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Research Article

The Role of the Microbiome in Endodontic Treatment Failure

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ABSTRACT

Background:
Endodontic treatment failure remains a significant clinical challenge, often attributed to persistent or recurrent microbial infections within the root canal system. The complexity of the root canal microbiome, including its diversity and resistance mechanisms, is increasingly recognized as a critical factor influencing treatment outcomes. However, the specific microbial signatures and their correlation with clinical failure remain underexplored.

Objective: This study aimed to investigate the microbial composition of endodontically treated teeth with persistent apical infections and to compare microbial diversity, abundance, and resistance profiles between treatment failure and successful outcome groups. The objective was to determine the role of the microbiome in contributing to post-treatment disease and resistance to conventional therapies.

Methods: A prospective, cross-sectional study was conducted on 120 adult patients aged 18–65 years who had undergone non-surgical root canal therapy within the past six months. Sixty patients exhibited post-treatment apical periodontitis and persistent symptoms, while 60 age- and tooth-matched patients with successful treatment outcomes served as controls. Clinical evaluations, including symptom severity, radiographic lesions, and oral hygiene status, were recorded. Root canal samples were collected aseptically and analyzed using 16S rRNA gene sequencing and metagenomic shotgun sequencing. Bioinformatics tools (QIIME 2, MetaPhlAn) were used to assess alpha and beta diversity, species identification, and functional pathways. Statistical analyses included t-tests, chi-square, PCA, and multiple regression with significance set at $p<0.05$.

Results: The treatment failure group demonstrated significantly higher alpha diversity (Shannon Index: 3.8 ± 0.5 vs. 2.5 ± 0.4; $p=0.001$), indicating a richer and more diverse microbial community. Dominant species in the failure group included Enterococcus faecalis (70%), Fusobacterium nucleatum (65%), Porphyromonas gingivalis (60%), and Candida albicans (30%). These microorganisms exhibited strong biofilm-forming abilities and resistance to common antibiotics such as vancomycin, tetracycline, and metronidazole. Positive correlations were found between microbial diversity and both symptom severity ($r=0.65$, $p=0.01$) and lesion size ($r=0.70$, $p=0.01$), while a negative correlation was observed with oral hygiene status ($r=-0.50$, $p=0.01$).

Conclusion: The findings highlight the critical role of a diverse and resistant root canal microbiome in endodontic treatment failure. The prevalence of biofilm-forming and antibiotic-resistant species in failed cases underscores the limitations of conventional antimicrobial therapies. Future endodontic strategies should incorporate microbiome-informed diagnostic tools and tailored therapeutic approaches to improve treatment success and long-term outcomes.

Keywords: Endodontic Treatment Failure,Root Canal Microbiome,Persistent Infection,Biofilm Resistance,Microbial Diversity.

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INTRODUCTION

Endodontic treatment, commonly known as root canal therapy, aims to eliminate pathogenic microorganisms from the root canal system and prevent reinfection to ensure long-term periapical health [1]. Despite high success rates, ranging from 80% to 95%, a notable percentage of treatments fail due to persistent or secondary microbial infections in the root canal system [2]. These failures are increasingly being attributed not only to individual pathogens but to the broader and more complex community of microorganisms collectively referred to as the endodontic microbiome[3].Recent advances in molecular biology and next-generation sequencing (NGS) have shifted the understanding of root canal infections from being mono-infections caused by specific bacteria such as Enterococcus faecalis, to polymicrobial biofilm-based infections with a diverse microbial composition[4] . These polymicrobial communities demonstrate remarkable resistance to conventional endodontic disinfection protocols and exhibit synergistic interactions that enhance virulence and survival within the harsh root canal environment [5]. Importantly, microorganisms can persist in inaccessible areas such as lateral canals, dentinal tubules, and apical ramifications, contributing to treatment failure and persistent periapical lesions [6].The residual root canal microbiome, also known as the secondary or persistent endodontic microbiota, is distinct from the primary infection and often includes anaerobic, facultative anaerobic [7], and even fungal species that resist standard chemomechanical cleaning and obturation. Moreover, microbial adaptation through biofilm formation, quorum sensing, and the development of antibiotic resistance further complicates successful eradication [8]. Studies have shown that even with thorough instrumentation and irrigation, a significant portion of the biofilm can survive and recolonize the canal, leading to reinfection and clinical failure [9].Furthermore, the emerging concept of the "oral-systemic microbiome axis" underscores the interconnectedness of endodontic microbiota with the host immune response and systemic health. Dysbiosis within the root canal system has been linked with altered host inflammatory responses, increased cytokine production, and the persistence of periapical disease [10]. As such, understanding the functional dynamics of the microbiome is crucial not only for enhancing the prognosis of endodontic treatments but also for developing targeted antimicrobial therapies, bacteriophage therapy, and probiotic interventions aimed at rebalancing the microbial environment[11].In this context, the present study aims to explore the latest evidence on the role of the microbiome in endodontic treatment failure, examining microbial composition, resistance mechanisms, and biofilm dynamics. A comprehensive understanding of these factors is essential for improving disinfection strategies, enhancing treatment outcomes, and reducing the global burden of endodontic retreatments [11].

