Review Article

Oral microbiome: A paradigm shift in dental diagnosis

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Abstract

For decades, the diagnosis of oral diseases primarily relied on clinical symptoms presented by patients. This traditional approach, which included visual inspection, palpation, and assessment of discomfort, provided limited insights into the underlying microbiological factors contributing to oral diseases. Recent studies have identified specific microorganisms believed to play pivotal roles in oral disease development, particularly in conditions such as dental caries, periodontal disease, and pulpitis. The challenge lies in the dynamic nature of the oral microenvironment, where the microbial community can shift rapidly due to changes in diet, hygiene practices, and overall health, complicating attempts to establish direct causative links between specific pathogens and oral diseases. The aim of this study was to explore the role of the oral microbiome in advancing dental diagnostics and to assess how integrating microbial analysis can improve early detection and personalized treatment of oral diseases. Research into the oral microbiome has brought about a paradigm shift in understanding dental diagnostics. Advances in molecular biology techniques, such as next-generation sequencing (NGS) and polymerase chain reaction (PCR), have enabled more detailed examination of microbial communities within the oral cavity. This shift from a purely symptom-based diagnostic approach to one that incorporates microbial analysis represents a significant advancement in dental care. For instance, identifying specific bacterial profiles associated with caries could facilitate the implementation of personalized preventive measures, such as tailored oral hygiene regimens or dietary recommendations. Moreover, integrating microbiological data into clinical practices could lead to improved diagnostics for conditions that are traditionally difficult to assess. By adopting a more comprehensive view that includes microbial assessments, clinicians can better understand the interplay between oral microbiota and systemic health, as oral diseases are often linked to broader health issues. Another challenge is that this approach requires interdisciplinary collaboration among dental practitioners, microbiologists, and public health experts. This collaboration is essential to translate abstract microbiological findings into practical diagnostic indicators that can be utilized in clinical settings. Furthermore, with the advent of new technologies, maintaining accurate interpretations of microbiome data presents another layer of complexity, as variations in sample collection, processing, and analysis can lead to differing results. In summary, the elevated role of the oral microbiome in dental diagnostics marks a significant transition from traditional, symptom-focused approaches to more holistic methodologies that consider the underlying microbial communities. By leveraging advanced technologies, dental practitioners can enhance their diagnostic capabilities, leading to improved outcomes for patients suffering from various oral diseases.

Keywords: Diagnosis, oral microbiome, microorganism, sequencing, 16S

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Introduction

From year to year, diagnosis of the oral diseases focused on the clinical symptoms of the patients. Earlier research found some dominant microorganisms that we think are responsible for the diseases to occur. For example, in 1924, *Streptococcus mutans* involved in dental caries were first declared (**Figure 1**). It is easy to study *S. mutans* outside the oral cavity, and researchers found that it can attach to the tooth surface and produce acid from sugar intake, thus promoting demineralization [1,2]. A similar shift occurred in periodontal research during the 1970s, when bacteria were initially recognized as the primary etiological agents of periodontal disease [3]. *Porphyromonas gingivalis* is frequently highlighted as a significant contributor to periodontal disease [3]. This bacterium is often referred to as a "keystone pathogen" due to its ability to disrupt the balance of the oral condition and destruction of periodontal tissues [4,5].

The pathogenicity of *S. mutans* or *P. gingivalis* is influenced by the presence of other species. Every time, there are changes in these microbial niches. In fact, the oral cavity contains a diverse group of microorganisms, with more than 700 species documented to date, and an average individual hosting approximately 250 species. These microorganisms adjust their microenvironment accordingly to the environment, resulting in dynamic interactions among microorganisms and between microorganisms and their hosts [6-8].

