification time for small amplicons to 3 minutes and 48 seconds [28], and the minimum detectable bacterial concentration was 125 CFU/µL [26]. Thus, LOC devices offer distinct advantages in processing speed and operational convenience.

Table 1 further details the range of materials and microfabrication techniques utilized in producing these microfluidic systems. Silicon wafers with nanoscale features were commonly fabricated using photolithography. Typical LOC designs incorporated components such as reservoirs, chambers, and microchannels, often supplemented by functional units like valves, mixers, and pumps to enable precise fluid manipulation. According to this review, polydimethylsiloxane (PDMS) was the most frequently used material for constructing microfluidic devices in laboratory settings.

Among the red complex bacteria, P. gingivalis received the greatest research emphasis. Nonetheless, the majority of studies investigated all three pathogens—P. gingivalis, T. denticola, and T. forsythia—

simultaneously [26, 28, 29, 31–33]. Most studies also used the same bacterial strains: P. gingivalis (ATCC 33277), T. denticola (ATCC 35405), and T. forsythia (ATCC 43037).

Despite comprehensive methodological reporting, several critical elements were often missing across studies, including sample size determination, operator calibration, randomization techniques, and blinding procedures. Nevertheless, most provided adequate details regarding research aims, control groups, experimental design, data analysis, and results presentation. Based on the in vitro bias assessment tool [24], all studies received a score of 6, corresponding to a moderate risk of bias. The diversity in study objectives, model frameworks, and methodological approaches prevented any meta-analytical synthesis of quantitative data.

Since its introduction, polymerase chain reaction (PCR) has served as a cornerstone in molecular diagnostics [35]. PCR is widely used for genomic studies, including the identification of infectious and hereditary diseases, as well as various bacterial agents [26]. The process involves in vitro amplification of genetic sequences through temperature cycling—denaturation, annealing, and extension—resulting in large-scale replication of target DNA fragments [32]. However, traditional thermocyclers are limited by slow heating and cooling rates, which restrict their use for point-of-care diagnostics. Consequently, there has been growing emphasis on the development of rapid PCR techniques [31, 32].

Over the years, multiple high-speed PCR innovations have emerged. For instance, ultrafast photonic PCR was introduced, completing 30 thermal cycles in 5 minutes using plasmonic photothermal light-to-heat conversion through photon–electron–phonon coupling [36]. Another advancement, loop-mediated isothermal amplification (LAMP), employs four or more primers to achieve amplification without requiring temperature cycling. Despite eliminating thermal variation, it presents challenges related to primer design and potential false-positive outcomes from cross-reactivity or aerosol contamination [37–39]. Additionally, a Rayleigh–Bénard PCR cell was created, utilizing natural convection to move liquid between temperature zones. While this design is simple, maintaining stable convection flow imposes strict constraints on the reaction chamber size [40–42].

A continuous-flow PCR (CF-PCR) system was introduced that successfully amplified the Neisseria gonorrhoeae DNA gyrase gene within 18.7 minutes [43]. This method functions by circulating the reaction mixture through microchannels exposed to alternating temperature zones. One key advantage of this system is its ability to regulate the PCR product yield by adjusting the flow rate [32]. As summarized in this systematic review, several research teams have investigated CF-PCR as a rapid diagnostic approach for pathogen detection [26–34]. Notably, CF-PCR was integrated with an electrophoresis-based microfluidic chip, creating a compact unit capable of detecting T. denticola in just 8 minutes. This platform also included an automated injection module, removing the need for external syringe pumps [27]. Moreover, multiplex PCR performed in serpentine microchannels enabled the simultaneous amplification of P. gingivalis, T. denticola, and T. forsythia [28]. To reduce cross-reaction problems, a CF-PCR array chip was designed to amplify all three genes concurrently, although an additional off-chip capillary electrophoresis step was still necessary for detection [29]. Ultimately, amplification of up to 12 target genes was achieved by directing the PCR mixture into separate chip compartments, enhancing CF-PCR efficiency and enabling on-site, point-of-care detection [32].

Although lab-on-a-chip (LOC) systems offer numerous advantages, certain limitations persist. Despite the versatility of polydimethylsiloxane (PDMS), a widely used fabrication material, its tendency to absorb small hydrophobic compounds or biomolecules can interfere with analytical accuracy, especially in cell-based studies [44]. To address this, various surface modification techniques have been explored to improve PDMS performance [45]. Furthermore, standardized experimental protocols for microfluidic applications are still lacking [46]. Detection limits in microbial assays using these systems can vary significantly depending on factors such as chip design, target species, and detection strategy. Nonetheless, LOC technologies continue to demonstrate the potential for extremely sensitive microbial detection [46, 47].

This systematic review also presents certain limitations. The evidence is primarily derived from a small number of laboratory-based experiments, which may constrain its clinical relevance. Additionally, the included studies exhibited considerable variability in objectives, methodologies, and experimental setups, along with a moderate level of bias. Despite these constraints, the findings highlight the strong potential of LOC systems for identifying and enhancing the detection of red complex microorganisms.

Conclusion

Microchip-based diagnostic systems can deliver results within minutes, allowing for rapid detection of red

complex bacteria. Such quick turnaround is particularly critical in clinical contexts where prompt diagnosis guides effective treatment decisions. Many of these platforms also achieve high analytical sensitivity, enabling identification of very low bacterial concentrations—essential for early infection detection and disease monitoring. Furthermore, microfluidic assays can be customized to selectively target specific red complex pathogens, thereby minimizing false-positive outcomes and improving diagnostic accuracy. Advancing the development of compact, portable microfluidic devices suitable for point-of-care use could expand their practical application in dental clinics and underserved regions. To ensure their clinical reliability, it is vital to conduct thorough validation and large-scale clinical trials to confirm the diagnostic performance and utility of lab-on-a-chip technologies in real-world dental healthcare settings.

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Ethics Statement: None

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