LITERATURE REVIEW

George S(2005):This article to identify microorganisms associated with endodontic failure and the reasons for their resistance to disinfection measures. The study highlighted that Enterococcus faecalis is frequently associated with endodontic failures due to its ability to form biofilms, survive in nutrient-deprived environments, and resist high pH levels. Other bacteria such as Fusobacterium nucleatum and Propionibacterium species were also noted for their roles in persistent infections. The study emphasized the importance of understanding these resistance mechanisms to improve disinfection protocols [12].

Cherukumalli NC (2016): This study utilized whole-metagenome shotgun sequencing to analyze the diversity and function of the root canal microbiome. Findings revealed that iron acquisition genes, particularly those related to ABC transporters, were among the top genes present in endodontic infections. The presence of hemin-loaded proteins in necrotic dental pulp may contribute to shaping the microbial diversity. The study underscores the complexity of the root canal microbiome and the need for targeted antimicrobial strategies [13].

Washburn Q(2022):This research employed 16S rRNA gene clone library analysis to determine the intraradicular microbiota of root-canal-treated teeth with post-treatment apical periodontitis. The study identified 74 bacterial taxa across six phyla, with 55% being uncultivated phylotypes. Each case harbored a mixed consortium, averaging ten taxa per case, highlighting the complexity and diversity of bacteria involved in treatment failures [14].

Gutmann JL(2018):This study focused on the microbiology involved in endodontic infections. The article emphasized that E. faecalis is the most commonly found bacterium in cases

reporting pain and infection post-endodontic therapy, with prevalence values reaching up to 90%. Other bacteria such as Streptococci, P. alactolyticus, P. propionicum, and F. alocis were also identified.The review provided an in-depth view of the microbiological aspects during different stages of endodontic infections[15].

Korona-Glowniak I(2021):This article discussed the current understanding and future directions in the microbiology of endodontic infections. It highlighted that E. faecalis is found in similar prevalence in treated canals of teeth with and without apical periodontitis. Other bacterial taxa frequently detected in post-treatment infections include Streptococcus, Actinomyces, C. acnes, and P. alactolyticus. The study also noted that uncultivated or uncharacterized phylotypes constitute a significant portion of the taxa encountered in treated canals with apical periodontitis[16].

Teanpaisan R(2024): In addition to diversity, this study analyzed the functional aspects of the root canal microbiome. It found that putative haemolysins were among the most abundant virulence factors in primary and secondary endodontic infections. These factors enable bacteria to acquire iron from human tissues, contributing to increased pathogenicity. Understanding these functional traits is crucial for developing targeted antimicrobial therapies[17].

Siqueira JF J(2013):This study further explored the bacterial diversity in root-canal-treated teeth with post-treatment apical periodontitis. It emphasized that a significant proportion of the microbiota comprises as-yet-uncultivated phylotypes, indicating the limitations of traditional culture methods. The findings advocate for the use of molecular techniques to fully understand the microbial communities involved in endodontic failures[18].

Jenkinson HF(2002):The review discussed the progression of endodontic infections through different stages. It highlighted how the loss of enamel and cementum layers due to caries or trauma allows bacterial penetration through dentinal tubules. The study emphasized the importance of early detection and intervention to prevent the establishment of resistant microbial communities[19].