Identifying the specific microorganisms responsible for oral diseases is challenging due to the dynamic nature of the microenvironment. Current research optimizes the use of broad-range polymerase chain reaction (PCR) techniques targeting the 16S rRNA gene combined with DNA sequencing. In the early 2000s, a comprehensive database, known as the Human Oral Microbiome Database, was established using sequencing technologies focused on 16S rRNA genes [9]. A microbiome is defined as a community of microbes occupying a reasonably well-defined habitat, forming a dynamic and interactive micro-ecosystem integrated with the macro ecosystem [10,11]. This database provides us with an interesting fact, such as oral pathogens were present in the oral cavity, whether they have the disease or not [6,7]. Microbiome dynamics are now used in the study of infectious diseases to explore the relationship between microbiome, disease and host-pathogen interactions [12,13]. From this point of view, we can start to re-examine our understanding of the diseases, and how they were diagnosed. This review summarizes the oral of the oral microbiome related to paradigm-shift in dental diagnosis [14,15].

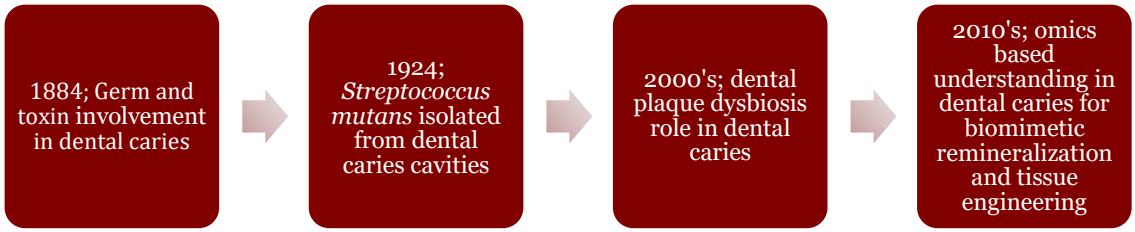

Figure 1. History of etiopathogenesis of dental caries.

Microbial interaction with the host

Improving and updating knowledge of microbiome has been an uprising topic for more than 10 years since the Human Microbiome Project in 2005 [10]. Microbiome research gives us a foundation of application in human health research. Microbiome research gives a consideration of natural microbial diversity and interaction with other organisms, including humans, as a host [13].

Historically, research about microbial activity began with the invention of microscope (**Table 1**). Using microscope, scientists uncovered the diversity of microorganisms, including bacteria, fungi, and even dental plaque [16]. Analysis of dental plaque samples opened the gate to a complex community of microbes, known as biofilm. The formation of biofilms on dental surfaces begins with the adherence of initial colonizers, which paves the way for the later recruitment of secondary colonizers, leading to a complex and dynamic microbial community [17-

21]. These findings continued to the point where scientists investigated the interactions between microbes and their hosts [22].

Host-microbial interaction networks are increasingly recognized as well-established and coordinated systems that significantly impact human health and disease. Recent studies have found correlations between the development of a complex microbiota and adaptive immune system. Humans, with their complex and sophisticated immune, can easily promote symbiotic connections between the host and microbiota as a way to preserve homeostasis, with the microbiota playing a key role in educating and promoting the immune system [23,24]. Oral homeostasis is maintained by the balance of oral microbial communities, which prevents dental caries, periodontal disease, and other oral conditions [25]. Disruption of this symbiotic balance can lead to a shift toward a parasitic or pathogenic state, ultimately causing disease in the host [26].

Table 1. Oral microbiome composition in supragingival plaque

Clinical conditions	Results	References
Children with severe early childhood caries (SECC)	*Streptococcus mutans* were significantly enriched in dental plaque samples	[27]
Adults with healthy teeth or caries	Operational Taxonomy Units (OTUs) at 97% diversity: 453 species, 122 genera, 68 families, 34 orders, 21 classes, and 12 phyla	[8]
Children with and without dental caries	Microbiome dominated by *Streptococcus* and *Neisseria*	[28]
Adults and children crowdsourced population study	Youth oral microbiomes are more diverse than adults; Adult oral microbiomes are affected by oral habits; Youth oral microbiomes are affected by sex and weight status; Participants from the same family have more similar oral microbiomes; No oral microbiome differences in adults or youth based on sweet or beverages consumption	[29]
Adults supragingival biofilms in health and caries	123 genera formed “core microbiome”, with four dominant phyla were Actinobacteria, Firmicutes, Bacteroidetes, and Fusobacteria (more than 10% abundance)	[30]
Adolescents with fixed orthodontic treatment, gingivitis and healthy	Patients with gingivitis have significantly higher bacteria such as *Saccharibacteria* (TM7), *Selenomonas*, or *Actinomyces*	[31]

Single-acting organism

Bacterial involvement in dental diseases has been recognized since 1880s. Black, in his book, stated that the acids causing tooth decay were indeed a result of bacterial fermentation [32]. Since the first isolation of *S. mutans* from dental caries by Clark in 1924, it is often said that dental caries was caused by *S. mutans* [2]. The ability of this bacterium to produce acid from dietary glucose intake supported this idea for decades. However, the role of other bacteria has often been ignored despite their presence in the oral cavity.