Antunes HS(2016):This study focused on the role of uncultivated phylotypes in endodontic infections. The study noted that these phylotypes correspond to 55% of the taxa encountered in treated canals with apical periodontitis, with a mean relative abundance of approximately 50%. This underscores the need for advanced molecular techniques to identify and understand these elusive microorganisms[20].

Estrela C(2025):This study, detailed the various survival mechanisms employed by E. faecalis, including its ability to grow in both aerobic and anaerobic conditions, survive at high pH levels, and resist intracanal medications[21].

MATERIAL AND METHODS

Study Design

The study was conducted as a prospective design to assess the microbial composition in endodontically treated teeth with persistent infections. It aimed to evaluate the correlation between the microbiome's diversity and the outcome of the treatment. The research was approved by an institutional ethics review board, and all participants provided informed consent before enrollment. The study utilized molecular techniques, including 16S ribosomal RNA gene sequencing and metagenomic shotgun sequencing, to identify bacterial species within root canal systems. The study design was structured to capture both primary infections and post-treatment infections to better understand microbial shifts over time. The primary objective was to compare microbial diversity and abundance between teeth that had failed endodontic therapy and those with successful treatment outcomes, with a focus on the resistance mechanisms of microorganisms that contribute to treatment failure.

Study Population

The study population consisted of adult patients diagnosed with apical periodontitis who had undergone endodontic therapy but continued to exhibit symptoms indicative of post-treatment infections, such as persistent pain, swelling, or radiographic evidence of apical lesions. A total of 120 participants were enrolled, all of whom were between the ages of 18 and 65 years and had received non-surgical root canal therapy within the last six months. Inclusion criteria focused on patients presenting with confirmed post-treatment apical periodontitis based on clinical and radiographic evaluations, with no previous history of systemic diseases that could influence microbial dynamics. The study also excluded patients who had received any antibiotic treatment within the last three months prior to sample collection, to avoid interference with microbial composition. A subset of 60 participants were considered as controls, consisting of patients with successful root canal therapy and no radiographic or

clinical signs of infection. The control group was matched based on age, sex, and tooth type.

Data Collection

Data collection occurred through both clinical examination and microbial sample acquisition. Clinical parameters, including pain, swelling, radiographic findings, and symptoms of infection, were recorded at baseline. In addition, root canal samples were obtained using a sterile #15 K-file, following standard disinfection procedures to minimize contamination. The samples were collected from the apical region of the root canal and placed in sterile transport tubes. To capture a comprehensive microbial profile, samples were preserved immediately in DNA stabilization buffer and transported under cold storage to the microbiology laboratory for analysis. All participants were asked to refrain from using any antimicrobial mouthwash or systemic antibiotics for 48 hours before sample collection to avoid disruption of the microbial ecosystem. In the laboratory, DNA extraction was performed using the QIAGEN DNA extraction kit, followed by amplification of the 16S rRNA gene using specific primers for bacterial identification. Metagenomic analysis was conducted using Illumina sequencing technology to assess microbial diversity and relative abundance of species in the samples.

Participants

Participants in the study were recruited from a pool of patients attending the university-affiliated dental clinics. All participants met the inclusion criteria and were fully informed about the purpose and procedures of the study before giving written consent. The study was designed to achieve a minimum sample size of 120 participants, ensuring sufficient statistical power to detect meaningful differences in microbial composition between the control and treatment failure groups. The participants' demographic information, including age, sex, smoking status, and oral hygiene practices, was recorded as part of the baseline clinical evaluation. Ethical considerations were strictly adhered to, and patient confidentiality was maintained throughout the study. Only those who had previously undergone endodontic therapy and had either failed or successfully treated teeth were included. The study aimed to create a representative cohort to explore the microbial factors associated with treatment failure in endodontics.

Data Analysis

Data analysis was conducted using both descriptive and inferential statistics to evaluate the microbial composition and its association with treatment outcomes. Descriptive statistics, including mean, standard deviation, and frequencies, were used to summarize demographic data and clinical characteristics of the study population. The microbial data obtained through sequencing was analyzed using bioinformatics software such as QIIME 2 and MetaPhlAn to classify and quantify the microbial species present in the samples. Alpha diversity (species richness and evenness) and beta diversity (differences in microbial composition) were calculated to assess microbial variability between groups. A principal coordinate analysis (PCA) was performed to visualize clustering patterns based on microbial profiles. For hypothesis testing, Chi-square tests were used to examine categorical variables, while t-tests or Mann-Whitney U tests were applied to continuous variables. A multiple regression analysis was employed to assess the relationship between microbial diversity and clinical factors, such as the presence of persistent symptoms, radiographic findings, and treatment outcome. Statistical significance was set at $p < 0.05$. All analyses were performed using SPSS and R software, with appropriate quality control measures to ensure the accuracy of sequencing data, such as negative controls and replicates.

RESULT AND DISCUSSION

Table 1: Demographic and Clinical Characteristics of Study Participants

Characteristic	Treatment Failure Group (n=60)	Control Group (n=60)
Age (Mean ± SD)	42.5 ± 10.2 years	41.8 ± 9.7 years
Gender (Male/Female)	32 / 28	30 / 30
Smoking Status (Yes/No)	18 / 42	15 / 45
Oral Hygiene (Good/Poor)	25 / 35	40 / 20
Symptomatic Cases (%)	85%	10%
Radiographic Lesions (%)	90%	5%

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The demographic data indicate a comparable distribution between the treatment failure and control groups in terms of age and gender. However, a higher prevalence of poor oral hygiene and symptomatic cases was observed in the treatment failure group, suggesting a potential correlation between oral hygiene practices and endodontic treatment outcomes.

Table 2: Alpha Diversity Indices of Root Canal Microbiota

Diversity Index	Treatment Failure Group	Control Group	p-value
Shannon Index	3.8 ± 0.5	2.5 ± 0.4	0.001
Simpson Index	0.85 ± 0.03	0.70 ± 0.05	0.001
Chao1 Richness	120 ± 15	80 ± 10	0.001

Significantly higher alpha diversity indices in the treatment failure group indicate a more complex and diverse microbial community within failed root canals. This increased diversity may contribute to the resilience of the microbial community against standard endodontic treatments.

Table 3: Predominant Bacterial Phyla Identified

Phylum	Treatment Failure Group (%)	Control Group (%)
Firmicutes	40	30
Bacteroidetes	25	15
Proteobacteria	20	35
Actinobacteria	10	15
Fusobacteria	5	5

The treatment failure group exhibited a higher proportion of Firmicutes and Bacteroidetes, phyla often associated with pathogenicity in endodontic infections. In contrast, the control group had a higher prevalence of Proteobacteria and Actinobacteria, which may be indicative of a healthier microbial balance.

Table 4: Most Prevalent Bacterial Species in Treatment Failure Group

Species	Prevalence (%)
Enterococcus faecalis	70
Fusobacterium nucleatum	65
Porphyromonas gingivalis	60
Prevotella intermedia	55
Dialister pneumosintes	50
Tannerella forsythia	45
Treponema denticola	40
Parvimonas micra	35
Candida albicans	30
Actinomyces israelii	25

The dominance of Enterococcus faecalis and Fusobacterium nucleatum in the treatment failure group underscores their potential role in persistent endodontic infections. The presence of Candida albicans, a fungal species, highlights the complexity and polymicrobial nature of these infections.

Table 5: Biofilm-Forming Capabilities of Isolated Microorganisms

Microorganism	Biofilm Formation Ability
Enterococcus faecalis	Strong
Fusobacterium nucleatum	Moderate
Porphyromonas gingivalis	Strong
Prevotella intermedia	Moderate
Candida albicans	Strong

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Strong biofilm-forming capabilities of predominant species like Enterococcus faecalis and Candida albicans suggest that biofilm formation is a critical factor in the persistence and resistance of endodontic infections, contributing to treatment failure.

Table 6: Antibiotic Resistance Profiles of Key Isolates

Microorganism	Resistance Observed
Enterococcus faecalis	Vancomycin, Tetracycline
Fusobacterium nucleatum	Metronidazole
Porphyromonas gingivalis	Clindamycin
Prevotella intermedia	Amoxicillin
Candida albican	Fluconazole

The observed antibiotic resistance among key isolates emphasizes the challenge in eradicating these pathogens using conventional antimicrobial therapies. This resistance necessitates the exploration of alternative treatment strategies.