This concept was gradually reconsidered following the discovery of dental plaque as a biofilm—a structured community of bacteria adherent to a surface and embedded within a self-produced extracellular matrix. The biofilm is formed by selective, reproducible, sequential colonization rather than random colonization [33]. Biofilm formation occurs progressively over time, beginning with the adhesion of early colonizers such as *Streptococcus* and *Actinomyces* species to specific receptors within the acquired pellicle that coats the tooth surface [34]. In the initial stages of biofilm formation, *Streptococcus* species play a key role by producing organic acids through carbohydrate metabolism. These acids contribute to environmental acidification and serve as substrates for secondary colonizers such as *Veillonella*, which utilize lactate as a carbon source, forming a rudimentary trophic interaction. As the biofilm matures, microbial accumulation and structural development promote the establishment of an increasingly anaerobic environment, favoring the growth of obligate anaerobes. This microbial succession leads to greater biofilm complexity and increased plaque thickness over time [35].

While lactic acid is recognized as a critical metabolic substrate for certain oral bacteria in pure cultures, its role in supporting the growth of complex bacterial communities on whole saliva

remains poorly understood. Attempts to cultivate mixed-species biofilms on whole saliva have largely failed, likely due to the need for specific spatial organization and cooperative interactions among select microbial populations. These communities may require a combination of complementary metabolic pathways to convert the latent nutrients present in saliva into bioavailable forms, enabling sustained growth and biofilm development [34]. Whole saliva contains a variety of antimicrobial components, nutrients, and signaling molecules that modulate microbial growth, viability, and interspecies interactions. Its composition can differentially influence the metabolic activity of oral bacteria, with certain species demonstrating enhanced proliferation in response to specific salivary constituents. However, the intricate ecological dynamics within salivary environments—driven by spatial arrangement, metabolic cooperation, and host factors—are challenging to replicate in standard culture media. This limitation contributes to discrepancies between in vitro findings and in vivo microbial behavior [23,36].

Polymicrobial network interaction

Humans are considered "superorganisms," with at least ten times the number of microbes as our cells. Oral cavity, particularly, is a unique environment with hundreds of microorganisms confirmed to be one of the parts with the highest microbial diversity in the human body. This unique ecosystem is characterized by its high microbial diversity, which includes bacteria, fungi, viruses, and archaea [25]. Most of these microorganisms coexist in a complex relationship. These interactions form a distinct microbiome defined as the oral microbiome[26]. However, microbial diversity can change as a result of selective pressures such as dietary changes, diseases, and antibiotic exposure [14].

In the oral cavity, such as on tooth surface or in the periodontal tissues, these bacteria often form a biofilm. These biofilms, consisting of microorganisms, are important causative factors in oral diseases. Biofilm maturation progress happened in stages, from the early initial phase, building the extracellular matrix called extracellular polymeric substances (EPS) [37], until forming a fully mature biofilm (**Figure 2**) [38,39]. According to several studies, oral biofilm formation started from *S. mutans* adherence to solid surfaces. The adhesive mechanisms in the initial stages typically involve facultative anaerobic bacteria using sucrose-dependent pathways mediated by glucosyltransferases (Gtfs) or sucrose-independent pathways involving salivary agglutinins [37-40].