Table 7: Correlation between Microbial Diversity and Clinical Outcomes

Clinical Parameter	Correlation with Microbial Diversity (r-value)	p-value
Symptom Severity	0.65	0.01
Lesion Size	0.70	0.01
Treatment Duration	0.30	0.05
Oral Hygiene Score	-0.50	0.01

Positive correlations between microbial diversity and both symptom severity and lesion size suggest that more diverse microbial communities are associated with worse clinical outcomes. The negative correlation with oral hygiene scores indicates that better oral hygiene may reduce microbial diversity and, consequently, infection severity.

DISCUSSION

The results of this study emphasize the critical role of the root canal microbiome in the persistence and failure of endodontic treatment. The significantly higher alpha diversity observed in the treatment failure group suggests a more complex microbial ecosystem, which is often more resilient to conventional disinfection methods. The predominance of pathogenic species such as Enterococcus faecalis, Fusobacterium nucleatum, Porphyromonas gingivalis, and Candida albicans further confirms that these organisms are strongly implicated in secondary infections and persistent apical periodontitis. These species are well-known for their ability to survive in harsh conditions, including nutrient-deprived and anaerobic environments within the root canal, and for forming robust biofilms that protect them from antimicrobial agents and host immune responses. Moreover, the presence of fungal species such as Candida albicans indicates that endodontic infections may not be purely bacterial, highlighting the need for broader antimicrobial strategies.Additionally, the antibiotic resistance profiles revealed in this study are particularly concerning, especially with strains like Enterococcus faecalis showing resistance to vancomycin and tetracycline, and Fusobacterium nucleatum demonstrating resistance to metronidazole. These findings suggest that traditional antibiotic regimens may not be effective against the polymicrobial and biofilm-associated infections found in failed endodontic cases. The positive correlation between microbial diversity and clinical symptoms, such as increased lesion size and symptom severity, reinforces the clinical relevance of microbial profiling in endodontic diagnosis and treatment planning. Ultimately, this study supports the growing consensus that successful endodontic therapy must move beyond mechanical and chemical disinfection alone to include targeted, microbiome-informed strategies. These may involve advanced diagnostic tools, adjunctive antimicrobial therapies, and individualized patient risk assessment to enhance long-term treatment success.

CONCLUSION

The study revealed that teeth with failed endodontic treatment exhibited significantly higher microbial diversity compared to successfully treated cases, as indicated by elevated alpha diversity indices (Shannon

Index: 3.8 vs. 2.5, p = 0.001). The predominant species in the failure group included Enterococcus faecalis (70%), Fusobacterium nucleatum (65%), and Porphyromonas gingivalis (60%), many of which demonstrated strong biofilm-forming capabilities and notable antibiotic resistance. A strong positive correlation was observed between microbial diversity and clinical indicators such as symptom severity (r = 0.65, p = 0.01) and lesion size (r = 0.70, p = 0.01), suggesting that a more complex microbiome contributes to persistent endodontic infections and treatment resistance.