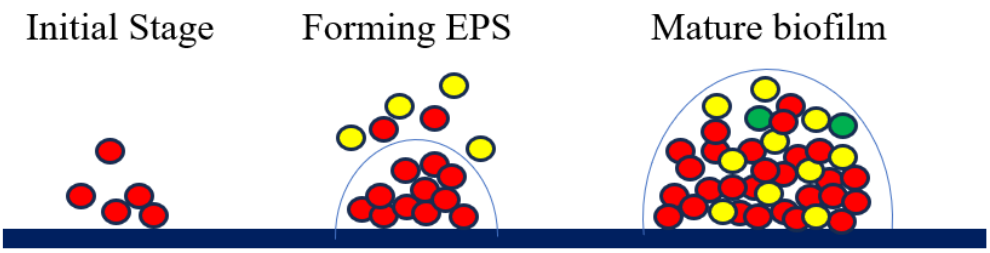

Figure 2. Oral biofilm formation stages.

Microbiome network

Oral microbiome is a complex microbial community in the human oral cavity [14], which is described as a group of microorganisms in the oral cavity that includes bacteria, viruses, fungi, archaea, and protozoa [26]. Longitudinal studies have demonstrated that the oral microbiome remains stable over time in healthy adults. For example, a study by Maruyama *et al.* indicated stability in an individual's oral microbiome over several weeks, reinforcing the idea that healthy individuals maintain a consistent microbial composition [41]. Additionally, Burcham reported that adult's oral microbiomes are strongly influenced by their oral health status [29].

Recent advanced sequencing analyses predict this ecosystem's total diversity is around 700 species, including opportunistic pathogens, harmless symbionts and commensals [9,14]. These microbiome ecosystems will change dynamically due to dysbiosis or homeostasis phase in oral environment [29,38,42,43]. In dental caries alone, some bacteria are affecting the development of the diseases (**Figure 3**) and the microbiome can shift in each caries stages [42].

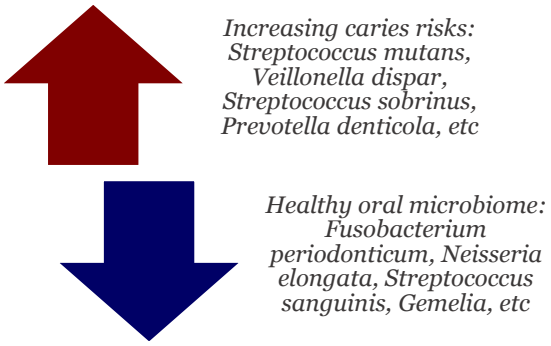

Figure 3. Oral bacteria role in dental caries (dysbiosis) and haemostasis

Other oral microbiomes, including fungi, protozoa, and viruses, remain relevant; however, literature on these microorganisms is still limited [26]. The human oral microbiome is a highly individualized and preserved community depending on the host's sex. Bacteriophages make up the vast bulk of oral cavity viruses, connected with several major commensal bacterial genera in the oral cavity, such as *Veillonella* spp. and *Streptococcus* spp. [26].

Fungi and yeast also inhabit the human mouth cavity. *Candida* genera are present in about 70% of healthy individuals. They are the most prevalent commensal fungi and members of the basal oral microbiota, followed by *C. albicans*, which is also present in 40%–80% of healthy individuals. Under some circumstances, *C. albicans* and *C. glabrata* are significant pathogens. The oral cavity's microbial biogeography and fungal colonization succession, as well as their impact on host health and disease, are still poorly understood [26].

Protozoa have traditionally been regarded as parasitic organisms detrimental to their hosts. *Trichomonas tenax* and *Eubacterium nodatum,* as well as *P. gingivalis* and *Treponema denticola*, have recently been linked in studies. However, these findings were interpreted as being unrelated to host health and related to protozoal nutritional parameters [26].

Archaea are organisms that appear the same as bacteria but have many similarities in their molecular when compared to eukaryotes. Archaea species have also been associated with oral diseases due to their ability to form biofilms and interact with human immune system. *Methanobrevibacter oralis* has been found in periodontitis, peri-implantitis, and root canal necrosis cases, which makes it possible that they might implicate these diseases. These findings point to archaea as potential terminal degraders of host components that enable the continuation of the catabolic series [26].