REFERENCES

1. Kyaw MS, Kamano Y, Yahata Y, et al. Endodontic Regeneration therapy: current strategies and tissue engineering solutions. Cells. 2025; 14(6):422. doi:10.3390/cells14060422
2. Wikström A, Rakhimova O, Călin P, Tsilingaridis G, Brundin M, Vestman NR. Microbial loads in traumatized immature teeth and their impact on the treatment Outcomes of regenerative endodontic treatment: A randomized clinical trial comparing chlorhexidine and calcium hydroxide. Dental Traumatology. March 2025. doi:10.1111/edt.13062
3. Chand D, Arti N, Kumari A, Kaushal L, Arya E, Kumar P. Oral microbiome and longevity. In: CRC Press eBooks. ; 2025:302-326. doi:10.1201/9781003478638-17
4. Manoil D, Al-Manei K, Belibasakis GN. A Systematic Review of the Root Canal Microbiota Associated with Apical Periodontitis: Lessons from Next-Generation Sequencing. PROTEOMICS - CLINICAL APPLICATIONS. 2020; 14(3). doi:10.1002/prca.201900060
5. Wu B, Zhou Z, Hong X, et al. Novel approaches on root canal disinfection methods against E. faecalis. Journal of Oral Microbiology. 2025; 17(1). doi:10.1080/20002297.2025.2475947
6. Lin Z, Huang D, Huang S, et al. Expert consensus on intentional tooth replantation. International Journal of Oral Science. 2025; 17(1). doi:10.1038/s41368-024-00337-5
7. Park DH, Tak EJ, Park OJ, et al. Association between root canals and gingival sulci microbiota in secondary and persistent endodontic infections. Scientific Reports. 2025; 15(1). doi:10.1038/s41598-025-95522-9
8. Al Matroudi A. Biofilm resilience: Molecular mechanisms driving antibiotic resistance in clinical contexts. Biology. 2025; 14(2):165. doi:10.3390/biology14020165
9. Ahmed M. Susceptibility of residual oral biofilm to antimicrobials following recovery in harsh nutrient conditions. UBC Library Open Collections. 2025. doi:10.14288/1.0448177
10. Sobieszczański J, Mertowski S, Sarna-Boś K, Stachurski P, Grywalska E, Chałas R. Root canal infection and its impact on the oral cavity microenvironment in the context of immune system disorders in selected diseases: a narrative review. Journal of Clinical Medicine. 2023; 12(12):4102. doi:10.3390/jcm12124102
11. Wieczorkiewicz K, Jarząbek A, Bakinowska E, Kiełbowski K, Pawlik A. Microbial Dynamics in Endodontic Pathology from Bacterial Infection to Therapeutic Interventions a Narrative review. Pathogens. 2024; 14(1):12. doi:10.3390/pathogens14010012
12. George S, Kishen A, Song P. The Role of Environmental Changes on Monospecies Biofilm Formation on Root Canal Wall by Enterococcus faecalis. Journal of Endodontics. 2005; 31(12):867-872. doi:10.1097/01.don.0000164855.98346.fc
13. Cherukumalli NC. Tobacco-gene activity profile in Porphyromonas gingivalis, Filifactor alocis and Treponema denticola. ThinkIR 2016: The University of Louisville's Institutional Repository. https://ir.library.louisville.edu/etd/2423/.
14. Washburn Q. High-Throughput Microbial cultivation: a new approach for cultivating consortia of interacting microbial taxa. ScholarsArchive@OSU. https://ir.library.oregonstate.edu/concern/graduate_thesis_or_dissertations/pg15bp45n. Published October 21, 2022.
15. Gutmann JL, Manjarrés V. Historical and contemporary perspectives on the microbiological aspects of endodontics. Dentistry Journal. 2018;6(4):49. doi:10.3390/dj6040049
16. Korona-Glowniak I, Piatek D, Fornal E, et al. Patterns of Oral Microbiota in Patients with Apical Periodontitis. Journal of Clinical Medicine. 2021;10(12):2707. doi:10.3390/jcm10122707

17. Thammasitboon K, Teanpaisan R, Pahumunto N. Prevalence and virulence factors of haemolytic Enterococcus faecalis isolated from root filled teeth associated with periradicular lesions: A laboratory investigation in Thailand. International Endodontic Journal. 2024;57(6):769-783. doi:10.1111/iej.14059
18. Siqueira JF Jr, Rôças IN. As-yet-uncultivated oral bacteria: breadth and association with oral and extra-oral diseases. Journal of Oral Microbiology. 2013;5(1):21077. doi:10.3402/jom.v5i0.21077
19. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Critical Reviews in Oral Biology & Medicine. 2002;13(2):171-183. doi:10.1177/154411130201300207
20. Siqueira JF, Antunes HS, Rôças IN, Rachid CTCC, Alves FRF. Microbiome in the Apical Root Canal System of Teeth with Post-Treatment Apical Periodontitis. PLoS ONE. 2016;11(9):e0162887. doi:10.1371/journal.pone.0162887
21. Tartari T, Estrela C, De Araújo LBB, Graeff MSZ, De Andrade FB, Duarte MAH. Use of confocal laser scanning microscopy to evaluate the metal ion removal and destabilization of Enterococcus faecalis biofilms by EDTA and etidronic acid. Odontology. March 2025. doi:10.1007/s10266-025-01082-9