Oral dysbiosis

According to several studies, lifestyle modifications, including dietary shifts away from plant-based diets to high-energy foods, are responsible for the decrease of microbial diversity in modern humans [24]. Even though the oral microbiome has the ability to cope with disturbances without disrupting its symbiotic state, insults or modifications can alter the eubiotic balance from mutualism or commensalism to an unbalanced parasitic or pathogenic state, causing disease in the host [14]. This decline in variety is likely to contribute to dysbiosis, a condition that affects the makeup of bacterial communities and, in turn, microbial metabolism, which can either promote health or sickness [24]. Dysbiosis can be defined by three distinct possibilities that are not reciprocally exclusive and may occur concurrently: overall microbial diversity loss, loss of beneficial microorganisms, and expansion of microbial pathogens [26,44-46]. In order to prevent any local diseases and systemic consequences, researchers must first understand the complex microbial interactions that contribute to the transition from healthy to disease [14].

Oral dysbiosis has been implicated in the pathogenesis of a range of systemic and local diseases, including dental caries, periodontitis, diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, atherosclerosis, head and neck cancers, and Alzheimer's disease [24,26,29,47]. These studies confirm the presence of oral microbes in various parts of the human body, linking systemic diseases to oral dysbiosis and, as a result, providing new insights into those diseases [26]. *S. mutans*, *Veillonella*, *Actinomyces*, *Lactobacilli*, *Bifidobacterium*,

Propionibacterium, and *Scardovia* have previously been reported as caries-associated bacteria [16,25,48].

Microbial profiling

Human microbiota consists of about 100 trillion microbial cells that outnumber our human cells by a ratio of 10 to 1. Completing the human genome means characterizing the microbiome through profiling techniques. Currently, sequencing techniques targeting rRNA are employed to understand the functions encoded within these microbial genomes [7,49,50].

Bacterial profiling has evolved over many years through the development of various techniques. In the early time of bacterial profiling, researchers used cultivation techniques, which involved isolating specific bacteria from clinical samples and growing them in selective media culture. However, this approach is limited by the inability to culture multiple bacteria, or even if it is doable, with so many resources [51].

By the 1980s, microbial profiling began to advance with the introduction of modern techniques such as polymerase chain reaction (PCR), enabling more precise identification and characterization of oral microbial communities. Moreover, this method allows detection and identification of organisms that are hard to isolate using the amplification of DNA. Subsequent advancements in microbial profiling introduced gene sequencing technologies capable of simultaneously identifying multiple bacterial taxa within complex environments, such as dental plaque or saliva [52-55].

16S rRNA Sequencing Techniques

Due to its ability to amplify DNA from diverse sources, PCR has been widely applied in various fields, including the diagnosis of genetic disorders and the detection of low-level viral infections. In dentistry, PCR has been utilized for a broad range of applications, including the detection of periodontal and cariogenic pathogens, identification of microorganisms associated with endodontic infections, detection of viral genomes within host cells, identification of diagnostic genetic markers, and quantification of specific microbial populations [55,56].

Since 1996, Parra and Slots have provided evidence of viral presence within gingival crevicular fluid in advanced periodontal disease, highlighting the role of viruses in periodontal pathogenesis. Subsequent PCR-based studies have continued to yield significant findings, such as the work by Sakamoto *et al.* [57], which demonstrated higher quantities of *Treponema socranskii* in subgingival plaque compared to salivary samples. The utility of PCR has also enabled significant advancements in understanding the pathogenesis of periodontal disease, including the detection of human cytomegalovirus in crevicular fluid by Saygun *et al.* [58], and the identification of elevated expression of macrophage inflammatory protein-1 alpha (MIP-1α), interferon-gamma-inducible protein (IP-10), and their receptors C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 3 (CXCR3) in periodontal tissues, as reported by Garlet in 2003 [59,60]. Microorganisms such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *T. denticola*, and *Aggregatibacter actinomycetemcomitans* have been found during diagnosis of periodontal disease using the technology [56].

Prior to the advent of high-throughput sequencing technologies, PCR-amplified 16S rRNA gene sequences were commonly used to generate operational taxonomic units (OTUs), which were then compared to reference databases to infer the likely taxonomic identity of microbial constituents. Even though functional and beneficial, such use of 16S has necessitated some assumptions, such as the now-defunct notion that sequences with greater than 95% character represent the same genus and sequences with greater than 97% identity recognize the same species. Nevertheless, until recent times, high-throughput sequencing platforms were unable to generate accurate, full-length 16S sequences [61-63].

One thing that could affect the outcome of 16S rRNA sequencing is the DNA extraction methods [64-66]. A published report stated that DNA extraction methods highly affected the microbiome structure, with the best method being enzymatic-mechanical-lysis-based DNA [66]. Choosing the observed region also affects the diversity of the microbiome, in which V3-V4 and V4-V5 regions gave more accurate structure than the V1-V3 [66].

Circular consensus sequencing (CCS), in conjunction with sophisticated denoising algorithms 5-8 to remove PCR and sequencing error, allows the bias of millions of sequences reads that differ by as little as one nucleotide across the entire gene [67-69]. As a result of these technological and methodological advancements, it is now possible to exploit the full discriminatory potential of 16S in a high-throughput manner for the first time. A study published in 2019 revealed that the usage of full-length 16S sequence data from elevated sequencing has the opportunity to allow precise results of individual organisms at quite high taxonomic resolution [61].

Advanced technology in microbiome research

The application of innovative proteomic technology characterized by mass spectrum simplifies the creation of protein profiles, the acquisition of sequence information, and the analysis of acquired enamel pellicles. Label-free proteomics, which has been utilized in the past to do proteomic analysis on acquired enamel pellicles, is a promising method for estimating the changes in protein abundance in various disorders because of its widespread availability and high proteome coverage. Additionally, a cutting-edge focused quantification technique called parallel reaction monitoring (PRM) has been used to confirm relative protein abundance with high resolution and mass accuracy. The researcher used the label-free proteomics and PRM test combination to identify and validate specific protein biomarkers. Even so, it has not been widely used in the proteome and in vivo investigation of acquired enamel pellicle proteins at various time points under different caries stages [70,71].

The multi-omics procedure is a comprehensive transcriptome-proteome strategy for investigating the contribution of each organism in mixed biofilms, which is largely unknown in cross-kingdom biofilms [72,73]. Ellepola *et al.* (2019) found a unique cross-feeding pathway for GtfB in regard to caries, as well as novel clues into the synergistic cross-kingdom interaction between *S. mutans* and *C. albicans* in biofilms, using a multi-omics method [73]. They used lately established isobaric tags for relative and absolute quantitation (iTRAQ) [73,74]-based quantitative proteomics complemented by RNA sequencing (RNA-Seq)-based transcriptomics to assess the molecular pathways mediating *C. albicans* and its interactions with *S. mutans* in this virulent cross-kingdom relationship. The multi-omics approach combined with gene ontology (GO) pathway analysis and biochemical methods reveals that *C. albicans* play a significant role in carbohydrate metabolism and environmental acidification when interacting with *S. mutans*. In contrast, the presence of *C. albicans* altered the proteome of *S. mutans*, specifically those involved in carbohydrate usage and glucan biosynthesis. Furthermore, we revealed a fascinating cooperative mechanism in which the bacterial GtfB can contribute directly to C. albicans growth and metabolism by providing glucose and fructose from sucrose breakdown. These results highlight the significance of establishing therapeutic approaches that focus on bacterial interactions and fungal contributions related to a common illness [73].

Conclusion

Traditionally, oral disease diagnosis has relied on clinical symptoms, but advances in microbial research have revealed the complex role of host-microbe interactions in disease development. The dynamic nature of the oral microenvironment complicates the identification of causative agents, requiring a shift in our understanding of disease etiology. The oral microbiome—comprising bacteria, fungi, viruses, archaea, and protozoa—forms a tightly interconnected community where changes in host behavior or environment can lead to dysbiosis. Factors such as diet-induced microbial shifts and species like *S. mutans* and *Lactobacilli* have been implicated in caries and other oral diseases. Technological advancements such as PCR, proteomics, and multi-omics approaches have enhanced our ability to detect pathogens and explore microbial functions. These tools underscore the need for diagnostic and therapeutic strategies that address the complex, multispecies nature of oral biofilms, including contributions from fungal and viral members.

Ethics approval

Not required.

Acknowledgments

None to declare.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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